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Light Emissions fromthe Firefly at Low Temperatures

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Abstract

Light emissions fromthe fireflyhave been investigated for drawing conclusions on its light-emitting system and the very efficient light-emitting reaction. From these conclusions, attempts have also been made to explain certain behavioural patterns of this insect. Reasons have been given for some species being early-starting while the other ones late-starting, and also for a particular species selecting a particular habitat for dwelling. It has been observed that females of the widely available Indian species of firefly *Luciolapraeusta* generally disappear a bit earlier than males as the winter season sets in. Steady-state emission spectra and pulses from both males and females of this species have been analysed to find the reason for this happening, and the results obtained are presented in this article.

Key words :Firefly bioluminescence, female emission, peak shift, pulsewidth increase

1. Introduction

Fireflies flashing in a dark summer evening make for an enchanting view, which is the source of many a poem. Along with poets, the light of the firefly has been attracting the attention of scientists for over a century—the very efficient light-producing reaction being the chief reason for this interest.This reaction involves oxidation of the luciferin molecule (substrate)in presence of the enzyme luciferase (catalyst) and Mg-ATP. An unstable dioxetanone intermediate is formed, which decomposes to form an electronically excited oxyluciferin ($OxyLH_2$) species [McElroy et al., 1969]. Visible light is emitted during the decay of excited luciferin to its ground state. The reaction is called a chemiluminescence reaction.

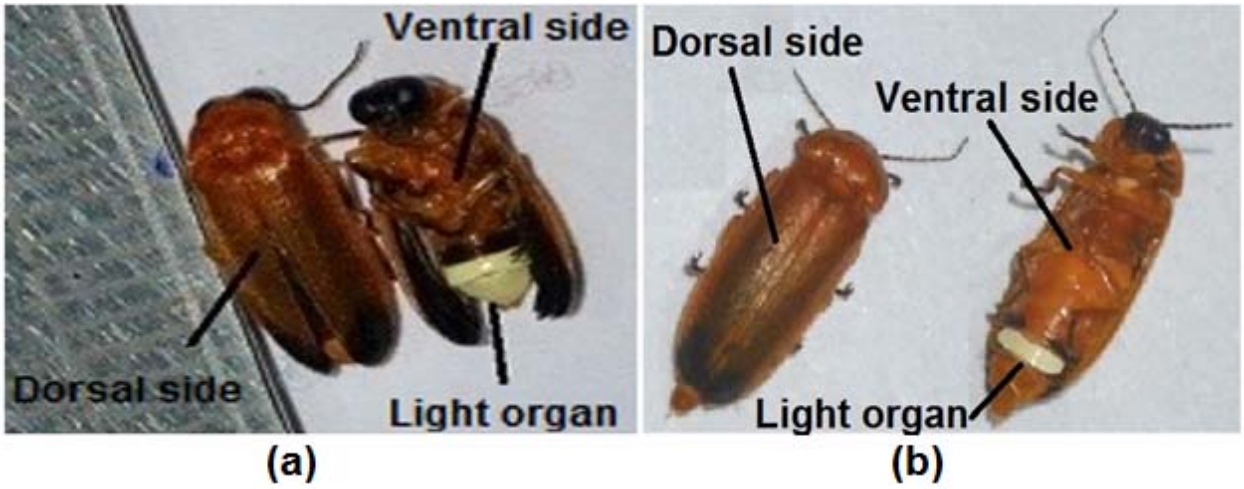

Figure 1. Widely available Indian species of firefly *Luciolapraeusta*. (a) Dorsal and ventral sides of a male specimen. (b) Dorsal and ventral sides of a female specimen.

The fireflyspecies found in plenty in our part of the globe is *Luciolapraeusta*; a male and a female specimen of this species are shown in Figure 1.In recent times, a few investigationshave been carried out on the light emitted from this speciesfor havingsome knowledge on the light-emitting reaction and the light-emitting systeminside the body of a *live* firefly. There have been studies on emissions from the male fireflies vis-à-vis female ones.The emission spectra of the male and the female have been observed to be similar. However,flashes of female fireflies, of average duration of 197 ms, have been found to be considerably longer than those of males, having average duration of 97 ms, which imply longer-lasting or slower proceeding reaction [Sharma, et al., 2014, Rabha, et al., 2017].Vapours of ethyl acetate anaesthetize the firefly, producing a glow in the lantern. An investigation of this ~~continuous~~ light with a preamplifier circuit and a digital storage oscilloscope has revealed a continuous train of microsecond-duration pulses, where a set of three such pulses has appeared to be a mirror image of the preceding set of three. Hence the conclusion that the light of the firefly is the manifestation of an oscillating chemical reaction [Gohain Barua and Rajbongshi, 2010]. Because of the triangular shape of the waveforms, it has been hypothesized that apassive component exists in the firefly system whose action on photons is analogous to that of a condenser on electrons. The obvious corollary has been that the rising half of the pulse indicated the

ON state and the falling half of the pulse indicated the OFF state of the light production; that is, this light has possibly represented a rectangular clock waveform, sampled both in amplitude and time — manifesting both pulse amplitude modulation (PAM) and pulse width modulation (PWM) [Gohain Barua, 2013].At temperatures lower than 17 °C for males and 24 °C for females of the species *L. praeusta*, a flashhas been observed to split into two and three, revealing the three luminescent forms of the emitter molecule oxyluciferin [Goswami, et al., 2019]. Lifetimes of these three forming a compound or combination flash have been measured to be of the order of tens of milliseconds which pointed towards phosphorescence, not fluorescence, as the process of light emission*in vivo*. With the increase in the temperature, the pulse width has been observed to decrease in a substantially linear manner in the temperature range of 20 – 40 °C, indicating an almost linear increase in the speed of the light-producing reaction in this range[Sharma, et al., 2014]. A sharp intense laser-like line has been found to exist in the emission spectrum of this species the presence of densely packed uric acid granules in the light organ suggestinga random laser-like emission [Gohain Barua et al., 2013]. At the temperature of approximately 42 °C, the emission peak has shifted towards red and the pulseduration has become the shortest, increasing rapidly thereafter for small increase in temperature [Rabha, et al., 2017]. This has indicated thermal denaturation of the enzyme luciferase, which should be the reason behind this species becoming an early-starting or dusk-active one [Rabha, et al., 2021].

2. Results and Discussion

2.1 Light Emissions at Low Temperatures

In the normal range of temperature, roughly 26 – 33 °C, at which the firefly-species *L.praesuta*flashes, peaks in emission spectra of both male and female fireflies lie at 562 nm. When the temperature is gradually lowered to 11.5 – 11 °C for males and 16.5 – 15 °C for females, the peaks get shifted towards the lower wavelength-side by about 5 nm, that is, those appear at approximately 557 nm. This blue shift is made evident in Figure 2(a) and (b) for a male and a female, respectively, by a vertical line drawn in each of the two spectra. This phenomenon is reversible; the position of the wavelength peak becomes 562 nm again as the temperature is increased to values greater than these peak-shifting ones. It has been proposed that a slightdifference in the enzyme structure isthe reason for different species emitting in slightly

different spectral regions, measured from 548 nm for the species *Diaphanes* sp. [Rabha, et al., 2020] to 582 nm for *Pyrophorus plagiophthalamus* [Seliger, et al., 1964]. Therefore, the structures of male and female firefly luciferases must have changed slightly at such low temperatures. Hence forwarded the hypothesis that denaturation of the enzyme luciferase has probably occurred for males and females of *L. praeusta* at 11.5 – 11 °C and 16.5 - 15 °C, respectively [Borah,et al., 2023].

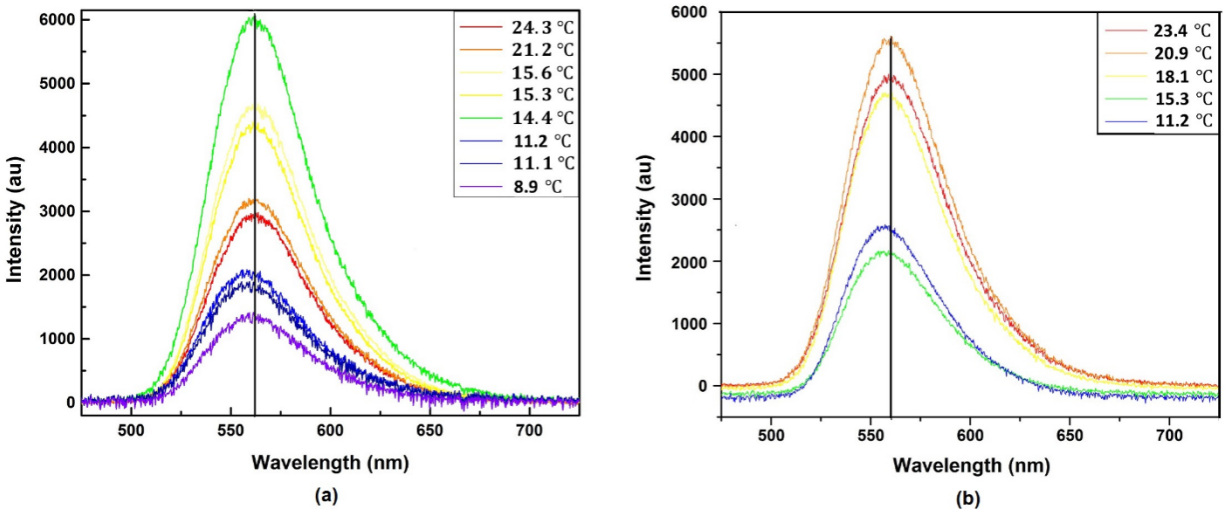

Figure 2. Emission spectra of the firefly *Luciolapraeusta* at different temperatures. **(a)** For the male emission, the wavelength peak appears at 562 nm at temperatures of 24.3°C, 21.2°C, 15.6°C, 15.3°C and 14.4 °C. The peak is clearly shifted towards the lower wavelength side at 11.2°C, 11.1°Cand 8.9 °C. **(b)** For the female firefly, the blue shift is clear at 15.3°C and 11.2 °C. The vertical lines drawn here and in (a) make the shifting clear.

Flashes from a male specimen and a female oneare presented in Figure 3 at temperatures of 10.5°C and 15.5 °C, respectively. Single-peaked flashes at such low temperatures are a rarity: those usually get split into two- or three-peaked ones [Goswami et al., 2016]. It has been reported earlier that at 30 °C the average duration of a male fireflypulse is about 97 ms [Sharma et al., 2014], while that of a female pulse is about 197ms [Rabha, et al., 2017]. It could be easily noticed that the male flash in Figure 3(a) isconsiderably longer than a typical flash at the normal flashing temperature. At this low temperature, unlike a normal temperature-flash, the end of the falling half isfar from being sharp; it deviates sharply away from the point towards which it was heading, making the complete duration more than 1 sec. The two female pulses presented in Figure 3(b) at 15.5 °C are also

considerably longer than the typical flashes at the normal temperature. Because of the splitting of peaks into two or three, the flash duration has been observed to vary widely, making a statistical analysis almost pointless. Still, ithas been shown that the flash duration not only increases abnormally but also varies greatly at peak-shifting and lower temperatures [Borah et al., 2023].

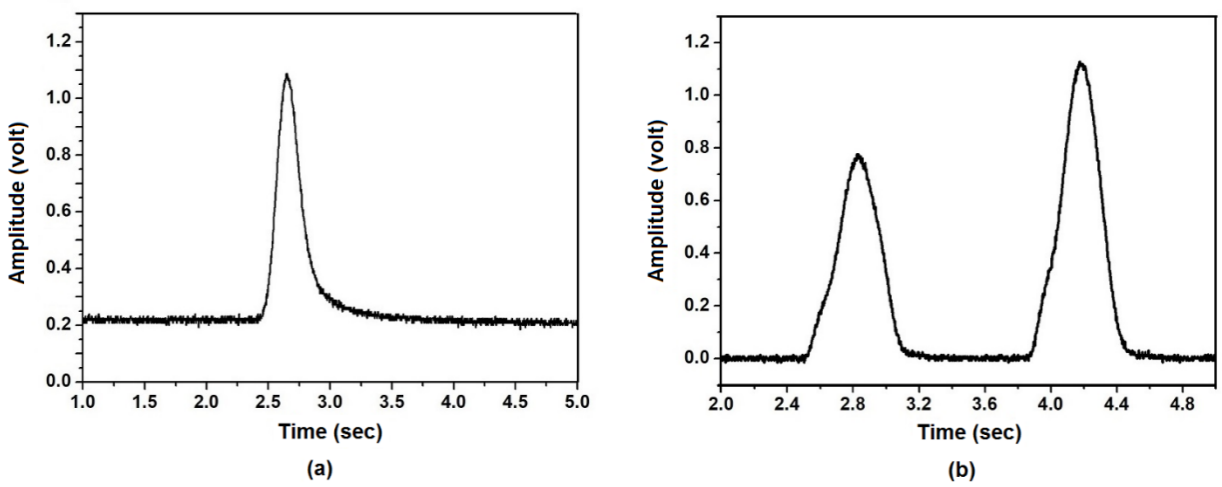

Figure 3. Flashes from the firefly at low temperatures. (a) For a male firefly at 10.5°C, the pulse is broadened abnormally. The falling half is anything but sharp; it deviates away from the approaching point, which makes the complete duration much longer than the average duration of 97 ms at 30 °C.(b)For a female firefly at 15.5 °C, the durations of the two pulses are about 5 times greater than the average duration of 197 ms at 30 °C.

Till now, only four proteins — yeast frataxin homologue 1 (Yfh1) [Pastore et al., 2007], the C-terminal domain of ribosomal protein L9 [Li et al., 2007], the scaffold protein for iron-sulfur (Fe-S) cluster biosynthesis in *Escherichia coli*, IscU [Bothe et al., 2015], and the human immune deficiency virus-1 (HIV-1) protease [Rösner et al., 2017] — have been found to undergo cold denaturation above the freezing point of water in the absence of denaturants. In our earlier study, the enzyme luciferase of the firefly *S. substriata* in the live condition has been proposed as an addition to this list [Rabha, et al., 2021]. This proposition has also been apparently supportedin the present study by the luciferase of the firefly *L. praeusta* [Borah et al., 2023].However, it has beena tentative assignment, as only the determination of the relevant thermodynamic parameters would confirm the denaturation.

2.2 Disappearance of Fireflies

The number of flashing fireflies decreases rapidly as the winter approaches in this region from the beginning of the month of November. Though a few flying males are observed till the end of November, grass or bush-lying females could be noticed only with great difficulty even in mid-November. From the observation of a dozen years, it appears that most of them disappear at that time of the year. With great difficulty, a female or two could be found in holes or pits in the land, usually covered with grass or straw. In mid-November, the temperature goes down to a minimum of about 18-19°C in late night which is close to the temperature of possible cold denaturation of the female luciferase (15 – 16.5°C). As this particular temperature for the males is considerably higher (11 - 11.5 °C), they could appear without much difficulty at that time. A male or twocouldbe sighted flashing even in the first couple of days of December just after the sunset. The ~~minimum~~ temperature on those days comes down to a minimum of 14-15 °C— about 4 °C above thepeak-shifting temperature of male fireflies. Therefore, shifting of the emission peakwavelength and abnormal broadening of the flash indicate the temperature-tolerance of both male and female fireflies, which is the probable reason for the females generally disappearing a little earlier than the males at the beginning of the winter.

3. Conclusion

In conclusion, the emission peak wavelength of the firefly *L. praeusta* shifts towards the lower wavelength side, and the emitted pulses broaden abnormally with considerable standard deviationsin duration at significantly low temperatures.This implies that the relative probabilities of transition to different vibrational levels in the lower electronic state get affected at these temperatures, which is probably due to slight changes taking place in the structures of enzyme luciferases of male and female fireflies of this species. The higher value of this temperature for females ndicates their lower level of tolerance of low temperatures, and this could be the probable reason for them generally disappearing earlier than males at the onset of the winter season.

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