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Bioluminescent Countershading in Midwater Animals: Evidence from Living Squid

Abstract. *Midwater squid respond to overhead illumination by turning on numerous downward-directed photophores; they turn off the photophores when overhead illumination is eliminated. The squid are invisible when the intensity of the photophores matches the intensity of the overhead illumination. These results strongly support the theory of ventral bioluminescent countershading.*

Bioluminescence is undoubtedly the most characteristic feature of the midwater fauna of the open ocean. Numerous functions have been suggested to explain luminescent structures (*1*). One function, camouflage, would seem especially important in the open ocean, where an animal has no holes in which to hide. An opaque animal in the dimly lit midwaters, silhouetted against the highly directional downwelling light, will be visible to predators below. Animals under these conditions might conceal themselves by eliminating their silhouettes with ventrally directed bioluminescent light (*2*). This theory of ventral bioluminescent countershading is supported for various squid, fish, and shrimp by vertical distributional patterns, photophore patterns, photophore structure, the radiance pattern of emitted light, and luminescent feedback mechanisms (*3–5*). However, the most critical evidence, direct observations of living animals, is almost totally lacking (*6*). Hastings (*7*) found that a flashlight stimulated a luminous response in the shallow-water ponyfish, *Leiognathus equulus*, which he attributed to countershading behavior, and Lawry (*5*) noted that some myctophids in a shipboard tank luminesced coincident with overhead illumination but gave few details. We have made observations related to ventral countershading which warrant reporting at this time even though data on light intensities are not yet available.

Midwater squid were maintained in a portable shipboard laboratory designed to simulate the low-temperature and dim-light characteristics of their midwater habitat. Shipboard observations were made in a small cylindrical tank supplied with cooled running seawater. A large mirror placed beneath the tank at a 45° angle permitted convenient observation through the bottom of the tank. Overhead illumination was provided by a 25-watt rheostat-controlled incandescent blue light with a broad reflector. Three diffusers placed between the light and the tank eliminated bright spots but did not make the illumination completely uniform. The tank and mirror were encased in a black box to reduce stray light, and access to the mirror was provided through a black curtain. All captures were made at night, and precautions were taken to ensure that specimens were not exposed to bright light.

The squid most suitable for observations was an undescribed, short-bodied species of *Abraliopsis* (Fig. 1) (*8*). This species occupies depths primarily between 500 and 650 m during the day and 50 and 100 m at night (*9*), depths at which bioluminescent countershading can be expected to occur off Hawaii (*10*).

Squid, observed either singly or in pairs, were exposed to alternating periods of dim light and complete darkness. Neither the light spectrum nor intensity was measured, but the light levels selected were well below those obtainable by the animal's photophores, the maximum intensity of which was determined by adjusting the overhead light to match the bioluminescent output of captured specimens. Readings taken from the rheostat then allowed selection of appropriate intensities for experiments with squid captured later. Six squid exhibited distinct responses to the overhead light regime while several others did not respond, probably due to trauma and damage resulting from capture.

Each of the six squid was examined during four to six trials (Table 1) (*11*). Each trial usually consisted of a 10-minute period with the light on followed by a 10-minute period with the light off (*12*). In nearly all cases the squid consistently responded to the overhead illumination by producing a steady downward-directed glow and to the absence of illumination by extinguishing the luminescence. Luminescence by a

Table 1. Responses of squid to overhead illumination. Symbols: +, luminescence initiated during and continued through test period; 0, no luminescence, or luminescence extinguished during test period; G, ghost stage present at end of test period.

Animal	Overhead lights											
	On	Off	On	Off	On	Off	On	Off	On	Off	On	Off
1	+	0	+	0	+	0	+					
2	+	0	+	0	+	0	+	0	+	0		
3	+	0	+	0	0*	0	+	0	+	0	+	0
4	+	0	+	0	+	0	+	0	+	0	+	0
5	0	0	+	0	+	0	+	G	+	G	0	0
6	+	0	+	0	+	0	+	0	+	0	+	0

* Overhead light on for only 2 minutes.

squid was confirmed by observing either (i) the animal glowing against the dimmer fringes of the overhead illumination (16 trials) or (ii) the continuation of the existing glow as the light was turned off (18 trials). Response to the changing overhead light was not immediate. Animals began to glow 1/2 to 5 minutes after the overhead light was turned on (median time, 1 minute; $N = 13$). When the overhead light was turned off the luminescence decreased rapidly until it was barely detectable to our dark-adapted eyes. The barely visible squid, which we call "ghosts," could not be reliably detected with foveal (central) vision, but they were visible when viewed with slightly peripheral vision. After the overhead light was extinguished, the luminescence diminished over a period of 3/4 to 5 minutes (median time, 2 minutes; $N = 29$) until the onset of the ghost stage, which lasted from 0 to 9 minutes (median time, 1 minute; $N = 24$). The ghost stage was not extinguished in two trials.

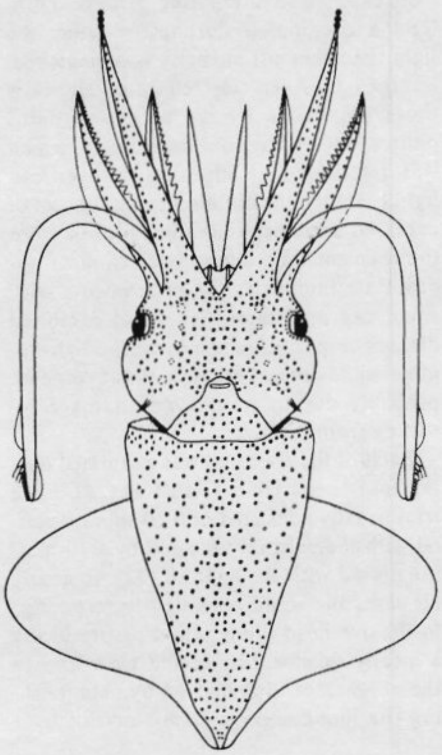

Fig. 1. Arrangement of photophores on ventral surface of *Abraliopsis* sp.

We were unable to determine how closely the squid matched in intensity the wavelengths of light appropriate for countershading in their natural habitat because of presumed differences in the absorption spectrum of the observers' eyes and of the emission spectra of the squids' photophores and the overhead light. However, not all specimens luminesced equally brightly since one squid matched the intensity of the brightest portion of the overhead illumination, while two matched intermediate portions, and three did not match the dimmest parts. We assume this variability is an artifact of the experimental situation.

The value of ventral countershading was apparent even though we could not determine how precisely the squid matched the appropriate illumination. The silhouettes of the squid were distinct when the overhead light was on and the photophores were not yet lighted. With photophores dimly glowing, the contrast between silhouette and background was greatly diminished, and the squid was difficult to see. A squid would disappear from view completely when it swam beneath light of the same intensity as its luminescence. On one such occasion a glowing squid flashed its armtip photophores brilliantly, revealing its location, although nothing but the flashing lights could be detected.

Our observations demonstrate that *Abraliopsis* sp. responds to on-off sequences of overhead illumination by appropriately luminescing and extinguishing their photophores. The observations also demonstrate the effectiveness of ventral countershading in completely eliminating the animal's silhouette. Thus, the results of this study support the theory of ventral bioluminescent countershading (*13*).

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4. R. E. Young, *Pac. Sci.* **27**, 1 (1973).
5. J. V. Lawry, Jr., *Nature (London)* **247**, 155 (1974).
6. Bioluminescent countershading never has been observed from submersibles in spite of numerous observations of midwater animals [for example, R. H. Backus, J. E. Craddock, R. L. Haedrich, D. L. Shores, J. M. Teal, A. S. Wing, G. W. Mead, W. D. Clarke, *Science* **160**, 991 (1968); E. G. Barham, in *Proceedings of an International Symposium on Biological Sound Scattering in the Ocean*, G. B. Farquhar, Ed. (Government Printing Office, Washington, D.C., 1971), pp. 100–118; R. Church, *Oceans* **3**, 20 (1970)]. The failure to observe bioluminescent countershading in nature could be considered as evidence against the theory. We found that countershading was extremely difficult to observe in the aquarium with the observers' dark-adapted eyes only 30 to 45 cm from the animals. With faint overhead illumination in the laboratory, squid could be confirmed to luminesce only when seen against the dimmer portions of the illumination. Such uneven illumination certainly is unlikely to occur in the midwaters of the open ocean. Countershading, such as we observed, is so effective that under natural conditions the animals would be invisible to the observer from all angles. Thus, one should not expect casual observations from a submersible to reveal countershading luminescence. For the present, at least, bioluminescent countershading is best studied in the laboratory.
7. J. W. Hastings, *Science* **173**, 1016 (1971).
8. The ventral surfaces of the mantle, funnel, head, and the ventral two pairs of arms bear numerous, small ventrally directed photophores of three basic types. One type, numbering about one-half of the total photophores, possesses a distal color filter indicative of the countershading function. (The structure of these photophores is currently being investigated by R. E. Young, C. F. E. Roper, and J. Arnold.) In addition, five flattened photophores occur on each eyeball, and a series of three large, spherical photophores occurs on the tip of each ventral arm.
9. R. E. Young, in preparation.
10. ——, *Symp. Zool. Soc. London*, in press.
11. Observations were made separately by each investigator on a total of six squid during a 10-day cruise off Oahu, Hawaii, in November 1975.
12. Time periods with the light on varied from 2 to 16 minutes (mean, 9.7 minutes; 73 percent of trials were between 9 and 11 minutes). Periods with the light off ranged from 5 to 25 minutes (mean, 10.1 minutes; 76 percent of trials were between 9 and 11 minutes). Specimens were placed in the observation tank with the overhead light off at least ½ hour before testing; the observer dark adapted during this period. In all six cases, the squid at this stage did not luminesce.
13. Bioluminescent countershading may be a continuous process, or it may be an intermittent display as part of an escape reaction involving retreat and then concealment (*4*). The consistent reaction of squid in this study to the overhead illumination was unrelated to any apparent escape reaction, supporting the likelihood of a continuous and automatic countershading behavior in these animals. The squid *Heteroteuthis hawaiiensis* under constant overhead illumination in the aquarium (i) produce a luminous cloud, (ii) dart quickly across the tank, (iii) produce a ventral glow for several minutes, and finally (iv) extinguish the glow. This four-step sequence was observed about 20 times in two specimens when apparently they were disturbed repeatedly. In spite of difficulties introduced by the experiment, these observations suggest that intermittent countershading (as part of an escape reaction) remains a distinct possibility for some species.
14. We thank the officers and crew of the R.V. *Kono Keoki*, University of Hawaii, and the members of the scientific party who participated in cruise *Fido IV* for their assistance and Alan Hart, who prepared the illustration. This work was supported in part by National Science Foundation grant DES 72-01456 AO2 (R.E.Y.) and by the Smithsonian Institution (C.F.E.R.). This is Hawaii Institute of Geophysics contribution No. 738.

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