

AN ABSTRACT OF THE THESIS OF

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The nature of the bioluminescence of Gonyaulax catenella is similar to that observed for other dinoflagellates in culture, showing photoperiod-entrained rhythms of luminescence and stimulability with relatively constant luminescent capacity during scotophase. G. catenella is very sensitive to stimulation and photoinhibition. The nature of the response to stimulation by bubbling appears similar to that of G. polyedra, and comparisons of the total stimuable light with G. polyedra indicate that G. catenella emits approximately  $6 \times 10^7$  photons per cell, during exhaustive scotoptic stimulation.

Over a range of cell concentrations, the rates of cell removal and filtration for Calanus pacificus when grazed on G. catenella were considerably lower when the dinoflagellates were in a relatively non-luminescent phase as opposed to a highly sensitive and luminescent phase. These differences could not be attributed to differences in particle size, culture age, distribution of dinoflagellates, ambient

light intensity, rhythms in copepod feeding activity, or other factors reported to affect copepod grazing. Possible mechanisms of the effect are discussed.

It is proposed that bioluminescence in dinoflagellates serves as a protean display type of defense mechanism against copepod grazing, has selective value, and is of adaptive and ecological significance.

**Ecological Implications of Dinoflagellate Bioluminescence**

by

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# ECOLOGICAL IMPLICATIONS OF DINOFLAGELLATE BIOLUMINESCENCE

## INTRODUCTION

The bioluminescence of dinoflagellates has received considerable attention over the past decade, with emphasis on the distribution and identification of luminescent species (Kelly, 1968; Seliger and McElroy, 1968), the physiology and nature of their luminescence (Biggley et al., 1969; Eckert, 1966), and the biochemistry of their luminescent systems (Hastings et al., 1966; Lee and Winans, 1968). A review by Loeblich (1967) and the works cited above will acquaint the reader with the major aspects and state of the art.

The ecological significance and adaptive value of bioluminescence is readily apparent or has been shown for many organisms, but functional uses of the light in some lower organisms, including the dinoflagellates, are not so obvious. For this reason some workers are of the opinion that bioluminescence in the dinoflagellates is fortuitous, a by-product of some metabolic process essential to the cell, and shows no adaptive significance (Nicol, 1967; Harvey, 1952; McElroy and Seliger, 1962; Sverdrup, Johnson and Fleming, 1942).

Several characteristics of dinoflagellate luminescence indicate that there is a great degree of control placed upon it and associated processes, which indicates adaption of the phenomenon for a functional use in the environment. Among these are the requirements



for stimulation rather than continual emission, maximum capability for luminescence only throughout periods of darkness (when the relatively weak light can be of significant value), a fast recovery rate following stimulation, and its non-essential quality in regards to cell growth and maintenance. It can be argued that the color of the luminescence has been determined by selective pressures, since it lies in that region of the spectrum which is attenuated least by oceanic water and to which the majority of marine visual pigments are most sensitive (Nicol, 1967).

Kelly (1968) has noted that apparently there are strong selective pressures on luminescence in the sea, and that the expenditure of the energy required for luminescence and its associated systems would be unlikely if it had no adaptive value.

There has been no previous experimental work dealing with possible selective advantages or adaptive values of dinoflagellate bioluminescence, although such a function is suggested by its characteristics. In a free living, photosynthetic unicellular organism, a protective function would appear to be the most likely use of luminescence. It is therefore relevant to study the effects of the bioluminescence on the interactions of planktonic herbivores and dinoflagellates, since grazing organisms are the major consumers of the phytoplankton.

The purpose of this work was to investigate the effects of

luminescence in dinoflagellates on copepod grazing. Copepods are rather easily obtainable, and graze effectively under laboratory conditions. In order to minimize the discrepancy between laboratory flasks and the natural environment, a dinoflagellate and copepod grazer were chosen which are known to coexist and presumably interact in nature.

## MATERIALS AND METHODS

### Experimental Organisms

The photosynthetic luminescent dinoflagellate Gonyaulax catenella Whedon and Kofoid (1936) was isolated from phytoplankton collections taken 5 miles off Newport, Oregon, in January of 1969. The individual cells, or chains of cells, were washed three times in small volumes of autoclaved sea water using a mouth-controlled micropipette, and inoculated into 13 x 80 mm test tubes containing 5 ml of medium diluted 1:25 with sea water, and incubated under constant light at 15 °C. Care was taken to avoid temperature, salinity, and nutrient shocks as suggested by Nordli (1957). Success of isolants was best using the above tube sizes and volumes similar to the findings of Wall et al. (1967). Three clones of the organism were obtained in persistent unialgal cultures.

Gonyaulax catenella was identified on the basis of the overall size and shape of the cell, its tendency to form chains, shape and arrangement of the apical plates, and the presence of a girdle curtain on the right ventral epicone of some individuals.

A culture of G. polyedra was the gift of Dr. H. H. Seliger.

Both species were grown in an enriched sea water medium (Table 1) very similar to that reported by Swift and Taylor (1967).

Table 1. Culture Medium

Millipore filtered sea water	750 ml	
Distilled water	250 ml	
NaNO <sub>3</sub> (150 g/liter)	1 ml	(1.76 mM)
Na Glycerophosphate (.1M)	2 ml	(0.20 mM)
Fe sequestrene* (10 mg/ml)	1 ml	(23.2 μM)
NTA (35 mg/ml)	2 ml	(0.37 mM)
Metal mix "a"***	1 ml	
Vitamin mix ***	0.5 ml	

\* Sodium iron salt of ethylenediaminetetracetic acid  
(EDTA) 13% Fe

\*\* Metal mix "a"

CuSO <sub>4</sub>	0.079 mM
ZnSO <sub>4</sub>	0.015 mM
CoCl <sub>2</sub>	0.085 mM
MnCl <sub>2</sub>	1.82 mM
NaMoO <sub>4</sub>	0.052 mM

\*\*\*

Vitamin mix	mg/liter
Thiamine·HCl	120
Pyridoxamine·2HCl	20
Riboflavin	8
Ca Pantothenate	60
Nicotinic acid	80
Folic acid	5
Biotin	0.2
Cobalimine	0.02
Putrescine:2 HCl	8
PABA	0.2
Choline	200
Inositol	200
Thymine	200
Orotic acid	60
D, L-Thioctic acid	66
Folinic acid	0.4

The diluted seawater was autoclaved at 15 lbs gauge pressure for 10 - 15 minutes, and the nutrients added aseptically after cooling.

Cultures were normally illuminated by cool-white fluorescent lights (2600 uw cm<sup>-2</sup>) at 15 or 20°C.

Female Calanus pacificus were collected at frequent intervals with a half-meter net 3 miles off Newport, and were kept in one gallon wide-mouth jars half filled with Millipore\* filtered sea water at a maximum density of 150 animals per jar. They survived well if kept in dim light at 15°C and fed periodically with phytoplankton.

#### Cell Counting

Replicate 2 ml samples of cultures were taken while stirring gently with a magnetic stirrer, and placed in 3 ml test tubes. A small drop of I<sub>2</sub>KI solution (Soli, 1966) was added to immobilize and preserve the cells if counting was to be delayed, and the tube sealed with Parafilm\* to retard evaporative volume changes. The contents of these samples were counted in a Sedgewick-Rafter cell by making several lengthwise scans to obtain an average cell density per sample based on no fewer than 300 individuals when possible.

Counting three samples per culture yielded an average coefficient of variation (s. d. /mean of three samples) of about 0.08. In grazing experiments from six to eight samples of each culture were counted, and coefficients of variation of about 0.05 were obtained. The dilution caused by adding the drop of stain was estimated to be less than 0.5%, and was considered to be negligible.

### Cell Sizes

The transapical and transverse diameter of cells were measured with an ocular shearing micrometer, with an estimated precision of  $\pm 2\%$ .

### Photometer

The apparatus used for recording the flashing rates, total stimuable light, and intensity of luminescence consisted of an RCA 1P21 photomultiplier tube in a Gamma Scientific housing, a Keithley 240A High Voltage Power Supply, a Keithley 301K electrometer amplifier powered by an Acopian dual power supply and equipped with a shielded switch containing the feedback elements, and a Moseley 680 strip chart recorder. The amplifier feedback elements consisted of 10 and 100 megohm precision resistors, and 0.15 and 0.01 uf low leakage polystyrene capacitors, allowing measurements of luminescence intensity and total emitted light. A shorting switch was installed to discharge the capacitors and zero the recorder at unity gain. Coupled with the wide sensitivity range of the recorder, the apparatus was fairly versatile, although the relatively slow response time of the Moseley (0.5 sec. full scale) did not allow an accurate measurement of flash peak heights.

The amplifier was calibrated with a millivolt potentiometer.

All light measurements were made relative to a one volt deflection at 1000 volts potential using the 0.15 uf capacitor, which was defined as one relative light unit. Gain calibrations were made with a constant low-level emission standard fabricated from parts of a self-luminous watch, which was also used to check the overall sensitivity of the apparatus with time.

The phototube housing, which was provided with a variable neutral density filter, was fixed to a light-tight box in which was mounted a holder which received 16 mm test tubes in a fixed position 3 cm in front of the photomultiplier tube. A 2" diameter concave mirror behind the holder focused light from the test tube on the photomultiplier tube to give increased sensitivity. When assaying bright cultures, the neutral density filter was adjusted to insure that the ratio of anode/dynode current never exceeded 0.05 to insure a linear response to incident light.

#### Luminescence Assays

Samples containing from 3000 to 15,000 cells in 3.0 ml were removed approximately 6 hours prior to darkness from log phase cultures while stirring gently to insure a uniform distribution. The samples, in 16 x 100 mm plastic tubes, were placed in racks and taken to a dark room in a light-tight box at darkness, and at the appropriate time gently inserted into the assay geometry. When recording the

total stimuable light (TSL), the cells were stimulated by bubbling air through a No. 15 canula at a rate of 1.5 liters per minute, with the tip 1 cm below the surface of the sample. The current from the photomultiplier was integrated for two minutes, at which time the cells were essentially exhausted. During scotophase the majority of the light was emitted during the first fifteen seconds of stimulation.

When measuring spontaneous luminescence, samples of a culture in tubes or in 30 - 50 ml beakers were placed in front of the phototube; Aluminum foil was wrapped around the opposite side of the beakers to increase the sensitivity when necessary. The effects of copepod feeding and swimming activities on spontaneous luminescence were determined in a similar manner.

While the photomultiplier and assay geometry was not calibrated absolutely, we have been able to perform an approximate calibration using the known photon yield of Gonyaulax polyedra (Biggley, et al., 1969). The TSL/cell of this particular organism has been shown to be fairly constant during scotophase regardless of the manner of stimulation, culture age, time, and over a range of growth illumination. The culture of G. polyedra obtained from Dr. Seliger has been grown here under very similar conditions of light, temperature, and nutrient medium, and the luminescence assayed by similar techniques, and it is felt that the procedure is justifiable until more rigorous calibration is performed.



### Copepod Grazing Rates

The methods used to determine copepod grazing or feeding rates are very similar to those used by Conover (1956) and Mullin (1963). A suspension of phytoplankton is dispensed into several jars or beakers. Copepod grazers are added to some of these containers, the remainder serving as non-grazed controls. At the end of a suitable time period the concentrations of phytoplankton in the containers is determined, and the grazing is measured by a decrease in the cell concentrations of the samples containing copepods relative to the non-grazed controls.

The rate of cell removal (R) and the filtration rate (F) are computed according to the formulae:

$$R = \frac{(C_c - C_g) \cdot v}{N \cdot t} \quad \text{units} = \text{cells copepod}^{-1} \text{day}^{-1}$$

$$F = \frac{(\ln C_c - \ln C_g) \cdot v}{N \cdot t} \quad \text{units} = \text{mls copepod}^{-1} \text{day}^{-1}$$

where  $C_c$  is the final cell concentration of the control,  $C_g$  is the final cell concentration of the grazed sample,  $v$  is the volume of the grazing sample,  $N$  is the number of copepods used, and  $t$  is the time in days of the grazing experiment.

There are problems associated with these parameters. The rate of cell removal does not consider the effects of the exponential decrease in cell concentration and its effect on the grazing rate, and

the filtration rate gives the connotation that a copepod is an indiscriminate minimum porosity filter and not the selective and variable feeder which it has been shown to be (Mullin, 1963). These parameters are used because they enable us to compare experiments run under different conditions, and with values given in the literature, and as indications of bioluminescence effects on grazing.

## RESULTS

Growth and Luminescence of *G. catenella*

A knowledge of the characteristics of growth and bioluminescence of the dinoflagellate is necessary prior to using it to determine the effects of bioluminescence on copepod grazing. Details of these characteristics have not been reported in the literature, although Sweeney (1963) confirmed earlier reports of its luminescent nature with cultured material. Therefore the first part of this work concerned pertinent aspects of the physiology and luminescence of the dinoflagellate.

Growth

Growth curves for *G. catenella* at 20°C under LD 12:12 and constant light (Figure 1) show initial doubling times on the order of 3.3 and 1.8 days, respectively, which are in the range reported for other dinoflagellates (Loeblich, 1967). Growth appeared severely limited at temperatures above 23°C; cultures grew best at 15°C, the lowest temperature at which they were grown. Cell densities on the order of  $4 \times 10^4$ /ml were the maximum attained.

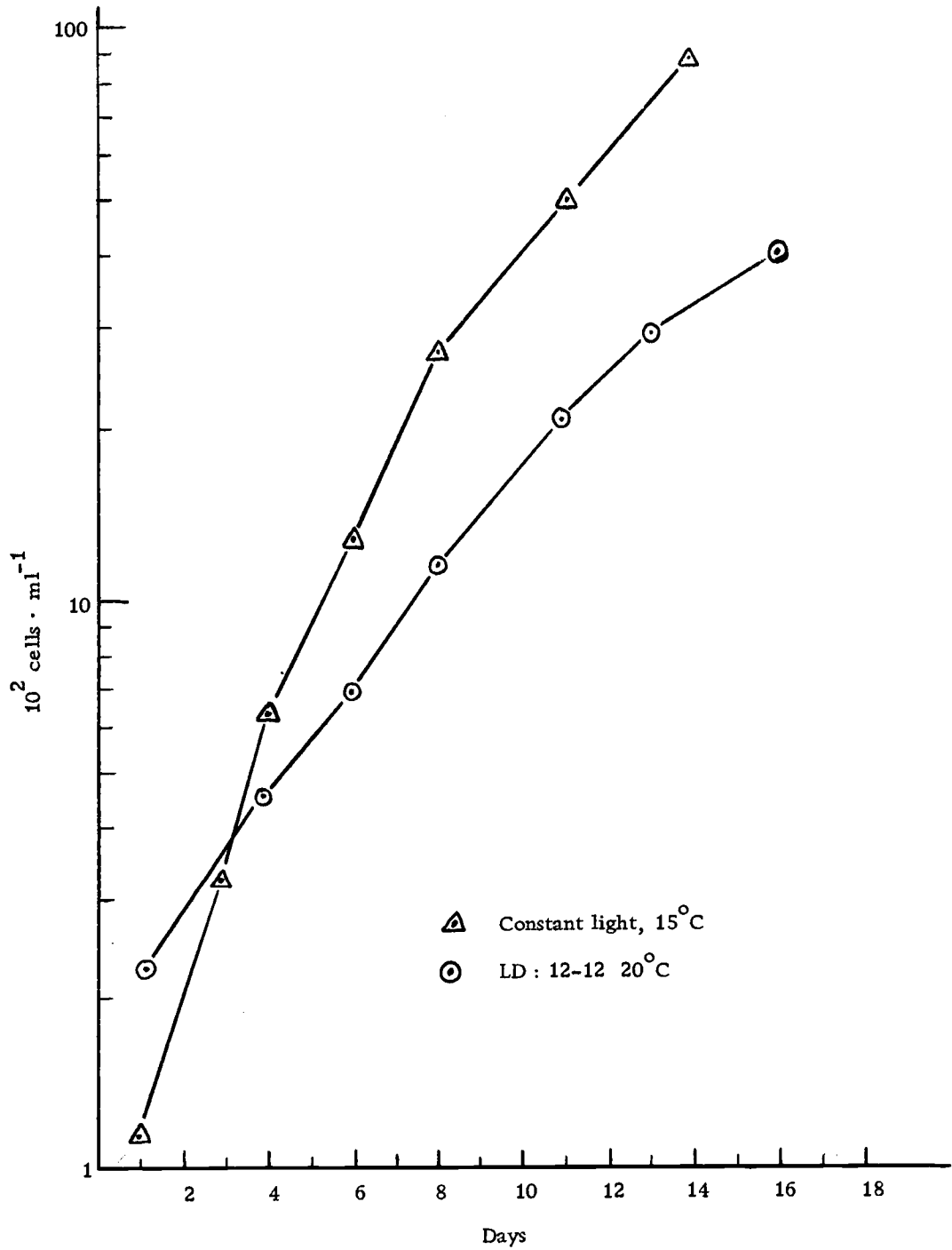


Figure 1. Growth of *Gonyaulax catenella*

### Chain Formation

The number of two and four cell chains was small ( $\sim 10\%$ ) in all cases using the reported culture medium, and seemed to remain constant through log and stationary phase. Dupuy (1968) observed that a similar but unidentified dinoflagellate of the genus Gonyaulax did not form chains in a medium similar to that used here, but formed chains of up to eighteen cells when grown in a soil extract enrichment. He further noted that the cells in the soil extract culture detached themselves from chains and reformed chains while under observation.

Gonyaulax catenella formed more and longer chains when grown in a soil extract medium, and chains of up to twelve individuals were noted. Although lengthy observations were made on cultures of various ages and cell densities, no recombination was seen, even when cells of the same size collided in the correct juxtaposition, and it is believed that chain formation is the result of cell division with a lack of separation.

### Time of Cell Division

Gonyaulax catenella apparently does not divide during periods of darkness, as is the case with some other dinoflagellates (Sweeney and Hastings, 1958). Cell counts of a culture grown on a LD 12:12 light regime show increases in cell density only during the

photoperiod (Figure 2). The time of cell division could not be detected by relative increases in the number of double cells, presumably due to the high normal percentage of two cell chains. There was only a very slight increase (<8%) in cell densities of cultures following the initiation of darkness in the grazing experiments, over a period of 30 hours.

### Luminescence

A discussion of the various rhythms and responses of stimuable and spontaneous luminescence is contained in a recent publication by Biggley et al. (1969), concerning the dinoflagellates Gonyaulax polyedra, Pyrodinium bahamense, and Pyrocystis lunula. The methods that were used in this study are very similar to theirs, including lighting, culture medium, and bubble stimulation for some of their results, which makes comparisons possible.

### Stimulable Luminescence

Samples of cultures of the three clones of G. catenella grown under constant light ( $2600 \text{ uw cm}^{-2}$ ) at  $20^{\circ}\text{C}$  were assayed for total stimuable luminescence (TSL) at various times after being placed in darkness. The results shown in Figure 3 demonstrate the existence of an exogenous rhythm in TSL for this organism. The increase in TSL following initiation of darkness is very fast, with a doubling time

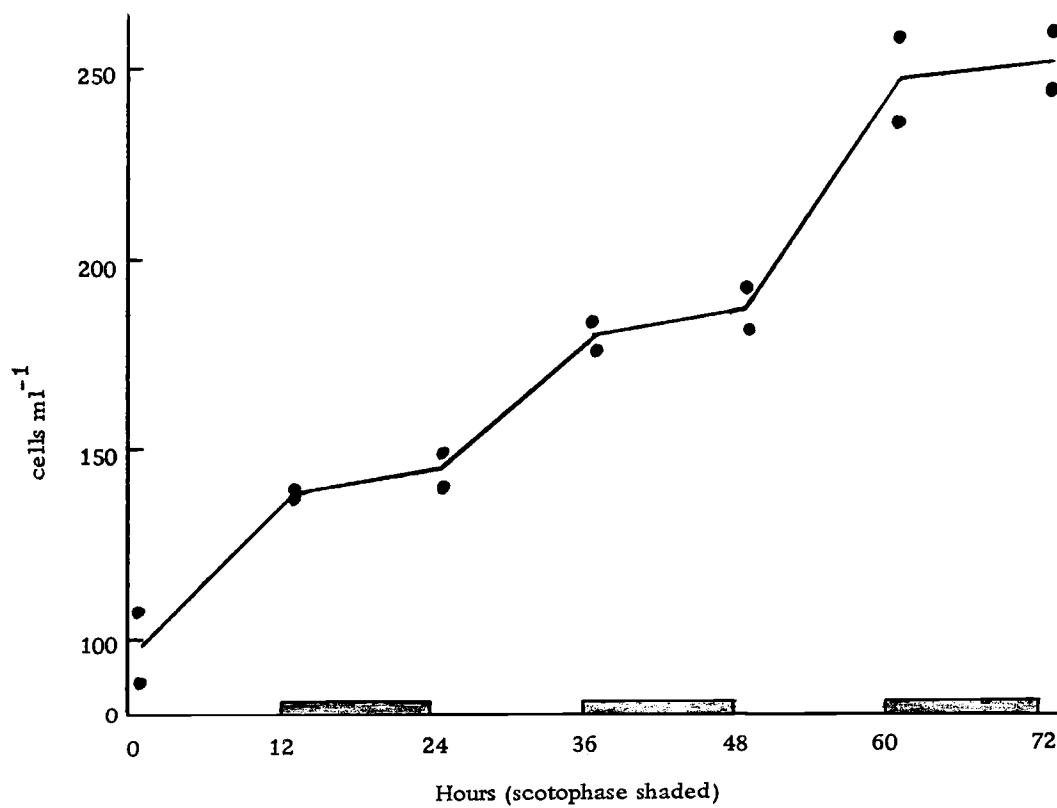


Figure 2. Time of Cell Increase

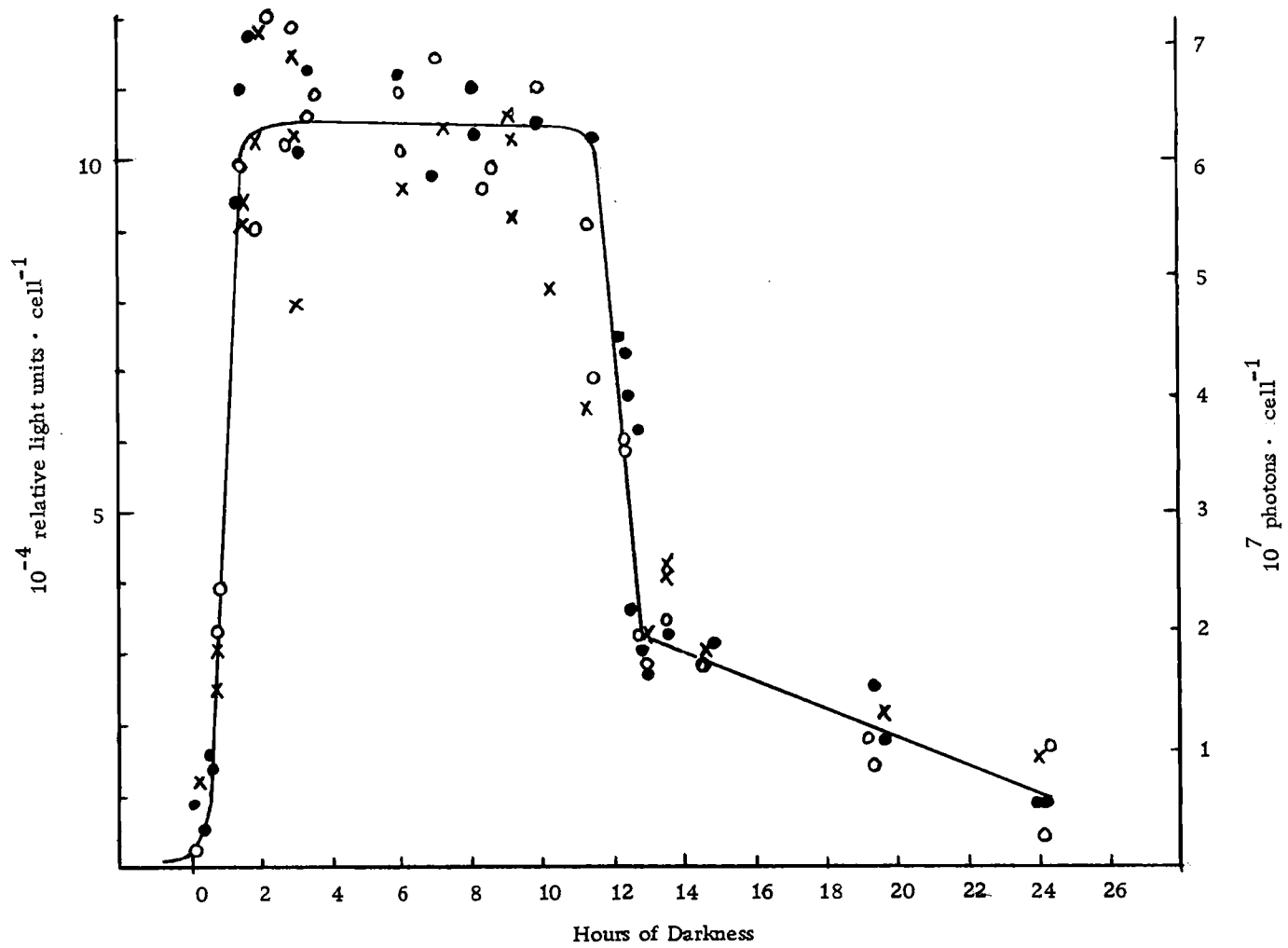


Figure 3. Total Stimulable Light for Three Clones of Gonyaulax catenella.



of ca. 9 minutes, and reaching a plateau in approximately 80 minutes. The decay of TSL at 12 hours ( $D_{12}$ ) for cells held in continued darkness is less rapid than the rise, and decreases to approximately 20% of the plateau value in two hours. Maximum scotophase/photophase ratios of TSL observed were on the order of 200. Decay of TSL at  $D_{12} = L_0$  for cells grown in a LD 12:12 regime is very rapid, and reaches values equal to those observed at  $D_0$ .

On the basis of the average plateau values, there is no detectable difference in TSL/organism during scotophase for the three clones isolated (t test,  $P < .01$ ).

Cells of G. catenella are extremely sensitive to any mechanical stimulation during scotophase, more so than G. polyedra from subjective measurements, and for this reason the data show a good bit of scatter. Biggley et al. (1969) found that P. bahamense was more sensitive and gave more variable results than G. polyedra. In addition, G. catenella appears to be much more sensitive to photo-inhibition than G. polyedra.

The output of the TSL measurements for cells which had been kept in the dark for more than 12 hours was consistently bimodal. Figure 4 shows actual traces of the TSL and intensity at  $D_6$  and  $D_{18}$ , indicating a second peak in the  $D_{18}$  recordings. A similar observation was made by Biggley et al. for G. polyedra only, during the first several minutes of scotophase.

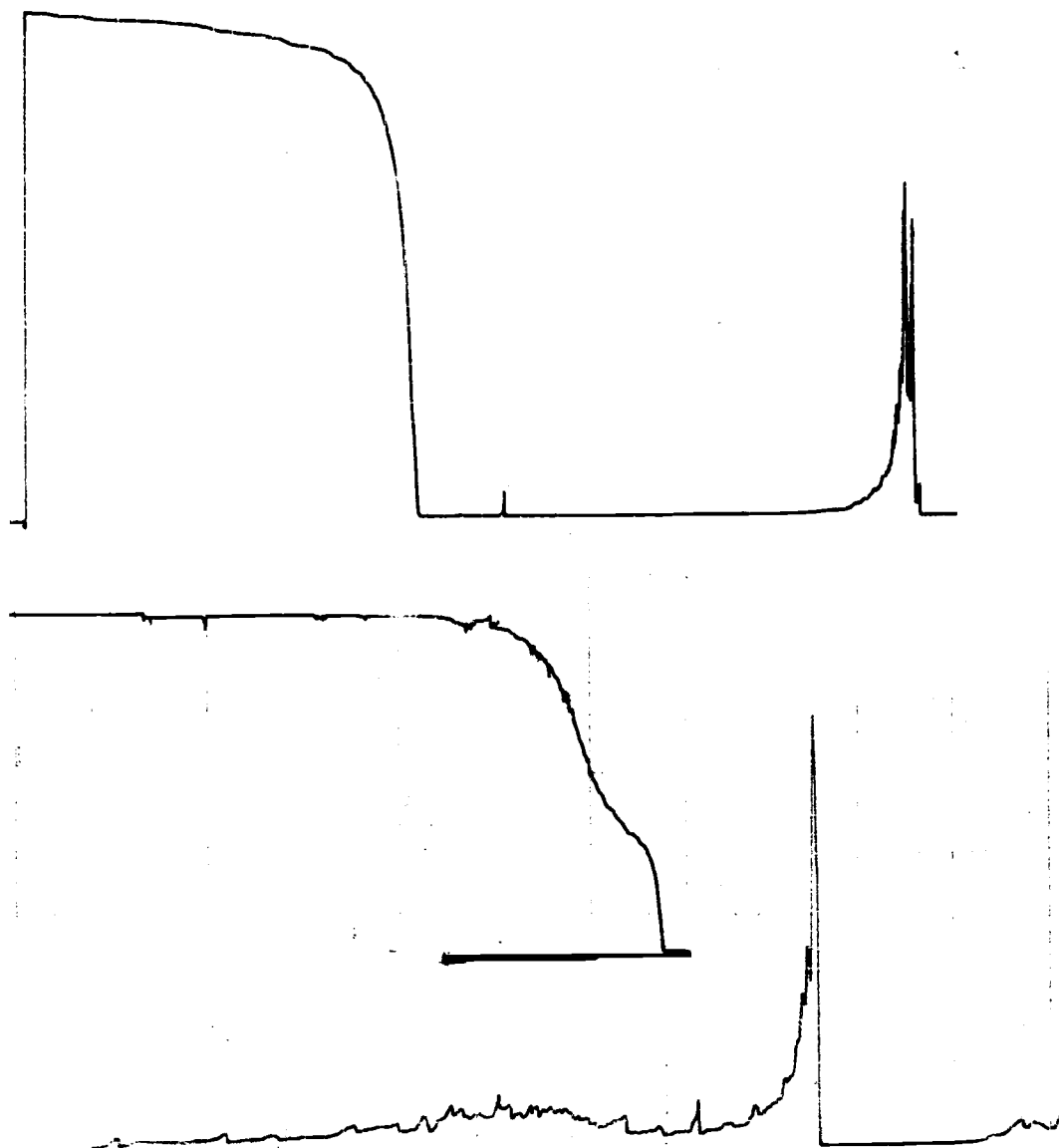


Figure 4. Upper trace - record of bubble stimulated flash and integrated TSL for G. catenella at  $D_6$ . Time scale 2 in/min, from right to left.

Lower traces - same at  $D_{18}$ . Time scales: flash - 8 in/min, integrated TSL - 2 in/min.

### Spontaneous Luminescence

G. polyedra exhibits a circadian rhythm of unstimulated intensity which peaks at  $D_{11}$  and corresponds to a period of maximum cell division (Sweeney and Hastings, 1958). Three attempts failed to show any indication of the "glow" rhythm in G. catenella, but did show an increasing rate of spontaneous flashing until  $D_5$  (Figure 5).

### Calibration Using G. Polyedra

Several determinations of maximum scotophase TSL/cell were made using cultures of G. polyedra and G. catenella which had been grown under identical conditions. The average ratio of the TSL between G. catenella and G. polyedra was found to be 0.566. Seliger *et al.* (1969) have found that G. polyedra grown under similar conditions emits  $1.15 \pm 0.07 \times 10^8$  photons/cell during scotophase. If we assume identical spectra and that minor variations in growth conditions have no great effect on the scotophase emission of G. polyedra, then G. catenella emits on the order of  $6 \times 10^7$  photons/cell during exhaustive stimulation in scotophase (Table 2).

The emission spectrum of G. catenella has not been determined yet, but in view of the nearly identical spectra obtained for the three dinoflagellates previously studied, and since no differences in the

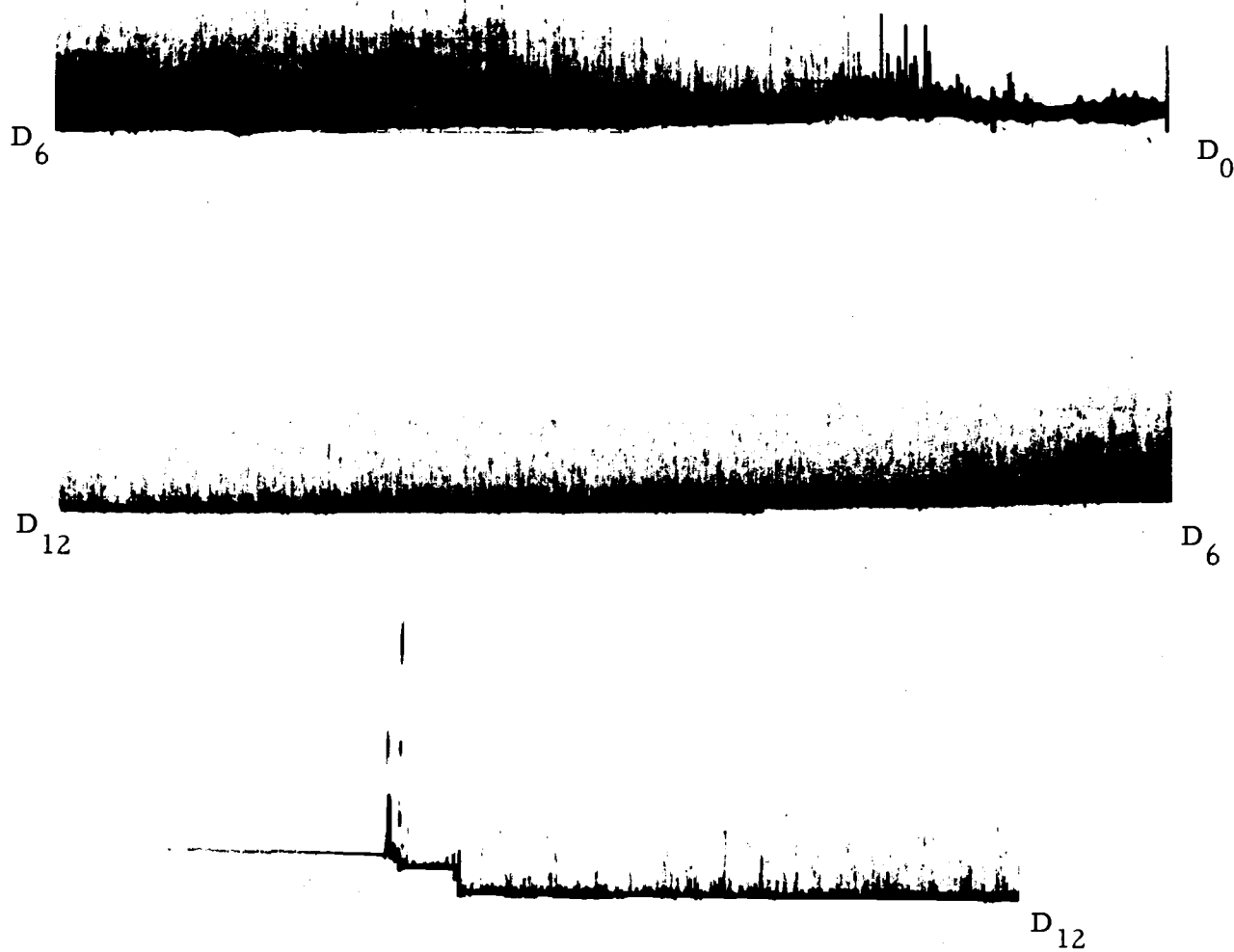


Figure 5. Record of the spontaneous luminescence of a 4.0 ml sample of Gonyaulax catenella throughout the scotophase. The baseline drifted considerably during this record, but was never "pinned." Chart speed: 1 inch/hour.

overall color of the light was detected by a dark adapted observer, there are probably no great differences in the spectra of the organisms used here.

Table 2. TSL Ratios for G. catenella and G. polyedra

Exp.	Organism		Ratio	Mean
I	<u>G. polyedra</u>	17.71 ± 1.77	0.619	
	<u>G. catenella</u>	10.98 ± 1.59		
II	<u>G. polyedra</u>	18.9 ± 1.70	0.524	0.566
	<u>G. catenella</u>	9.88 ± 0.89		
III	<u>G. polyedra</u>	18.47 ± 1.79	0.556	
	<u>G. catenella</u>	10.3 ± 1.32		

#### Effects of Copepods on Dinoflagellate Luminescence

Observations of copepods in cultures of luminous dinoflagellates showed that the turbulence produced during swimming and feeding activity was sufficient to produce very bright luminescence. In fairly concentrated cultures swimming movements gave the appearance of a streak of light, and stationary activity, probably feeding, was evident as a bright and continuous spot of light. At low concentrations representing those found under natural conditions (< 10 cells per ml) actions of copepods were very difficult to distinguish from spontaneous flashing.

Figure 6 is a copy of the record of flashing before and after

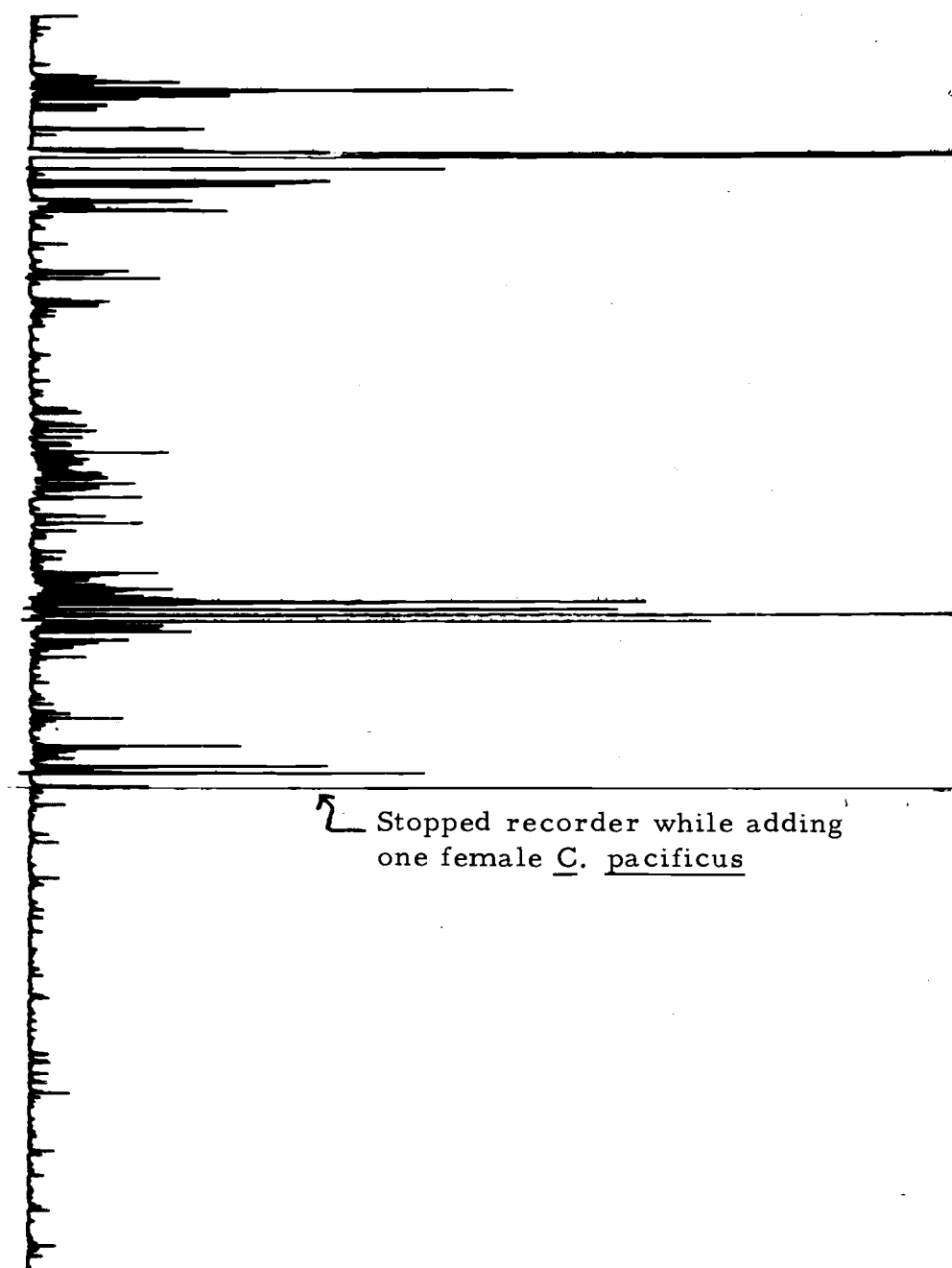


Figure 6. Record of copepod stimulation of Gonyaulax catenella.  
30 ml sample of scotophase ( $D_4$ ) culture, 2000 cells/ml.  
Chart speed: 1 in/minute.

the addition of a female Calanus pacificus. The cell concentration was  $2 \times 10^3$ /ml, and a 50 ml beaker containing 30 ml of culture was used. An increased rate of flashing was noted after the addition of the copepod, with variations in the rate and magnitude of the flashes depending on the behavior of the copepod.

### Effects of Luminescence on Copepod Grazing

#### Experimental Design

Previous studies (Marshall and Orr, 1955; Mullin, 1963; Conover, 1956) have shown that a variety of factors affect copepod grazing, in addition to the species and life stage of the animal. Copepods are selective for large food particles, and graze at a higher rate in darkness. The rates are inversely proportional to the age of the phytoplankton culture and cell density, and are affected by the algal species and temperature. A procedure was developed which allowed comparisons between conditions of high and low luminescence capability of the dinoflagellate food organism, and minimized differences in other factors known to affect grazing rates.

Sweeney and Hastings (1958) first reported that constant bright light suppressed the rhythmic variation of TSL in G. polyedra, and that the endogenous rhythm could be initiated, beginning with a luminescent phase, by placing such an arrhythmic culture in darkness

at any time of day. In this procedure, an arrhythmic culture of G. catenella was divided in half, and luminescence initiated by placing the samples in darkness at staggered intervals, to give samples of the same initial culture which were nearly 180° out of phase.

A week old, log phase, arrhythmic culture of the dinoflagellate, grown under continuous light at 15°C and having been inoculated with an arrhythmic parent culture grown under the identical conditions, was stirred gently to insure a uniform distribution of cells, and samples designated as type A removed. These consisted of approximately one dozen 3 ml samples for luminescence assays and counting, and larger samples to serve as the grazed and non-grazed conditions, and were placed in darkness at 15°C at a time  $t = 0$  hrs.

At  $t = 11$  hrs., samples designated as type B were removed from the still arrhythmic culture in a similar manner, and immediately placed adjacent to the A type samples in darkness and at 15°. At approximately  $t = 12$  hrs a number of randomly selected C. pacificus females were added to the samples which were to serve as the grazed conditions of both type samples, and were allowed to graze for from 10 to 12 hours. After this period, all samples were filtered through a 110 u Nitex\* screen to remove copepods and fecal pellets, the number and condition of the copepods recorded, the volume measured accurately, and from six to eight samples from each beaker taken for counting. The luminescence (TSL) for each

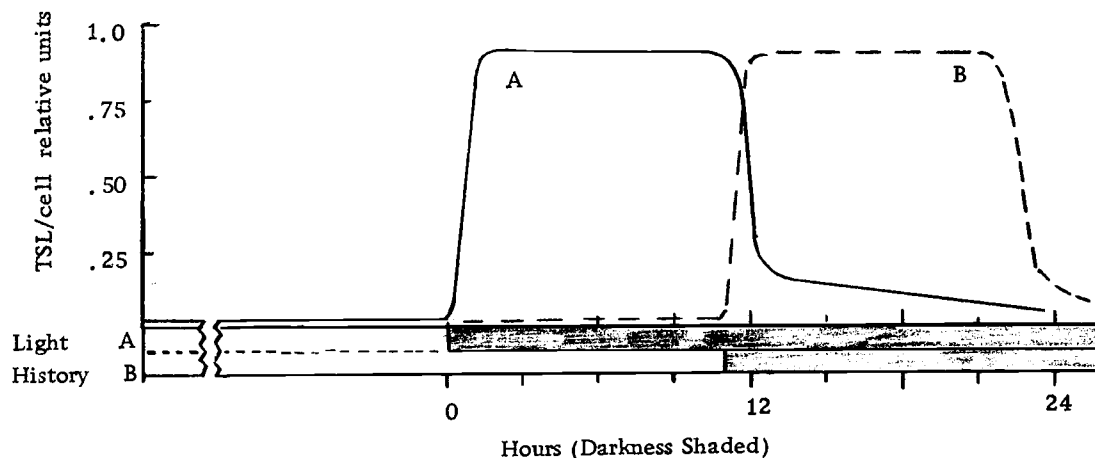


type sample was determined during the hours of grazing.

Figure 7 depicts the expected time course of luminescent capability, as predicted from a knowledge of the response of the organisms and the light assays. It is seen that from approximately  $t = 12$  to  $t = 23$  hrs that the luminescent capability of the cells in the A type samples will be at a minimum, and that of the B type samples at a maximum, while under identical conditions of darkness and temperature, and nearly identical cell concentrations and culture age. Thus, grazing during this period will occur when the cells in A are relatively non-bioluminescent compared to those in the B type samples.

No significant differences could be found in the size of the cells or percentage of double cells between the A and B type samples during the period of grazing, as shown in Table 3, using the  $t$  statistic to test the difference between the means. It is assumed that particle size differences between the A and B samples has no effect on the grazing rates.

The uniform distribution of food organisms in previous grazing studies was insured by various mixing processes. This was not possible in the present study, as mixing or stirring would induce turbulence and cause luminescence in the B series. The motile dinoflagellates used here assumed a relatively random distribution, but concentrated to some degree around the side of the beakers near the



## PROCEDURE

t = 0 A samples removed from arrhythmic cultures and placed in dark box

t = 11 B samples removed from arrhythmic culture and placed next to A samples in dark box

t = 12 Copepods added to A and B grazing samples

t = 12 Grazing period. TSL, cell size, percent doubles, and to distribution of cells determined.

t = 23 Bioluminescent capability is low in A samples and high in the B samples

t = 23 Grazing period ended. Cell concentrations ( $C_c$ ,  $C_g$ ) determined

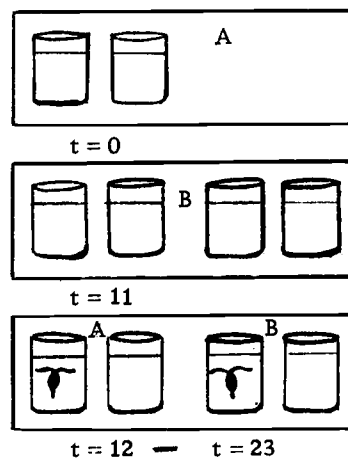


Figure 7. Expected Course of TSL for A and B Series

Table 3. Particle Sizes

Trial	Series	Averaged Mean Diameter in micra	N	"t"	Percent Doubles	N	"t"
I	A	29.3 ± 1.3	20	.198	10.5 ± 1.1	5	.493
	B	29.5 ± 2.7	20		10.8 ± 0.8	5	
II	A	28.8 ± 2.2	20	.438	12.3 ± 0.9	5	.848
	B	29.3 ± 1.6	20		12.9 ± 1.3	5	

Mean diameter is the average of the transapical and transverse diameters

A total of 400 cells were counted in each determination of the percent of doubles for each sample N.

Student's "t" statistic was used to test the difference between the two means. The null hypothesis ( $U_1 = U_2$ ) could not be rejected at the 90% level of significance in any instance.

meniscus in both A and B type cultures. Sampling near the bottom, middle and surface at five sites in the beakers at  $t = 18$  hours in a trial series showed no differences in the relative distribution of cells between the A and B series. This result was found qualitatively, and for all grazing experiments when examined visually at the termination. It is assumed that relative distribution of cells was not different in the two series.

The possible presence of endogenous rhythms in copepod feeding behavior will have no effect on these experiments, since grazing occurs at the same time for both series. The primary assumption is that the only difference between grazing in the A and B type cultures is the degree of luminescence of the dinoflagellates.

#### Comparative Grazing Rates

Following the procedure given above, five grazing experiments were conducted using G. catenella over a range of concentrations, and one experiment using G. polyedra as the food organism. Values and ratios of the TSL of each type culture for all experiments are given in Table 4. The experimental conditions and results of the experiments are listed in Table 5.

Grazing occurred in all instances, as shown by positive values for R and F. At similar cell concentrations, grazing was always lower in the B series, as shown by values less than 1.0 for the ratios

of grazing in the B series to grazing in the A series ( $R_B/R_A$ ,  $F_B/F_A$ ). Since no differences have been found between the two series other than the luminescence capacity, the results given here strongly indicate that the rate of copepod grazing is decreased when the dinoflagellate is highly luminescent.

Table 4. Total Stimulable Luminescence During Grazing

Experiment	Series	TSL cell <sup>-1</sup>		N
		(10 <sup>-3</sup> rel. light units)	TSL <sub>A</sub> /TSL <sub>B</sub>	
1	A	2.4 ± 0.3	0.221	12
	B	10.9 ± 1.6		10
2	A	2.7 ± 0.25	0.26	15
	B	10.4 ± 0.95		17
3	A	2.9 ± 0.3	0.257	13
	B	11.3 ± 1.3		14
5	A	3.0 ± 0.2	0.297	11
	B	10.1 ± 1.4		10
6	A	2.5 ± 0.3	0.236	9
	B	10.6 ± 1.1		13

Data for G. polyedra experiment not available.

A significant ( $P < .01$ ) negative correlation of  $\bar{F}$  to cell concentration for the A series is shown in Figure 8. Similar negative correlations have been presented by Mullin (1963) for C. hyperboreus grazing on Ditylum brightwellii and by Marshall and Orr (1955) for C. finmarchicus grazing on a number of phytoplankton including

Table 5. Grazing Results

Exp.	$C_c$	$C_g$	v	N	t	R	$R_B/R_A$	F	$F_B/F_A$
1A	2160	1890	340	17	11	10.7		5.39	
1B	2340	2270	330	17	11	2.96	.277	1.70	.317
2A	1230	1030	300	9	11	11.8		13.4	
2B	1290	1250	300	9	11	2.91	.247	2.29	.171
3A	466	288	750	38	12	7.04		19.0	
3B	480	373	750	38	12	5.44	.774	11.5	.605
4A	640	411	600	27	10.5	13.3		23.2	
4B	670	530	600	30	10.5	6.61	.498	11.1	.478
5A	600*	454	430	15	10.5	9.52		18.3	
5A	600*	430	410	15	10.5	10.6		20.9	
5A	600*	411	400	15	10.5	11.5	.699*	23.1	.693*
5B	615*	474	400	16	10.5	8.07		14.7	
5B	615*	498	400	16	10.5	6.68		12.1	
6A	191	137	412	14	9.5	4.01		23.9	
6A	197	152	430	13	9.5	3.77	.355	21.7	.302
6B	205	186	410	14	9.5	1.41		7.20	
6B	195	182	420	13	9.5	0.96	.379	5.65	.261

\* = mean value;  $C_c$ ,  $C_g$  in cells/ml; v in mls; N = No. copepods; t in hrs. R in  $10^3$  cells/copepod/day; F in ml/copepod/day. Experiment No. 4 used G. polyedra as the dinoflagellate.

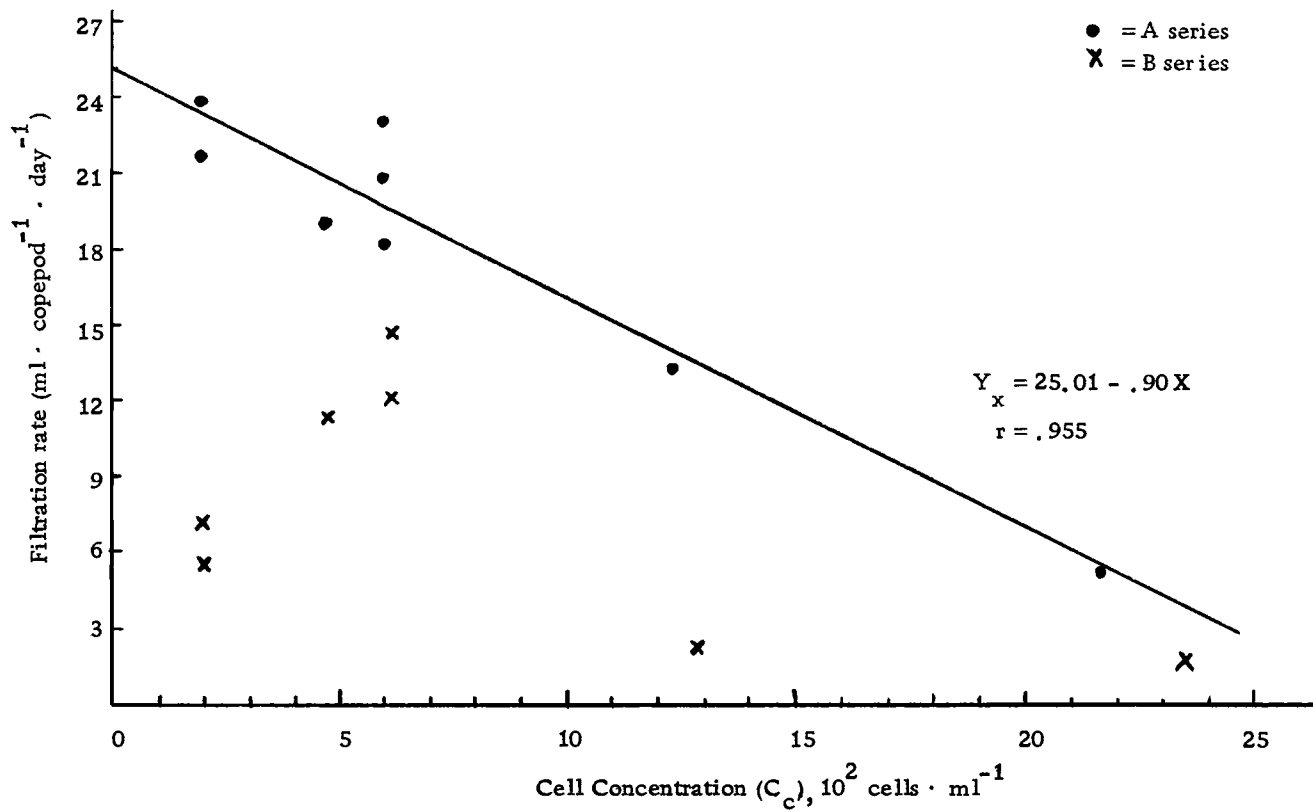


Figure 8. Filtration Rate of Calanus pacificus Grazing on Gonyaulax catenella

dinoflagellates. The maximum filtration rate found here is within the range of values reported by the above authors also.

Figure 9 shows the relationship of  $\underline{R}$  with cell concentration, which is of the same type presented by Conover (1966) in a plot of  $\underline{R}$  as a function of cell carbon concentration, for the A series.

The fact that these parameters for the A series show dependencies on cell densities and values similar to those reported by other workers indicates that the copepods behaved in the expected manner and there was normal laboratory grazing with the relatively non-bioluminescent cultures. When the dinoflagellates were capable of bright luminescence, there was less grazing, and no clear cell density effect on either  $\underline{R}$  or  $\underline{F}$ .

Since the dinoflagellates were in different physiological states in the A and B series, there is the possibility that some unmeasured parameter which affected copepod behavior was also different. If an extracellular metabolite or exotoxin was released differentially according to the physiological state, it would necessarily have to have been relatively labile in order to affect the results differentially, since each state was preceded by the alternate no less than twelve hours prior to any grazing. The copepods appeared normal in all respects when examined at the end of the grazing period, and little mortality was observed.



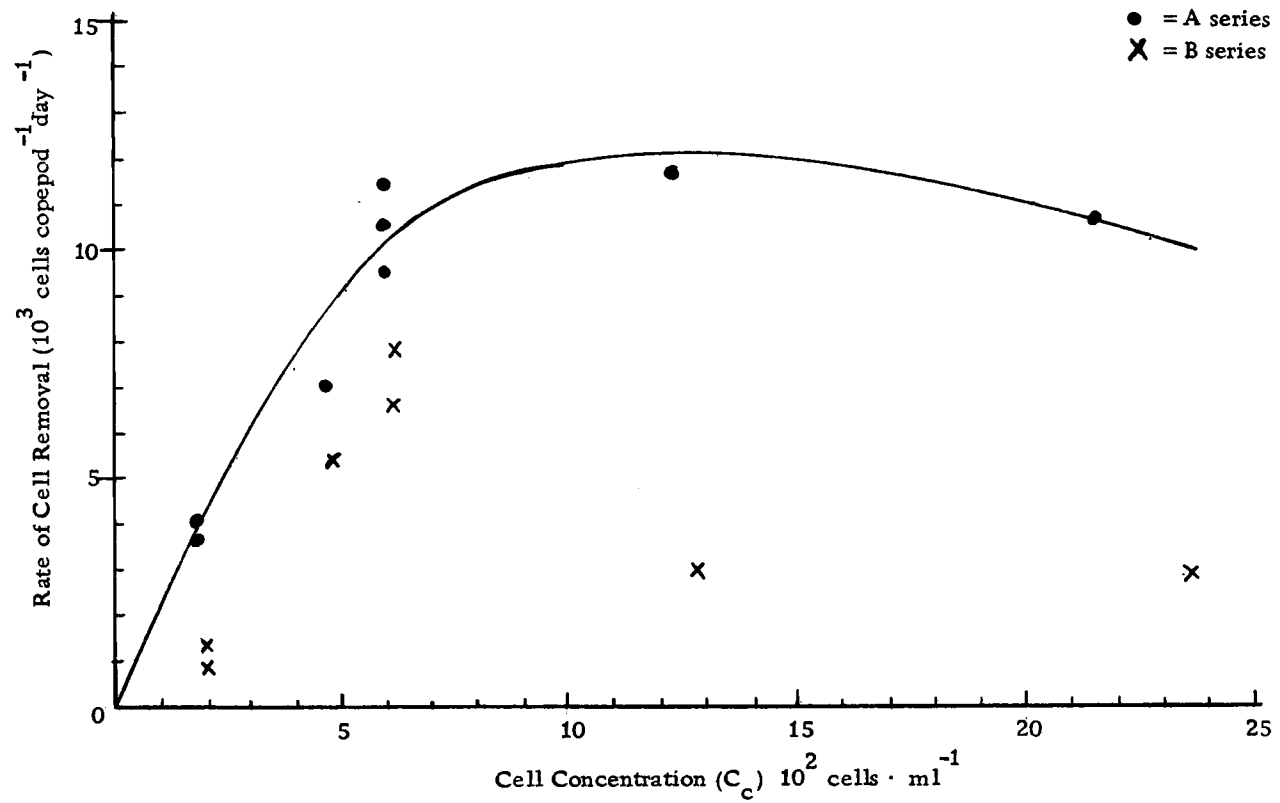


Figure 9. Rate of Cell Removal by Calanus pacificus Grazing on Gonyaulax catenella

## DISCUSSION

The four species of luminescent dinoflagellates which have been studied so far exhibit many similar properties, including photoperiod entrained rhythms of luminescence and sensitivity to stimulation, spectral qualities, and photoinhibition. Maximum scotophase/photo-phase TSL ratios are all above 200, and are relatively constant throughout the scotoperiod. Variations between genera and species in the amount of light emitted during exhaustive stimulation and the amount of stimulation required to produce luminescence are apparent. A correlation between the average ambient sunlight intensity and the amount of light required to effect photoinhibition, indicated by the studies of Biggley, et al. (1969) appear to be supported qualitatively by the marked sensitivity of G. catenella to light during scotophase.

There appear to be variations of luminescent capability in some species of dinoflagellates with time and space (Nordli, 1957, Kelly, 1968), and further work is required to ascertain the significance of this. There was no difference in the total stimuable emission of the three clones of G. catenella studied here, indicating that differences between these individual clones is small in regards to TSL.

While a great deal more work is required on the ecology of dinoflagellates, it appears that there is no direct correlation between

luminescence in these organisms and their distribution, optimal temperatures, salinities, nutrients, mode of nutrition, toxicity, or other physical and hydrological parameters. Not all red-tide organisms are bioluminescent, nor is the reverse always true; permanent blooms are not uniquely luminescent. Luminescence as found in dinoflagellates shows a high degree of organization, and apparent adaptation to the environment.

There are several possibilities for functions of bioluminescence which could have protective value for dinoflagellates or populations. Burkenroad (1943) proposed a "burglar alarm" function, in which fish predation on herbivores would be facilitated if the herbivore stimulated luminescence in a dinoflagellate prey, thereby revealing its position to the fish. This predator removal function would benefit the entire phytoplankton population, but depends on a relatively complex series of events. Observations on copepods swimming in dinoflagellate cultures have indicated that a high concentration of cells is necessary to obtain an accurate positional fix on a copepod, and that at low, more natural cell concentrations spontaneous flashing obscures copepod activity. This hypothesis may have value under bloom conditions, when increased predation of any sort would increase nutrient recycling.

A second possibility is that luminescence continues after the dinoflagellate has been consumed, and makes the herbivore vulnerable

to predators by shining through the gut wall. The opaque stomachs of some fish which are known to feed on luminescent animals is explained as a protection against this sort of an effect (Nicol, 1967). Although C. pacificus is transparent, an effect of this type was not noticed in the observations.

A more immediate and effective benefit to an individual dinoflagellate, or a population, would be obtained if luminescence prevented or decreased the selection of a luminescent dinoflagellate by an herbivore grazer, as the results of this study indicate. There are three possible explanations for the observed decrease in grazing due to luminescence of the dinoflagellate.

The luminescent flash could be a type of protein display. Chance and Russell (1959) first used this term to denote any sudden or unexpected behavior by a prey organism which serves to startle, confuse, or disrupt the attack of a predator, and allow the prey to escape. Humphries and Driver (1967) discuss the adaptive value and natural selection of these behavior patterns. The bright (relative to a dark-adapted herbivore) flash of luminescence at close range might cause the grazer to suspend its filter-feeding processes and allow the motile dinoflagellate to escape, or cause it to reject the luminescent particle. Several workers have reported that copepods of the Genus Calanus are able to reject particles while filtering (Esterly, 1916; Cannon, 1928).

Copepod grazing and vertical migration has been shown to depend on the ambient light level (Marshall and Orr, 1955) with maximum grazing occurring in darkness. It is possible that due to the high cell densities used, especially in the first experiments, the copepod stimulation resulted in an ambient light level high enough to inhibit grazing to some degree. This cannot be evaluated until the effect of light on C. pacificus behavior is more clearly understood.

The third possibility is that selection, according to a Burkenroad or similar type process, has resulted in a copepod population which does not graze in the presence of luminescent dinoflagellates. Since grazing did occur in all cases to some degree, this seems unlikely.

The results obtained in the grazing study can be most easily explained by a protean display type mechanism, although none of the above hypotheses have been completely disproven. A protean display-rejection type function is also consistent with aspects of phytoplankton ecology and the characteristics of dinoflagellate bioluminescence. The period of maximum stress in a phytoplankton species probably occurs under non-bloom conditions. In a mixed population of phytoplankton with a low percentage of luminescent dinoflagellates, the protean display function would be most effective and also of great value in preserving the species. Many heterotrophic dinoflagellates, which are not known to form blooms, are capable of bioluminescence.

A selective advantage to explain the retention of bioluminescence in these forms must function only at low cell densities. The short latency (5-10 msec for Noctiluca; Eckert, 1966) between the stimulus and flash response, the nature of the flash response, short recovery time, and color of luminescence are of significance in the defensive function. Photoperiod controlled rhythms in luminescence and sensitivity of the cells, the photoinhibition effect, and the requirement for stimulation limit energy expenditures to periods when the light can be most effective. The fast rise in luminescent capability following the onset of darkness and the plateau of maximum luminescent capability insure a maximum response to stimulation during scotophase.

Luminescence probably functions advantageously in several ways depending upon the algal species, the grazer, and phytoplankton concentrations. Clearly, more work is needed before the mechanisms of its function can be fully explained. When technical problems are solved, tests will be made to determine whether copepods preferentially select non-luminescent phase dinoflagellates over highly luminescent cells. The reactions of different dinoflagellates, copepods, and other grazers must also be explored. The single experiment conducted with G. polyedra and C. pacificus indicates that the same relationship of luminescence and grazing found with G. catenella holds for other copepod-dinoflagellate predator-prey relationships.

Dinoflagellates account for most of the stimuable bioluminescence in the surface regions of the oceans (Kelly, 1968) and has several ecological significances other than the one demonstrated here. Luminescence stimulated by nets has reported to be a factor in net avoidance by fishes (Nicol, 1963). Stimulation by schools of fish has been used as a means of locating schools of tuna at night (Scott, 1969, Whitney, 1969a) and remote sensing of this luminescence by aircraft appears feasible (Douglass and Gorenbein, 1968). Whitney (1969b) indicates that bioluminescence may be a factor in maintaining fish schooling at night.

The results reported here are of importance in evaluating herbivore grazing effects on phytoplankton populations, and should be considered in studying primary production and energy transfer in the lower trophic levels.

## CONCLUSIONS

1. Gonyaulax catenella exhibits photoperiod-entrained rhythms in luminescence typical of other luminescent dinoflagellates, including a relatively constant scotophase capacity for luminescence, variation in the sensitivity of the cell to stimulation, and photoinhibition.
2. Gonyaulax catenella emits approximately  $6 \times 10^7$  photons per cell during exhaustive scotophase stimulation, based on a comparison with G. polyedra.
3. Bioluminescence as found in dinoflagellates, shows a high degree of organization and apparent adaptation to the environment.
4. Swimming and feeding activity of copepods is sufficient to stimulate luminescence of dinoflagellates.
5. Grazing of Calanus pacificus on Gonyaulax catenella is partially dependent on the luminescent capacity of the dinoflagellate; grazing rates were considerably lower when the cells were highly luminescent when compared to relatively non-luminescent cells.
6. Luminescence in dinoflagellates may have selective values in reducing copepod predation on luminescent forms.
7. It is proposed that luminescence functions as a type of protean display as a possible mechanism of its defensive value.



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