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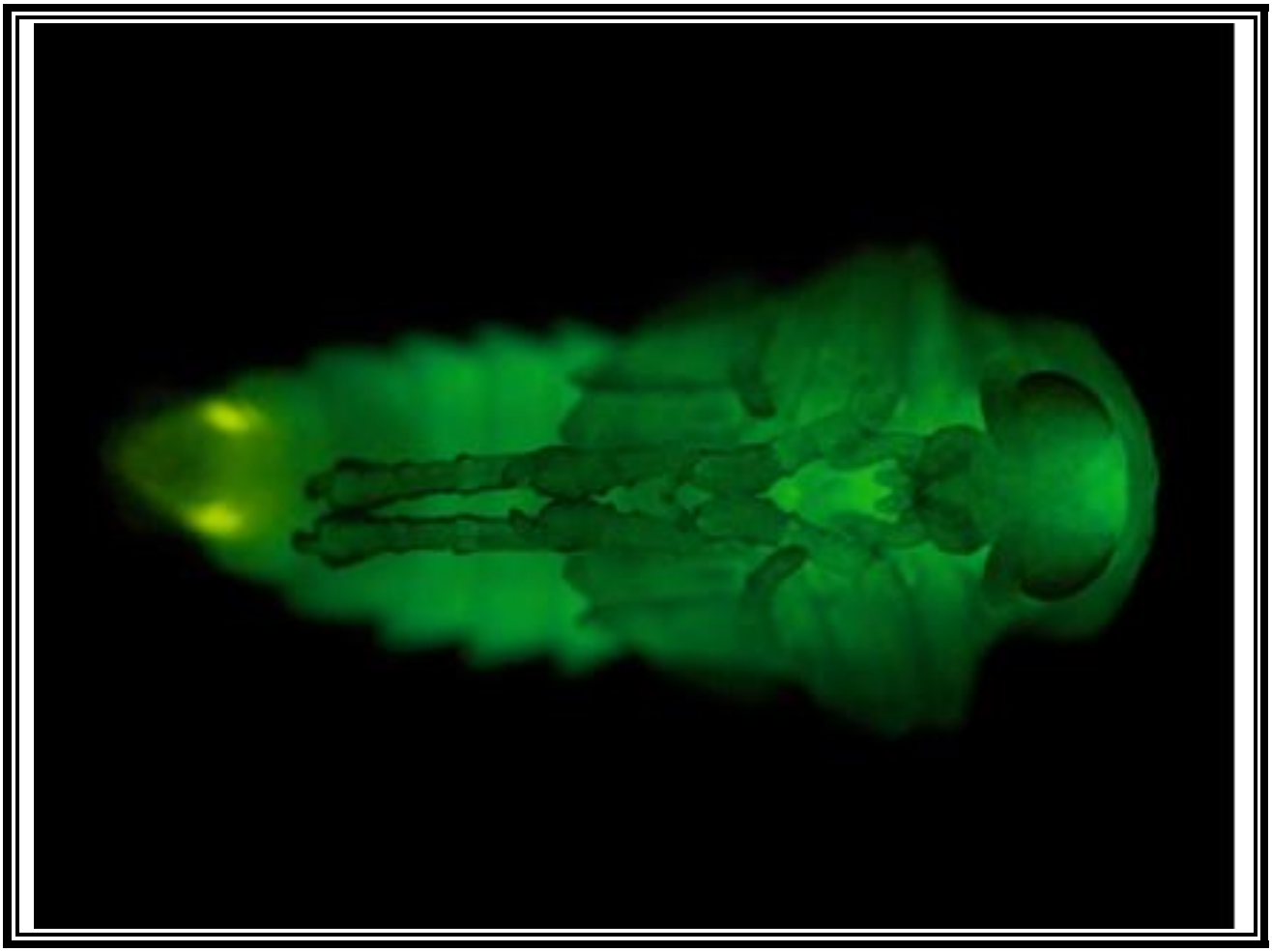

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Department of Bioagricultural Sciences and Pest Management

Colorado State University

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11. BIOLUMINESCENT BEHAVIOR OF NORTH AMERICAN FIREFLY LARVAE (COLEOPTERA: LAMPYRIDAE) WITH A DISCUSSION OF FUNCTION AND EVOLUTION

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Cover: Image: A photograph of a *Photuris* pupa showing the glow coming from two oval light organs and bright body glow from the body. (Photo by David Liittschwaer, extended time exposure, used with permission).

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Bioluminescent Behavior of North American Firefly Larvae (Coleoptera: Lampyridae) with a Discussion of Function and Evolution

ABSTRACT: Observations were made on the ecology, natural history, and glowing behavior of five North American species of firefly larvae, two *Pyractomena* LeConte, two *Photuris* LeConte, and one *Photinus* Laporte. These observations focused on response and periodic glowing. Response glows were long-lasting glows produced by resting/hiding larva in response to a threatening stimulus. Periodic glows were short spontaneous glows produced by actively crawling larva. Durations of three short periodic glowers averaged 0.8 to 3.5 seconds with a duty cycle of 30 to 46%. Durations of five long periodic glowers averaged 4.1 to 6.5 seconds with a duty cycle of 40-52%. Larvae started glowing ca. 1 hr. after sunset and glowed all night until about 20 minutes before sunrise. Some 72-87% of periodic glows were produced during locomotion. Glowing and locomotion were significantly affected by time in the laboratory and by feeding status. Larvae seemed to switch between response and periodic glowing as though these were two alternative physiological conditions. When firefly larvae were crawling and glowing periodically, the first defensive response to disturbance was to freeze and stop glowing periodically. When similar larvae were hiding the first response to disturbance was to glow responsively. Response glowing appears to be part of a package of defensive behaviors that includes: nocturnal activity, camouflage, freezing or fleeing, response glowing, and emitting defense chemicals. Periodic glowing appears to be part of a second package of defensive behaviors that includes: nocturnal activity, camouflage, stopping periodic glowing, and freezing or fleeing. Glowing of firefly larvae did not seem to be involved in prey capture or feeding. The interaction between larvae and ants was unexpectedly non-hostile, as though larvae had chemicals to pacify ants. Vertebrate predators were probably the driving force in the evolution of aposematic defenses. No evidence was found to support any of the non-defensive functions for bioluminescence in firefly larvae. The function of bioluminescence in firefly larvae can best be understood in the context of the evolution of bioluminescence. The forces that may have driven the evolution of bioluminescence may still be active in modern firefly larvae.

Key Words: bioluminescence, aposematic behavior, defensive behavior, periodic glows, response glows, natural history, predation, defense chemicals

GENERAL INTRODUCTION

This study was conducted to develop a better understanding of the function and/or survival value of larval bioluminescence in firefly larvae. There has been a lot of speculation about this and many interesting and sometimes contradictory ideas have been offered. Most of these ideas were based on fragmentary and/or anecdotal behavioral observations. In order to address this topic effectively, we need to consider available information on the biology and ecology of firefly larvae. Initial reports on the natural histories of North American *Photuris* LeConte 1851, *Pterotus* LeConte 1859 and *Pyractomena* LeConte 1850 larvae have been presented by Williams (1917), Hess (1920), Mclean *et al.* (1972), Dean (1979) and Buschman (1984a; 1984b; 1988). However, there is much less information available on the natural history of *Photinus* Laporte 1833 larvae, which are some of the most important fireflies in North America. In Europe, there are reports of the natural histories of *Lampyris noctiluca* (Linnaeus 1758), *Lamprohiza splendidula* (Linnaeus 1767) and *Phosphaenus hemipterus* (Goeze 1777) (Dreisig 1974; Schwalb 1961; Tylor 2002; De Cock 2004a; 2009). Natural histories of several species of Asian and African fireflies have also been reported and those published in English include: Okada (1928); Kaufmann (1965); Wang *et al*. (2007) and Fu *et al*. (2006; 2009).

To effectively address the survival value or function of bioluminescence in firefly larvae, we also need to understand the glowing behavior of firefly larvae. Therefore, in **Chapter 1** of this report, observations and data describing firefly larval bioluminescent behavior will be presented. In **Chapter 2,** observations and data describing the ecological and physiological factors that affect larval glowing will be presented. In **Chapter 3,** observations and data describing the larval bioluminescent behavior of firefly larvae during their interactions with other organisms including prey, natural enemies and competitors will be presented. Then in **Chapter 4** the evolution of bioluminescence in firefly larvae and the potential survival value or function of bioluminescence for firefly larvae will be discussed.

CHAPTER 1.

DESCRIPTION OF LARVAL GLOWING BEHAVIOR

INTRODUCTION

Early observations of glowing behavior of firefly larvae have been summarized by Sivinski (1981). Those observations, as well as my initial observations, were confusing. Sometimes larvae glowed in response to disturbance, but then at other times they stopped glowing in response to disturbance. As I continued my observations of this behavior, I realized that larvae that were actively crawling would glow spontaneously in a periodic manner and would respond to disturbance by stopping their spontaneous glowing. However, larvae that were resting and/or hiding were normally dark, but they would glow brightly when disturbed. This meant that it was important to recognize the type of larval activity that was associated with observations of larval glowing behavior. Therefore, it became important to recognize two types of larval glowing behavior, "**response glows"** and "**periodic glows**.**"** Response glows were long-lasting glows that were produced by resting and/or hiding larvae in response to a threat stimulus (like vibrations in the substrate). Periodic glows were shorter spontaneous glows that were produced by larvae that were actively crawling and hunting. In this chapter, I will present observations and data describing response and periodic glowing for five different types of North American firefly larvae.

MATERIAL AND METHODS

Study Sites

The larvae in these studies were observed in the field at several sites around the University of Florida (UF) campus, including Lake Alice and the Medical Plant Garden, Gainesville, Alachua Co., Florida, 1969-1976. Some larvae were also collected for observations in the laboratory. A few observations were made in 2011 at a wooded site near Knoxville (Knox Co.), Tennessee. Most of these larvae were "large" larvae—indicating last instars or near maturity (ca. 1.2 cm long).

Study Species

The identity of firefly larvae in these studies was recorded but the specific identity was sometimes impossible to determine. Most firefly larvae have not been described or associated

with the adult. *Pyractomena* LeConte 1850 larvae that were found climbing on emergent vegetation on Lake Alice (Fig. 1A–B) were identified with confidence as *P. lucifera* (Melsheimer 1846), because hundreds of these larvae were reared to the adult stage and identified by J.E. Lloyd (Buschman 1984b, 1988). Glowing observations were made on these larvae as they climbed on floating common water hyacinth, *Eichhornia crassipes* (Mart.) Solms. 1883 (Pontederiaceae), that covered large areas of Lake Alice. Some observations were made from a wooden catwalk that extended over the aquatic vegetation on Lake Alice. Other observations were made while wading in the shallow waters of Lake Alice. *Pyractomena* larvae that were found climbing on branches and vines in a lake-side forest (near Newnans Lake) were identified by J.E. Lloyd as *P. limbicollis* Green 1958 (Fig. 1D). These larvae did not accept food (snails) so they may have been climbing to find pupation sites. *Photinus* Leporte 1833 larvae (Fig. 2C) that were found on mats of floating dead vegetation on Lake Alice were identified as belonging to the "*Photinus ardens* LeConte 1851 species group (=complex)" (Green 1956). There were three different flash patterns produced by *P. ardens* complex fireflies on Lake Alice, suggesting there were three different species (Buschman 1977). These larvae were identified as *P. consimilis* Green 1956 in Buschman (1977). However, I have subsequently ascertained that the *Photinus* fireflies at the type locality in Missouri are more like *P. carolinus* Green 1956, a woodland/riverside firefly and not at all the marshland firefly observed in Florida. Therefore, these southern fireflies will be referred to the higher species group—the *P. ardens* complex of Green (1956). These three Florida populations have not been taxonomically described.

Adult *Photuris* LeConte 1851 fireflies cannot be identified with much confidence morphologically so neither can their larvae (Lloyd 2018, Buschman 1984a). Most *Photuris* fireflies are identified by their species-specific flash behavior. However, during this study it was not possible to have larvae or their reared adults produce their species-specific flash patterns (in captivity). Most of them could not be identified to species with confidence although a few of them were tentatively identified by J.E. Lloyd. The seasonal occurrence of these *Photuris* fireflies has been reported in Buschman (1984a). With experience it was found that *Photuris* larvae could be sorted into two categories, "red" and "non-red *Photuris* larvae." Red *Photuris* larvae had a rufous-brown dorsal color pattern (Fig. 1E). Hundreds of red *Photuris* larvae were reared, but only one pupated, producing a partially eclosed adult that was identified as *P. congener* LeConte 1852 by J.E. Lloyd (Buschman 1984a). They were found in more xeric leaf

litter, compared to non-red *Photuris* larvae. Non-red *Photuris* had a black or dark-brown dorsal color pattern (Fig. 1F). This category includes all the other *Photuris* species known to occur in these habitats. The non-red *Photuris* larvae occurred in wetter habitats, often along drainage areas and streams. Hundreds of "non-red *Photuris* larvae" were reared and many of them pupated and produced adults, but many would not pupate (Buschman 1984a). The category non-red *Photuris* larvae must be considered a mixture of about six species known to occur in these Florida habitats (Buschman 1984a). The size of field collected larvae was recorded as "large", "medium" and "small" to indicate the size relative to the largest and smallest larvae observed of each species.

Observations of Glowing Behavior

Observations of the glowing behavior of firefly larvae were made while using a headlamp covered with red cellophane. This red light appeared to be less disruptive to their activity than white light, but it also had some subtle effects, particularly when the light was shone directly on larvae. This red light also contrasts with the greenish-white larval glow, so the glow can be seen in the red light. An effort was made to collect observations from as many larvae as possible. Care was taken to avoid disturbing them, either with the light or by making movements or vibrations in the soil or water during the approach. Most observations were made from about one meter, close enough to observe larvae clearly and document activity.

Initially laboratory observations did not seem to work because the larvae did not seem to glow periodically in the laboratory. They seemed to only glow responsively. However, with experience it was found that periodic glowing would continue for a short time in the laboratory, but it tended to decrease over a one- to two-week period. Therefore, behavior observations in the laboratory were made only with larvae that had recently been collected from the field.

Larvae were held in various containers including aquariums (38 liter (10 gal.)) with soil and branches or in large petri dishes (15.5 cm diam. x 2.5 cm) with moist filter paper (soil was added for *Photuris* larvae). The containers were watered regularly to keep the humidity high. The larvae were given food about once a week (cut up earthworms (Oligochaeta) for *Photuris* and *Photinus* larvae and snails (Gastropoda) for *Pyractomena* larvae).

The periodic glowing of firefly larvae was recorded on a voice recorder while watching the larva. The glows were recorded as simple on—off events. These observations do not reflect the gradual on and off the typical larval glow or the glow modulations that occur during longer

glows. Some of the measurements of the length of each glow were timed with a stopwatch during play back of the voice recording. Other measurements were made by transferring the events to paper with an event recorder and measurements were made with a ruler and converted to time. Direct recording of events was not possible because the event recorder was not portable and behavior notes could not have been recorded in the dark. The terminology that Lloyd (2018) uses for describing firefly flash behavior will be adopted for use with larval glowing behavior: "glow duration" is the length of the glow, "glow period" is the time between the beginning of one glow and the next and "glow pause" is the time interval between two glows.

The frequency of glow durations and glow pauses (dark time) were plotted as the percent of glows or pauses occurring within 11 or 16 one-second increments, <1, 2, 3…10 seconds or <1, 1, 2, 3…15 seconds. The means and standard deviations were calculated for each type of larvae. These calculations were subject to a somewhat arbitrary limit of 10 or 15 seconds (longer glows were considered continuous glows and not included, longer pauses were considered inactive larvae and also not included). Therefore, it did not seem appropriate to proceed with mean separation statistics. The means will be treated as descriptive observations. The "duty cycle" was the glow time as a percent of total time.

RESULTS AND DISCUSSION

Introduction to Glowing Behavior

The two types of glowing behavior will be called **"response glows"** and **"periodic glows."** "**Response glows**,**"** are long sustained glows produced by the light organs of larvae that are motionless, resting or hiding. These glows seem to occur in response to external stimuli, such as vibrations in the substrate, which the larvae probably interpret as approaching danger. Response glows are relatively bright and can last a long time, often many minutes. "**Response glows**,**"** have also been called "induced" or "defensive" glowing (Dreisig 1974; Schwalb 1961; De Cock 2004a; 2009).

"Periodic glows" were usually short, only seconds long (1-20 seconds), and they are bright—similar to the response glow. They appeared to be spontaneous since there were no apparent external stimuli. They were produced by the light organ of larvae that are actively crawling or hunting. **"Periodic glows"** have also been called "spontaneous glowing" (Dreisig 1974; Schwalb 1961; Sivinski 1981, De Cock 2004a; 2009), "intermittent glowing" (Buck 1978)

or “lighthouse glowing” (Tyler 2002). I am calling these glows **“periodic glows”** to emphasize their recurring nature. The term “spontaneous glowing” does not give the sense of repeated or recurring glows.

Periodic Glowing

Periodic glowing by firefly larvae has been described as “irregular” with no regular pulse period (Dreisig 1974) or as “rhythmic” with a regular pulse period (Kaufmann 1965). This difference seems to be largely a function of glow duration and the species being observed. Dreisig was observing *Lampyris noctiluca* (Linnaeus 1758), a species with long glows (ca. 10 second) and long periods (up to 27 second) with a lot of variation, particularly in glow periods. He perceived the glows as “irregular”. On the other hand, Kaufmann was observing *Luciola discicollis* Castelnau 1833, a species with short glows (one to three seconds) and equally short periods with more limited variation. He described the glowing as rhythmic. The term “periodic glows” allows for some glows to be more rhythmic than others. The distribution of glow durations and pauses were different across different types of firefly larvae and across different larval growth stages. The periodic glowing behavior of five different types of North American firefly larvae will now be presented.

Pyractomena lucifera: The typical periodic glowing behavior of a *P. lucifera* larva is illustrated in Fig. 2A, B and H. These larvae were actively crawling on the aerial portions of aquatic vegetation. These glows appeared to be quite rhythmic. When the frequency of glow durations was plotted there was a relatively narrow distribution of glow lengths with a peak at one or two seconds (Fig. 4A and C). The frequency of glow pauses was a little wider with peaks between two and four seconds (Fig. 4B and D). The mean glow durations averaged 1.1 to 2.4 seconds and the glow pauses averaged 2.7 to 3.6 seconds for large or small larvae in the field or laboratory, respectively (Table 1A-B). In general, the measurements were a little shorter for glows in the laboratory than in the field. The duty cycle (glowing time as percent of total time) for periodic glowing was 40-41% for *P. lucifera* larvae in the field and 30 to 36% for larvae in the laboratory (Table 1A-B). Periodic glowing of *P. lucifera* larvae was faster and more rhythmic than glowing of most other larvae studied.

Pyractomena limbicollis: The typical periodic glowing behavior of a *P. limbicollis* larva is illustrated in Fig. 2C. These larvae were actively crawling and climbing on low branches and vines in the woods. These glows appeared to be less rhythmic than those of *P. lucifera*. When the

frequency of glow durations was plotted there was a relatively wide distribution with peaks from two to six seconds (Fig. 5A). When the frequency of glow pauses was plotted there was a wide distribution with peaks between two and seven seconds (Fig. 5B). The mean glow durations averaged 4.1 to 4.3 seconds and the glow pauses averaged 4.3 to 6.5 seconds for large larvae in the field or laboratory, respectively (Table 1C-D). In general, the measurements were a little longer for glows in the laboratory than in the field. The duty cycle for periodic glowing was 49% for *P. limbicollis* larvae in the field and 40% for larvae in the laboratory (Table 1C-D). The periodic glowing of *P. limbicollis* larvae was slower and less rhythmic than periodic glowing of *P. lucifera*.

***Photinus ardens* complex:** The typical periodic glowing behavior of a *Photinus* larva is illustrated in Fig. 2D. This larva was actively crawling on filter paper in the laboratory. These glows appeared to be somewhat rhythmic. When the frequency of glow durations was plotted there was a relatively wide distribution with peaks between two and seven seconds (Fig. 5C). When the frequency of glow pauses was plotted there was a wide distribution with peaks between two and five seconds (Fig. 5D). The mean glow durations averaged 6.5 seconds and the glow pauses averaged 6.1 seconds for large larvae in the laboratory (Table 1E). The duty cycle for periodic glowing was 52% for *Photinus* sp. larvae in the laboratory (Table 1E). The periodic glowing of *Photinus* sp. larvae was slower and less rhythmic than glowing of any of the other larvae.

Non-red *Photuris*: The typical periodic glowing behavior of a non-red *Photuris* larva is illustrated in Fig. 2E and G. This larva was crawling in the leaf litter on the forest floor. These glows appeared to be rhythmic at times and non-rhythmic at other times. When the frequency of the glow durations was plotted there was a relatively narrow distribution with peaks at one and four seconds (Fig. 6A). The frequency of glow pauses was a little wider with peaks between two and four seconds (Fig. 6B). The mean glow durations averaged 1.6 to 3.7 seconds and the glow pauses averaged 4.4 to 5.9 seconds for large larvae in the field or laboratory, respectively (Table 1F-G). In general, the glow duration measurements were shorter in the laboratory than in the field while field glow pause measurements were longer than laboratory measurements. The duty cycle for periodic glowing was 46% for non-red *Photuris* larvae in the field and 21%for larvae in the laboratory (Table 1F-G). The periodic glowing of non-red *Photuris* larvae was slower and less rhythmic than periodic glowing of *P. lucifera.*

Red *Photuris*: The typical periodic glowing behavior of a red-*Photuris* larva is illustrated in Fig. 2F. This larva was crawling in the leaf litter on the forest floor. These glows appeared to be non-rhythmic. When frequency of glow durations was plotted there was a relatively narrow distribution with a peak at two seconds (Fig. 6C). However, the plot of glow pauses was a little wider with peaks between two and four seconds (Fig. 6D). The mean glow durations averaged 2.8 seconds and the glow pauses averaged 4.7 seconds for large larvae in the field (Table 1H). The duty cycle for periodic glowing was 37% for red-*Photuris* larvae in the field (Table 1H). The periodic glowing of red-*Photuris* larvae was slower and less rhythmic than the glowing of *P. lucifera*.

Much of the variation in glow pauses seemed to be caused by larvae coming to obstacles and the glow rate seemed to slow down while they investigated the obstacle. Periodic glowing seemed to resume when larvae resumed crawling. This can be seen in Fig. 3 A-B where *P. lucifera* larvae that were glowing periodically but stopped glowing while they investigated the water's edge ("I" in Fig. 3A-B). Periodic glowing also seemed to stop when a *P. lucifera* larva stopped to groom itself ("Gl" in Fig. 3C). This behavior will be described in more detail in Chapter 2.

Periodic glowing also seemed to stop when larvae encountered a defensive situation. For example, when a *P. lucifera* larva was touched ("T" in Fig. 3C) it stopped periodic glowing, but it glowed continuously when it was picked up ("H" in Fig. 3C). When it was released it remained motionless for 39 seconds, but then started glowing periodically again as it began to crawl away. A non-red *Photuris* larva that was glowing periodically (Fig. 3D), also glowed continuously for 27 seconds when it was touched, but then it went dark and remained motionless. When it was picked up ("H" in Fig. 3D) it again glowed continuously for 18.5 seconds. However, when it started crawling away it glowed periodically. This behavior will be described in more detail in Chapter 2.

Periodic glowing also seemed to stop when a larva attacked its prey. In Fig. 3E, a *P. lucifera* larva attacked a snail ("At" in Fig. 3E) after which there were only occasional short glows as the larva pulled the snail out of the water and up the vegetation ("Dr" in Fig. 3E). Glowing stopped almost completely when the larva settled down to feed on the snail ("Fe" in Fig. 3E). In Fig. 3F, a non-red *Photuris* larva glowed periodically until it reached an earthworm. This larva went dark as it continued to feed at "Fe". In Fig. 3G, another non-red *Photuris* larva

was glowing continuously until it reached an earthworm and it also went dark as it continued to feed at "Fe". This behavior will be described in more detail in Chapter 3.

There was a physiological component to larval glowing behavior. For example, larvae that were held in the laboratory over a period of time seemed to stop periodic glowing, although they continued to glow responsively. In Table 1A-B, field *P. lucifera* larvae seemed to glow more freely than laboratory larvae, but it was not clear if these differences were meaningful. Larvae also seemed to switch between glowing periodically and response glowing. This can be seen in the glowing of a non-red *Photuris* larva (Fig. 2G) and in the glowing of a *P. lucifera* larva (Fig. 2H). These larvae seemed to be in an intermediate stage between the two physiological states associated with periodic and response glowing. This behavior will be described in more detail in Chapter 2.

In contrast to the above terrestrial observations, the *P. lucifera* larva that crawled into the water in Fig. 4B glowed continuously until it emerged from the water ("Uw" in Fig. 3B). It is interesting that Asian aquatic larvae also glow continuously when they are under water, but they are reported to glow periodically when they crawl on shore (Fu et al. 2006).

Since there is behavioral information for the periodic glowing of 12 species (or groups) of fireflies (Table 2), there appears to be two trends developing. First, the duty cycle (glowing time as a percent of total time) for glowing seems to remain constant across the species. Larvae glowed for 30-50% of the time, regardless of whether the glows were short or long. Second, the glow length seemed to fall into three categories; long periodic glows, short periodic glows and no periodic glows (Table 2). There was a group of 5 species that produced long glows (4-20 seconds) and even longer glow pauses (4.3-24.1 seconds) (Table 2). Then there was a second group of 6 species that produced short glows (0.8-4.3 seconds) and short glow pauses (ca. 1.0-6.5 seconds) (Table 2). There also seemed to be a group of larvae that did not produce periodic glows (*Lamprohiza splendidula* (Linnaeus 1767) (and possibly other members of the genus *Lamprohiza* Motshulsky 1853) (De Cock 2009)). The following suggestions are presented to explain these trends.

Phylogenetic Relationship: There seemed to be no phylogenetic relationships within or between the larval groups with long or short periodic glows (see the phylogenetic relationships presented by Branham and Wenzel 2000, 2003, Martin et al. 2017). However, the species that **do not** produce periodic glows, *Lamprohiza* spp. may be part of a more primitive clade (De Cock 2009).

Periodic glowing may be characteristic for most lampyrid larvae, but it is not known for most other related bioluminescent cantharoid beetles of the superfamily Elateroidea (Lloyd 1978; De Cock 2004a), although Buck (1948) describes "intermittent glows" in *Photuris* larvae and in *Phrixothrix* E. Olivier 1909 larvae (Lampyridae and Phengodidae). In any case, phylogenetic position does not explain the occurrence of most short or long periodic glowing among the lampyrids. We clearly need more information on the occurrence of periodic glowing among the bioluminescent cantharoid beetles.

Habitat: Sivinski (1981) suggested that the length of larval glows could be related to the "opacity" (=transparency?) of the environment and that they should be longer in more opaque environments. De Cock and Matthysen (1999) suggested that, with higher opacity, the time between glows should decrease and the duration of each glow should increase. However, my observations suggest the duration of glows and the pause between glows changed together so that the duty cycle remained at 30-50% for long and short glows. We don't have detailed information on the opacity of the habitats of the fireflies in Table 2; however, woodland habitats are expected to be more transparent than meadows and marshes which are covered with grasses (based on personal observations that larval glows are easier to observe in woodland rather than grassland habitats). In addition, both long and short periodic glows occur in woodland and in grassland habitats (Table 2). Sivinski (1981) also suggested that subterranean larvae (an opaque environment) will glow infrequently. *Photinus* larvae are normally thought to be subterranean, but the species complex in this study appeared not to be as subterranean as usual, since they were found on mats of decaying vegetation floating on the lake. These *Photinus* larvae produced very long glows with long pauses, but the duty cycle was similar to that of other larvae. My observations do not seem to support the opacity prediction. Sivinski (1981) suggested larvae would glow more frequently or more intensely when going into dangerous situations. He notes that larvae frequently glow periodically while crawling or hunting in open spaces or they glowed longer when crawling into the water (*P. lucifera*, Buschman 1977) versus crawling out of the water (*Aquatica cruciata* (Malschulsky 1854) and *A. lateralis* (Molschulsky 1860)) (Sivinski 1981). My observations somewhat support this hypothesis. Sivinski (1981) also suggested short periodic (or spontaneous) glows might be associated with surface dwelling fireflies. This may be the case. Additional observations are needed to fully evaluate these ideas.

Physiological or Developmental Factors: Small larvae tend to have shorter glows (*P. lucifera*, Table 1, and Kaufmann 1965). A reasonable number of small *P. lucifera* larvae were observed and recorded (Buschman 1988, Table 1), but small larvae of other species were seldom seen, so later instar larvae are the ones that were recorded in the field (De Cock 2009). The size of larvae does not explain the larger trends in Table 1 and 2. There seem to be small differences between individuals based on feeding history and developmental stage (De Cock 2009). These differences may relate to the relative need for periodic versus response glowing during different stages of development (Chapter 2).

Effects of Temperature: Bioluminescent behavior of adult fireflies has often been described as negatively correlated with temperature (Lloyd 1966; Buschman 1984b). It is likely that larval glowing behavior will show a similar negative correlation with temperature. I have not investigated the temperature correlations with larval behavior, but differences in temperature may be responsible for the small differences observed between laboratory and field observations (Table 1). Fu *et al.* (2006) report changes in glowing behavior of an aquatic larva over a 10^{o}C temperature range. Wang *et al.* (2007) report that in *Pyrocoelia pectoralis* (E. Oliver 1883) the duration of periodic glows increased while the pauses decreased over time during the night. It seems possible that these behavior changes could be due to temperature differences. The effects of temperature on larval glowing need to be investigated further.

Palatability: Sivinski (1981) suggested that aposematic displays should differ between species due to differences in palatability—levels of toxins or deterrents in the body. This is hard to evaluate now because we have no information on the levels of toxins or deterrents in different types of larvae. However, Marek and Moore (2015) were able to show a positive correlation between bioluminescent brightness and the toxicity of millipedes. This suggests bioluminescence in lampyrids could also be related to the levels of toxins in their bodies (Eisner et al. 1978; Day 2011; Chapter 4).

Predator Interactions: De Cock and Matthysen (1999) suggested that the timing of periodic glows could be related to the time it takes a predator to orient and attack. They reported that in dark conditions, the time the toad needed to focus and launch its attack was ca. 38 seconds. They reported that the glows of *L. noctiluca* averaged 6-8 seconds. All species in Table 2 have glows that are less than the 38 seconds. Periodic glows that were less than 38 seconds would allow larvae to produce the bioluminescent warning signal for general predators to warn them of their

unsuitability as food, but they would also be able to avoid specialized predators by not presenting the bioluminescent signal long enough for the predators to focus for an attack.

Function: It is likely that glow patterns are related to the function(s) of glows. There may even be several different functions at work. As explained above, the glows are probably a warning to general predators of the larval unsuitability as food. As stated above, the periodic glow may also help them avoid specialized predators by not presenting the signal long enough for predators to focus and launch an attack. This may explain the function of long glows, but it does not explain why the glows of other larvae are so much shorter. Larvae with short glows may be adding a third function to their repertoire. This third function (Flash Display and/or Enhanced Visibility) will be discussed in more detail in Chapter 4 in a more extensive discussion of the functions of bioluminescence in firefly larvae. Apparently, not all larvae need this third function, so they continue producing the long glows—glows just short enough to avoid predation. These two types of larvae may have different predators or different predator pressures.

Sivinski (1981) and De Cock and Matthysen (1999) suggest that periodic glowing represented a "facultative aposematic display." This may very well be the case; however, the evidence supporting this conclusion is tenuous. The facultative nature of the aposematic display will be discussed in more detail in Chapter 4. There needs to be more careful observation of larval behavior and natural history to associate larval glowing behavior with other ecological conditions. In my observations, periodic glowing was strongly associated with larval crawling activity-which was probably hunting activity (Chapters 2 and 3). Additional observations will need to be done to see if we can build a case for periodic glowing being a part of a second suite of defensive behaviors (discussed further in Chapter 3).

Response Glowing

In addition to periodic glowing, firefly larvae also produce response or defensive glowing. This occurs when larvae are sedentary, motionless and/or hiding. This glowing continues for many minutes, making it sometimes difficult to identify the stimulus that was responsible for the initiation of the glow. These glows are bright and continue for many seconds or minutes, but they were not often actually timed in this study. Occasionally, it was observed that active larvae exhibit a mixture of periodic glows and continuous bright glowing (Fig. 4G). Response glowing occurs within the context of several other defensive activities, so it is easy to

conclude that these glows are part of a defensive suite of behaviors that includes the aposematic display (discussed further in Chapter 3).

Other Glowing Behaviors

Response and periodic glowing have often been observed in the same population, sometimes at the same time (Fig. 4F-G). This was seen most clearly in red *Photuris*. After a long gentle rain event in fall, all the red larvae were observed glowing periodically while crawling. As time passed (several days) and the habitat dried out, fewer and fewer larvae would be observed glowing periodically. At the same time, one would see increasing numbers of larvae glowing responsively as the larvae became quiescent. They seemed to switch to responsive glowing—glowing in response to various stimuli—when in this quiescent state. The larvae were observed to respond to vibrations in the ground or leaf litter from my footsteps. At times, I could see a string of continuous glowing larvae, following my footsteps into the habitat. Dean (1979) also reports observing a string of glowing larvae following his footsteps in the habitat. Although not conclusive, this suggests that larvae go through a change in motivational or physiological status as the habitat dries out. The disturbance threshold for response glowing probably changes with hunting conditions, feeding and/or developmental status. De Cock (2009) suggested that newly molted larvae did not glow readily, but larvae that were ready to molt or pupate glowed readily. However, he also suggested that some species may glow only when ready to molt or pupate. These changes are probably associated with physiological changes associated with changing from periodic to response glowing (discussed further in Chapter 2).

Not all the glowing behavior of larvae can be categorized as either periodic or response glowing. In eastern Tennessee, larvae were observed that were glowing continuously while they crawled about (Fig. 3G). These glows appeared to be response glows, but there did not seem to be any stimulus. These glows may represent an intermediate physiological stage between response glowing and periodic glowing.

There were also times when larvae that were known to glow periodically were seen crawling actively, but not glowing periodically. Active non-glowing larvae have been reported by Hess (1920), Schwalb (1961), McLean *et al.* (1972), Dreisig (1974), Tyler (2002) and De Cock (2009). This behavior may be common in the field at night, but we would not know about it because we normally locate firefly larvae by their glowing in the dark (discussed further in Chapter 2).

There may be additional types of larval bioluminescence. I have observed *Luciola* larvae in Africa that appeared to produce flare-like glows. There may also be additional glowing behaviors associated with the aquatic larvae in Asia. I would encourage other workers to carefully record responsive versus periodic glowing when they report on the bioluminescent behavior of firefly larvae.

CONCLUSIONS

In summary, several observations are reported for response and periodic glowing in firefly larvae. Response glows were long-lasting glows produced by the light organ of resting and/or hiding larva in response to a stimulus, like vibrations in the substrate. Response glowing seemed to be an aposematic signal warning of toxic or deterrent chemicals in their bodies (see also Chapter 3 and 4). Periodic glowing was a string of shorter spontaneous bright glows that were also produced by the light organ, but by larvae that were actively crawling and hunting. The survival value or function of larval glowing will be discussed in Chapter 4.

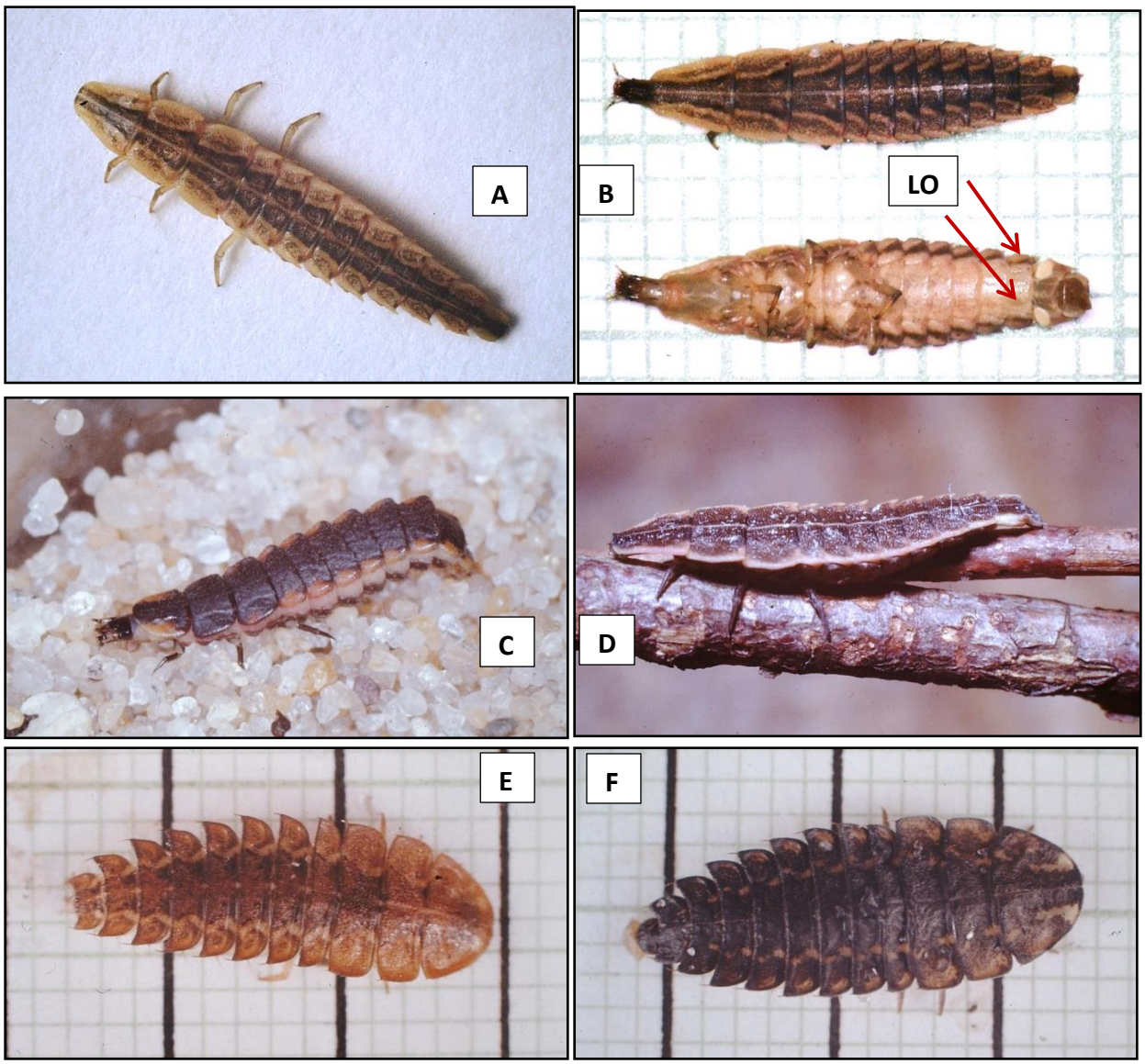


Figure 1. Firefly larvae that were studied. **A.** *Pyractomena lucifera*. **B.** *Pyractomena lucifera*, dorsal and ventral views showing the light organs (LO). **C.** *Photinus* sp. **D.** *Pyractomena limbicollis*. **E.** Red *Photuris*. **F.** Non-red *Photuris*. All larvae are about the same scale. See the vertical dark lines in **E.** and **F.** which are 5 mm apart.

Figure 2. Examples of glowing by different kinds of larvae: Time wraps after 40 seconds one to four times for up to 160 seconds. The number at the left indicates first to fourth cycle of 40 seconds. **A.** *Pyractomena lucifera* third instar: periodic glowing larva, 26 Nov. 1975. **B.** *Pyractomena lucifera* fifth instar: periodic glowing larva, 26 Nov. 1975. **C.** *Pyractomena limbicollis* fifth instar: periodic glowing larva climbing on a branch, 26 Jan. 1976. **D.** *Photinus* sp. larva: periodic glowing larva crawling in a petri dish, 10 Apr. 1975. **E.** Non-Red *Photuris* larva: periodic glowing larva crawling in the field, 25 Oct 1975. **F.** Red *Photuris* larva: periodic glowing larva crawling in leaf litter, 18 Oct 1975. **G.** Non-Red *Photuris* larva: mixture of continuous glowing and periodic glowing, 4 Nov. 1975. **H.** *Pyractomena lucifera* fifth instar: mixture of continuous glowing and periodic glowing, 26 Nov. 1975.

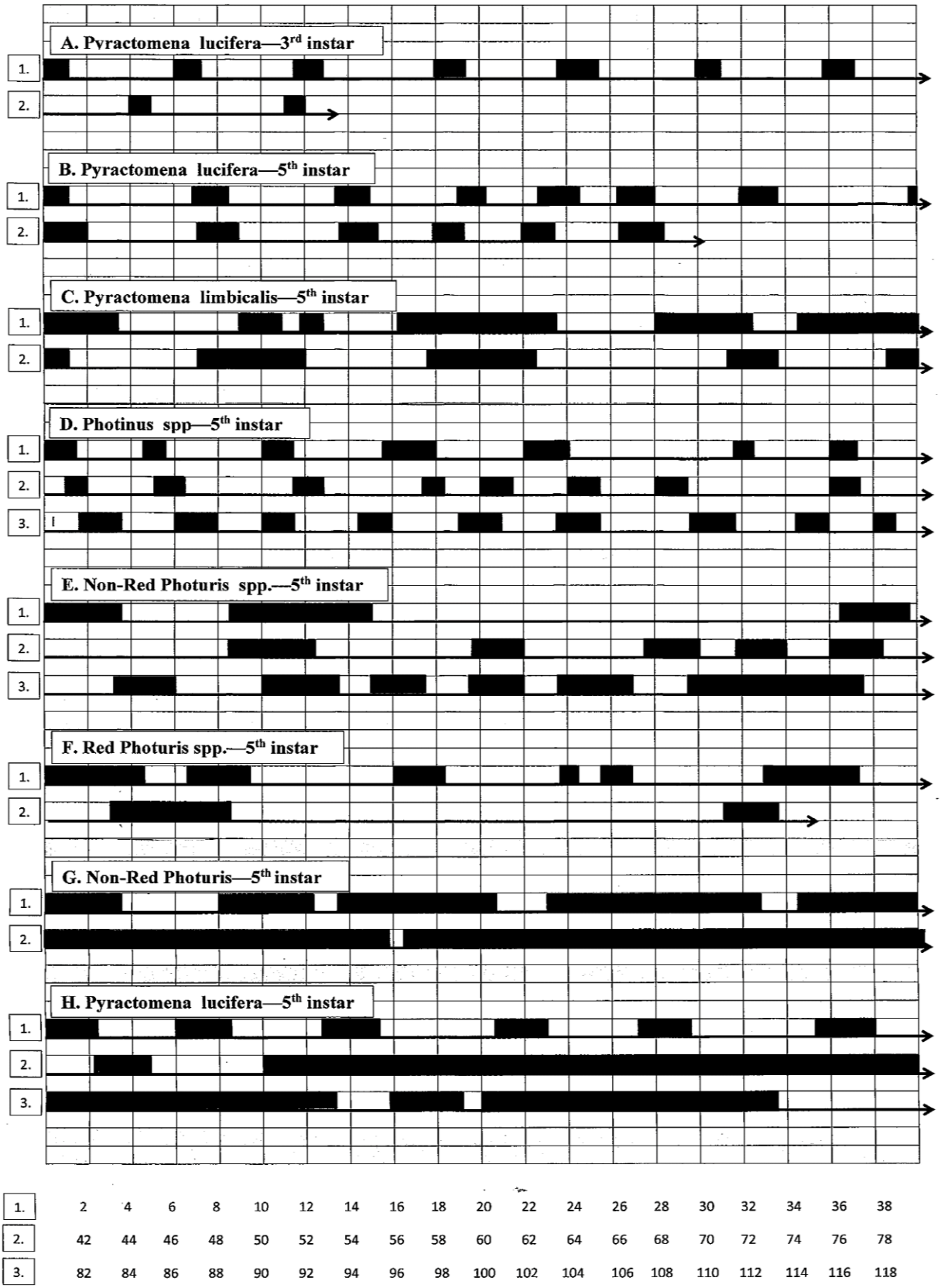
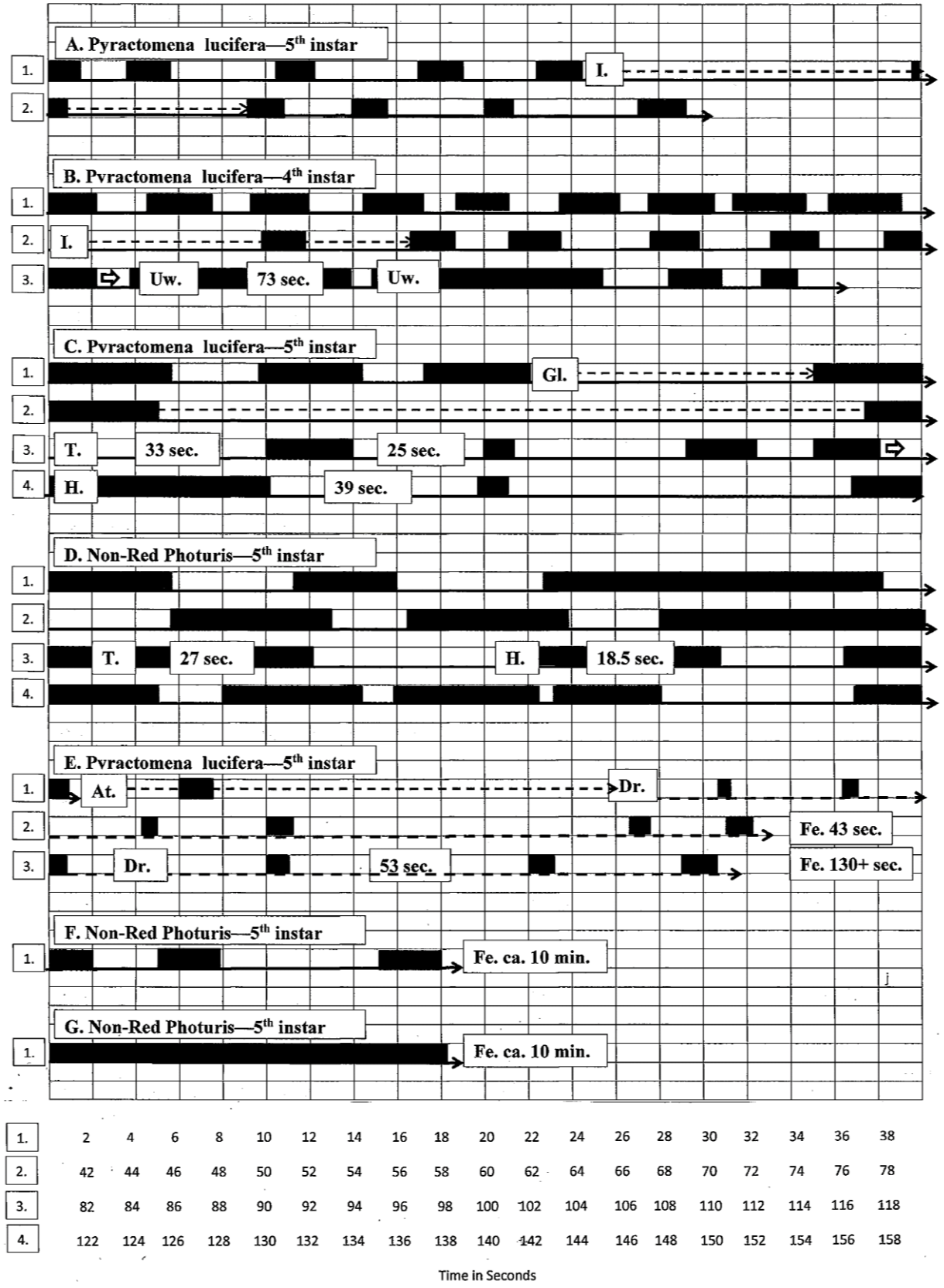

A. Pyractomena lucifera—3rd instar
1.
2.
B. Pyractomena lucifera—5th instar
1.
2.
C. Pyractomena limbicalis—5th instar
1.
2.
D. Photinus spp—5th instar
1.
2.
3.
E. Non-Red Photuris spp.—5th instar
1.
2.
3.
F. Red Photuris spp.—5th instar
1.
2.
G. Non-Red Photuris—5th instar
1.
2.
H. Pyractomena lucifera—5th instar
1.
2.
3.
1. 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38
2. 42 44 46 48 50 52 54 56 58 60 62 64 66 68 70 72 74 76 78
3. 82 84 86 88 90 92 94 96 98 100 102 104 106 108 110 112 114 116 118

Figure 3. Examples of larvae glowing in various situations: Time wraps after 40 seconds one to fourth times for up to 160 seconds. The number at the left indicates first to fourth cycle of 40 seconds. **A.** *Pyractomena lucifera* larva crawling and glowing periodically. It then investigates the water's edge ("I" and dashed line) but then continues crawling and glowing periodically, 6 December 1975. **B.** *Pyractomena lucifera* larva crawling and glowing periodically and then investigates the water's edge ("I" and dashed line). It then continues crawling and glowing periodically. Then it crawls under water (UW) and glows continuously. When it emerges, it continues to crawl while glowing periodically, 26 Nov. 1975. **C.** *Pyractomena lucifera* larva crawling and glowing periodically until it stops to groom itself (Gl and dashed line) and it stops glowing. It then continues crawling and periodic glowing and is touched (T) with bristles of a brush and it becomes motionless and stops glowing for 33 seconds. Then it crawled away glowing periodically. When the larva is picked up ("H") it glows continuously in the hand and then goes dark and motionless (39 seconds). Then it crawled off glowing periodically, 15 Nov. 1975. **D.** Non-Red *Photuris* larva is crawling and glowing periodically. When the larva is touched ("T") with the bristles of a brush it glows continuously and then goes dark. When it is picked up in the hand ("H") it glows continuously and then goes dark but soon starts crawling and glowing periodically, 14 Nov. 1975. **E.** *Pyractomena lucifera* larva is crawling and glowing periodically when it attacks a snail ("At") and the glowing slowed down. Then, later when the larva started dragging the snail up the vegetation ("Dr" twice) there were short periodic glows. When the larva settled down to feed ("Fe" twice) there was no glowing, 26 Nov. 1975. **F.** Non-Red *Photuris* larva is crawling and glowing periodically until it comes to an earthworm. It starts feeding and goes dark, 27 Sept. 2011. **G.** Non-Red *Photuris* larva is crawling and glowing continuously until it comes to an earthworm. It starts feeding and goes dark, 27 Sept. 2011.


A. Pyractomena lucifera—5th instar
1.
I.
2.
B. Pyractomena lucifera—4th instar
1.
2.
I.
3.
Uw.
73 sec.
Uw.
C. Pyractomena lucifera—5th instar
1.
Gl.
2.
3.
T.
33 sec.
25 sec.
4.
H.
39 sec.
D. Non-Red Photuris—5th instar
1.
2.
3.
T.
27 sec.
H.
18.5 sec.
4.
E. Pyractomena lucifera—5th instar
1.
At.
Dr.
2.
Fe. 43 sec.
3.
Dr.
53 sec.
Fe. 130+ sec.
F. Non-Red Photuris—5th instar
1.
Fe. ca. 10 min.
G. Non-Red Photuris—5th instar
1.
Fe. ca. 10 min.
1. 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38
2. 42 44 46 48 50 52 54 56 58 60 62 64 66 68 70 72 74 76 78
3. 82 84 86 88 90 92 94 96 98 100 102 104 106 108 110 112 114 116 118
4. 122 124 126 128 130 132 134 136 138 140 142 144 146 148 150 152 154 156 158
Time in Seconds

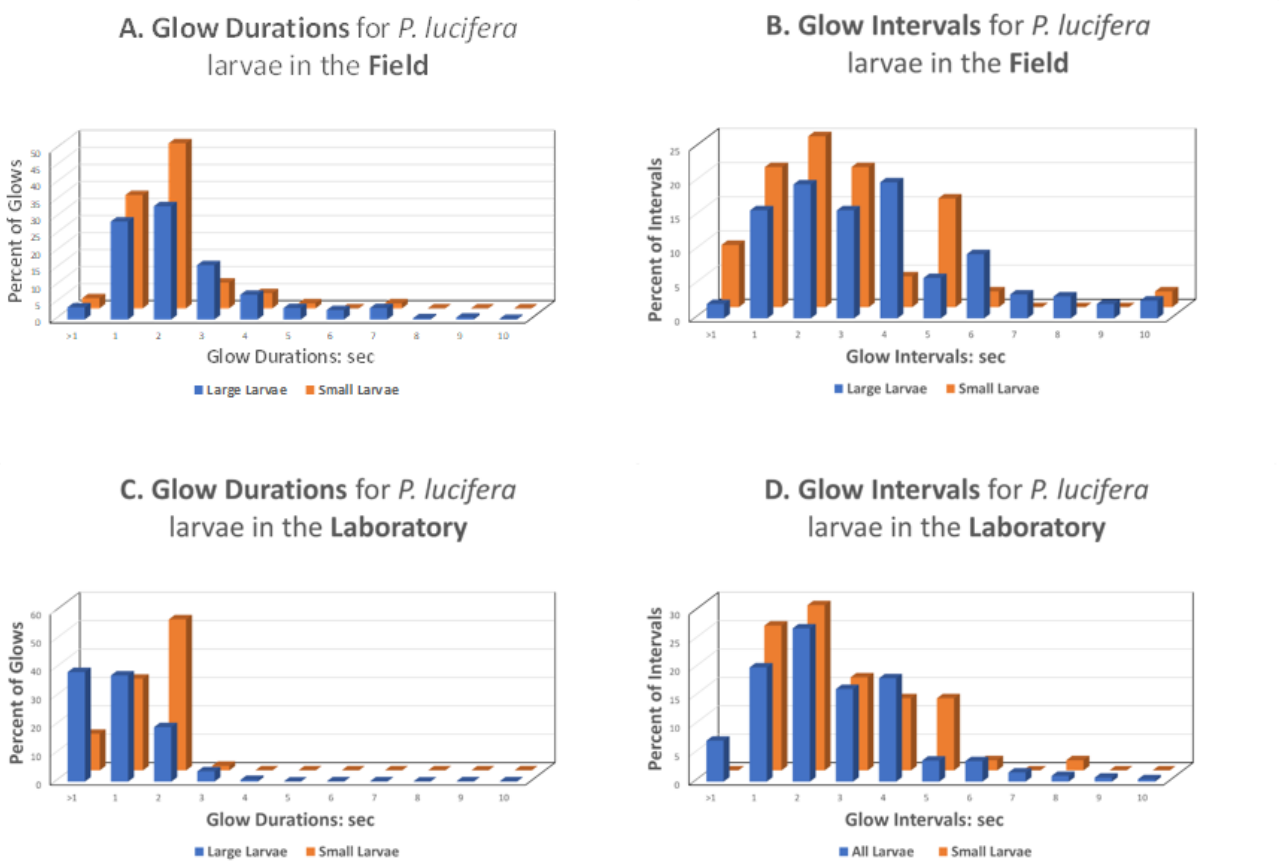


Figure 4. A. and B. Periodic glow durations and pauses for *Pyractomena lucifera* larvae in the field; **C. and D.** Periodic glow durations pauses for *P. lucifera* in the laboratory. The data are presented in two categories: small is ≤ half cm, large is ≥half cm.

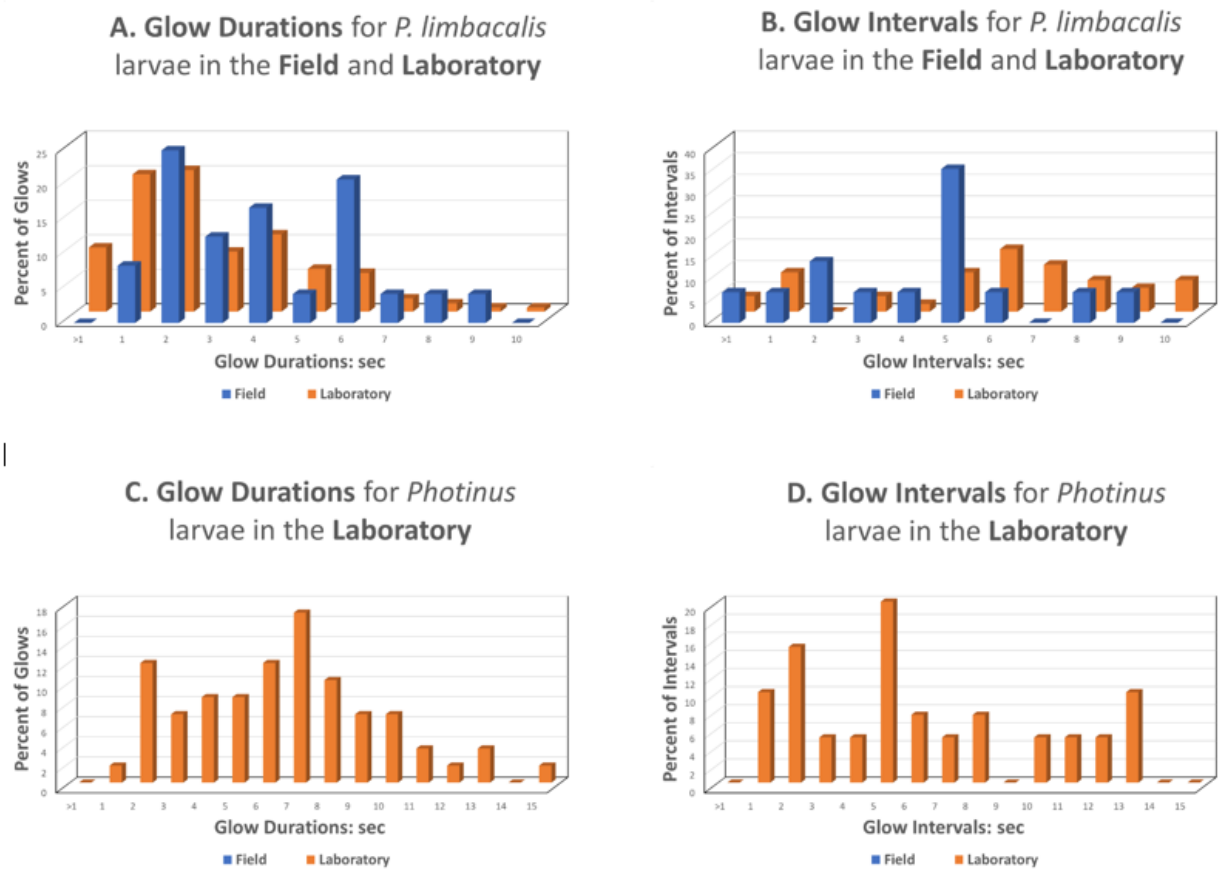


Figure 5. A. and B. Periodic glow durations and pauses for large *Pyractomena limbicollis* larvae in the field or laboratory; **C. and D.** Periodic glow durations and pauses in the laboratory for *Photinus* sp. larvae.

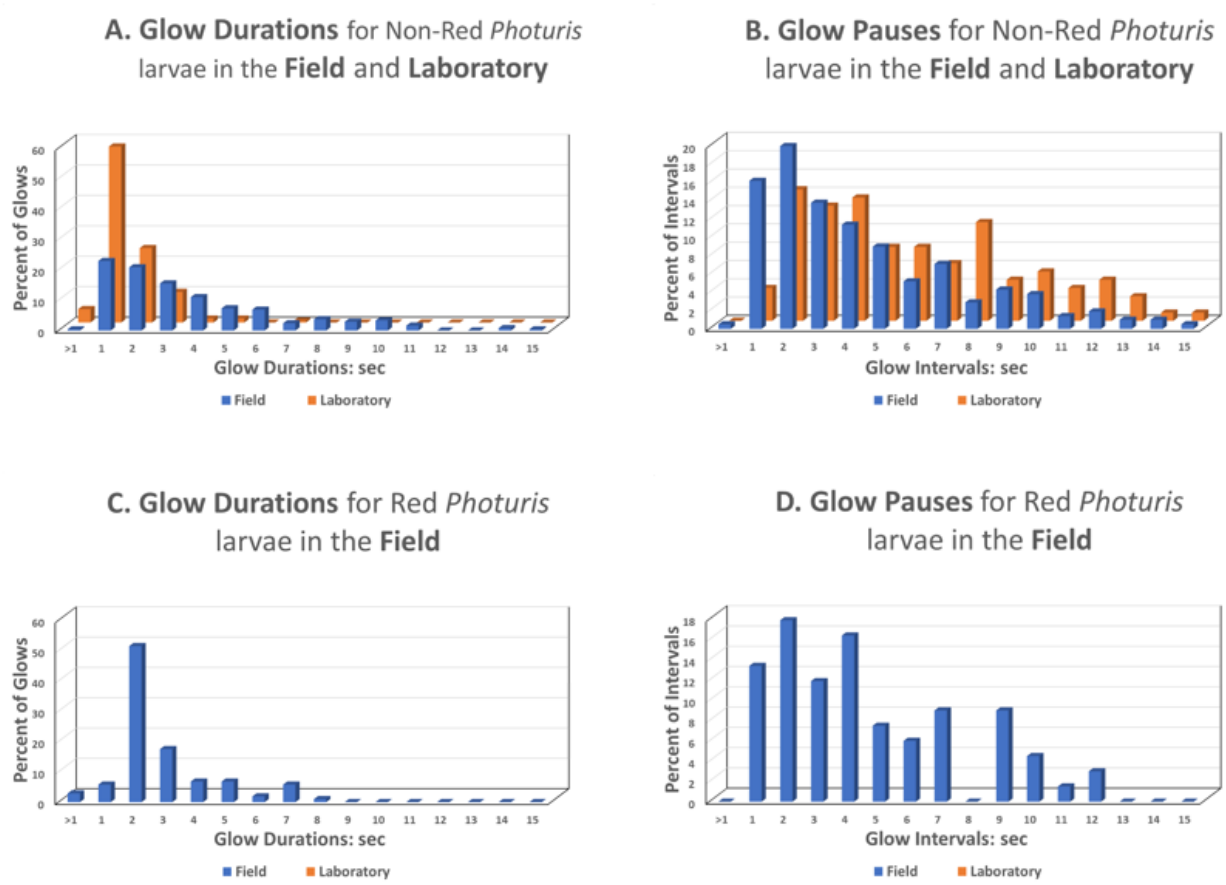


Figure 6. A. and B. Periodic glow durations and pauses for large Non-Red *Photuris* larvae in the field or laboratory; **C. and D.** Periodic glow durations and pauses in the field for Red *Photuris* larvae.

Table 1. Means and standard deviations (SD) for glows and inter-glows of five different taxa of firefly larvae).

Firefly Studied	Number of larvae	Number of glows	Number of glows pauses	Glow duration Mean-sec	SD for glow duration	glow pause Mean-sec	SD for glow pause	% of Total Time glowing
A. *P. lucifera*—Field								
Large larvae	43	468	341	2.4	1.68	3.6	2.29	40
Small larvae	6	66	44	1.9	1.08	2.7	1.88	41
B. *P. lucifera*—Lab.								
Large larvae	16	671	670	1.1	0.88	2.7	1.76	30
Small larvae	3	62	55	1.5	0.61	2.7	1.58	36
C. *P. limbicollis*—Field								
Large larvae	3	24	14	4.1	2.20	4.3	2.37	49
D. *P. limbicollis*—Lab.								
Large larvae	12	161	55	4.3	4.20	6.5	2.91	40
E. *Photinus*—Lab.								
Large larvae	5	59	40	6.5	3.14	6.1	3.85	52
F. *Photuris* non-red—Field								
Large larvae	20	245	210	3.7	2.86	4.4	2.55	46
G. *Photuris* non-red—Lab.								
Large larvae	11	159	111	1.6	0.95	5.9	3.45	21
H. *Photuris* red—Field								
Large larvae	9	103	72	2.8	1.61	4.7	2.95	37

Table 2. Summary of known examples of periodic glowing in firefly larvae.

Species	Source	Glow duration means—sec.	Pause duration means--sec	Duty cycle %	Season	Habitat activity
Short Periodic Glows						
Pyrocoelia pectoralis	Wang *et al.* 2007	1-5	3-7	25-42%	May-Sept.	Meadows-Climbs on soil in abandoned farm fields
Pygoluciola qingyu	Fu & Ballantyne 2008	2.5-4	--	--	--	Marsh and river bank
Luciola discicollis	Kaufmann 1965	0.8-2.0	ca. same	ca. 50%	Rainy season	Meadows Climbs on grass
Pyractomena lucifera	This report	1.1-2.4	2.7-3.6	30-41%	All year	Marsh Climbs on vegetation
Photuris red	This report	2.8	4.7	37%	Fall	Woods and meadows Climbs in leaf litter
Photuris non-red	This report	1.6-3.7	4.4-5.9	21-46%	All year?	Woods Climbs in leaf litter

Table 2. (continued).

Species	Source	Glow duration means—sec.	Pause duration means--sec	Duty cycle %	Season	Habitat activity
Long Periodic Glows						
Luciola leii[1]	Fu *et al.* 2006	11-16	9-20	42-56	All year ??	Aquatic—Climbs on substrate or vegetation
Lampyris nociluca	De Cock 1999 De Cock 2009 Dreisig 1974	-- 6-8 4.5-8.8	24.8 -- --	-- -- 20-32	Fall and Spring	Meadows Climbs on grass
Phosphaenus hemipterus	De Cock 2009	8-16	--	--	Fall	Woods Climbs in leaf litter
Pyractomena limbicollis	This report	4.1-4.3	4.3-6.5	40-49	Nov.	Woods Climbs on shrubs
Photinus sp. complex	This report	6.5	6.1	52	Early Spring	Marsh—Climbs on Floating mats vegetation
No Periodic Glows						
Lamprohiza splendidula	De Cock 2009	--	--	--	--	Woods Climbs in leaf litter

[1] This Fu et al. (2006) report on larval glowing behavior is confusing. The text says larvae glow continuously under water, but they present periodic glowing data in a table (I wonder if this periodic glowing was for larvae that were climbing on land?). In personal communication with X. Fu, he stated that there was no fixed glowing pattern.

CHAPTER 2.

ECOLOGICAL AND PHYSIOLOGICAL EFFECTS ON LARVAL GLOWING BEHAVIOR

INTRODUCTION

A description of the glowing behavior of several North American firefly larvae has been presented in Chapter 1. Now we need to discuss how ecological and physiological factors affect the glowing behavior of firefly larvae. As stated earlier, larval glowing behavior must be discussed in terms of **response glowing** or **periodic glowing**. **Response glows** were long-lasting glows produced by resting and/or hiding larvae in response to a threat stimulus (like vibrations in the substrate). **Periodic glows** were shorter spontaneous glows produced by larvae that were actively crawling and hunting.

Firefly larvae are known to be active during the night; however, the details of this activity are poorly documented. In North America, McLean *et al.* (1972) observed that *Photuris* larvae started glowing at dusk and continued glowing until midnight—when observations ended. This is all the information we have for the daily activity of North American firefly larvae. In Europe, *Lampyrus noctiluca* (Linnaeus 1767), *Phosphaenus hemipterus* (Goeze 1777) and *Lamprohiza splendidula* ((Linnaeus 1767) larvae were found to be active for many hours each night (Schwalb, 1961; Tylor, 2002; De Cock, 2004a). The species differed as to when their activity started and when the activity peaked. This contrasts with the many observations of the activity of many North American adult fireflies which are active for only 15 to 60 minutes each evening (Lloyd, 1966; Buschman, 1977), although Buschman (2017b) and Lloyd (2018) have reported that some adult *Photuris* fireflies can be active throughout the night. Knowing the daily activity cycle of firefly larvae will inform us on when we need to make behavior observations. It will also inform us as to when not seeing larvae really means they are not present. Early in my studies, I searched for larvae immediately after the adult flash activity was over and was usually unsuccessful. Later I realized that most larvae were not active until later in the evening. Here I will report several all-night observations of the glowing activity of firefly larvae.

The seasonal occurrence of adult fireflies in North America has also been discussed by a number of authors (McDermott, 1958; Barber, 1951; Lloyd, 1966), but the seasonal occurrence of larvae is not well-known. Preliminary observations have been reported for the seasonal occurrence of *Photuris* (Buschman, 1984a), and *Pyractomena* (Buschman, 1984b; 1988) larvae,

but there is currently little information available for *Photinus* larvae (Buschman, 1977). In Europe, there are three species that have been observed to glow spontaneously, both in fall and spring, but there are another three species that have been observed to glow spontaneously only in fall (De Cock, 2009). Information on the seasonal occurrence of periodic glowing of larvae will allow us to know when to go to the field to make larval observations. Here I will be summarizing the seasonal observations of glowing activity for several North American firefly larvae.

Early in my observations, I noted that the light from crawling firefly larva illuminated the area around and ahead of the crawling larva, and it seemed possible that it could be involved in illuminating the surroundings for the larva. I therefore tested the illumination hypothesis by recording the glowing behavior of larvae that were free crawling compared with that of larvae that were stopped at an obstacle. It was assumed that larval glowing would increase when they were investigating an obstacle if they were using the glow for illumination.

In my early observations, it was noted that there were differences in the glowing behavior of individual larvae of the same species. It appeared that these larvae were in different physiological states. For example, I noted that larvae seemed to stop periodic glowing as they approached pupation. They also had day-to-day variation in crawling and glowing activity, and there were differences in glowing of freshly caught larvae and larvae that had been held in the laboratory for a week or two. It seemed possible that some of these differences might be related to differences in feeding status. A laboratory experiment was organized to observe larvae on a day by day basis to determine if there were changes in glowing and locomotion over time, in relation to feeding status and/or other physiological events.

The overall objective of this Chapter will be to describe the relationship between response glowing and/or periodic glowing and several ecological and physiological factors

MATERIAL AND METHODS

The study sites and study species have been described in Chapter 1.

Daily Activity Cycle

On 30 March 1976 an unusually large number of glowing firefly larvae were observed on the floating vegetation on Lake Alice. This presented an opportunity to make observations on the levels of larval glowing activity throughout the night. Observations were made while walking slowly back and forth on the catwalk (four times over a 25 m section for each observation) and

counting larval glows from two to three meters on each side of the catwalk. The surface area included in the sample was ca. 150 m^2 per trip. On this date the emergent aquatic vegetation was unusually short (5-10 cm) which meant it had been killed during the winter, either by frost or treatment with herbicide. Most larval glows were periodic glows from larvae crawling on the short aquatic vegetation. Observations were made ca. every hour during the night, but more frequently at dusk and at twilight when glowing activity was changing rapidly. Observations were repeated on 31 March 1976. Since the dusk observations were missing for the first night, they were repeated on a third evening (4 April 1976). Each night some glowing larvae were also inspected with a flashlight to determine which species was glowing. These observations were made at 4:30 a.m. the first night, at 8:30 p.m. and 5:00 a.m. the second night and 9:30 p.m. the third night for a total of 80 larvae identified. The moon was new (all dark) on 31 March, but the sky was also overcast so there was considerable light pollution (light produced by human activity coming from the city and reflected back from the low overcast skies). There was a light rain at ca. 1:30 a.m. on both nights.

Seasonal Occurrence

Observations on the seasonal occurrence were compiled for each species. This data was summarized together with information from the literature.

Illumination Hypothesis

A one factor experiment was set up in the laboratory to test the illumination hypothesis. A group of thirty-nine large field collected larvae were set up individually in large petri dishes (15.5 cm diam. X 2.5 cm) containing a moist filter paper and a wood dowel (3 mm diam. X 15 cm.). There were 14 *P. lucifera* larvae, 9 *P. limbicalis* larvae, 5 *Photinus* larvae and 11 non-red *Photuris* larvae (Table 3). The photophase was shifted so that the room went dark at 12:00 pm in order that these observations could be made during the afternoon. The air temperature was not recorded, but the room was in a poorly heated/air-conditioned building, air temperatures were probably ca. 24 ^oC ($\pm$ 2 or 3 ^oC). Larvae were observed under indirect dim red light. The start and ending of each glow was recorded on a voice recorder together with notes as to what the larva was doing: locomotion, investigating, grooming or inactive. When crawling larvae reached an obstacle they stopped and seemed to search for a way around the obstacle. This was termed "investigation". Larvae sometimes stopped crawling and used the caudal grasping organ to brush different parts of the body. This was termed "grooming". Inactive larvae lay flat on the substrate

and were motionless. The number of glows produced during each activity were totaled up and the number of glows per minute of total observation time was calculated to standardize the glowing activity for the amount of time spent doing each activity.

Physiological Events

A second two-factor experiment was set up to test the effects of *time in the laboratory* ("veteran" versus "new-comer" larvae) and *feeding status* ("fed" versus "unfed" larvae). The "veteran" larvae were collected on 27 March and had been held in the laboratory for almost two weeks. The "new-comer" larvae were collected the night before the experiment was set up on 9 April. On each collection date three types of larvae were collected. The larvae that were included in the experiment were uniformly "large" larvae. There were 11 *P. lucifera*, 5 *Photinus* and 4 *Photuris* larvae in the "Veteran" group. There were 12 *P. lucifera*, 4 *Photinus* and 8 *Photuris* larvae in the "new-comer" group. The target number for the experiment was 12, but the lack of availability limited the numbers for some groups. Each of these 6 groups was then subdivided into "fed" and "unfed" larvae, so there were 2 to 6 larvae in the 12 groups to start the experiment. There was a total of 47 larvae in the 12 treatment groups. The fed groups were given food 7-13 April (Table 4 & 5). The *P. lucifera* larvae were given snails and the *Photinus* and *Photuris* larvae were given earthworm pieces. The photophase was shifted so that the room went dark at 12:00 pm and these observations could be made during the afternoon. The room was illuminated with very dim indirect red light. The dishes were arranged in a pattern on a table allowing each larva to be identified by position in the dim red light. The temperature was not recorded, but the room was in a poorly heated/air-conditioned building, air temperatures were probably ca. 24 ^{o}C ($\pm$ 2 or 3 ^{o}C).

Behavior observations were made for the following 12 consecutive days. Each larva was observed in turn for 10 to 15 seconds under indirect red light to record glowing and activity (locomotion, investigating, grooming and crawling) on a voice recorder. Observations were made on each larva five times during the next hour. Five observations for the 47 larvae took 30-40 minutes. These observations were then repeated on the hour three more times for a total of four sets of observations to give a total of 20 observations for each larva on each date. The data were compiled as the number of times out of 20 observations that each larva was observed glowing and which of the other activities were present during that observation. This data was organized by collection date and then again for fed or unfed larvae. Some larvae pupated during

the experiment and the activity and the glowing of those larvae were different from the rest of the larvae. Those observation records were not included in the analysis. However, the data for these pupating larvae were then organized by the date of pupation. Removing the observations for the pupating larvae reduced the number of larvae in the statistical analysis of variance. The sample sizes were small and unbalanced; therefore, a 2-way ANOVA could not be used. Therefore, two one-way ANOVA analyses were conducted. First, a one-way ANOVA was done to compare the "veteran" versus "new-comer" larvae and then a second analysis was done to compare "fed" versus "unfed" larvae (MSTAT Development Team, 1988).

RESULTS AND DISCUSSION

Daily Activity Cycle

The density of glowing larvae observed on the 30 and 31 of March was the highest ever observed by this author (180 glows/600 m^2). The larvae started glowing ca. 1 hr. after sunset or 10-20 minutes before darkness at 8:07 p.m. (Fig. 7). The number of glows increased steadily, reaching a plateau about two hours later. The number of glows was high for about six hours and then declined, slowly at first, and then rapidly during the one and a half hours before sunrise. Glowing ended about 20 minutes before sunrise. Glowing peaked at 3:00 a.m. and 4:00 a.m. on the two nights. Glowing seemed to be suppressed slightly during the rain which started at ca. 1:30 a.m. on both nights. There were many large larvae that had matured during the winter and were ready to pupate to become adults (Buschman, 1988). Adult *P. lucifera* were just beginning to fly on these dates. The larvae were also more visible than usual because the vegetation was short, and this increased the visibility and concentrated the larvae on the smaller plants.

The glowing larvae were identified as 78% *P. lucifera* larvae and 22% *Photinus* sp. larvae. The *Photinus* larvae were usually observed close to shore, crawling on mats of floating dead (non-green) vegetation. *P. lucifera* larvae were observed crawling and glowing both on the aerial plant parts and on the submerged plant parts. They were observed across the lake on the living floating water hyacinth. They were good climbers, but they did not swim. Water hyacinth leaf petioles are enlarged and serve as flotation devices for the plants. The plants were partially submerged and were interconnected with stolons which extended from plant to plant. Larvae could be seen climbing across the tangled bridges between the plants. There were no *Photuris* larvae in the observation area (but were observed in the woods near the lake).

Several authors have stated that firefly larvae are active during all hours of darkness, from dusk until dawn (Schwalb, 1961; Kaufmann, 1965; De Cock, 2004a). De Cock (2004a) made 14 all-night observations for *L. noctiluca* and 16 all-night observations for *P. hemipterus.* He was able to use statistical analysis and modeling to document the suppressive effects of light pollution (excessive human produced light) and other environmental conditions on larval glowing activity. He found that in *L. noctiluca,* larval glowing activity increased over ca. 2-3 hr from dusk to a peak and then it remained high or declined slowly over the next 4-5 hr. Then it declined rapidly for 1-2 hr. to zero before sunrise. However, in *P. hemipterus* the glowing activity increased more slowly to a peak some 6-7 hr. after dusk and then declined rapidly to zero before dawn. Both Dreisig (1974) and De Cock (2004a) present evidence that larval glowing activity was reduced during bright moonlight and bright human light pollution. *L. noctiluca* started glowing a little earlier in the evening than did *P. hemipterus* (De Cock, 2009).

Most adult fireflies were active for a fairly short period, but the activity of larvae was more extended. Adult *P. lucifera* were active for 15 to 30 min at dusk while adult *P. ardens* complex fireflies were active for several hours after dusk (Buschman, 1977; 1988). Larval glowing activity barely started during the period when the adult activity occurred. This means that observations on larvae must be done late at night, well after adult activity is over.

Active *L. noctiluca* and *P. hemipterus* larvae have been observed crawling but not glowing during the low light of early morning or evening hours (Schwalb, 1961; De Cock, 2004a). De Cock (2004a) reports that active *P. hemipterus* larvae have been observed crawling but not glowing in spring. This seems to mean that larvae can be active even when glowing is not observed. Taylor (2002) reports that *L. noctiluca* larvae go into a "walkabout" stage where larvae wander about during all times of the day, even during bright daylight hours. I assume the larvae do not glow during this diurnal activity, but this is not stated.

Seasonal Occurrence

All stages of *P. lucifera* larvae were present on the floating vegetation on Lake Alice (Buschman 1988). The larvae were found on warm nights in all months of the year (Buschman, 1977; 1984b; 1988). Gainesville, Florida, sometimes had freezing weather during the winter, but cold spells were followed by warm periods when larvae became active. Small larvae were present during the spring and summer following adult emergence and presumed egg laying. There was a buildup of large larvae in spring just before they pupated to produce the large surge

of adults in March and April (Buschman, 1988). Large glowing larvae could be observed in all warm weather conditions, but larger numbers could be seen after a day of gentle rain.

Large larvae of *P. limbicollis* were observed climbing on vines, shrubs and tree trunks in fall (November). They were not observed at other times of the year and it unknown where they spent the rest of the year. The mature fall larvae did not seem to take food, so they were probably searching for pupation sites. However, it should be noted that these larvae did not pupate when held in captivity, like most other large larvae collected in fall or spring. A similar climbing behavior in the winter leading to pupation and adult emergence has been described in more detail for a closely related species, *P. borealis* (Faust, 2012).

Medium to large *Photinus* larvae were observed mainly in spring. They were found crawling on mats of decaying vegetation floating on Lake Alice (Buschman, 1977). These larvae would feed on earthworm sections. Two larvae pupated in the laboratory. These were the most observable of the *Photinus* larvae that I have found. Most *Photinus* larvae are thought to be subterranean, since they are only collected accidently while digging in the ground or while tearing rotten logs apart. In addition, some female *Photinus* females are observed to flash or glow near burrows in the soil, suggesting they have emerged from these burrows.

Large red *Photuris* larvae were observed only in August and September. They were found crawling in leaf litter in relatively xeric woodland habitats, compared to the habitats where the non-red *Photuris* larvae were found (Buschman, 1984a). They fed on earthworm sections in the laboratory, but seldom pupated.

Medium to large non-red *Photuris* larvae were observed year-round in leaf litter in moist woodland habitats (Buschman, 1984a). This was a species complex so there may have been cryptic seasonal patterns of occurrence for individual species. Two of the four identifiable species were only collected in spring while the other two were collected all year (Buschman, 1984a). Some of these larvae would feed on earthworm sections and develop into adults, while others would feed, but would not pupate. Adults of most *Photuris* species cannot be identified with confidence to species using morphological characteristics.

Gunn and Gunn (2012) were able to make repeated observations on the glowing larvae of a *L. noctiluca* population and observed that there were major seasonal peaks and dips in larval glowing activity. They reported that for one summer, there were four major peaks of larval glowing activity which occurred when there was a new moon (dark—no moonlight) and there

were three major lows that occurred when there was a full moon (lots of moonlight). The suppressed glowing activity during the full moon did not increase when clouds reduced light levels. Larval glowing activity seemed to have an intrinsic lunar rhythm rather than a simple response to light. Gunn and Gunn (2012) observed no strong response to temperature or moisture conditions. In contrast, they report that adult female glowing started during the full moon, in July, and showed no signs of following the lunar cycle as did larval glowing.

Certain types of larvae were observed only during specific times of the year. Red *Photuris* larvae were observed glowing periodically or continuously only in the fall—August to October. It is possible that red *Photuris* are active only in fall, however, it seems likely that these larvae may have been active at other times of the year, but not glowing periodically. *P. limbicollis* larvae were found only in the fall (November). They also could be active at other times of the year, but not glowing. Non-glowing larvae would escape notice. They may have been in the leaf litter rather than climbing on branches during the rest of the year. *P. lucifera* were observed glowing all year long. Non-red *Photuris* were also observed all year long; however, in this case there may have been species within that mix that could have shown up at different times of the year without being recognized. We will need to figure out a good way to demonstrate when larvae are active in the field even when they may not be glowing. Currently, all field observations of firefly larvae are based on observations of glowing larvae. Additional sampling techniques will need to be developed—like using food baits (Chapter 3).

Illumination Hypothesis

During locomotion, the larva held the body up off the substrate with the six thoracic legs and the tip of the abdomen (Fig. 8). The tip of the abdomen was extended down from the body to hold onto the substrate using the caudal grasping organ (Fig. 8A-D). The light organs, which are located on the ventral side of the eighth abdominal segment actually faced forward during locomotion. The light from the light organ was directed to the front and laterally. Crawling larvae seemed to move in a purposeful direction when crawling freely. However, when crawling larvae reached an obstacle they stopped and waved the head back and forth as they seemed to search for a way around the obstacle. If glowing was being used as illumination, to see the surroundings, then glowing should increase during this investigation activity.

In the illumination experiment, *P. lucifera* larvae were observed for a total of 103.5 min (Table 3). During this time, there were a total of 887 glows, with 74.5% occurring during

locomotion and 19.5% during investigation (Table 3, Fig. 9). There were few glows during grooming or inactivity. The glowing frequency was lower for investigation (9.7 glows per min) than for locomotion (13.2 glows per min).

Pyractomena limbicollis larvae were observed for a total of 45.8 minute (Table 3). During this time, there were a total of 99 glows, with 87% occurring during locomotion and 5.1% during investigation (Table 3, Fig. 9). There were few glows during grooming or inactivity. The glowing frequency was 3.1 and 0.5 per min for locomotion and investigation, respectively.

Photinus larvae were observed for a total of 33.8 min (Table 3). There was a total of 69 glows during that time, with 72% occurring during locomotion and 22% during investigation (Table 3, Fig. 9). There were few glows during grooming or inactivity. The glowing frequency was 3.1 and 1.5 per min for locomotion and investigation, respectively.

Non-red *Photuris* larvae were observed for a total of 52.9 minute (Table 3). There was a total of 178 glows during this time, with 84% occurring during locomotion and 12% during investigation (Table 3, Fig. 9). There were few glows during grooming or inactivity. The glowing frequency was 5.9 and 2.2 per minute for locomotion and investigation, respectively.

In all four types of larvae the periodic glowing was clearly associated with locomotion. However, there were some glows that were recorded with other activities, but these were probably due to the difficulty in judging the transition between locomotion and the other activities. Larvae were sometimes doing two activities at the same time or in quick succession and a decision had to be made quickly as to which of the activities should be assigned to the glow. For example, a larva could be investigating an obstacle and then it moved a half cm and continued investigating. If there was a glow during this period it was probably recorded as investigation, but it could have been associated with the brief locomotion. In any case, it was clear that there was no increase in glowing during investigation, by both measures (total glows or glows per min). In fact, glowing during investigation appeared to be less frequent (rather than more frequent) than during locomotion. These results clearly do not support the illumination hypothesis.

Several situations in the literature appear to be related to bioluminescence being used as illumination. Sivinski (1981) cites Schwalb (1961) as saying that *L. noctiluca* larvae glowed when they came across a snail slime trail. The larva then proceeded to follow the slime trail. This could imply that the larva might be able to follow snail trails using reflections from their own

bioluminescence. However, I'm not sure that this observation can be verified. In addition, Schwalb (1961) himself describes the slime trail following behavior of these larvae and showed that it was based on chemical sensory information collected by the maxillary palps which were held in contact with the substrate during slime trail following. I was not able to find trail following behavior in North American fireflies (Chapter 3).

Sivinski (1981) also suggested that the red "headlights" of *Phrixothrix* Olivier 1909 and *Astraptor* Murray 1868 larvae (Coleoptera: Phengodidae) might be used for illumination. The red light would be invisible to prey and predators. However, we need more evidence to support this hypothesis. Illumination has been attributed to some *Photuris* adults, since their flashes are used like "landing lights" (Lloyd, 1968). Illumination is also suggested as the function of continuous glows of male *Phausis riticulata* (Say 1825) (De Cock *et al.,* 2014). However, there are no similar reports for firefly larvae. It needs to be noted that adult fireflies have large eyes that seem to be able to take advantage of bioluminescence for illumination. However, firefly larvae have eyes that are small and simple, seeming unlikely that they have the visual acuity to see objects in the dark (McLean *et al.,* 1972; De Cock, 2004a). Therefore, the probability that illumination is a function for bioluminescence in firefly larvae seems quite low.

Effects of Physiological Events

In the experiment on physiological effects on larval behavior, the veteran *P. lucifera* larvae had significantly less glowing and less locomotion activity than similar new-comer larvae (Table 4, Fig. 10A-B). In addition, both glowing and locomotion activity was significantly higher in unfed compared to fed larvae for the veteran larvae (Table 5). In contrast, the glowing and crawling activity of the new-comer group was significantly higher for the fed compared to the unfed larvae (Table 5). It was not clear why the glowing and locomotion activity of the new-comer larvae had different trends than did the veteran larvae or why this activity declined so much at the end of the experiment.

The veteran *Photinus* larvae had less glowing and locomotion activity than similar new-comer larvae (Table 4, Fig. 11A-B). Glowing activity was higher in the fed than the unfed larvae; however, locomotion activity was higher for new-comer fed larvae compared with veteran unfed larvae (Table 5). I need to point out that the surviving number of larvae in each sample group was small ((n=4, 1, 2, and 2, Table 5). I am reporting these results because these larvae are difficult to obtain this data may be the only information available for the near future.

The veteran *Photuris* non-red larvae had similar glowing and locomotion activity as new-comer larvae (Table 4, Fig. 12A-B). Glowing activity was higher in the fed than the unfed veteran larvae, but the opposite trend was present for the new-comer larvae (Table 5). In addition, locomotion activity was higher for the fed as compared to the unfed veteran larvae, but not in the new-comer larvae (Table 5). The new-comer larvae were extremely active in locomotion, but the frequency of glowing seemed low. However, I need to point out that the surviving number of larvae in some samples was small ((n=2, 2, 6, and 2, Table 5).

These results demonstrate that glowing and crawling of firefly larvae was affected by time in the laboratory, but the trends were contradictory. As noted earlier, the longer the larvae were in the laboratory the less likely they were to glow periodically. To some extent, this may have been a response to a lack of proper feeding. However, feeding did not fully restore glowing to normal. Feeding also had an impact on the glowing and crawling of the larvae. In the laboratory, some larvae were very active, but did not glow periodically. This suggests that larvae in the field may also be active even when one does not see larval periodic glowing.

During these observations 4 of 23 *P. lucifera* larvae pupated during this experiment. As these larvae approached pupation they gradually stopped glowing and crawling over a period of several days (Fig. 13A). When these observations were assembled according to days to pupation, these larvae were active and glowing up until four days before pupation (Fig. 13A). They went dark one day before they could be identified as being in the prepupal state (a bloated condition with the abdomen glued to the substrate). Larvae in the genus *Pyractomena* are known to pupate on various plants where they are exposed and not hidden (Lloyd 1973b; Buschman, 1984b). These pupae are cryptically colored for camouflage. They do not glow readily (Lloyd 1973b). The glowing of *Pyractomena* larvae would be expected to change from periodic glowing to the dark condition at pupation, since they do not normally glow even in response to disturbance.

During these observations, one of 10 *Photinus* larvae pupated. This larva was active and glowed until the day before it sealed itself in a cell that it made from chewed up filter paper (Fig. 13A). Two of 14 *Photuris* larvae pupated during this experiment. They were active and glowed until the day before they sealed themselves in the soil cell which they made (Fig. 13B). There probably was a transition from periodic glowing to response glowing during this process, but unfortunately this was not recorded. Most non-*Pyractomena* firefly larvae are known to pupate in earthen cells. They are white colored with transparent cuticle, seeming to project their

bioluminescence into the chamber around them. These larvae include *Photuris* and *Photinus* that are known to glow responsively when disturbed (Lloyd 1973b; Fig. 13B). These larvae would be expected to change from periodic glowing to response glowing as they approached pupation.

There are probably similar changes in glowing and crawling activity associated with molting, but these have not been documented. Changes associated with molting could be responsible for some of the changes in crawling and glowing behavior of larvae over time in the laboratory experiment.

In the field, larvae seemed to switch between response and periodic glowing as though these were two alternative physiological conditions (Chapter 1). For example, under wet conditions, after rain, most larvae seemed to be in an active physiological state and they appeared to be hunting while they glowed periodically. When things dried out, they became inactive as they stopped hunting, so they switched to glowing responsively. The reaction of crawling larvae to disturbance was different from that of hiding larvae (Chapter 3). Larvae that were crawling and glowing periodically would freeze and go dark after a slight disturbance, but a few days later when they were hiding and sedentary they glowed responsively after a slight disturbance.

Periodic glowing was clearly associated with crawling and locomotion which appeared to be related to hunting. Periodic glowing did not appear to be associated with illumination. The ecological and physiological factors may affect the timing features of periodic glowing behavior so these effects will need to be better documented in the future.

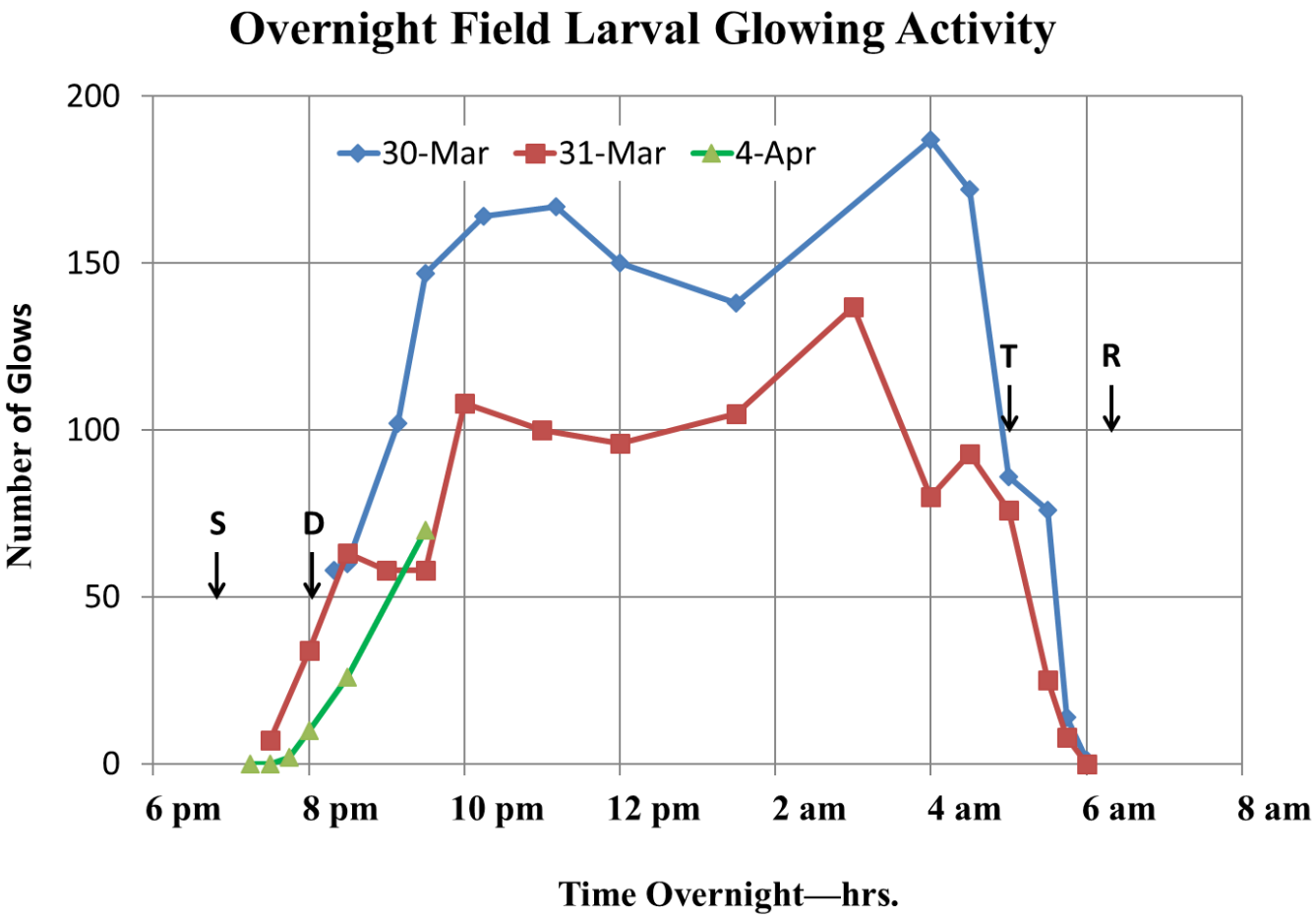


Figure 7. Occurrence of glowing firefly larvae during the night, 30 and 31 March and 4 April 1976, S=sunrise at 6:47 p.m., D=darkness at 8:07 p.m., T=twilight 4:58 a.m., R=6:18 a.m.

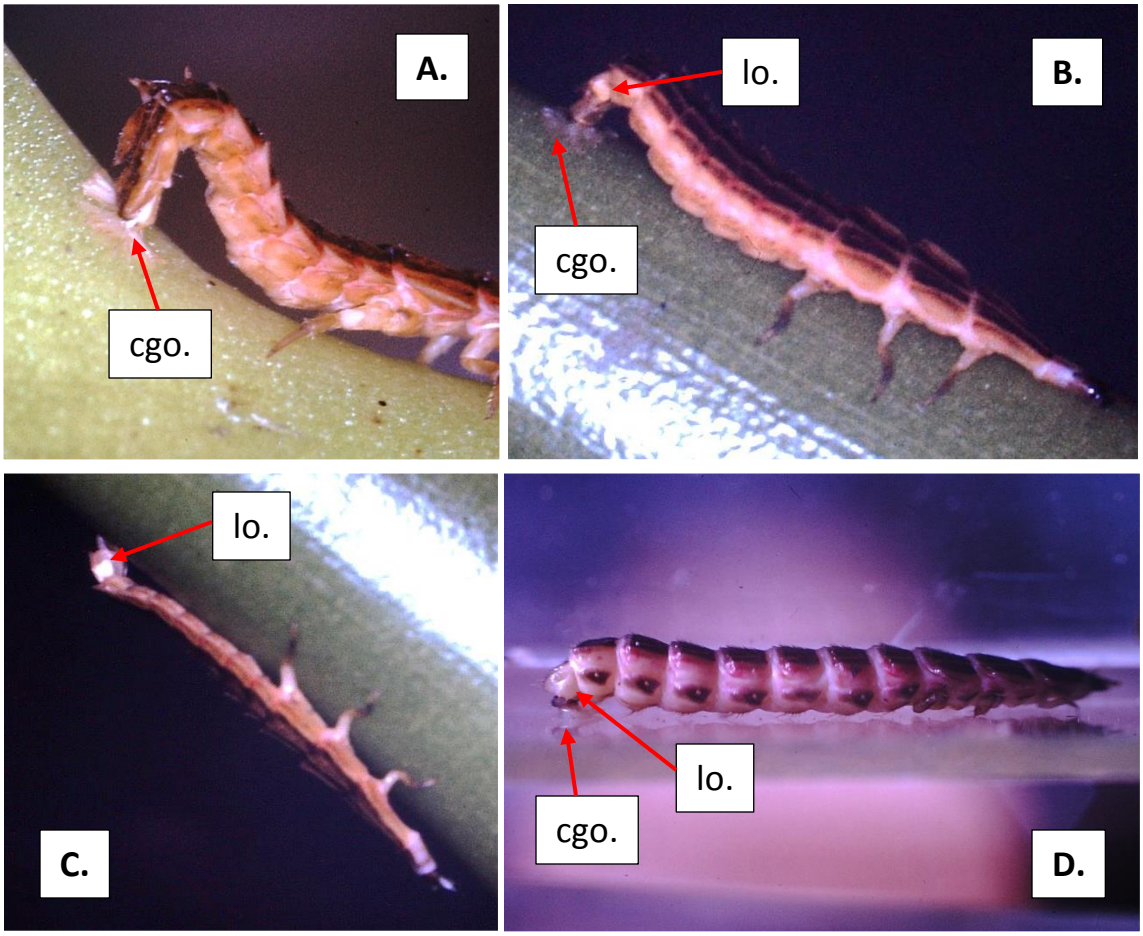


Figure 8. A-C. Side views of *Pyractomena lucifera* larvae showing various crawling positions with the tip of the abdomen pointed down and attached to the substrate with the caudal grasping organs (CGO). Note that the light organ (LO) faces forward and laterally. The light organ in **C** is reflecting light from the camera flash. **D.** Side view of *Photinus* sp. larva in crawling position. Note the light organ at the tip of the abdomen.

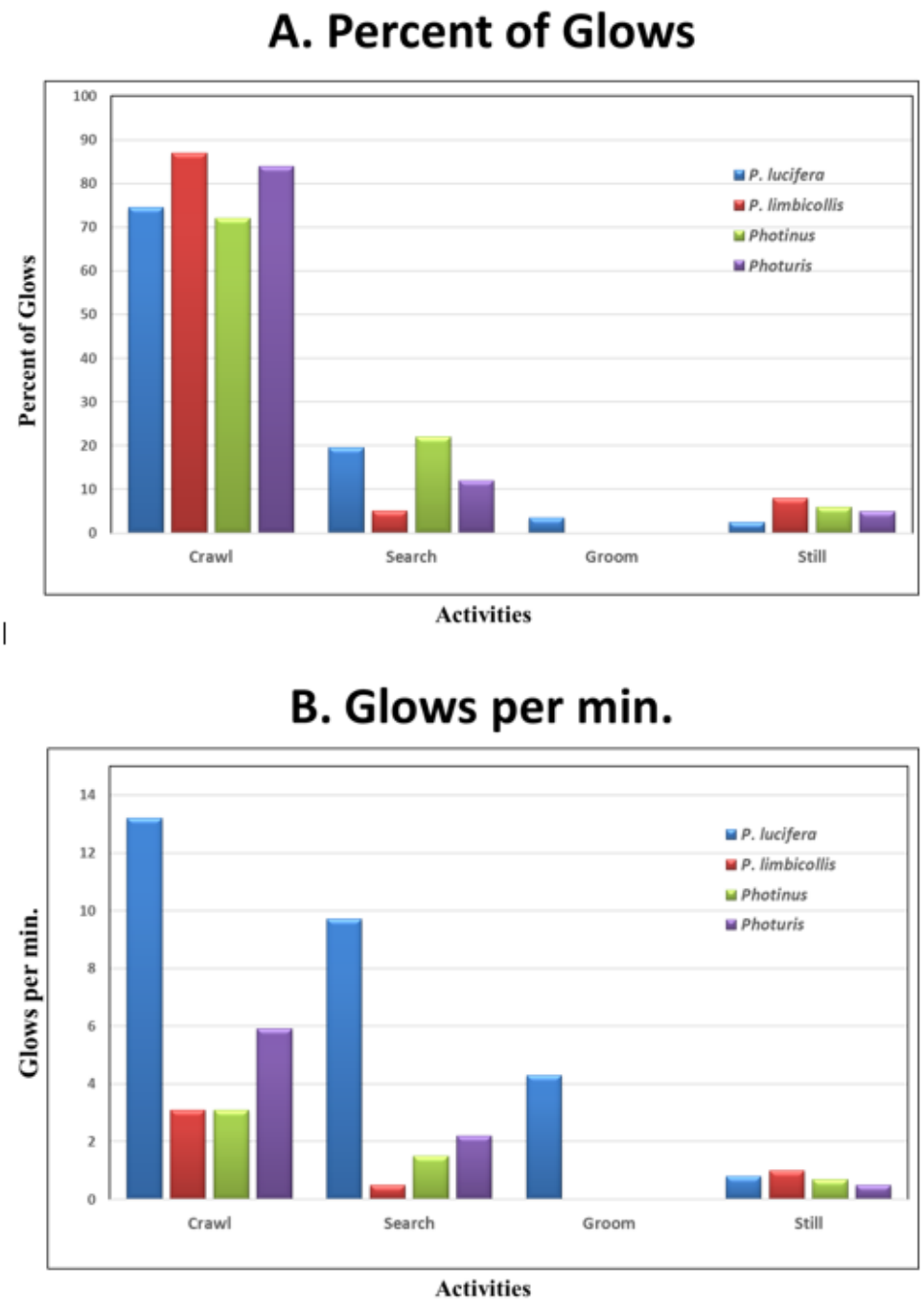


Figure 9. Occurrence of glows during various activities, crawling, searching grooming and resting: **A.** Percent of glows; **B.** glows per 100 seconds.

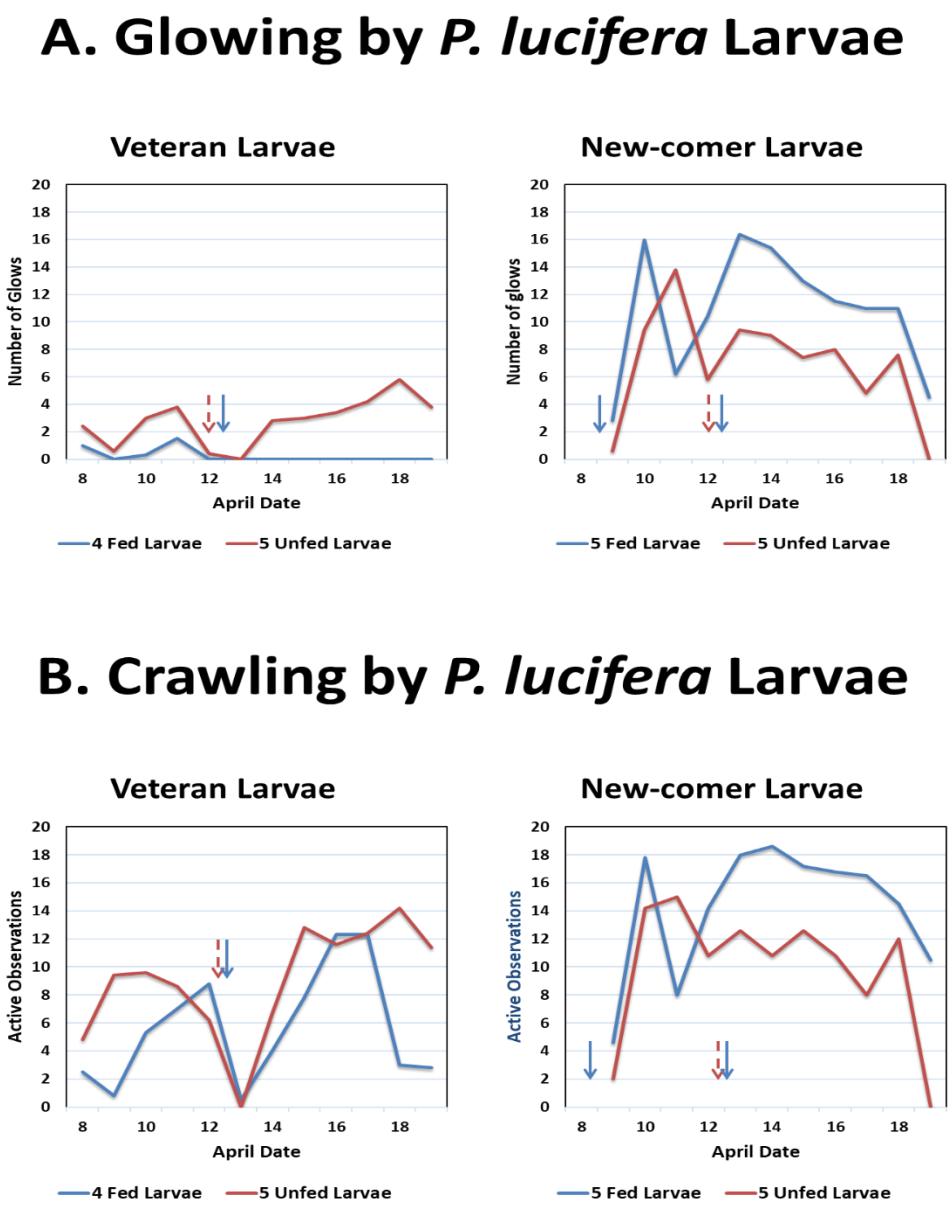
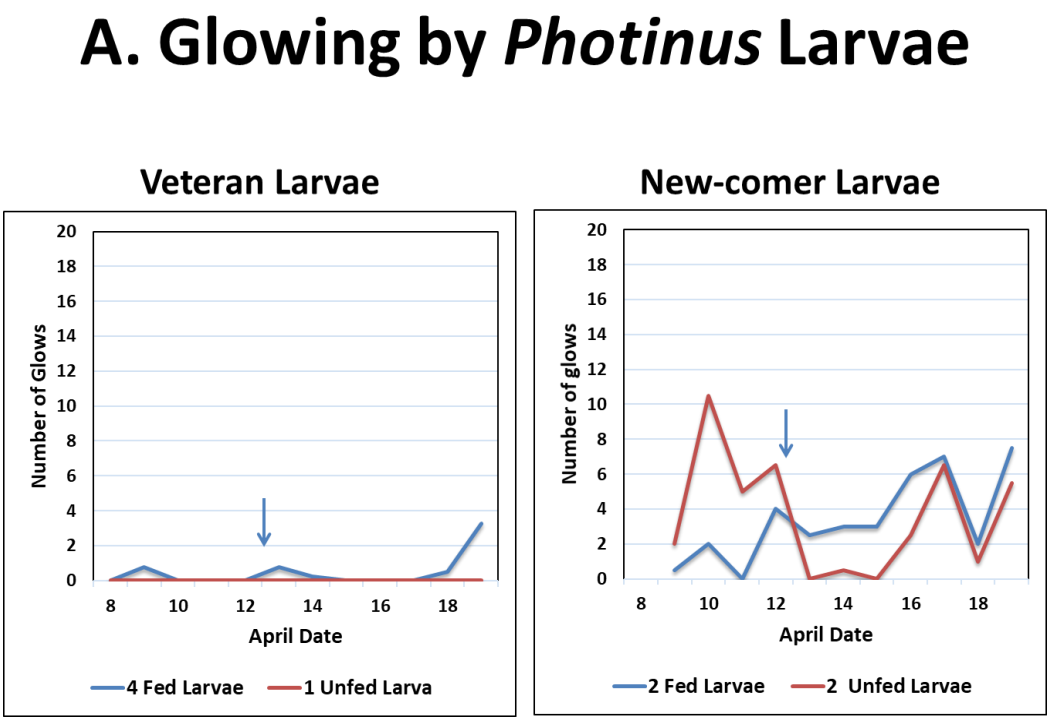


Figure 10. Occurrence of glowing and locomotion activity in two groups of fed and unfed *Pyractomena lucifera* veteran and new-comer larvae: **A.** Glowing; **B.** Activity. The arrows indicate feeding events: the fed larvae were fed 8 and 13 April and the unfed larvae were fed 13 April.



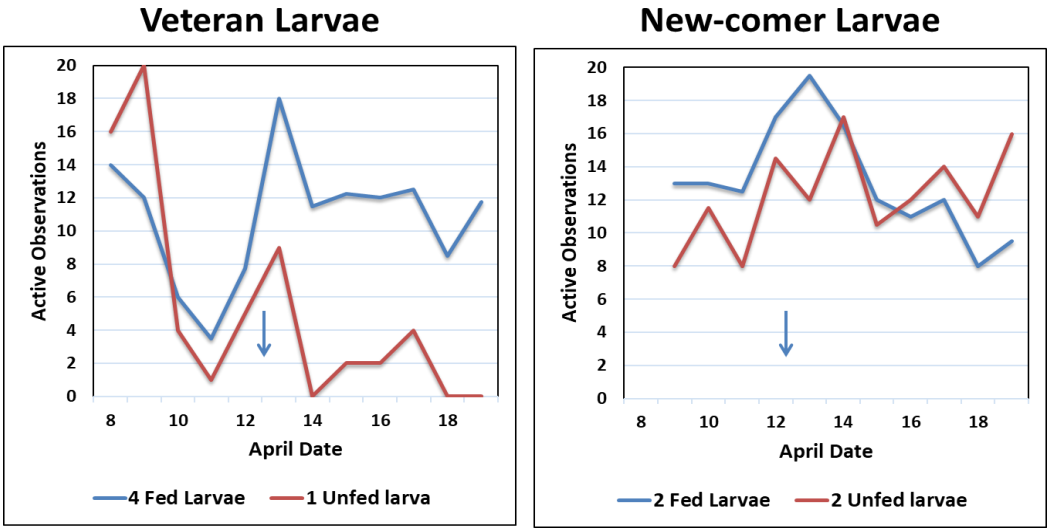


Figure 11. Occurrence of glowing and locomotion activity in two groups of fed and unfed *Photinus* veteran and new-comer larvae: **A.** Glowing; **B.** Activity. The arrows indicate feeding events: the fed larvae were fed 12 April.

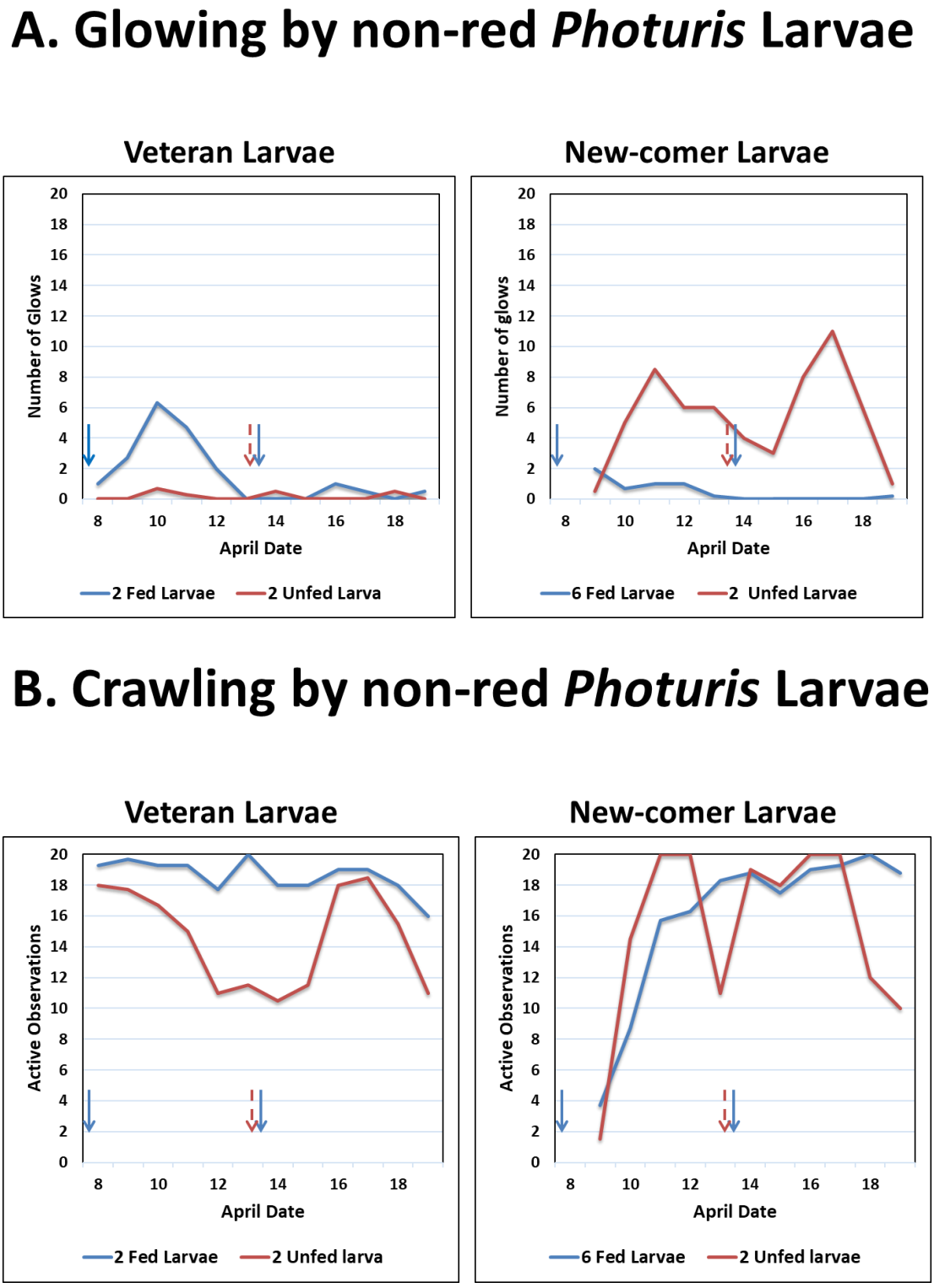
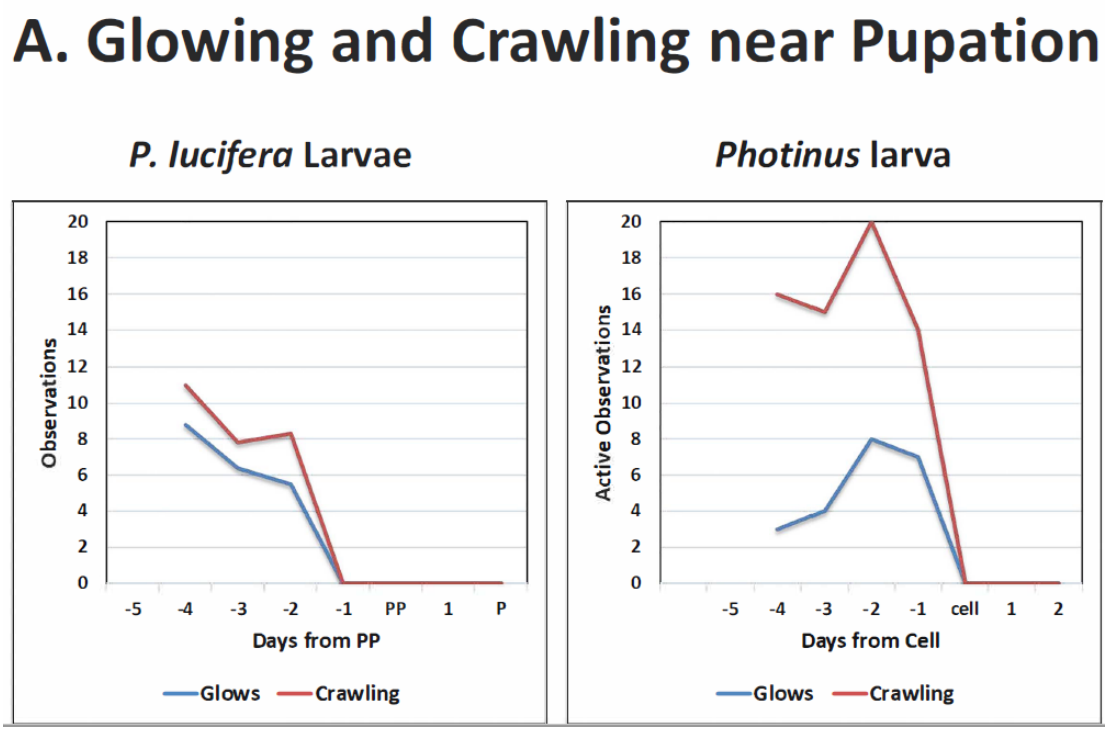


Figure 12. Occurrence of glowing and locomotion activity in two groups of fed and unfed non-red *Photuris* veteran and new-comer larvae: **A.** Glowing; **B.** Activity. The arrows indicate feeding events: the fed larvae were fed 7 and 13 April and the unfed larvae were fed 13 April.



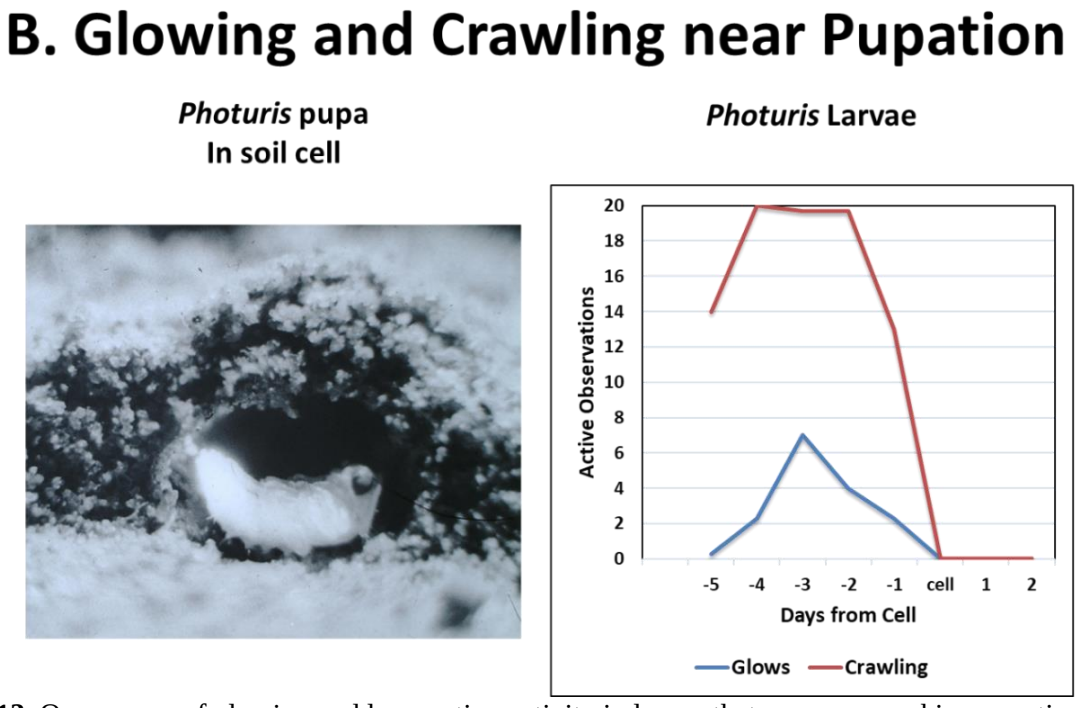


Figure 13. Occurrence of glowing and locomotion activity in larvae that were approaching pupation: **A.** *Pyractomena lucifera* and *Photinus* sp. larvae; **B.** non-red *Photuris* larvae. B. also including a photo of a glowing *Photuris* pupa in its soil igloo with some background light to show the outlines of the soil igloo cell.

Table 3. Larval periodic glowing during different activities for four different kinds of firefly larvae in the illumination experiment.

Firefly Studied	Number	Number of	Total Obs.	Activity--% of total/glows/min.			
Measurement	of larvae	glows	time-min.	Locomotion	Investigation	Grooming	Inactive
P. lucifera							
No. glows	14	887	103.5	74.5	19.5	3.5	2.5
Glows/min.	--	--	--	13.2	9.7	4.3	0.8
P. limbicollis						r	
No. glows	9	99	45.8	87.0	5.1	0.0	8.0
Glows/min.	--	--	--	3.1	0.5	0.0	1.0
Photinus							
No. glows	5	69	33.8	72.0	22.0	0.0	6.0
Glows/min.	--	--	--	3.1	1.5	0.0	0.7
Photuris non-red							
No. glows	11	178	52.9	84.0	12.0	0.0	5.0
Glows/min.	--	--	--	5.9	2.2	0.0	0.5

Table 4. Results from the first analysis for glowing and locomotion activity for veteran and new-comer larvae in the physiology experiment.

Species	Dates included in Analysis	Glowing Mean			Locomotion Mean		
		Veteran larvae[1]	New-comer larvae[1]	Probability	Veteran larvae[1]	New-comer larvae[1]	Probability
P. lucifera	Data: 9-15 Apr.	1.2	9.7	<0.0001	6.5	12.6	<0.0001
		n=9 (12)	n=10 (12)		n=9 (12)	n=10 (12)	
Photinus	Data: 9-19 Apr.	0.4	3.3	<0.0001	9.3	12.7	0.0063
		n=5 (6)	n=4 (4)		n=5 (6)	n=4 (4)	
Photuris	Data: 9-19 Apr.	1.1	1.3	>0.50	16.5	15.7	>0.50
		n=6 (6)	n=8 (8)		n=6 (6)	n=8 (8)	

[1] Number of larvae included in the analysis (and number of larvae that entered the experiment in parenthesis).

Table 5. Results from the second analysis for glowing and locomotion activity for veteran and new-comer larvae that were fed and unfed in the physiology experiment.

		Glowing Mean			Locomotion Mean		
Species Larval group	Date(s) Fed Data Analyzed	Fed[1]	Unfed[1]	Probability	Fed[1]	Unfed[1]	Probability
P. lucifera Veteran	Fed: 8 Apr. Data: 9-19 Apr.	0.2 n=4 (5)	2.9 n=5 (6)	0.0004	5.8 n=4 (5)	9.5 n=5 (6)	0.0036
P. lucifera New-comer	Fed: 10 Apr. Data: 12-19 Apr.	12.9 n=5 (6)	7.0 n=5 (6)	0.0002	16.8 n=5 (6)	10.9 n=5 (6)	0.0003
Photinus Veteran	Fed: 12 Apr. Data: 14-19 Apr.	0.7 n=4 (4)	0.0 n=1 (2)	>0.50	11.4 n=4 (4)	1.3 n=1 (2)	0.0003
Photinus New comer	Fed: 12 Apr. Data: 14-19 Apr.	3.9 n=2 (2)	2.7 n=2 (2)	>0.50	11.5 n=2 (2)	13.4 n=2 (2)	>0.50
Photuris Veteran	Fed: 7 Apr. Data: 8-12 Apr.	3.3 n=2 (3)	0.2 n=2 (3)	0.0057	19.1 n=2 (3)	15.7 n=2 (3)	0.0066
Photuris New-comer	Fed: 13 Apr. Data: 14-16 Apr.	0.03 n=6 (6)	5.5 n=2 (2)	--[2]	18.9 n=6 (6)	16.5 n=2 (2)	--[2]

[1] Number of larvae included in the analysis (and number of larvae that entered the experiment in parenthesis).

[2] Data omitted because there were only two larvae in the unfed group and one was an usually active glower (perhaps a response glower).

CHAPTER 3.

THE ROLE OF BIOLUMINESCENCE IN FIREFLY LARVAL INTERACTIONS WITH OTHER ORGANISMS: INCLUDING PREY, NATURAL ENEMIES AND COMPETITORS

INTRODUCTION

In Chapters 1 and 2, I presented a preliminary analysis of glowing behavior and the ecological and physiological factors that affect larval glowing behavior. In this Chapter, I will present additional observations on the glowing behavior during interactions with other organisms such as prey, natural enemies and competitors. Initial observations suggested that it was important to recognize two types of glowing behavior, response glowing and periodic glowing (Chapter 1). Response glows are long-lasting glows produced by resting and/or hiding larvae in response to stimuli like vibrations in the substrate. Periodic glows are shorter spontaneous glows that were produced by larvae that were actively hunting/crawling.

The bioluminescent behavior of firefly larvae is often said to be defensive behavior (Sivinski, 1981; De Cock and Matthysan, 1999). It is therefore important to better understand this defensive behavior. We need to know which predators are being defended against. Numerous anecdotal reports of natural enemies of fireflies can be found in the literature going back to the natural history reports from the 1800's. Lloyd (1973a) has compilated many of these reports. Day (2011), De Cock and Matthysen (1999) and De Cock (2009) have added some additional observations. In this report, I will summarize and discuss available observations of natural enemies. I will also describe the defensive behavior for several types of firefly larvae. Since preliminary observations indicated that larvae that were crawling and glowing periodically responded to disturbance differently from larvae that were hiding and glowing responsively, observations comparing the glowing responses to disturbance will be reported for these two situations. It has been suggested that bioluminescence must have developed first in immature fireflies before being adapted for use by adults in sexual communication (Branham and Wenzel, 2003; De Cock and Matthysen, 1999; Lewis and Cratsley, 2008). Therefore, it is important to discuss these bioluminescent behaviors specifically in immature fireflies.

Larval glowing is sometimes thought to be associated with prey capture (Sivinski, 1981; De Cock and Matthysan, 1999). I will compile what we know about the feeding habits of firefly

larvae and the role of glowing in prey capture and feeding of North American firefly larvae. I will compare these with observations of Schwalb (1961) and Taylor (2002) who have given extensive accounts of this behavior for *Lampyris noctiluca* (Linnaeus 1758) and *Lamprohiza splendidula* (Linnaeus 1767).

There are very few records of interactions between firefly larvae and other organisms in their environment as larvae are usually only active in the dark. In this study, food baits were tested to see if they would attract firefly larvae. When baits were placed in firefly habitat, they successfully attracted *Photuris* LeConte 1851 larvae. The baits also attracted several other organisms. This resulted in several interesting interactions between *Photuris* larvae and the various organisms attracted to the baits.

MATERIAL AND METHODS

Study Sites and Study Species

The study sites and study species have been described in Chapter 1.

Defensive Behavior and Associated Glowing Behavior

Field observations were made on the defensive activity of some North American firefly larvae by carefully approaching them (to ca. 1 m), while trying not to disturb them. The initial or starting state of activity (crawling vs. motionless) and glowing (periodic vs. continuous) was determined. Then a slight disturbance was created and the leaves in the area (or soil when it was bare ground) were disturbed using a finger. The subsequent crawling and glowing behaviors were determined. Then a heavy disturbance was created, (the larva was picked up with the fingers—handled) and the glowing activity was observed. Finally, the larva was released, and the crawling and glowing activity was observed for the next 10-30 seconds. Since this was done in the field, there were times when not all three observations could be obtained for each larva (i.e. larva escaped into leaf litter). Many early observations had to be discarded because the larvae had only been recorded as "glowing" when they should have been recorded as "glowing responsively" or "glowing periodically." The responses of larvae to each stimulus were analyzed statistically to see if the frequencies were statistically different for larvae that had been active and glowing periodically versus larvae that had been glowing responsively or continuously when first observed. The Chi-square analysis of two-by-two contingency tables was used to compare the two types of larvae (MSTAT Development Team, 1988).

Firefly Natural Enemies

Larval natural enemy observations were compiled in the field. These observations and those reported in the literature are summarized. Most of these observations were made on *Photuris* larvae, but there are also a few observations on other types of larvae.

Prey Records, Prey Capture and Feeding Behavior

A list of known published and unpublished prey records for some common firefly larvae was developed. Behavior observations focused on *Photuris* and *Pyractomena lucifera* (Melsheimer 1845) larvae because they live in habitats where observations on predation were easier to make.

Interactions with other Organisms

Baits, such as small pieces of insects, earthworm (Oligochaeta) or canned tuna (fish) were placed on three by three cm sections of white paper. The sticky baits were easier to handle with forceps when they were on the paper sections. The paper sections also were easier to see in the dark when I was using a dim red light. Up to 40 baits were placed in a bait trail in *Photuris* habitat at dusk. The baits were placed about 30 cm apart. The bait stations were then visited repeatedly over the next one to two hours, to observe visitors. These observations included mostly visitors on the papers, because those not on the paper were not visible in dim red light. The observations were momentary, long enough to record the visitors, unless something interesting was happening. After two hours some of the baits had been removed and/or they were no longer attractive (they seemed to dry up or lose attractiveness), so observations were terminated at this time.

On three occasions, 24, 25, and 26 Sept. 1975, 20 field collected tent caterpillars (no ID) (three to four cm long and three to four mm thick) were killed by freezing and placed on paper squares. On each evening at dusk, the baits were placed in the field, twice in a red *Photuris* habitat and once in non-red *Photuris* habitat. The three-night total numbers of visitors are reported.

On two occasions, 28 and 29 Sept 2011, observations were made in East Tennessee. Four different baits were tested: a) canned tuna, b) earthworm section, c) insect (grasshopper) section and d) a blank paper (as control). Again, the baits were placed on the ground in leaf litter in a bait trail along a gravel road that was non-red *Photuris* habitat. The baits were replicated six

times and they were visited seven times (the first evening) and three times (the second evening) for a total of 60 observations (for each bait type) over the two evenings.

RESULTS AND DISCUSSION

Defensive Behavior and Associated Glowing Behavior"

Defensive behavior observations were made on 64 non-red *Photuris* larvae, on 60 red *Photuris* larvae and on 28 *P. lucifera* larvae (Table 6). Among the non-red *Photuris* larvae, 53 were crawling and glowing periodically and 11 were glowing continuously when found (probably glowing responsively to the disturbance of my approach). Of the non-red *Photuris*, only 21% of the periodically glowing larvae glowed, whereas 73% of the continuously glowing larvae glowed after slight disturbance. This difference was statistically significant (P=0.0021). Among the red *Photuris* larvae, 30 were crawling and glowing periodically and 30 were motionless and glowing continuously when found. Of these, 30% of the periodically glowing larvae glowed, while 77% of the continuously glowing larvae glowed after slight disturbance. This difference was statistically significant (P=0.0008). Among the *P. lucifera* larvae, 15 were crawling and glowing periodically and 13 were glowing continuously. After slight disturbance, 27% of the of the first group and 38% of the second group glowed or continued glowing. This difference was not statistically significant (P=0.79). Most larvae of the two larval glowing backgrounds tended to glow during heavy disturbance (63-93%) although *P. lucifera* larvae glowed at a lower frequency than the others (43-54%) (Table 6).

Most non-red *Photuris* larvae of both larval glowing backgrounds glowed on release (83-100%) (Table 6). In the red *Photuris* larvae, only 41% of the periodically glowing larvae and 71% of the continuously glowing larvae glowed after release. This difference was not statistically significant (P=0.87). *Pyractomena lucifera* larvae glowed at a slightly lower frequency after release (14-50%). Most larvae of all three types and glowing backgrounds tended to “freeze” (remain motionless) after slight disturbance (73-93%). Most larvae fled after release (63-86%).

It seemed clear that when *Photuris* larvae were crawling and glowing periodically, the first response to a slight disturbance was to freeze and stop glowing periodically. When similar larvae were hiding, the first response to slight disturbance was to remain still, but to glow responsively. When the disturbance became more severe, as when they were handled, both types

of larvae glowed brightly in the hand and glowed on release—especially the response glowers (some of the periodically glowing larvae went dark and fled) when released.

These observations suggest that larvae that are crawling and glowing periodically are in a different motivational or physiological state than larvae that are hiding and glowing responsively. This is also supported by the observation that after a rainy period most of the larvae were found hunting, crawling and glowing periodically. However, several days later, when the habitat was drying out, many of the same larvae were hiding and glowing responsively. Larvae seemed to move between the two motivational states as the habitat conditions changed. The glowing pattern appeared to be an indication of the motivational or physiological status of the larvae and not an indication of different kinds of larvae. In future studies, it will be important to make behavior observations with larvae that are better standardized for motivational status.

The change in motivational status may explain some of the confusion on glowing behavior reported in the literature. Some authors report that larvae glow when disturbed while others report that they stop glowing when disturbed. Sivinski (1981) reviews the literature and gives a list of elaterid, phengodid, and lampyrid species that glow when stimulated mechanically, and a shorter list of species that do not glow after being prodded. Then he lists some species that would glow spontaneously but stopped glowing when disturbed. It is helpful to note that the same larvae can behave differently depending on several environmental and physiological conditions. This change in behavior was observed in several species, so it will be interesting to see if this behavior will be confirmed more broadly in other species and in other areas.

In Florida, responsive glowing larvae were relatively common among the red *Photuris*, but not among the non-red *Photuris*. Since the red *Photuris* live in slightly dryer habitats, they may be more prone to the habitat drying out. However, it is possible the non-red *Photuris* larvae simply hide more effectively when the habitat dries out. There may also be times when *Photuris* larvae are active but move about without glowing—we would not know if this was happening because all our observations are based on glowing larvae. Such non-glowing active larvae have been reported by Schwalb (1961), De Cock (2009) and in Chapter 2. It should be noted that most of my recorded non-red continuous glowing larvae were observed in Tennessee where the larvae were crawling and not hiding like those of red *Photuris*.

Continuous glowing, in *P. lucifera* larvae, was probably not physiologically equivalent to continuous glowing in *Photuris* larvae. In this case, continuous glowing seemed to be associated

with crawling under water. During these observations, the responses to disturbance of continuously and periodically glowing *P. lucifera* larvae were similar (not significantly different). These larvae live next to the water (on vegetation floating on Lake Alice) and may not be exposed to habitat drying out as are the red *Photuris* larvae living on the sandy soil at slightly higher elevations. A 1 m difference in elevation on sandy Florida soil often makes a large ecological difference.

Firefly Natural Enemies

Since glowing is thought to be defensive behavior, we need to consider which natural enemies the larvae are defending against. The most common and perhaps most important natural enemies encountered in these studies were the pathogens: fungi and bacteria (Lloyd, 1973a; Day, 2011; Buschman, 1984a; 1984b; Buschman and Faust, 2014). Lloyd (1973a) and Day (2011) reviewed many of these anecdotal observations, but there is no in-depth research on firefly pathogens. Fungal pathogens have been catastrophic in my efforts to rear *Photinus* fireflies (Buschman and Faust 2014; Fig. 14G). They appear to be less common in *Photuris* and *Pyractomena* larvae (personal observations). When rearing firefly larvae, there always seem to be larvae that wither away and die. These may be examples of bacterial and/or virus infections, but this has not been verified. I have no evidence that fungal pathogens affect the bioluminescence (response and periodic glowing) of firefly larvae.

Various invertebrates are encountered when observing or rearing firefly larvae. Invertebrate parasites of fireflies include parasitic insects, Phoridae, Tachinidae and Staphylinidae (Lloyd, 1973a; Buschman, 1977; Day, 2011) and nematodes and mites (Lloyd, 1973a; Buschman, 1977). Lloyd (1973a) lists some 16 records of dipterous parasites, but these appear to be single occurrence reports. This seems to be a relatively short list compared to the parasitism I have observed when rearing other field collected insects (personal observations). However, Lewis and Monchamp (1994) recorded up to 86% (n=7) fireflies infested and Faust (2010) recorded up to 63% (n=8) fireflies infested with phorids. These parasites have been recovered from adult fireflies. It is not clear if the fireflies were infested as larvae or as adults. Therefore, it is not clear if they would have been affected by the bioluminescence of firefly larvae.

Invertebrate predators of fireflies include insects and spiders. Spiders are commonly encountered (Lloyd, 1973a; Day, 2011; Lewis *et al.*, 2011; personal observation), but most of

these records are as predators of adult fireflies. The list of predators of firefly larvae is shorter: a wolf spider and a giant water bug (Belostomatidae) were recorded feeding on *P. lucifera* larvae (Buschman, 1984b); a harvestman was recorded feeding on a glowing *Photuris* pupae (personal observation); Faust (2010) reports twice finding harvestmen (Opiliones) feeding on glowing pupae of *Photinus carolinus* Green 1956; Lloyd (1973a) lists a pillbug (*Armadillidium* sp.) (Isopoda: Armadillidiidae) that was feeding on a *Pyractomena* pupa. Lloyd (1973a) lists several other invertebrate predators that could attack either larva or adult fireflies but does not identify them as larval predators. He also lists several records of ants feeding on fireflies, but again, it appears they were attacking dead adults. Firefly adults and larvae are known to glow responsively and flash spontaneously while being attacked and/or eaten by spiders (Lewis *et al.*, 2011; personal observation).

De Cock and Matthysen (1999) suggest firefly bioluminescence and chemical defenses must have evolved in the context of visually hunting predators. Vertebrates would be high on the list of visually hunting predators. Vertebrates would have the visual acuity to hunt using visual signals such as larval bioluminescence and have the memory to learn avoidance of chemical defenses. These are two components of a successful aposematic defense strategy.

Lloyd (1973a), Sivinski (1981) and De Cock (2009) present long lists of vertebrates preying on fireflies in the literature (more than 60 by my count). There were 34 reports of vertebrates accepting fireflies as prey (sometimes eagerly) and 26 reports of vertebrates rejecting fireflies as prey (sometimes emphatically). However, when the acceptance of fireflies was tested, as was done in lizards, mice, birds and toads (Lloyd 1973a; Underwood *et al.*, 1997; De Cock and Matthysen, 1999; De Cock, 2004a), it was clear that prey acceptance was not an absolute response. Acceptance depended on factors such as how hungry the predator was, the lighting conditions during prey presentation, the sequence of the prey presentation, etc. For our purposes, we can acknowledge that there is a lower likelihood of acceptance of fireflies than non-fireflies when offered as prey to these predators. This would be enough to give an evolutionary advantage to fireflies with bioluminescence and defensive chemicals.

For the purposes of this discussion, only the predators of firefly larvae will be listed. Domestic ducks are reported to eat firefly larvae (Table 7; Obs. #15 and Lloyd 1973a). Up to 100 firefly larvae were found in the crop of an American robin, *Turdus migratorius* Linnaeus 1766 (Lloyd 1973a). American robins are known to feed on the ground and hunt using sound and

movement clues to identify prey. A firefly larva was found in the stomach of a frog, *Lithobates pipiens* (Schreber 1782) complex (Lloyd 1973a). In the laboratory, *Luciola* Laporte 1833, *Photinus* Laporte 1833, *Photuris* LeConte 1851 and *Pyractomena* larvae are reported to attack and feed on other molting, pupating or dead firefly immatures (Fig. 14G; Lloyd 1973a). However, these are laboratory reports and it is not clear that such predation occurs at a significant frequency in the field.

De Cock and Matthysen (1999) list toads, hedgehogs and shrews as additional vertebrates that could be important predators of firefly larvae in Europe. Birds that scratch around in the leaf litter like chickens, pheasants, quail, thrushes, thrashers and juncos etc. and mammals that root around in the soil and leaf litter like pigs, armadillo, skunks and opossums would probably be potential predators of firefly larvae. Unfortunately, we have little information on the interaction between these predators and firefly larvae. In 2012, much of the firefly habitat in the Great Smokey Mountain National Park, Tennessee, was dug up by feral pigs. However, I did not recognize a suppression of the fireflies in spring (personal observation, one season).

In summary, there are more field predation records for invertebrates than for vertebrate predators. Perhaps the firefly camouflage and aposematic defenses are so effective that interactions with vertebrate predators are rare and we no longer see them even though they were important in driving the evolution of these defenses. It should be recognized that we are more likely to observe the specialized predators that are not sensitive to aposematic defenses of firefly larvae than we are to see the predators that are sensitive to these defenses and therefore may be responsible for the selection pressure that maintains these defenses.

Detailed experimental evaluations of firefly palatability or avoidance have been conducted with mice (Underwood *et al.*, 1997), toads (DeCock and Matthysen, 1999; De Cock, 2004a), starlings (De Cock and Matthysen, 2001) and bats (Moosman *et al.*, 2009). In addition, Marek *et al.*, (2011) have documented the aposematic function for bioluminescence in millipedes (Diplopoda: Polydesmida: *Motyxia* Chamberlin 1941) exposed to field mice as predators. These trials have firmly established the aposematic function of bioluminescence in firefly larvae and adults. They have demonstrated that these predators could be important in the evolution of bioluminescence, but its aposematic function still needs to be documented in field trials. Marek *et al.*, (2011) did field trials with florescent millipede models and tethered millipedes to verify the aposematic function of bioluminescence when exposed to predation by field mice.

Firefly larvae seem to maintain bioluminescence in many different habitats. Bioluminescent larvae can be found on the ground in leaf litter, climbing on plants and trees, tunneling underground and in rotten logs, swimming or crawling in fresh water and even climbing on rocks on a marine seashore. As far as we know, all these larvae continue to utilize bioluminescence, at least in defensive situations. It is not clear what predator or group of predators would be common in all those habitats and would provide selection pressure to maintain this larval bioluminescence. Is it possible that one function, even the aposematic defense function, could be so uniform across all those habitats? The function(s) of bioluminescence in the lives of firefly larvae will be discussed in more detail in Chapter 4.

Specialized Defense Structures

Tyler (2001) first reported that some lampyrid larvae had specialized defensive organs. Fu *et al.,* (2009) reported that these defensive organs could be found on nine species of fireflies. Aquatic *Luciola* (now *Aquatica* Fu, Ballantyne and Lambkin 2010) larvae had longer white eversible organs and produced a "pine oil smell". Terrestrial *Diaphanes* Motschulsky 1853 and *Pyrocoelia* Gorham 1880 and the European *L. noctiluca* had shorter pigmented eversible organs and the larvae also produced a "weak mint smell" (no smell in *L. noctiluca*). They also produced bioluminescent glows during the defense display, but the glows of aquatic larvae were fainter than those of the same larvae when they emerged from the water and were climbing about on land looking for pupation sites.

I have not observed such specialized defense structures, as reported by Fu *et al.* (2009), in North American firefly larvae. However, Vencl *et al.* (2012) used pH paper to show that *Photuris* larvae released defensive fluids from openings along the pleural cuticle of the abdomen when squeezed. I have not tested this technique.

Fu *et al.* (2009) also report that when *Aquatica* larvae were presented four increasing levels of threat stimuli: they froze and withdrew the head when approached (S1); they glowed when touched (S2), they curled up and glowed stronger when rolled over (S3); they glowed, everted defensive organs and released the pine-oil smell when squeezed (S4). Small larvae preferred to flee at S1. The odors appeared to be released from openings on the plural cuticle (separate from the eversible organs) and this opening could be seen opening and closing (Fu *et al.,* 2007). When terrestrial *Diaphanes* and *Pyrocoelia* larvae were tested with increasing levels of threat stimuli the results were similar to those of *Aquatica*, but they had brighter glows (Fu *et*

al., 2009). However, the *Pyrocoelia* larvae were less mobile, especially when well fed. Again, small larvae preferred to flee.

Fu *et al.* (2007) also reports that in the aquatic *Aquatica leii* (Fu and Ballantyne 2006) immature larvae (fifth instars taken from the water) had weaker responses to the graded levels of threat. They curled up (and glowed) but did not evert their organs or produce the odors when touched or turned over (S2-S3). When squeezed (S4) they produced the odors but did not evert the glands. However, more mature larvae (fifth instars collected on shore when they were preparing to pupate) gave strong responses: they froze and retracted the heads (S1); they froze (S2), they curled up, glowed, produced the odors and everted their organs (S3-S4). They also everted the organs for longer periods.

Fu *et al.* (2009) also report some observations for *L. noctiluca* larvae that were placed on an ant nest. The larvae crawled normally even with a carpet of ants crawling over them. They had none of the expected defensive responses. Occasionally a larva stopped and withdrew its head when the head was touched by an ant. The defensive organs were everted only occasionally when touched by an ant. Rarely, when ants bit the larva, they produced droplets of hemolymph. The ants recoiled immediately on contact with the hemolymph. All the *L. noctiluca* larvae were able to crawl away from the ant nest. The *L. noctiluca* larva interaction with ants was similar to the interactions I will describe below for *Photuris* larvae.

Vencl *et al.* (2012) did a physiological analysis of the defensive behavior of *Photuris* larvae. He found that the defensive neurotransmitter, "octopamine", was involved both with glow behavior and with the release of defensive chemicals. Both glow responses and defensive chemical releases were stimulated by manipulation of setae on the back of the larvae. Octopamine plays a defensive role in arthropods that is similar to the defensive role that noradrenalin plays in vertebrates.

Prey Records

Buschman (1984b) reported 46 prey records for *P. lucifera* larvae: 38 snails, five fresh water limpets, one small jumping spider, one small damselfly nymph and one small leech (Table 7, Obs. #5). There are another four records of *Pyractomena* larvae feeding on snails in the literature (Farnworth, 1973; Lloyd, 1973a, 2018). In the laboratory, there is only the report by Buschman (1984b) that *P. lucifera* larvae were fed chicken liver and snails. Most other field collected *Pyractomena* larvae did not feed in the laboratory (personal observation). These larvae

may have been collected when they were in the pre-pupal state looking for a place to pupate and no longer interested in food. *Pyractomena lucifera* appear to be primarily snail predators (Fig. 14A-C), but they will take other food items. It is unclear whether other *Pyractomena* larvae are also snail predators—in most cases we don't even know where they hunt for food (only when they climb up trees to pupate (Faust, 2012)).

Buschman (1984a) reported 21 prey records for *Photuris* larvae: 17 for red *Photuris* larvae and four for non-red *Photuris* larvae. Of the 17 records for red *Photuris* larvae, 11 were for various insects (dead and alive), five for snails and slugs, and one for a fruit (wild grape) (Table 7, 10 of 15 Obs.). Of the four records for non-red *Photuris* larvae, three were for fruit and one was for earthworm. In addition, I have four new records of non-red *Photuris* larvae feeding on earthworms. There are another three records for *Photuris* larvae feeding on earthworms and two records for them feeding on snails in the literature (Williams, 1917; Hess, 1920). There is an extensive list of living and non-living items that *Photuris* larvae will attack or eat in the laboratory (McLean *et al.,* 1972; Buschman, 1984a). Some species of *Photuris* larvae have been reared from eggs to adults by feeding them earthworm pieces (Fig. 14E, Buschman, 2017b). However, other species of *Photuris* will eat and develop into larger larvae but then fail to pupate (Buschman, 1984a; Buschman, 2017b). I conclude that *Photuris* larvae are scavengers that will take a variety of items on the forest floor, but they will attack-kill-and feed on soft-bodied insects, snails and earthworms when these are encountered. They are not specialist snail predators. Faust and Faust (2014) report that *Photuris* larvae fed eagerly on milkweed rhizome sections. This suggests that they might be feeding on the plant material to gain defensive chemicals. This will need further research.

Buschman (1977) reported two prey records for *Photinus* sp. larvae. One was a small leach that was being pulled across a mat of decaying vegetation and another was a small earthworm being eaten by a larva on a mat of decaying vegetation. There are no additional field prey records in the literature. In the laboratory, *Photinus* larvae feed on earthworms (live or pieces) (Fig. 14F) (Table 7, Obs. #7) (Wing, 1988; Buschman and Faust, 2014; Buschman, 2017b). Lynch (2013) reports that first instar *Photinus* larvae will feed on *Drosophila* Fallén 1823 pupae. *Photinus* larvae have been difficult to rear because we did not know what to feed them. They can now be reared into large larvae on earthworm, but they still will not pupate to produce adults (Buschman and Faust, 2014). This suggests something is still missing in the

rearing procedure. Two field-collected *Photinus* larvae pupated in the laboratory and produced *Photinus ardens* LeConte 1851 complex adults. *Photinus* larvae are also difficult to rear because they are very susceptible to a fungus disease (no identification; Fig. 14E). Most *Photinus* larvae are seen and/or collected so seldom that it is generally assumed that they must be subterranean, possibly feeding on earthworms. It is interesting that the group for which we know so much about their adult biology and flash courtship communication (Lewis and Cratsley, 2008) is the group we know so little about their larval biology and natural history.

In Europe, most firefly larvae, including *L. noctiluca*, are reported to prey on snails, but Photinini larvae, including *P. hemipterus,* are reported to prey on earthworms (De Cock 2009). Taylor (2002) lists 37 snail species that *L. noctiluca* will eat but only six of them have been recorded as prey in the field. Schwalb (1961) and Taylor (2002) report that these larvae will eat several other items in the laboratory. In Asia, we have records that several species of *Luciola, Aquatica* and *Pyrocoelia* fireflies prey on aquatic and land snails (Fu, *et al.,* 2007; Fu and Ballantyne, 2008; Ho *et al.,* 2014), but there are also recent reports that some Asian firefly larvae prey on ants (Fu *et al.,* 2007; Fu and Ballantyne, 2008; Ho *et al.,* 2014). Glowing is not reported to be important in prey capture or feeding of any of these firefly larvae.

Prey Capture and Feeding Behavior

I observed that when a *Photuris* larva became active, it slowly extended the head and waved it from side to side (Table 7, Obs. #12). It slowly started locomotion and glowed periodically. However, when there was a food item in the vicinity (5-10 cm), the larva appeared to recognize an odor coming from it. The larva would wave its fully extended head side to side and crawl slowly towards it. This movement was directional and suggested that the larva was following the odor gradient coming from the food. When the larva reached the food item it simply extended the head and started chewing on it. It continued to feed in this position for some time, with only the head and/or mouthparts in contact with the food. Sometimes the larva was observed to hold on to a snail with the mouth and the first pair of legs (Fig. 14A-C). Feeding larvae glowed infrequently and the glows were dim and seemed half-hearted. In the field, these glows were sometimes recognizable as glows from feeding larvae because they were noticeably dimmer and more irregular than periodic glows of crawling larvae.

When the food item was alive, the larvae approached in a manner similar to the previous description. If the prey did not move, the larva simply continued to chew and feed in that

position. It often appeared as though the prey did not sense that it was being bitten. There may be salivary secretions that were injected during this feeding activity that acted as an anesthetic. The mandibles of firefly larvae appear to be hollow (Fig. 14D) and may be connected to a toxin storage vesical (Schwalb, 1961). The chewing action also probably allowed the regurgitated digestive enzymes from the midgut to digest the prey tissues before they are sucked or pulled into the mouth. There was only occasional dim glowing during active feeding.

When the living prey reacted with movement to being bitten, the larva simply withdrew and waited until the prey became inactive again. It then approached again as described previously. However, on four occasions, twice in the field and twice in the laboratory, *Photuris* larvae were observed climbing quickly onto a moving caterpillar, wrapping themselves around the caterpillar and then proceeding with the chewing and feeding actions (Table 7, Obs. #1, #4). In each case the larva was able to hang on with the caudal grasping organ and legs while the caterpillar thrashed around for a period of several minutes. These larvae glowed continuously during the thrashing. This glowing appeared to be like response glowing. When the caterpillar stopped thrashing the glowing stopped and feeding proceeded as described earlier.

After the prey was subdued, the *Photuris* larva fed for several minutes at the kill site. However, many larvae, (ca. 30-50% based on laboratory observations), would move the prey item away from the kill site. *Photuris* larvae were observed to release the prey and crawl away to explore the surroundings (Table 7, Obs. #4, #5, #12, #13). They then returned to the prey item and dragged it to a location that the larva had just visited. The larva dragged the prey backwards, holding the prey with the head and mouthparts (and sometimes with the first pair of legs), pulling with the muscles of its abdomen. The larva reached back with the caudal grasping organ for new holds on the substrate. It pulled the prey some 5-10 cm. These larvae would glow occasionally while dragging the prey (Chapter 1), but the glows were dim.

Pyractomena lucifera larvae seemed to hunt and search for food by climbing about on the aquatic vegetation. They also repeatedly visited the water's edge and stuck the head into the water where they appeared to be smelling/tasting the water, possibly checking for chemical evidence of snails in the water nearby. Larvae also completely entered the water, either to climb to another plant or to seek a snail under the water. When larvae captured a snail under the water, they dragged it to a feeding site above water. Fig. 14A shows a larva holding a snail with the mouthparts and the first pair of legs under water. Fig. 14B shows a larva feeding on a snail after

pulling it above the water. These larvae normally feed with most of their feet on the substrate and the head and mandibles extended into the food item (Fig. 1A-C). I once watched a *P. lucifera* larva drag a snail up a cattail reed (Table 7, Obs. #5). There was a frog perched on the same reed and the larva pulled the snail up and over the frog. The frog probably sat motionless because of the red light I was using to watch what was happening. I have seen *Photinus* sp. larvae dragging a prey item on a mud flat.

The purpose of dragging the prey to a new site may be to move the prey away from the kill site where there could be a lot of blood and other body fluids that could attract other predators or scavengers. Larvae may be moving the prey to keep it safe for themselves to feed on in peace. My larvae did not seem to hide the prey or themselves, however Schwalb (1961) describes more of a hiding action by *L. noctiluca*. *Pyractomena lucifera* larvae must need to get out of the water to avoid aquatic predators and scavengers since they always seem to drag their prey out of the water. The larvae and their food were clearly vulnerable to thievery from other odor sensing predators and scavengers (Table 7, Obs. #6, #9-14).

My feeding observations differ in several ways from those reported for *L. nociluca* and *Phosphaenus splendudula* (=*Lamprohiza splendudula* (Linnaeus 1767)). Schwalb (1961) presents strong experimental evidence that *L. nociluca* and *P. splendudula* follow the slime trails of snails and earthworms. During my study, time was spent looking for evidence that *P. lucifera* and *Photuris* larvae would follow slime trails of their prey. There was no such evidence found. The slime trail following idea was not tested experimentally. Schwalb (1961) also described larvae following the trail by continually touching the substrate with the mouth parts (maxillary palps?). North American firefly larvae do not approach their prey this way; they hold the head high and wave it back and forth as though they are checking the air for odor. This behavior was consistent with following odor emanations from the prey item. Schwalb (1961) tested his larvae for odor-following and got negative results. I believe he was using too much air flow—which diluted the odor from the prey. However, his evidence for slime-trail-following appears sound. There appear to be real differences in the prey searching and capture behavior of European and North American firefly larvae.

Schwalb (1961) described the firefly larval attack on snails and slugs as being directed to the head end of the animal. My observations were that larvae attacked anywhere on the prey body—often at the tail-end of the caterpillar or earthworm. Schwalb (1961) also describes a bite and

release behavior, which I have not seen in the attacks of *P. lucifera* or *Photuris* larvae, but I have seen this bite and release behavior in a video of a North American firefly (possibly *Pleotomus* sp.) (Ben Pfeiffer, personal communication). My observations were that the firefly larva only release the prey if it struggles or when the larva was ready to go find a place to which it could drag the prey item. *Pyractomena lucifera* larvae do not release their prey, probably because they would lose it into deep water if they did so. Schwalb (1961) describes *L. noctiluca* riding the snail shell during the attack and waiting for the snail to succumb to the effects of the toxin. In my observations, there was some waiting for the prey to become quiet, but usually the attack and feeding was all one continuous action.

Schwalb (1961) presents evidence that head extracts and midgut extracts kill a snail when injected into it. He found that the head extract appeared to be more potent. He also presented evidence that the head extract was exhausted after the larva inflicted a few bites on a snail. He suggested that there may be two toxins, one in the head that can be exhausted after a few bites and another from the midgut which may simply be digestive enzymes. My observations suggest one of the toxins (probably the head toxin) had something of an anesthetic action because the prey sometimes did not seem to sense they were being attacked.

Schwalb (1961) discusses extra-intestinal digestion which has been suggested for firefly larvae by several workers (see references in Schwalb 1961). He downplays the role of extra-intestinal digestion because he found cells and tissue in the larval midgut. However, I believe this evidence only suggests that extra-intestinal digestion is not complete digestion. I have watched the action of larval mandibles during feeding. They move smoothly back and forth so there was no contact between the two mandibles and therefore there was no chewing action. There was also no tearing action. The mandible action apparently opened the tissues to digestive enzymes which then degraded them so that the larvae could suck and pull them into the mouth. The digestive enzymes may be regurgitated from the midgut and forced into the tissue of the prey ahead of the mouth. The action of the mandibles also operates a set of setae mounted on the inside base of the mandibles (Fig. 14D). These setae extend across the pharyngeal opening and seemed to act like two brooms sweeping and pulling the loosened tissue into the mouth.

Some authors have suggested that firefly larvae hunted and/or fed as a group (reviewed by Sivinski 1981; 1998). In the field, I seldom observed multiple *Photuris* or *P. lucifera* larvae feeding on the same prey. Only once in this study were multiple larvae observed feeding on the

same food item and that was when two *Photuris* larvae were feeding on a 15-cm earthworm (Table 7, Obs. #6). The earthworm was probably killed by the larva that was there when I arrived. I observed a second *Photuris* larva arrive and join in the feeding. The second larva apparently did not cooperate in killing the worm. The two were feeding ca. 3-4 cm away from each other and there was no apparent interaction between them. The earthworm was simply too large for one larva to drag away. Dean (1979) reports that he found six larvae feeding on a prey item in the field, but that was not a common occurrence. I believe that most observations of multiple larvae feeding on the same food item occur in the laboratory (Fig. 14E—F). In this confined space the first larva can't prevent other larvae from feeding on their prey. The prey dragging behavior, described above, appears to be intended to prevent competition from other larvae. We need more evidence of group hunting and feeding to verify its occurrence in the field.

There was usually little glowing during the attack or during the feeding process, so it is unlikely that glowing plays any role in predation. However, a group of ca. 30 *Photinus* larvae (second and third instar) were observed to glow faintly while feeding on a piece of earthworm (Table 7, Obs. #7). These glows may have been an indication of competition for access to the food. Sivinski (1981) states that *Photuris* larvae did not seem to do an unusual amount of luminescence while attacking snails.

There are both daily and seasonal rhythms in the glowing behavior of firefly larvae. De Cock (2004a) suggests that these different seasonal rhythms may be associated with the rhythms of their prey. *Lampyris nuctiluca* glow spontaneously in fall and spring while *P. hemipterus* (Goeze 1777) glow spontaneously only in fall. Presumably their prey is active at these times. *Phosphaenus hemipterus* and earthworms are both active during cooler, darker and more humid autumn nights. The seasonal rhythms could also be timed for when their predators were or were not present. All these possibilities will need further research.

Interactions with other Organisms

In a laboratory feeding trial, an intact uninjured caterpillar was added to a container with about a dozen *Photuris* larvae. The caterpillar crawled around but drew little attention from the *Photuris* larvae. However, when the caterpillar was accidently wounded (exposing some hemolymph), it attracted *Photuris* larvae from all over the container. The larvae seemed to sense "odors" released from the hemolymph. This could be repeated as needed. When similar wounded caterpillars were taken to the field, *Photuris* larvae were attracted over short distances (5-10 cm).

When other baits (like canned dog food; cut up insects; cut up earthworm; and canned tuna) were tested, they all attracted larvae. Initially, small numbers of baits were tested, usually placed near active *Photuris* larvae. When this worked, a larger number of baits were tested in a "bait trail". Again, *Photuris* larvae were attracted. There were also many other organisms that were attracted to these baits and the interactions between these organisms and the *Photuris* larvae were interesting.

The bait stations set out in Sept. 1975 attracted arthropods to the 60 caterpillar bait stations over the three nights as follows: five *Photuris* larvae (four red and one non-red), nine ants (Hymenoptera: Formicidae), seven harvestmen (Opiliones), two wolf spiders (Araneae: Lycosidae), one carabid larva (Coleoptera: Carabidae), one cricket nymph (Orthoptera: Gryllidae) and one cockroach nymph (Blattodea: Ectobiidae). The attraction rate for *Photuris* larvae was 8% (five *Photuris* on 60 caterpillar baits).

The bait stations set out in Sept. 2011 attracted arthropods to the 36 bait stations with four different baits over two nights as follows. These observations will be presented as "observations" because it was usually not possible to identify which animals were present for only one observation and which were present for several observations. The blank papers had two ant observations. The canned tuna had three *Photuris* observations (probably one larva), 362 ant (up to 60 small ants or one or two larger ants per observation), nine cockroach, three cricket, two pill bugs, one harvestman, one millipede (Diplopoda: Juliformia) and one slug (Gastropoda) observation(s). The attraction rate for *Photuris* larvae was 13% (one *Photuris* on 8 tuna bait stations). The earthworm pieces had 10 *Photuris* observations (probably two larvae), 36 ants, three crickets, one harvestman, one large millipede and one slug observation(s). The millipede removed the bait which surprised me since I thought millipedes were detritivores (Table 7, Observation #11). Three of the 12 sections of earthworm were removed. The attraction rate for *Photuris* larvae was 25% (two *Photuris* on eight earthworm baits). The insect sections had 85 ant and one millipede observation(s). Five of the 12 insect sections were removed, usually early during the observations, thus reducing the total number of active stations. Both the tuna and the earthworm pieces attracted *Photuris* larvae. *Photuris* larvae were not attracted to insect pieces in this trial, probably because many of the baits were removed early in the evening and they also may have dried up and were no longer attractive. *Photuris* larvae are known to eat insects (as described earlier). The assortment of other taxa attracted to these bait stations gives an indication

of the competitive environment around the *Photuris* larvae. Ants and harvestmen were the major competitors.

The interaction between *Photuris* larvae and ants was interesting. The larvae were expected to show some of the defensive behaviors, described earlier, when ants approached. However, the *Photuris* larvae seemed content to have the ants crawling over them, as though they were comfortable together (Table 7, Observation #9-10, #14). Taylor (2002) was also surprised when his firefly larvae appeared to be content to be submerged in a carpet of ants. Occasionally, when an ant would try to bite the larva, it would respond in three ways. It could bleed reflexively, and this would repel ants and sometimes glue the mouthparts or legs together (Blum and Sannasi, 1974: Fu et al., 2009). It could evert the eversible defensive organs that occur along the sides of the body (Taylor, 2002; Fu et al., 2009) and/or it could emit plant-like odors from glands that also occur along the sides of the body. There does not appear to be any glowing associated with these interactions with ants. It is interesting to remember that some firefly larvae are known to live in ant nests as inquilines in Africa (Cros, 1924; Lheritier, 1955) and in Florida (Sivinski *et al.,* 1998). The above *Photuris* observations appear to be those of an inquiline, although the *Photuris* larvae are not known to live in ant nests. They probably interact with ants often enough that they may have had to produce chemicals to pacify the ants. In Asia, there are also firefly larvae that prey on ants (Fu and Ballantyne, 2008; Ho *et al*., 2014).

The interactions of firefly larvae with ants (Fu *et al.,* 2006; 2009) appear to suggest the existence of a special chemical relationship between firefly larvae and ants. Firefly larvae are known to have chemical defenses that help them deal with predators. Perhaps the widely recognized chemical defenses of fireflies were originally developed within the context of living with ants—either in the nest as inquilines or outside the nest as competitors. Firefly larvae seem to be vulnerable to many arthropod predators, but they are not as vulnerable to ants (although they may be more vulnerable to the exotic red imported fire ants *Solenopsis invicta* Buren 1972). There is no indication that bioluminescence is involved in any of these interactions.

The interaction between *Photuris* larvae and the harvestmen was more like what would be expected from two competitors (Table 7, Observation #12-13). Both arthropods held onto the food item and tugged to try to gain control. Glowing did not seem to be an important component of the interaction with any of the other taxa at the bait stations.

The bait station concept seemed to work reasonably well with 8-25% of the bait items attracting *Photuris* larvae in good *Photuris* habitat. In the future, bait stations could be deployed at various times of the year to record *Photuris* larval activity (bait station visits) and associated glowing activity. Bait stations could also identify the presence of hunting larvae even when they were not glowing. We need to know if larvae hunt at times of the year when they are not observed glowing spontaneously. Bait stations did not work in a *Photinus* habitat (one trial) and would not be practical in the aquatic or marsh *P. lucifera* habitat. In the future, it may be desirable to monitor or video the stations to record whether the larvae that come are glowing periodically as they approach. In this study, larvae seemed to appear out of nowhere because I was moving from station to station and was not watching when larvae arrived.

CONCLUSIONS ON NATURAL HISTORY OF LARVAL BIOLUMINESCENCE

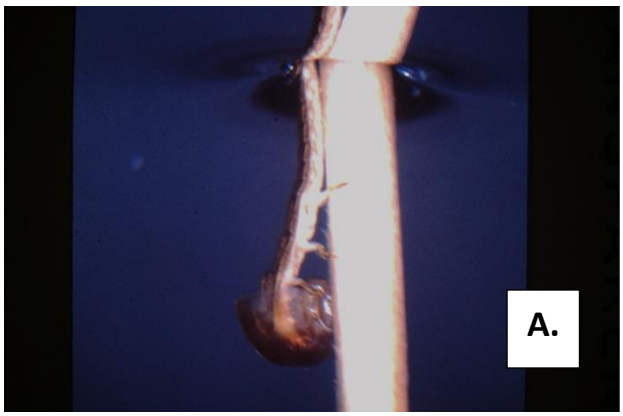
To summarize the results of this and previous reports on glowing behavior of firefly larvae (Chapters 1, 2, and 3), firefly larval bioluminescence did not seem to be involved in illumination of the surroundings, prey capture, feeding or interactions with competitors. However, larval bioluminescence was clearly involved in several defensive situations. De Cock (2009) and De Cock and Matthysen (1999) list 10 defensive behaviors/situations that are associated with bioluminescence: 1. fireflies are unpalatable to many predators; 2. fireflies have a low attack rate in experiments; 3. fireflies have toxic or deterrent chemicals in their bodies; 4. firefly bioluminescence allows predators to learn to avoid attacking them; 5. Fireflies have defensive plant-like odors; 6. fireflies have reflexive bleeding which produces repulsive droplets of blood; 7. fireflies have eversible defense glands; 8. fireflies have warning color patterns—black-red-yellow in both adults and larvae; 9. fireflies have many mimicry complexes (Batesian and/or Müllerian); and 10. Fireflies use the neurotransmitter "octopamine", which is the arthropod defensive neurotransmitter, to activate many of the above defensive activities (bioluminescence and the toxic and repellent chemical release). All these situations argue for bioluminescence being part of the firefly larval defense.

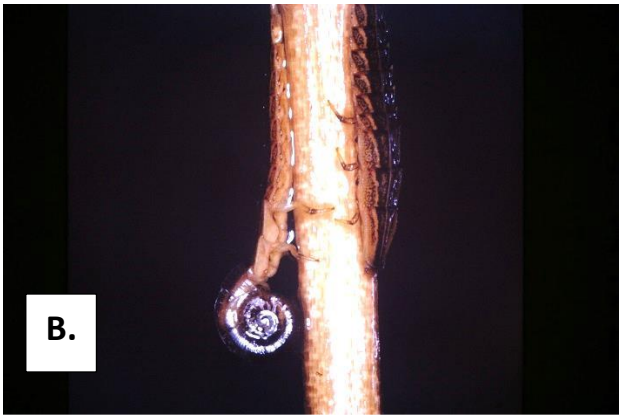
However, although the evidence is strong that glowing is associated with defensive activities, the evidence is not as strong for periodic glowing as it is for response glowing. If we consider the evidence carefully, we can see that we need to distinguish between the two types of bioluminescence.

Response glowing clearly occurs during defensive situations. Everything seems to fit the suggestion that glowing is an aposematic signal warning of toxic or deterrent chemicals in firefly larvae. Response glowing appears to be part of a package of defensive behaviors that include: nocturnal activity, camouflage, freezing or fleeing, response glowing, and emitting defense chemicals.

Periodic glowing is not as strongly associated with other defensive behaviors. It is associated with locomotion and hunting. Periodic glowing occurs before larvae are in imminent danger or feel threatened. It could still be an aposematic signal warning of toxic or deterrent chemicals, but this conclusion is not obvious. I suggest that periodic glowing is a component of the second suite of defensive behaviors that include: nocturnal activity, camouflage, stopping periodic glowing, and freezing or fleeing. The evidence that periodic glowing is part of a defensive package is somewhat circumstantial, but it is bolstered by the process of eliminating alternative explanations. A more complete discussion of the function(s) of the various types of bioluminescence in firefly larvae is a large undertaking and will be presented in Chapter 4.

Figure 14. A. Fifth instar *Pyractomena lucifera* larva with captured aquatic snail underwater pulling it up to the surface. **B.** Fifth instar *Pyractomena lucifera* larva with captured aquatic snail it has pulled up above the water surface where it is feeding. **C.** Fifth instar *Pyractomena lucifera* larva with captured aquatic snail it has pulled up above the water surface where it is feeding. **D.** One mandible of a fifth instar *Pyractomena lucifera* larva showing the internal channel and the setae on the medial base of the mandible. **E.** Third instar *Photuris* spp. larvae that were feeding on a piece of earthworm. **F.** Fourth instar *Photinus carolinus* larvae that are feeding on a piece of earthworm. **G.** *Photinus carolinus* 3rd and 4th instars with five larvae dead with fungus growth. **H.** Three *Pyractomena lucifera* larvae feeding on a fourth larva that had just entered the prepupal stage (because the tip of abdomen looks like it had been glued to the substrate). Light organs are showing on the killed larva.


A.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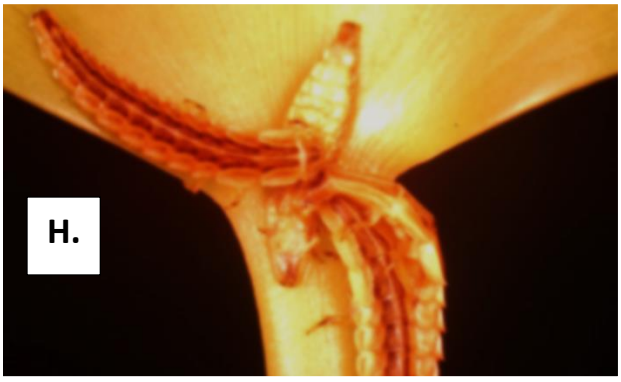
H.

Table 6. Number of larvae glowing and/or fleeing in response to a slight disturbance (moving leaf litter near larva), being picked up between the fingers and being released after being handled, together with Chi-Squared probability (2 by 2 contingency tables).

	Non-red *Photuris*			Red *Photuris*			*Pyractomena lucifera*		
Stimulus / Reaction	Periodic glows	Continuous glows	X^2 Prob.	Periodic glows	Continuous glows	X^2 Prob.	Periodic glows	Continuous glows	X^2 Prob.
Disturbed									
glow	11	8	0.0021	9	23	0.0008	4	5	0.79
dark	42	3		21	7		11	8	
Flee	7	3	0.48	2	5	0.42	4	3	0.83
freeze	46	8		28	25		11	10	
Handled									
glow	43	7	0.89	16	16	0.36	10	3	0.79
dark	3	1		11	5		7	4	
Released									
glow	39	7	0.54	11	15	0.87	8	1	0.25
dark	8	0		16	6		8	6	
Flee	39	6	0.72	17	17	0.30	11	6	0.74
freeze	8	1		10	4		5	1	

Table 7. Selected observations **(Obs.)** from my field notes to illustrate predation, feeding behavior and interactions between lampyrid larvae with other organisms in the environment. The following abbreviations are used for locations: **MPG**=Medical Plant Garden on the UF campus; **LA**=Lake Alice on the UF campus, **SU**=Student union on the **UF campus**, **TN**=Eastern Tennessee, **Lab**=Laboratory.

Obs. #1. 20 Aug. 1975: Red *Photuris* larva at the **MPG**: Inspected a glowing red *Photuris* larva. Found that the larva was attacking a caterpillar (4 mm by 4 cm--no ID). The caterpillar was thrashing with the larva wrapped around the mid-section of the caterpillar. Fifteen minutes later the caterpillar had stopped thrashing and the larva was feeding. Glowing was continuous while the caterpillar was thrashing but it became occasional during feeding like glowing while dragging prey.

Obs. #2. 20 Aug. 1975: Red *Photuris* larva at the **MPG**: Inspected a dim glow by a red *Photuris* larva. The larva had its head stuck down into the leaf litter. When I carefully pulled the leaf litter away, I found that it was feeding on a lovebug larva (*Plecia neartica* Hardy 1940) (Diptera: Bibionidae). There was a whole aggregation of lovebug larvae under the leaf litter and they scattered when exposed. Lovebug larvae feed on leaf litter.

Obs. #3. 21 Sept. 1975: Red *Photuris* larva at the **MPG**: Inspected a large red *Photuris* larva that was glowing continuously. Found that it was attacking a slug by crawling slowly up to the slug with extending head and it began biting/feeding on the slug.

Obs. #4. 23 Sept. 1975: Red *Photuris* larva at the **MPG**: Placed a freshly wounded thrashing caterpillar (3mm by 2 cm tent caterpillar) on a leaf near a large red *Photuris* larva. The larva retracted at the disturbance of my approach. After several minutes it extended its head and moved slowly towards the caterpillar. When it got close, it moved quickly to wrap itself around the middle of the caterpillar, biting and holding on with the thoracic legs and the caudal grasping organ while the caterpillar thrashed around. The larva glowed continuously during the thrashing. The glowing seemed to be associated with the movement. After 3-4 minutes the caterpillar was subdued, and the continuous glowing stopped. Eight minutes after the attack started the larva left the caterpillar and crawled about 2.5 cm away. It then returned and pulled the caterpillar to the place it had previously visited. It glowed periodically during the search but only occasionally during the dragging. Then it settled down to feed on the caterpillar and there was little glowing during the feeding stage.

Obs. #5. 27 Nov. 1976. *P. lucifera* larva at **LA**: Inspected a continuously glowing large *P. lucifera* larva. Found that it was dragging a snail. There was also a small bull-frog on the stem it was on. It dragged the snail over a bull frog all the way over his back, his head and off his head onto the stem they were on. The frog stayed still—perhaps frozen by the red light I was using to see the activity.

Obs. #6. 27 Sept. 2011. Non-red *Photuris* larva in **TN**: Inspected a weak glow and found a non-red *Photuris* larvae feeding on a freshly killed earthworm (ca 10 cm long). Then observed a second non-red *Photuris* larva approaching the earthworm glowing continuously. It stopped 1 cm away and then approached slowly with head extended, still glowing continuously. It started biting and feeding about 3 cm from the first larva. There was no observable interaction between the two *Photuris* larvae. Thirty min later, one larva (probably the first) left leaving the secondsond larva feeding. Another 30 min later, a continuously glowing larva (perhaps the first larva returning?) approached the earthworm and started feeding about 2 cm from the other larva. When feeding, there were only occasional faint glows from either larva.

Obs. #7. Fall 2012. *Photinus* larvae in the **Lab:** Observed a group of *Photinus carolinus* 2nd and 3rd instars feeding on piece of earthworm. There were many larvae that all climbed onto the piece of earthworm and it looked like a pin-cushion with larvae tails pointing in all directions. They glowed dimly and continuously while feeding.

Obs. #8. 5 Sept. 1970. *Pyractomena lucifera* larva at **LA**: Inspected some continuous glowing in a hyacinth leaf role. Found a large *P. lucifera* larva, apparently in response to the disturbance of an active hyacinth weevil that was present in the leaf roll.

Obs. #9. 23 Sept 1975. Red *Photuris* larva at the **MPG**: A red *Photuris* larva was feeding on a caterpillar. It left when disturbed by my movement. A trigger ant picked up the caterpillar and carried it away. Larva glows periodically while searching for the caterpillar.

Obs. #10. 23 Sept 1975 non-red *Photuris* larva at the **MPG**: A medium non-red *Photuris* larva was feeding on a caterpillar and it glowed only occasionally while feeding. Ants were observed crawling over both larva and caterpillar, but there was no glowing. Thirty minutes later the larva was still feeding and glowing occasionally, and ants were still present.

Obs. #11. 23 Sept 1975 Red *Photuris* larva at the **MPG**: Wounded caterpillars were placed on the ground in red *Photuris* habitat. A large cylindrical millipede (Diplopoda: Juliformia) came and removed one caterpillar—I was surprised because I thought they were herbivores.

Obs. #12. 24 Sept 1975 Red *Photuris* larva at the **MPG**: Wounded caterpillars were placed on the ground in non-red *Photuris* habitat. A large *Photuris* larva crawled straight to a caterpillar glowing periodically. It bit the caterpillar with head extended. Glowing stopped during the feeding. A harvestman came and grabbed the caterpillar and began pulling it away from the *Photuris* larva. They both tugged at the caterpillar. The *Photuris* larva glowed briefly (as when dragging food). The harvestman then released the caterpillar (because of the glow?). The *Photuris* larva continued feeding in the same spot. Some 10 minutes later the *Photuris* larva crawled away from the caterpillar and then returned about a minute later and dragged the caterpillar to the location the larva visited 5 cm away. The movement seemed well directed and intentional and not a random search.

Obs. #13. 24 Sept 1975 Red *Photuris* larva at the **MPG**: Placed 10 wounded caterpillars on paper squares on the ground in red *Photuris* habitat. A harvestman (with shorter legs) came to feed on a caterpillar. A red *Photuris* larva approached the same caterpillar slowly with head extended and glowing periodically. The *Photuris* lunged at the caterpillar and began biting/feeding. The harvestman and the *Photuris* larva tugged on the caterpillar. The *Photuris* larva was glowing periodically as it does when dragging prey. The harvestman eventually released its hold on the caterpillar. The *Photuris* larva dragged the caterpillar away holding caterpillar with the head while glowing periodically. Some 10 min later the harvestman came back and tried to feed on the caterpillar. They tugged on it, but the harvestman soon released its hold on the caterpillar.

Obs. #14. 26 Sept 1975 non-red *Photuris* larva in the **MPG**: Wounded caterpillars were placed on the ground in non-red *Photuris* habitat. A medium non-red *Photuris* larva was feeding on a caterpillar. An ant approached, and the larva retreated and then crawled away glowing periodically.

Obs. #15. Nov. 1976 non-red *Photuris* larvae at the **SU** pond. Tried to feed lampyrid larvae to semi-domesticated ducks at a pond where people commonly fed the ducks. Day 1: one duck picked up several larvae and swallowed them but seemed to do some extra mandibulation. Day 2: Two ducks picked up several larvae, mandibulated them a little, and then discarded them. I collected the discarded larvae and reared them. One of the three larvae died and two survived. Day 3: one duck ate seven larvae without any sign of discomfort. Day 4: The ducks were not hungry and ignored the larvae.

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CHAPTER 4.

THE EVOLUTION AND FUNCTION OF BIOLUMINESCENT BEHAVIOR IN FIREFLY LARVAE

INTRODUCTION

The function and survival value of bioluminescent behavior in firefly larvae is a subject that has fascinated biologists for many years (Sivinski 1981; De Cock 2009 and citations therein). It seems like every biologist who has ever seen larval glowing has felt compelled to comment on its potential function. Many interesting and sometimes contradictory hypotheses have been offered (Buck 1978; Sivinski 1981; De Cock and Matthysen 1999; De Cock 2009). However, most of these ideas are based on fragmentary and/or anecdotal behavioral observations. Recently, several workers have described the potential biochemical mechanisms that may have been involved in the evolution of bioluminescence (McElroy and Seliger 1962; Oba *et al.* 2013; Marek and Moore 2015) while others have tested the aposematic hypothesis (the idea that larvae are using the bioluminescence to warn predators of their defensive chemicals) for the function of bioluminescence (Underwood *et al.* 1997; De Cock and Matthysen 1999; 2003; Tyler 2002, Marek and Moore 2015). However, these results have been difficult to discuss more fully because our understanding of bioluminescent behavior of firefly larvae is so limited and our knowledge of larval natural history and ecology is so incomplete.

The goal of this Chapter is to discuss firefly larval bioluminescent behavior in the light of observations reported in Chapters 1, 2 and 3 explaining the behavior and natural history of several different North American firefly larvae. This discussion will be done in the context of reviewing the evolution of bioluminescence in firefly larvae. This will allow us to discuss the functions of bioluminescence together with the selection forces that may have driven their evolution—historically. I believe that the functions that were involved in this evolution in the past are probably still active today. A table will be constructed (Table 8) to help us grade the evidence supporting the various suggestions for the evolution and function of bioluminescence in firefly larvae.

I believe it is important to recognize, at the outset, that bioluminescence in firefly larvae is not a single entity—it is in fact a spectrum of different bioluminescent entities. Each of these entities will need to be discussed individually, because they probably have different

functions. In the literature several different types of larval bioluminescence have been described in a preliminary way, beginning with Kaufmann (1965), McLean *et al.* (1972) and Dreisig (1974). As reviewed in Chapter 1, there are two types of bioluminescence that must be reviewed: response glowing and periodic glowing. In the following sections, I will be describing a few more types of larval bioluminescence bringing the total to six types of firefly larval bioluminescence: **incidental luminescence**, **faint body glowing**, **bright body glowing**, **bright continuous glowing**, **response glowing** and **periodic glowing**.

This discussion is offered to build a working hypothesis for the evolution of bioluminescence that can be used to organize our thinking about firefly larval bioluminescence. This discussion should also help us develop workable and testable hypotheses that can then be tested in future research. It is also hoped that this discussion will stimulate additional field and laboratory observations that will help us understand larval bioluminescent behavior a little better.

Early Evolution of Bioluminescence

Bioluminescence appears to have evolved independently in some 30-different major taxonomic groups in the plant and animal kingdoms (De Cock and Matthysen 1999). The widespread occurrence of bioluminescence suggests that it has considerable adaptive value for organisms that live in many different habitats. Within the Coleoptera, bioluminescence is present in the related families of Elateroidea: Lampyridae, Cantharidae, Elateridae and Phengodidae (Sivinski 1981; Branham and Wenzel 2000; 2003). Martin *et al.* (2017) suggest there may have been one to six origins of bioluminescence followed by five to ten losses of bioluminescence in adult Lampyridae. The evolution of larval bioluminescence among the Elateroidea has not been addressed in the same way, because of lack of information across these families. My default hypothesis would be that there was probably a single origin of bioluminescence in the Lampyridae, and so far, we have no documented losses of bioluminescence among these larvae (there are, however, many genera within the other Elateroidea families that appear to lack larval bioluminescence).

Incidental Luminescence. Bioluminescence in fireflies is known to be based on biochemical reactions that involve luciferin and luciferase together with several co-factors (Lewis and Cratsley 2008, Lloyd and Gentry 2009). Primitive luciferases appear to have evolved from enzymes that helped the cell utilize oxygen as an electron acceptor (Seliger 1975). The resulting highly reactive molecules, i.e. peroxides, emit light photons to release this surplus

energy (McElroy and Seliger 1962; De Cock and Matthysen 1999; Marek and Moore 2015). This produces a low-level luminescence that is not visible to vertebrates and will be termed **"incidental luminescence"**. (I am using the term luminescence because the light itself does not seem to have a biological function. I will use the term bioluminescence when the light seems to have a biological function.) This luminescence can be detected by special instruments and can be found in many animal tissues, even human tissues (Kobayashi *et al.* 2009). Since the biochemical reactions that produce luminescence are widespread in the Animal Kingdom, bioluminescence can develop independently in many different taxonomic groups.

Incidental luminescence will be rated as having strong observational support for the biochemical function (Table 8), since we have some basic experimental information about it. It may continue to be present in many modern organisms, but usually in the background. In fireflies it probably occurs together with other brighter forms of bioluminescence. There seems to be no functional or selective advantage for this light, although the biochemical reaction does seem to have a biological function.

Physiological Bioluminescence. There must have been an increased demand for the detoxification function, perhaps when more energy was needed to deal with environmental stresses (Marek and Moore 2015). This would have caused an increase in incidental luminescence. This luminescence is produced throughout the body (the light organ will come later) (Fig. 15) and can be called a **"body glow**,**"** (Buck 1948; 1978; Buschman 1984a; De Cock 2009; Tisi *et al.* 2014).

The very dim forms of luminescence can be called **"faint body glow**.**"** This is a very faint glow that is hard to see. For example, the glow of *Pyractomena lucifera* (Melsheimer 1845) eggs can't be seen until the eyes have dark adapted for 15 to 20 min (see photo in Buschman 1984b). The very faint body glow of larvae, pupae and adult fireflies has also been photographed by Tisi et al (2011) (Fig. 15). The pigmented sclerites of the insect body seem to hide much of this internal luminescence. Since this luminescence does not seem to be directed to the outside of the body, the function of this luminescence must be on the inside of the body, there must be a **"physiological function**.**"** This very faint body glow is unlikely to be active in warning predators since it is hard for the predator to see it. I have not found a published function that would work internally within the body, so I am suggesting that the light may function in **"immunology**.**"** Light is known to have negative effects on microorganisms, such as fungus

pathogens (Page 1965; Idnurm and Heitman 2005). The faint body glow would likely make it hard for internal pathogens to develop and/or reproduce within such bioluminescent cells. This would provide a selective advantage for larvae that had such faintly bioluminescent cells. Such a physiological function would then provide a selective advantage for the larva and this could lead to the development of a brighter body glow. When the glow became bright enough to be seen easily by predators it could take on the function of warning predators of defensive chemicals.

The faint body glow appears to be present in all firefly life stages (Buschman 1984b, Tisi *et al.* 2014) and it may be present in most types of fireflies. It may also be present in other groups within the Elateroidea (De Cock 2009; Tisi *et al.* 2014). Viviani *et al.* (2008) and Tonolli *et al.* (2011) locate the body glow in the lobes of the fat body and suggest that this tissue may have contributed to the development of the light organ.

The faint body glow will be rated as having minimal observational support for the physiological function, because we have little evidence for this function (Table 8). However, I am also suggesting that this glow is probably present together with the brighter types of bioluminescence in modern fireflies (Table 8), but I recognize that this is an extrapolation.

There are serious disadvantages for an organism that has bioluminescence, because it makes them more "apparent" or "visible". Increased bioluminescence will cause predator pressure to increase so there would have been selection pressure against developing brighter bioluminescence. Firefly larvae appear to have used three types of adaptations to deal with increased visibility caused by bioluminescence and the associated increased predator pressure: **a.** develop an opaque cuticle to hide the internal body glow, **b.** accumulate defensive toxic and/or repellant chemicals to deter potential predators and **c.** develop a mechanism that allows larvae to turn the bioluminescence off when it is not needed so larvae can use camouflage for defense.

Many fireflies have developed thick sclerotized opaque cuticle that seem to hide the faint body glow (Fig. 15) (Tisi *et al.* 2014; De Cock 2009). De Cock (2009) suggests that the pink or magenta coloration that is often found in firefly larvae may actually function in absorbing the faint body glow (pink is the complementary color of yellow-green, the color of bioluminescence in most firefly larvae) (De Cock 2004b; 2009). Most fireflies have sclerotized opaque cuticles as larvae and also as adults, however many eggs and pupae have transparent cuticles which allows the world to see the bioluminescence. This body glow is very faint, but in some pupae, it is brighter, particularly when the cuticle is transparent. However, most of these eggs and pupae are

found in the soil where the bioluminescence may not be as likely to attract visually hunting predators (see also the "false surfacing" function below).

Bioluminescence Takes on a Defensive Aposematic Function. Bioluminescent larvae that had accumulated toxic or repellant defensive chemicals would have had a selective advantage over their non-toxic relatives, becoming more common. At first defensive chemicals were probably obtained by feeding on other organisms that contained these chemicals. Faust and Faust (2014) report that several fireflies were observed to feed on milkweed, *Asclepios* Linnaeus 1753. Milkweeds are known to contain alkaloid chemicals that are toxic to other organisms. They surmise that these fireflies may be feeding on milkweed to obtain defensive chemicals. Eisner and his group showed that *Photuris* LeConte 1851 fireflies can obtain defensive chemicals by feeding on *Photinus* Leporte 1833 fireflies that have different defensive chemicals (Eisner et al. 1978). Fireflies such as *Photuris* have also developed metabolic mechanisms to modify these acquired chemicals (Gonzales et al. 1999a). Eventually fireflies would have developed the metabolic mechanisms to produce defensive chemicals themselves. Fireflies in the genus *Photinus* are known to produce steroidal pyrones (cardiotonic steroids) which are structurally similar to toad venoms, so the chemicals were named lucibufagins (Eisner et al. 1978). Fireflies in the genus *Photuris* are known to produce a betaine, N-methylquinolinium 2-carboxylate (Gonzales et al. 1999). The chemistry of these defensive chemicals is reviewed by Day (2011).

Eventually bioluminescence would have become a marker for larvae that had defensive chemicals. Animals that had toxic and/or repellant chemicals often have bright colors which they show off to warn potential predators of their defensive chemicals. This is known as an **"aposematic signal."** The presence of aposematism in firefly larvae is supported by the observed unpalatability of fireflies, reflex bleeding, aposematic color patterns, and other insects that mimic fireflies (De Cock 2009). Bioluminescence has also been shown to have an aposematic function in experimental tests (Underwood *et al.* 1997; De Cock and Matthysen 2003). The suggestion that larval bioluminescence had an aposematic function was developed by Sivinski (1981) and then by De Cock (2009). The aposematic function of bioluminescence has also been demonstrated in millipedes (Marek *et al.* 2011).

The brighter forms of bioluminescence could not have developed without the development of the aposematic defense. The presence of bioluminescence almost demands the

presence of toxic or repellant chemicals to repel potential predators (Day 2011). Once larvae developed defensive chemicals, there would have been selection for brighter bioluminescence to provide a better warning. This would have led to the development of the **"bright body glow"** (Fig. 17A). This is a glow that is bright enough to be seen readily by potential predators, so it can act as an aposematic signal (Fig. 17B) (Viviani *et al.* 2008; Tonolli *et al.* 2011; Oba *et al.* 2010; 2013).

De Cock (2009) has postulated that visually hunting predators, such as toads, frogs, lizards, birds, mammals and insectivorous arthropods, that can see bioluminescence while hunting would begin to target bioluminescent larvae. These predators have the visual acuity to see the bioluminescence and the memory to remember the results of attacking these chemically defended larvae. The evidence for firefly natural enemies is summarized in Chapter 3. De Cock (2009) also summarizes seven reports of firefly unpalatability to lizards, frogs, toads, starlings, spiders, centipedes and carabid beetles. Lewis and Cratsley (2008) and Day (2011) summarize the evidence for chemical defenses in fireflies as presented by Lloyd (1973a); Eisner *et al.* (1978); Gonzalez *et al.* (1999). The various glands and mechanisms used to present these chemicals to their predators are described by Tyler (2001); Fu *et al.* (2007; 2009); Vencl *et al.* (2012). These chemicals may have evolved in firefly larvae initially during interactions with ants, since some fireflies are known to be inquilines in ant nests and firefly larvae seem to be tolerated by ants (and *visa-versa*), even around food (Chapter 3).

I am rating the bright body glow as having very strong observational support for aposematism, mostly by extrapolating from the experimental support for the aposematic function of continuous glowing and the evidence that all stages of fireflies have defensive chemicals (Table 8).

There has also been some progress in understanding the chemical and physiological mechanisms that could have been involved during the evolution of bioluminescence. Oba *et al.* (2003) described two different functions for two luciferases, one was synthesis of fatty acyl-CoA and the other was oxidation to produce bioluminescence (the body glow). There was only a single amino acid difference in the active site of these two luciferase enzymes (Oba *et al.* 2009). Oba *et al.* (2010; 2013) documented that light produced as a body glow and light produced by the light organ were produced by different luciferases and that the two lights had two different wave lengths (colors). Day (2009) found that there were many different luciferase-like genes in

beetles and he suggested there may have been several gene duplication events. Each duplicated gene could then become associated with a different function and thus there could be a family of luciferase genes with different functions (some genes may also be non-functional). These biochemical and genetic observations provide some insight into the question of "How" bioluminescence developed. However, we must also address the question of "Why" bioluminescence developed: the physiological and ecological functions of larval bioluminescence.

Development of Light Organs. When bioluminescent larvae with defense chemicals became common and the aposematic function was established, there would have been selection for brighter bioluminescence as well as stronger and more effective defensive chemicals. This relationship between an increased brightness and an increased toxicity has been demonstrated in millipedes (Marek and Moore 2015), but not in firefly larvae.

The demand for brighter bioluminescence would have led to the development of specialized cells that could produce the brighter bioluminescence. Then there would have been selection to bring these specialized cells closer together to form groups of bioluminescent cells and thus further increase the apparent brightness of the light. These groups of cells would thus become loosely organized light organs. These light organs would have produced a **"bright continuous glow**,**"** often on specific parts of the body—like the spots and bands observed in many larvae and adults within the Elateroidea (Fig. 16: #17, 18) (Lloyd 1971; Halverson *et al.* 1973, Sivinski 1981).

I have rated the aposematic defense as having experimental support for bright continuous glowing (Table 8). Most of the experimental work on aposematism has been done with continuous glowing so there is strong observational and experimental support for it (Underwood *et al.* 1997; De Cock and Matthysen 2003), but we still need field validation. Within the scientific community, the aposematic function of bioluminescence in firefly larvae appears to be a well-accepted theory (De Cock 2009; Vencl *et al.* 2012). However, while it is easy to agree that bioluminescence has an aposematic function, it is not so easy to understand how, exactly, firefly bioluminescence and the bioluminescent behavior function in this aposematic defense.

Nervous Control. Until this point in our discussion, bioluminescence has been produced continuously with no option of turning it off (except hiding it with an opaque cuticle). When specialized predators developed that could overcome the defensive chemicals, they would have

targeted bioluminescent larvae, and this would put heavier predation pressure on them. There must have been selection pressure for larvae to develop additional defensive strategies. The ability to turn the bioluminescence off would help these larvae hide more effectively. This would require either hormonal control or nervous control of light production.

The neural activating hormone, "octopamine", is known to be used by arthropods to activate defensive responses when insects are threatened (Christensen and Carlson 1982). This neurotransmitter produces defensive responses such as the release of defensive chemicals (Vencl *et al.* 2012). With increasing predator pressure, there would have been selection for light organ cells to also respond to octopamine, to activate the bioluminescence during defensive situations. This would allow the light organ to glow when stimulated by octopamine. Halverson *et al.* (1973) report that based on behavioral observations, the light organs in three genera of Phengodidae appeared to be controlled by the neural system while those in the fourth genus were not (possibly controlled through hormones?). When bioluminescence was not needed, such as during the day when larvae are resting or hiding, it could be turned off by turning off the hormonal signals.

Then there would be selection for a more direct neural connection between the nervous system (where the octopamine is produced) and the light organ. This would allow the light organ to operate faster and in coordination with other defensive behaviors. Eventually the neurons that produce octopamine would extend into the light organs to activate it directly as they do today in lampyrid larvae. Thus, larvae would continue to use the neurotransmitter, octopamine, to activate the light organ. Modern firefly larvae have nervous control of bioluminescence and can turn it on or off over a period of one to several seconds. The nervous system of fireflies is described in more detail by Buck (1948), Christensen and Carlson (1982) and Robertson and Carlson (1976).

Nervous control of bioluminescence appears to be widespread and may be universal among the lampyrids. Buck (1948) described six types of larval light organs based on the morphology of the tracheal and nerve supply. Modern larval light organs occur universally as two oval spots on the 8th abdominal sternite (Fig. 16 A, B, E, F) (Sivinski 1981; Buschman 1988; Branham and Wenzel 2000; 2003; De Cock 2009). Nervous control would have allowed larvae to go dark when they were hiding so their camouflage could be more effective. Thus, larvae developed what Sivinski (1981) has called **"facultative aposematism**,**"** bioluminescence that could be used in specific situations when needed. De Cock (2004a; 2009) and Chapters 1, 2 and

3. I have identified two types of facultative aposematism: "**response glowing"** and "**periodic glowing**."

Response glowing. Response glowing (also called "induced glowing" (De Cock 2004a, 2009) or "disturbance" and "defense" related glowing (Sivinski 1981; Taylor 2002)) is a long-lasting bright glow that is produced by the light organ of a larva in response to the approach of a potential predator, for example when there are vibrations in the substrate (Chapter 1 and 3). These glows can be a few seconds to many minutes long. They can be so long that they can be confused with continuous bright glowing. Response glowing appears to be widespread among firefly larvae and it may be universal (Buck 1948; Lloyd 1973a; Sivinski 1981; De Cock 2009). In my study of North American firefly larvae, response glowing was observed in larvae that were in a resting and/or hiding physiological condition or when larvae were being handled (Chapter 1 and 3).

Response glowing appears to be part of a package of defensive behaviors including; nocturnal activity, camouflage, freezing (becoming motionless), fleeing, response glowing, and emitting defense chemicals (Chapter 3). This appears to be a defensive package for larvae that are in the hiding and/or motionless physiological condition. The larval body color patterns in many (maybe most) fireflies match the surroundings creating an effective camouflage. However, there are some firefly larvae that have contrasting aposematic color patterns (De Cock and Matthysen 2001). Such larvae are sometimes active during daytime when there is light, allowing predators to see the aposematic color patterns (Tyler 2002).

I am rating response glowing as having very strong observational support for aposematism, mostly by extrapolating from the experimental support for continuous glowing and the evidence that all stages of fireflies have defensive chemicals (Table 8). My experience is that the hiding and camouflage defenses of firefly larvae are extremely effective, and I have seldom been able to locate firefly larvae when they were not glowing. Later, I will be discussing four defensive behaviors which appear to be "enhancements" of the response glow.

Periodic Glowing. Periodic glowing (also called "spontaneous glowing" (De Cock 2004a; 2009), "intermittent glowing" (Buck 1948) or "light house glowing" (Taylor 2002) are bright glows produced by the larval light organs while larvae are in the crawling and hunting physiological condition (Chapters 1 and 2). These glows are short, usually only a few seconds long (1-20 seconds). These glows are being called **"periodic glows"** because they are produced

repeatedly. Some authors describe these glows as “rhythmic”, while others describe them as “non-rhythmic” (Chapter 1). Periodic glowing is not as widespread within the fireflies as is response glowing (De Cock 2004a; 2009; Chapter 1), but our knowledge of its occurrence is limited, because observers have not reported which type of glowing behavior was observed.

Periodic glowing appears to be part of a second suite of defensive behaviors which include: nocturnal activity, camouflage, stopping periodic glowing, etc. (discussed further in the next section). Larvae seem to feel exposed (to potential predators), but don’t sense the immediate danger of a predator attack. In this situation the defensive response to danger is to stop periodic glowing (Chapter 3).

Although the existence of these two types of facultative glowing behavior have been known for several years, observers have not generally associated them with different ecological and behavioral observations. In this study, many of my early observations were made without making this distinction and the glowing behavior did not make sense. However, when the type of glowing was identified and associated with specific defensive behaviors the glowing behavior made more sense. For this reason, many of my early observations had to be discarded. In the same way, many observations in the literature cannot be interpreted because we do not know which type of glowing behavior was being observed.

Now we need to consider the selective advantage of periodic glowing—as opposed to continuous glowing or response glowing. The first suggestion was that periodic glowing could save on metabolic energy (De Cock and Matthysen 1999). In my observations (Chapter 1), the periodic glows occupied 32-52 % of total time (“duty cycle”). Therefore, periodic glowing would appear to reduce glowing time 48-68% and this should represent a similar level of energy savings. However, the direct cost of light production appears to be relatively low—it uses less energy than walking (Wood *et al.* 2007). The indirect cost due to predation may be more significant than the energy cost (Wing 1988). In addition, we need to consider that there would be considerable metabolic cost in producing the specialized innervated light organs that are able to produce periodic glows. There would also need to be enhanced energy and oxygen supplies. It appears that periodic glowing may require a greater cost than continuous glowing.

Not only does there not seem to be much increased energy cost, but it does not make sense that larvae would save energy precisely when they are most vulnerable, while hunting and thus exposed to predators. I would expect that they would use some sort of enhancement to their

defensive system. I believe that periodic glows must provide some advantage over continuous glows for the larva. I am therefore proposing that periodic glowing represents an enhancement of the basic aposematic signal (discussed further below).

Periodic glowing will be rated as having very strong observational support for aposematism, mostly by extrapolating from the experimental support for the aposematic function of continuous glowing and the good evidence that all stages of fireflies have toxic and repellent chemicals (Table 8). There are also two defensive behaviors that will be considered enhancements of the aposematic periodic glow and they will be discussed below. The conclusion that periodic glowing has an aposematic function is also supported by the process of eliminating alternative explanations.

In summary, response and periodic glowing could not have developed without the advanced innervated light organ. It is my conclusion that response and periodic glowing should be considered enhancements of larval bioluminescence. I believe that these different forms of glowing behavior not only function in aposematic defense, but they also add to the impact of the aposematic signal.

Enhancement Functions for the Aposematic Defensive

Although bright continuous bioluminescence in larval fireflies probably developed as an aposematic warning display, bioluminescence may also have developed additional functions over time (without necessarily abandoning the original aposematic function). Buck (1978), Sivinski (1981), Taylor (2002), and De Cock (2009) list more than 20 different proposals for the function of bioluminescence. These various proposals are difficult to harmonize because the terminologies are different, the ideas are not always well developed, and some ideas simply do not apply to fireflies. I will be discussing those proposals that I believe have potential application for fireflies. First, I will discuss a list of six proposed defensive functions which I believe function as enhancements of the basic defensive aposematic signal. Later, I will also discuss a similar list of four non-defensive functions.

According to Robinson (1969), defensive behavior can be understood as two types of defense: the first type includes defensive behaviors that prevent a predator from initiating an attack; and the second type includes defensive behaviors that operate during an interaction with the predator. The first line of defense in Robinson's proposal normally includes things such as camouflage and nocturnal behavior. These defenses are often indirect and non-obvious. Periodic

glowing of firefly larvae seems to fit this non-obvious category of defensive behaviors. It seems to be an aposematic signal acting in a prophylactic way during locomotion (Chapter 1 and 2). It will be considered a part of the first line of defense—preventing a predator from initiating an attack. Larvae that are moving about and hunting give up much of their camouflage defense through this movement. This exposes them to predators, so this is when one would expect larvae to do everything possible to enhance their defenses. We observe that this is when they produce the periodic glow (Chapters 1 and 2). This glow must be a stronger and/or a more effective aposematic signal than the continuous glow. Therefore, periodic glowing will be interpreted to be an aposematic signal that deters predators when larvae are active and crawling in the environment. This glow appears to function like the bright colors of butterflies that fly about displaying their bright aposematic colors (Blest 1957; Cott 1957). These colors are recognized as aposematic displays and they have been shown to deter predators (Blest 1957). The effectiveness of the periodic glow as an aposematic display appears to be enhanced in two ways over continuous glows: first as a "flash display" and second as an "enhanced visibility" display.

Flash Display: The flash display is a flash of color that an animal shows when it flees a predator that gets too close (also called the "distraction defense)". The role of the flash display in firefly larvae has been discussed by Buck (1978) and De Cock (2009). Periodic glows may act like the "flash coloration" observed in moths, butterflies and grasshoppers (Blest 1957; Cott 1957, De Cock 2009). This display is also observed in the white-tailed deer and the cotton tail rabbit. All these animals are cryptic initially, but when disturbed they suddenly flash bright colors while they flee. When they eventually settle down and become motionless; they hide their color. The fleeing animal has created a bright flashing search-image for the predator, but when the animal hides the color, the animal seems to disappear because the bright colors that the predator has been following have disappeared. However, the animal may still be visible and in plain sight. In the case of firefly larvae, the periodic glow creates a bright search image for the predator. When the larva turns off the glow, the larva seems to disappear into darkness and/or the leaf litter.

The periodic glow may also interfere with the predator visual system (De Cock 2009; Ce Cock and Matthysen 1999) to make it difficult for a predator to track the position of the larva. Visually hunting predators need a lot of time to focus under low light conditions. It seems to take the toad some 38 seconds to focus for an attack on a prey item in low light conditions (De Cock

and Matthysen 1999). When the glow goes off, the visual processing of the predator has to start over again. It is interesting that the duration of all known periodic glows average less than 38 seconds (Chapter 1).

The flash display seems to be a reasonable hypothesis because it has been effective on me (as a predator collecting larvae), but there is little experimental evidence to support or refute it. This hypothesis will be rated as having very strong observational support for periodic glowing, but it is non-applicable for continuous and response glows (Table 8).

Enhanced Visibility: Enhanced visibility is proposed as a defensive strategy for increasing the visibility of larval bioluminescence. Periodic glowing increases visibility the same way that flashing warning lights on bicycles, motorcycles, radio towers and lighthouses increase their visibility. Humans (and possibly other predators) habituate to a steady light, but a blinking light breaks up this habituation. Lloyd and Wing (1983) found that decoys with continuous glows were easier targets for a predator than decoys with intermittent short glows or decoys that were dark.

The enhanced visibility defense seems to be a reasonable hypothesis and it has been very effective on me (as a predator collecting larvae), but there is little experimental evidence to support or refute it. This hypothesis will be rated as having very strong observational support for periodic glowing, but it seems to be non-applicable for response or continuous glows (Table 8).

The second type of defense in Robinson's system operates during the interaction with a predator--after an attack has been initiated. It often includes defensive behaviors such as startle, bluffing, eye spot displays, etc. Firefly larvae use a number of these strategies in their response glowing. There appear to be four enhancements of the response glow: "startle", "blinding", "false surfacing", and "eye spot/false-head" displays.

Startle: The startle defense involves a bright conspicuous color that is exposed when the insect is threatened, as when it is being disturbed or handled by a predator (Buck 1948; 1978; Blest 1957; Sivinski 1981; De Cock 2009). These bright colors are initially kept hidden, but they are exposed when the organism is threatened, as is commonly observed in Lepidoptera (Blest 1957). In the dark, a glow would act like a sudden exposure of a bright color and would be unexpected for a predator, particularly if the predator had negative phototropic tendencies. The response glow would be expected to produce a momentary hesitation in the attack which could allow the larva to escape. Blest (1957) also presents data showing that this "flash" defense in

butterflies is effective against passerine bird predators. Lloyd (1973a) presents anecdotal reports of horses, raptorial insects, rats, geckos, and chickens showing startle behavior to firefly bioluminescence. He also presents reports of toads, frogs, spiders and bats showing no startle behavior to bioluminescence. Long et al. (2012) report that *Photuris* fireflies flashed when attacked by a jumping spider, *Phidippus princeps* (Peckham and Peckham 1883). However, the spider showed no signs of "startle" when the firefly flashed.

The startle defense seems to be a reasonable hypothesis and it has been effective on me while collecting fireflies and larvae, but there is limited experimental evidence to support or refute it. This hypothesis will be rated as having very strong observational support for response glowing, but it appears to be non-applicable for continuous and periodic glowing (Table 8).

Blinding: A nocturnally active predator must have its eyes dark-adapted to see in the dark. A sudden bright glow at close quarters could destroy this dark adaptation and could have a blinding effect on the predator (Buck 1978; De Cock 2009). Nocturnal predators could also have negative phototropic tendencies. Buck (1978) suggested calling this the "jamming" defense. De Cock and Matthysen (1999) suggested that predators may get a false "after-image" from such a response glow and that this could cause the predator to misdirect its attack. Buschman (2017b) suggested that the flicker flash may interfere with the predators' vision by forcing the eyes to light adapt and dark adapt in quick succession—essentially blinding the predator.

The blinding defense seems to be a reasonable hypothesis, but there is little experimental evidence to support or refute it. This hypothesis will be rated as having very strong observational support for response glows, but it appears to be non-applicable for periodic and continuous glows (Table 8).

False surfacing: Lloyd (1973b) and I suggested the false surfacing defense as a potential function for larval bioluminescence. Many firefly eggs and pupae are found in the soil or in subterranean cells where the transparent cuticle allows the light to shine into the underground chamber. A subterranean predator, that is probably negatively phototropic, might come to a chamber containing a glowing pupa or larva while burrowing in the soil. The predator would probably withdraw because the light indicates that it was surfacing unintentionally. The subterranean predator avoids the surface because there are predators and environmental conditions above ground that they are not equipped to handle. There is one group of fireflies, the genus *Pyractomena* LeConte 1850, which do not pupate in the soil, but instead pupate in the

open, glued to stems or branches (Lloyd 1973b). These pupae have developed an opaque cuticle with camouflage coloration. These pupae do not readily use their light organs, although there may be some internal body glow (Lloyd 1973b). In this situation, it must not be advantageous to broadcast the fact that they have bioluminescence.

The false surfacing defense seems to be a reasonable hypothesis, however, there is little observational or experimental evidence to support or refute it. This hypothesis will be rated as having strong observational support for bright continuous and response glowing, but it appears to be non-applicable for periodic glowing (Table 8).

Eye-Spot/False-Head Display: Dean (1979) suggested that larval light organs might function as eye-spot displays. Buschman (1988) developed the idea more extensively and proposed that the eye-spot/false-head defense would account for the fact that firefly light organs occur almost universally as a pair of oval spots on the eighth abdominal segment, at the tip of the abdomen (Fig. 16A, B, E. F.) (Sivinski 1981; Buschman 1988; Branham and Wenzel 2000; 2003; De Cock 2009). The only known variation in this morphology occurs in the genus *Lamprohiza* Motschulsky 1853 (and perhaps a few other related genera) where larvae have additional pairs of oval light organs (De Cock 2009). The eye-spot is a common hypothesis for round bright spots of color on the wings of various lepidopterans (Blest 1957; Robbins 1981). They also have multiple pairs of spots that are also considered eye-spots (Blest 1957). Blest also suggests that larger eyespots are likely used to intimidate their enemies while smaller eyespots are more likely to be used to deflect an attack to a less vulnerable part of the body or to a source of defensive chemicals. Firefly light organs are clearly small and therefore more likely to be used to deflect an attack.

The shape of larval light organs must be important to its function, otherwise there would be more variation in its morphology. In adults, the shape and size of light organs is extremely variable (Fig. 17C, D, G, H, Fig. 16 #1-23). There seems to be ample genetic plasticity for shape and size of light organs. In addition, the aposematic function does not require any specific light organ morphology—it simply needs to be visible.

The fact that the "eye spots" occur near the tip of the abdomen on the eighth abdominal segment, suggests that they are there to create a **"false-head."** The false-head would direct a predator attack to the tail rather than to the head. Robbins (1981) reports that lycaenid butterflies with classic or complete false-head wing patterns had five times more bird beck shaped holes in

the rear margins of their wings (where the false-head is located), compared to moths with less complete false-head markings. He suggests that these moths had escaped bird attacks, which were directed at the false-heads on the rear margin of the wings. In firefly larvae the tail is probably less vulnerable than the head, but more importantly, the known defense organs that release toxic and repellant chemicals are in the lateral membranes of the abdominal segments (Fu *et al.* 2009; 2007; Tyler 2001; Vencl *et al.* 2012). The eye-spot/false-head display appears to direct the predator attack to the abdomen where the predator will be exposed to the defensive chemicals. I have observed ducks pick up and then reject firefly larvae (Chapter 3). Two of three rejected larvae survived (Chapter 3). Dean (1979) also reported that severely damaged larvae could survive. There appears to be survival value for the eye-spot/false-head display.

In addition, the caudal grasping organ is located near the anus. It is used in locomotion and in "grooming" (i.e. removing mucus and dirt from the body), but it may also be utilized to "paint" the body with liquids from the tail (possibly from the anus?). These liquids may aid in grooming, but they may also contain the toxic or repellant defensive chemicals. The larvae may increase their defenses by painting the defensive chemicals onto the rest of the body.

I have repeatedly experienced the impulse to release a larva, when I was handling it in the dark with a pair of forceps. The larva would curl up and the false head seemed to bear down on the forceps (and my fingers). I can just imagen how a bird would react when holding a larva in its bill and seeing this false head with bright glowing eyes approaching its beak and/or face.

The eye-spot/false-head hypothesis is the only hypothesis that explains and even requires the specific combination of morphological characteristics that are found in the light organs of firefly larvae. This hypothesis is also supported by the experimental evidence of bird marks on butterflies. This is a compelling hypothesis having very strong observational support for bright continuous and response glowing, but it appears to have minimal support for periodic glowing (Table 8).

In conclusion, it will probably be difficult to experimentally test each of these enhancement behaviors separately. However, aposematism can be tested with and without the enhancements. The enhancements should increase the effectiveness of the basic aposematic defense.

Non-Defensive Functions

Once the defensive aposematic function of bioluminescence was established for firefly larvae, there could have been selection for bioluminescence to function in other contexts (without necessarily giving up the aposematic function). Many non-defense functions have been suggested, including prey attraction, illumination, mimicry, and larval communication. The burden of proof for these non-defensive functions should be somewhat higher than for the defensive enhancements, because it would probably take a longer series of evolutionary adaptive steps to develop these functions. In contrast, the defensive function is already well-established, so the defensive enhancements can be added with only a few new evolutionary adaptations.

Prey Attraction. Bioluminescence is well-known to be involved in prey attraction in several non-lampyrid larvae, so several authors have suggested it could apply to firefly larvae (Buck 1978; Sivinski 1981; Sivinski *et al.* 1989; De Cock 2004a; 2009). However, De Cock (2009) points out that when prey attraction occurs with bioluminescent organisms, the insect remains stationary and practices ambush behavior. This behavior is observed in bioluminescent elaterid beetles (Coleoptera: Elateridae) which attract and prey on adult termites (Sivinski 1981) and in bioluminescent fungus gnats (Diptera: Mycetophilidae) which attract and prey on small insects that are attracted to the bioluminescence and get trapped in their sticky webs (Sivinski 1981). Female *Photuris* fireflies, "*femmes fatales*", also use response flashes that mimic female response flashes of other firefly species to attract and prey on them (Lloyd 1965, 1969). However, firefly larvae do not practice ambush behavior (Chapter 3). They hunt by crawling about in their habitat. Prey attraction seems unlikely for most of them. However, there is one report of a firefly larva that floats upside-down under the surface of a pond in Malaysia while glowing continuously (Annandale 1900). This could be an example of prey attraction (but the larva could also be feeding on something else in the water and the glow may be aposematic). There are several other examples that are listed as possible prey attraction by Sivinski (1981) and Day (2011), but since those larvae did not practice ambush behavior, they will not be included in this discussion. My observations of North American firefly larvae do not suggest a meaningful role for bioluminescence in prey capture or feeding (Chapter 3).

I do not consider the prey attraction hypothesis to be a compelling hypothesis for glowing in firefly larvae and there is little observational evidence to support it. This hypothesis will, therefore, be rated as having minimal observational support for bright body glow and bright

continuous glowing, and it appears to be non-applicable for response and periodic glowing (Table 8).

Illumination. Several authors have listed illumination as a possible function for larval bioluminescence (Buck 1978; Sivinski 1981; De Cock and Matthysen 2003; De Cock 2009; Chapter 2). There are four examples of bioluminescence possibly being used as illumination in bioluminescent beetles. The first example is the non-lampyrid larva from South America known as the "railroad worm," *Phrixothrix* sp (Coleoptera: Phengodidae) (Viviani and Bechara 1997). These larvae have two sets of light organs, a series of yellow-green dorsolateral glowing bars along the thorax and abdomen and a red glowing light organ on the head and prothorax. The two sets of organs operate independently. The yellow-green light organs operate as expected for an aposematic display while the red-light organ operates as expected for illumination. The color sensitivity of the eyes of these larvae is red-shifted as though they are adapted for seeing in red light. This would allow these larvae to illuminate the surroundings in red light allowing visual detection, but predators and prey would not be able to see them because they are not as sensitive to red light. However, we need to know more about their prey capture behavior and the visual acuity of their eyes—can they see objects in the dark.

The second example of bioluminescence potentially being used as illumination is that of *Lampyris noctiluca* (Linnaeus 1758) larvae. Larvae are reported to glow when they come across a snail slime trail (Sivinski (1981) citing Schwalb (1961)). The larva then seems to follow the snail slime trail (apparently using their bioluminescence to do so?). However, I'm not sure this observation can be verified, especially since Schwalb (1961) experimentally demonstrated that these larvae follow slime trails using chemical sensory information collected by the maxillary palps. They hold the palps close to the substrate during slime trail following. They probably glow periodically as they follow the slime trail (Chapter 1 and 3). I have not been able to find evidence of trail following behavior in any of North American fireflies that I have studied (Chapter 3).

The third example of bioluminescence potentially being used as illumination is my observation that firefly larvae seem to produce light primarily during locomotion, as though they were illuminating the path they were traveling (chapter 2). In addition, when they moved about, they held the body up off the substrate, supported by their feet in front and by the tip of the abdomen in the back. The tip bends down to contact the substrate so the larvae could hold on

using the caudal grasping organ. The light organs, which are located near the tip of the abdomen, ended up facing forward like head-lights (Chapter 2). I, therefore, tested the hypothesis that periodic glowing was being used in illumination. I assumed that glowing should increase when larvae reached an obstacle because it would be searching for a way around the obstacle. However, there was no such increase in glowing at an obstacle (Chapter 2), so there was no experimental support for the illumination hypothesis.

The fourth example of bioluminescence being used for illumination is that of adult *Photuris* fireflies (Lloyd 1968). These fireflies appear to use their bioluminescence like "landing lights", to light up the substrate when they land. However, these fireflies have large compound eyes which likely can take advantage of the light from the light organs for illumination. Firefly larval eyes are small and simple. It is not clear that such simple eyes could have the visual acuity needed to see obstacles in the dark or to follow slime trails (McLean *et al.* 1972; De Cock 2004a).

Overall, the illumination hypothesis for firefly larvae is no longer a compelling hypothesis for me. There is little observational and no experimental evidence for it. This hypothesis will be rated as having minimal observational support for periodic glowing, but it appears to be non-applicable for bright continuous and response glowing (Table 8).

Mimicry and Camouflage. Lloyd (1966; 1973a) suggested that glowing could be "mimicry" or "camouflage" related to the faint bioluminescence of fungi and bacteria. Many fungi are known to be toxic so there could be mimicry of fungal bioluminescence. However, we have no information on the toxicity of bioluminescent fungi, so it is not clear that a model for mimicry exists. Since fungi are motionless, any mimicry or camouflage related to them would need to be motionless. This could apply only to continuous body glow or to response glowing of larvae hiding in a cell.

Many firefly adults and a few larvae are brightly colored with contrasting black, yellow, red and brown bodies. These color patterns, as well as their behavior, make them quite visible during the day. This seems to suggest that this should be considered evidence of aposematism (De Cock 2009). These bright contrasting color patterns are also seen in a number of non-firefly insects, so they can be considered "Müllerian mimics" of the fireflies (Lloyd 1973a; 1989; McDermott 1964; De Cock 2009). There may also be "Batesian mimics" among the fireflies—individuals lacking or having less effective defensive chemicals (De Cock 2009)

but benefiting from the color pattern that suggests they have defensive chemicals. However, the only evidence we have of individual variation in chemical defenses would be in the genus *Photuris* where we know that some females have extra defensive chemicals that they accumulate by feeding on *Photinus* prey fireflies. Chemical variation among fireflies needs to be better documented before these hypotheses can be considered further.

I am not aware of any supportive evidence for the role of bioluminescence in mimicry or camouflage hypotheses. Therefore, these hypotheses are not very compelling for most fireflies and there is little evidence to support them. These hypotheses will be rated as having minimal observational support for bright body glow and bright continuous glowing, but they would appear to be non-applicable for response and periodic glowing (Table 8).

Larval Communication. Larval communication has been suggested as a possible function of bioluminescence by several authors (Kaufmann 1965; Buck 1978; Sivinski 1981; De Cock and Matthysen 2003; De Cock 2009). At times larvae appear to glow in response to glows by nearby individuals so researchers have wondered if larvae could see each other's glows (Sivinski 1981; Viviani 2001). However, I and others have tried to repeat such observations and have failed (McLean *et al.* 1972; Annandale 1900; Keiper and Solomon 1972). Perhaps these larvae are responding to the same unknown stimulus in the background. Lloyd and Minnick report that they collected a *Pyractomena* larva when it responded repeatedly to flashlight flashes (Sivinski 1981).

On the other hand, larval glowing could be a competitive signal aimed at other larvae to keep them at a distance—a territorial signal (Kaufmann 1965; Dreisig 1974). Kaufmann (1965) suggested that larval glows could provide larval density information that could be used by females to determine when and where to oviposit. However, these authors have offered no evidence in support these ideas and I know of no other supporting information.

Other authors have suggested that larval glows could be altruistic calls to other larvae (perhaps kin?) to help subdue and feed on larger prey (Buck 1978; Sivinski 1981). My field observations on North American firefly larvae are that more than one larva are seldom seen feeding on the same prey item (Chapter 3). However, Dean (1979) reports once finding 6 larvae feeding together on a food item. In opposition to this hypothesis some larvae are known to move the prey, apparently in order to avoid sharing it with competitors (Chapter 3). I believe most observations of group feeding have been made in the laboratory where larvae are not able to keep

the prey away from other larvae. My field observations, as well as other observations in the literature, do not support glowing as an altruistic call to other larvae.

We need repeatable observations on larval communication before these hypotheses can be taken seriously. The larval communication hypotheses will be rated as having minimal observational support for response glowing and non-applicable for bright continuous and periodic glowing (Table 8). In addition, it is difficult to imagine how a visual communication system could be based on the poorly developed visual system we see in firefly larvae (De Cock 2009). We really need more information on the sensitivity of the larval visual system before we can give much weight to these hypotheses. In summary, the non-defensive hypotheses are not compelling and there is little observational evidence to support any of them.

Adult Bioluminescence

These observations on adult bioluminescence are included in this discussion, because, adult bioluminescence appears to be a secondary adaptation of larval bioluminescence (Branham and Wenzel 2000; 2003). In some adult fireflies, the light organs appear to be morphologically similar to the larval light organs and also appear to be carried over from larvae through the pupal stage into the adult stage. However, in most firefly groups, the adult light organs are morphologically new structures developed during the pupal stage (Figs. 15-17). Some adult fireflies have lost light organs and have reverted to using pheromone sexual communication. It is interesting to note that although sexual communication occurs in all fireflies, the shape, position and even the presence of adult light organs is extremely variable (Figs. 16, 17). This contrasts with the situation in larval fireflies where the light organs occur uniformly as a pair of oval spots on the eighth abdominal segment (Fig. 17) (additional pairs occur in a few species).

Sexual Communication. The use of bioluminescence in sexual communication is well-established. Biologists have known for hundreds of years that glowing lavaform flightless females (known as glowworms) attract flying non-glowing males and the glowing allows males to locate the female (Branham and Wenzel 2000). However, biologists puzzled for a hundred years about the flashing of North American fireflies. Finally, McDermott (1911) realized that *Photinus* firefly females flashed in response to a specific male advertising flash. The female responded to that flash pattern at a specific response interval and this allowed the male to recognize the female response flash (Lloyd 1966). There are also several more complex forms of

sexual communication that are still being described, but our basic understanding that flashing is involved in courtship communication is not in question.

The role of bioluminescence in sexual communication will be considered a proven function for adult fireflies (Table 8). We have laboratory and field experimental evidence to support sexual communication in flashing fireflies and in glowworms (McDermott 1911; Lloyd 1966; Branham and Wenzel 2003).

Aposematism of Adult Bioluminescence. De Cock and Matthysen (1999) pointed out that courtship flashes of adult fireflies disclose the location of flashing individuals and can lead to increased predator pressure. In fact, this suggests that courtship communication probably could not have evolved without protection from visually hunting predators. Since both larvae and adults carry toxic and deterrent chemicals (as documented earlier) we have to assume that both larvae and adults practice aposematism. The presence of aposematism in adult fireflies is supported by the unpalatability of adults, presence of reflex bleeding, presence of aposematic color patterns, and presence of other insects that mimic fireflies (De Cock 2009).

Many fireflies in genera like *Photinus* and *Pyractomena* have flash patterns that we identify as species specific "advertising flash patterns", because they are known to function in courtship communication. However, firefly flashes appear to have more than one function. They clearly function in courtship flash communication, but it may be more important that they also function as aposematic defensive signals (Moosman *et al.* 2009, Buschman 2017a). It might be more useful to think of firefly flashing first as aposematic defensive behavior and then consider whether they also function as courtship flashes.

Potentially dedicated aposematic firefly flashes can be observed in *Photuris* fireflies (Buschman 2017b). These fireflies often have several different flash patterns. The multiple flash patterns have caused a lot of confusion among biologists when they have tried to identify these fireflies (Barber 1951; McDermott 1958; Lloyd 1969). Buschman (2017a-b) was able to show that only one of the three known flash patterns of *Photuris quadrifulgens* Barber 1951 appeared to be used in sexual communication, so that flash pattern was identified as the advertising flash pattern. The function(s) of the other two patterns, periodic flashes and flicker flashes, remained uncertain. Flicker flashes have developed independently in 30 of the 130 species with known flash patterns (Lloyd 1981). Lloyd pointed out that some of these male flashes were like flashes of the fireflies on which their females' prey (Lloyd 1969, 1981). He suggested that these males

were mimicking the advertising flashes of prey males to elicit aggressive mimic response flashes from their females. He suggested that males might be able to convert these hungry aggressive mimic females into courting females and thus gain a mating. Another suggestion by Buschman (2017a-b) was that the rapid alternation between bright flashes and darkness in the flicker flash probably made vision difficult on vertebrate predator's eyes. The eyes must dark adapt to see in the dark and then light adapt to see in the light in quick succession. The flicker rate is faster than the time the eyes need to change their adaptation to the light conditions. The predator may lose vision when exposed to a flicker flash. More recent observations also suggest that fireflies may use flicker flashes in active defensive against predator fireflies (personal observations). Periodic and/or cruising flashes appear to be dedicated aposematic flashes, directed against predators including flying bats (Lloyd 1989; Moosman *et al.* 2009; Buschman 2017a).

The courtship communication of the North American *Phausis reticulata* (Say 1825) fireflies is by pheromones with a little help from the female glow when he gets closer (De Cock *et al.* 2014). The continuous glow of the flying male does not seem to function in sexual communication (De Cock and Matthysen 2005). These glows may function in aposematism and/or defense (De Cock *et al.* 2014; personal observation).

The aposematic hypothesis of adult bioluminescence will be rated as having proven support for adult glows and flashes (Table 8). There are observational and experimental results that support this function (Moosman *et al.* 2009). There are also several other functions for adult flashes that have strong or very strong observational support including enhanced visibility (Buschman 2017a, 2017b), prey attraction (Lloyd 1965; 1969; 1981), and illumination (Lloyd 1968; Chapter 2).

CONCLUDING COMMENTS

The possible function of bioluminescent behavior in firefly larvae has been a subject that has fascinated biologists for centuries and there are hundreds of comments and suggestions in the literature. In the past, discussion of this behavior was difficult because there was so little information available on the bioluminescent behavior and on the natural history of firefly larvae.

In this review, I have addressed the function of larval bioluminescence with the benefit of several recent reports of the bioluminescent behavior and natural history of firefly larvae (Chapters 1, 2 and 3). In these reports it was concluded that larval bioluminescent behavior was

clearly associated with several defensive behaviors, probably as an aposematic warning of defensive chemicals (Chapter 3). It was also concluded that larval glowing behavior did not appear to be associated with any of the non-defense hypothesis such as illumination (Chapter 2), prey capture (Chapter 3), competitive interactions (Chapter 3) or larval communication (Chapter 4). These conclusions reduce the number of hypotheses that need to be considered further. They also allow us to focus on the details of how bioluminescent behavior works in larval defense.

It is important to recognize that there are several different types of larval bioluminescence, potentially with different functions. The function of larval bioluminescence was discussed in the context of the evolution of bioluminescence in firefly larvae. This allowed us to discuss the functions in the context of the selection forces that may have driven the evolution of bioluminescence together with the description of the different types of larval bioluminescence. There appear to be six types of bioluminescent behavior that can be listed as: incidental luminescence, faint body glow, bright body glow, bright continuous glowing, response glowing and periodic glowing.

The evolution of bioluminescence apparently started with light photons that were released as a by-product of certain biochemical reactions--a biochemical function. This light can be termed **"incidental luminescence**." When there was increased demand for these specific biochemical reactions, the light production increased to produce a **"faint body glow"**. This faint luminescence was first associated only with the biochemical function, but then it may have gained a "**physiological function"**—as for example, protecting the cell from fungus infections. As this physiological function became more important to the organism there was selection for a brighter bioluminescence, and this became the **"bright body glow**." This glow was now bright enough so that predators could see it and they would have begun targeting bioluminescent larvae as prey. Larvae that also had toxic or deterrent chemicals would have had a selective advantage and this would have led to selection for bioluminescent larvae that had effective toxic or deterrent chemicals. Bioluminescence therefore came to be associated with defensive chemicals—producing the "**aposematic defense**." As the aposematic defense became established, there would have been increased selection for brighter bioluminescence and more or better chemical defenses. This would have led to the development of light organs that could produce the **"bright continuous glow**." Specialized predators continued to adapt to the chemical defenses of these larvae, so larvae would have needed additional defensive behaviors. This led to

the development of the nervous system which allowed larvae to turn the bioluminescence on and off. This allowed larvae to use camouflage in the first line of defense, but when attacked they could still turn the bioluminescence on for the second type of defense. The nervous system then allowed larvae to develop new adaptations that could improve the effectiveness of bioluminescent defense; **"response glowing"** for larvae that were hiding and **"periodic glowing"** for larvae that were hunting. Response glowing was further enhanced by the startle, blinding, false surfacing, and eye-spot/false head defenses. Periodic glowing was further enhanced by the flash display and enhanced visibility defenses. All of these defensive behaviors appear to be active in modern firefly larvae.

Many of these defensive behaviors will need to be evaluated experimentally. This can be done using the protocols of Underwood *et al.* (1997); De Cock and Matthysen (2003) and testing the effectiveness of continuous light versus the other light patterns that have been suggested as enhancements for aposematic signals. Researchers should also carefully record the type of bioluminescent behavior for firefly larvae, specifically recording response versus periodic glows.

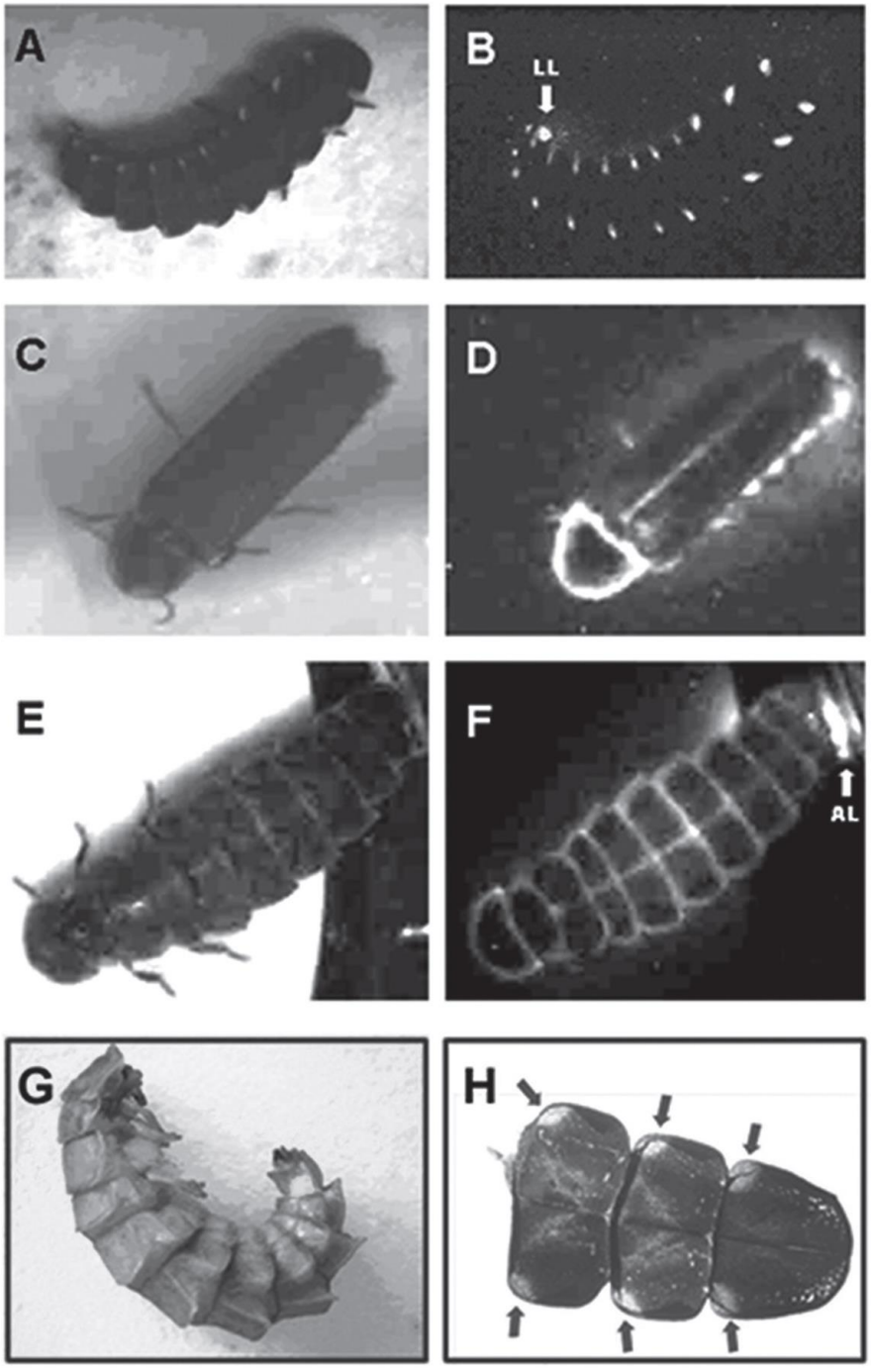

Figure 15. Light emission in the glow-worm *Lampyris noctiluca*. (A) and (B) Dorsal view of a mature larvae. (C) and (D) Dorsal view of a male. (E) and (F) Dorsal view of a female. (G) Pupa emitting light from abdominal lanterns. (H) Larval exuviae with posterolateral transparent spots indicated with arrows. (B), (D) and (F) 43-minute exposures of light emission from larval and adult glow-worms. (LL) Larval lanterns. (AL) Adult lanterns. (Reproduced from Tisi et al. 2014 with permission).

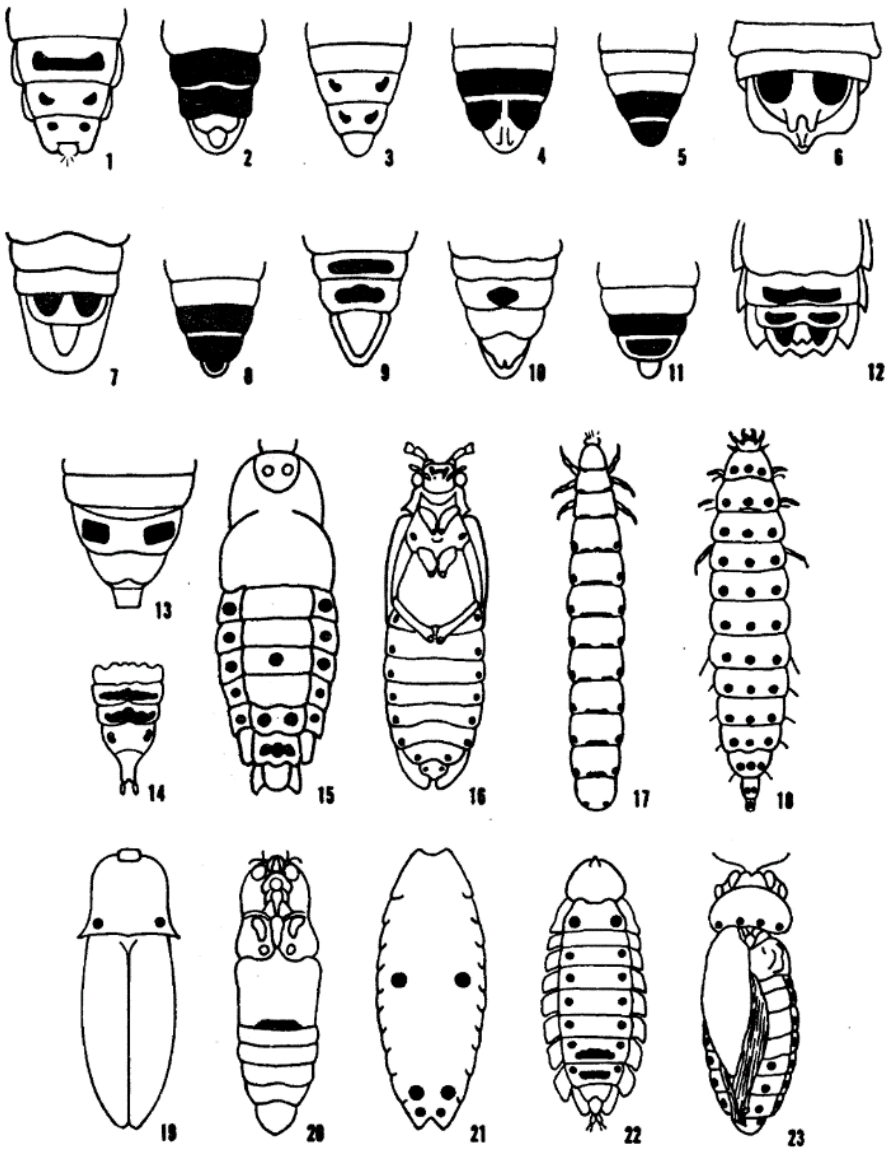

PLATE I. Figures 1–23, light organs of various luminescent beetles, shown in black. 1. *Lychnuris rufa,* female. 2. *Photinus scintillans,* male. 3. *Pyractomena* sp., female. 4. *Luciola* sp., male. 5. *Luciola chinensis,* male. 6. *Callopisma* sp., female. 7. *Robopus montanus,* male. 8. *Luciola cruciata,* male. 9. *Photuris* sp., female. 10. *Photinus scintillans,* female. 11. *Luciola lateralis,* male. 12. *Pleotomus* sp., female. 13. *Luciola lusitanica,* female. 14. *Lampyris noctiluca,* female. 15. *Lamprohiza splendidula,* female. 16. *Harmatelia bilinea,* male. 17. *Phengodes* sp., female. 18. *Diplocladon hasselti,* female. 19. *Pyrophorus* sp., male and female, dorsal. 20. *Pyrophorus* sp., male and female, ventral. 21. *Phausis reticulata,* female. 22. *Lamprohiza mulsanti,* female. 23. *Dioptoma adamsi,* male. Figures 1–5, 8–11, 13–15, 19, 20, and 22 redrawn from Buck (31) from various sources; Figures 7 and 17 redrawn from Buck (31); Figures 12 and 21 redrawn from Lloyd (107); Figures 16 and 23 redrawn from Green (77); Figure 18 redrawn from Harvey (89); Figure 6 original. Figures by P. Laessle.

Figure 16. Plate I reproduced from Lloyd (1971) with permission.

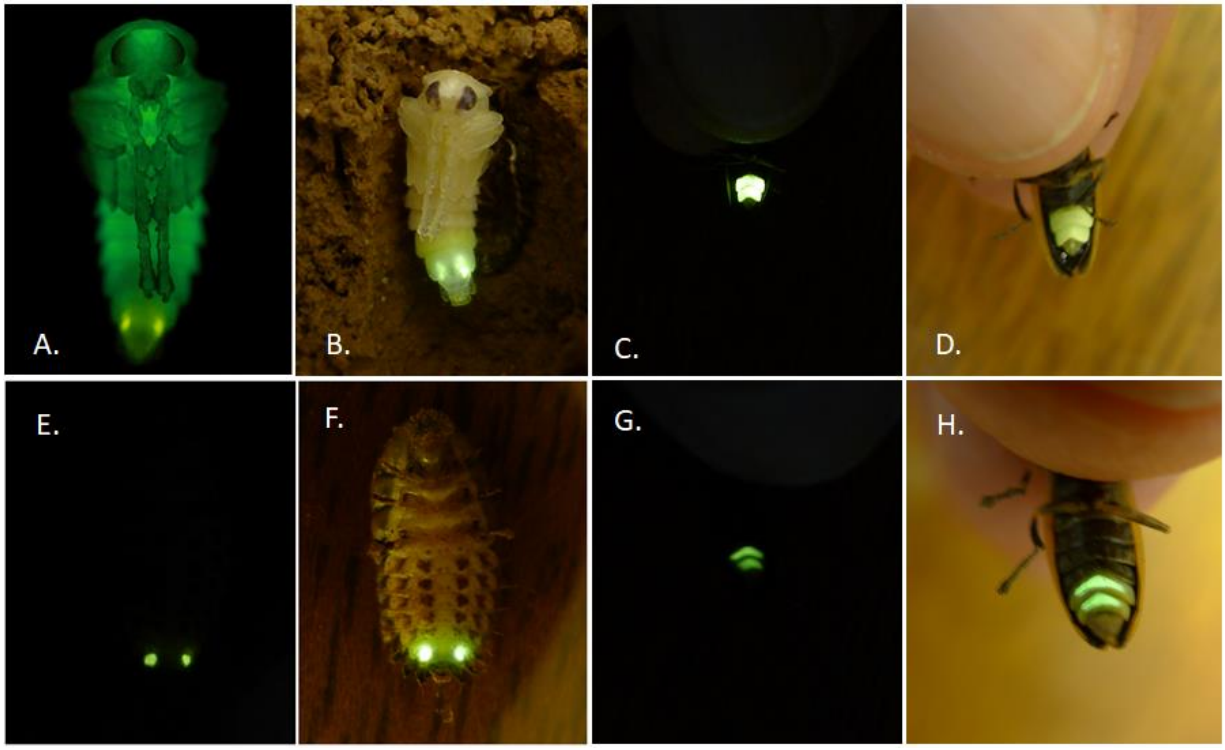

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Figure 17. Firefly light organs and body glow bioluminescence photos: **A.** *Photuris* pupa in the dark showing the two glowing oval light organs and the bright body glow from the body of the pupa (Photo by David Liittschwager, extended time exposure), **B.** *Photuris* pupa with some ambient light showing the pupa and the two glowing oval light organs, **C.** *Photuris* adult male in the dark showing the two segments of the glowing light organ, **D.** *Photuris* adult male with some ambient light showing the firefly and the two glowing segments of the light organ, **E.** *Photuris* larva in the dark showing the two glowing oval light organs, **F.** *Photuris* larva with some ambient light showing the larva and the two glowing oval light organs, **G.** *Photuris* adult female in the dark showing glowing light organ as two strips, **H.** *Photuris* adult female with some ambient light showing the firefly and the glowing light organ as two strips. (Photos B-H by author, one eighth seconds time exposures).

Table 8. Bioluminescent behavior of lampyrids and the proposed functions rated according to author's judgment of observational and experimental support (see text for justification). "Minimal" observational support, "Strong" observational support," Experimental" support, and "Proven" support.

	Immature Stages						Adult Stage	
Types of Glow and Proposed Functions	Incidental Lumen-escence	Faint Body Glow	Bright Body Glow	Bright Continuous Glow	Response Glow	Periodic Glowing	Male and Female Glows	Male and Female Flashes
A. Biochemical	Minimal	Minimal	Minimal	Minimal	Minimal	Minimal	Minimal	Minimal
B. Physiological	--	Minimal	Minimal	Minimal	Minimal	Minimal	Minimal	Minimal
C. Aposematic Defense	--	--	Strong	Experi-mental	Strong	Strong	Strong	Experi-mental
Flash Display	--	--	--	--	--	Strong	--	Minimal
Enhanced visibility Defense	--	--	--	--	--	Strong	--	Strong
Startle Defense	--	--	--	--	Strong	--	Minimal	Minimal
Blinding Defense	--	--	--	--	Strong	--	--	Minimal
False Surfacing	--	--	--	Minimal	Minimal	--	--	--
Eye spot/False-head Defense	--	--	Minimal	Strong	Strong	Minimal	--	--
D. Non-Aposematic Functions								
Prey attraction	--	--	Minimal	Minimal	--	--	--	Experi-mental
Illumination	--	--	--	--	--	Minimal	--	Strong
Mimicry	--	--	Minimal	Minimal	--	--	--	--
Larval Communication	--	--	--	--	Minimal	Minimal	--	--
E. Adult Bioluminescence								
Sexual Communication	--	--	--	--	--	--	Proven	Proven
Aposematic Defense	--	--	--	--	--	--	Proven	Proven

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