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CORRELATION OF *IN SITU* FLUORESCENCE AND BIOLUMINESCENCE WITH BIOTA IN THE NEW YORK BIGHT

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Two aspects of optical oceanography appear to offer the possibility for *in situ* optical observations that may be correlated with concurrently existing specific biota. One technique is *in situ* measurement of stimulated bioluminescence, and the other is *in situ* measurement of visible fluorescence from ultraviolet excitation. These methods are of great interest because their use permits detailed vertical profiling with immediate information access. Synoptic information may be acquired by deploying unattended instrumentation at a number of locations.

Modern *in situ* photometric measurements of bioluminescence began with the use of a bathyphotometer by Clarke and Wertheim (1956), Boden and Kampa (1957), and Hardy and Kay (1964), who describe observations in Lock Fyne in 1957. The bathyphotometer designed by Clarke and Wertheim (1956) was used by Clarke and Breslau (1959, 1960) and Clarke and Backus (1956) to show that large scale bioluminescence exists at all depths of the sea and is an important factor in the ecology of marine life. Clarke and Kelly (1964) were able to estimate the distance over which one subsurface organism is able to communicate with another by measuring the intensity of the bioluminescent flashing and the transparency of the Indian Ocean. Clarke and Kelly (1965) also developed an *in situ* photometer usable day and night in contrast to previous units that could be used at night or only at great depths. This new photometer was light-tight and permitted water to be pumped through and to be subjected to controlled physical agitation to produce bioluminescence. Egan (1969) described an *in situ* photometer used aboard the submersible *Ben Franklin* during its 30-day drift mission, that was capable not only of measuring bioluminescence, but fluorescence as well.

Boden, Kampa and Snodgrass (1960) describe instrumentation similar to that of Clarke and Wertheim (1956) that was used by Boden and Kampa (1957). They found layers of strong luminescence associated with the sonic scattering layer.

These instruments used for bioluminescence measurements differ in detail in the electrical and optical characteristics. The device described by Boden, Kampa and Snodgrass (1960) was carefully calibrated and used a 931-A (response between 0.36 and 0.54 μm) photomultiplier, and 5 interference filters with peaks presumably at 434, 460, 491, and 514 nm. Switching time between filters was 10 s, and the system time of response to bioluminescent flashes was essentially limited by the recorder to about 50 cycles per minute (Kampa and Boden 1956). There was a serious question as to whether the measurement was of spontaneous bioluminescence or stimulated bioluminescence caused by motion of the photometer

in the water. In answer to this criticism, Boden, Kampa and Snodgrass (1965) developed a coincidence sensing technique using two photometers viewing a common volume included in the intersection of two 10 degree cones viewed by the photometers at a distance of one meter. The sensor was a type 6472 photomultiplier (response between 0.36 and 0.54 μm). The system time of response to bioluminescent flashes is about 20 milliseconds. The experiment is still open to question because sympathetic flashing may occur in biota in the sensed area due to undetected flashes near the sensor caused by physical agitation of the water from sensor motion.

The photometer of Hardy and Kay (1964) used a 931A photomultiplier, but in association with a 200 cps rotating shutter and an ac amplifier for fast response. No optical filters were used, and the system was calibrated at one level of illumination to check the gain stability. The system time of response was limited by the Fielden Servograph to about 1 cps for bioluminescent flashes.

Clark and Kelly (1965) used an end window type 5819 photomultiplier (response between 0.34 and 0.55 μm) with no optical filtration in their *in situ* measurements of stimulated bioluminescence from a motor driven vane. Graphical records of radiance from bioluminescent flashing are presented with the impeller on and off. The system time of response appears to be of the order of a fraction of a second.

All of the foregoing instrumentation responds to bioluminescent flashes in a way that is limited by the time of response of the system, and the flashes are recorded as pulses. Another approach to the sensing of bioluminescence is that of recording the time average of the radiance from bioluminescent organisms. This time average is proportional to the energy output of the bioluminescent organisms which should be related to the number and species present. By including stimulated bioluminescence, some causes of natural variations in light emission are eliminated and a more realistic representation is obtained. An integrating type *in situ* sensor of this type was used to sense bioluminescence in the Gulf Stream (Egan, 1969), and in the New York Bight (Egan, Cassin and Hair, 1972). However, the correlation of the *in situ* bioluminescence with biota remains to be presented.

Fluorescence in sea water from ultraviolet excitation is another optical phenomena that can be used to advantage for *in situ* measurements of chlorophyll and Gelbstoff. Long wavelength ultraviolet red fluorescence from chloropigments is related to total chlorophyll and chlorophyll *a* (Yentsch and Menzel, 1963). Since certain species of phytoplankton contain chlorophyll, and if the distribution of these species is known, the amount of long wavelength fluorescence of chlorophyll *a* is proportional to the biomass of these species. Another aspect of fluorescence is that short wavelength ultraviolet fluorescence produces a blue-green radiation in luminescent organisms (Kelly, 1968). Another source of fluorescence in sea water in Gelbstoff (Kalle, 1966). Gelbstoff is a collective term that includes humic acids and melanoidines that are formed whenever decomposition of living cells occurs. The decomposition releases carbohydrates and amino acids (Kalle, 1966). Gelbstoff is found in areas where coastal waters from rivers and estuaries flow into the sea. Representative areas are the North Sea and the Baltic Sea (Jerlov, 1955; Kalle, 1966) and the Dutch Wadden Sea (Otto, 1966; Postma and Rommets, 1970). The New York Bight, similarly, is typical of coastal waters fed by highly

fluorescent fresh water (Egan, Cassin and Hair, 1972). A blue fluorescence occurs from organic substances present during the formation of the melanoidines during ultraviolet radiation (Kalle, 1966). This fluorescence is then proportional to the quantity of these substances present, and for a given area, related to the Gelbstoff present.

In order to make *in situ* investigations of bioluminescence and fluorescence in estuaries and deep sea environments, the photometric equipment used in the Gulf Stream drift experiment (Egan, 1969) was assembled in a geometrical configuration identical to that on the submersible used in the Gulf Stream. Subsequently additional instrumentation was added (Fig. 1). The other instruments on the assembly (Fig. 1) were used to gather information for a previous paper (Egan, Cassin and Hair, 1972). Thus, k is a flow type pH electrode, and b is its preamplifier; e is a lamp used to measure transmission in the red spectral region with sensor c; j is a dissolved oxygen probe; h is a Beckman conductivity probe for salinity measurements in association with the temperature sensing thermistor 1; a is a thin walled stainless steel pivoted tube containing a thin film type water velocity probe (a fin for aligning the tube in the direction of maximum water velocity is not shown); and m is the hoist cable. The multiconductor cable from the instrumentation to the surface is not shown to emphasize clarity. Of the many instruments in this assembly, only certain units are relevant to this paper.

These were the photometers c and d, the ultraviolet lamps f and g, and the electric motor driven propeller, i. A very detailed description of the optical properties of the ultraviolet lamps and the photometers will be presented in a subsequent paper (Egan, in preparation). However, briefly, photometer d is a wideband sensor for bioluminescence using a 6199 photomultiplier with optical filtration to produce a response between 0.46 and 0.55 μm, sensing blue-green bioluminescence expected from dinoflagellates (Kelly, 1968). The optical band-pass is limited by interference filters. This same photometer measures Gelbstoff fluorescence within the acceptance band resulting from short wavelength ultraviolet radiation. This is provided by an S4T5 short wavelength ultraviolet lamp (peak radiation at 254 nm), with a Corning CS7-54 filter to attenuate the visible radiation. The measurement sequence consists of a background dc measurement of the photomultiplier output; then a measurement of the output with the motor alone operated; then a measurement with the short wavelength lamp alone operated. After the subtraction of the background dc measurement, and using photometric calibrations against an Eppley thermopile with neutral density filters to attenuate the radiance sensed by the photomultipliers, a radiance level is determined. This is then proportional to the bioluminescence or Gelbstoff depending upon the measurement.

For chlorophyll *a* measurements by fluorescence, the upper sensor is used in a similar way. It is a type 6199 photomultiplier with a Corning Glass CS2–60 filter to sense red radiance (0.62 to 0.65 μm) as a result of long wavelength fluorescent radiation from an S4T5 ultraviolet lamp, g. The UV lamp has a radiation peak at 370 nm, achieved with a fluorescent coating and a Corning CS7-60 glass filter to attenuate the visual and red spectral regions. Chlorophyll calibrations are based on samples acquired during the measurement sequence and analyzed at the Adelphi Institute of Marine Science (Strickland and Parsons, 1968). Both ultraviolet lamps, f and g, were protected from the sea by pressure resisting housings with UV transmitting quartz windows capable of withstanding depths to 600

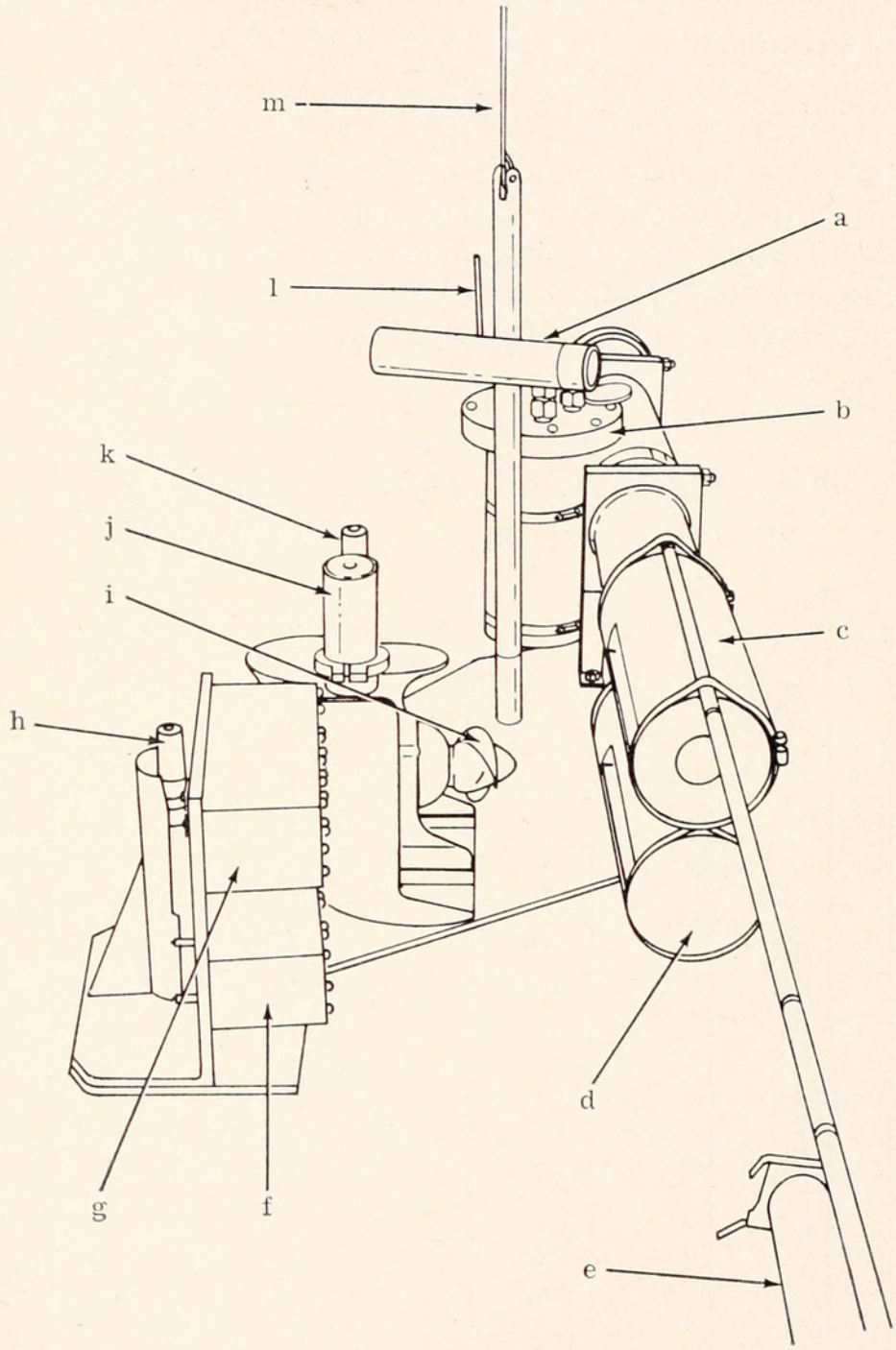

FIGURE 1. Sensor assembly; a, thin film anemometer unit; b, pH sensor preamplifier; c, red photometric sensor unit; d, broadband photometric sensor unit; e, transmissometer light source; f, 254 nm ultraviolet source; g, 370 nm ultraviolet source; h, conductivity cell; i, propeller agitator; j, dissolved O_2 sensor; k, flow type pH sensor; l, thermistor; m, hoist cable.

m. The photometers are of an extremely simple design with high voltage batteries within the housings, and gain variable by a set of relays also within the housings. Signals are transmitted to the surface through a multiconductor cable with vulcanized connections.

The utility of this *in situ* photometric instrumentation lies in the fact that bioluminescence and fluorescence measurements made *in situ* are not restricted by collection problems and are not handicapped by time lapse between sample collec-

tions and analyses. The instrumentation is well suited for unattended long term monitoring of estuarine areas.

To determine the applicability of this *in situ,* photometric instrumentation to estuarine research, a series of observations were made in the New York Bight.

By comparison of bioluminescence, short-wavelength ultraviolet-produced fluorescence, and long-wavelength ultraviolet-produced fluorescence with the laboratory determined biota distribution, the causative organisms may be inferred. The inferences are drawn on the basis of previous work on bioluminescent organisms, specifically dinoflagellates, and the known existence of chlorophyll *a* in certain phytoplankton.

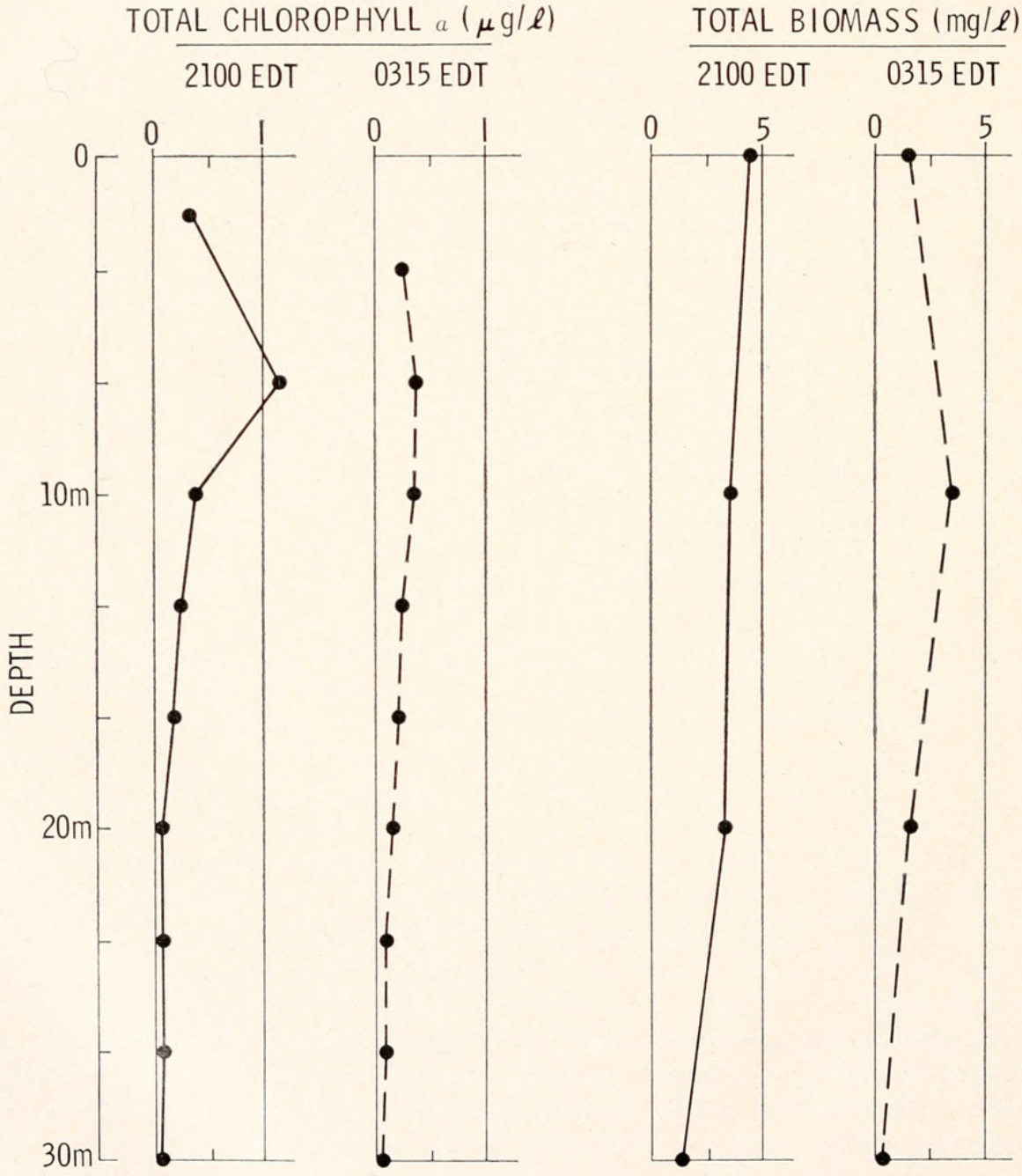

FIGURE 2. Comparison of *in situ* chlorophyll *a* measurements with total biomass at different depths and times.

Materials and Methods

There were two aspects to the experimental procedure: the first involves the *in situ* measurements, the second involves the laboratory methods for phytoplankton analysis. Both aspects of the experimental procedure were carried out concurrently.

A measurement program was conducted at night on October 19–20, 1970, one-half mile north of Ambrose Tower. *In situ* profiles were made using the assembly previously described. Profiles started at the surface with depth increments generally of 3 m. A background level was recorded on each photometer, and then each ultraviolet lamp was activated, and the photometer output and ultraviolet lamp input recorded. Photometer sensitivities were capable of remote incremental variation. For bioluminescence observations, input to the propeller agitator was adjusted to 250 w, and output of appropriate photometers recorded (with the

Table I

Standing crop from laboratory analyses

Date	Time EDT	Depth m	Zooplankton (total/l) $\times 10^3$	Phytoplankton (total/l) $\times 10^3$	Total biomass (mg/l)
19–10–70	2140	Surface	0	0.83	4.4
		10	60	3.15	3.53
		20	10	0.774	3.19
		30	50	0.64	1.2
20–10–70	0315	Surface	10	1.53	1.47
		10	60 (tintinnids)	1.63	3.34
		20	0	0.56	1.53
		30	110 (polychete larvae)	0.15	0.015

ultraviolet lamps off). The readings of the sensors, after the background level is subtracted, is then proportional to the fluorescence of total chlorophyll, the fluorescence of bioluminescent organisms, Gelbstoff or dissolved minerals, or bioluminescence.

Samples for laboratory analyses were collected concurrently with the *in situ* measurements at 2100 hours on October 19 and at 0315 hours on October 20, 1970. For phytoplankton, liter samples were collected in two 500 ml polyethylene bottles from Nansen bottles submerged to depths of 1, 10, 20, and 30 m. Samples were concentrated to a final volume of 10 ml, using continuous centrifugation (American Public Health Association, 1965; Kimball and Wood, 1965). After microscopic examination to identify forms that would be damaged by preservation, concentrate was fixed in 3 percent neutral formalin. Counts were performed in a Palmer nannoplankton cell. Raw counts were adjusted to cells per liter using appropriate dilution and efficiency factors (Cassin and McLaughlin, 1973).

Numbers of zooplankton were recorded with no attempt to classify by genus or species. Phytoplankton standing crop was recorded, and organisms identified to genus or when possible to species. Dimensions were measured by calibrated Whipple disc, and volumes (in cubic μm), derived by assigning one of three geometrical forms: rectangle, cylinder, or sphere. Estimated biomass (mg/l) was

derived from calculated cell volumes (Strickland, 1966; Willin, 1959). Paasche (1960) noted a 0.62 correlation between cell volume and productivity.

Results

Results of *in situ* fluorescence and bioluminescence measurements are shown in Figures 2 and 3. Laboratory analyses for standing crops are presented in Table I. *In situ* measurements indicate that total chlorophyll is high at the surface and decreases with increased depth, as expected. This correlates with laboratory determinations of total biomass. Total chlorophyll pigments are related (in a variable way) to total biomass, and would be expected to correlate with it. Laboratory

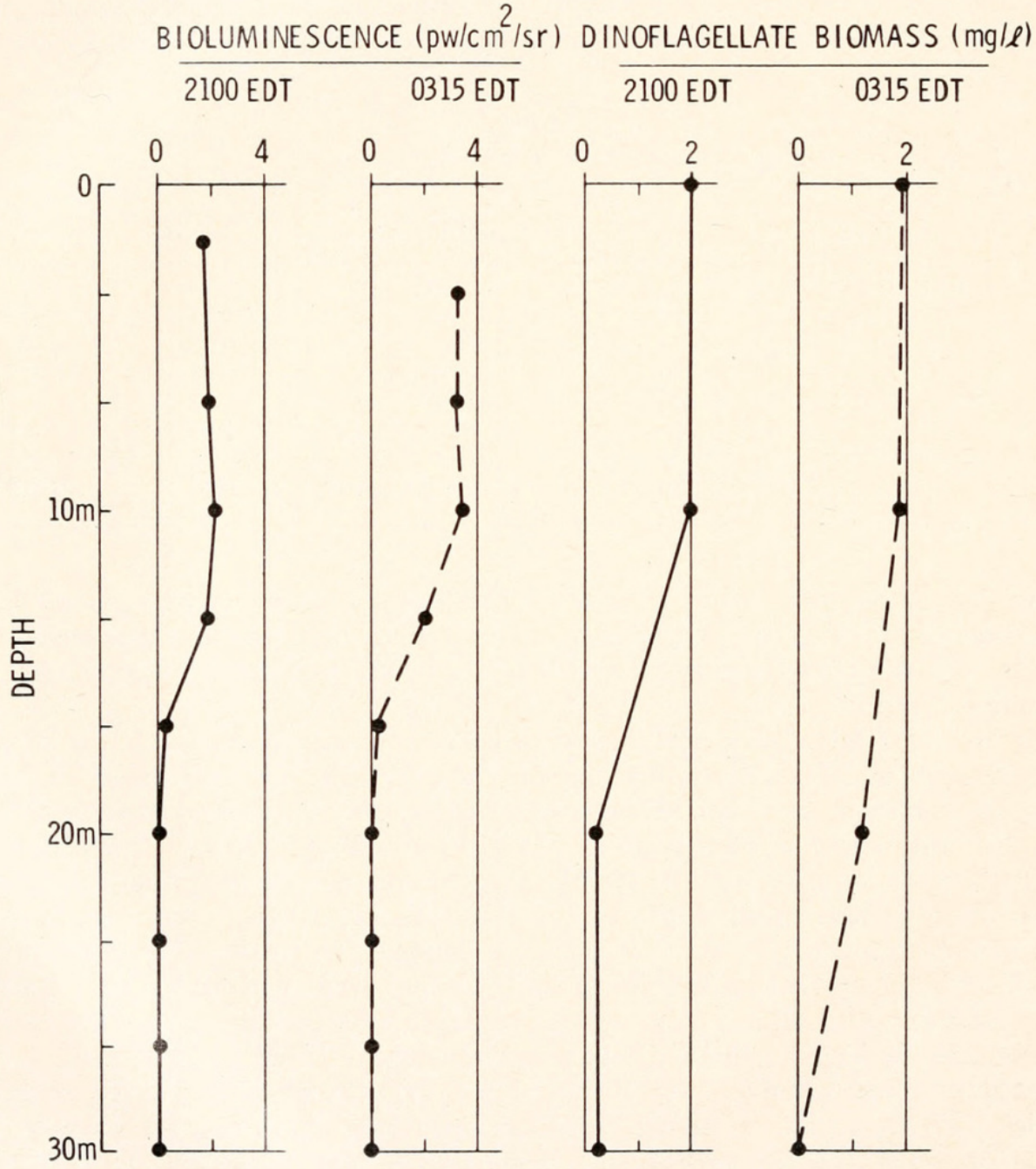

Figure 3. Comparison of *in situ* bioluminescence measurements with dinoflagellate biomass at different depths and times.

TABLE II

Phytoplanktonts occurring in regions of high long wavelength (370 nm) ultraviolet fluorescence; depths: A-surface, B-10 m, C-20 m, D-30 m

Species	Date - Time		Chlorophyll *a*
	19–10–2140	20–10–0315	
Cyclotella caspia Grunow		C	yes
Skeletonema costatum (Greville) Cleve	A, B, C	A, B, C	yes
Thalassiosira condensata Cleve		B	yes
T. gravida Cleve	A, B, C		yes
T. nordenskioeldi Cleve	A	C	yes
Actinocyclus sp.		C	yes
Chaetoceros decipiens Cleve	A		yes
Biddulphia pulchella Gray	B		yes
Thallassiothrix frauenfeldi Grunow		B, C	yes
Cylindrotheca clostridium (Ehrenberg) Reimann and Lewis	A, C, D	B	yes
Nitzschia seriata Cleve	A, D		yes
N. affinis Grunow	B, D	A, C	yes
Exuviella marina Cienowski	A		yes†
Prorocentrum micans Ehrenberg	A, C	B	yes†
P. scutellum Schroeder	A, B, C, D	A, B, C	—
Gymnodinium simplex (Lohmann) Kofoid and Swezy	B		yes*
Goniaulax diegensis Kofoid	A, D		—
G. polygramma Stein	A, D		—
G. spinifera (Claparede and Lackmann) Diesing		A	yes*
Peridinium depressum Bailey	A, B, C	B, C	no*
P. minusculum Pavillard	A	A	no*
P. ovatum (Bouchet) Scheutt		C	yes†
P. trochoideum Lemmerman	A, B	A, B, C	yes*
Dinophysis ovum Schuett	B	A, B	yes*
Glenodinium sp.	A		—
Ceratium lineatum Ehrenberg	B		—
C. arcticum Ehrenberg	B	A, B	—
Olisthodiscus luteus Carter		A, B	yes
Eutreptiella marina de Cunha	A		yes
Cryptomonas salina Wisloch	B		yes

† Raymont, 1967.
* Lebour, 1925.
—undetermined

analyses of the phytoplankton (Table II) indicate the major portion of the biomass to be made up of Bacillariophyceae and Dinophyceae. Also listed in Table II are the phytoplankton occurring in the regions of high long-wavelength fluorescence (surface and 10 m at 2100 hours, the surface and 10 and 20 m at 0315 hours), and the concurrent depth locations where they were found; there is a slight amount of overlap at the greater depths. Also indicated in Table II are those organisms that contain chlorophyll *a* and thus would be expected to fluoresce in long-wavelength ultraviolet light, permitting detection by this technique. In Figure 2, at 2100 hours at 7 m there is a chlorophyll increase not picked up in the bottle sampling.

The *in situ* observations of bioluminescence correlate with the dinoflagellate biomass (Fig. 3), as would be expected from the work of Kelly (1968). Haxo and Sweeney (1955) and Hastings and Sweeney (1958) have demonstrated an endogenous diurnal rhythm in laboratory cultures of *Gonyaulax polyedra*. An anomalously high value of dinoflagellate biomass occurs in the bottle sampling at 0315 hours at a depth of 20 m; this appears to be the result of local turbulence occurring at about 18 m depth since it does not agree with the *in situ* measurement. Turbulence in the water was observed with the thin film anemometer located on the platform (Fig. 1, sensor a). Note that the bioluminescence at 0315 hours is 1/3 higher than at 2100 hours, even though the dinoflagellate biomass is the same near the surface; this is presumably the result of the continuing increase subsequent to normal daylight inhibition of the luminescence of dinoflagellates, or an endogenous diurnal rhythm reported by Kelly and Katona (1966).

Correlation of the strong *in situ* bioluminescence occurring down to the thermocline (at 18 m) with laboratory determinations of dinoflagellates in the same region is presented in Table III. Known luminescent dinoflagellates are indicated and those with unknown characteristics denoted by a dash. It can be seen from the distribution of phytoplankton and known bioluminescent organisms that the bioluminescence observed is the result of the Dinophyceae: Goniaulaceae, and Peridiniaceae; many of the species recovered by us enhance and confirm the work of Kelly (1968) and Tett (1971), but we had certain dinoflagellates (Prorocentraceae) not reported as being bioluminescent, but observed within the population.

It appears that the zooplankton do not contribute to the total bioluminescence.

Augmenting factors that affect the efficiency of a luciferin-luciferase bioluminescence are water salinity, pH, pressure, and temperature, all of which varied with depth, and thus could cause some effect. The temperature of the water dropped from 16.4° C to 9.8° C below the thermocline, which would tend to decrease the bioluminescence.

There was no correlation of biomass, or dinoflagellate biomass, with the short wavelength ultraviolet fluorescence, indicating the dominance of Gelbstoff and/or dissolved minerals over biomass.

Discussion

Much of our present knowledge of the phytoplankton physiology arises from indirect sources such as laboratory experiments on marine cultures, or studies of general plankton ecology emphasizing primary production. However, it is most desirable for the environmental physiologist to conduct experiments at sea utilizing natural populations. However, in natural populations the investigator generally must work with low biota concentrations and techniques that the insensitive for experimentation. We have approached this problem with our *in situ* instrumentation, whereby a large volume of water is subjected to the experimental investigation to achieve a higher sensitivity. The importance of *in situ* measurements in this regard has been emphasized by Yentsch (1962) and Kelly (1968). This situation existed at the time of our study. Our observations were made during a transition time when the typical temperate diatom population was taking dominance over the typical summer flora, as evidenced by the appearance of Thallasiosira species and other diatoms (Table II).

Our technique for the measurement of bioluminescence differs from that of Kelly (1968) in that our index of bioluminescence is a photometric measurement of total integrated energy during agitation of the media. Kelly (1968) measured a flashing rate with his undersea photometer during agitation. The flashing rate does not measure the energy unless a time-intensity integration is performed on the luminescent flash record. The technique used by Kelly is applicable for areas

TABLE III

Dinoflagellates occurring in region of high bioluminescence; depths of samples: A-surface, B-10 m, C-20 m, D-30 m

Species	19–10–2140	20–10–0315	Luminescent
Prorocentraceae:			
Exuviella marina Cienowski	A		—
Prorocentrum minimum var. triangulatum (Martin) Hulbert	A, C	B	no*
P. scutellum Schroeder	A, B, C, D	A, B, C	—
Gymnodiniaceae:			
Gymnodinium simplex Kofoid and Swezy	B		—
Goniaulaceae:			
Goniaulax diegensis Kofoid	A, D		probable*
G. polygramma Stein	A, D		yes*
G. spinifera (Claparede and Lackmann) Diesing		A	yes*
Peridiniaceae:			
Peridinium depressum Bailey	A, B, C	B, C	yes*
P. minusculum Pavillard	A	A	—
P. trochoidium Lemmerman	A, B	A, B, C	no*
Dinophysiaceae:			
Dinophysis ovum Schuett	B	A, B	no*
Glenodiniaceae:			
Glenodinium sp.	A		no*
Ceratiaceae:			
Ceratium lineatum Ehrenberg	B		no*
C. arcticum Ehrenberg	B	A, B	no*

* Kelly (1968, Ph.D. thesis, Harvard University) and Kelly, 1969; Tett, 1971.

where the bioluminescence is weak, whereas when strong bioluminescence or a wide range of bioluminescence occurs, our technique, responding to the integrated light intensity, appears to be the best solution. Flashing rate measurements require a pulse counting technique, which is more complicated than the integrated light energy approach. The circuitry must be of the fast response, low time constant type, in order to respond to light pulses occurring at rapid rates, and to distinguish between nearly coincident pulses. Further, the interpretation of physiochemical reactions requires energy balance considerations, and the integrated light measurement furnishes the pertinent information directly. It is important to note

that this technique requires that there be an adequate supply of luminescent organisms and the necessary oxygen (McElroy and Seliger, 1963) which would not generally exist in a laboratory environment. A further consideration in the measurement of bioluminescence is that the spottiness noted by Kelly (1968) was also observed in our *in situ* measurements. This requires an averaging be made over a period of time that is long compared to the variations.

If measurements of bioluminescence are made without intentional agitation, either by virtue of a motor-driven impeller or by the motion of the photometer through the water, there is an uncertainty as to the physical cause of the bioluminescence (Tett, 1971).

Our fluorescence measurements of chlorophyll *a,* bioluminescent organisms, Gelbstoff or dissolved minerals similarly are made *in situ,* in a large volume of water. This has a limitation of application in highly turbid, estuarine water, because the ultraviolet radiation from the source lamps will be scattered and absorbed very strongly, as will be the fluorescent radiation. In an application of this sort, an alternative would be a flow through a Turner fluorometer because it has shorter light path lengths (Strickland and Parsons, 1968). However, the sensitivity would generally be severely decreased because of the smaller volume of turbid water with its increased absorption both of the ultraviolet and fluorescent radiation.

A criticism of the present instrumentation is that the configuration (Fig. 1) has the highest sensitivity when the background light level is lowest. This is no great problem for our measurements because the bioluminescence is greatest at night, and chlorophyll or Gelbstoff fluorescence measurements may be made over a night tidal cycle. However, if a 24 hour operational cycle is desired, a light tight shroud can be placed around the system and a recalibration made.

The ultimate use of *in situ* measurements is for mathematical modelling of a specific area. One application of *in situ* measurements for mathematical modelling has been made for Bahia Fosforescente by Seliger, Carpenter, Loftus, Biggley and McElroy (1971), although not without extensive associated laboratory investigations. They proposed three conditions as sufficient for modelling this bioluminescent bay, with two parameters to define the equilibrium state of the bay (the concentrations of *Pyrodinium bahamense* Margalef and *Ceratium furca hircus* Margalef). Effects are attributed to salinity, tidal flow, wind conditions, rain runoff, and geology. Nutrient concentrations are treated in a gross manner, since the detailed physiology of dinoflagellates and their relation to nutrients is as yet poorly determined (Kelly, 1968).

It is of interest to note that *Olisthodiscus luteus* Carter and *Eutreptiella marina* Cunha (Table II) appeared in high concentrations, although not indicated in the tabular data. Typical concentrations were 5000/liter at 0315 hrs at 30 m depth for *Olisthodiscus* and 1000 to 50,000/liter between 1735 and 2100 hrs at various depths. The appearance of these organisms has been noted by one of us as appearing at Bridgeport Harbor, Hempstead Harbor, and other small eutrophic embayments of Long Island. Palmer (1969) has indicated the Euglenoid group as a prime indicator of nutrient rich waters. As shown in Table II, both of these organisms fluoresce when irradiated with long wavelength ultraviolet radiation. Hence an *in situ* fluorescence technique offers possibilities for the monitoring of water quality.

The use of fluorescence as an indicator of chlorophyll, Gelbstoff, or dissolved

minerals for mathematical modelling is not yet tractable. It appears that an approach utilizing selective wavelength excitation and sensing would be advantageous to determine the form of chlorophyll *a in situ* (Goedheer, 1964). Further, any fluorescent dissolved minerals would have excitation and emission bands determined by their atomic and molecular bonds, which would characterize them. However, spectral resolutions necessary would be of the order of a nanometer, which requires a monochromator, with associated higher instrumental complexity. Nevertheless, for ecological monitoring of estuarine areas in terms of primary production and algal blooms, *in situ* fluorescence instrumentation permits rapid evaluation and subsequent monitoring of estuary conditions.

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Summary

We have described a series of measurements emphasizing the *in situ* aspects of bioluminescence and fluorescence photometric observations. The fundamental question of isolating a particular organism and determining whether it fluoresces or bioluminesces has not been answered; however, we have observed a phenomenon and sought to deduce the probable causative organisms. It appears that the causative organisms may be inferred by a comparison of the *in situ* bioluminescence, short-wavelength ultraviolet-produced fluorescence, and long-wavelength ultraviolet-produced fluorescence with the laboratory determined biota distribution.

Using this approach, we find that strong bioluminescence occurs above the thermo/halicline and appears to be caused mainly by Peridiniaceae and Gymnodiniaceae.

The long-wavelength ultraviolet fluorescence is correlated with the total chlorophyll and consequently with the total biomass.

It is evident that *in situ* bioluminescence and fluorescence instrumentation is not restricted by collection problems, and is not handicapped by the time lapse between sample collections and analyses; it is well suited to long term monitoring of estuarine areas.

To understand the energetics of a bioluminescent reaction, it is necessary to express the transformation in terms of an average light energy output rather than a flash rate.

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