Studies at Oyster Bay in Jamaica, West Indies. I. Intensity Patterns of Bioluminescence in a Natural Environment[1]

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ABSTRACT

Night and day patterns in the intensities of stimulable bioluminescense in *Pyrodinium bahamense* were measured during the period of April 1966 through May 1967 in Oyster Bay, Falmouth Harbor, Jamaica, West Indies. During sunset and subsequent to dawn, rapid changes of more than a factor of 1000 in the stimulable bioluminescent intensities resulted from changes in stimulable bioluminescence per organism. During the night—from one hour past sunset through dawn—the total number of photons emitted per individual *P. bahamense* upon stimulation remained constant; it has been possible, therefore, to assay *P. bahamense* concentrations by measuring the stimulable bioluminescent intensities during the night. A seasonal variation in the occurrence of the evening increase in stimulable bioluminescent intensity has been correlated with the times of sunset. In nature, the nocturnal rhythm in stimulable bioluminescence is entrained by the photoperiod. Samples maintained in constant darkness exhibited an endogenous circadian rhythm of stimulable bioluminescence. *P. bahamense*, stimulated until no further light was emitted, recovered its capacity to luminesce in the dark in as little as 30 minutes.

Introduction. Although previous studies have contributed interesting and useful observations on bioluminescence and pertinent organisms, it has become increasingly apparent that the known information and data are wholly inadequate for a comprehensive understanding of the phenomenon of bioluminescense. Therefore, in April 1966 an organized program was launched at Oyster Bay, Falmouth Harbor, Jamaica, W.I., to study pertinent biological and physical parameters over a prolonged period of time. This Bay, which contains a persistently high concentration of the bioluminescent photosynthetic dinoflagellate, *Pyrodinium bahamense*, is especially well suited for such a study.

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In this report, covering a period of more than one year (April 1966 through May 1967), the diurnal intensities of stimulable bioluminescense in *P. bahamense* in the Bay have been correlated with the concentrations of this organism as determined in our laboratory, using Bay samples obtained concurrently with the field observations. Also reported are observations on concentrated cultures of *P. bahamense*. In this issue also, Carpenter and Seliger (1968) have described the physical oceanography of the Bay and adjacent areas while Seliger and Fastie (1968) have described and discussed the spectral distributions of ambient sunlight in the Bay.

Description and Methods. *P. bahamense* exhibits a nocturnal (diurnal) rhythm in stimulable bioluminescence. During the night, when the organism is maintained undisturbed, there is a very low level of spontaneous emission—less than 10^{-4} of the stimulable intensity. However, with the slightest disturbance, such as the tapping of a beaker containing the organisms, the shear produced by a gas bubble rising from the bottom of the Bay, or the motion of a fish or a finger through the water, the organisms flash so brightly that, in a natural concentration, a continuous glow is observed during the stimulation.

The equipment for assaying the bioluminescence has already been described (Seliger et al. 1962). Briefly, a motor-driven impeller pump housed in clear plastic is used to agitate the organisms and replenish the system continuously with unstimulated organisms. The impeller pump and a phototube that views the plastic housing are enclosed in a light-baffled container so that day and night intensity measurements can be made without interference from ambient sunlight or moonlight. For calibration, a miniature lamp with a blue-light-transmitting filter has been incorporated as an integral part of the impeller housing.

Under gentle conditions of agitation, the light intensity emitted by a given volume of *P. bahamense* is dependent upon the degree of stimulation. We have determined from visual observation of the impeller pump effluent that the agitation under our conditions of measurement is sufficient to stimulate the organisms to emit essentially all of their available light within the impeller housing. Oscilloscopic observation of individual light flashes showed an initial rapid rise in light intensity (less than 10 m sec) and an exponential decay having a mean lifetime of 50 m sec. Upon stimulation, the organism emits a succession of flashes. The observed bioluminescent intensity is the superposition of a large number of these flashes from a large number of stimulated organisms, resulting in a steady glow.

The data reported here cover the period from April 1966 through May 1967. On the basis of measurements of stimulable bioluminescent intensities, organism identifications, salinities, temperatures, and circulation patterns at various points in the Bay, a location was chosen that was considered to be representative of the Bay. This location was approximately 150 m out from

a point of land adjoining our laboratory trailers and is referred to here as the fixed-station site. Marker stakes served as navigational aids for day and night transect measurements and served to relate other locations at which comparative measurements were made.

Daily samples of seawater along east-west and north-south transects, taken at a depth of 1 meter during the midafternoon (when the water of the inner Bay is uniformly mixed), revealed average concentrations in the distribution of the organism that were in good agreement with the concentrations at the fixed-station site.

From April through December 1966, stimulable bioluminescent intensities, salinities, and temperatures as functions of time of day and of depth were measured manually at the fixed-station site at weekly intervals. Comparative measurements were made at other points in the Bay, though not as frequently. In January 1967, a floating automated sampling station was installed at the fixed-station site. Since January 1967 we have collected data that show the depth distribution of stimulable bioluminescent intensity for every half hour throughout each day; our data also include printed records of salinity, ambient-light intensity, temperature, water level, wind speed, and wind direction.

The temporal pattern of bioluminescence observed at any fixed position in the Bay is a function of the magnitudes and relative phases of wind, tide, and river flow (Carpenter and Seliger 1968). As a result of these oceanographic and meteorologic fluctuations, the Bay water is in constant circulation, hence the same volume is never sampled twice at any fixed position. Therefore, observations of stimulable bioluminescent intensities, if they were to be at all meaningful, had to be correlated with (i) the identification and concentrations of species in the same volume of water that was stimulated and (ii) the Bay circulation pattern at the time of measurement. Furthermore, there are peripheral shallow areas in the Bay that are not representative of the main circulation pattern; in such areas there are sporadic concentrations of organisms that range from 10 to 100 times the concentrations found in the deeper parts of the Bay. The shallow areas are subject to large perturbations in both influx of low-salinity water and layered outflow of seawater.

Observations and Data. An example of stimulable bioluminescent intensity as a function of time of day is given in Fig. 1. The data were obtained at the fixed-station site. For clarity of presentation, only the values for depths of 15, 30, and 60 cm have been plotted. To show the total distribution, the sum, Σ, of the intensities at the 15, 30, 60, 90, 120, and 150-cm depths is also plotted; this sum is used as an index of the total stimulable bioluminescence. Since the observed intensity of stimulable bioluminescence varies over many orders of magnitude, the intensities are plotted on a logarithmic scale. During the initial portion of the night period, the intensities at the 15, 30, and 60-cm depths are coincident. During the later night period, a tongue of water having

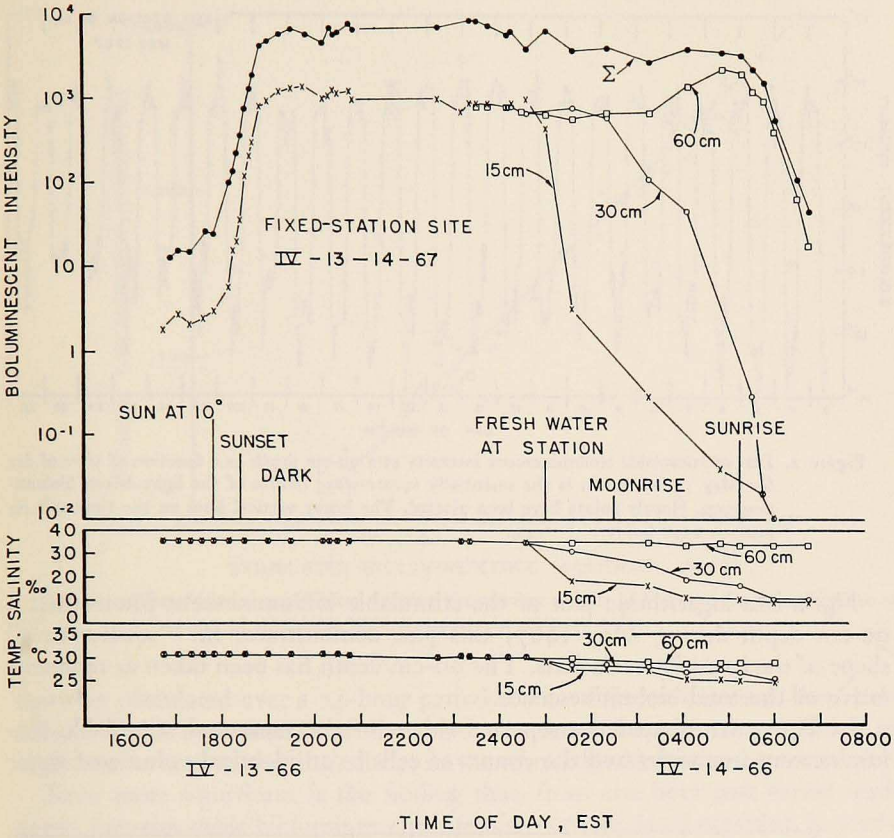

Figure 1. Stimulated bioluminescent intensities at 15, 30, and 60-cm depths as functions of time of day, April 13–14, 1966; shown is the decrease in measured intensity at the 15 and 30-cm depths during the advance of a low-salinity, low-density, and low-temperature tongue of river water across the inner Bay. Corresponding salinity and temperature readings are plotted below.

low salinity, low density, and low temperature and containing no *P. bahamense* proceeds eastward across the inner Bay (Carpenter and Seliger 1968). Fig. 1 shows that the thickness of this low-salinity layer at 0100 h reached 15 cm, therefore the 15-cm reading of stimulable bioluminescent intensity dropped precipitously. In another hour this low-salinity layer had become 30 cm thick, and the 30-cm bioluminescence reading decreased. The low-salinity layer never became 60 cm thick, so the 60-cm reading did not drop. The rise in the intensity of stimulable bioluminescence around dawn at the 60-cm depth is related to the circulation pattern of water in the Bay. In this case, higher concentrations of *P. bahamense* were carried past the fixed station. In Fig. 1, these lateral movements of low-salinity and low-temperature surface layers are demonstrated in plots of the associated salinities and temperatures.

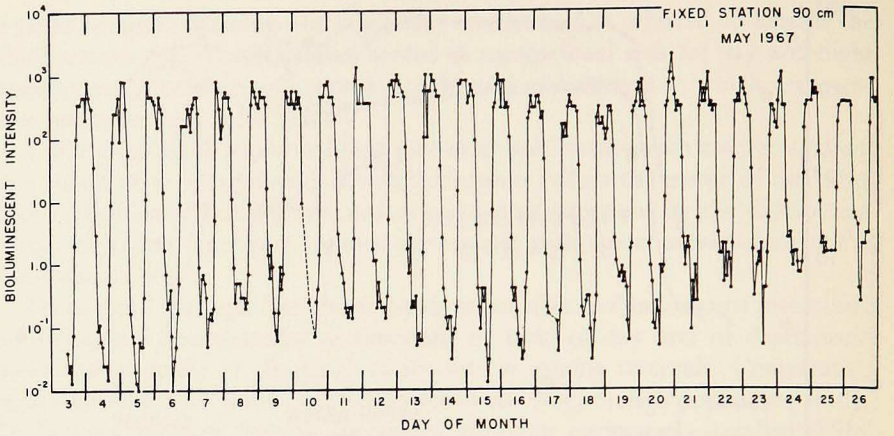

Figure 2. Plot of stimulable bioluminescent intensity at a 90-cm depth as a function of time of day for May 1967; shown is the essentially square-wave nature of the light-driven bioluminescence. Hourly points have been plotted. The heavy vertical lines on the time axis represent 2400 hours.

Fig. 2 is a logarithmic plot of the stimulable bioluminescent intensity at a 90-cm depth during May 1967; this plot demonstrates the "square-wave" shape of the intensity variations. The 90-cm depth has been taken as representative of the total bioluminescence.

In Fig. 3 are plotted the separate values of the measured stimulable bioluminescent intensities and the counts of cells in an identical volume of water

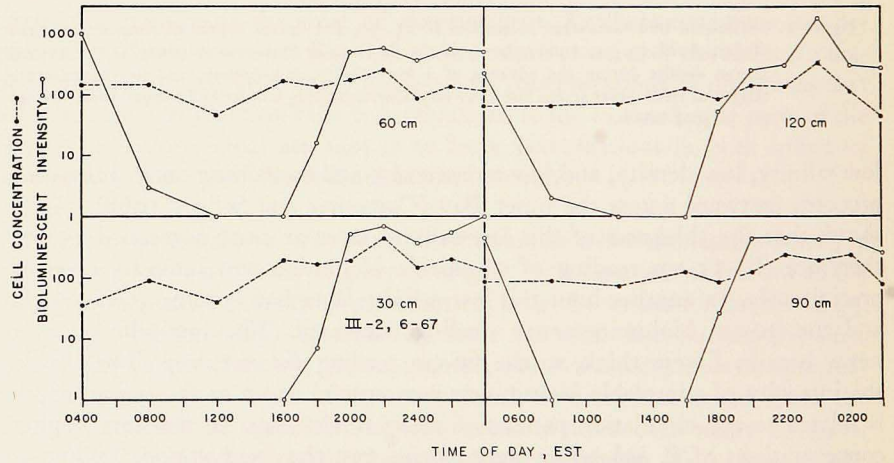

Figure 3. Direct verification of natural diurnal variations in the stimulable bioluminescence of *P. bahamense*. Counts of cells from 30, 60, 90, and 120-cm depths, covering a 24-hour period, are plotted separately.

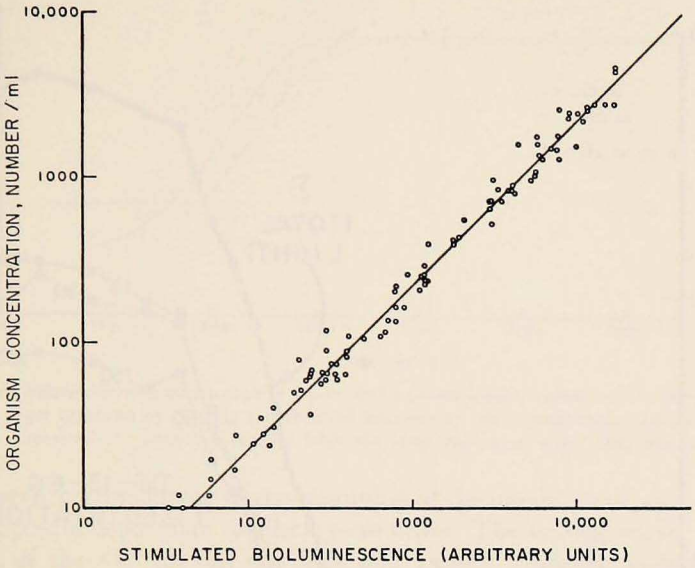

Figure 4. Plot of log-log coordinates showing the number of cells of *P. bahamense* per millimeter as a function of stimulable bioluminescence in arbitrary units.

that was stimulated over a 24-hour period at four different depths at the fixed-station site. These data show the relative constancy in the concentration of organisms as well as the diurnal rise in the stimulable bioluminescent intensity.

Even more significant is the finding that, from one hour past sunset until dawn, the stimulable bioluminescent intensity per individual organism is nearly constant. A summary of our data is shown in Fig. 4. These data were taken in the Bay over a period of several months at different depths, at different locations, and at different times during the night. The manually operated portable underwater photometer (Seliger et al. 1962) was used for measurements at locations other than the fixed-station site. Both the portable photometer and the fixed-station photometer were calibrated so that the intensity readings, though in arbitrary units, were identical for identical *P. bahamense* concentrations. The stimulable bioluminescence per individual organism is shown to be constant over the entire concentration range of the *P. bahamense* measured—i.e., from 10 cells/ml to 4800 cells/ml—so long as the measurements were made from one hour past sunset to dawn.

At certain times of the month, the wind may stop around 1600 h when the tide is at low ebb. At such times the inner Bay is more or less uniformly mixed and additional mixing is at a minimum; the measurements of stimulable bioluminescent intensities as functions of depth and time under these conditions show that the rise in stimulable bioluminescence occurs several minutes earlier at the 150-cm depth than at the 15-cm depth, with the increases at

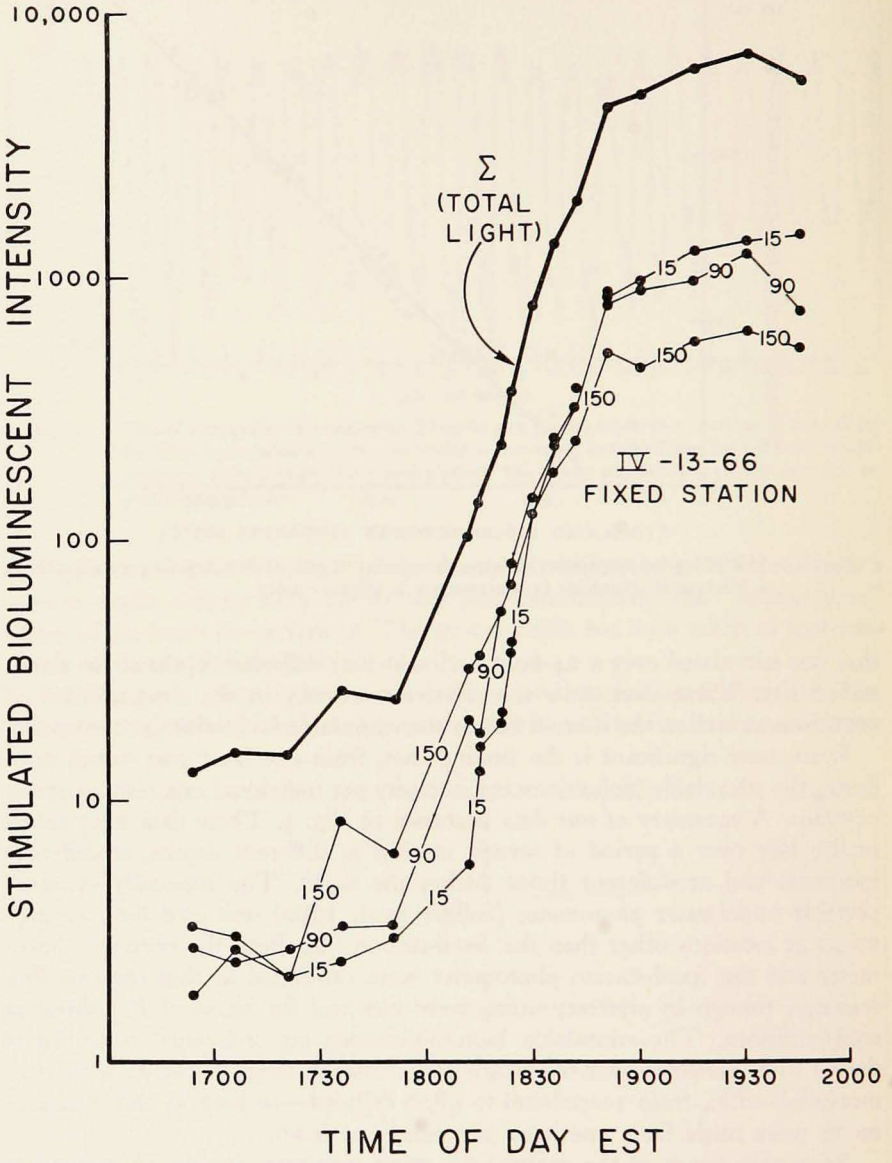

Figure 5. Intensities of stimulable bioluminescense at 15, 90, and 150-cm depths over the time period of the rise in bioluminescence. EST = Eastern Standard Time.

intermediate depths falling between these two extremes. An example of these observations is shown in Fig. 5; the readings for the 15, 90, and 150-cm depths are plotted over the time period of the rise in bioluminescence. The flexion

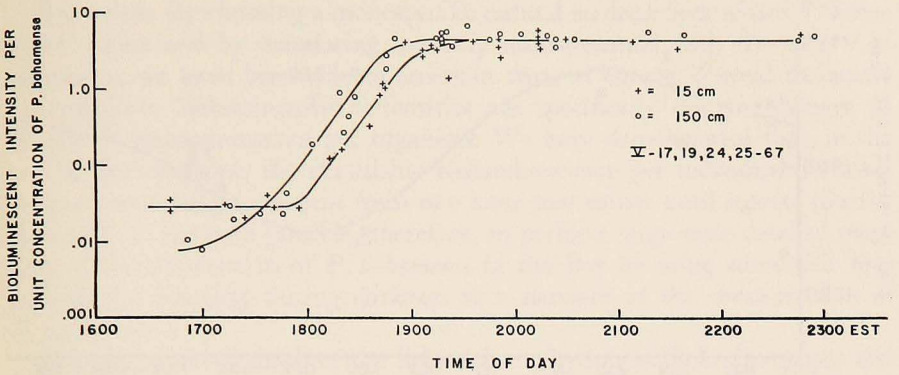

Figure 6. Stimulable bioluminescence at 15-cm and 150-cm depths, normalized to bioluminescence per organism by dividing the observed intensity by cell concentrations determined microscopically by counting samples from the same volume of water that was agitated.

in the curve, corresponding to the beginning of the intensity rise, occurs earlier for the 150-cm depth than for the 15-cm depth. The average curve, based on the sum of the 15, 30, 60, 90, 120, and 150-cm readings, shows that the general shape of the rise is not affected by this rather small perturbation.

Since concurrent *P. bahamense* microscopic counts were not made for the data in Fig. 5, we have plotted in Fig. 6 the results of four separate experiments to show that the data in Fig. 5 are not artifacts due to patchiness in sampling. In these experiments, samples from the impeller pump effluent were counted under the microscope. The ordinates are in units of intensity of stimulable bioluminescence divided by the number of *P. bahamense* per milliliter.

Under the same conditions noted above—a uniformly mixed Bay—it is seen in Fig. 5 that the stimulable bioluminescent intensity subsequent to 1900 h is greater at the 15-cm depth than at the 150-cm depth, with intermediate intensities at intermediate depths. Since these differences occur at a time when the stimulable bioluminescence per organism is constant, it would appear that there are larger concentrations of *P. bahamense* at the surface than at lower depths. By microscopic counting we have ascertained in separate experiments at the fixed-station site that, during daylight, natural samples collected at different depths as a function of time exhibit an increase in cell concentration at the surface and a decrease in cell concentrations at lower depths, corresponding to a relative migration. In additional experiments, initially homogeneous natural concentrations of *P. bahamense* were enclosed in long clear plastic cylinders that were submerged vertically at the fixed-station site to simulate the natural ambient light environment; again, microscopic counting of small samples drawn off as functions of depth and time demonstrated a strong positive phototaxis during daylight. The same experiments, repeated under a full moon, showed no net migration.

For a quantative measure of a natural time to be associated with the rise in

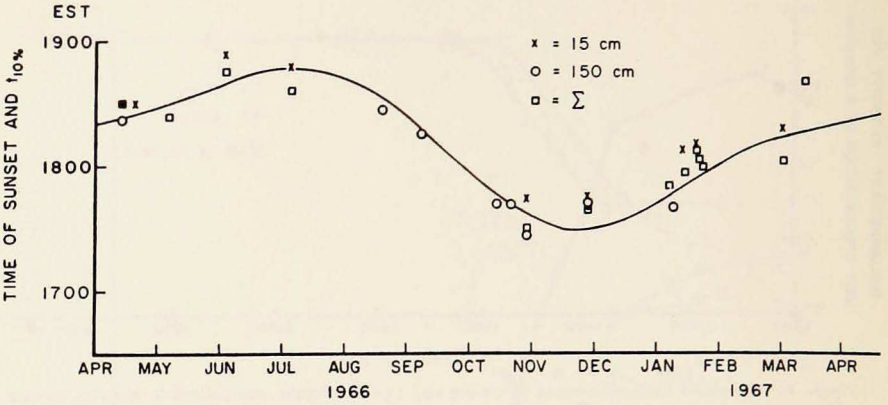

Figure 7. Seasonal variation in the time of rise in stimulable bioluminescence correlated with the time of sunset. EST = Eastern Standard Time.

bioluminescence, we have arbitrarily taken the time at which the stimulable bioluminescent intensity has reached 10% of its maximum value. This is always on the exponential portion of the curve and can be determined readily. In Fig. 7 we have plotted these values of $t_{10\%}$ as well as the times of sunset as a function of the time of year (solid curve) at this location from April 1966 through May 1967.

In addition to the data presented, we have made the following qualitative observations:

a. *P. bahamense* taken directly from the Bay at one or more hours before sunset and kept under bright artificial light did not exhibit an observable increase in stimulable bioluminescent intensity throughout the natural night period.

b. *P. bahamense* taken directly from the Bay at around noon and placed immediately in complete darkness and kept there exhibited two types of stimulable bioluminescence: (i) an initial rise in intensity by a factor of 50–100 that reached a plateau within 60 minutes; (ii) a second rise (very rapid), again by a factor of approximately 100, that followed the same pattern as that observed in the Bay.

c. These same organisms, maintained in total darkness for 2 to 3 days, exhibited an endogeneous circadian rhythm of stimulable bioluminescence, but the changes with time were not as rapid.

d. In the dark, *P. bahamense* can be stimulated to exhaustion, with no further bioluminescence emitted, but after a rest period of as little as 30 minutes, they could be stimulated again to emit almost as much light as that emitted initially. Although this process can be repeated several times, less and less total light is emitted after each rest period.

Discussion. By choosing a monospecific natural environment where *P. bahamense* occurs and by correlating intensity measurements with the counts of organisms, we have been able to ascertain that, in nature, diurnal variations in stimulable bioluminescent intensities are specifically due to changes in stimulable bioluminescence per organism. We have demonstrated that, in the natural environment, the stimulable bioluminescence per individual *P. bahamense* is essentially a constant from one hour past sunset until sunrise (during darkness). It has been possible, therefore, to perform large-scale detailed mapping of the distribution of *P. bahamense* in the Bay by using stimulable bioluminescent intensity during darkness as a measure of the concentration of *P. bahamense.*

The square-wave shape of the light-driven rhythm in bioluminescence and the large ratios of stimulable bioluminescence from night to day are different from the data reported by Hastings and Sweeney (1958) and Sweeney et al. (1959) for luminescence in laboratory cultures of *Gonyaulax polyedra.* There may be physiological differences between these two species of dinoflagellates in both diurnal rhythm of stimulability and synthesis of luciferin and luciferase.

We can offer no explanation for the large disparities between our own measurements of stimulable bioluminescense and those reported by Soli (1966) for the same species in the same Bay. We found that there is: (i) a very rapid rise in stimulable bioluminescence at dusk and a rapid decrease subsequent to dawn, (ii) a constancy in the stimulable bioluminescence per organism during the night period, and (iii) a night-to-day ratio of at least one thousand to one in the stimulable bioluminescence intensities. Hundreds of similar daily observations have verified these observations.

We have never observed in *P. bahamense* any rhythn of downward and upward migration during the night, as reported by Soli. When similar decreases and increases in stimulable bioluminescence were observed, they could be accounted for completely by the influx and outflux of low-salinity dinoflagellate-free surface layers produced by flood and ebb tides, respectively. We feel that this also explains the peculiarly shaped curve for the diurnal variation in stimulable bioluminescence that was reported in our earlier paper (Seliger et al. 1962); we mistakenly proposed a night-time migration of *P. bahamense.*

The data in Fig. 5 point up an important physiological mechanism. There appears to be a threshold of light intensity at which the rise in bioluminescence is triggered, since, in the turbid Bay, the ambient light intensity will be lower at the 150-cm depth than at the surface. This increase in stimulable bioluminescence is triggered while the sun is still bright, up to approximately one hour before sunset. The rate of decrease in the intensity of stimulable bioluminescence subsequent to sunrise is also rapid, but in some cases we have observed slower rates of decrease. Since the Bay is turbid at the time of trig-

gering, a spectral dependence, such as a two-wave-length two-pigment equilibrium system (analogous to the phytochrome pigment system) cannot be excluded.

B. M. Sweeney has isolated *P. bahamense* from Oyster Bay, and she has furnished us with an inocculum of *P. bahamense* and of *Gonyaulax polyedra*; both species are now growing well in our laboratory. They are being compared under identical conditions of stimulation and growth. On the basis of preliminary measurements on laboratory cultures of *P. bahamense*, there appears to be a variability in the stimulability with time during the dark period, as judged by the fraction of total bioluminescence observed under conditions of less than maximal stimulation. The question arises as to whether the night-to-day ratios of stimulable bioluminescence observed are due to enzyme induction, substrate synthesis, or both, or whether the components are always present but require that a stimulable (shear-sensitive) structure be formed. We do not know the mechanism for the recovery of stimulable bioluminescence in the dark.

We have ascertained that there is an endogenous rhythm in the stimulable bioluminescence of *P. bahamense*. However, the rhythm observed in nature is a light-driven rhythm, with a time of increase modified by seasonal variations in time of sunset, and with a decrease that occurs after sunrise. Since the endogenous rhythm does exist, it is obvious that the stimulable bioluminescence, even in darkness, will decrease after approximately 12 hours of darkness. This rate of decrease, however, is different from the light-driven rate of decrease. The drop in stimulable bioluminescence after sunrise is most probably a combination of an endogenous rhythm and a photo-inhibition of bioluminescence. The latter has been reported by Sweeney et al. (1959) for *G. polyedra*.

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