Deep-Sea Research, Vol. 38, No. 2, pp. 201–217, 1991.
Printed in Great Britain.

0198–0149/91 $3.00 + 0.00
© 1991 Pergamon Press plc

Mechanical stimulation of bioluminescence in the deep Pacific Ocean

M. S. WEBSTER,* C. E. ROOS,* A. ROBERTS,† A. OKADA,‡ Y. OHASHI,‡
D. O'CONNOR,† R. MITIGUY,§ S. MATSUNO,† R. MARCH,‖ J. G. LEARNED,†
D. KARL,¶ J. CLEM,* G. BLACKINTON,**†† H. BRADNER‡‡ and J. BABSON†

(*Received* 5 *January* 1990; *in revised form* 7 *August* 1990; *accepted* 15 *August* 1990)

Abstract—Measurements of bioluminescence have been made at depths of about 4500 m with (1) freely falling, (2) ship-suspended and (3) bottom anchored instruments. A comparison of these measurements shows that most of the light recorded by ship-suspended measurements at great depths is bioluminescence stimulated by the motion of the ship on the surface translated to the detector at depth. Most of the light observed in all of these measurements is from flashes. An estimate of the intensity distribution of source brightness of flashes is presented. The observed flashes were produced more frequently within a few meters of the detector than in an equal volume of water at greater distance in the ocean.

INTRODUCTION

LIGHT in the deep ocean is contributed by physical and by biological sources. The physical sources are discussed by BRADNER *et al.* (1988), who conclude that Cherenkov light from electrons emitted in the decay of ^{40}K is the dominant source of light from physical effects and that the flux from this source is about 120 visible photons $cm^{-2}\,s^{-1}$. The median light level recorded in Bradner's ship-suspended measurements at 4500 m is about a factor of 20 higher. Much of the light came as flashes and the flux during the quiet times between flashes dropped to less than about three times the estimate of Cherenkov light produced by ^{40}K decay.

Bioluminescence from marine organisms at great depths is well known, and is often induced by mechanical stimulation (CLARKE and BACKUS, 1956; CLARKE and WERTHEIM, 1956; HASTINGS and SWEENEY, 1958; CLARKE and HUBBARD, 1959; NICOL, 1967; KELLY and TETT, 1978; KRASNOW *et al.*, 1980, 1981; HAAS, 1980; YOUNG *et al.*, 1980; NEALSON, 1981; SWEENEY, 1981; YOUNG, 1981; ANDREWS *et al.*, 1984). CLARKE and HUBBARD (1959)

*Department of Physics and Astronomy, Vanderbilt University, Nashville, TN 37235, U.S.A.
†Hawaii DUMAND Center, University of Hawaii, Manoa, HI 96822, U.S.A.
‡Institute of Cosmic Ray Research, University of Tokyo, Japan.
§Hawaii Institute of Geophysics, University of Hawaii, Manoa, HI 96822, U.S.A.
‖Physics Department, University of Wisconsin, Madison, WI 53706, U.S.A.
¶School of Ocean and Earth Sciences and Technology, University of Hawaii, Manoa, HI 96822, U.S.A.
**Hawaii Institute of Geophysics, University of Hawaii, Manoa, HI 96822, U.S.A.
††Present address: Seafloor Surveys, Int'l. Inc., 1221 Kapiolani Blvd., PH40, Honolulu, HI 96814, U.S.A.
‡‡Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92093, U.S.A.

reported a 6 s periodicity in the observation of flashes at a depth of about 1 km and associated it with the roll of the ship. LOSEE (1982) reported seeing bioluminescence around the propellers of a deep submersible at 3500 m any time the submersible moved, and BRADNER *et al.* (1988) reported significant differences between light levels when raising the instrument and when holding it at constant cable length. These reports of stimulated bioluminescence clearly demonstrate the near omnipresence of bioluminescent organisms of unidentified type, even at 4500 m depth, but do not yield estimates of the relative rates of stimulated and spontaneous bioluminescence. AOKI *et al.* (1986) found a factor of 10 between the rates while the detector was suspended from the ship and while it was anchored from the ocean floor. Their bottom-tethered rates are at most a factor of two greater than the ^{40}K estimate. Their result was the first quantitative comparison of bioluminescence observed with ship-suspended instruments to measurements with bottom-tethered instruments, and it indicated a dramatic difference between the two. We reinforce their observations with records of the light intensity measured by our instrument as it came to rest above the ocean floor, and we also compare the intensity measured during fall, just before coming to rest, with that measured by BRADNER *et al.* (1988).

Most previous measurements have used single detectors. Consequently it was not possible to determine either the distance to the organism which produced a flash or the source strength. In the experiments reported here we used two detectors which were sensitive to sources in a common volume of ocean; this permitted a rough determination of distance and source strength in the experiment. Comparison of the number of flashes which occurred in the volume of water viewed by both detectors with the number seen by one of the detectors in a larger volume of ocean enables us to show that the rate of occurrence of flashes per unit volume of water was at least four times greater within a few meters of the apparatus than at greater distance in the ocean.

MATERIALS AND METHOD

Light detection apparatus

The light detectors for this experiment consisted of two photomultipliers, one 5.5 m above the other (Fig. 1). The sensitivity of the lower photomultiplier (BRADNER *et al.*, 1988) was such that a uniform illumination of 300 photons $cm^{-2}\ s^{-1}$, several times that expected from ^{40}K, was detectable. This photomultiplier faced upward in its cylindrical baffle and was sensitive to sources within a cone of about 25° from the vertical. The larger photomultiplier was about 10 times as sensitive for point sources on the axis of the tube (30 photons $cm^{-2}\ s^{-1}$). It was housed in transparent material, and its sensitivity was approximately linear in the cosine of the angle between the downward direction and the location of the source. It was mounted with its direction of greatest sensitivity toward the other tube and the sensitivity in the backward (that is, from above) direction was 5% of the sensitivity for sources directly below the tube.

The instrument used for our measurements is a modification of that described by BRADNER *et al.* (1988), which used only the 5-inch phototube. The second, 16-inch, photomultiplier was added, the integration times were increased to 1 s, the anode currents were each read once per second, and the readings from the two photomultipliers were interleaved on the cassette tape. The response time of the detectors was very fast (microseconds), so a fast pulse would be recorded as a step with a 1 s decay set by the

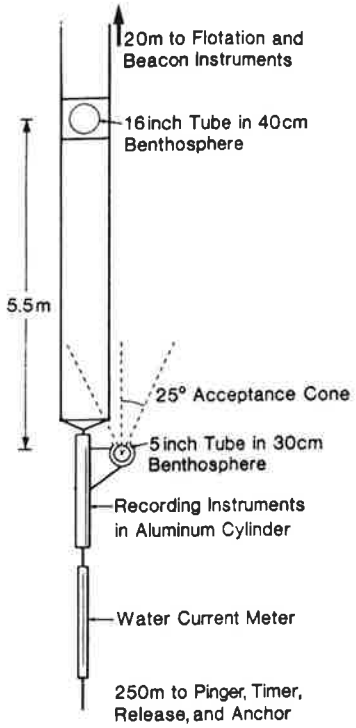


Fig. 1. The arrangement of the detectors and recording instruments in the central portion of the deployed string. The upper detector was mounted in a transparent housing with its direction of greatest sensitivity facing downward so that both detectors recorded flashes produced within the conical volume of water defined by the 25° acceptance of the lower detector and extending above the upper detector.

integration time constant. A light-emitting diode was mounted in the benthosphere with the 5-inch photomultiplier to provide a short, weak flash every 20 s to verify proper response from the photomultiplier. (The response to this input is shown with the data in Figs 2 and 4d.) The electronics, packaging and calibration of the 5-inch photomultiplier are described in more detail by BRADNER *et al.* (1988). The anchor, timer and release, and pinger were attached with 250 m of plastic rope to a current meter which was attached with a short cable to the bottom of the cylinder which housed the electronics. The 5-inch photomultiplier, inside a Benthosphere and cylindrical baffle, was mounted to the side of the cylinder looking upward. The 16-inch photomultiplier in its transparent plastic housing and Benthosphere was attached to the top of the electronics cylinder with 5 m of stainless steel cable. The floats, radio beacons, and strobe flashers were attached to the top of the housing of the 16-inch photomultiplier by a 20 m length of stainless steel cable. The anchor and flotation were adjusted so that the anchor would rest on the bottom with the photomultipliers about 250 m above the anchor and the floats and recovery beacons 20 m above the photomultipliers. The radio beacons and strobe lights were active only when near the surface. A 12 kHz, 1 s period, acoustical pinger was attached 25 m above the anchor.

Deployment

This instrument was deployed from the R.V. *Kana Keoki* in the basin west of Keahole Point, off the Island of Hawaii, at a depth of 4700 m. The precise location was determined from shore transponders as (19°37.9345'N, 156°34.986'W). This is the region selected for the DUMAND (Deep Underwater Muon and Neutrino Detector) array. After free-falling for 44 min, the anchor struck bottom at 0235, local time, 11 January 1984. This event was identified by the ship sonar, which showed no rebound of the detector package. The batteries and the cassette recorder were adequate for 2 days of operation, and the timers were set to release the anchor at approximately 1730 the following day. The electronics package was in operation at the time of launch, and the high voltage was applied to the photomultipliers by action of a pressure-activated switch at approximately 500 m.

The explosive bolts fired as scheduled but failed to release the anchor. Initial attempts to recover the instruments by trawling with a hook on the bottom succeeded in making contact with the anchor rope of the instrument, but did not hook on to it so that it could be brought to the surface. Consequently it was not recovered until 18 months later, in July 1985, with the aid of a new, side-scan sonar device from the R.V. *Melville*. The instrument had sustained little damage despite the prolonged exposure to the ocean, and the data were successfully recovered from the cassette tape. There was no evidence of any biological growth on the package.

RESULTS AND DISCUSSION

Stimulation of bioluminescence by motion

The most striking result from this experiment is the abrupt decrease in the level of light recorded by both photomultipliers when the instrument came to rest 250 m above the bottom. These data are shown in Fig. 2 for both photomultipliers. The zero of the time axis has been taken from the ship sonar determination of the time at which the anchor struck bottom. The error in locating this zero on the axis for these plots is about half a minute and is due primarily to uncertainty in the synchronization of the internal clock of the instrument with the ship clock. It has been cross-checked against several other events, such as the application of high voltage at 500 m. For negative times on the axis, the instrument was falling at its equilibrium or limiting velocity of 100 m min^{-1}. Thus it was 1000 m above the bottom, at a depth of 3500 m, at the time labeled as -10 min.

The regular pulses which are evident in the 5-inch tube data shown in Fig. 2 for times greater than about 2 min are calibration pulses from a photodiode mounted in the collimator which surrounds the tube. The appearance of these pulses with the expected height and frequency gives us confidence that the instrument was operating properly. They were overwhelmed by the bioluminescence from outside while the tube was falling. The dramatic decrease in the signals from both tubes, accurately coincident with the anchor coming to rest on the bottom, is strong evidence that most of the signal recorded during the fall at great depth was, in fact, stimulated by the motion of the device through the water. Much of the light recorded during descent is clearly flashes, and the principal difference between falling and at rest is the much higher rate of occurrence of the flashes while in motion. Detailed study of the flashes while at rest shows that they are produced by organisms some distance from the detector, and analysis to be presented of ship-suspended measurements shows that the response to mechanical stimulation may occur

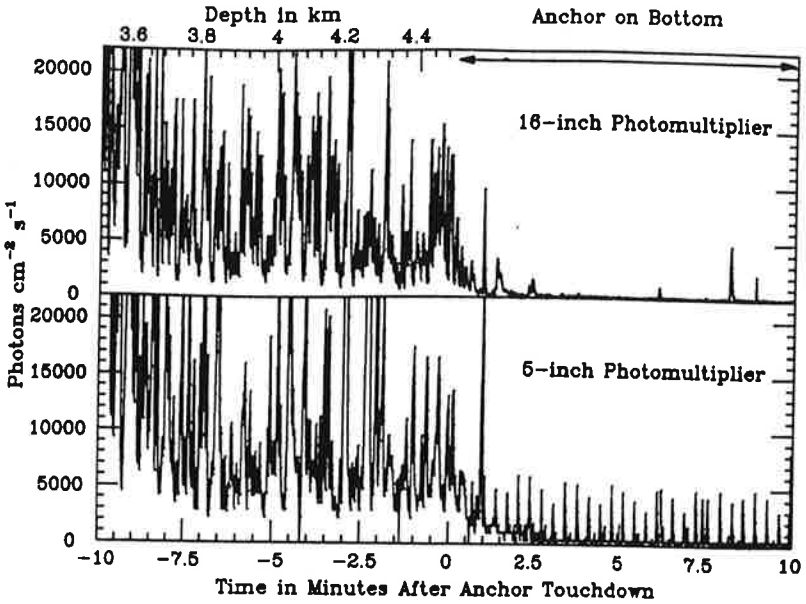


Fig. 2. The sharp decrease in bioluminescence at the end of the descent. The zero on the time axis is the time at which the anchor hit bottom.

after the stimulation. During descent the anchor preceded the instruments through the water by about 2.5 min. The water was again disturbed by the 5-inch tube which looked at the disturbed water above it. The 16-inch tube was oriented so that its direction of greatest sensitivity was toward the 5-inch tube. It was 5.5 m above the 5-inch tube, and thus went through the water which had been disturbed 3 s earlier.

The differences between freely falling, ship-suspended and bottom-tethered measurements also were reported recently by an experiment (Aoki *et al.*, 1986) which counted photoelectrons for brief intervals rather than integrating and continuously recording the anode current of the photomultipliers. Because our method of recording the data gave much more detailed information about the time structure of the pulses and we collected data for a much longer time, we consider our results to be an important confirmation of this earlier report of stimulation of bioluminescence at free-fall speeds of approximately 100 m min^{-1}. In order to compare data from this experiment with data from ship-suspended measurements, we have reanalysed data from Bradner *et al.* (1988). These data were taken with the same 5-inch photomultiplier and electronics except that the recording and integrating times were only 0.1 s in the earlier experiment. The data have been integrated numerically and are plotted at 1 s intervals in Fig. 3 to simulate the data-recording procedures of the present experiment and thus to permit direct comparison with Fig. 2. Figure 3 covers the time at which the recovery was started, and the zero on the plot is the time at which winching was begun. Since the photomultiplier was used pointing downward in the earlier experiment, the visibility of water in the wake of the device is similar for fall in this experiment and recovery in the Bradner *et al.* (1988) experiment. The plot is qualitatively similar to the data from the 5-inch tube in the part of Fig. 2 before the anchor struck bottom, strongly suggesting that most of the bioluminescence at great depth

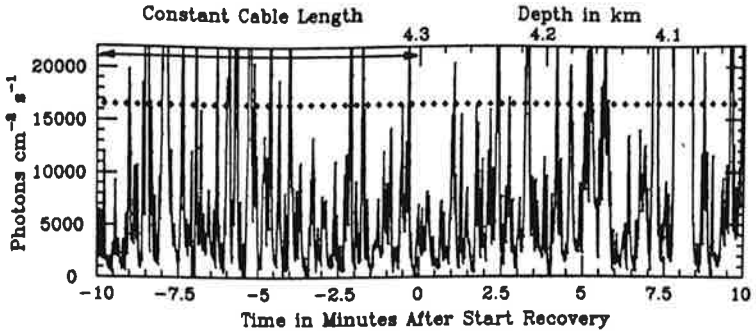


Fig. 3. Reanalysed data from BRADNER *et al.* (1988). The data have been numerically integrated and plotted at 1 s intervals to simulate the response of the tube with the method of recording data which was used in the present experiment. The small diamonds show the times at which the calibration pulses were evident in the data before the integration.

reported by BRADNER *et al.* (1988) was stimulated. The analysis of medians showed an increase of less than a factor of two between the constant cable length data and the retrieval at 40 m min^{-1}. This small difference is not readily discernible in Fig. 3, and both parts are qualitatively similar to the free-fall data of Fig. 2.

CLARKE and HUBBARD (1959) observed a component with 6 s period in part of their data, but argued that the presence of a much larger component without obvious periodicity showed that mechanical stimulation was responsible for only a small part of the light they observed. The data of BRADNER *et al.* (1988) while the detector was at depth have been reanalysed for periodicity by studies of the Fourier coefficients of intensity and of the time correlation of the identified pulses. The conclusion is that there is weak evidence for a component with a period of about 17 s, and that such a period is compatible with the expected swells on a calm day, such as that of the deployment of the experiment. The data are not consistent with more than about a 30% periodic component. Thus both the CLARKE and HUBBARD (1959) data at 1 km and the BRADNER *et al.* (1988) data at 4.5 km are consistent with the interpretation that at least part of the observed bioluminescence shows the periodic structure of the motion of the ship which supports the detector. However, by comparing the Bradner data with the present data, we conclude that most of the light measured in ship-suspended experiments, such as Bradner's is stimulated by coupling of the instrument to the motion of the ship. The evidence that most of the observed bioluminescence has been stimulated is very clear in the comparison of ship-suspended and bottom-tethered experiments, but does not show clearly in the analysis of the time structure of the ship-suspended data. The analysis of the time structure of the bioluminescence to search for evidence of periodic stimulation requires the further assumption that the response to the stimulation is prompt, like the response of surface microorganisms to a net which is pulled through the water. Evidently, the distribution in time of the response to the stimulation of the organisms at depth is so broad that it effectively averages out most of the periodic structure of the stimulation due to the motion of the ship on the waves. An example of the simple mechanism for producing such an increase not correlated with ship motion would be attraction of free-swimming organisms to the detector because of its oscillatory motion in the water and the secondary stimulation of other bioluminescent

organisms by the presence of the organisms attracted to the neighborhood of the disturbance.

Exponential decrease with depth

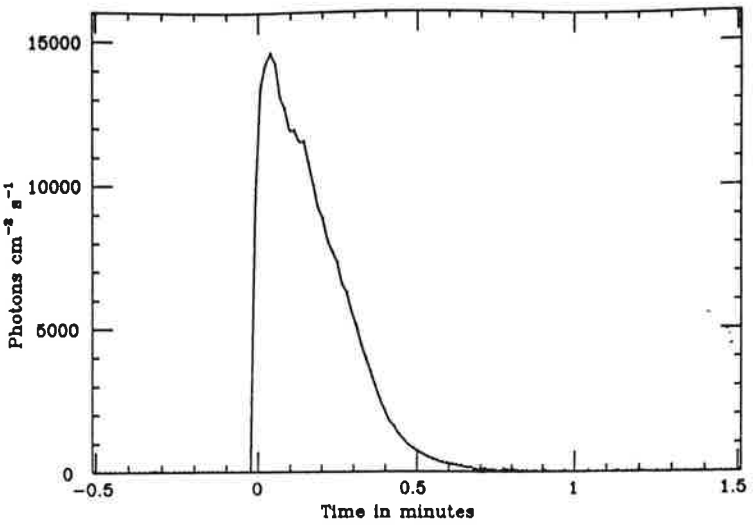
Even though most of the bioluminescence observed during free fall or by ship-suspended measurements is stimulated, the variation with depth does give information on the presence of organisms that can be stimulated to produce bioluminescence. BRADNER *et al.* (1988) and AOKI *et al.* (1986) found an exponential decrease with depth and that the *e*-fold reduction distance was approximately 1 km for depths greater than about 2 km. At shallower depths, the bioluminescence was larger than the values given by the extrapolation of this fit. The data presented here confirm this decrease of bioluminescence with depth. Our instrument reached its limiting velocity of 100 m min^{-1} within a few seconds after release from the ship, and we have computed the median light intensity at 500 m intervals during the free fall. The large photomultiplier was more sensitive and remained saturated most of the time above 2.5 km, so only data below 2.5 km from the large photomultiplier have been used in the calculation. We find that the data from the large photomultiplier are well represented by an exponential decrease, with a $1/e$ decrease in 0.8 km while the corresponding rate of decrease for the small tube is 1 km. Thus the general rate of decrease with depth is consistent with the previous measurements.

The observed exponential decrease in bioluminescence with increasing water depth was not unexpected. Because the primary energy source for the oceanic biota, solar radiation, is restricted to only the upper 200 m of the water column, the biomass of living organisms also decreases with increasing water depth in response to food availability (LONGHURST *et al.*, 1989). Based on data for the distribution of dissolved oxygen, WYRTKI (1962) developed an advection–diffusion model to describe an exponential decrease in oxygen consumption rates for the Atlantic Ocean. His *e*-fold reduction distance was 300 m for depths less than 1500 m. The limited data at greater depth (RILEY, 1951) show greater oxygen consumption than the simple extrapolation of WYRTKI's (1962) fit, and require an *e*-fold reduction distance approximately twice as large for depths greater than 1500 m. More recently, MARTIN *et al.* (1987) have used a normalized power function to describe the downward flux of particulate organic carbon in the northeast Pacific Ocean. These non-linear decreases in (a) total animal biomass, (b) the availability of particulate organic matter, (c) oxygen consumption, and (d) bioluminescence with increasing water depth are undoubtedly manifestations of the same environmental forcing function.

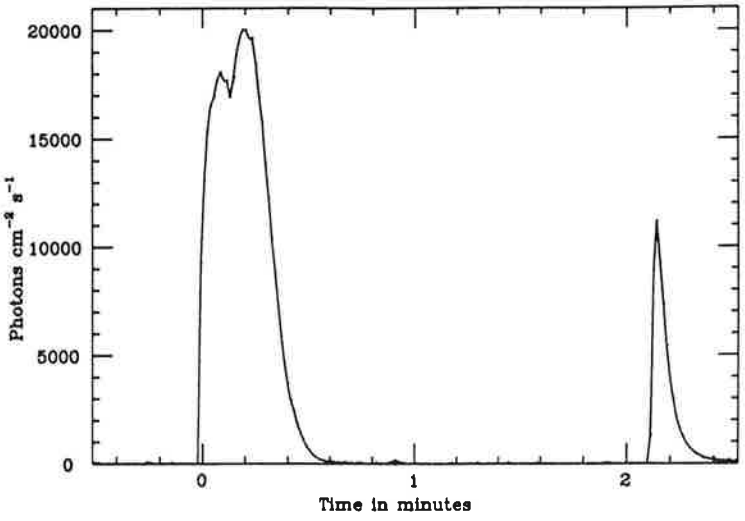
Brief, infrequent flashes dominate bioluminescence

The major contributor to light in the deep ocean is the flashes, which are much brighter than the ambient background. Examples that illustrate the type of pulses observed are shown in Fig. 4a–d. The brightest and longest duration pulse observed during the 52 h is shown in Fig. 4d. Note the almost periodic ripple structure as it diminishes. The upper detector is saturated at the peak of this flash. Thus the much sharper fall-off shown by the lower detector may be due to the source moving out of the sensitive cone of the lower detector about half a minute after the beginning of the flash, or the very bright part may simply have been suppressed by the saturation of the upper detector.

Three of these pulses were observed during time intervals which show particularly high bioluminescent activity, but during most of the time there was little activity. This contrast

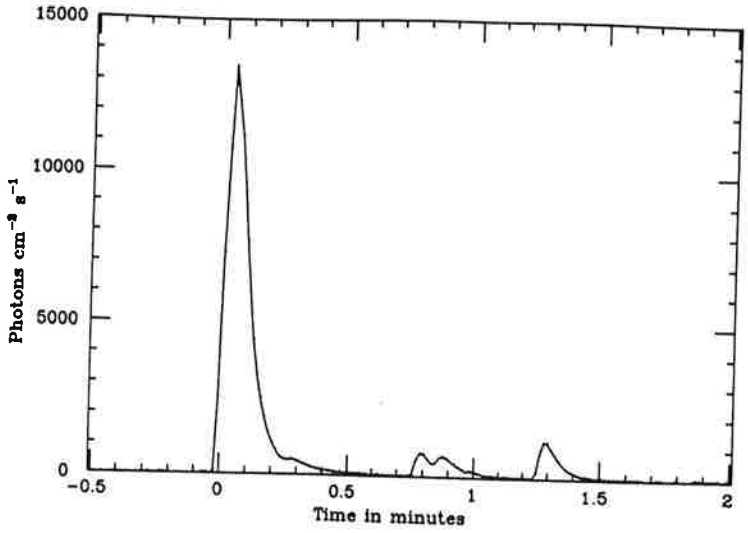


(*a*)

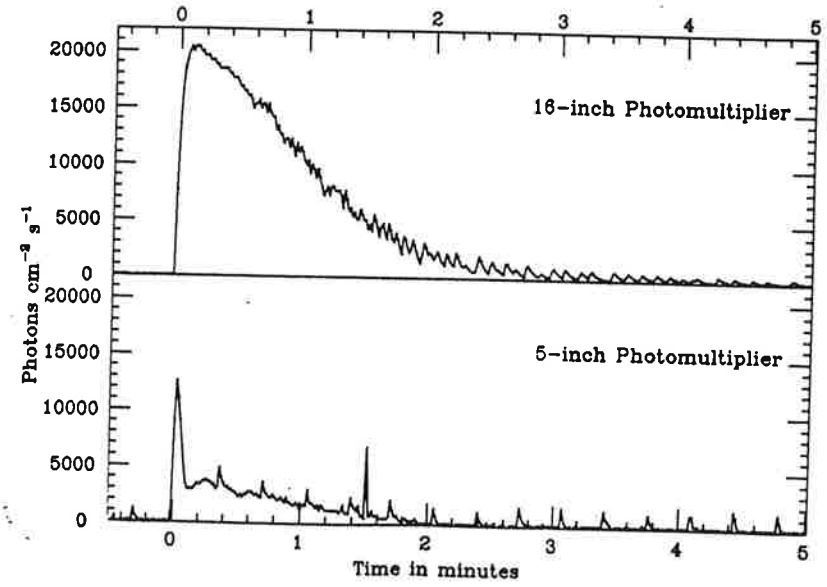


(*b*)

Fig. 4. Examples of observed pulses. Typical large flashes recorded by the 16-inch phototube are shown in (a), (b) and (c). The flash shown in (a) occurred during a quiet time 19.7 h after hitting bottom. The flash sequences shown in (b) and (c) occurred at 32.0 and 32.7 h after hitting bottom, during a relatively busy time. The largest flash seen in this experiment occurred 40.6 h after hitting bottom and the signals recorded by both tubes are shown in (d) with a common time scale. This large flash is one of a burst of flashes lasting an hour which occurred at this time.


15000
10000
5000
0
Photons cm^{-2} s^{-1}
−0.5
0
0.5
1
1.5
2
Time in minutes

(*c*)


0
1
2
3
4
5
20000
15000
10000
5000
0
16-inch Photomultiplier
5-inch Photomultiplier
Photons cm^{-2} s^{-1}
Time in minutes

(*d*)

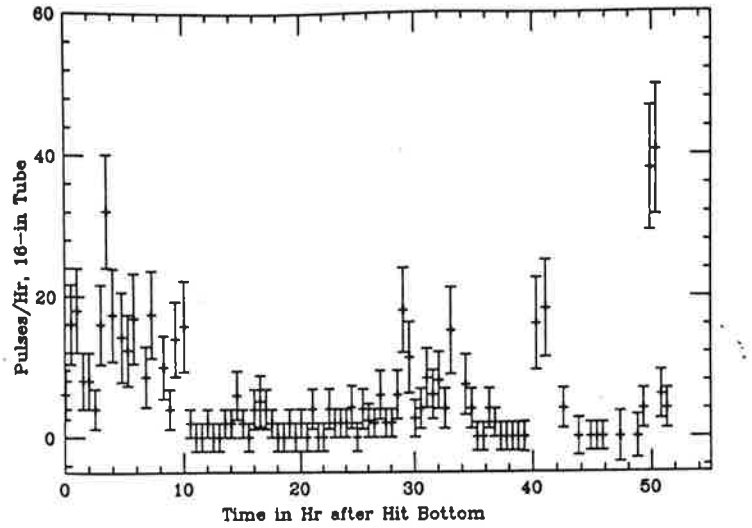


Fig. 5. The number of pulses per hour detected by the 16-inch photomultiplier.

is best illustrated by the plot of frequency of flashes detected by the 16-inch photomultiplier (Fig. 5). The pulses that rise above the low, constant background by 270 photons $cm^{-2} s^{-1}$ for more than 2 s are clearly identifiable and are counted to make this plot. This threshold for definition of a pulse is so low that a pulse too weak to be counted is barely visible on the scales chosen for Fig. 4, and the small pulses in Fig. 4c are about four times greater than this threshold. The average width at half height of these identified pulses is 6 s, so the widths of even the large pulses shown in Fig. 4 are fairly typical. The time is measured from the time at which the anchor hit bottom, and the very high rate during free-fall is not shown on the plot. The very high pulse activity at 52 h is the most striking feature of the plot and is positively identified with trawling to recover the instrument after it failed to appear on the surface. At 52 h, during the trawling operation, the separation in time between the direct signal from the acoustical pinger and its reflection from the bottom decreased, indicating that the trawling cable had intersected the anchor cable and had pulled the pinger down nearer the bottom. At approximately this same time, the current meter, which recorded extremely low current velocities (0.02–0.05 cm s^{-1}) during the rest of the time, showed a brief period of high current velocities, up to 20 times greater. This incident clearly explains the high pulse rate at 52 h and further substantiates the stimulation of bioluminescence by motion through the water.

The sound of the exploding bolts was heard on the ship's sonar system at 39 h. The burst of activity at 40 h shown in Fig. 5 begins an hour later, and there is no evidence that the explosions stimulated any bioluminescence. We may speculate that the high level of pulse activity at 1–10 h may be associated with surface organisms ensnared in the apparatus and carried down with it or that the disturbance created by the descent and landing of the instrument attracted organisms from a large region around the mooring site. The distribution of intensities of the flashes during this interval is not noticeably different from the distribution of intensities at other times, and the relative intensities of those pulses recorded by both detectors indicate that the organisms were not localized on either

detector housing. Thus attraction of free-swimming organisms from the region around the mooring site appears to be the more likely explanation, and the data presented here do not discriminate between models in which the attracted organisms are bioluminescent or stimulate bioluminescent organisms which are already present. While the model of ensnared surface organisms appears unlikely, the burst of activity at approximately 30 h would be consistent with the circadian rhythm (KRASNOW *et al.*, 1980) observed for some organisms.

Distribution of brightness of flashes

The intensity observed with a single detector from a flash in the ocean depends upon the source brightness as well as the distance of the source from the detector. The distribution of intensity recorded by a single detector is not generally sufficient information to determine the distribution of brightness of the population of flash sources. The additional information available from detection of a flash of light in two detectors some distance apart is very helpful in resolving this ambiguity between brightness and distance. As a simple example, this data sample includes concurrent flashes of approximately equal intensity recorded by both detectors. Since an organism would have to be approximately equidistant from the two detectors to produce such an event, it is not consistent with the hypothesis that the organism was stimulated to flash by contact with the housing of the detector. For two detectors with uniform angular sensitivity and for distances small compared with the attenuation length of the light in ocean water, the ratio of observed intensities determines the square of the ratio of distances from the two sources and thus defines a surface of possible source locations. For this experiment, an additional constraint was supplied by a mask which limited the angular sensitivity to 25° for the lower detector, the interior of the cone shown in Fig. 1. Because of the strong decrease in sensitivity of the upper detector for sources above it, the locus of possible locations in the ocean for a flash detected by both tubes is a closed surface surrounding the upper detector, and only the portion of that surface within the cone is acceptable as a solution. That is, identical detector responses would have been obtained for flashes of different brightness at different points on the solution surface, and only one of these points corresponds to the actual flash which was recorded. Because of the remaining ambiguity of the family of solutions for each flash detected in both detectors, we first present the data from the upper, more sensitive detector. The distribution of intensities observed in the upper detector is used to constrain the parameters, (a) density of organisms in the ocean and (b) distribution of flash brightness, of a model of organisms which are distributed uniformly throughout the ocean and which emit light isotropically during a flash. This constrained model is then used to predict the distribution of intensities expected at each detector and the number and locations of flashes which should be detected in both. The resulting conclusion is that the number of flashes observed in both detectors is much too large to be consistent with any model of uniform distribution throughout the ocean which is consistent with the intensity distribution and number of flashes seen in the upper detector. Thus we are forced to speculate that most of the pulses seen in both detectors result from a concentration of relatively weak flashers which have been attracted to the apparatus or of the resident population of bioluminescent organisms which has been stimulated to flash more frequently by another organism which has been attracted to the location.

The distribution of intensity at the upper detector for flashes detected during the 48 h of

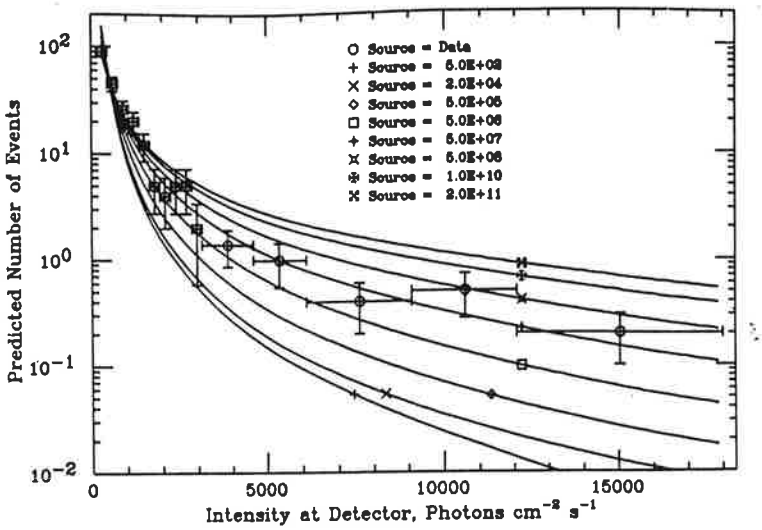


Fig. 6. Intensity spectrum of flashes observed at 16-inch phototube. Each curve is the simulation of the expected spectrum for an ocean uniformly populated with sources of the indicated brightness. The number of flashes plotted for each population has been normalized to the first 10 data points, so that a weighted sum of these should be considered to account for the data.

observation after the anchor hit bottom is compared with a simple model in Fig. 6. This model assumes that the ocean is uniformly populated with sources which emit light isotropically during a flash, and the intensity at each detector is then computed using the inverse square law of intensity, exponential attenuation of the light in the ocean water, and the angular sensitivity of the detectors. This simplest form of such a model uses the same source brightness for all flashes, and consequently is characterized by two parameters, the flash brightness and the product of source density in the ocean and rate of flashing. Each curve in Fig. 6 is the distribution of intensities expected for a population of flashing organisms of the labeled brightness, and the number of flashes per unit time per unit volume of ocean water has been adjusted, for each brightness, to the number of observed flashes in the first 10 bins, 150–3125 photons $cm^{-2} s^{-1}$. This lower limit is the intensity of the smallest flash which can be clearly distinguished from background, and 10 bins encompass the region in which the number of observed flashes is large enough so that the statistical error in the data is small. The upper limit shown in the plot is the intensity which produces saturation of the detector. Additional information on the maximum distance at which a flash of each brightness could be detected, on the number of flashes per second per cubic meter of water required by the normalization condition, on the number of events predicted above the normalization and in the saturation regions, and on the number of events predicted to be detected by both detectors is shown in Table 1. The observed numbers are presented on the last line of the table. The intensity distribution expected from a source population with a brightness of 5×10^7 photons $cm^{-2} s^{-1}$ at 1 m is in rough agreement with the observed distribution, and also with the number of flashes which saturate the detector. Obviously even better agreement could be obtained by a weighted combination of sources of greater and lesser brightness, but even this simple observation shows that a model of uniform population of flash sources throughout the ocean would

Table 1. *Comparisons of single brightness models with data*

Source brightness (photons $cm^{-2} s^{-1}$ at 1 m)	Source frequency ($m^{-3} s^{-1}$)	Maximum distance (m)	Events		Both detectors	
			High	Saturate	Between	Above
5.0×10^{2}	7.0×10^{-5}	1.8	3.1	0.0	0.0	0.0
2.0×10^{3}	9.3×10^{-6}	3.5	3.4	0.2	0.2	0.0
4.0×10^{3}	3.5×10^{-6}	4.9	3.5	0.3	3.3	0.0
8.0×10^{3}	1.3×10^{-6}	6.8	3.7	0.3	3.4	0.0
1.5×10^{4}	5.5×10^{-7}	9.0	3.9	0.3	3.3	0.1
3.0×10^{4}	2.2×10^{-7}	12.2	4.2	0.3	1.3	2.1
5.0×10^{4}	1.1×10^{-7}	15.2	4.5	0.4	0.6	2.9
1.0×10^{5}	4.8×10^{-8}	20.2	5.1	0.4	0.2	3.5
5.0×10^{6}	9.8×10^{-10}	73.4	13.9	2.0	0.0	3.0
5.0×10^{7}	2.1×10^{-10}	124.0	25.8	6.1	0.0	4.0
5.0×10^{8}	6.8×10^{-11}	185.0	40.6	16.4	0.0	5.4
1.0×10^{10}	2.4×10^{-11}	273.0	58.9	41.6	0.0	7.3
5.0×10^{11}	9.0×10^{-12}	400.0	76.7	93.0	0.0	9.4
1.0×10^{13}	5.2×10^{-12}	500.0	85.9	142.0	0.0	10.5
Data			28.0	6.0	38.0	

require a substantial contribution from sources of at least 5×10^{7} photons $cm^{-2} s^{-1}$ at 1 m because sources of lesser brightness simply cannot produce the high tail seen on the distribution. Additional data from the second detector are required to impose more significant constraints on such a model for bioluminescent pulses.

In order to be observed by both detectors, the source of the flash must be within the 25° cone in which the lower detector is sensitive, and the intensities at each detector must be above the detection thresholds for each of the detectors. No model of single brightness sources or any linear combination of them constrained by the normalization condition is able to give anywhere near enough flashes seen by both detectors. The disagreement is sharpened by dividing the model calculation into source locations between the two detectors and above the upper detector. Only sources below about 10^{5} photons $cm^{-2} s^{-1}$ at 1 m give a significant number of flashes seen by both detectors for sources between the two detectors. The number seen by both does increase for brighter flashes, but the contribution is from the ocean region above the upper detector. As discussed below, the observed distribution of the ratio of intensities in the two detectors is not consistent with a large contribution from sources above the detectors. Most of the flashes detected by both photomultipliers were produced by sources within a few meters of the apparatus, and any model of this sort, with a uniform distribution of flash sources throughout the ocean, fails by approximately a factor of 10 to account for the number of flashes seen by both detectors.

Since most of the flashes are produced in the water near the detectors, it is useful to analyse the data as if the sources were on the line joining the two detectors and to investigate the errors which result from this simplification. A source of brightness B, located on the line joining the detectors and at a distance r from the upper detector, will produce intensities I_u and I_l at the upper and lower detectors, respectively, where

$$I_u = \frac{Be^{r/\lambda}}{r^2} \text{ and } I_l = \frac{Be^{(s-r)/\lambda}}{(s-r)^2}.$$

The separation of the detectors is s and the attenuation length of light in the water is λ. The water at this site is very clear. BRADNER and BLACKINTON (1984) measured the attenuation length of 480 nm light at this site at depths of 780 and 1200 m and obtained 24.4 ± 1 and 25.9 ± 1 m, respectively. BABSON *et al.* (in press) obtained 45^{+40}_{-15} m at 420 nm, approaching that of the clearest natural waters (SMITH and BAKER, 1981). A value of 40 m has been used for these calculations. Since photon fluxes are given in $cm^{-2}\ s^{-1}$, it is convenient to use units of $cm^{-2}\ s^{-1}$ at 1 m for the source brightness. For a pair of observed or simulated intensities, the elimination of B is straightforward and yields a transcendental equation which can be solved iteratively for r.

The solutions of this pair of equations for the observed data are shown in Fig. 7. The opening angle of the mask on the lower detector restricts the positions of sources so that the approximation that the source is on the line between centers introduces only a small, less than about 25%, error in the source brightness for sources below the plane of the upper detector. Sources which are above the plane give (false) solutions with a characteristic distribution of r, and we use this feature of the solutions for the simulated pulses to show that the data are inconsistent with the solutions produced by the very large flashes which do yield a significant number of events visible to both detectors.

The contribution of simulated pulses detected by both detectors is predominantly from sources between the detectors for sources in the brightness range 2×10^3 to 3×10^4 photons $cm^{-2}\ s^{-1}$ at 1 m, and primarily from sources above the upper detector for pulse brightness above 3×10^4 photons $cm^{-2}\ s^{-1}$ at 1 m (Table 1). For flashes in the lower of these two ranges, these yield solutions of the simple, on-axis, equations for the distance

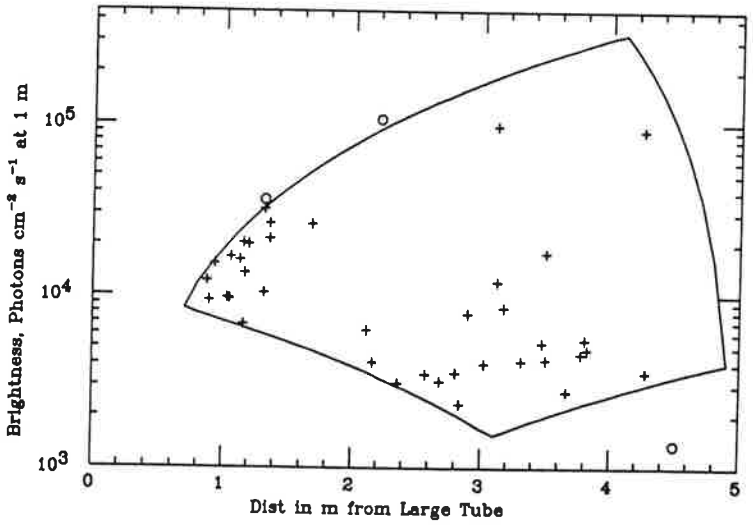


Fig. 7. Brightness and location along the line between the detectors for sources which would produce the intensity pairs observed for flashes detected by both detectors. The four boundary lines correspond to intensities at the upper and lower limits of the two detectors. The points represented by circles are not used in the analysis because they are outside the range which can be detected reliably or because only a lower limit was obtained for one of the pair of intensities.

from the upper detector which are in good agreement with the correct value for points within the 25° cone. For very large pulses, the solutions of the simple equations lie in a very narrow spike at 3.6 m below the upper detector. This spike broadens as the brightness of the source population decreases and extends from 2.8 to 3.6 m at 5×10^6 photons $cm^{-2}\,s^{-1}$ at 1 m. Since the data (Fig. 7) show no such concentration, we conclude that very large flashes by sources above the detectors do not contribute significantly to the data.

The relatively high pulse rate during the first 10 h may be an indication of an anomalous distribution of organisms immediately after the impact, so we have repeated the analysis using only the pulses observed after 10 h. The single source brightness that best matches the distribution of intensity in the upper detector, as in Fig. 6 for the entire sample, is approximately a factor of 10 smaller, and the model predicts only two pulses from the region between the detectors while 21 pulses are detected in both detectors. Thus these conclusions about the spectrum of intrinsic brightness of the sources and their clustering near the instrument also characterize the data after impact transients have subsided.

The observation that the flashing organisms are concentrated within a few meters of the detectors indicates that the data will not yield reliable flash rates for the undisturbed ocean but this observation also shows that the source brightness is calculated approximately correctly by the simple, on-axis equations for most of the events detected in both tubes. Consider first the concentration of points within about 1.5 m of the large detector. The solution surface for an event which in this region closes around the large detector, intersecting the line between the detectors at the distance shown and approaching close to the large detector on the opposite side. Thus the brightness estimate is essentially fixed by the approximately constant distance of the entire surface from the lower detector. In contrast, the events which could be incorrectly reconstructed in the region from 2 to 4 m from the upper detector would be from very much brighter flashes produced in the very large, conical volume of water several tens of meters above the detectors. As argued in the preceding paragraph, these would reconstruct into a very narrow region at 2.8–3.6 m below the upper detector. The absence of such a concentration in the data (Fig. 7) shows that most of the events plotted in the region of 2–4 m actually occurred in the volume of the acceptance cone between the two detectors and thus had a brightness very nearly equal to the brightness calculated from the simple equations.

Most of the flashes observed were produced by sources with brightnesses in the range 2×10^3 to 5×10^4 photons $cm^{-2}\,s^{-2}$ at 1 m, or 2×10^8 to 6×10^9 photons s^{-1} if the light is radiated isotropically. In order to put these numbers into perspective, we note that a single protozoan at a distance of 1 m produces a flash of intensity (Nicol, 1967) about 10^{-15} W cm^{-2} (2.5×10^3 photons $cm^{-2}\,s^{-1}$) and more recent measurements of flashes from *Gonyaulax* (Krasnow *et al.*, 1980, 1981) give intensities in the range $4–10 \times 10^3$ photons $cm^{-2}\,s^{-1}$ at 1 m and of duration 25–75 ms. These flash brightnesses are typical of the smaller pulses recorded by both detectors, but the durations, and hence total light output, are very much greater in the flashes recorded here. The flashes produced by pelagic jellyfish, siphonophores and ctenophores are about 10^4 times (Nichol, 1967) as intense and are much brighter than any observed by both detectors in this sample.

CONCLUSIONS

We have shown the very striking decrease in the light flux measured by our instrument when it came to rest 250 m above the bottom. A comparison of data taken in this

experiment with data taken by the same detector in an earlier, ship-suspended experiment is nearly as striking. We conclude that most of the light measured during free-fall and most of the light measured by ship-suspended instruments at great depth is stimulated bioluminescence. The response of the bioluminescent organisms to the stimulation appears, however, to be very loosely correlated in time with the actual motion. Consequently the periodicity of the stimulation is not very evident in the light production. Most of the flashes detected by both tubes while bottom moored are from sources with brightness in the range 2×10^3 to 5×10^4 photons $cm^{-2} s^{-1}$ at 1 m, and the concentration of flash sources is several times greater within a few meters of the instrument than at greater distance in the ocean. The distribution of the flash intensities observed by the upper detector alone would require some contribution from much brighter sources if the sources were uniformly distributed throughout the ocean, but this distribution could also be produced by weaker sources concentrated near the detectors. Thus both observations indicate that the sources were either attracted to the instrument or stimulated, perhaps indirectly, to flash more frequently by its presence.

Acknowledgements—We would like to express our appreciation to the Hawaii Institute of Geophysics and to the crew of the R.V. *Kana Keoki* for their assistance in the deployment of the instrument, Hawaii Institute of Geophysics Contribution no. 2214. We would also like to thank the Scripps Institution of Oceanography, Prof. F. N. Spiess and D. E. Bogeman, and the crew of the R.V. *Melville* for recovery of the instrument after the failure of the anchor release mechanism. We are indebted to W. W. Broenkow, J. W. Hastings and R. E. Young for reading the manuscript critically and for several valuable suggestions. This work is part of the evaluation of the light background in the deep ocean for the DUMAND array, which has been designed to measure the flux of very high energy (hundreds of GeV) neutrinos, and much of this work was supported by the particle physics programs of the DOE (Hawaii and Wisconsin) and the NSF (Vanderbilt). Additional support was obtained from the Japan Society for Promotion of Science (Institute of Cosmic Ray Research, Tokyo) and the Foundation for Ocean Reseach, La Jolla, CA (Scripps Institution of Oceanography).

REFERENCES

ANDREWS C. C., D. M. KARL, L. F. SMALL and S. W. FOWLER (1984) Metabolic activity and bioluminescence of oceanic fecal pellets and sediment trap particles. *Nature*, **307**, 539–541.

AOKI T., T. KITAMURA, S. MATSUNO, K. MITSUI, Y. OHASHI, A. OKADA, D. R. CADY, J. G. LEARNED, D. O'CONNOR, S. DYE, P. W. GORHAM, M. MCMURDO, R. MITIGUY, M. WEBSTER, C. WILSON and P. GRIEDER (1986) Background light measurements in the deep Ocean. *Il Nuovo Cimento*, **9C**, 642–652.

BABSON J., R. BECKER-SZENDY, R. CADY, S. DYE, P. GORHAM, J. G. LEARNED, S. MATSUNO, D. O'CONNOR, V. PETERSON, A. ROBERTS, V. STENGER, J. CLEM, C. ROOS, M. WEBSTER, J. GAIDOS, C. WILSON, H. BRADNER, W. KROPP, L. PRICE, F. REINES, H. SOBEL, R. MARCH, B. BARISH, T. KITAMURA, K. MITSUI, Y. OHASHI, A. OKADA and P. K. F. GRIEDER (in press). Detection of muons in the deep Pacific Ocean. (DUMAND Collaboration). *Physical Review D*.

BRADNER H. and G. BLACKINTON (1984) Long base line measurements of light transmission in clear water. *Applied Optics*, **23**, 1009–1012.

BRADNER H., M. BARTLETT, G. BLACKINTON, J. CLEM, D. KARL, J. LEARNED, A. LEWITUS, S. MATSUNO, D. O'CONNOR, W. PEATMAN, M. REICHLE, C. ROOS, J. WATERS, M. WEBSTER and M. YARBOROUGH (1988) Bioluminescence profile in the deep Pacific Ocean. *Deep-Sea Research*, **34**, 1831–1840.

CLARKE G. L. and R. H. BACKUS (1956) Measurements of light penetration in relation to vertical migration and records of luminescence of deep-sea animals. *Deep-Sea Research*, **4**, 1–14.

CLARKE G. L. and G. K. WERTHEIM (1956) Measurements of illumination at great depths and at night in the Atlantic Ocean by means of a new bathyphotometer. *Deep-Sea Research*, **8**, 189–205.

CLARKE G. L. and C. J. HUBBARD (1959) Quantitative records of the bioluminescent flashing of oceanic animals at great depths. *Limnology and Oceanography*, **4**, 163–180.

HAAS E. (1980) Bioluminescence from single bacterial cells exhibits no oscillation. *Biophysical Journal*, **31**, 301–312.

HASTINGS J. W. and B. M. SWEENEY (1958) A persistent diurnal rhythm of luminescence in *Gonyaulax polyedra*. *Biological Bulletin*, **115**, 440–458.

KELLY M. G. and P. TETT (1978) Bioluminescence in the ocean. In: *Bioluminescence in action*, P. J. HERRING, editor, Academic Press, New York, pp. 399–417.

KRASNOW R. A., J. C. DUNLAP, W. TAYLOR, J. W. HASTINGS, W. VETTERLING and V. GOOCH (1980) Circadian spontaneous bioluminescent glow and flashing of *Gonyaulax polyedra*. *Journal of Comparative Physiology*, **B138**, 19–26.

KRASNOW R. A., J. C. DUNLAP, W. TAYLOR, J. W. HASTINGS, W. VETTERLING and E. HAAS (1981) Measurements of *Gonyaulax* bioluminescence, including that of single cells. In: *Bioluminescence—current perspectives*, K. H. NEALSON, editor, Burgess Publishing Co., Minneapolis, Minnesota, pp. 52–63.

LONGHURST A. R., A. BEDO, W. G. HARRISON, E. J. H. HEAD, E. P. HORNE, B. ERWIN and C. MORALES (1989) NFLUX: a test of vertical nitrogen flux by diel migrant biota. *Deep-Sea Research*, **36**, 1705–1719.

LOSEE J. R. (1982) Bioluminescence in the deep ocean. In: *Proceedings of the 1982 DUMAND Signal Processing Workshop*. A. ROBERTS, editor, Hawaii DUMAND Center, University of Hawaii, Honolulu, pp. 19–29.

MARTIN J. H., G. A. KNAUER, D. M. KARL and W. W. BROENKOW (1987) VERTEX: carbon cycling in the northwest Pacific. *Deep-Sea Research*, **34**, 267–285.

K. H. NEALSON, editor (1981) *Bioluminescence—current perspectives*, Burgess Publishing Co., Minneapolis, Minnesota, 163 pp.

NICHOL J. A. C. (1967) *Biology of marine animals*, Pitman, New York, 699 pp.

RILEY G. A. (1951) Oxygen, phosphate, and nitrate in the Atlantic Ocean. *Bingham Oceanographic Collection Bulletin*, **13**, 1–126.

SMITH R. C. and K. S. BAKER (1981) Optical properties of the clearest natural waters. *Applied Optics*, **20**, 177–184.

SWEENEY B. M. (1981) Variations in the bioluminescence per cell in dinoflagellates. In: *Bioluminescence—current perspectives*, K. H. NEALSON, editor, Burgess Publishing Co., Minneapolis, Minnesota, pp. 90–94.

YOUNG R. E. (1981) Color of bioluminescence in pelagic organisms. In: *Bioluminescence—current perspectives*, K. H. NEALSON, editor, Burgess Publishing Co., Minneapolis, Minnesota, pp. 72–81.

YOUNG R. E., E. M. KAMPA, S. D. MAYNARD, F. M. MENCHER and C. F. E. ROPER (1980) Counterillumination and the upper depth limits of midwater animals. *Deep-Sea Research*, **27**, 671–691.

WYRTKI K. (1962) The oxygen minima in relation to ocean circulation. *Deep-Sea Research*, **9**, 11–23.