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Bioluminescence in the Monterey Submarine Canyon: image analysis of video recordings from a midwater submersible

E. A. Widder [1], S. A. Bernstein [1], D. F. Bracher [1], J. F. Case [1], K. R. Reisenbichler [1], J. J. Torres [2] and B. H. Robison [1]*

[1] University of California, Marine Science Institute, Santa Barbara, California 93106, USA
[2] University of South Florida, Department of Marine Science, St. Petersburg, Florida 33701, USA

Abstract

Video images of bioluminescence were recorded in situ during a 1985 study of the midwater environment of the Monterey Canyon, using a single-person, untethered submersible. Gelatinous organisms were responsible for the most brilliant bioluminescent displays, often exhibiting elaborate kinetics in response to mechanical stimulation. Images of bioluminescent displays recorded from identified organisms are shown and display patterns are described. All bioluminescence emission spectra from captured specimens were blue, with peak emissions between 460 and 494 nm. Image-analysis of recordings of mechanically stimulated bioluminescence revealed source densities between 43 and 175 m^{-3} and intensities between 2.5 and 37.3 $\mu W\ sr^{-1}\ m^{-3}$. The predominant display type at all depths studied (between 100 and 560 m) was luminous secretions. Despite high intensities of mechanically stimulated bioluminescence, no spontaneous light production was recorded in the absence of mechanical stimulation.

Introduction

Bioluminescence occurs throughout the world's oceans (Tett and Kelly 1973). The number and variety of bioluminescent organisms (Herring 1987) as well as the presence of well developed eyes in so many organisms inhabiting depths where sunlight never penetrates (Locket 1977, Hiller-Adams and Case 1988), are evidence of the importance of bioluminescence in the visual ecology of earth's largest habitat, the ocean below the photic zone. The nature of this visual environment remains obscure. What is known of deep oceanic bioluminescence is based on in situ radiometer measurements, on specimens from net sampling, and on a few accounts of observations from submersibles. The present report substantially extends this body of knowledge by providing the first images of in situ midwater bioluminescence as well as quantitative data on spontaneous light production compared with stimulated bioluminescence. In this context, spontaneous light production refers to the natural background bioluminescence which is unstimulated by the observer (Tett and Kelly 1973).

Radiometer measurements have generally been designed to measure stimulated light (for examples: Clarke and Kelly 1965, Losee and Lapota 1981, Swift et al. 1985), and rarely, spontaneous bioluminescence (Boden et al. 1965). Although pump-through systems can provide information about the amount of stimulated light per sampled volume, such systems typically have problems related to undefined flow fields (Case et al. 1987), identification of bioluminescent sources, and destruction of fragile gelatinous organisms. Nonetheless, measurements of stimulated bioluminescence are more reliable than any that have been made for spontaneous bioluminescence. Data on the extent of spontaneous emissions in the midwater environment are critical to assessing the ecological role of bioluminescence. Whether or not a luminescent display is a comparatively rare event or if it occurs against a constant background of bioluminescent activity has been a subject of considerable interest and speculation (Rudyakov 1968, Tett and Kelly 1973, Young 1983). However, attempts at measuring such undisturbed bioluminescence have been compromised by inadvertent mechanical stimulation of organisms by the detector systems. Any motion by a detector lowered from a ship, or of water currents around a moored detector will stimulate bioluminescence mechanically. Efforts to circumvent these difficulties with two coincident counting radiometers viewing a volume, common to both detectors, 1 m away (Boden et al. 1965, Boden 1969) still suffer from the fact that the measurements are not made in an undisturbed environment. Bioluminescence, mechanically stimulated by motion of the apparatus through the water, may elicit responding flashes from organisms in the undisturbed volume; or an organism contacting the appara-

* Present address: Monterey Bay Aquarium Research Institute, 160 Central Avenue, Pacific Grove, California 93950, USA

tus may enter the coincident, "undisturbed" volume while still luminescing. Here we demonstrate that use of a small, neutrally buoyant, untethered submersible eliminates these difficulties and allows for direct comparison of unstimulated and stimulated bioluminescence in the same body of water, while at the same time providing otherwise unobtainable and invaluable data on spatial distributions and species contributions to the signal.

Net sampling has provided bioluminescent organisms for laboratory investigations. While trawling is generally destructive of gelatinous sources, some information on bioluminescence of gelatinous organisms has come from specimens collected at the surface in regions of upwelling. The recordings described here relate such laboratory studies to the behavior of organisms in situ as well as providing new information about organisms whose bioluminescence has not been described previously.

Observations of bioluminecence from submersibles have been largely anecdotal, with the most consistent observation being the inability to identify the sources of the bioluminescence (Boden and Kampa 1964, Tett and Kelly 1973, Baguet et al. 1983, Young 1983). Such observations are also consistent with the results of efforts directed at photographing the sources of bioluminescence in situ using a strobe triggered by luminescent flashes (Clarke and Breslau 1959, Breslau et al. 1967). The majority of photographs revealed no identifiable source which might have triggered the strobe.

The images of bioluminescence described here were made with an intensified video camera from a small, midwater submersible, as part of a study of the mesopelagic environment of the Monterey Submarine Canyon. Originating in Monterey Bay on the central California coast, the canyon is comparable in size to the Grand Canyon of the Colorado River (Shepard 1973) and, as a rich, deep-water habitat located in a sheltered, near-shore region, it provided an ideal site for in situ midwater research.

Materials and methods

During August and September 1985, three of us (B.H.R., J.J.T. and E.A.W.) alternately piloted the single-person submersible "Deep Rover" in studies of the mesopelagic environment of the Monterey Submarine Canyon. The submersible, which previously had been used only for commercial diving, was designed by Deep Ocean Engineering (San Leandro, California, USA) and leased from Can-Dive Services (North Vancouver, British Columbia, Canada). It is a 1.5 m diam acrylic sphere attached to a battery sled that supports horizontal and vertical thrusters and electrically controlled manipulators. The pilot, seated in the center of the sphere, has maximum visibility and easy access to submersible controls located in the seat base and arm rests. The submersible is untethered, operates to a depth of 1 000 m, and is small enough to be launched from most research vessels. R.V. "Wecoma" was the vessel used once the submersible was trimmed to neutral buoyancy, there was no motion relative to the encompassing water mass, and mechanical stimulation of bioluminescence occurred only with use of thrusters or a change of ballast. All bioluminescence recordings were made by one of us (E.A.W.) using an intensified silicon-intensified target (ISIT) video camera (Dage 66) mounted inside the sphere during 12 of our 51 dives. Recordings were made on a portable VHS videocassette recorder (Hitachi Model VT-TU7A). The camera was focused on a 5 mm mesh screen stretched across a 1 m transect hoop mounted 1.9 m away from the camera lens in front of the submersible (see Fig. 5A). All lights on the submersible were extinguished during these recordings and the ISIT camera was set to maximum sensitivity with the automatic gain control disabled.

Bioluminescent displays were recorded from organisms large enough to be identified by eye by first maneuvering the submersible until the organism was between the screen and the camera lens. The lights were then extinguished and the submersible was reversed until the organism struck the screen directly in front of the camera, producing a mechanically stimulated bioluminescent display. These recordings were made with the camera lens (Panasonic 28–210 mm zoom) set at a focal length of 210 mm for maximum magnification. Whenever possible, the organism was then captured using a low-speed suction sampler and brought to the surface for further study, identification and preservation. Surface studies included ISIT video (Nikon 55 mm macro-lens) recordings of mechanically or KCl-stimulated bioluminescence in chilled sea-water aquaria. Still photographs were taken with a strobe and side or back illumination with the same macro-lens on a 35 mm Nikkormat camera back. Bioluminescence emission spectra were measured with an intensified optical multichannel analyzer (OMA) system, and computer-corrected and smoothed as previously described (Widder et al. 1983).

In order to assess stimulated bioluminescence as well as the undisturbed background, video-recordings were made of horizontal transects (see Fig. 5) during which organisms were mechanically stimulated to luminesce as they struck the screen mounted in front of the submersible. Prior to each transect, with the submersible motionless in the water, a period of undisturbed background was recorded (see Fig. 5B).

Each transect was begun by activating the single-speed horizontal thrusters. At a forward speed of 1 knot, each 2 min transect represented a horizontal distance traveled of 62 m. Data provided by Can-Dive indicated that forward speed varies less than 10%, except when the batteries are over-charged or nearly depleted. On one occasion, forward speed was checked at the very beginning and end of a particularly long dive by video-recording the rate of passage of particles in the water along an exterior mounted scale. Image analysis revealed forward speeds of 1.33 and 0.75 knots, respectively. For our estimates of stimulated bioluminescence and source concentrations a forward speed of 1 knot was assumed.

ISIT video-recordings were analyzed with a Megavision 1024XM image-analysis system. A Quasar video-cassette

recorder was interfaced to the Megavision system for automatic frame-advancing, digitizing and analysis. Bioluminescence intensity levels during each transect were quantified by calculating the gray level in a designated region of interest. The region selected formed a 2000 cm^2 rectangle in the upper hemicircle of the 1 m transect hoop (see Fig. 5A). The camera was calibrated at 450 and 500 nm by exposure to point-sources over three orders of magnitude of known intensity. The radiometric calibration source was an NBS-referenced, Optronic Laboratories Model 310 multi-filter calibration source. Brighter sources produced increased bloom, which extended the dynamic range of the intensity measurements beyond the 256 gray values permitted by the 8 bit analog to digital converter. Gray values were converted to average radiance in W cm^{-2} sr^{-1}. A 50-frame average of the undisturbed background intensity recorded prior to each transect was subtracted from the stimulated bioluminescence values. All absolute values are minimum estimates, since point-sources emitting less than 1×10^{-11} W sr^{-1} were not detected and no correction was made for losses due to reflections or absorption over the 1.9 m between the camera lens and the screen. The average intensity for each transect is presented as μW sr^{-1} m^{-3} plotted against depth (see Fig. 6). Estimates of the average number of luminescent sources per cubic meter were made by counting the luminescent strikes against a 500 cm^2 region of the screen surface in the upper hemicircle of the transect hoop during slow-motion playback of each transect. Camera resolution varied between 100 and 600 lines, depending on field illumination. Video-recordings made with far red illumination and maximum zoom (210 mm), prior to recording individual bioluminescent displays, just resolved the 5 mm mesh of the transect screen. At the lower zoom factor used during horizontal transects, the mesh was not resolvable (see Fig. 5A). Hydrographic data was collected at each dive site using a Neil Brown standard CTD.

During the dive schedule, a second vessel, the R.V. "Cayuse", made trawls at discrete depths in the area of the dive sites using an opening/closing, modified rectangular midwater trawl (RMT-8: Roe et al. 1980). Net mesh-size was identical to that used on the transect screen. Towing speeds were between 1.5 and 2 knots, duration was approximately 30 min, and tows were conducted around the clock for a period of 8 d. Animal concentrations from these net hauls were correlated with visual observations of animal abundance seen from the submersible as well as to regions of high stimulated bioluminescence.

Results

Identified displays

Of the 30 different species of identified organisms from which mechanically stimulated bioluminescent displays were recorded, 29 were gelatinous. Many of the displays from these gelatinous sources were noteworthy because of their exceptional brilliance and often elaborate kinetics. Examples of the spatial patterns of bioluminescence recorded from four specimens are shown in Figs. 1, 2 and 4 along with the 35 mm photographs and emission spectra which were taken following capture. Fig. 3 illustrates bioluminescent displays from three specimens which were too fragile to survive capture for surface studies and were noteworthy because of the kinetics of their displays.

Hydrozoa

In all but one of the hydrozoan jellyfish from which luminescent displays were recorded in situ, the luminescence originated from the margin of the bell. The exception was *Aegina citrea*, which produced a series of flashes from one of its four tentacles. These flashes, which became progressively shorter in duration, initially were confined to the base of the tentacle, until the final flash, which propagated distally at approximately 20 cm s^{-1}.

In *Halistaura cellularia* (Fig. 1A), *Tima saghalinensis* and *Solmissus marshalli*, the luminescence was seen as a continuous ring of light around the margin of the bell (Fig. 1B). This pattern of luminescence has been described for captured specimens of *H. cellularia* by Davenport and Nicol (1955). They concluded that the luminescence was entirely intracellular and that discharges of luminescent material into the sea water with rough handling was not a normal physiological process. However, video-records of a specimen of *H. cellularia* whose tentacles, but not the bell, brushed the screen, revealed streaks of glowing luminescence left along the screen which continued to glow for more than 3 s. No evidence of luminescent discharge was ever observed in captured specimens, suggesting that this form of luminescence is exhausted during capture, while the intracellular luminescence of the bell margin is not.

In *Cunina globosus* (Fig. 1D) and *Colobonema sericeum*, the bioluminescence was confined to point-sources around the bell margin (Fig. 1E). This display pattern has also been described in *Aequorea forskalia* and *Phialidium gregarium*, two leptomedusae in which the luminescent tissue resides either beside or in the tentacular bulbs (Davenport and Nicol 1955).

The bioluminescent emissions of all these medusae were blue, with emission maxima between 460 and 494 nm (Fig. 1C, F). The distinctive short-wavelength shoulder in the emission spectrum of *Halistaura cellularia* is not common in midwater bioluminescence spectra, and was apparently not due to masking since this species is transparent. Very similar spectra have been recorded from the amphipod *Parapronoe crustulum* and the penaeid shrimp *Sergestes similis* (Herring 1983, Widder et al. 1983).

Siphonophores were responsible for the most brilliant bioluminescent displays recorded. An outstanding example was the calycophore *Praya dubia* (Fig. 2A). The in situ recording shown in Fig. 2B of a chain that was more than 6 m long, was made during a dive when the transect screen was not in place. The submersible brushed against one end of the colony, and when lights were extinguished the whole

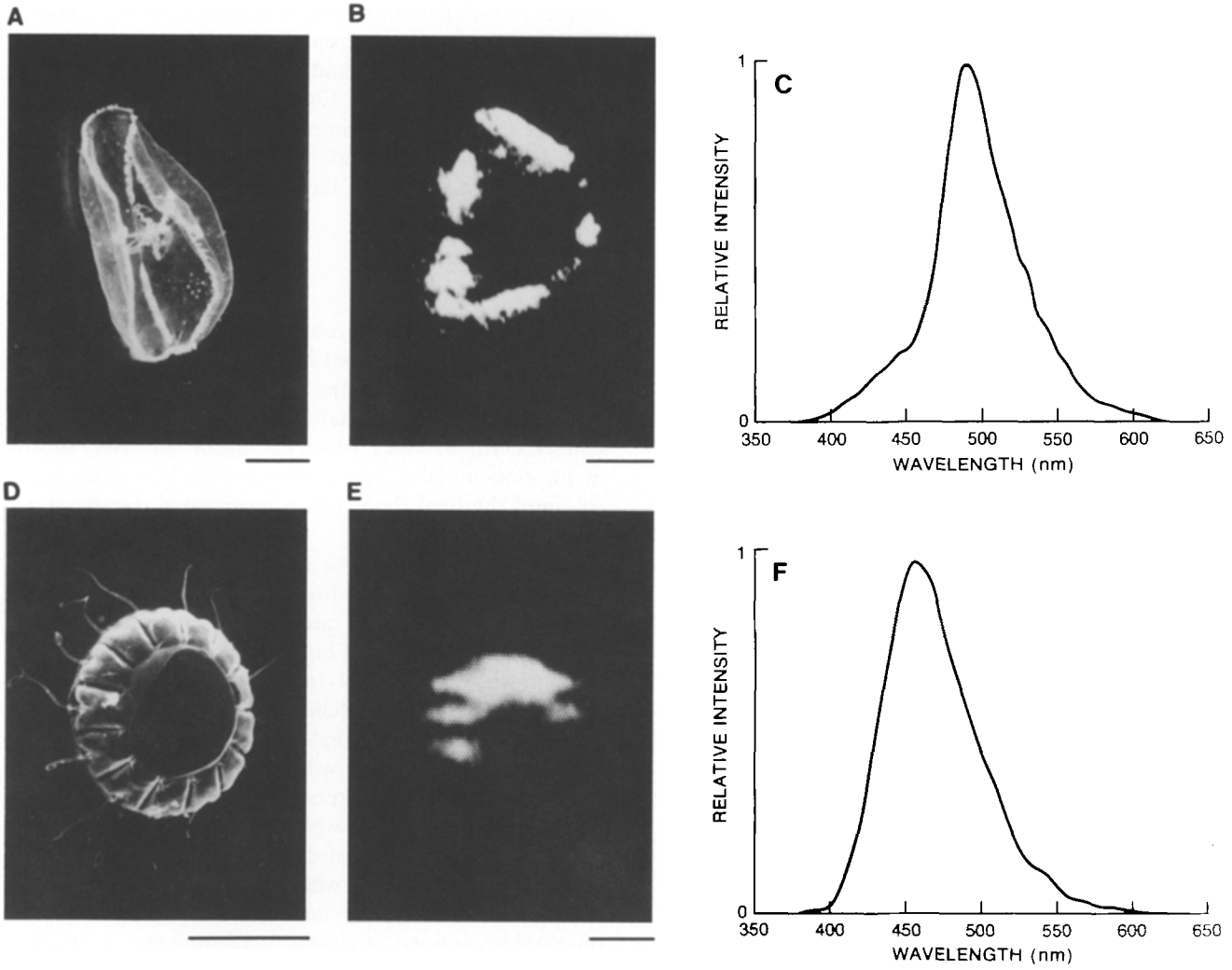

Fig. 1. Hydrozoan jellyfish *Halistaura cellularia* (A, B, C) and *Cunina globosus* (D, E, F). (A) *H. cellularia* photographed in laboratory; the acentric attitude of the bell margin was a common attribute of specimens observed in situ. (B) Mechanically stimulated bioluminescent display of specimen in (A) taken with ISIT video in situ prior to capture; video image was digitized, enhanced and sharpened with image-processing routines and photographed from the video monitor; note that bioluminescence was essentially continuous around bell margin. (C) Bioluminescent emission spectrum of *H. cellularia* plotted as relative intensity vs wavelength; wavelength of maximum emission was 494 nm with a full-width at half maximum (FWHM) of 53 nm; signal-to-noise ratio (S:N) of recording was 91. (D) *C. globosus* photographed in laboratory. (E) Mechanically stimulated bioluminescent display of specimen in (D) taken with ISIT video in situ prior to capture; video image was processed as in (B); note that bioluminescence originated from discrete sources around bell margin. (F) Bioluminescent emission spectrum of *C. globosus* plotted as relative intensity vs wavelength; wavelength of maximum emission was 460 nm, FWHM = 69 mm, S:N = 34. All scale bars = 1 cm

chain was seen to be glowing. This glow, which persisted for more than 45 s after mechanical excitation had ceased, originated from the bracts of the siphosome. No nectophores were apparent. The intensity of the bioluminescence was such that even though the chain was at least 3 m from the camera, the lens aperture had to be reduced two stops from the typical setting of f/1.8 to f/4 to avoid overloading the tube. ISIT recordings made of captured specimens of *P. dubia* stimulated mechanically and with KCl identified the origin of the bioluminescent emission as two sharply defined lines corresponding to the two edges of the transverse groove which runs along the dorsal margin of the bract (Fig. 2C, D). The bioluminescent emission spectrum (Fig. 2E) peaked at 476 nm.

The predominant siphonophore throughout the water column from the surface down to 550 m was the physonect *Nanomia bijuga*. The spatial and temporal pattern of mechanically stimulated bioluminescence which was recorded in situ 32 times, both as controlled strikes and during transects, was sufficiently unique to make it readily identifiable. Bioluminescence originated from both the nectosome and the siphosome (Fig. 3A). Nectosome emission appeared as a steady glow following stimulation, lasting approximately 4 s. Siphosome emission began as a steady glow of about 2 s duration, followed by a brief period of scintillation in which individual bracts produced 200 to 300 ms flashes. Bracts, which readily separated from the siphosome, continued to luminesce. Freeman (1987) identified the sources of emission

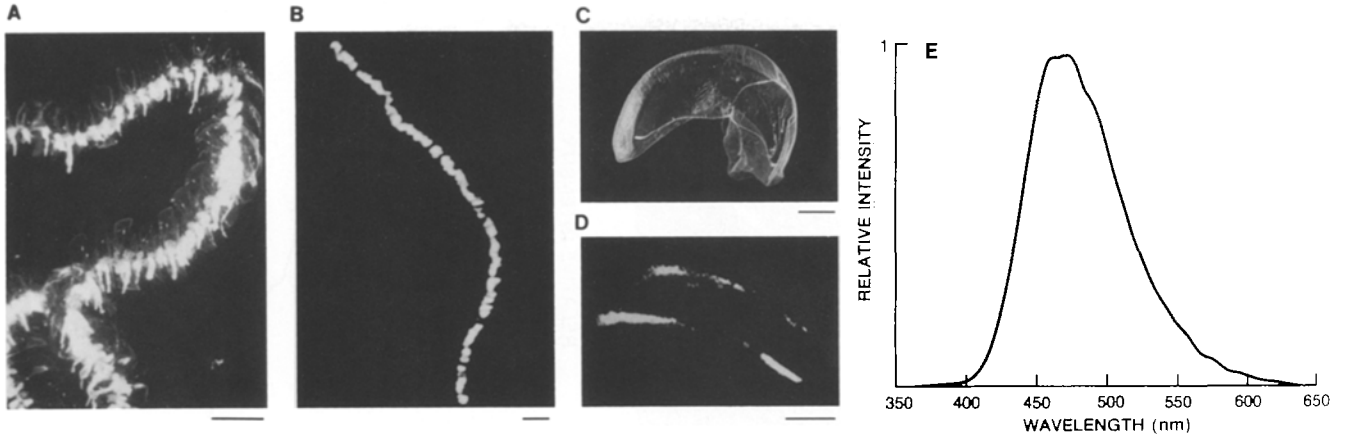

Fig. 2. Calycophore siphonophore *Praya dubia*. (A) Specimen photographed in laboratory; note transparent bracts which provide flotation for the colony; scale bar 2 cm. (B) Bioluminescent emission display recorded in situ with ISIT video; unlike other in situ recordings, mechanical stimulation was not result of direct contact of whole colony with the transect screen, but apparently occurred when submersible brushed against one end of the colony, causing the entire chain to bioluminesce; light emission persisted for more than 45 s after mechanical stimulation had ceased; scale bar = ~4 cm. (C) Photograph of preserved bract; only one edge of dorsal transverse groove is visible; scale bar = 0.5 cm. (D) Recording of KCl-stimulated bioluminescence in isolated bract, localizing emission to two sharply defined lines which correspond to edges of dorsal transverse groove; bract in this recording is rotated approximately 45° toward the viewer, relative to photograph in (C), and both edges of the dorsal groove are visible; scale bar = 0.5 cm. (E) Bioluminescence emission spectrum from KCl-stimulated bracts plotted as relative intensity against wavelength; emission maximum was 476 nm, FWHM = 80 nm, S:N = 87

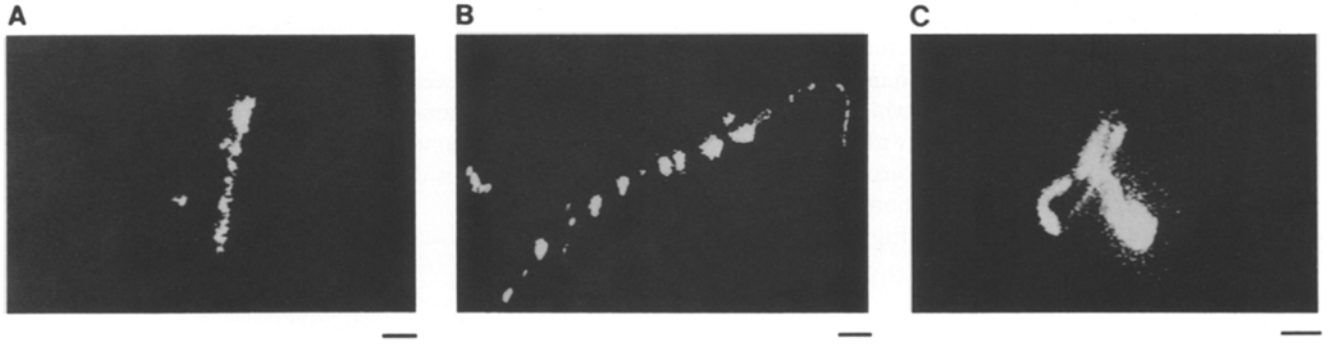

Fig. 3. Physonect siphonophores *Nanomia bijuga* (A) and *Apolemia* sp. (B) and lobate ctenophore *Bolinopsis infundibulum* (C). Video images of gelatinous sources too fragile to survive capture for surface studies. Recordings were digitized and frame-averaged in order to reveal spatial rather than temporal features of displays, and then enhanced and sharpened prior to photographing video monitor. (A) In situ video-recording of bioluminescent display pattern of *N. bijuga*; nectosome, at the top of image, glowed for approximately 4 s following contact; bracts, which were the sources of luminescence in the siphosome, seen here as linear chain below nectosome, glowed for approximately 2 s and then began to scintillate; a single bract which has separated from siphosome is seen on left. (B) *Apolemia* sp. produced different displays from different parts of the colony, with brightly glowing sources originating from siphosome (left) and rapidly propagating bands of light along nectosome stem (upper right); a single bract separated from siphosome is seen on left. (C) *B. infundibulum* produced bands of luminescence which propagated along the comb rows; frame-averaging reveals full spatial extent of display along the comb rows of both lobes (bottom). All scale bars = 2 cm

as paired bilaterally symmetrical organs located on both nectophores and bracts in a recent investigation of *N. cara*.

The siphonophore *Apolemia* sp., another long-stemmed physonect, demonstrated a more dramatic difference in kinetics between the bioluminescent responses of the siphosome and nectosome (Fig. 3 B). The bioluminescence of the nectosome orginated from the stem rather than the nectophores, and propagated as bands of light at 50 cm s^{-1}. Initially, a flash propagated from the distal tip to the middle of the nectosome stem, followed by a series of flashes from the middle to the distal and proximal ends of the stem simultaneously, and ended with single flashes that alternately propagated from distal to middle and middle to distal portions of the stem. Propagated flashes in the nectosome stem lasted for approximately 2 s. Bioluminescence from the siphosome lasted more than 3.5 s and, as with *Nanomia bijuga*, began as individual glowing sources along the length of the siphosome which, after 2 s began to scintillate, producing asynchronous flashes of about 600 ms each.

Scyphozoa

Bioluminescence of the coronate *Atolla parva* (Fig. 4 A) was distinctly different in situ than in the laboratory. Mechanical stimulation in situ caused the release of a bioluminescent

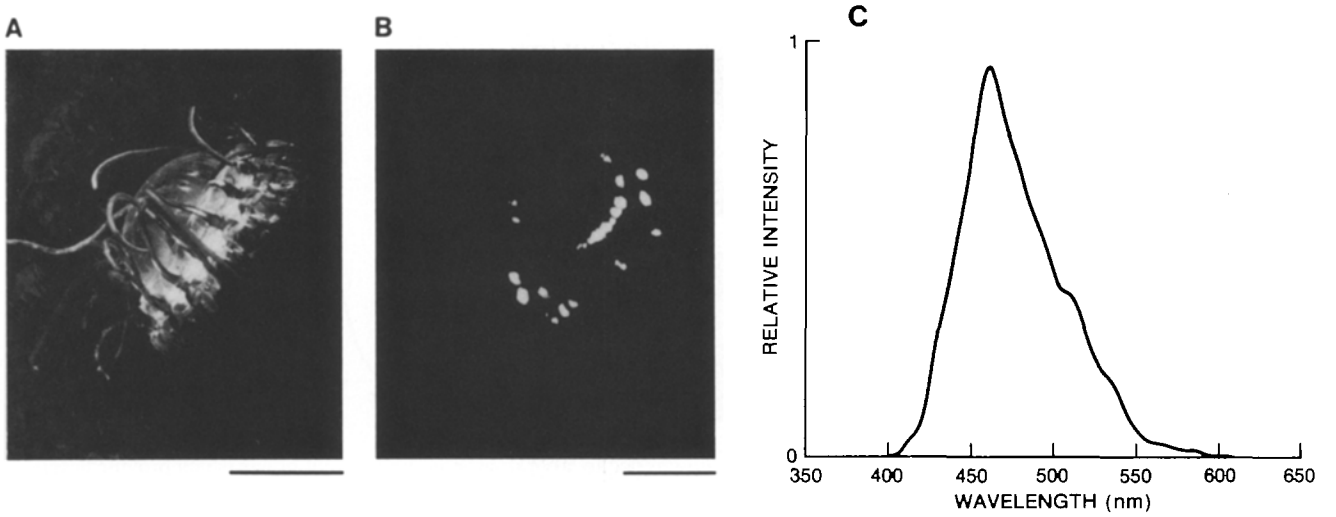

Fig. 4. Scyphozoan jellyfish *Atolla parva*. (A) Specimen photographed in laboratory; scale bar = 1 cm. (B) ISIT video recording of KCl-stimulated bioluminescence in captured specimen, showing ring of point-sources around bell margin and second ring near coronal furrow; this display was very different from that recorded in situ, which consisted of a contact-induced luminescent secretion; scale bars = 1 cm. (C) Bioluminescence emission spectrum of KCl-stimulated specimen; relative intensity is plotted against wavelength; wavelength of maximum emission was 465 nm, FWHM = 61 nm, S:N = 13

secretion which made it appear that the jellyfish had disintegrated on contact with the transect screen. However, with lights on it was found to be intact. No further bioluminescence was observed with additional mechanical stimulation in situ. In the laboratory, this specimen, which was captured at 440 m, responded to KCl stimulation with 300 ms flashes originating from a ring of point-sources around the margin of the bell as well as a second ring of more closely spaced point sources near the coronal furrow (Fig. 4B).

Ctenophora

Ctenophore bioluminescent displays were all multiple flashes which propagated along the comb rows. Displays were recorded from *Beroe abysicola, Beroe* sp., and *Bolinopsis infundibulum*. The latter, an extremely fragile, lobate ctenophore, which broke apart on contact with the net, produced the most outstanding displays, with flashes propagating around the lobes (Fig. 3C) at approximately 50 cm s^{-1}.

Other identified displays

Larvacean houses, which were seen at depths greater than 150 m, were not intrinsically bioluminescent. However, what appeared to be older, unoccupied houses, which had a tattered appearance and a considerable amount of particulate matter adhering to them, often produced bioluminescent flashes from small, disparate sources scattered over the web. These sources, which flashed in response to mechanical stimulation, were presumably from organisms either stuck to or residing on the web.

Transects in regions where the only visible organisms were euphausiids exhibited a large number of displays from small sources that did not pass through the net but were seen to swim rapidly while glowing for 2 to 3 s. These sources generally exhibited lower emission intensities than most of the other displays seen during transects, and were most easily recognized by rapid changes in swimming direction. The resolution of the camera, even at full magnification, was not sufficient to permit direct correlation between the display and the source.

Unidentified displays

The majority of bioluminescent displays recorded during transects could not be correlated with identifiable sources. Although these displays were highly variable in form, several distinctive classes were recognizable.

The predominant displays at all depths were luminous secretions, which passed through the screen as structureless clouds or discrete particles. Within this class, the considerable variability in intensities and kinetics of the emissions suggested that a variety of different organisms were responsible for these displays. It was the combination of relatively high emission intensities and large numbers which made these sources primary contributors to the stimulated bioluminescence.

Small swimming sources correlated with high concentrations of euphausiids were often difficult to distinguish from secretion particles unless they were seen to change swimming direction. The intensity of emission from these sources was low compared to other displays, so that this category represented a minor contribution to the stimulated bioluminescence even when present in high concentrations.

Another distinctive display type that did not contribute significantly to the stimulated bioluminescence originated from small discrete sources which appeared to pass through the net and produced very fast flashes of approximately

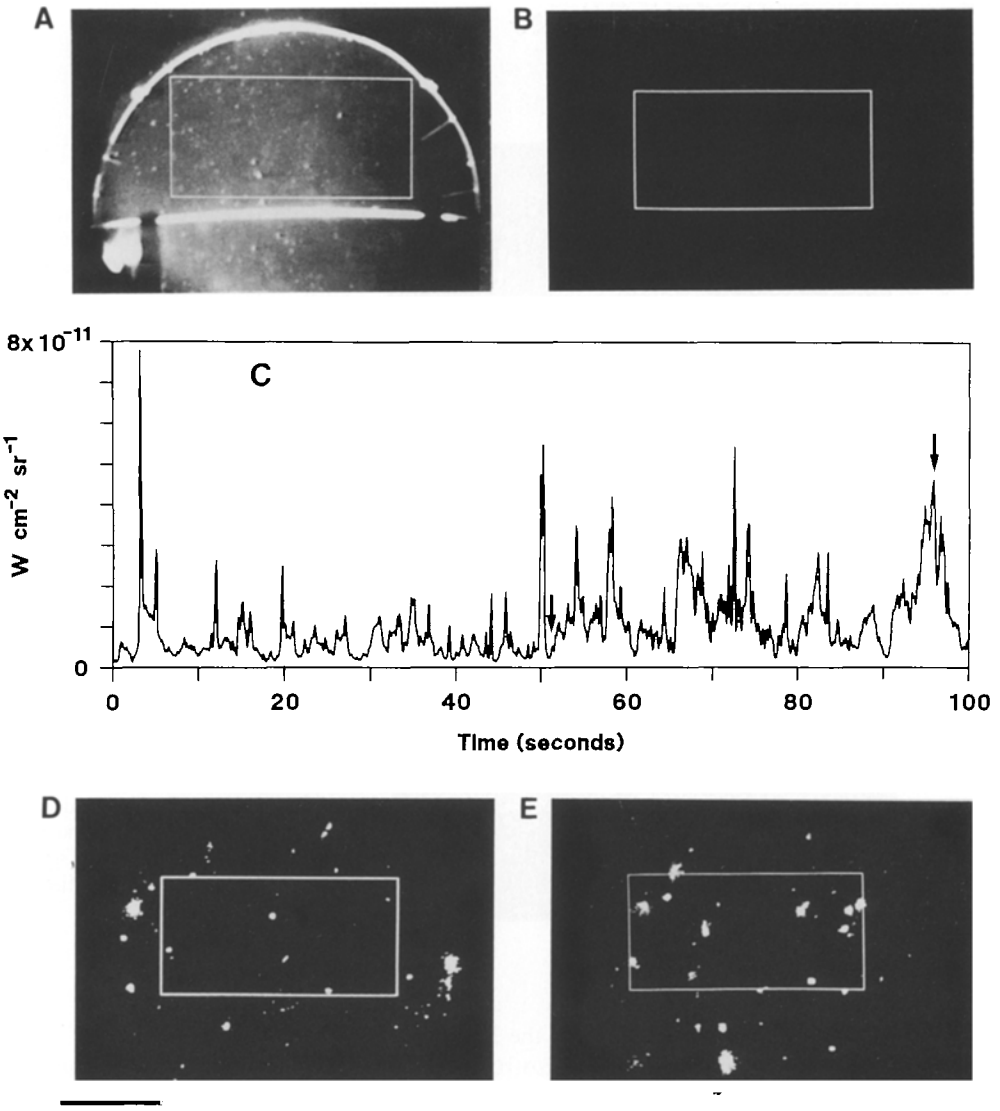

Fig. 5. (A) 1 m transect hoop with 5 mm mesh screen, mounted 1.9 m in front of ISIT video-camera lens; horizontal bar, for structural support, is 1 m long; computer-generated rectangle in upper hemicircle of hoop designates 2000 cm^2 region from which gray values in each video-frame were calculated. (B) Fifty-frame average of undisturbed background recorded at 430 m prior to horizontal transect. (C) Screen radiance plotted against time for horizontal transect at 430 m; the 100 s shown is equivalent to transect distance of 52 m; average stimulated bioluminescence for this transect was 6.9 $\mu W\ sr^{-1}\ m^{-3}$ (see Fig. 6 B). (D) Single video-frame from point of low bioluminescence intensity in transect indicated by first arrow in (C); display to right of rectangle is characteristic of siphonphore *Nanomia bijuga* (cf. Fig. 3 A). (E) Single video-frame from point of high bioluminescence intensity in transect indicated by second arrow in (C)

100 ms. This display was only apparent when the camera lens was set for maximum magnification (210 mm), and was only seen at depths of less than 125 m. Similar displays have been imaged from dinoflagellates (Widder and Case 1982); therefore, these were referred to as "dinoflagellate-like" flashes.

Among those few sources which did not pass through the screen was one distinctive class of "glowers" which, adhering to the screen, produced prolonged and intermittent flashes sometimes lasting 90 s. Although rare relative to other display types, the high intensities and long duration of these emissions could result in significant contributions to the stimulated bioluminescence (See Fig. 6 A).

Background bioluminescence

Mechanical stimulation of bioluminescence ceased when the submersible was trimmed to neutral buoyancy, thus providing an excellent platform for recording background bioluminescence. A total of 46 min of background was recorded in this manner, including the 1 to 2 min of background recorded prior to each transect (Fig. 5 B). Within this time over the depth range sampled (between 100 and 560 m), no spontaneous luminescence was ever recorded. This remarkably low background was not due to a scarcity of bioluminescent organisms, since the slightest motion of the submersible stimulated flashes of bioluminescence all around the hull.

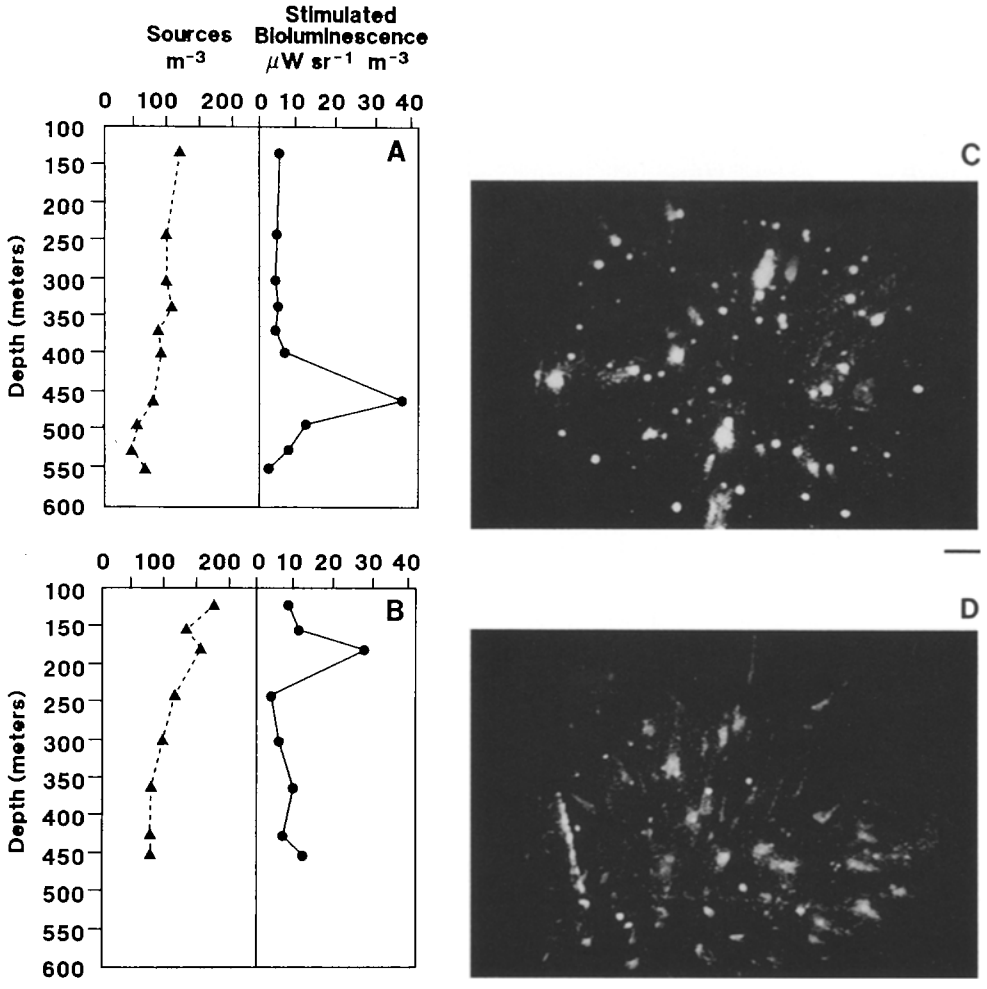

Fig. 6. (A) Depth profile of average concentration of bioluminescent sources m^{-3} (▲) and average stimulated bioluminescence in $\mu W\ sr^{-1}\ m^{-3}$ (●) calculated from transects on 7 September 1985. (B) As (A), at same location on following night. (C) Average of 8 video-frames from transect at 460 m on first night; bioluminescence passing through screen appeared as streaks compared to well defined boundaries of "glowers" which stuck to the screen; scale-bar = 10 cm. (D) As (C) for transect at 180 m on second night, showing large number of luminescent secretions as well as a display characteristic of siphonphore *Nanomia bijuga*, seen at left with nectosome pointed down (cf. Fig. 3A); several "glowers" are also present. Note that character of the secretions in (D) is very different from those in (C), which were fewer in number but more intense and long lasting; the faint horizontal line visible in (D) is the horizontal structural support illuminated by the intense bioluminescence

Transects

Mechanically excited bioluminescence was quantified from image analyses of video-recordings made during horizontal transects. Thirty five transects were run at depths from 100 to 560 m. Image-analysis data of a typical transect is shown in Fig. 5. The computer-generated rectangle, which designates the 2 000 cm^2 region from which gray values were calculated, was always positioned in the upper hemicircle of the screen, above the horizontal structural support (Fig. 5A).

The fifty-frame average of background recorded prior to each transect revealed no spontaneous bioluminescence (Fig. 5B).

Fig. 5C shows the stimulated bioluminescence recorded within the 2 000 cm^{-2} region of interest as screen radiance plotted against time. Two sample video-frames from the transect (Fig. 5D, E) show regions of low and high intensity, respectively.

The average intensity measured over the course of each transect provided a measure of the average stimulated bioluminescence at each depth. For example, the average intensity for the transect in Fig. 5C was 6.9 $\mu W\ sr^{-1}\ m^{-3}$, and this value is shown as a function of the depth at which the transect was run (430 m) in Fig. 6B. Bioluminescence intensities from the 35 transects ranged from 2.5 to 37.3 $\mu W\ st^{-1}\ m^{-3}$. For isotropic sources emitting at 480 nm these intensities equal 7.6×10^7 to 1.1×10^9 photons $s^{-1}\ cc^{-1}$.

Transect data collected during two dives made at one location (36°45′17″N; 121°58′77″W) on two consecutive nights are shown in Fig. 6. Both dives began at 02.00 hrs and were conducted in the same manner: a slow descent was made with the lights on, noting organism types and relative concentrations. The first transect started 60 m above the bottom, as indicated by the surface ship's echo sounder, and subsequent transects were run at decreasing depths. Each dive lasted approximately 5 h. Periods between transects were devoted to observations and recordings of animal behavior using far-red light, recording bioluminescence from known organisms, and the capture of animals for studies at the surface.

Concentrations of bioluminescent sources measured over the 35 transects ranged from a high of 175 m^{-3} at a depth of 124 m (Fig. 6B), to a low of 43 m^{-3} at 525 m (Fig. 6A). These values are equivalent to average distances between sources of 20 and 32 cm, respectively (Mackie and Mills 1983). Although the number of luminescent sources per cubic meter decreased with depth, stimulated bioluminescence did not decrease proportionately because of variations in source intensities and kinetics (Fig. 6).

On each of the dives shown in Fig. 6, a region of very high bioluminescence was observed. The high intensities recorded at 460 m (37.3 μW sr^{-1} m^{-3}) in Fig. 6A were largely due to two different display types with long emission kinetics. One of these was a particulate luminous secretion which remained luminescent for several seconds. The other was from "glowers" which did not pass through the screen and produced long intermittent flashes for almost the entire transect. Although "glowers" represented less than 10% of the 76 sources m^{-3} counted for this transect, their accumulated light output contributed significantly to the average intensity recorded.

The peak at 180 m on the second night (28.5 μW sr^{-1} m^{-3}) was due to a high concentration of unusually bright luminous secretions. The emission kinetics of these small structureless clouds were much briefer (300 ms) than those of the particulate secretions seen at 460 m, but their high intensity and high concentrations dominated the mechanically stimulated bioluminescence. The difference in the display types from these two regions is seen in Fig. 6C and D, where an average of eight consecutive video-frames causes bioluminescence passing through the screen to appear as streaks as compared to the well-defined boundaries of "glowers" which stuck to the screen.

It is possible that both these regions of high bioluminescence were present on both nights. No transects were run near 180 m on the first night, and on the second night an increase was observed at 455 m, although not of the magnitude seen previously.

Following each transect with lights on, no obvious source of luminescence was ever apparent which could be directly related to the stimulated bioluminescence. Sources which could be identified by the spatial and temporal patterns of their displays represented only 1% of the displays recorded during these transects. The most commonly seen identifiable source, *Nanomia bijuga* (Figs. 3A, 5D, 6D), was seen 18 times during the transects of Fig. 6, with one or two per transect between 180 and 490 m.

Correlations with trawling data revealed very high concentrations of euphausiids near the deeper of the two high-intensity regions of Fig. 6, with as many as 9 256 *Euphausia pacifica* captured during a 35 min trawl at 430 m (ca. 540 1 000 m^3). Other bioluminescent sources collected in high concentrations near this depth included the gonostomatid fish *Cyclothone signata* (mean of 2.8 collected min^{-1} for night-time tows between 400 and 500 m, ca. 5.7 1000 m^{-3}) as well as the myctophid *Stenobrachius leucopsaurus* (mean of 1 min^{-1} for the same tows, ca. 2.1 1000 m^{-3}). Also present were the decapod *Sergestes similis* (140 captured during the same 35 min tow as above, ca. 8.1 1000 m^{-3}), the copepod *Gaussia princeps* (54 captured, ca. 3.1 1000 m^{-3}), as well as several luminescent mysid shrimp and amphipods. Gelatinous organisms collected from the net tows have not been identified, but were collected in similar amounts at both depths of interest.

For the region of high-stimulated bioluminescence at 180 m, the major difference was a lower concentration of *Euphausia pacifica* (1 058 captured during a 31 min night trawl at 160 m, ca. 69.1 1000 m^{-3}) a higher concentration of *Sergestes similis* (1606 captured in the same tow at 160 m, ca. 104.9 1000 m^{-3}) and a much lower concentration of *Cyclothone signata* (mean of 0.02 collected per minute for nighttime tows between 100 and 200 m, ca. 0.04 1000 m^{-3}). The relative difference in concentrations of euphausiids compared to sergestids at the two depths was apparent from the submersible. Very high concentrations of sergestids were seen around 180 m.

It is unlikely that any of these organisms were responsible for the regions of high stimulated bioluminescence. None of these species emit bioluminescent secretions and the correlation of high concentrations of *Cyclothone signata* with the glowers seen at 460 m seems unlikely, since these fish would probably not stick to the transect screen as the glowers did and they are large enough to have been visible when the lights were turned on.

Conductivity-temperature-depth profiles revealed no outstanding hydrographic features associated with the regions of high bioluminescence at 460 and 180 m. However, a narrow finger of slightly lower salinity water was found between 155 and 162 m which may have acted as a boundary to the luminescent organisms responsible for the higher intensity seen at 180 m (Fig. 6B). Temperatures at 460 and 180 m were 6.6° and 9.3 °C, respectively.

Discussion

Considerable effort in biological oceanography is directed at localizing and identifying organisms in the water column. Bioluminescence, which is very common in mesopelagic organisms, is a potentially valuable indicator of animal distributions and may actually have an important influence on such distributions due to bioluminescence-mediated behavioral interactions. The importance of bioluminescence both as an indicator and effector of animal distributions cannot be realized until the primary sources are identified.

The video images of in situ bioluminescence recorded during our investigations indicated that the dominant form of bioluminescence mechanically stimulated by the transect screen at all depths sampled below 100 m was luminous secretions. It is probable that many of these displays originated from small crustaceans such as ostracods, and copepods which are known to emit luminous secretions (Herring 1985) and are small enough to pass through the 5 mm mesh of both the trawl net and the transect screen. Direct contact with the screen or hydrodynamic shear stress as the organism passed through the 5 mm mesh at 1 knot was apparently sufficient to stimulate emission. The small size of such sources would account for the general failure of observers to correlate regions of stimulated bioluminescence with identified organisms and could also account for the large number of bioluminescence-triggered photographs of Clarke and Breslau (1959) and Breslau et al. (1967), which lacked any identifiable organisms. Direct correlation of this very important display type with identified sources will require higher

magnification and greater resolution of in situ-imaging systems.

The data presented here indicate that such correlations are critical to bioluminescence measurements because merely correlating a region of bright bioluminescence with the presence of high concentrations of a known bioluminescent source such as *Euphausia pacifica* may be misleading. A smaller source, present in lower concentrations, could actually be the predominant source of light due to a large disparity in emission capabilities.

The patterns of displays recorded during our submersible-based investigations were all characteristic of zooplankton, with the exception of the "dinoflagellate-like" flashes seen above 120 m. It has been generally assumed that bioluminescence in the "upper few hundred metres" is dominantly dinoflagellate in orgin (Kelly and Tett 1978), and good correlations have been demonstrated between regions of high bioluminescence and high concentrations of bioluminescent dinoflagellates (Kelly 1968). However, recent investigations of epipelagic bioluminescence in the Sargasso Sea with a pump-through bathyphotometer and concomitant organism collections demonstrated a strong correlation between stimulated bioluminescence and the presence of zooplankton such as bioluminescent larvaceans, colonial radiolarians and small crustaceans (Swift et al. 1985). During our investigations with "Deep Rover", in situ-imaging of bioluminescent sources was not attempted above 100 m because of difficulties in tracking the submersible from the surface ship at these shallower depths. However, at the only depths where dinoflagellate-like flashes were recorded, between 100 and 120 m, the zooplankton-like flashes still dominated the stimulated bioluminescence.

Correlations of bioluminescent displays with organisms was accomplished here for larger gelatinous sources. Although smaller sources dominated the stimulated bioluminescence in numbers of organisms, it was the gelatinous sources which were dominant in the intensity of individual displays. Many of these sources were so fragile that they broke apart on contact; consequently, in situ-imaging would appear to be the only means of documenting the luminescent capabilities of such diaphanous creatures. Furthermore, our data clearly indicated that displays recorded in situ can differ radically from those recorded from captured specimens, suggesting that correlations carried out in the laboratory between source and display types may not always be transferable to in situ recordings.

Given the high intensities of bioluminescence stimulated during horizontal transects, the absence of any recorded spontaneous bioluminescence was surprising. There is a considerable body of morphological and physiological data as well as laboratory observations of midwater organisms to suggest that spontaneous bioluminescence does occur in the mesopelagic environment. Examples of such data are the presence of sexually dimorphic light organs in euphausiids (Herring and Locket 1978) and fishes (Herring and Morin 1978), evidence of counterillumination in fishes (Case et al. 1977), shrimp (Warner et al. 1979), and squid (Young and Roper 1976), and observations of apparently unstimulated flashes from captured euphausiids (Hardy and Kay 1964). Dim, ventrally directed counterillumination would not be easily recorded, nor would it be expected in the absence of downwelling light. The lack of any recorded spontaneous flashes during our investigations suggests a time scale of minutes to hours for the occurrence of such events within the viewing range of the camera.

Longer viewing intervals are clearly called for, and must be carried out from an untethered platform such as a submersible in order to avoid unwanted mechanical stimulation of bioluminescence. This great advantage of the submersible was augmented by the ability to mechanically stimulate bioluminescence in a controlled manner, demonstrating that the absence of spontaneous bioluminescence was not due to an absence of bioluminescent organisms.

Bioluminescence has been assigned many functions including camouflage, sexual recognition, courtship, schooling, illumination and attraction of prey, and defense (Tett and Kelly 1973, Buck 1978, Young 1983). The majority of the mechanically stimulated bioluminescence described here probably falls into the latter category. However the variety of displays which were recorded undoubtedly encompassed a number of different defensive strategies such as distraction, blinding, warning and exposing primary predators to secondary predators.

In analyzing the defensive nature of stimulated bioluminescence, it seems significant that the most eye-catching displays in terms of both intensity and kinetics originated from the most fragile sources. While the emission intensities of these sources might suggest blinding, this seems a poor defensive strategy if the predator then blunders into further contact. Also, elaborate kinetics of emission would be wasted on a blinded predator. Discouragement of destructive contact with predators or even non-predators must be a primary defensive strategy for such fragile organisms, and several lines of defense may be employed. For example initial contact with the tentacles of *Halistaura cellularia* which left streaks of glowing luminescence on the screen could mark a predator for capture by other predators. Use of luminescent secretions as a first line of defense to mark or distract a predator might then be followed by more intense and elaborate intracellular emissions as a second line of defense. The propagated flashes of the ctenophores and of the siphonophore *Apolemia* sp. readily attract the attention of the human observer, but whether the adaptive significance of such a display is to attract secondary predators as postulated in the "burglar alarm" theory of Burkenroad (1943) or to confuse or perhaps warn the primary predator remains a matter for debate (Morin 1974).

In this environment, where spontaneous luminescence is low and stimulated bioluminescence potentially high, it seems probable that any spontaneous emissions or movements by an animal would risk revealing its presence to visual predators. This is undoubtedly a major influence on animal behavior, on community-wide activity levels, and thus on the dynamics of the midwater environment.

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