Cryptic Bioluminescence in a Midwater Shrimp

Abstract. *The mesopelagic shrimp* Sergestes similis *emits ventrally directed bioluminescence that closely matches the intensity of downward-directed illumination and is able to rapidly modify its light output to match changes in background intensity. Masking experiments show that the photoreceptors involved are the compound eyes or adjacent tissues. Light emission originates from modified portions of the hepatopancreas and is similar to oceanic light in angular distribution and spectral characteristics. Normally oriented animals respond minimally to upward-directed light.*

The bioluminescence of mesopelagic animals is commonly directed downward from the ventral surface (*1*), which suggests that it functions to obscure the silhouette by replacing downwelling light that has been reflected or absorbed by the body with equivalent bioluminescence (*2*). There have been few quantitative tests of this concept of cryptic bioluminescence (*3, 4*) because of the difficulty of making measurements on animals that typically will not perform while restrained. We have been able to make such measurements on the midwater shrimp *Sergestes similis* Hansen, which has proved to function well under the conditions of restraint necessary for comparisons of bioluminescent output with changes in background light.

Sergestes similis is almost transparent except for the digestive organs, eyes, scattered chromatophores, and the organs of Pesta (*5*). Foregut and hepatopancreas are bordered anteriorly by a pair of laterally placed organs of Pesta and posteriorly by a single large organ of Pesta. Two small mediolateral organs of Pesta lie on the ventral surface of the hepatopancreas (*6*). Visual observation after mechanical stimulation reveals bluish luminescence originating from these organs (*7*).

The opacity of the digestive organs, which tends to make the otherwise nearly transparent animal visible against the background, appears to mask luminescence of ingested prey and perhaps to mask luminous bacteria resident in the digestive tract (*8*). The presence of potentially luminous material in the gut was occasionally confirmed in our experiments by deposition of luminescing fecal pellets (Fig. 1D). The presence of the organs of Pesta beneath the major opaque parts of the digestive tract might therefore be an adaptation that minimizes the visual contrast produced by the masking of luminous gut contents (*9*).

Specimens of *S. similis* were trawled at depths of 25 to 400 m in the Santa Barbara Channel. Animals collected at night were sorted under dim white light and thereafter shielded from all but dim red light until the initiation of testing. Animals collected in the daytime and exposed to ambient light proved to be unusable. Animals were transferred to the shore laboratory and maintained in filtered seawater at 10°C. Starvation for 24 hours before testing reduced the amount of luminescent material in the gut. Six adult males and six adult females were used. No sexual difference in photic behavior was observed.

The experimental apparatus previously described (*4*) was used with modifications to the test chamber and calibration apparatus. Specimens were clamped by the posterior portion of the cephalothorax and placed in an 18-cm-diameter Lucite sphere half full of seawater. In experiments with downwelling light, the upper hemisphere was treated to provide radiance with an angular distribution approaching that of deep ocean water (*10*) when illuminated by a Sylvania R1166 glow modulator tube. Use of a blue pass filter produced a spectral distribution centered on 520 nm with a half-bandwidth of 40 nm. Irradiances measured at the dorsal surface of the test specimen could be varied between 0 and 3×10^{-4} μW/cm^2. These values bracket the irradiances measured at depths within the middle to upper portions of the daytime habitat occupied by *S. similis* in the Santa Barbara Channel (*11*).

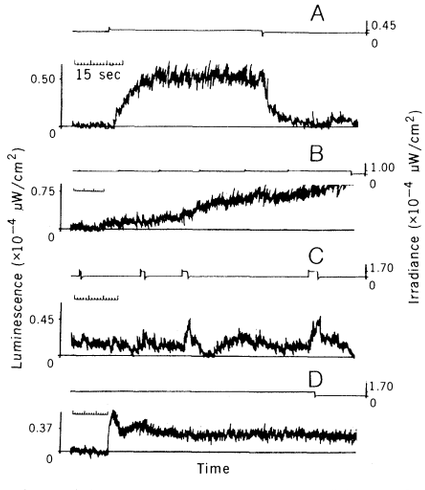

Fig. 1. Bioluminescent responses of *S. similis* to downwelling light. (A) Typical response to on-off downwelling stimulus with a maximum intensity of 0.45×10^{-4} μW/cm^2. (B) Step increments in bioluminescent response during step increments of light to a maximum of 1×10^{-4} μW/cm^2. (C) Luminescent responses to short pulses of light. (D) Record of spontaneous release of a luminescent fecal pellet. In each case the upper trace registers stimulus light intensity (right ordinate) and the lower trace the bioluminescent response (left ordinate). Time markers indicate 15 seconds.

Luminescence was detected 4 cm below the specimen by an upward-viewing EMI 9781B photomultiplier. To prevent artifacts induced by illumination of the photocathode by the background light and to permit high-sensitivity recording in the presence of background light, the photomultiplier was masked by a rotating shutter operating out of phase with the "on" period of the 180-Hz on-off cycle of the glow modulator tube. This allowed the photomultiplier to view the specimen against a dark background while the specimen was illuminated by effectively continuous light (*12*). For tests of the effect of upwelling light, the test sphere was illuminated from below to produce irradiance at the ventral surface of test specimens of 0 to 24×10^{-4} μW/cm^2.

Downwelling light induced bioluminescence at intensities as low as 0.06×10^{-4} μW/cm^2, and stimuli greater than 1×10^{-4} μW/cm^2 typically produced responses beyond our calibrated scale. Half-amplitude response times to stimulus "on" ranged from 1 to 16 seconds (4.2 seconds was the mean of 70 measurements). Bioluminescence was sustained and stable during long periods of steady illumination, 130 minutes in one instance. The intensity of bioluminescence rapidly followed sudden decreases in stimulus intensity. Half-amplitude response times to "off" stimuli ranged from 0.7 to 9 seconds (2.4 seconds was the mean of 70 measurements). Step changes in background intensity evoked equivalent changes in the responses (Fig. 1B). Bursts of light of 1.7×10^{-4} μW/cm^2 as short as 2 seconds led to measurable bioluminescence (Fig. 1C). When data such as those in Fig. 1, A and B, were plotted as light production versus background irradiance (Fig. 2), the slope of the linear regression was not significantly different from 1 (Student's $t = 1.6$, $P > .2$), indicating that the test animals were precisely matching the background. To our knowledge, this requirement for cryptic bioluminescence was not previously shown quantitatively.

Illumination of the eyes or adjacent tissues is essential to the light-matching response. Transparent eye shields (Fig.

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3) had an insignificant effect on response intensity ($t = 2.1$, $P = .05$), whereas identical but opaque eye shields reduced the response essentially to zero (Fig. 2). To eliminate the possibility that our experiments measured background excited fluorescence rather than bioluminescence, the eyestalks of an actively responding animal were removed and tested along with the animal. No signal was obtained at our highest background intensity. In another experiment, upwelling light at intensities up to 23.8×10^{-4} μW/cm^2 resulted in responses that were not significantly different from those in the opaque eye-shield tests ($t = .99$, $P = .4$). Whether determination of the directionality of the background light involves the gravitational sense remains to be ascertained. The failure of day-collected animals to respond was probably due to the illumination to which they were exposed, since one responsive night-collected specimen did not respond for at least 2 hours after exposure to white light at 1.25×10^5 μW/cm^2 for 130 seconds (*13*).

These experiments show that *S. similis* satisfies the requirements for demonstration of bioluminescent counterillumination; it (i) is appropriately directionally sensitive to background illumination, (ii) produces bioluminescence with angular and spectral characteristics similar to those measured for sunlight in deep ocean water, (iii) precisely matches overhead irradiance through an intensity range characteristic of its normal environment, (iv) sustains a constant intensity output over long periods of time, and (v) can respond to short-term variations in the intensity of downwelling light. To our knowledge these criteria have not previously been met in studies of a single species.

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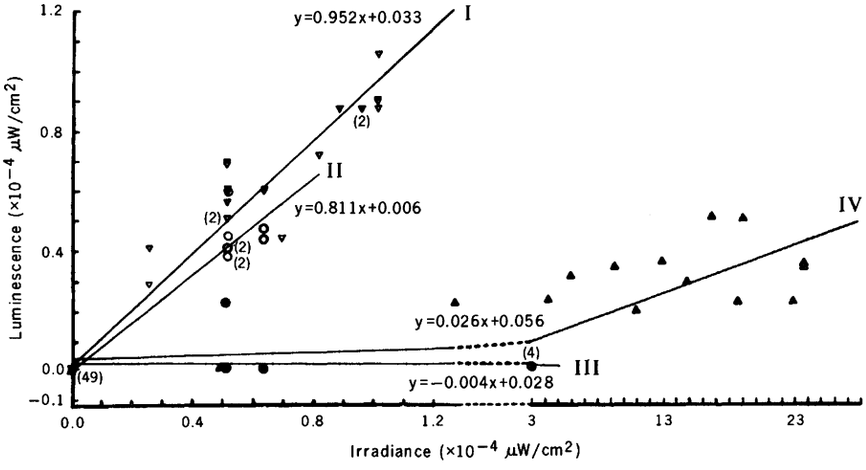

Fig. 2. Bioluminescent output plotted against stimulus irradiance as linear regressions in four types of experiments. (▽) Normal animals responding to downwelling light. (○) Animals with transparent eye shields responding to downwelling light. (●) Animals with opaque eye shields responding to downwelling light. (▲) Normal animals responding to upwelling light. Stimulus intensities in the upwellling light experiment were as much as an order of magnitude greater than those in the downwelling experiments, as indicated on the abscissa to the right of the dotted lines. The slope of line IV is 0.026 throughout.

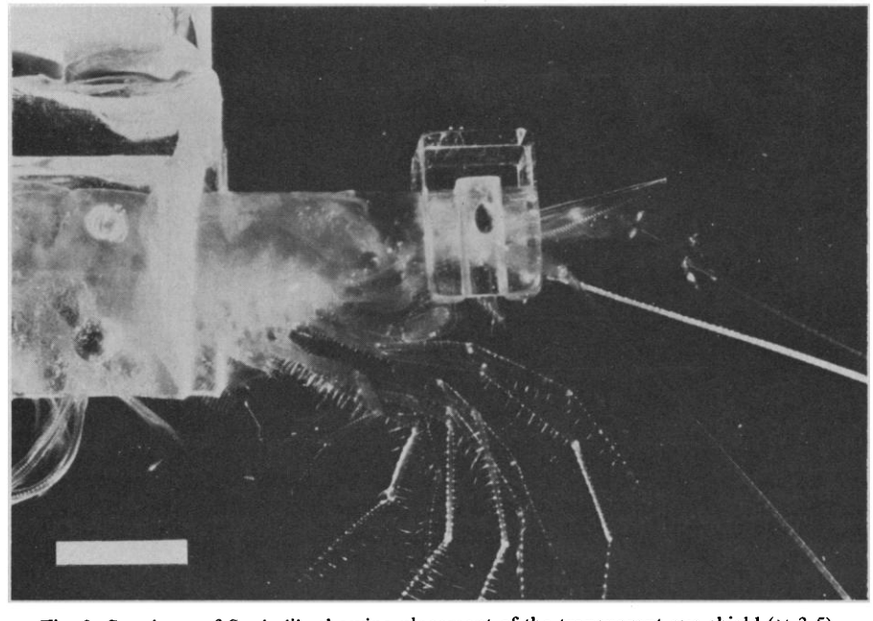

Fig. 3. Specimen of *S. similis* showing placement of the transparent eye shield (× 3.5).

References and Notes

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14. Supported by Office of Naval Research contract NOOO-75-C-0242 and by University of California faculty research funds. We are most grateful for the support and facilities provided by the Quantum Institute and Marine Science Institute, University of California, Santa Barbara, and for the assistance of S. Anderson, J. Torres, R. K. Zimmer, and C. H. Seaman of the Jet Propulsion Laboratories, Pasadena, Calif. Emission spectra were determined by G. T. Reynolds and S. M. Epstein, Department of Physics, Princeton University.

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