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Bioluminescence Emissions from the Indian Winter Species of Firefly *Diaphanes* sp.

Mana Mohan Rabha
Department of Physics, Pandit Deendayal Upadhyaya Adarsha Mahavidyalaya, Amjonga, Assam, India

Abstract

Bioluminescence, the emission of visible "cold" light by living organisms, represents one of the most efficient biochemical light-producing processes in nature. While numerous studies have characterized the emission spectra and flash dynamics of tropical, summer-active fireflies, little is known about species that remain active during winter. This chapter presents the first detailed spectroscopic and temporal analysis of ***bioluminescence in the Indian winter-active firefly Diaphanes sp.****, discovered in Assam, India.*

Steady-state emission spectra were recorded at varying temperatures, and temporal characteristics were analyzed both in captive and free modes. The emission spectra showed a green peak at ***548 nm****, slightly blue-shifted compared to those of summer species such as Luciola praeusta and Asymmetricata circumdata. Increasing the temperature to ~28°C caused a distinct red-shift, suggesting the onset of luciferase enzyme denaturation. Uniquely, Diaphanes sp. exhibited* ***continuous light emission patterns*** *rather than discrete flashes, indicating sustained reaction activity in the lantern tissue. The study demonstrates how cold-adapted biochemical mechanisms support light emission at low temperatures, revealing new insights into the diversity and adaptation of firefly bioluminescence.*

1. Introduction

1.1 Bioluminescence: An Overview

Bioluminescence refers to the biochemical emission of visible light by living organisms, where the enzyme **luciferase** catalyzes the oxidation of **luciferin** in the presence of oxygen, magnesium ions (Mg^{2+}), and adenosine triphosphate (ATP). The resulting excited-state oxyluciferin emits photons upon returning to the ground state. This light production is distinct from fluorescence or phosphorescence because it arises from a **chemical reaction within the organism itself**. The process exhibits remarkably high efficiency—quantum yields exceeding 40% have been reported (Ando *et al.*, 2008)—making it one of the most energetically efficient biological reactions known.

1.2 Firefly Bioluminescence and its Roles

Fireflies (Coleoptera: Lampyridae) use bioluminescence primarily for **communication and mating**. Light patterns differ between males and females, and even among species, functioning as a reproductive isolation mechanism. Some species employ light for predation or defensive mimicry (Lloyd, 1965). Beyond natural ecosystems, bioluminescent systems have found wide applications in

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biotechnology, **molecular imaging**, and **biosensor development** (Fan *et al.*, 2008; Gabriel & Viviani, 2014).

1.3 The Gap in Research: Winter Fireflies

Globally, most fireflies are active during summer months when ambient temperatures favor enzymatic reactions. However, **winter-active fireflies** represent a rare ecological and biochemical adaptation. The genus *Diaphanes*, distributed across Asia, includes such cold-tolerant species. Prior to this work, no detailed **spectroscopic study** had been conducted on any Indian winter firefly species.

The discovery of a new *Diaphanes* species active during cold months in Assam provided an opportunity to explore how **biochemical light production** adapts to **low temperatures**. This chapter focuses on the emission spectra, temperature dependence, and time-domain behavior of this winter species.

2. Materials and Methods

2.1 Study Area and Specimen Collection

Specimens of *Diaphanes* sp. were collected from the **banks of the Dikrong River**, Lakhimpur District, Assam (27°03′N, 93°57′E), approximately 330 feet above sea level.

The region experiences night time temperatures ranging from **18°C to 7°C** between late November and early February, coinciding with the activity period of the species.

Adults were captured using light traps and hand nets between **6:30 p.m. and 7:30 p.m.** Active males showing intense flashes were selected for analysis.

2.2 Morphological Features

A specimen and its normal flash of this winter firefly is shown in Fig. 1. The average length and width of the firefly were **15 mm** and **4 mm**, respectively. The lantern consisted of two light-emitting segments separated by a small gap (~0.8 mm), a feature not observed in other Indian Species *Luciola praeusta* or *Asymmetricata circumdata*.

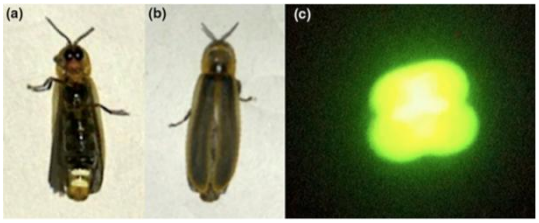

Figure 1. A specimen of the Indian winter firefly Diaphanes sp. showing (a) ventral view, (b) dorsal view, and (c) the luminous abdominal light organ.

2.3 Experimental Setup for Spectroscopy

Each specimen was gently immobilized on soft foam using sellotape and cotton supports. The lantern was oriented toward the input face of a **fiber-optic cable (QP200-2-UV-VIS)** connected to a **calibrated HR4000 Ocean Optics spectrometer**. Emission spectra were recorded via **SpectraSuite software** with an integration time of **2000 ms** due to low light intensity.

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Temperatures were controlled using a **2 kW heater**, adjusted to maintain stability within ±0.2°C. Room temperatures ranged from **14°C to 20°C**, and spectra were recorded at **16°C, 20°C, 24°C, 28°C, 35°C, and 38°C.**

2.4 Flash Waveform Recording

Temporal flash profiles were recorded using a **Hamamatsu H10722 photomultiplier tube (PMT)** linked to a **Tektronix TDS 2022C digital oscilloscope**. Specimens were studied under both **captive** and **free-flight** conditions inside a transparent chamber to compare natural flashing behavior.

3. Results

3.1 Spectral Characteristics

The steady-state emission spectrum at 16°C displayed in Fig. 2. The emission **peak appears at wavelength at 548 nm**, within the **green region of the visible spectrum**, with a **full width at half maximum (FWHM)** of 63 nm (523–586 nm).

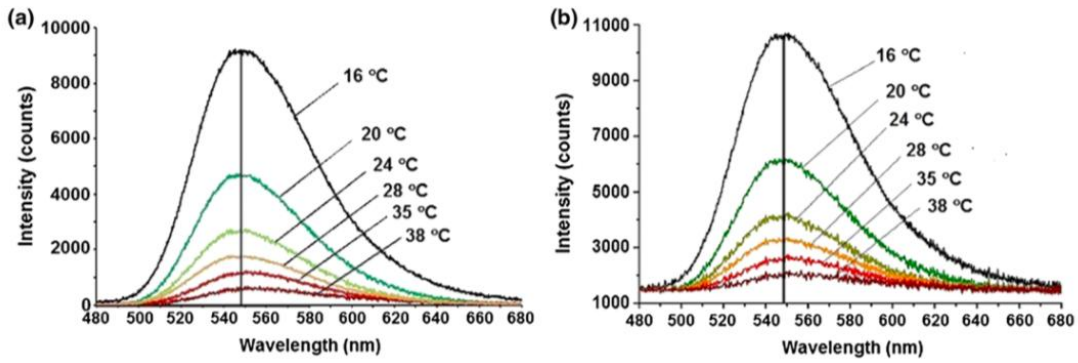

Figure 2. Emission spectra of the firefly *Diaphanes* sp. recorded in the captive mode at temperatures of 16, 20, 24, 28, 35, and 38°C. (a) Spectrum obtained after subtracting the background (no-signal) data. (b) Spectrum without background subtraction. The emission peak appears at 548 nm at 16°C, with a measured FWHM of 63 nm, which remains unchanged up to 24°C. From around 28°C onward, the peak gradually shifts toward longer wavelengths, and at 38°C—the highest temperature used in the experiment—a maximum red shift of 7 nm is observed. A vertical reference line at 548 nm highlights the spectral shift.

3.2 Effect of Temperature on Emission

The emission characteristics remained stable up to 24°C, beyond which a **red-shift** in peak wavelength became apparent. At **38°C**, the maximum observable peak shift was **7 nm**, indicating **partial denaturation of luciferase**. Additionally, overall light intensity declined with increasing temperature, consistent with the reduced quantum efficiency of the enzyme reaction at higher thermal levels.

3.3 Time-Domain Emission Behavior

The time-resolved spectra of this firefly are shown in Fig.3 and Fig. 4. The time-resolved profiles revealed **long-duration flashes** with incomplete dark intervals— a behavior previously unrecorded in Indian species.
In the **captive mode**, flashes appeared as continuous wave trains rather than distinct

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pulses, implying sustained reaction kinetics within the photocytes (Fig. 3). In the **free mode**, flashes were smoother, with durations between **0.9–1.8 seconds (Fig.4)**, indicating longer reaction times and slower oxyluciferin decay rates compared to summer species.

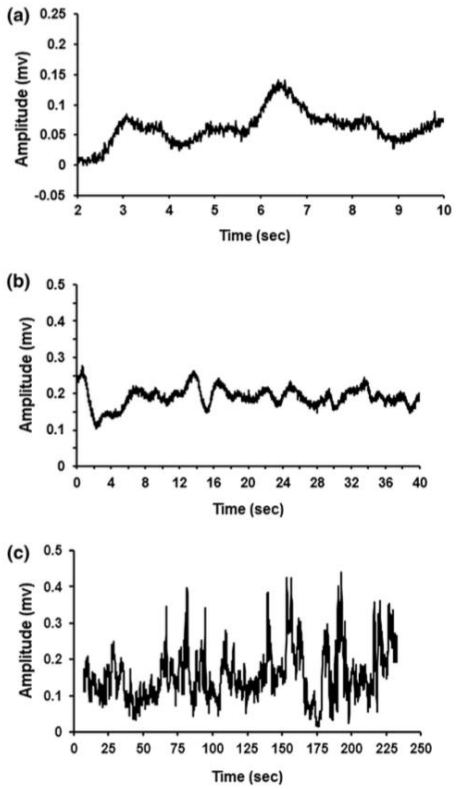

Figure 3. Flashes emitted by the firefly *Diaphanes* sp. under captive conditions at the normal flashing temperature of 16°C. (a) Recorded over a duration of 10 s, (b) over 40 s, and (c) over an extended period of approximately 4 min. The recorded flashes exhibit a noticeably noisy or irregular appearance.

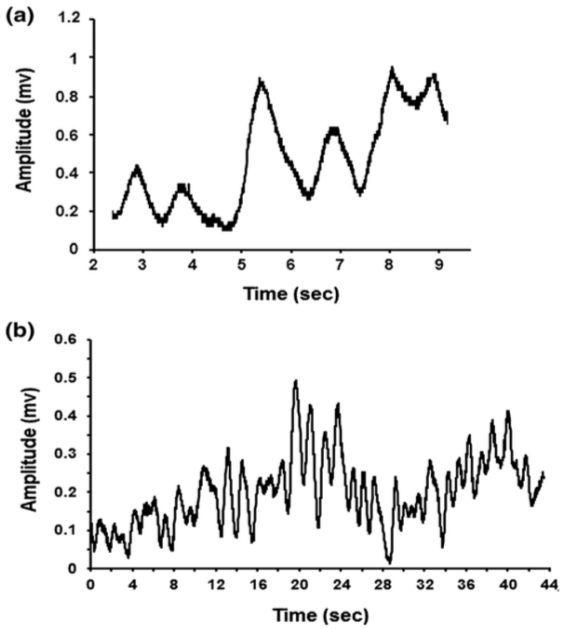

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Figure 4. Typical flashes from a specimen of the firefly *Diaphanes* sp. recorded in free-flight mode at 16°C. (a) Recorded over a 10-s timescale and (b) over a longer duration of approximately 30 s. In contrast to the flashes observed under captive conditions, these signals appear smoother and exhibit longer durations.

3.4 Response to Temperature Variation

The time-resolved spectra at different temperature are shown in Fig. 5. At **28°C**, flash durations shortened noticeably, while at **37–38°C**, flashes became broader and irregular. This confirmed the **thermal sensitivity** of the luciferase-luciferin system and its degradation beyond physiological limits.

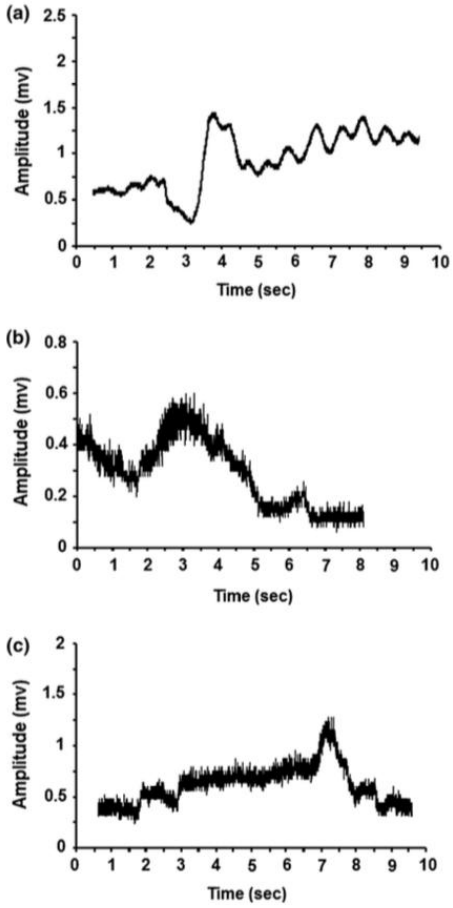

Figure 5. Flashes emitted by a specimen of the firefly *Diaphanes* sp. at elevated temperatures. (a) At 28°C, the flash durations are distinctly shorter than those observed at the typical winter flashing temperature. (b) At 37°C, the flashes become considerably longer compared to those at normal winter conditions. (c) At 38°C—the highest temperature used in the experiment—the flash pattern, similar to that at 37°C, deviates markedly from the usual winter pattern, with some flashes appearing abnormally broadened.

4. Discussion

4.1 Spectral Adaptation

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The green-peaked emission (548 nm) corresponds to wavelengths most efficiently perceived by the **compound eyes of fireflies (540–560 nm)**, suggesting optimal visibility for nocturnal communication.
Such emission profiles are typical of **dark-active species**, corroborating field observations that *Diaphanes* sp. is active only during the darkest hours of the night.

4.2 Temperature and Enzyme Dynamics

Temperature dependence of the emission pattern indicates that *Diaphanes* sp. is adapted to function efficiently at **low ambient temperatures (10–20°C)**. Luciferase denaturation above 28°C may explain the absence of this species in summer. This biochemical adaptation allows successful communication and reproduction in cooler winter environments, where other species are inactive.

4.3 Unique Flashing Pattern

The continuous flash train pattern observed is unprecedented among Indian fireflies. It may arise from **overlapping emission events** of multiple oxyluciferin intermediates or sustained neuronal stimulation of lantern photocytes. Neurochemical control involving **octopamine release** is likely slower at low temperatures, prolonging the light pulses and leading to quasi-continuous luminescence.

4.4 Ecological and Evolutionary Implications

The winter activity of *Diaphanes* sp. reduces predation risk and competition with summer-active species. This temporal isolation could be a form of **seasonal niche differentiation**, enabling coexistence of multiple firefly species in the same region.

4.5 Comparison with Other Indian Species

Species	Season	Peak (nm)	Flash Duration	Remarks
Luciola praeusta	Summer	562	0.3–0.5 s	Bright, rhythmic flashes
Asymmetricata circumdata	Summer	570	0.4–0.6 s	Yellow emission
Diaphanes sp.	Winter	548	0.9–1.8 s	Continuous glow pattern

The lower emission wavelength and prolonged flash of *Diaphanes* sp. clearly distinguish it from the tropical fireflies studied earlier.

5. Conclusion

The **Indian winter firefly *Diaphanes* sp.** exhibits several unique bioluminescent characteristics:

- A **green emission peak at 548 nm**, narrower and cooler than summer species.
- **Temperature-sensitive emission**, with a red-shift beyond 24°C and luciferase denaturation near 28°C.
- **Continuous flash-train emission pattern**, suggesting slow enzymatic turnover and persistent neural control.

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- Adaptation to **cold environments**, facilitating activity in the winter months when competitors are absent.
- This study establishes *Diaphanes* sp. as a **cold-adapted bioluminescent model** and opens new avenues for biochemical and ecological research on thermal adaptations in luminescent organisms.

6. Future Perspectives

- **Molecular characterization** of *Diaphanes* luciferase to identify structural motifs responsible for cold adaptation.
- **Comparative genomics** with tropical species to reveal evolutionary divergence.
- **Applications** in low-temperature biosensing and luminescent tagging due to the enzyme's stability at reduced temperatures.
- **Conservation efforts**, as habitat disruption and artificial lighting threaten the survival of firefly populations.

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