Reference: *Biol. Bull.* **179:** 340–350. (December, 1990)

Patterns of Stimulated Bioluminescence in Two Pyrosomes (Tunicata: Pyrosomatidae)

MARK R. BOWLBY[1], EDITH A. WIDDER[2], AND JAMES F. CASE

Marine Science Institute and Department of Biological Sciences, University of California, Santa Barbara, California 93106

Abstract. Pyrosomes are colonial tunicates that, in contrast with typical luminescent plankton, generate brilliant, sustained bioluminescence. They are unusual in numbering among the few marine organisms reported to luminesce in response to light. Each zooid within a colony detects light and emits bioluminescence in response. To investigate the luminescence responsivity of *Pyrosoma atlanticum* and *Pyrosomella verticillata,* photic, electrical, and mechanical stimuli were used. Photic stimulation of 1.5×10^9 photons $\cdot s^{-1} \cdot cm^{-2}$, at wavelengths between 350 and 600 nm, induced bioluminescence, with the maximum response induced at 475 nm. The photic-excitation half-response constant was 1.1×10^7 photons $\cdot s^{-1} \cdot cm^{-2}$ at 475 nm for *P. atlanticum; P. verticillata* had a significantly higher half-response constant of 9.3×10^7 photons $\cdot s^{-1} \cdot cm^{-2}$. Individual zooids within a colony, however, appeared to have different half-response constants. Stimulus strength influenced recruitment of zooids and, in turn, luminescent duration and quantum emission. Image intensification revealed saltatory propagation of luminescence across the colony, owing to photic triggering among zooids. Repetitive, regular mechanical or electrical stimulation elicited rhythmic flashing characterized by alternating periods of high and low light intensities.

Introduction

Pyrosomes are holoplanktonic colonial tunicates found at depths to 1000 m (Soest, 1981). Their remarkable capacity to luminesce and their occasional presence in very large numbers at the ocean surface occasioned T. H. Huxley to write in his diary in 1849: "I have just watched the moon set in all her glory, and looked at those lesser moons, the beautiful Pyrosoma, shining like white-hot cylinders in the water" (Huxley, 1936).

Colonies may be of highly variable size, reaching lengths of 30 m in some species (Griffin and Yaldwyn, 1970), owing to their growth habit of budding successive rings of zooids around the periphery of an elongating cylinder. The zooids are arranged so that their exhalent currents are conducted into the hollow core of the cylinder to generate a communal locomotor current. Each zooid contains a pair of luminescent organs, bilaterally flanking the incurrent siphon, and lying at the periphery of the colonial cylinder (Panceri, 1873). The organs are external to the pharyngeal epithelium, but protrude into the pharyngeal cavity (Mackie and Bone, 1978). Closely packed cells in the luminescent organs are filled with luminous organelles, which may be intracellular luminescent bacteria (Pierantoni, 1921; Neumann, 1934; Buchner, 1965; Mackie and Bone, 1978). Bacterial luciferase activity similar to that of the luminescent bacteria *Photobacterium* has been found in *Pyrosoma* sp. (Leisman *et al.,* 1980).

The mechanism of luminescence propagation within the colony is not neural. Neither innervation nor an epithelial conduction pathway is in evidence (Mackie and Bone, 1978). Photic stimuli are presumably received by a photoreceptor lying just above the brain, triggering brain-induced arrests of gill basket cilia (Buchner, 1965; Mackie and Bone, 1978). This ciliary arrest, and the concomitant gill collapse, might reduce blood flow and decrease the supply of oxygen or metabolites to the light organ, thereby indirectly controlling light emission (Mackie and Bone, 1978; Mackie, 1986).

Received 18 May 1990; accepted 25 September 1990.

[1] Present address: Department of Neurobiology, Harvard Medical School, 220 Longwood Ave., Boston, MA 02115.

[2] Harbor Branch Oceanographic Institution, Fort Pierce, FL 34946.

Pyrosomes have the remarkable ability to detect external light flashes and to respond by luminescing (Polimanti, 1911). Colonies respond to conspecifics (Burghause, 1914) and simulated bioluminescence (Mackie and Bone, 1978). Localized stimulation of one area of a colony produces a wave of light that travels across the colony from the point of stimulation (Panceri, 1873). Intact tissue connections are not necessary among zooids, indicating that wave propagation within the colony may occur photically (Burghause, 1914; Mackie and Bone, 1978).

Luminescence may also be photically induced in other organisms, including ctenophores (Lábas, 1980), ostracods (Tsuji *et al.*, 1970), copepods (Lapota *et al.*, 1986), euphausiids (Kay, 1965; Tett, 1969, 1972), and a decapod shrimp (Herring and Barnes, 1976). Pulsed, colored light at 700 m *in situ* enhanced bioluminescence activity from unidentified organisms (Neshyba, 1967). Some of these organisms undergo inhibition of their luminescence when exposed to constant, "bright" illumination (Burghause, 1914; Nicol, 1960).

The organisms used in this study were *Pyrosoma atlanticum* Peron, a cosmopolitan form, and *Pyrosomella verticillata* (Neumann), which occurs in tropical and subtropical waters (Soest, 1981). Although we investigated bioluminescence produced by photic, electrical, and mechanical stimulation, photic stimulation was our major concern because it is the least well understood among the bioluminescence excitatory modes, has never been investigated quantitatively, and has significance in the interpretation of the roles of bioluminescence in the behavior of marine animals. In our work, photic stimuli varying in wavelength and irradiance were related to their bioluminescent responses and compared with the effects of other stimulus modes. The pattern of the luminescent wave and the mode of its transmission through the colony are discussed. A preliminary report of this work has appeared (Bowlby and Case, 1988).

Materials and Methods

Specimen collection

Mature specimens of *Pyrosoma atlanticum* and *Pyrosomella verticillata* were studied during July 1986 and 1987, aboard the R.V. *New Horizon* off the southwest coast of Oahu, Hawaii. Collections were made at approximately 21° N 158° W, with an opening-closing Tucker Trawl (length, 30 m; mouth, 10 m^2). The trawl was equipped with an insulating cod end (Childress *et al.*, 1977), towed at depths ranging from 400 to 800 m, and brought to the surface every 4 to 6 hours. Specimens were sorted under ambient light, and maintained in darkness in 12°C seawater for 8 to 24 hours. The colonies studied ranged in length from 1.4 to 7.7 cm; surface area was calculated from measurements of colonial length and diameter. Colonies up to 30 cm in length were captured, but were not included in the investigation due to space limitations in the experimental apparatus.

Experimental procedure

Individual colonies were placed in covered Plexiglas chambers holding 25 to 125 ml of filtered seawater. The dimensions of the chamber substantially exceeded those of the colony to minimize luminescence induced by contact with the container. A 25-cm diameter integrating sphere, coated internally with white Polane polyethylene paint (97% reflectance at 500 nm), surrounded the animal to insure maximal reflectance and detection of bioluminescence irrespective of orientation (Latz *et al.*, 1987). Bioluminescence was detected by a photon counting photomultiplier tube (RCA Model 8850), which viewed the interior of the sphere through a 4.5-cm diameter port. A baffle between the source and detector only allowed light that had undergone multiple reflections within the sphere to be measured. This apparatus provides a directionally unbiased, quantifiable measure of bioluminescence from non-isotropic sources. Radiometric calibrations were made with an Optronics Laboratory Model 310 multifilter calibration source referenced to an NBS standard. At sea, the system calibration was maintained with a C^{14} phosphor referenced to the Optronics source. The calibration corrected for the spectral responsivity of the sphere and photomultiplier tube, as well as for the bioluminescence spectrum of both pyrosome species, as measured by an optical multichannel analyzer (Widder *et al.*, 1983).

The photomultiplier signal was monitored for 40 to 200 s with a Norland Model 5400 multichannel analyzer (MCA) and stored on a diskette in a microcomputer for subsequent analysis. Temporal resolution ranged from 10 to 50 ms per channel.

Bioluminescence was stimulated by light, electrical, and mechanical excitation. Electrical stimuli (0.5–50 Hz, 5 ms duration, 50 V) from a Grass S48 stimulator were delivered by tungsten electrodes projecting into the chamber. A 1-cm diameter fiberglass rod driven by a solenoid to produce a displacement of 1 cm in 0.5 s applied mechanical stimulation. In some trials the colony was stimulated with the rod manually until bioluminescence was no longer produced. Photic stimuli, produced by a Bausch and Lomb monochromator with a tungsten light source, were delivered through a 5-mm diameter fiber optic into the sphere. Stimuli entering the sphere were deflected by a stimulus baffle placed at 45° to the fiber optic, providing a uniform stimulus illumination over the entire colony. Stimulus wavelength (FWHM = 21 nm) was either varied between 350 nm and 800 nm (in 25 nm

Table I

Kinetics and intensities of pyrosome flashes stimulated at effective wavelengths (350–550 nm) and irradiances (6.8×10^6 to 4.3×10^{10} photons · s^{-1} · cm^{-2})

Species	Latency (s)	Rise time (s)	98% Duration (s)	Maximum flux (photons · s^{-1})	Mean emission (photons · s^{-1})	Quantum emission (photons · $flash^{-1}$)
Pyrosoma atlanticum (n = 6)	1.4 ± 0.2	4.9 ± 1.6	16.0 ± 3.8	1.2×10^{11} $\pm 1.0 \times 10^{11}$	4.8×10^{10} $\pm 3.8 \times 10^{10}$	1.4×10^{12} $\pm 1.1 \times 10^{12}$
Pyrosomella verticillata (n = 9)	1.4 ± 0.1	4.3 ± 1.1	11.6 ± 2.9	1.1×10^{10} $\pm 4.3 \times 10^{9}$	4.5×10^{9} $\pm 2.1 \times 10^{9}$	1.0×10^{11} $\pm 5.0 \times 10^{10}$

Values represent the mean ± standard error of the mean. Means are not significantly different between species (*t*-test, $P > 0.05$).

increments) at constant quantal irradiance, or irradiance was varied with neutral density filters, between 6.8×10^6 and 4.3×10^{10} photons · s^{-1} · cm^{-2}, at constant wavelength. The stimulus duration was controlled by a Uniblitz electronic shutter at 0.5 s for all trials. Individual colonies were allowed to dark adapt for a minimum of one hour before testing. In preliminary trials to determine the optimum interstimulus period, colonies produced less consistent responses (flash strength and number) to interstimulus periods of less than 3 min. Increasing the interstimulus interval beyond 5 min made no further improvement in response uniformity. Consequently, stimuli were delivered every 4 to 5 min, with the colony remaining undisturbed in the light-tight sphere during the interstimulus periods. The temperature of the seawater gradually increased from 12 to approximately 18°C during an experimental session, but no change in excitability was observed. Colonies produced few flashes in the absence of applied stimuli.

Stimulus irradiance was calibrated with a radiometer (United Detector Technology, Model S370) equipped with a silicon photodiode detector and a 180° cosine diffuser in the test specimen position. The presence of the stimulus baffle created a uniform diffuse stimulus; therefore, the radiant energy arriving at the surface of the sphere, as measured with the silicon photodiode cosine collector, is a measure of spherical irradiance. Because the pyrosome tissue is very clear, the zooid light receptor receives input from all directions, so the measured irradiance was multiplied by four to convert to scalar irradiance which is the energy per area arriving at a point from all directions about the point (Tyler and Preisendorfer, 1962). Stimulus irradiance was finally converted into quantal units, as (1) the number of photons may be more important than total energy in stimulating pyrosomes to produce light, and (2) to aid in comparisons with bioluminescence measurements.

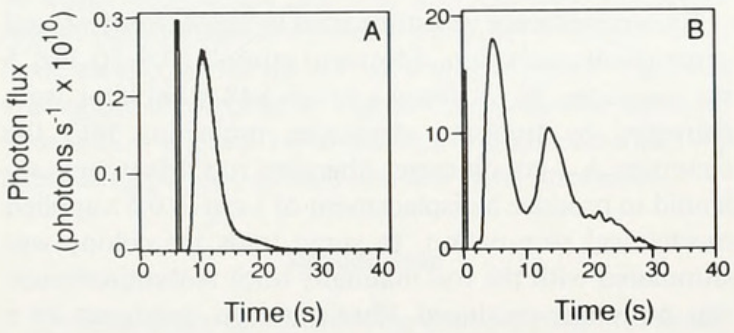

Figure 1. Varying luminescent responses of pyrosome colonies to photic stimuli at 475 nm, measured in an integrating sphere. The first flash is the stimulus artifact; it is followed by the bioluminescent response after a brief latency. The height of the stimulus flash does not represent the true intensity of the stimulus, due to detector saturation. (A) A simple response from *Pyrosomella verticillata*, due to simultaneous zooid light production. (B) A complex response from *Pyrosoma atlanticum* with two distinct peaks of luminescence.

Measured flash characteristics were:

(a) Latency—time from stimulus onset to flash onset;

(b) Rise time—time from flash onset to maximum photon flux of the flash;

(c) 98% response duration—time from flash onset to when photon flux has declined to 2% of maximum;

(d) Maximum flux—maximum flash intensity;

(e) Quantum emission—total integrated photons emitted over 98% response duration; and

(f) Mean emission—average integrated photons per second emitted during 98% response duration.

Images were intensified with an ISIT (Dage) low light level video camera with a 105 mm Nikon f/4 lens. Specimens were placed in Plexiglas chambers and enclosed in a light-tight container with white reflective internal surfaces. A photon counting photomultiplier system viewed the interior of the box, permitting simultaneous recording of relative flash kinetics and intensified video images. In some cases, specimens were examined with a dissecting microscope, with the ISIT camera recording the image through the photographic tube. Stimuli identical to those described above were used to elicit bioluminescence.

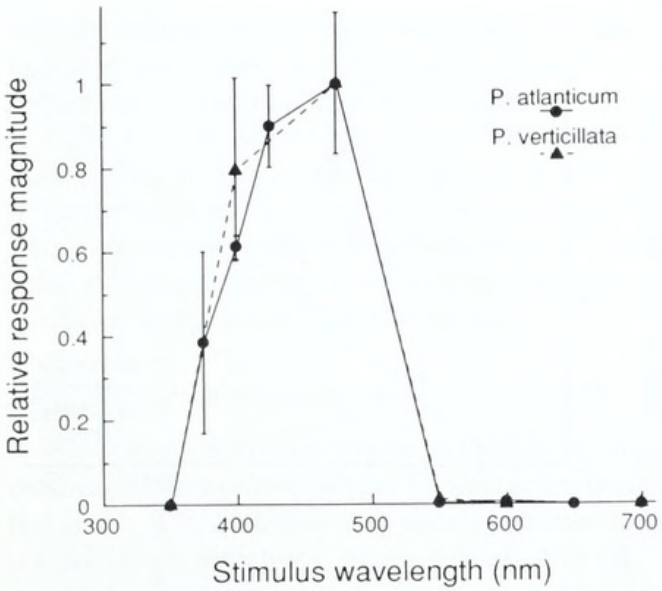

Figure 2. Normalized spectral responsivity of *Pyrosoma atlanticum* and *Pyrosomella verticillata*. Mean relative response magnitude is plotted as a function of stimulus wavelength; error bars represent standard errors of the mean. The stimulus scalar irradiance (500-ms pulse) for *P. atlanticum* was 1.5×10^9 photons $\cdot s^{-1} \cdot cm^{-2}$, and 7.1×10^8 photons $\cdot s^{-1} \cdot cm^{-2}$ for *P. verticillata*. Peak response for both was at approximately 475 nm. Data fit a quadratic regression for both species, according to the equation $y = -19.07 + 0.0887x - (9.82 \times 10^{-5})x^2$, $r^2 = 0.98$ for *P. atlanticum*; and $y = -8.34 + 0.039x - (4.32 \times 10^{-5})x^2$, $r^2 = 0.98$ for *P. verticillata*. n = 4 colonies of each species.

The resulting MCA recorded waveform was stored and analyzed as previously described, while the video images were viewed at slow speed to analyze the propagation of signals. Video images were enhanced with a Megavision 1024XM image-analysis system for final presentation. The relative light emission of individual zooids was also examined with the image analysis system. In this analysis, the gray scale of the luminescent signal indicated the relative flash intensity of the region measured. This analysis was performed only on: (1) data collected with the ISIT video camera set to the manual gain setting, and (2) data not saturating the gray scale levels.

Results

Photic stimulation

In response to light stimuli, colonies often produced 25 to 30 flashes over approximately a 2-h period. Characteristic flashes had long latencies and durations and large quantum emissions (Table I). Flash latency and rise time were much less variable than quantum emission. Kinetic values for the two species (Table I) were not significantly different (ANOVA, $P > 0.05$). Light emission was independent of the colony surface area.

Colonies responded to spatially diffuse photic stimulation with varying flash displays, ranging from the most commonly observed simple flash (Fig. 1A), in which the responding zooids react approximately simultaneously, to more complex emission patterns (Fig. 1B). Such patterns may result from a variable latency in response to the initial stimulus, or to zooid reexcitation after a refractory period.

The spectral responsivity curves for both *P. atlanticum* and *P. verticillata* lay between 400 and 550 nm, and are described by a quadratic regression (Fig. 2). The spectral responsivity maxima were approximately 475 nm.

The half-response constant of photic excitation was determined by exposing specimens to between three and eight stimuli of identical scalar irradiance. The percentage of stimuli eliciting a response to 475 nm, regardless of magnitude, was plotted as a function of stimulus scalar irradiance (Fig. 3). Most stimuli elicited a response in either 0 or 100% of trials, except in a narrow range of irradiances. Three wavelengths were examined (graphs not shown for 400 nm and 600 nm), with similar response patterns observed.

Investigations of single visual receptor cells in insects produce similar results (Laughlin and Hardie, 1978; Hardie, 1979). In insects, the intensity response function follows the form

$$V/V_{max} = \mu I/(\mu I + 1), \quad (1)$$

where I is the stimulus intensity, V is the response amplitude, V_{max} is the maximum response amplitude, and

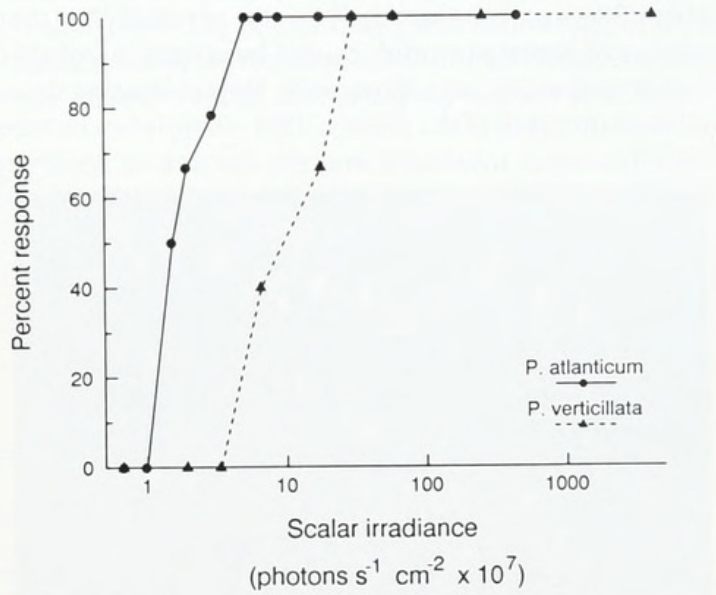

Figure 3. Bioluminescent response to 475 nm photic stimulation. The percentage of stimuli that elicited a response is shown as a function of the log of stimulus scalar irradiance. Data are grouped for all specimens. Calculated half-response constants are shown in Table II. Responses between 0 and 100% fit the linear regression $y = 10.13 + (1.9 \times 10^{-6})x$, $r^2 = 0.73$ for *Pyrosoma atlanticum*; and $y = 4.12 + (3.2 \times 10^{-7})x$, $r^2 = 0.91$ for *Pyrosomella verticillata*. n = 3 colonies of each species.

μ is the sensitivity parameter and equals the reciprocal of the intensity required to produce a response 50% of maximum (Laughlin, 1975). The half-response constant is the level at which the slope of the V/log I curve is maximal, and is defined in this study as the threshold level of photic stimulation that produces a bioluminescent response. Using formula (1), the half-response constant for *P. atlanticum* to 475 nm was significantly lower than for *P. verticillata* (Table II; *t*-test of slopes and points of linear regressions, $P < 0.05$). Half-response constants to 400 and 600 nm stimuli were not significantly different (Table II). The half-response constant to photic stimulation was independent of the overall colony length.

Colony quantum emission and flash duration were proportional to stimulus irradiance. Relative quantum emission (Fig. 4A) and 98% flash duration (Fig. 4B) varied logarithmically with stimulus scalar irradiance. The maximum and mean emissions, though, did not vary consistently with the stimulus scalar irradiance. Thus the scalar irradiance effect on flash duration may account for the change in quantum emission. To clarify these relationships, the relative light emission of individual zooids was examined with the image analysis system. Individual zooids in unvarying orientation during flash events elicited by photic, mechanical, and electrical stimuli had remarkably constant flash intensities. The quantum emission per zooid varied by less than 10% among flashes, independent of the colony quantum emission. Differences between flashes were often less than 2%. Light emission of zooids began to decrease only after about 10–15 flashes spaced about 30 s apart. Image analysis also revealed that more intense or repeated stimuli caused increasing numbers of zooids to respond asynchronously, thus increasing the total flash duration of the colony. This relationship between stimulus scalar irradiance and the fraction of zooids responding is evidence for a variation in the half-response constant among zooids. Thus, the dependence between quantum emission and stimulus scalar irradiance is due to the asynchronous triggering of greater numbers of zooids, leading to longer colonial flash durations.

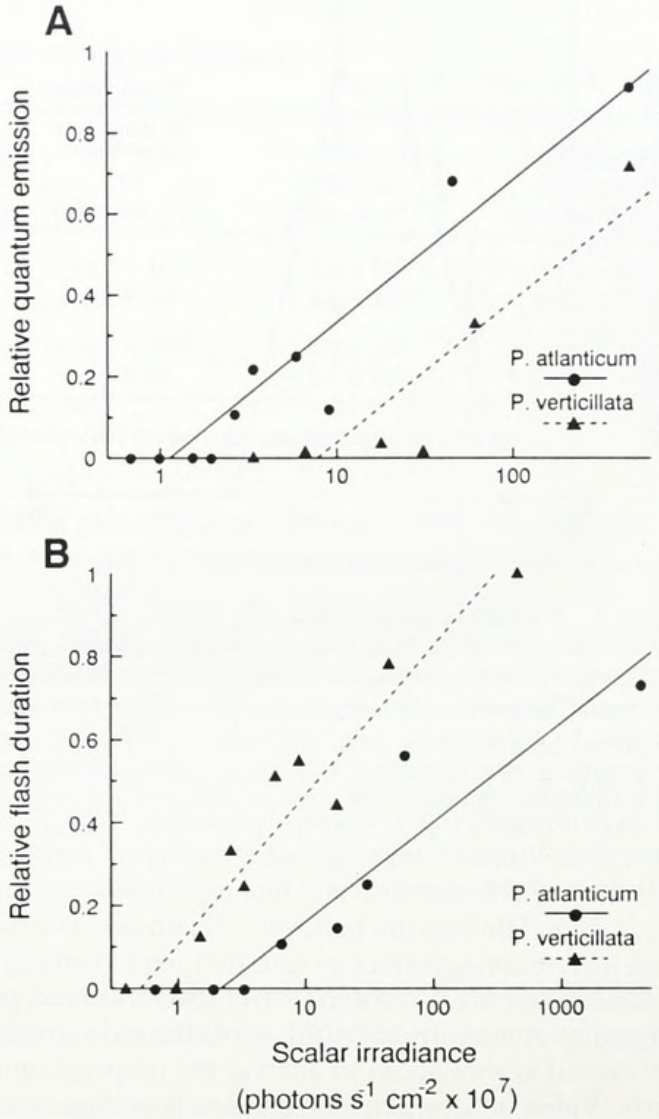

Figure 4. Quantum emission and flash duration elicited by 475 nm photic stimuli of varying scalar irradiance. The increase in quantum emission with the increase in stimulus scalar irradiance was due to greater flash durations caused by the asynchronous triggering of zooids. (A) Relative quantum emission of colonies from 2 to 7 cm length. The slope of the logarithmic regression for *Pyrosoma atlanticum* is 0.15 ($r^2 = 0.92$); that for *Pyrosomella verticillata* is also 0.15 ($r^2 = 0.83$). There is no significant difference between the regression slopes or elevations (*t*-test, $P > 0.05$). (B) Relative 98% flash durations. The slope of the logarithmic regression for *P. atlanticum* is 0.10 ($r^2 = 0.86$); that for *P. verticillata* is 0.16 ($r^2 = 0.91$). These slopes are significantly different (*t*-test, $P < 0.05$).

Table II

Half-response constants (photons · s^{-1} · cm^{-2}) to photic stimulation (V_{max} = 50% response) calculated from the V/log I function of Laughlin, 1975

	Stimulus wavelength (nm)		
	400	475[a]	600
Pyrosoma atlanticum	1.1×10^8	1.1×10^7	9.7×10^8
Pyrosomella verticillata	2.6×10^8	9.3×10^7	3.3×10^9

[a] Slopes and points on linear regression significantly different between species (*t*-test, $P < 0.05$). Variances are not significantly different (F test, $P > 0.05$).

Image intensification revealed strikingly different colonial patterns of luminescence in the two species investigated. In *P. atlanticum*, small and large zooids are intermixed throughout the colony. This pattern is evident as an irregular pattern of small and large luminescent sources distributed over the colony surface (Fig. 5A). The zooid light organs lie close together, often producing ap-

parent single points of light, which are resolved under higher magnification into pairs of luminous sources. *P. verticillata*, in contrast, possesses zooids of uniform size in distinct rows, with a wider spacing between the pair of light organs in each zooid. This results in uniform rows of luminescent sources over the colony surface (Fig. 5B). These obvious differences in light patterns, rooted in the colony morphology, make the two species easily distinguishable by their luminescent patterns.

Bioluminescence propagation

ISIT video records of events caused by a single mechanical stimulus revealed that luminescence begins from the point of stimulation and slowly spreads across the colony in all directions, at an overall rate of 2.1–4.1 $mm \cdot s^{-1}$, at temperatures of 12 to 16°C (Fig. 6). The light usually travels across the colony by saltatory conduction, the nodes being either single zooids or groups of zooids 0.5 to 1.5 cm apart, with a latency between nodes of about 3 s. Zooids between the responsive regions begin to luminesce after being bypassed, while the wave continues to the next responsive site. This disjointed wave of bioluminescence is characteristic of both species examined.

Electrical stimulation

Single electrical stimuli produced flashes of simple shape, with few irregularities in the waveform (Fig. 7A; Table III). Temporal summation of luminescence was induced by electrical pulses at 0.5 and 1 Hz (Fig. 7B). Video analysis revealed that this summation was due to increasing recruitment of zooids with successive stimuli and, to a lesser extent, an increase in zooid light emission. In trials with constant stimulus rates of 5 to 50 Hz, luminescence was produced initially, and was often followed by a series of shorter, repeating flashes (Fig. 7C) with an average interflash period of 18 s. In a few trials, however, light was elicited at a similar initial rate, but no repetitive flashing pattern was observed (Fig. 7D). Multiple stimulation elicited a significantly larger response duration, maximum flux, and mean emission than single pulses (*t*-test, $P < 0.05$; Table III). Rise time, quantum emission, and flash duration in response to electrical stimuli were significantly different from these parameters for photic excitation (Tukey test, $P < 0.05$). The maximum flux and quantum emission were again independent of colony surface area.

Mechanical stimulation

Repetitive mechanical stimulation induced a significantly greater light emission than any other stimulus method employed in this study (Tukey test, $P < 0.05$; Table IV). The total duration of light emission in all cases exceeded the 200-s collection period, with a mean flash duration of 59 s. Within this period a repeating flash pattern, with similar kinetics to that for electrical stimuli, was observed (Fig. 8A).

Many colonies also produced bioluminescent flashes with simple kinetics (Fig. 8B) in the absence of any obvious external stimuli except ship movement (Table IV). Bioluminescent events of this type occurred randomly during the interstimulus resting periods and were thus easily separated from flashes elicited by photic or other stimuli. Unlike photically or electrically stimulated organisms, the light emission for mechanically induced bioluminescence was directly related to the colony surface area (Fig. 9).

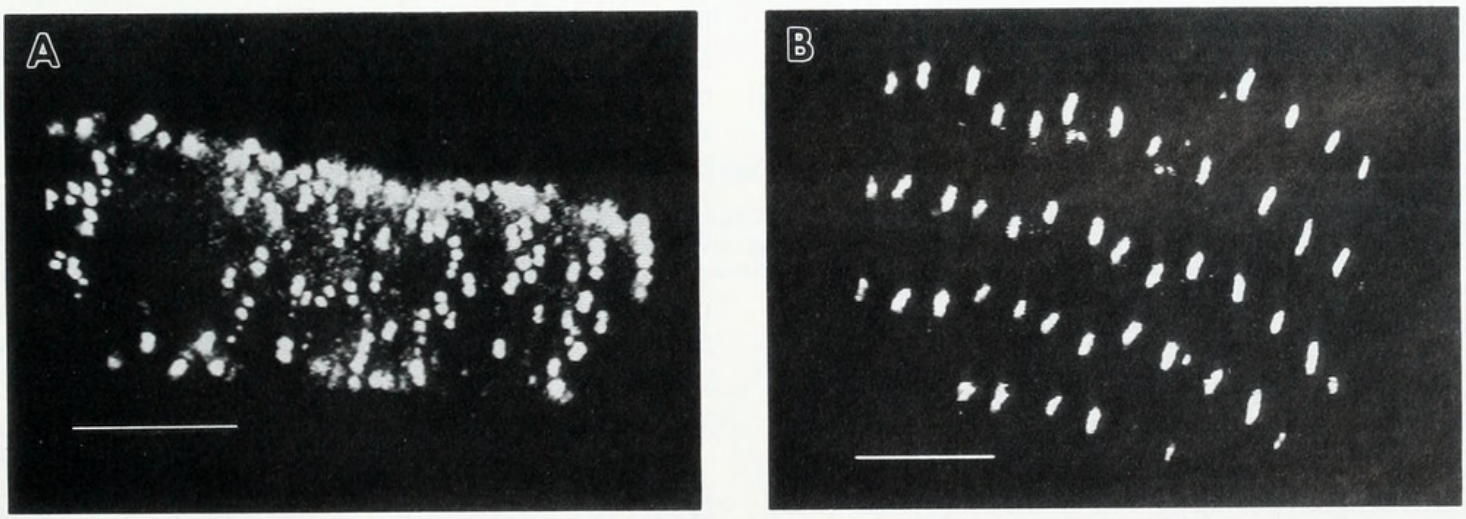

Figure 5. Single ISIT video frames of patterns of luminescence in response to photic stimuli. Arrangement of luminous sources is based upon colony morphology. (A) *Pyrosoma atlanticum*, showing an irregular pattern of luminescent sources. Bar = 1 cm. (B) *Pyrosomella verticillata*, exhibiting uniform distribution of luminescent sources. Bar = 0.5 cm.

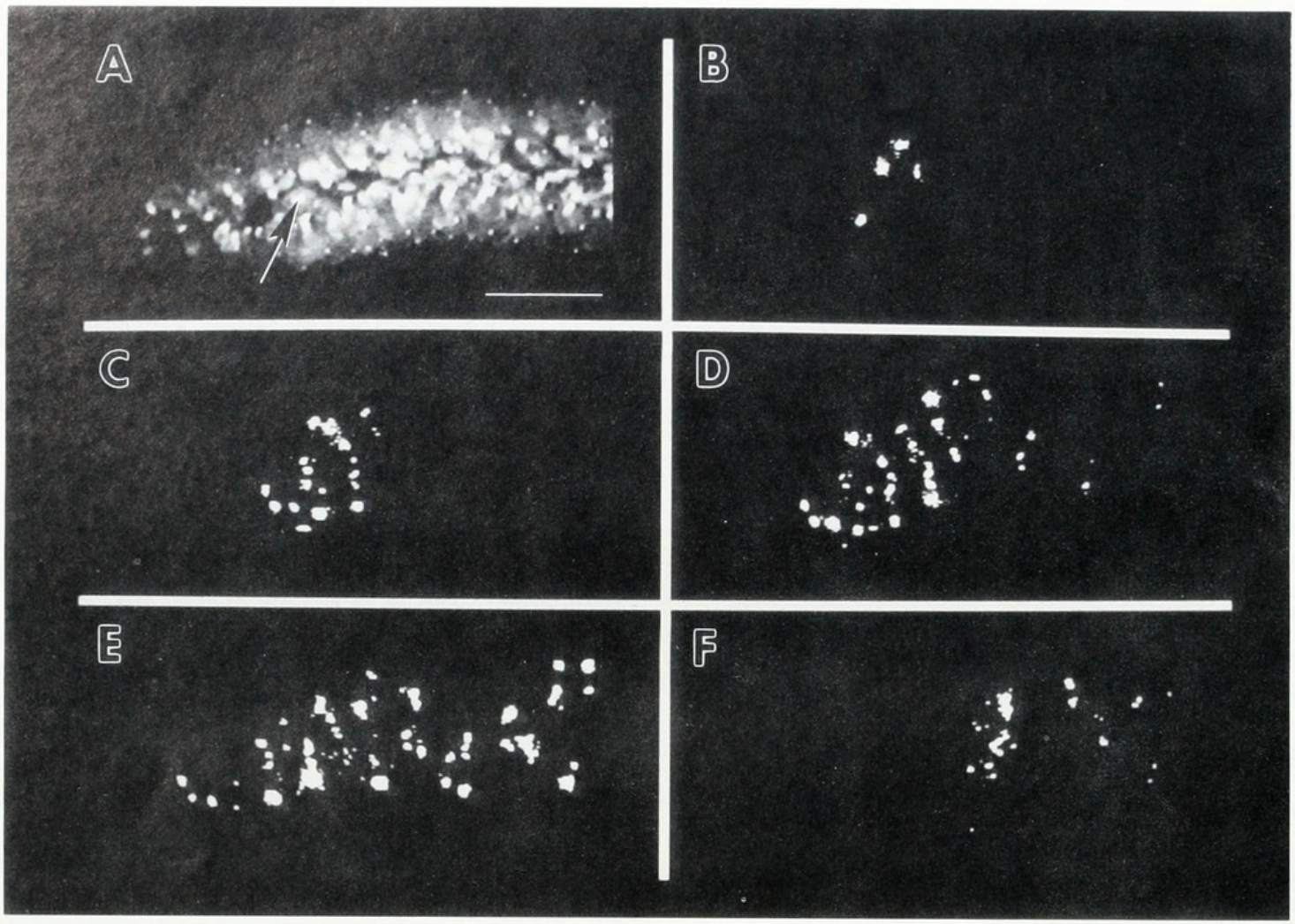

Figure 6. A bioluminescent wave traveling across *Pyrosoma atlanticum*. The colony is oriented in the same position in (A) through (F). (A) Image of the colony with red illumination. Arrow indicates the point of mechanical stimulation. Bar = 1 cm. (B) The flash begins at the point of stimulation (time = 0 s). (C) After 3 s the luminescent response has spread to other nearby zooids. Progressive bidirectional conduction of the light wave across the colony at (D) 6 s and (E) 9 s after the beginning of the response. (F) Decay in intensity of the response (time = 15 s).

Therefore, most zooids in the colony probably responded to mechanical stimuli, in contrast to responses to the other stimulation methods.

Discussion

Bioluminescence of pyrosome colonies begins at the location of the stimulation, and slowly propagates by a photic, saltatory conduction process in all directions. The photic propagation of light along the colony is supported by several pieces of evidence. First, the similarity between our action spectrum and the luminescent emission spectrum (Swift *et al.*, 1977; Widder *et al.*, 1983) indicates that the colony responds to, and emits, the same wavelengths of light. Second, light from one zooid is also easily able to surpass the half-response constant of other nearby zooids. According to Allard's law

$$E_x = Ie^{-cx}/x^2, \qquad (2)$$

light of intensity I will be attenuated in the sea, at a distance x, to an irradiance E, where c is the light attenuation coefficient (Jerlov, 1968). Using formula (2) and an attenuation coefficient of 0.05 for Type I Hawaiian waters (Jerlov, 1968), the approximate luminescent output of an individual *P. verticillata* zooid, calculated from the colony maximum flux and total number of zooids, decays to the colonial photic half-response constant at 2.6 m, assuming no absorption or scattering due to pigments in the colony. Finally, the variation among zooids in their half-response constants reflects their ability to respond independently to light.

Progression of the colonial luminescent wave is not dependent upon intact connections between the zooids, clearly indicating that the wave propagates by a photic process (Burghause, 1914). Mackie and Bone (1978) photically stimulated the tetrazooid of *P. atlanticum* and calculated that, if the luminescent wave were propagated

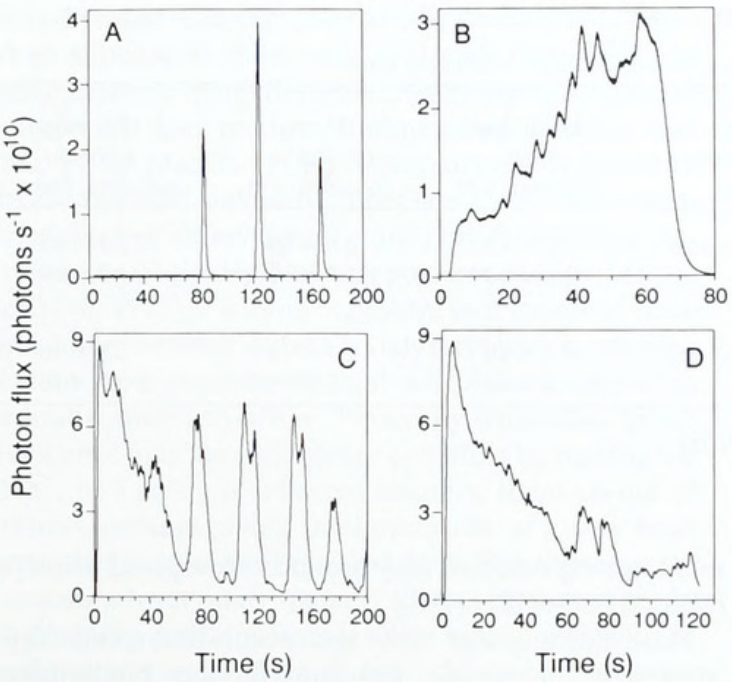

Figure 7. Examples of electrically induced bioluminescence of *Pyrosomella verticillata*. Fifty-volt, 5-ms duration pulses were applied to the medium. (A) Single stimulus delivered at 80 s, 120 s, and 165 s; each produced a simple response. (B) Stimuli delivered at 1 Hz for 60 s, starting at time = 0, showing temporal summation. (C) Constant 5 Hz stimulation, yielding a regular flashing pattern, perhaps due to an 18-s refractory period characteristic of the zooids. (D) Constant 10 Hz stimulation, eliciting light at a similar initial rate to (C), but without a regular flashing pattern.

by the serial excitation of zooids, the colony propagation velocity, taking into account the response delay and the distance between zooids, would be 2.0–4.0 mm · s^{-1}. This value is remarkably similar to the observed saltatory propagation rate of 2.1–4.1 mm · s^{-1} found in this study of mature colonies. Mackie and Bone also found no nerves or gap junctions associated with the light organ, and deemed it unlikely that conducting epithelia could propagate this activity, because the normal rate of epithelial conduction in tunicates is about 20 cm · s^{-1}. In addition, no specific cellular depolarizations were associated with flashing, and the mantle epithelium, to which the light organ is attached, is not a conducting type in the taxonomically similar ascidians. Luminescent waves typically propagate in the colonial coelenterate *Renilla* at 6–10 cm · s^{-1} (Nicol, 1955; Morin and Cooke, 1971), and 20–50 cm · s^{-1} in hydrozoa (Widder *et al.*, 1989). These high propagation rates are enabled by the underlying nervous tissue. These data indicate that the luminous wave is propagated by a photic chain reaction.

The mechanism underlying the saltatory conduction of luminescence may derive in part from the different photic half-response constants of the zooids. This is a reasonable finding, as each zooid contains its own light detection and production organs (Bone and Mackie, 1982; Mackie, 1986). The zooids thus seem to act independently of one another, rather than as a single, integrated colonial receptor.

The variation in colony half-response constant may be due in part to colony size. Larger colonies necessarily absorbed a larger number of photons entering the sphere; less light is therefore incident per zooid for a given number of photons. Thus, half-response constants may have been artificially elevated for larger colonies.

Continuous excitation of a colony often produced a rhythmic colonial flashing pattern, characterized by alternating periods of high and low light emission. Although some light is produced between flashes under these conditions, the majority of zooids are quiescent. Zooids thus appear to possess a refractory period of about 18-s duration, during which their half-response constant is greater than the stimulation that they receive.

The existence of a refractory period is further supported by the quenching of a single wave of luminescence elicited by a local mechanical stimulus. In long colonies, light

Table III

Kinetics and intensities of Pyrosomella verticillata *flashes stimulated with single or repetitive electrical pulses. 50-V, 5-ms pulses were applied to the medium. Values represent the mean ± standard error of the mean*

Stimulus type	Latency (s)	Rise time (s)	98% Duration (s)	Maximum flux (photons · s^{-1})	Mean emission (photons · s^{-1})	Quantum emission (photons · flash^{-1})
Single (n = 4)	5.6	2.1 ± 0.3	8.8 ± 1.6[a]	2.3×10^{10} $\pm 6.6 \times 10^{9}$	6.1×10^{9}[a] $\pm 1.3 \times 10^{9}$	5.9×10^{10} $\pm 2.1 \times 10^{10}$
Multiple (n = 11)	3.4 ± 1.2	10.1 ± 2.7	78.3 ± 21.8[a,b]	6.6×10^{10} $\pm 1.4 \times 10^{10}$	2.7×10^{10}[a] $\pm 5.7 \times 10^{9}$	3.7×10^{12} $\pm 1.5 \times 10^{12}$

[a] Means are significantly different (*t*-test, $P < 0.05$).

[b] Mean underestimates the actual value, because some responses persisted beyond the data collection period.

Table IV

Kinetics and intensities of mechanically stimulated pyrosome flashes. Values represent the mean ± standard error

Species	Stimulus type	Rise time (s)	98% Duration (s)	Maximum flux (photons · s^{-1})	Mean emission (photons · s^{-1})	Quantum emission (photons · $flash^{-1}$)
Pyrosoma atlanticum	Constant prodding (n = 9)	20.0 ± 1.6	59.2 ± 14.6	3.3×10^{12} [a] $\pm 3.1 \times 10^{11}$	6.6×10^{11} $\pm 6.0 \times 10^{11}$	2.3×10^{13} $\pm 1.9 \times 10^{13}$
Pyrosome sp.	Ship movement (n = 6)	8.3 ± 3.0	25.2 ± 6.7	7.5×10^{11} [a] $\pm 6.8 \times 10^{11}$	1.7×10^{11} $\pm 1.3 \times 10^{11}$	3.8×10^{12} $\pm 2.7 \times 10^{12}$

[a] Means are significantly different between stimulus methods (*t*-test, $P < 0.05$).

from the stimulus area was extinguished when the wave of light was at the far end of the colony. As a zooid's maximum flux decays to the colonial half-response constant at about 2.6 m, the wave of light should be able to reexcite the previously responsive parts of the colony. Reexcitation, however, is rarely observed, with a colonial response to a single stimulus usually subsiding after one pass across the colony.

The bioluminescent response to light flashes implies several uses of light for the colony. Using Allard's law for Hawaiian waters, the maximum flux for *P. atlanticum* (mechanical stimuli) would decline to the photic half-response constant at 78 m. Few zooids in a colony would respond, however, to this dim level of luminescence. The maximum quantum emission observed to photic stimuli in this study could be induced at a distance of 17 m, indicating that flash entrainment among colonies may occur at large distances. Roe *et al.* (1987) report a maximum of 85 colonies per 10,000 m^3 at 800 m in the Atlantic near the Canary Islands. The model of closest packing of equal spheres allows for one colony every 5.5 m, indicating that, in some areas of the ocean, flash entrainment among colonies may produce widespread displays (Mackie and Mills, 1983).

Flash entrainment may also occur interspecifically. Most other planktonic organisms produce bioluminescence of the same wavelengths as that of pyrosomes (Young, 1981; Herring, 1983; Widder *et al.*, 1983; Latz *et al.*, 1988); this light could also stimulate pyrosome luminescence if it were of sufficient intensity. For example, a flash from the common copepod *Pleuromamma xiphias* in Hawaiian waters would be sufficient to elicit luminescence in *P. atlanticum* at 14 m (Latz *et al.*, 1987).

Luminescence is often produced in response to a disturbance by a predator, and is conventionally thought to confer protection by startling or blinding the predator, or by attracting a secondary predator (David and Conover, 1961; Morin, 1983; Young, 1983; Buskey and Swift, 1983,

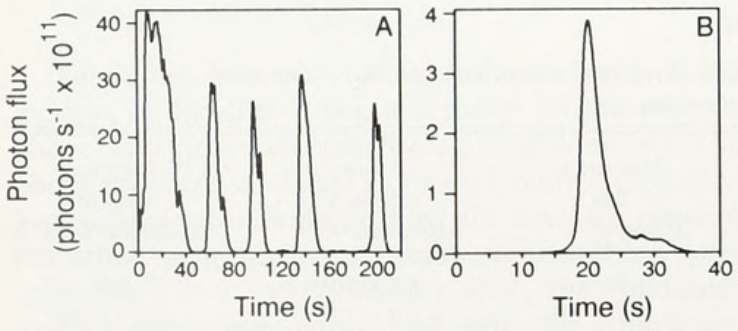

Figure 8. Examples of luminescence by *Pyrosoma atlanticum* induced by mechanical stimulation. (A) Periodic stimuli (1–2 Hz) produced a regular flashing pattern similar to that caused by repetitive electrical stimulation. Individual flashes within the pattern averaged 59-s duration. (B) A simple flash presumably induced by ship motion.

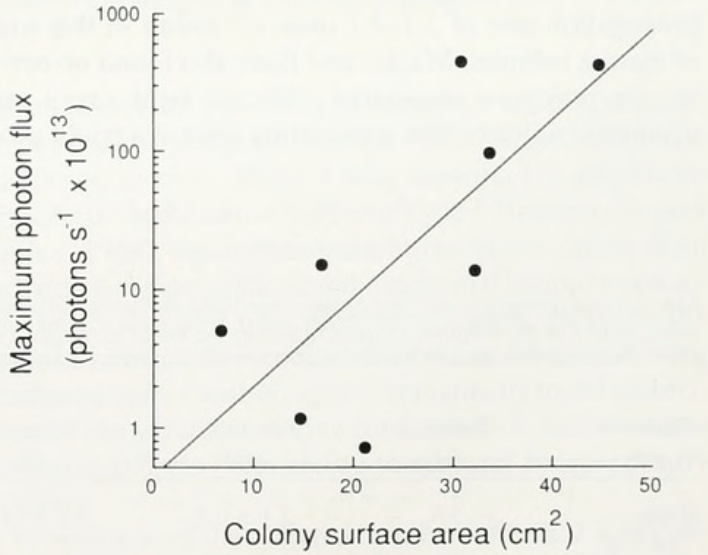

Figure 9. Maximum bioluminescence production as a function of colony surface area, including both species. Luminescence was induced by mechanical stimulation, as this method produced the greatest light emission and thus most closely approximated a colonial response of all zooids. The slope of the exponential regression is 0.15 ($r^2 = 0.55$).

1985). Pyrosomes also display an additional set of behaviors in response to photic stimuli; zooids close their oral openings, arrest their cilia (resulting in the suspension of locomotion), and produce luminescence (Mackie and Bone, 1978; Mackie, 1986). Being negatively buoyant, the colony would sink into deeper layers until the recommencement of ciliary action, thus perhaps evading predation by leaving a depth of high predator density (Mackie and Bone, 1978). Flashing may serve as a means of communication between distant zooids or colonies, enabling them to close protectively and sink before oncoming harmful stimuli can arrive. Photically stimulated bioluminescence may also discourage predators by making the colony, or a group of adjacent colonies, loom up out of darkness, perhaps giving the impression of a very large source that should not be trifled with. Simultaneous luminescence from many spatially separated sources might also distract a predator from a single target, analogous to the simultaneous displays of fish schools in the photic zone (Radakov, 1973; Morin, 1983).

Acknowledgments

The authors are grateful to the captain and crew of the RV *New Horizon* and to J. Favuzzi, T. Frank, M. Latz, A. Mensinger, and S. Bernstein for technical assistance. Ship time was generously provided by J. Childress. Supported by the Office of Naval Research (Contracts N00014-84-K-0314 and N00014-87-K-0044).

Literature Cited

Bone, Q., and G. O. Mackie. 1982. Urochordata. Pp. 473–534 in *Electrical Conduction and Behavior in 'Simple' Invertebrates,* G. A. B. Shelton, ed. Clarendon Press, Oxford.

Bowlby, M. R., and J. F. Case. 1988. Bioluminescence of photically stimulated pyrosomes. *Am. Zool.* **28**(4): 191A.

Buchner, P. 1965. Symbiosis in luminous animals, pp. 543–605 in *Endosymbiosis of Animals with Plant Microorganisms.* Interscience, New York.

Burghause, F. 1914. Kreislauf und Herzschlag bei *Pyrosoma giganteum* nebst Bemerkungen zum Leuchtvermogen. *Z. Wiss. Zool.* **108:** 430–497.

Buskey, E. J., and E. Swift. 1983. Behavioral responses of the coastal copepod *Acartia hudsonica* (Pinhey) to simulated dinoflagellate bioluminescence. *J. Exp. Mar. Biol. Ecol.* **72:** 43–58.

Buskey, E. J., and E. Swift. 1985. Behavioral responses of oceanic zooplankton to simulated bioluminescence. *Biol. Bull.* **168:** 263–275.

Childress, J. J., A. T. Barnes, L. B. Quetin, and B. H. Robison. 1977. Thermally protecting cod ends for the recovery of living deep-sea animals. *Deep-Sea Res.* **25:** 419–422.

David, C. N., and R. J. Conover. 1961. Preliminary investigation on the physiology and ecology of luminescence in the copepod *Metridia lucens. Biol. Bull.* **121:** 92–107.

Griffin, D. J. G., and J. C. Yaldwyn. 1970. Giant colonies of pelagic tunicates (*Pyrosoma spinosum*) from SE Australia and New Zealand. *Nature* **226:** 464–465.

Hardie, R. C. 1979. Electrophysiological analysis of fly retina. I: Comparative properties of R 1–6 and R 7 and 8. *J. Comp. Physiol.* **129:** 19–33.

Herring, P. J. 1983. The spectral characteristics of luminous marine organisms. *Proc. R. Soc. Lond. Ser. B.* **220:** 183–217.

Herring, P. J., and A. T. Barnes. 1976. Light-stimulated bioluminescence of *Thalassocaris crinita* (Dana) (Decapoda, Caridea). *Crustaceana* **31(1):** 107–110.

Huxley, J. 1936. *T. H. Huxley's Diary of the Voyage of H.M.S. Rattlesnake.* Doubleday, Garden City, New York. 301 pp.

Jerlov, N. G. 1968. *Optical Oceanography.* Elsevier Publishing, New York. 194 pp.

Kay, R. H. 1965. Light-stimulated and light-inhibited bioluminescence of the euphausiid *Meganyctiphanes norvegica* (G. O. Sars). *Proc. R. Soc. Lond. Ser. B.* **162:** 385–386.

Lábas, I. A. 1980. Luminescent signalling in the ctenophores. Pp. 41–49 in *The Theoretical and Practical Importance of the Coelenterates,* D. V. Naumov and S. D. Stepan'iants, eds. Zoological Institute, Academy of Sciences, U.S.S.R., Leningrad.

Lapota, D., J. R. Losee, and M. L. Geiger. 1986. Bioluminescence displays induced by pulsed light. *Limnol. Oceanogr.* **31**(4): 887–889.

Latz, M. I., T. M. Frank, M. R. Bowlby, E. A. Widder, and J. F. Case. 1987. Variability in flash characteristics of a bioluminescent copepod. *Biol. Bull.* **173:** 489–503.

Latz, M. I., T. M. Frank, and J. F. Case. 1988. Spectral composition of bioluminescence of epipelagic organisms from the Sargasso Sea. *Mar. Biol.* **98:** 441–446.

Laughlin, S. B. 1975. Receptor function in the apposition eye- an electrophysiological approach. Pp. 479–498 in *Photoreceptor Optics,* A. W. Snyder and R. Menzel, eds. Springer-Verlag, New York.

Laughlin, S. B. and R. C. Hardie. 1978. Common strategies for light adaptation in the peripheral visual systems of fly and dragonfly. *J. Comp. Physiol.* **128:** 319–340.

Leisman, G., D. H. Cohn, and K. H. Nealson. 1980. Bacterial origin of luminescence in marine animals. *Science* **208:** 1271–1273.

Mackie, G. O. 1986. From aggregates to integrates: physiological aspects of modularity in colonial animals. *Phil. Trans. R. Soc. Lond. B.* **313:** 175–196.

Mackie, G. O., and Q. Bone. 1978. Luminescence and associated effector activity in *Pyrosoma* (Tunicata: Pyrosomida). *Proc. R. Soc. Lond. Ser. B.* **202:** 483–495.

Mackie, G. O., and C. E. Mills. 1983. Use of the *Pisces IV* submersible for zooplankton studies in coastal waters of British Columbia. *Can. J. Fish. Aquat. Sci.* **40:** 763–776.

Morin, J. G. 1983. Coastal bioluminescence: patterns and functions. *Bull. Mar. Sci.* **33**(4): 787–817.

Morin, J. G. and I. M. Cooke. 1971. Behavioral physiology of the colonial hydroid *Obelia.* II. Stimulus-initiated electrical activity and bioluminescence. *J. Exp. Biol.* **54:** 707–721.

Neshyba, S. 1967. Pulsed light stimulation of marine bioluminescence *in situ. Limnol. Oceanogr.* **12:** 222–235.

Neumann, G. 1934. Pyrosomida. Pp. 226–323 in *Handbuch der Zoologie,* Vol. 5, pt 2, W. Kükenthal and T. Krumbach, eds. W. de Gruyter Publishing, Berlin.

Nicol, J. A. C. 1955. Nervous regulation of luminescence in the sea pansy *Renilla kollikeri. J. Exp. Biol.* **32:** 619–635.

Nicol, J. A. C. 1960. The regulation of light emission in animals. *Biol. Rev.* **35:** 1–42.

Panceri, P. 1873. The luminous organs and light of *Pyrosoma. Q. J. Microsc. Sci.* **13:** 45–51.

Pierantoni, U. 1921. Gli organi luminosi simbiotici ed il loco ciclo ereditario in *Pyrosoma giganteum. Pubbl. Staz. Zool. Napoli* **3:** 191–221.

Polimanti, O. 1911. Uber das Leuchten von *Pyrosoma elegans* Les. *Z. Biol.* **55:** 502–529.

Radakov, D. V. 1973. *Schooling in the Ecology of Fish.* J. Wiley, New York. 173 pp.

Roe, H. S. J., J. Badcock, D. S. M. Billett, K. C. Chidgey, P. A. Domanski, C. J. Ellis, M. J. R. Fasham, A. J. Gooday, P. M. D. Hargreaves, Q. J. Huggett, P. T. James, P. A. Kirkpatrick, R. S. Lampitt, N. R. Merrett, A. Muirhead, P. R. Pugh, A. L. Rice, R. A. Russell, M. H. Thurston, P. A. Tyler. 1987. Great Meteor East: a biological characterization. Institute of Oceanographic Sciences Deacon Laboratory, Report No. 248, 332 pp.

Swift, E., W. H. Biggley, and T. A. Napora. 1977. The bioluminescence emission spectra of *Pyrosoma atlanticum, P. spinosum* (Tunicata), *Euphausia tenera* (Crustacea) and *Gonostoma* sp. (Pisces). *J. Mar. Biol. Assoc. U. K.* **57:** 817–823.

van Soest, R. W. M. 1981. A monograph of the order Pyrosomatida (Tunicata, Thaliacea). *J. Plank. Res.* **3(4):** 603–631.

Tett, P. B. 1969. The effects of temperature upon the flash stimulated luminescence of the euphausiid *Thysanoessa raschii. J. Mar. Biol. Assoc. U. K.* **49:** 245–258.

Tett, P. B. 1972. An annual cycle of flash induced luminescence in the euphausiid *Thysanoessa raschii. Mar. Biol.* **12:** 207–218.

Tsuji, F. I., R. W. Lynch, and Y. Haneda. 1970. Studies on the bioluminescence of the marine ostracod *Biol. Bull.* **139:** 386–401.

Tyler, J. E., and R. W. Preisendorfer. 1962. Transmission of energy within the sea. Pp. 397–451 in *The Sea,* M. N. Hill, ed. Interscience, New York.

Widder, E. A., S. A. Bernstein, D. F. Bracher, J. F. Case, K. R. Reisenbichler, J. J. Torres, and B. H. Robison. 1989. Bioluminescence in the Monterey Submarine Canyon: image analysis of video recordings from a midwater submersible. *Mar. Biol.* **100:** 541–551.

Widder, E. A., M. I. Latz, and J. F. Case. 1983. Marine bioluminescence spectra measured with an optical multichannel detection system. *Biol. Bull.* **165:** 791–810.

Young, R. E. 1981. Color of bioluminescence in pelagic organisms. Pp. 72–81 in *Bioluminescence: Current Perspectives,* K. H. Nealson, ed. Burgess Publishing, New York.

Young, R. E. 1983. Oceanic bioluminescence: an overview of general functions. *Bull. Mar. Sci.* **33**(4)**:** 829–845.