INTENSITY REGULATION OF BIOLUMINESCENCE DURING COUNTERSHADING IN LIVING MIDWATER ANIMALS

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ABSTRACT

Nine species of midwater cephalopods, fish, and shrimp, examined in a shipboard aquarium, adjusted the intensity of their luminescence to match the intensity of the overhead light. Most animals tested could regulate this ventral countershading luminescence in response to a wide range of light intensities. A black anglerfish, *Cryptopsaras couesi,* also produced a faint later glow, indicating that bioluminescence may be important in lateral countershading in some species. Our observations indicate that ventral countershading is effective to depths of 750-775 m during the day off Hawaii. We suggest that the concealment strategy of ventral bioluminescent countershading is limited to depths greater than 350-400 m, largely because of the high visual acuity of predators and the high cost of producing countershading luminescence at lesser depths.

In a previous paper (Young and Roper 1976), we demonstrated that the midwater squid *Abraliopsis* sp. turned its photophores on in response to dim overhead illumination in a shipboard aquarium and turned them off when the light was extinguished. In addition, we noted that the squid became invisible from below when the luminescence of the squid had the same apparent intensity as the overhead illumination.

In this paper we examine in more detail bioluminescent countershading in living midwater animals. Tests were conducted on seven species of squids (*Abralia trigonura, Abraliopsis* sp., *Pterygioteuthis microlampas, Pyroteuthis addolux, Enoploteuthis* sp., *Octopoteuthis nielseni, Heteroteuthis hawaiiensis*), one black anglerfish (*Cryptopsaras couesi*), and one half-red shrimp (*Oplophorus gracilirostris*). We will demonstrate that these animals not only respond to on-off sequences of overhead illumination, but alter their luminosity (luminous intensity) in order to match comparable alterations in the overhead illumination. The implications of these and our other findings concerning bioluminescent countershading in the midwater environment are discussed.

Rauther (1927) first suggested that bioluminescent light from the ventrally directed photophores of teleost fishes might diminish their silhouettes when viewed from below. W. D. Clarke (1963) revived the idea and assembled supporting evidence. He suggested that opaque animals in the dimly lit midwaters of the open ocean will be silhouetted against the highly directional downwelling daylight, and they will, therefore, be visible to predators below. The production of downward luminescence of proper intensity would eliminate the silhouette and thereby conceal the animal. Considerable evidence now has accumulated to support the hypothesis of ventral bioluminescent countershading.

Denton et al. (1969) demonstrated that the photophores of the hatchetfish *Argyropelecus* were designed to distribute their luminescence in a manner consistent with countershading requirements. Denton et al. (1970) found that color filters in the photophores of the fishes *Argyropelecus* and *Sternoptyx* passed only blue light at about 480 nm. This wavelength is close to the transmission peak of sunlight in oceanic water. Such "skylight" filters also have been found in a number of squid (Arnold et al. 1974; Young in press), and they probably occur in sergestid shrimps with the photogenic organs of Pesta (Foxton 1972). Young (in press) suggested that all photophores bearing skylight filters were countershading organs. Best and Bone (1976), however, found that photophores with blue color filters are not all directed ventrally in the fish *Xenodermichthys* (and apparently *Photostylus*) and concluded that blue filters must have functions in addition to countershading.

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Young (1973) described extraocular photoreceptors in the midwater squid *Abraliopsis* sp. which seemed designed to detect downwelling sunlight as well as bioluminescent light from some of the animal's own photophores. This system could provide the animal with the information necessary to adjust the intensity of its photophores to match the downwelling light. Similar systems now have been described in a variety of squids (Young in press). Stomiatoid fishes have photophores directed into their eyes that might be a part of a similar system, and Lawry (1974) described a comparable mechanism in myctophid fishes. Arnold et al. (1974) described a possible mechanism for regulating the intensity of luminescence in the countershading photophores of the squid *Pterygioteuthis*.

Several workers have found that data on the vertical distribution of midwater animals support the ventral countershading hypothesis. Badcock (1970) noted a changeover in midwater fishes with increasing depth from predominately reflective species with large ventral photophores to predominately nonreflective species with small or no ventral photophores at a depth of 650 to 700 m off Fuerteventura, eastern Atlantic Ocean. Amesbury (1975) noted similar trends among midwater fishes off Hawaii. Foxton (1970) reported a changeover in the sergestid fauna off Fuerteventura from shallower-living species consisting of half-red shrimps with complex photophores (organs of Pesta) to deeper-living forms with all-red coloration and simple dermal photophores. He suggested that the latter group lived too deeply for countershading to be effective. Walters (1977), in a similar study off Hawaii, pointed out that ventral countershading may be effective 110 to 120 m below a depth where lateral countershading (i.e., countershading of the animal's sides) is no longer of use. While agreeing with Foxton (1970) that the red pigmentation aids in reducing reflection of bioluminescence from nearby animals, Walters suggested that ventral countershading still is necessary in these deep-living shrimps and that the simple dermal photophores are adequate for such low-level luminescence. Walters proposed that the lower limit of ventral countershading off Hawaii is 775 m, the depth where the lower distributional limit of common all-red sergestids with simple photophores approximately coincides with the upper limit of sergestids without photophores.

Young (1973) indicated similar trends in the reduction of photophore complexity with depth in midwater squids and noted (Young in press) that only species living above 700 m had photophores with skylight filters. Habitat data on midwater cephalopods also indicate that bioluminescent countershading may operate at night and at twilight as well as during the day in the open ocean (Young in press). Hastings (1971) suggested that a countershading function in the shallow-water pony fish, *Leiognathus*, was indicated by the luminous radiance pattern and the luminous response to light from a flashlight. If bioluminescent countershading occurs in clear water neritic environments, it probably operates against dim skylight or moonlight at night and not, as Hastings suggested, during the day.

Denton et al. (1972) demonstrated that the angular distribution of bioluminescence produced by the midwater fishes *Argyropelecus* and *Chauliodus* closely matched the radiance field of daylight in the midwaters of the open ocean.

MATERIALS AND METHODS

Animals studied during two 10-day cruises aboard the RV *Kana Keoki* off leeward Oahu, Hawaii, in the spring of 1976 were captured by a shortened version of the 3-m Isaacs-Kidd midwater trawl with a conical, aluminum cod-end bucket. The bucket was designed to reduce internal turbulence and to eliminate strong sunlight. On deck the bucket was wrapped in black plastic during the day and removed to a dark room. At night the catch was brought aboard under dim red light. In both cases the catch was quickly transferred to cold water and live squids were placed in vials with Nitex[3] screening at each end then transferred to holding tanks in a portable lab-van. The lab-van had a light-tight portion with cooled, running seawater and an adjoining dry lab. The temperature in the holding tanks was cycled day and night between 5°-7°C and 15°C to approximate the day-night temperatures in the habitats of the vertically migrating animals. For the most part we had little control over the selection of animals tested. We used only squids that were large enough and in good enough condition for reliable testing. Only one fish and

[3]Use of trade names does not imply product endorsement by the National Marine Fisheries Service, NOAA, or by the authors' institutions.

one shrimp were tested. Although many living specimens of the shrimp were available, time limitations prevented testing more than one specimen.

Tests were made in a tank 30 cm on a side supplied with running seawater. The tank lay inside a black box which supported a series of three Plexiglas light diffusers and a light above the tank, as well as a mirror tilted at a 45° angle beneath the tank. The 25-W light was covered by a Kodak No. 45 blue filter with peak transmission at 487 nm and a range of 430-540 nm. The black box prevented light from entering the tank except from above and from the front observation port. The observation port was draped with a dark cloth which allowed the observer to watch the animals without admitting stray light. The entire apparatus was placed on a vibration dampener which eliminated high-frequency but not low-frequency vibrations. The room in which observations were made was kept dark except for the enclosed overhead light directed into the tank. Observations were made by looking into the mirror beneath the tank at the ventral surface or downward directed aspect of the animal. Prior to each test series, the observer dark adapted for a minimum of 30 min. The squids generally were confined in small screened cages or placed beneath an inverted petri dish held slightly off the bottom to allow water circulation. The small containers enabled better viewing of the animals by restricting their movements but did not seem to affect their responses. The shrimp would not swim upright in the tank, so a supporting harness of monofilament line was tied around the animal at the junction of the thorax and abdomen. The line was suspended from a slender H-shaped float, and a shorter, ballasted line extended below the shrimp. This apparatus held the shrimp in the center of the tank and allowed it to swim in an upright position without noticeably affecting the overhead illumination.

Light measurements were made with an EMI 9558B photomultiplier. A 3.2-mm diameter fiber-optic light guide connected to the photomultiplier was secured in front of the mirror with a narrow crossbar. The entire bottom of the tank was within the acceptance field of the fiber-optic probe. This arrangement allowed the observer a nearly unobstructed view of the mirror and permitted simultaneous measurements of the overhead illumination. A picoammeter connected to the photomultiplier tube was operated by a second person in the adjoining, lighted lab. The light intensity above the tank (overhead illumination) was regulated with a variable transformer by the observer who depended on verbal feedback from the operator in the adjoining lab to set the light at a specific level.

Animals were subjected to the following light regime with only a few exceptions. Light levels were increased in a fixed series of steps by factors of 1, 2, 6.7, 20, 60, 200, and 300 (see Figure 1). A few animals were tested at levels corresponding to factors of 0.5, 0.17, and 0.067 of the step 1 value. Between each step the light was turned off for 10 min. The following regime was used at steps 0.067-3: the light was turned on for 10 min; at the end of 5 min and at the end of the period, measurements of the luminosity of the animal were made. The light was then turned off for 10 min, then the sequence was repeated, providing a total of four trials and four measurements at each step. Initial observations indicated that animals required longer exposures to light at higher light levels, so beyond step 3 the regime was increased to 20-min periods with measurements at 10 and 20 min. The dark time between periods, however, remained at 10 min (the same duration as between steps). Not all animals were subjected to the highest steps. Generally, at a step where the animal's luminosity was equivalent to or lower than its luminosity at the preceding step or when the observer concluded that the animal could no longer match the overhead illumination, the experiment was terminated. Total testing time approached 7 h, including the time given the animal to adjust to the countershading tank. After the highest step,

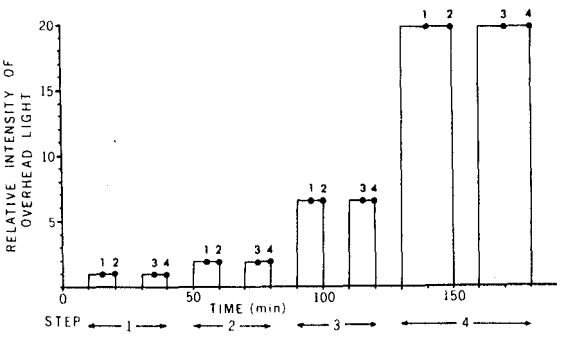

FIGURE 1.—Diagram of steps 1-4 of the testing regime, showing relative light intensities, exposure times during steps, and times (dots) at which countershading measurements were made.

occasionally a lower step was repeated to insure that the animal was still capable of responding. In a few cases, explained in the results, the standard testing sequence was varied slightly.

Measurements of the animal's luminosity at each step were made in the following manner. A luminescing animal, in a natural orientation, was completely invisible when it matched the overhead illumination. The observer quickly reduced the overhead light to nearly zero intensity, then rapidly increased the illumination until the animal became invisible. The observer called the match, and the intensity of the overhead illumination displayed on the current meter was noted by the operator. The measurements, therefore, represent the intensity of the overhead illumination which the animal was judged to be matching, and they provide an indirect measure of the animal's luminosity. As this technique lacks high precision, the four measurements at each step were averaged. The averaging has the disadvantage of combining readings on a squid that did not match the overhead illumination with readings that did match. Since an animal rarely became brighter than the overhead illumination, the effect of averaging usually reduces somewhat the final value of the luminosity of the animal or underestimates the animal's ability to match the overhead illumination. To determine the accuracy of this method, we examined 17 steps in which the animal matched the overhead illumination just prior to the measurement in at least three of the four trials. After the measurements the values for trials within each step were averaged. If accuracy were perfect, these values would be equal to the standard value of the respective step. The actual values in 70% of the cases were within 15% of the standard value of the respective step. Over 75% of the measured values were less than the standard values. Two difficulties contributed to this. When the overhead illumination was quickly increased for a matching reading and the animal started to become invisible against the overhead light, the exact point of disappearance became somewhat subjective, and the observer tended to call for the measurement before disappearance was complete. In addition, an animal occasionally began to lower its intensity immediately when the overhead illumination decreased, but before the measurement could be completed.

On the basis of this analysis, each measurement was assigned a nominal value that was a multiple of 30% of the standard value of the overhead illumination at a given step. Thus, if the measurement was over or under a nominal value by less than 15% it was assigned this nominal value. If the measurement was over or under by more than 15%, it was assigned the next upper or lower nominal value. Both methods of averaging and of assignment into nominal values tended to underestimate an animal's ability to match the overhead illumination. The most critical measure of an animal's ability to match the overhead illumination was the observer's visual determination that the animal was or was not matching just prior to a measurement.

This technique had strong limitations beyond the step at which the animal appeared to match the overhead illumination. Because of increased resolution the observer saw a silhouette with glowing photophores superimposed and he attempted to match the photophore luminosity with the overhead light intensity without losing sight of the silhouette. Such measurements became very subjective. In addition, at high light levels cone vision by the observer becomes significant and would compound any color mismatch between photophore and overhead light. Thus, beyond the level at which the animal matched the overhead illumination, the data were interpreted as an indication only of increase or decrease in luminosity from the previous step.

An animal was recorded to have turned off its photophores during dark periods when luminescence could no longer be detected by the observer (i.e., when the animal's luminosity decreased below the visual threshold of the observer).

We currently are unable to determine the oceanic depths to which our measurements correspond because we have been unable to confidently calibrate our light-measuring system. Our calculations of the energy cost of bioluminescence are based on light values in the ocean near Hawaii at lat. 19°44.5'N, long. 154°40.7'W (E. Kampa pers. commun.).

RESULTS

Enoploteuthidae

Abralia trigonura Berry, 1913

This species possesses numerous photophores of three basic types that are scattered over the ventral surfaces of the body, head, funnel, and arms; in addition, a series of photophores lies on the

ventral surface of each eyeball. This species occupies depths primarily between 450 and 560 m during the day and between 50 and 100 m at night (Young[4]). Four specimens were tested ranging from 28 to 37 mm ML (mantle length).

Three individuals matched the overhead illumination perfectly at most trials in steps 1 through 4, while one specimen did not quite match at step 3. At step 5, one specimen came very close to matching during one trial. While the other individuals could not match this level, they increased the intensity of their luminescence at step 5. The specimen that produced the highest luminosity had been brought through the first three steps at a rapid rate by eliminating two trials at each step. Only one of the two squid tested at step 6 increased the intensity of its photophores at this level; however, it could not begin to eliminate its silhouette at this step or subsequent higher steps. This resulted partly from the increased resolution in the observer's eyes at the high light level; individual photophores and the silhouette could be seen at the same time.

The squid extinguished their lights when the overhead illumination was turned off during the first four steps. Beginning at step 5, two specimens did not extinguish their lights completely during dark periods but always reduced their luminosity to a very low value (average intensity = 35% of the intensity at step 1). Two specimens continued to extinguish their luminescence through steps 6 and 7, but at these high light intensities our eyes began to lose dark adaptation which raised the intensity of the "turn-off" point. At steps 1 and 2 most specimens extinguished their luminescence within 2½ min after the overhead light was turned off, while beyond step 3 extinction times rose to 5 to 10 min. Turn-on times of the photophores following illumination of the overhead light were subjectively determined as the time when the silhouette began to fade. The average time for initial observation of luminescence through step 4 for three specimens was about 1½ min. In several cases initial luminescence was detected within ½ min after the overhead light was turned on.

A large complement of photophores of two different intensities was detected at step 6 through step 8. While the intensity of the dimmer photophores remained the same or decreased slightly at step 8 (see Table 1), the less numerous bright photophores increased in intensity at step 8. Ocular photophores seemed to luminesce at steps 4 and 5 in two specimens; however, these photophores were not detected in the other two specimens.

Abraliopsis sp.

This species has numerous, small photophores

[4]Young, R. E. Photosensitive vesicles and the vertical distribution of pelagic cephalopods off Oahu, Hawaii. Manuscr.

TABLE 1.—Bioluminescent response of midwater animals to overhead illumination. Testing regime included periods of darkness both between and during steps (see text) which are not indicated on chart. Step = category of each test level. Relative light value = intensity of overhead illumination relative to step 1. Matching values = measure of luminescent intensity of animals relative to step 1 in response to the overhead illumination. Solid bar = highest level at which animal was able to match overhead illumination during at least one of the four trials. Dotted bar = highest level at which animal was nearly able to match overhead illumination during at least one of the four trials. Superscripts = number of trials other than the standard four. Parentheses = relative light value of overhead illumination instead of standard step value. + = animal luminesced but measurement not possible. Series 2 = repeat of tests.

Step no.	Relative light value	*Abralia trigonura*	*Abralia trigonura*	*Abralia trigonura*	*Abralia trigonura*	*Abraliopsis* sp.	*Abraliopsis* sp.	*Pyroteuthis addolux*	*Pyroteuthis addolux*	*Pterygioteuthis microlampas*	Series 2	*Octopoteuthis nielseni*	*Heteroteuthis hawaiiensis*	*Cryptopsaras couesi*	*Oplophorus gracilirostris*	Series 2
		Matching values														
0.067	0.067											+				
0.17	0.17											0.20				
0.5	0.5															0.5
1	1.0	1.0	0.72[1]	1.0	0.72	1.0	1.0	0.12[1]		1.0		4.8	1.5		1.5	0.72[2]
2	2.0	2.0	2.0[2]	1.5	2.0	2.5	1.5	1.0		2.0		3.5	1.5	(1.7)2.0	1.5	2.0[2]
3	6.7	4.8	6.7[2]	4.8	6.7	4.8	4.8	3.5	3.5	4.8		10	6.7	7.5	3.5	
4	20	20	20	14	20	10	14	20	14	20		14	20	14[2]	1.5	
5	60	31	43	31	20	(33)20	43	43		60	43[2]	4.8	31			
6	120		75	60		10	43	31		4.8[2]	60					
7	200		75							1.0[2]	4.8[2]					
8	300		60													

of three basic types scattered over the ventral surfaces of the mantle, head, funnel, and arms (Figure 2). In addition a series of photophores lies on the ventral surface of each eyeball. *Abraliopsis* sp. occurs primarily between 500 and 600 m during the day (although it has been captured rarely at depths up to 400 m) and between 50 and 100 m at night (Young see footnote 4).

Two specimens were tested (18 and about 20 mm ML), but they were treated somewhat differently. One squid was tested through six steps with only two trials at each step, then the sequence was repeated through step 5. In both series the step 5 overhead illumination was only 56% of the standard value at this level, so the fifth level is called step 4½ for this specimen. There were no important differences in the animal's luminous responses between the two series and they are combined in Table 1. The second specimen was tested in the standard manner.

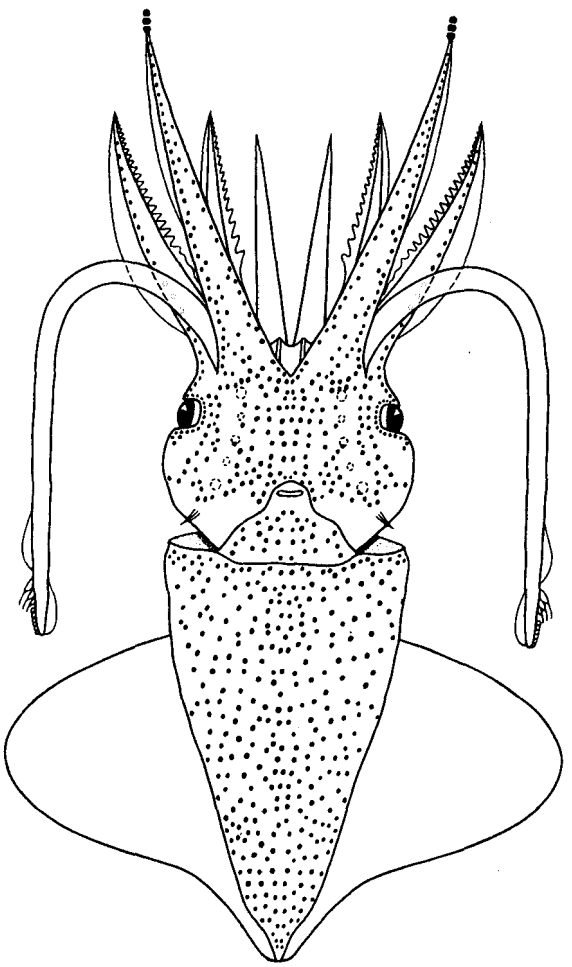

FIGURE 2.—Arrangement of photophores on ventral surface of *Abraliopsis* sp. (from Young and Roper 1976).

Perfect matches were recorded during trials at steps 1 through 4; although one specimen did not match at step 4, it produced a near-match at step 4½. One specimen was able to match the overhead light at step 5 with the observer 1.5 m back from the mirror, while at step 6 a silhouette was still visible when viewed from this distance. Each specimen extinguished its photophores only once during dark periods; however, their luminosity always decreased markedly during these periods. The average luminosity for all trials at the ends of dark periods was 45% of the step 1 intensity.

At least three types of photophores seemed to be involved in countershading at various light levels. Ocular photophores in one specimen seemed to be lighted first at step 4 and were clearly lit at step 4½, but they were never detected in the other specimen. Large numbers of photophores of two different intensities were first detected at step 4 and were apparent at steps 5 and 6. Individual photophores could be detected at step 4 but they could not be resolved with the observer 30 to 45 cm away from the mirror.

Pterygioteuthis microlampas Berry, 1913

This species carries an array of complex photophores on each eye (see Arnold et al. 1974; Young in press) and an equally complex pair in the mantle cavity near the anus. A series of four photophores is located farther posteriorly along the ventral midline in the mantle cavity. The midline photophores have a simpler structure than the others (Chun 1910). Photophores are also present at the bases of the gills and in the tentacles, but they play no role in countershading. *Pterygioteuthis microlampas* lives primarily between 450 and 500 m during the day and 50 and 100 m at night (Young see footnote 4).

One specimen (20 mm ML) was tested. Since this species orients obliquely to countershade (Young in press), a small cage was made to hold the specimen at approximately the correct angle; however, the animal rested on the bottom of the tank with its head tilted more than the body, and the arms were improperly held. Because of the imperfect orientation, the animal was not observed to disappear against the overhead illumination. However, with allowance for orientation, the observer concluded that the squid completely

matched the overhead illumination at each level through step 4, and this interpretation was confirmed by the measurements. By mistake, step 7 was run between steps 4 and 5, so at the completion of the test series, steps 5 through 7 were repeated. The jump from step 4 to step 7 in the first sequence resulted in almost no luminous response from the squid; its luminosity was only that of step 1. The squid came close to matching at the following step 5, but a dim silhouette could be detected by the observer 1.5 m from the mirror. At level 6 the squid again barely responded to the overhead illumination. The squid came even closer to matching the overhead light during one trial of the rerun of step 5. At step 6 in the second sequence, the squid responded to the overhead illumination but could not come close to matching it. Indeed, only a few of the brightest photophores could be detected. The squid barely responded at step 7.

The squid extinguished its lights during three of the four dark periods in steps 1 and 2, and in the remaining steps the luminosity always diminished greatly. The average value of the luminosity by the end of the dark periods was 50% of the step 1 intensity.

Individual photophores could be resolved at step 3, and at step 4, ocular, anal, and one of the midline photophores were visible. The midline photophore was not visible at the beginning of step 5 but was clearly visible by the end of the step. An additional midline photophore was visible at step 6. Some of the photophores were markedly brighter than the overhead illumination at step 5, and at step 6 some of the brightest photophores seemed equivalent in intensity to the overhead light.

Pyroteuthis addolux Young, 1972

This species has nearly the same complement of photophores as the closely related *Pterygioteuthis microlampas*. *Pyroteuthis addolux* occurs primarily between depths of 450 and 500 m during the day and between 150 and 200 m at night (Young see footnote 4).

Two specimens (21 and 23 mm ML) were tested, although we were able to test only one specimen through the entire series. Like *Pterygioteuthis, Pyroteuthis* appears to countershade normally in an oblique position. We placed the specimens in a small, bottomless cage to hold them at approximately the proper angle, but they were never well oriented and observations were difficult. Nevertheless, we were able to secure measurements from portions of the animals. Neither specimen performed well at step 1. One specimen was increased to step 3 and measurements were obtained at steps 3 and 4 but were discontinued because of poor orientation. The animal clearly responded to the overhead illumination during these two steps but, because of the orientation problem, the observer could not determine whether or not the animal matched the overhead illumination. The second specimen was better oriented, but the sides of the cage cast a shadow at low light levels, making it difficult to determine how well the animal matched the overhead light. In addition, the arms were held slightly away from the body axis exposing a dark silhouette of the large, heavily pigmented buccal membrane. Nevertheless, the data show that the animal was countershading. The observer concluded at step 4 that if the animal were properly oriented it would completely match the overhead light. The animal came close to matching at step 5, but at step 6 the intensity of its photophores was less than at the previous level.

The specimen tested only at steps 3 and 4 extinguished its lights during dark periods. The other specimen extinguished its lights during the dark periods of steps 1 through 3. At steps 4 through 6 its luminosity always decreased greatly during dark periods to an average value equivalent to the light intensity at step 1.

Enoploteuthis sp.

This animal has numerous photophores of two basic types distributed over the ventral surface of the mantle, head, funnel, and arms. The mantle photophores that contain skylight filters have a slightly different distribution than the nonfilter type. They form oblique strips that extend from the ventral midline of the mantle to its lateral margins. A series of photophores also is present on the ventral surface of each eyeball. Based on a few captures, *Enoploteuthis* sp. occurs at depths of 500 to 600 m during the day and in the upper 100 m at night (Young see footnote 4).

A single animal (37 mm ML) was tested before we established a standard testing procedure. The results, therefore, are not included in Table 1. The specimen increased its luminosity as the overhead light was increased from one level to the next, and it reduced its luminescence greatly when the

overhead light was extinguished. The squid matched the overhead illumination at levels comparable to step 1 through step 4½, and at a level comparable to step 5 it nearly matched when the observer was 45 cm from the mirror. The ocular photophores luminesced at a level comparable to step 4 and the oblique strips on the mantle were clearly seen to glow.

Octopoteuthidae

Octopoteuthis nielseni Robson, 1948

This species is a stocky squid with enormous, muscular fins. The animal is heavily pigmented and possesses a small number of widely spaced photophores that, in dissection, have a very simple structure. The photophores that were observed to luminesce on the one specimen tested (70 mm ML) are illustrated in Figure 3. Based on sparse data, Young (see footnote 4) indicated a depth distribution between 650 and 765 m during the day and between 100 and 200 m at night.

The testing sequence differed somewhat for this animal. Testing began at step 1, dropped to step 0.17, and then to step 0.067. Step 1 was repeated, then followed by steps 2 through 6 in the standard procedure. With the observer 30 cm back from the mirror at step 1 the squid came close but did not quite eliminate its silhouette, although the individual photophores were much brighter than the overhead light. The figures in Table 1 reflect matching of individual photophores and not of the animal as a whole. The squid typically folded its fins against its body, and it could countershade more effectively in this attitude. The folded fins reduced the distinctness of some of the photophores. At level 0.17 the squid matched the overhead illumination perfectly when the fins were folded against the body and the observer was 30 cm away from the tank. The overhead light at step 0.067 approached the threshold intensity for the observer and the silhouette of the large squid first appeared as a faint smudge then disappeared. Measurements were not possible because of the extremely low light levels. When the overhead light was extinguished at this step, the squid could be seen to be glowing and the luminescence faded rapidly.

The squid extinguished its photophores during dark periods at steps 0.067 through 1. The specimen diminished greatly in intensity during the dark periods at step 2, but turned upside-down on both occasions before the photophores were extinguished. Photophores were not extinguished at steps 3 through 6 but decreased to an average of 60% of the step 1 intensity by the end of the dark period.

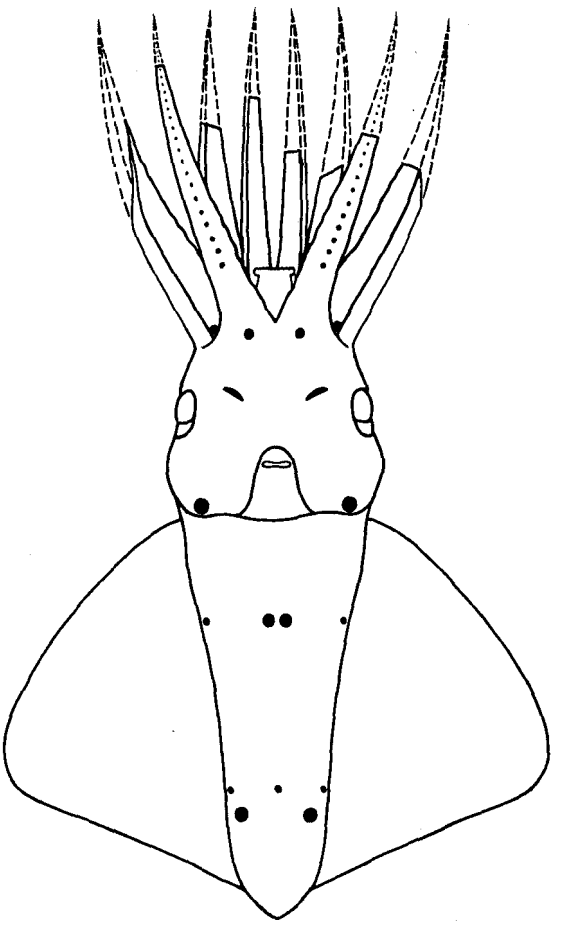

FIGURE 3.—Arrangement of photophores on ventral surface of *Octopoteuthis nielseni*.

The squid was unable to match the overhead light beyond level 0.17; however, it continued to increase the intensity of its photophores through step 4, while at step 5 the intensity decreased greatly. Step 3 was repeated to check the animal's condition and its response was the same as the previous test at that step.

The following photophores were visible at step 1: two large posterior mantle photophores, two ink sac photophores, two photophores at the posterior margin of the head, four photophores at the bases of arms III and IV, and, at the beginning of the

dark period, the axial photophores along the ventral arms. The remaining photophores on the mantle and head were detected at step 3.

Sepiolidae

Heteroteuthis hawaiiensis (Berry, 1909)

This species is a small, robust cephalopod with a single, large photophore embedded in the ink sac (see Young in press). Animals larger than 17 mm ML are found between 375 and 650 m during the day and between 100 and 550 m at night (Young see footnote 4).

A single specimen, 24 mm ML, was tested. *Heteroteuthis hawaiiensis* countershades in an oblique position. Even though the animal was placed in a small cage, the animal's orientation was not perfect. The animal did not countershade well at the first two steps. During this period the squid was upset and discharged luminous clouds several times. The animal nearly matched the overhead illumination at step 3 and did match at step 4. The specimen again was very disturbed during step 5; it discharged luminous clouds and did not match the overhead illumination. The animal extinguished its photophore very rapidly in the dark periods of all five steps.

Ceratiidae

Cryptopsaras couesi Gill, 1883

Only two juvenile specimens of *Cryptosaras couesi* have been taken previously in horizontal tows off Hawaii; both were captured at night at 180 and 195 m (T. A. Clarke pers. commun.). The adult specimen examined here came from a tow that descended to about 200 m at night. Bertelsen (1951) found this species to occupy lesser depths than any other anglerfish examined. Unfortunately, day and night captures were not distinguished. R. H. Gibbs, Jr. (pers. commun.) has taken this species at a minimum depth of 635 m in an opening-closing net during the day in the Atlantic.

The specimen examined was a female, 150 mm standard length, with a small parasitic male attached to its ventral surface. *Cryptopsaras couesi* is a small jet-black anglerfish whose luminescence was thought to be limited to its esca and caruncles. To our vast surprise, this specimen was capable of luminescent countershading. We would not have introduced this animal into our experimental tank had it not been glowing when the catch was sorted. The glow was very directional which suggested a countershading function, although it was directed posteriorly. We could not detect the source of the luminescence, but it appeared to originate from the skin; where the skin was abraded or purposely cut, there was no luminescence. Except for the anteriorly placed, blunt lower jaw, all of the black skin, including that on the fin rays and on the dwarf male, luminesced. After some minutes in a holding tank, the luminescence decreased greatly and the animal was placed in the experimental tank. The fish immediately adopted a head-up position directing the low-intensity glow downward (measured at one-third the intensity of step 1) (Figure 4).

The first test level was set well above step 1 to obtain an unambiguous response. The series, therefore, began at an intensity slightly lower than step 2. When the overhead light was turned on the fish was darkly silhouetted, but it rapidly increased its luminescence until it virtually disappeared from view. It continued to match the overhead illumination perfectly through all trials of step 2. In the initial two trials of step 3 the fish matched the overhead illumination perfectly, while on the last two trials its luminosity was slightly greater than the overhead illumination. While the fish never completely turned off its luminescence during dark periods, it decreased its intensity during step 2 to an average of 10% of the step 1 intensity and during step 3 to an average of 150% of the step 1 intensity. When the lights were extinguished during step 3, and the fish was observed through the side of the tank a faint glow could be seen from its lateral surfaces. The intensity of the glow was comparable to our "ghost stage" (see Young and Roper 1976) which generally measures between 2 and 4% of the intensity of step 1. This estimate indicates that the lateral glow was $^1/_{15}$ to $^1/_{30}$ of the intensity of the ventral glow.

The fish came close to matching the overhead illumination at step 4; but after 10 min the fish discharged luminous material from the caruncles, turned head downward, and beat its tail vigorously for 3 min while its head pressed against the bottom; finally it lay motionless on the bottom. Although the intensity of the overhead illumination was decreased, the fish did not resume the head-up position. The fish was preserved while still alive nearly 2 h later after considerable additional handling and observation.

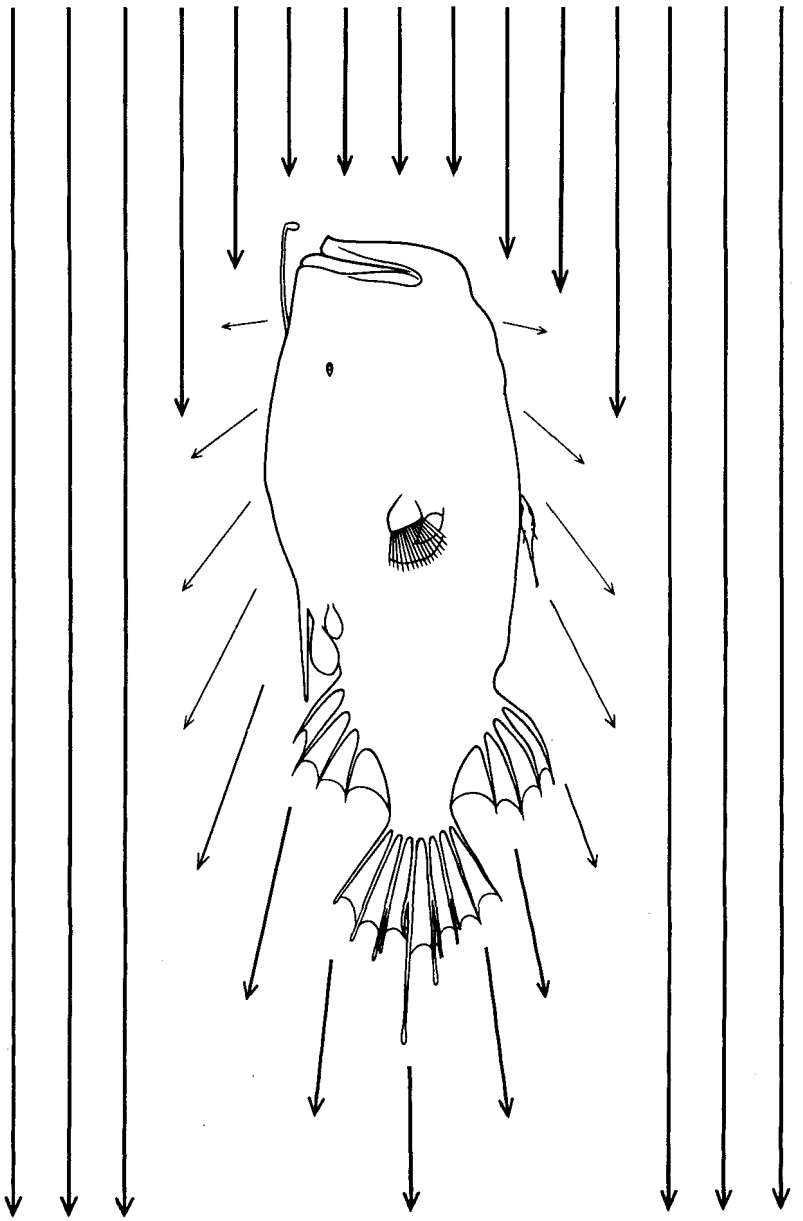

FIGURE 4.—Artist's rendition of luminescent countershading by *Cryptopsaras couesi*. Parallel vertical lines represent downwelling light; lines radiating from fish represent relative luminescence.

Oplophoridae

Oplophorus gracilirostris A. Milne-Edwards, 1881

This species is a half-red shrimp. Photophores that were observed to luminesce occurred on each side of the abdomen, near the joints of the last three thoracic appendages, and on the ventral side of the third maxillipeds. When the thoracic appendages are folded beneath the thorax, the photophores are aligned in two series that are continuous with the two series of photophores along the abdomen (Figure 5). Other photophores are present, but were not observed to luminesce. One specimen, 18 mm carapace length, was examined.

The shrimp matched the overhead illumination perfectly when the thoracic appendages were folded in during trials at steps 1 and 2. The animal, however, was unable to match the overhead illumination at higher steps, even though it clearly increased its luminosity at step 3. At step 4 its luminosity decreased below that at step 3.

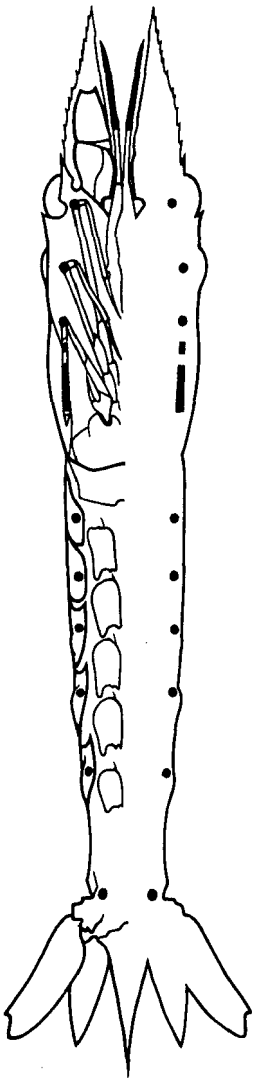

FIGURE 5.—Arrangement of photophores observed to luminesce on ventral surface of *Oplophorus gracilirostris*. (Right half of drawing shows only outline of shrimp and photophores.)

Steps 1 and 2 were repeated and the animal again matched the overhead illumination. The overhead light was then decreased to step 0.5 at which the shrimp also matched perfectly.

Oplophorus gracilirostris turned off its photophores during the dark periods of steps 0.5 through 2. The shrimp reduced its luminescence greatly (average intensity = 5% of the step 1 intensity), but it did not turn off during the first dark periods of steps 3 and 4. During the second dark period in these two steps, the animal's lights were extinguished.

DISCUSSION

Our studies demonstrate that the animals tested turned their photophores on in response to overhead illumination and turned them off or greatly reduced their luminosity when the overhead light was extinguished. The data also show that the animals in experimental tanks can increase and decrease their luminosity to match alterations of the overhead illumination. Further, the data demonstrate that under certain conditions photophores other than those bearing skylight filters can be involved in countershading. Our observations confirm that a luminescing animal disappears from view when it matches the overhead illumination.

The studies also show that bioluminescence can be used for lateral as well as ventral countershading. This was demonstrated by a black anglerfish. A lateral glow was observed from this animal with an intensity estimated to be approximately the value necessary for lateral countershading according to the radiance distribution of daylight in the ocean (Denton et al. 1972).

Black stomiatoids occasionally are captured between 500 and 600 m off Hawaii (T. Clarke 1974), within the realm of the half-red shrimps and the silvery fishes and squids. Black fishes do not reflect light well laterally (Nicol 1958), suggesting that they cannot countershade well at this angle. Even though some of these fishes have a slight lateral bronze iridescence, this iridescence reflects blue light well only at oblique angles (Denton et al. 1972). Such animals seemingly would be conspicuous at 500 to 600 m in the ocean where lateral countershading typically is required. Many of these black fishes also have numerous small photophores along their flanks, indicating that they may utilize bioluminescence for lateral countershading. Perhaps silvery fishes rely totally on reflected light for lateral countershading, while black fishes (at depths where lateral countershading is necessary) rely to varying degrees on bioluminescence. While the latter strategy is energetically more expensive, it has the advantage of simultaneously camouflaging the animal against downwelling light and the flashes or searchlights of nearby animals.

The animals tested matched light intensities indicative of their relative depth range during the day. Midwater animals that occur as near the surface as 450 m during the day (i.e., *Abralia trigonura*, *Abraliopsis* sp., *Pterygioteuthis micro-*

lampas, Pyroteuthis addolux, Heteroteuthis hawaiiensis, and probably *Enoploteuthis* sp.) were able to countershade at step 4 and, in some cases, nearly at step 5. *Oplophorus gracilirostris,* which has an upper distributional limit of 490 m (Zieman 1975), countershaded at step 2 but not at step 3. *Octopoteuthis nielseni,* which has not been captured at less than 650 m off Hawaii during the day, could not quite match the overhead illumination at step 1, but it could countershade effectively at the lower intensities of steps 0.17 and 0.067. The black anglerfish countershades at light intensities at step 3. Although one specimen has been taken at a depth of 635 m, the upper limit of the day habitat of this species is uncertain.

Bioluminescent countershading must have upper and lower depth limits beyond which it no longer is effective. We are unable to determine the depths in the ocean to which the light intensities in our experimental system correspond. We believe, however, that the maximum intensity at which the animals can effectively countershade corresponds approximately to that of the upper limit of their day habitat. For several of the species examined this depth is 375 to 400 m (Young 1977). Our observations indicate the critical role that increased resolution at these depths plays in the effectiveness of countershading. During testing at these high light levels, individual photophores often could be resolved and the outline of the silhouette of the animal became distinct. While resolution of the observer's eyes (and presumably those of a predator) increases with increasing light levels, at these higher intensities resolution becomes so acute that luminescent countershading becomes an extremely difficult task for the animals. This strategy remains effective only at increasingly greater distances from the countershading animal.

The energy required to countershade at less than 400 m must also affect the utility of this strategy. We have made rough calculations of this cost based on one of the squid tested (*Abralia trigonura*) with a silhouette of 4 cm^2 and a wet weight of 2.5 g. At 400 m off Hawaii during the day, this animal must produce a light flux in the range of 0.9×10^{16} quanta/h in order to countershade. In the absence of data on cephalopods,[5] we assume the squid has a luminescent system similar to the firefly in which 60 kcal/mole is required to obtain an excited state of the luciferin molecule (McElroy and Seliger 1961). Since the quantum yield is about unity, this figure corresponds to 60 kcal for the production of 6.022×10^{23} quanta (Avogadro's number). Therefore, 0.0009 cal is necessary to produce the required number of quanta at 400 m. Unfortunately, very little is known of the energy budget of midwater squids. Belman[6], however, reported oxygen consumption of 0.030 μl O_2/mg wet weight per h in the midwater squid *Histioteuthis heteropsis* at 5°C. (Childress (1975) gave rates of 0.006 to 0.011 (average 0.035) μl O_2/mg wet weight per h for a variety of midwater shrimp at temperatures between 4° and 7.5°C.) Using the average energy equivalents of oxygen consumption in carnivorous ammoniotelic animals of 3.24 cal/mg O_2 consumed (Elliott and Davison 1975), the energy required for countershading at 400 m by this squid is 0.3% of the energy consumed by the resting animal during the day. Since downwelling light intensity changes by a factor of approximately 30 per 100 m near Hawaii (k = 0.034), at 350 m the cost of countershading climbs to 1.6%, at 300 m it becomes 9%, and at 200 m it becomes 270%. These figures would increase by a factor of over 3 if we based the energy costs on the luminescent system of the ostracod *Cypridina* rather than that of the firefly (Shimomura and Johnson 1970). The limitations imposed on bioluminescent countershading apparently above 350-400 m by the apparent high visual acuity of predators and by the high energy costs, suggest that few animals are capable of countershading above these depths during the day.

Walters (1977) suggested that the lower limit for ventral countershading off Hawaii is about 775 m, based on the assumption that simple photophores in all-red sergestids are used for countershading. Our observations on countershading in *Octopoteuthis nielseni,* which has simple photophores, supports his assumption. In addition, we were able to detect the silhouette of *O. nielseni* at step 0.067, but we could not detect it with the overhead intensity reduced again in half. If we compensate for the distance of the observer from the specimen and the light loss in the mirror, our

[5]Recent estimates of activation energy and quantum yield by the flashing photophore of an epipelagic squid indicate a very high metabolic cost (Girsch et al. 1976) that presumably is not applicable to countershading luminescence.

[6]Belman, B. W. Respiration and the effects of pressure on the vertically migrating squid *Histioteuthis heteropsis.* Manuscr.

threshold would be about this latter intensity, or approximately 0.06% of the intensity at step 5. If we assume that light intensities at 400 m off Hawaii correspond to light intensities midway between steps 4 and 5, the depth of our visual threshold for detecting large silhouettes would be about 610 m. Denton and Warren (1957) suggested that the eyes of deep-sea fishes are 60 to 120 times more sensitive than the human eye. These figures indicate that a fish should have a visual threshold for detecting silhouettes somewhere between depths of 730 to 750 m.

At the greatest depths where ventral countershading occurs, simple photophores suffice (e.g., *Octopoteuthis nielseni*); however, at higher light levels photophores often possess skylight filters (e.g., *Abralia trigonura, Abraliopsis* sp., *Pyroteuthis addolux, Pterygioteuthis microlampas, Enoploteuthis* sp., *Heteroteuthis hawaiiensis*).Skylight filters on photophores apparently eliminate the "tails" of spectral emission bands that lie outside the spectral range of downwelling light. Presumably these tails develop or are detectable at high luminescent intensities only. Best and Bone (1976) suggested that not all photophores carrying such filters were involved in countershading. However, in view of the role of bioluminescence in lateral countershading demonstrated here, and the fact that some fishes may countershade in the head-up position (as *Cryptopsaras couesi*), one must be cautious in ruling out a countershading function based on photophore distribution. Indeed, we examined one of the species Bone and Best examined, *Photostylus,* and found that the photophores are clearly directed posteriorly, indicating a head-up countershading orientation. Apparently beyond an animal's normal upper limit of countershading, it may turn on nearly every ventrally directed photophore it possesses in an attempt to eliminate its silhouette, even though some of the photophores lack skylight filters (e.g., *Abralia trigonura, Abraliopsis* sp., *Pyroteuthis addolux, Pterygioteuthis microlampas, Enoploteuthis* sp.).

The evidence in support of the theory of bioluminescent countershading in the midwaters of the open ocean is now substantial. The only major evidence lacking is the experimental demonstration of reduced predation on countershading animals and field observations of the phenomenon. The apparent importance of bioluminescent countershading in midwaters cannot be overestimated. Countershading appears to operate from depths of 750-775 m to about 350-400 m during the day off Hawaii. This zone is inhabited during the day by the great majority of fishes (Amesbury 1975), shrimps (Zieman 1975; Walters 1977; Riggs[7]), and cephalopods (Young see footnote 4) that occur in Hawaiian midwaters. The upper limit of the midwater fauna off Hawaii occurs at approximately 400 m (Maynard et al. 1975; Amesbury 1975). Amesbury (1975) in comparing the upper depth limits of midwater faunas in various areas, concluded that these depths were related to light intensity. We suggest that the upper depth limit of the midwater fauna is a result, to a large degree, of the severe limitations placed on bioluminescent countershading at this level. We envision a changeover in the macrofauna at this depth from a deeper component in which opaque animals are able to hide, to an upper component in which opaque animals must rely more on speed, size, weapons, etc., than on hiding to avoid predation.

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[7]Riggs, F. Vertical distribution of the pelagic shrimps, *Gennadas* and *Bentheogennema* off Oahu, Hawaii. Manuscr.

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