

Fig. 2. Network of bowed-out screw dislocations in an olivine-rich xenolith from Salt Lake Crater, Hawaii. Scale bar, 50 µm.

1) The great depth of decoration provides an excellent impression of the threedimensional aspects of the dislocation structure. With the depth of field of the petrographic microscope reduced to a few micrometers, small changes in the focusing level allow a vertical tour through the thin section. A similar effect is obtained by TEM or x-ray topography only through stereophotography and serial sectioning.

2) The large lateral extent of a decorated thin section permits a study of the variation of the dislocation structure within a grain and from grain to grain in a polycrystalline aggregate. Dislocation structures resulting from partial recrystallization and from small thermal strains imposed near grain corners and the distribution and statistics of small-angle tilt boundaries are among the topics that can be studied in a practical way only by the decoration technique.

3) Since the depth of decoration in a decorated thin section is at least one order of magnitude greater than the thickness of a TEM specimen, at least ten times as many dislocations are visible in the former than in the latter for a particular dislocation density and viewing area. This effect, coupled with the fact that optical magifications are considerably lower than conventional TEM magnifications, can lead to spectacular results. Where a TEM view at a magnification of 10,000 might show a few dislocations in a frame, a decorated section from the sample viewed at a magnification of 200 would show several hundred dislocations, revealing the extended structures that result from long-range dislocation interactions. Such structures in

most mantle-derived olivine-bearing xenoliths, whose dislocation densities are less than 10⁸ cm⁻², would likely be overlooked in a TEM examination.

Three additional points deserve comment. (i) As the decoration reaction involves the oxidation of the iron-rich component of the olivine, the technique will not work in pure forsterite, quartz, or calcite. (ii) In decorated samples the Burgers vector of a dislocation can be determined with certainty only if it can be traced into a small-angle tilt boundary, where the Burgers vector is normal to the plane of the boundary, or into a twist boundary, where Burgers vectors are parallel to dislocation line directions. (iii) Lastly, economic factors favor optical microscopy. Preparing decorated thin sections involves little more effort than preparing a standard petrographic thin section, or about one-tenth the effort of preparing a good TEM specimen. There is a similar saving in both cost and time in viewing the specimen.

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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 dimlight 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 12 MARCH 1976 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.

Ani- mal	Overhead lights											
	On	Off	On	Off	On	Off	On	Off	On	Off	On	Off
1 2 3 4 5 6	+ + + 0 +	0 0 0 0 0 0	+++++++++++++++++++++++++++++++++++++++	0 0 0 0 0 0	+ + 0* + + +	0 0 0 0 0 0	+++++++++++++++++++++++++++++++++++++++	0 0 0 G 0	+ + + +	0 0 0 G 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



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

time, 1 minute; N = 24). The ghost stage was not extinguished in two trials.

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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- (1974). 6. Bioluminescent countershading never has been ob served from submersibles in spite of numerous ob-servations 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 Biological Sound Scattering in the Ocean, G. 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 bio-luminescent 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 ani-mals. With faint overhead illumination in the labo-ratory, squid could be confirmed to luminesce only . B ratory, souid could be confirmed to luminesce only when seen against the dimmer portions of the illu-mination. 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 lumi-nescence. For the present, at least, bioluminescent countershading is best studied in the laboratory. J. W. Hastings, *Science* **173**, 1016 (1971).
- The ventral surfaces of the mantle, funnel, head, 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 in-dicative 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 fure flattened photophores Arnold.) In addition, five flattened photophores occur on each eyeball, and a series of three large, spherical photophores occurs on the tip of each

- 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 of series of series of the ser light off ranged from 5 to 25 minutes (mean, 10.1 minutes; 76 percent of trials were between 9 and 11 minutes). Speciments were placed in the observa-tion tank with the overhead light off at least $\frac{1}{2}$ hour before testing; the observer dark adapted dur-ing this period. In all six cases, the squid at this stage did not luminesce.
- 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 dis-turbed 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. We thank the officers and crew of the R.V. Kana 14. *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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Heavy Methanes as Atmospheric Tracers

Abstract. Methane-21 (${}^{13}CD_4$) is potentially a useful nonradioactive tracer for testing atmospheric transport and diffusion models on a continental scale. In an experiment to demonstrate this long-range utility, the release of 84 grams of methane-21 was detected at distances of 1500 to about 2500 kilometers at concentrations of about 1 part in 2×10^{16} parts (by volume) in the air by a technique in which methane was separated and the methane-21 content was measured with a mass spectrometer.

There is a growing need to understand the details of atmospheric circulation as well as the details of the dispersion of pollutants on regional, continental, and global scales. Although much theoretical work has been done on these problems, there have been almost no successful controlled experiments on these scales partly because of the lack of suitable tracer materials. Tracers that have been used successfully at shorter distances are generally impractical for long-range applications because of cost, relatively high background concentrations, interference from industrial sources, or insufficient detection sensitivity. An ideal tracer would be nontoxic and nondepositing, would have virtually no existing background concentration in the atmosphere, would be detectable at extremely low concentrations, and would be economical in terms of the costs of tracer and sample analysis.

With the availability of relatively cheap separated isotopes of carbon (1), methane-21 ($^{13}CD_4$) would appear to meet many of these requirements for an ideal meteorological tracer. Natural methane, mostly methane-16 (${}^{12}CH_4$), is present in the troposphere (northern mid-latitudes) at a concentration of about 1.4 parts per million (ppm) (2), although significant variations in the abundance occur between urban and rural areas (3). The production rate of nat-



Fig. 1. Post-facto meteorological trajectories, 300 to 2000 m above the ground, starting time 14 May 1974, 1500 and 1800 M.D.T., designated in the figure as 14/15 and 14/18, respectively [see (7)], and methane sampling stations. The fractional symbols give the times of arrival of a trajectory at a location; for example, 15/06 means arrival on 15 May, 0600 local time.

ural methane is estimated to be (2) about $(0.5 \text{ to } 1.0) \times 10^{15} \text{ g per year, and is bal-}$ anced by destruction (2) in the troposphere and stratosphere, leading to an estimated mean residence time for methane in the troposphere of 4 to 7 years. The exchange of carbon or hydrogen atoms between the different isotopic forms of methane should be much slower than the apparent destruction rate (4). The statistical equilibrium abundance ratio of natural methane-21 to natural methane-16 has been calculated to be 7×10^{-18} (5). This figure implies that, before the large-scale production of deuterium starting in the 1940's, the world's atmosphere contained only about 50 mg of methane-21. Nuclear energy activities have presumably perturbed this number significantly.

The potentialities of methane-21 as a meteorological tracer derive from this low abundance of methane in the atmosphere and from the high sensitivity of an ultrahigh-vacuum, high-resolution mass spectrometer (6) fitted with an electrostatic retardation lens. In this instrument, the background in the methane-21 mass region is about 10⁻¹¹ of the signal in the methane-16 mass region.

In order to demonstrate the feasibility of large-scale meteorological tracing based upon the use of methane-21, 84 g were released on 14 May 1974, from 1520 to 1750 M.D.T. from a 70-m stack at the Allied Chemical Corporation processing plant at Idaho Falls, Idaho. The initial dilution with air in the stack produced a starting ratio of methane-21 to methane-16 of ≈ 0.1 .

Cryogenic air samplers, operating at 13 National Weather Service stations at locations extending from Minnesota to Oklahoma (see Fig. 1), were used to concentrate the methane and krypton in the ambient air. Samples were collected twice daily at each station, from 9 p.m. to 7 a.m. (P) and 9 a.m. to 7 p.m. (A). On the basis of meteorological predictions, about 25 samples from four locations were chosen as likely to contain fractions of the released tracer. Eight additional samples were chosen for background measurements, with the sampling done either before the release or at a place (Tulsa, Oklahoma) far from the predicted trajectories.

Methane and krypton were separated from the air samples and purified by SCIENCE, VOL. 191