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Light Emissions from the Firefly at High Temperature

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Abstract

The firefly light has been attracting the attention of scientists, along with poets, for over a century. A few in vivo studies have been carried out in recent times on the emitted light for having some knowledge on the light emitting reaction and the light emitting system in live fireflies. There are two categories of fireflies: one category begins flashing just after the sunset, while the other kind appears from about half an hour after the sunset. For finding the reason behind this, steady-state and pulsed emissions from male specimens of the Indian species *Luciola praeusta* and *Sclerotia substriata* have been investigated at temperatures considerably higher than the ones at which they normally flash. When the temperature had been raised to 42 °C for *L. praeusta* and 34 °C for *S. substriata*, the peak wavelengths red-shifted and the emitted pulses became the narrowest which broadened considerably thereafter for small increase in temperature. These happenings have been irreversible, which pointed towards thermal denaturation of the enzyme luciferase catalysing the light-producing reaction. This hot effect could be the probable reason for the species *L. praeusta* being early-starting or dusk-active, and the one *S. substriata* late-starting or dark-active in the summer. It has been inferred that these two happenings determined the temperature tolerance, which played a major role in the selection of the habitat for the fireflies.

Keywords : Peak shift; Pulse lengthening; Dusk-active firefly; Dark-active firefly

1 Introduction

Flashes of fireflies in dark summer evenings are a delight for human beings. This enchanting light has given birth to quite a few poems and scientific studies. As this light is 'cold', it incurs little loss-the main reason for the interest among the scientists. In this bioluminescent system, light results from the oxidation of an organic substrate, a luciferin, catalysed by an enzyme called luciferase. Visible light is emitted during the decay of excited luciferin to its ground state. The reaction is called the chemiluminescence reaction.

The species widely found in the northeastern region of India is *Luciola praeusta*. Quite a few scientific studies were carried out on the emitted light and flashes of this species. It was found that a sharp intense line existed in the emission spectrum of this species, and the presence of densely packed uric acid granules in the light organ suggested that this emission was akin to that of the random laser [Barua et al., 2014]. At temperatures much lower than the normal flashing ones, a pulse got split into three, revealing the three

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luminescent forms of the emitter molecule oxyluciferin; their lifetimes of the order of tens of milliseconds suggested that the process of light emission was analogous to phosphorescence [Goswami et al., 2019]. With increasing temperature, the pulse width got decreased in a substantially linear manner indicating that the speed of the light-producing reaction increased almost linearly in the temperature range $20°C$ – $40°C$ [Sharma et al., 2014]. Flashes of female fireflies of a species were considerably longer than those of males, implying longer lasting or slower proceeding reaction [Rabha et al., 2017] . When a firefly was anaesthetised with vapours of ethyl acetate, it emitted continuous light or a glow, not flashes. Investigation of this light with a preamplifier circuit and a digital storage oscilloscope revealed that this light consisted of a continuous train of microsecond-duration pulses. A set of three such pulses appeared to be a mirror image of the preceding set of three, which prompted the conclusion that the light of the firefly is the manifestation of an oscillating chemical reaction [Barua and Rajbongshi, 2010]. As the waveforms were clearly triangular in shape, a hypothesis was put forward that a passive component existed in the firefly system whose action on photons was analogous to that of a condenser on electrons. The obvious corollary was 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. In other words, the continuous light of the firefly possibly represented a rectangular clock waveform, sampled both in amplitude and time - manifesting both pulse amplitude modulation (PAM) and pulse width modulation (PWM) [Barua, 2013].

2 Analysis

2.1 Firefly groups based on appearance time

Depending on their appearance time after sunset, fireflies were categorised into two broad groups: Early-Starting or Dusk-Active and Late-Starting or Dark Active [Lall et al., 1980]. Fireflies belonging to the early-starting category began flashing in advance of 30 minutes after sunset, while the late-starting ones began flashing after this. 23 of the 32 specimens of dark-active species studied were found to emit green light (wavelength peak $\lambda_{max} \leq 558\,nm$), while 21 of the 23 specimens of dark-active species emitted yellow light ($\lambda_{max} \leq 560\,nm$).

In the campus of Gauhati University, two summer species of fireflies are identified by the well-known expert on Asian fireflies, Dr.Lezley Ballantyne of Australia; these are *Luciola praeusta* and *Sclerotia substriata*. The first species *L. praeusta*, as already mentioned, is widely available while the second one, *S. substriata*, is rare, found in the bank of a big pond in the campus. As the emission peak wavelength of *L. praeusta* is 562 nm, this species should come under the early-starting category; but *S. substriata's* peak being at 558 nm, it should fall in the late-starting category. Observations of several years have confirmed this.

2.2 Emissions at high temperature

For the males of the species *L. praeusta*, the peak of the emission spectrum remains at 562 nm up to approximately $42°C$. Above this temperature, it is observed that the wavelength peak shifts towards the side of the longer wavelength, and the spectrum widens, that is, the value of the full width at half maximum (FWHM) increases. This is shown in Figure. 1. This phenomenon is found to be irreversible. As it is known that different species of fireflies emit light at slightly different wavelength peaks because of slight differences in their enzyme structures [Seliger et al., 1964], it is concluded that the structure of the luciferase of the *L. praeusta* firefly changes, pointing towards denaturation, around $42°C$. The emitted pulses are observed to decrease in width with the increase in temperature, becoming the narrowest at approximately this temperature, and thereafter increase considerably with a small increase in temperature [Rabha et al., 2017]. Typical flashes and average durations are presented in Figure. 2. For the species *S. substriata*, on the other hand, the red shift of the wavelength peak with widening of the spectrum man-

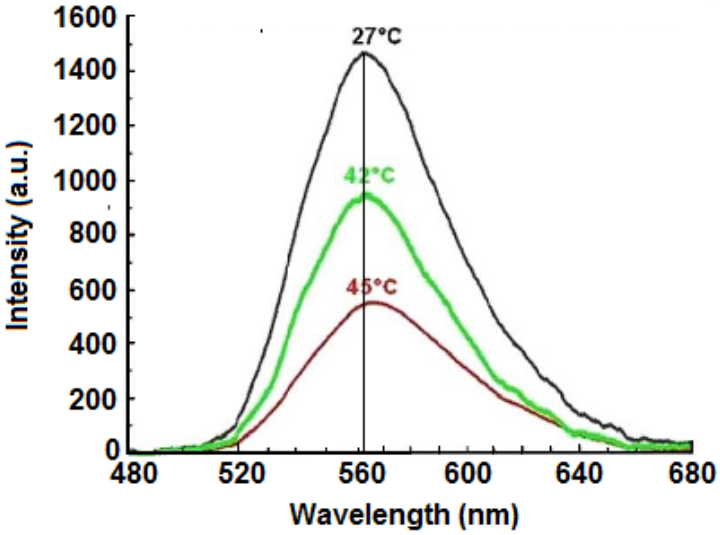

Figure 1: Emission spectra of a male specimen of the firefly *Luciola praeusta* at normal and considerably higher flashing temperatures. The peak shifts towards red and the spectrum widens just after $42°C$. A vertical line is drawn to make the shifting clear [Rabha et al., 2017]

ifests just above $34°C$, shown in Figure. 3. As in the first case, this should imply thermal denaturation of the enzyme luciferase. The pulse width is observed to be the narrowest at $34°C$, exhibiting a large increase thereafter for a small increase in temperature. The fluctuation in the pulse width also becomes considerable at those temperatures, as shown in Figure. 4 [Rabha et al., 2021].

Therefore, we could roughly consider the temperature to be high for *L. praeusta* from aboout $42°C$ and for *S. substriata* about $34°C$. This value for *L. praeusta* fireflies gives the reason why they could be active in the hot summer season just after sunset when the ambient temperature remains high. The temperature in their locality could reach $38-39°C$ in the hottest days, but that falls down to $35-36°C$ at the time of sunset in those days. As this value is sufficiently lower than the peak shifting or shortest pulse-duration temperature, these fireflies could come out just after sunset. For the species *S. substriata*, however, the ambient temperature needs to come down to a few degrees below their temperature optimum of $34°C$. So, they could become active only after one to two hours after sunset, usually. On the few cloudy or rainy summer days when the temperature at the time of sunset becomes $30°C$ or less, one or two specimens of *S. substriata* have been sighted just about half an hour after the sunset. It thus becomes almost dusk-active- there is hardly any difference between the appearance times of this species and the dusk-active *L. praeusta*. Generally, however, it is observed that the majority of *L. Praeusta* fireflies come out within half an hour after sunset on any day, while the majority of the *S. Substriata* fireflies come out roughly 45 minutes after sunset in those days. So it could be concluded that the peak wavelength, though generally determining a firefly's active time, does not determine its time of coming out; the temperature indicating denaturation of the luciferase is the chief deciding factor.

The peak-shifting temperature also gives the reason for a species' choice of the habitat. In this locality, temperatures of the hottest days during the summer go past the optimum denaturation temperature of $34°C$ of *S. substriata*. Shades from branches and leaves of the trees in the banks of the big ponds help to keep the temperature within the temperature optimum of this species. In hot days, they have been

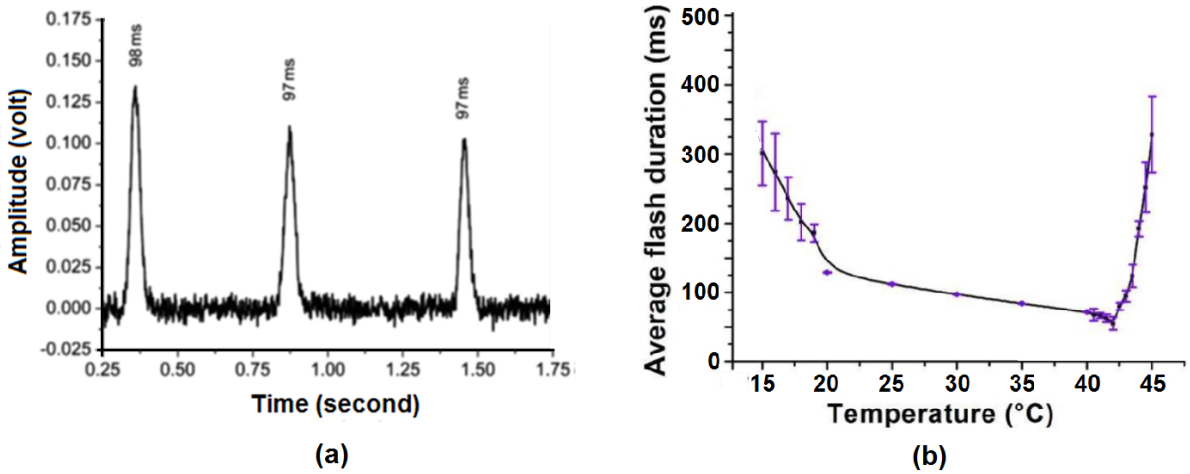

Figure 2: (a) Typical flashes from a male specimen of the species *L. praeusta* at $30°C$ [Sharma et al., 2014]. (b) Pulse width at different temperatures. The width goes on decreasing with increase in temperature, becomes the smallest at $42°C$, and increases considerably from that with slight increase in temperature [Rabha et al., 2017]

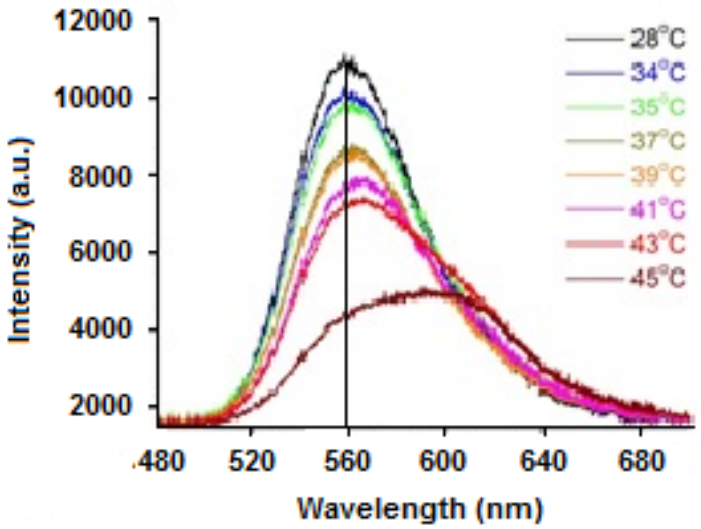

Figure 3: Emission spectra of a male *Sclerotia substriata* firefly at normal and different higher temperatures. The emission peak remains at 558 nm till $34°C$, and it shifts towards the longer wavelength side with an increase in the value of the full-width-at-half maximum from just above this temperature [Rabha et al., 2021]

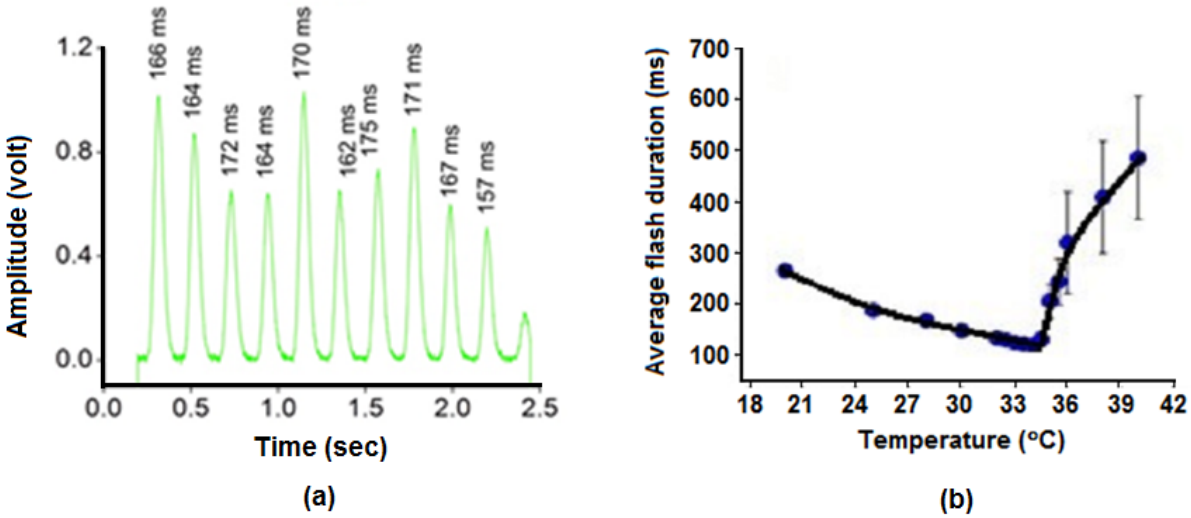

Figure 4: (a) Typical flashes from a male *S. substriata* firefly at $34°C$. (b)Pulse width at different temperatures. The width decreases with increase in the temperature and becomes the minimum at $34°C$; it increases considerably thereafter with a small increase in temperature [Rabha et al., 2021]

observed to start flashing two-three hours, sometimes more than three hours, after sunset, sitting on leaves or branches of trees. For the species *L. praeusta*, on the other hand, no such type of setting is required; availability of food is the only deciding factor.

3 Conclusion

When the temperature is raised to a value considerably higher than the ones at which the firefly normally flashes, the emission peak gets shifted irreversibly towards the higher wavelength side. The pulses also become abnormally broad with large fluctuations in the width. These indicate some change in the structure, probably denaturation, of the enzyme catalyst luciferase. This happening along with the availability of food determines the choice in the selection of the habitat of the firefly. Characterisations of the emitted light from all the species of fireflies will make the picture complete.

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