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The effect of photoperiod on the diurnal fluctuations in photosynthetic rate of two species of marine phytoplankton

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THE EFFECT OF PHOTOPERIOD ON THE DIURNAL FLUCTUATIONS IN PHOTOSYNTHETIC RATE OF TWO SPECIES OF MARINE PHYTOPLANKTON

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THE EFFECT OF PHOTOPERIOD ON THE DIURNAL FLUCTUATIONS IN OF TWO SPECIES OF MARINE PHYTOPLANKTON

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ABSTRACT

Photosynthetic rates were measured under constant conditions over a 24 hour period for cultures of *Skeletonema costatum* and *Amphidinium carterae* grown under various light - dark regimes. Photosynthetic rate fluctuated for both species under all photoperiods except for *A. carterae* under 8 hours light per day. Maximum photosynthetic rates occurred generally in the middle of the light period and minimum rates in the middle of the dark period. The ratio of maximum to minimum rates, or Pmax/min, differed significantly under the various photoperiods. Pmax/min values for *A. carterae* were significantly greater than for *S. costatum*. The greatest Pmax/min for *S. costatum* occurred under 12 hours light per day and for *A. carterae* under 14 hours light per day. Considering the differences in response of the two species to the same photoperiods, it is possible that species composition could have an effect on field data which relates Pmax/min to latitude and season.

ACKNOWLEDGMENTS

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INTRODUCTION

Studies by Doty and Oguri (1957), and Shimada (1958) show that photosynthetic rates of natural assemblages of phytoplankton measured under constant conditions may vary as much as ten fold during a 24 hour period. Experiments performed in fresh water give similar results to those involving marine plankton (Verduin, 1957). In certain cases changes in chlorophyll "a" concentration are associated with such fluctuations (Yentsch and Ryther, 1957), while in other instances (Holmes and Haxo, 1958), pigment levels are nearly constant and large changes in photosynthesis/chlorophyll are observed. It has been suggested (Newhouse, et al., 1967; J. D. H. Strickland, personal communication) that grazing by diurnally migrating zooplankton may be involved. The marine dinoflagellate Gonyaulax polyedra, however, has been shown to possess an endogenous photosynthetic rhythm when grown in light-dark cycles (Hastings, Astrachan, and Sweeney, 1961).

Doty (1959) notes that the ratio of maximum to minimum photosynthetic rate (Pmax/min) is related to the latitude at which samples are taken. Experiments conducted near the Equator give the highest degree of daily photosynthetic fluctuations, and the Pmax/min ratios decrease with increasing latitude. Lorenzen (1963) working at a single geographical location, but over a one year period, finds that Pmax/min ratios vary with the season, being highest in the spring and fall and lowest in the summer and winter. Both of these studies suggest that a photoperiod of twelve hours light per day yields maximum ratios and deviations toward either longer or shorter day lengths give lower values.

This hypothesis is tested in the present study by subjecting phytoplankton to a range of photoperiods in the laboratory and measuring the daily photosynthetic fluctuations. Two common marine species, Skeletonema costatum, a diatom, and the dinoflagellate Amphidinium carterae, are examined in order to ascertain to what extent species composition might also effect the Pmax/min values reported in field studies.

METHODS AND MATERIALS

Inocula for cultures were obtained from the Virginia Institute of Marine Science. The cultures were maintained at 15 liters in a medium consisting of Triton marine salts enriched with a nutrient solution (Table IV). Salinity was 17 ppt, pH 8.3 and temperature, 20 C. Fluorescent lights (cool white) provided a light intensity of 300 foot candles. The cultures were grown for at least one week under photoperiods of 8, 10, 12, 14, and 16 hours light per day. The following experiment showed that one week was adequate for the phytoplankton to acclimate to new photoperiods. Phytoplankton which were growing under a photoperiod of 12 hours light per day were introduced to a new photoperiod of 14 hours light per day. Diurnal fluctuations in photosynthesis were measured at the end of one week and again at the end of a second week. The fluctuations for phytoplankton under 14 hours light per day differed from those under 12 hours light per day, but there was no difference between the fluctuations for phytoplankton grown under a photoperiod of 14 hours light per day for one and two weeks (correlation coefficient for *S. costatum* =+0.867; *A. carterae* =+0.891).

Photosynthetic rates were measured for each culture over a period of 24 hours. At three-hour intervals, a 900 ml sample was removed from the culture and inoculated with 0.3 μ C carbon-14 solution ($NaHC^{14}O_3$, adjusted to a pH of 8.3). Three B.O.D. bottles, two light and one dark were filled from each sample. The bottles were held in a rotating rack in a light incubator at 1000 foot candles and a temperature of 20 C. After two hours the bottles were removed from the incubator and the contents filtered onto Millipore HA filters. The filters were washed with 0.003 N HCl and dried in a desiccator.

Filters were placed in scintillation counting vials with 20 ml fluor (Table V) and counted for 10 minutes in a liquid scintillation counter. Count rates were corrected for variations in counting efficiency (Table V) and were expressed as disintegrations per min, per liter of culture, per hour.

Relative photosynthetic rates were expressed as light bottle minus dark bottle count rates.

RESULTS

Photosynthetic rate fluctuated over a 24 hour period for both S. costatum and A. carterae under all photoperiods except for A. carterae under 8 hours light per day (Fig. 1 and 2; Table I). Maximum photosynthetic rates occurred generally in the middle of the light period and minimum rates in the middle of the dark period. The maximum and minimum rates for S. costatum occurred at 1200 and 2400 respectively in three of five experiments. For A. carterae, the maxima and minima occurred at those times in four of five experiments. Photosynthetic rates during the light period were significantly greater than during the dark period (S. costatum: $t = 23.1$, d.f. = 46; A. carterae: $t = 44.65$, d.f. = 46).

Ratios of maximum to minimum photosynthetic rates over 24 hours, varied from 1.37 to 2.45 for S. costatum, and from 1.41 to 6.67 for A. carterae (Fig. 3; Table II). The greatest Pmax/min occurred under 12 hours light for S. costatum and 14 hours light for A. carterae.

Differences in Pmax/min were examined in an analysis of variance test (Spence, et al., 1968) with species, photoperiods and interactions between the same as sources of variation (Table III). Significance to the .01 level of confidence was found in all three. The Scheffe multiple comparison test

(Spence, *et. al*, 1968) showed that greatest Pmax/min values differed significantly from those at the other four photoperiods (*S. costatum*: $F = 27.8$, $p < .05$; *A. carterae*: $F = 48.3$, $p < .01$).

DISCUSSION

The diurnal fluctuations in photosynthetic rate described here, as well as in related studies, are based on measurements made under constant light intensities, and consequently are called fluctuations in photosynthetic capacity (Strickland, 1960). The mechanisms responsible for this phenomenon are unclear, partially because of inconsistencies in results of several investigations. Considering evidence from his own study as well as from Yentch and Ryther (1957) and Shimada (1958), Lorenzen (1963) believes changes in chlorophyll "a" content brought about by photodestruction and synthesis of pigments are a direct cause of the photosynthetic fluctuations. Chlorophyll data from Holmes and Haxo (1958), on the other hand, do not parallel photosynthetic rates. Sweeney (1964) shows that changes in ribulose diphosphate carboxylase correlate closely with fluctuations in photosynthetic rate for Gonyaulax polyedra grown in light dark cycles, while the Hill reaction proceeds at a near constant rate. She concludes that the physiological mechanism of the periodicity is associated with the "dark" phase of photosynthesis, which is in disagreement with evidence for the importance of chlorophyll concentrations which directly affect only the "light" reactions.

Photosynthetic fluctuations in the present study are similar to those reported by Lorenzen (1963), in that maximum rates occur in the middle of the light period (1200) and minimum rates in the middle of the dark period (2400). In the earlier work of Doty and Oguri (1957), however, as well as that of Shimada (1958), maximum rates occur in the morning hours (0600 - 0800). Evidence from various sources suggests that the high incident radiation present at mid-day in the equatorial waters where these studies were conducted caused a lowering of photosynthetic rates (below those of 0600 - 0800), possibly through photodestruction of chlorophyll. Lorenzen (1963) finds greater fluctuations on overcast days than on bright days, and Yentch and Scagel (1958) report greater daily fluctuations in pigment concentrations from subsurface samples, where the radiation would be less, than from surface samples. Hastings, *et al*. (1961) show that bright light, even at levels not great enough for chlorophyll destruction, dampens the endogenous photosynthetic fluctuations of *G. polyedra*. Finally, Newhouse, *et al*. (1967) note that in studies conducted in oceanic waters, the photosynthetic maximum occurs in the morning, but near mid-day in neritic areas. It is perhaps only in the more transparent oceanic waters that light penetration is sufficient to inhibit the normally high mid-day photosynthetic rates both through the bleaching of chlorophyll and dampening of endogenous mechanisms. Conversely, increased light attenuation of inshore water columns would result in photosynthetic fluctuations that more closely resemble the laboratory data presented here.

Pmax/min values in the present investigation differ significantly for different photoperiods. In an attempt to coordinate the latitudinal relationship reported by Doty (1959) with the seasonal variations found in his own experiments, Lorenzen (1963) advances the idea that 12 hours light per day promotes higher Pmax/min ratios than either longer or shorter photoperiods. The response of S. costatum to various day lengths in the present study can be accommodated by this proposal. The Pmax/min ratio for A. carterae, however, is considerably greater under 14 hours light per day than under equal periods of light and darkness (6.67 compared to 2.81). It is perhaps significant that in Lorenzen's graph of Pmax/min vs. photoperiod (his Fig. 7) greatest ratios occur at 13-1/2 hours light per day.

The physiological basis of the relationship between photoperiod and Pmax/min is not clear. Algae grown under certain light-dark cycles become synchronous in cell division (Hoogenhout, 1963), and it is possible that photosynthetic rhythms are associated with the phasing of reproduction. Sorokin (1957) finds that photosynthesis is diminished during periods of division in Chlorella. Hastings, et. al. (1961) suggest that both reproduction and photosynthetic periodicity are under the control of an unknown factor. It follows that, if photosynthetic rate is closely associated with the course of cell development, large fluctuations in photosynthesis should be produced by phytoplankton cultures greatly synchronized with respect to cell division. Data from Eppley (1966) indicate that cultures of *Dunaliella tertiolecta* are most synchronous under a photoperiod of 12 hours light per day and are less synchronous when the day length is longer or shorter. In the present study the lowest Pmax/min ratios, and an apparent loss of the normal photosynthetic rhythm, are present under the shortest photoperiod, 8 hours light per day, perhaps reflecting a low degree of synchrony in those cultures.

An important aspect of the results given here is that *S. costatum* and *A. carterae* have been shown to respond differently to the various photoperiods. As mentioned above, greatest Pmax/min ratios for the two species occur under different day lengths. Moreover, Pmax/min values for *A. carterae* under all photoperiods are statistically greater than for *S. costatum*. The possibility therefore exists that species composition can have an effect on the magnitude of photosynthetic fluctuations observed in field studies, such as those describing relationships to season and latitude. In general, dinoflagellates become an increasingly important element in the phytoplankton of low latitudes and diatoms are more dominant in the colder waters of high latitudes (Raymont, 1963). If *S. costatum* and *A. carterae* are at all representative of their respective groups with regard to Pmax/min ratios, then the latitudinal relationship reported by Doty (1959) could be, in part, a reflection of geographical species succession.

SUMMARY

1. Photosynthetic rate fluctuated over a 24 hour period for both S. costatum and A. carterae under all photoperiods except for A. carterae under 8 hours light per day.
2. Maximum photosynthetic rates occurred generally in the middle of the light period and minimum rates in the middle of the dark period.
3. Pmax/min ratios differed significantly under the various photoperiods.
4. Pmax/min ratios for A. carterae were significantly greater than for S. costatum.
5. The greatest Pmax/min value for S. costatum occurred under 12 hours light per day, and for A. carterae under 14 hours light per day.
6. Considering the difference in response of the two species to the same photoperiods, it is possible that species composition could have an effect on field data which relates Pmax/min to latitude and season.

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Table I. Relative photosynthetic rates during a day for phytoplankton grown under different photoperiods. Relative rates taken as the difference between uptake of C^{14} in light and dark bottles.

Photoperiod (hours light per day)	Time (hour)	Relative Photosynthetic Rate (DPM per liter per hour) S. costatum	A. carterae
8	0900	7260	1570
		8170	1220
	1200	5790	1170
		4400	1230
	1500	5670	1310
		5940	1290
	1800	6100	1060
		6550	1130
	2100	5640	1100
		5430	1190
	2400	7070	1020
		6240	1010
	0300	5900	1050
		6280	1030
	0600	6490	1130
		7210	1160

Photoperiod (hours light per day)	Time (hour)	Relative Photosynthetic Rate (DPM per liter per hour) *S. costatum*	*A. carterae*
10	0900	5405 6088	5016 5352
	1200	5477 5327	8370 7930
	1500	7073 5233	6900 7250
	1800	4690 4710	5540 6060
	2100	4956 5746	4910 3920
	2400	4790 4116	4740 3780
	0300	4463 4806	5090 5780
	0600	5180 4443	4840 5080
12	1200	9910 7985	5890 6350
	1500	3377 5967	3660 4550
	1800	5370 2510	3540 4650
	2100	3703 3630	4120 3850
	2400	3970 3730	3160 3210
	0300	3960 3920	4140 4175
	0600	4980 4600	5060 5010
	0900	5050 4530	8990 9650

Photoperiod (hours light per day)	Time (hour)	Relative Photosynthetic Rate (DPM per liter per hour) *S. costatum*	*A. carterae*
14	0900	4350	6660
		4370	6380
	1200	5520	0540
		5620	0320
	1500	5010	5270
		5170	3936
	1800	4020	4770
		4460	4180
	2100	3260	1890
		3426	1610
	2400	3440	1470
		3280	1655
	0300	3320	1270
		3310	2590
	0600	4610	5790
		4860	5170
16	0900	3853	2520
		3620	2600
	1200	4566	3053
		4406	2860
	1500	3500	2220
		3646	2000
	1800	3253	1980
		2850	1596
	2100	3340	1943
		2346	1833
	2400	2256	1023
		2216	1103
	0300	2816	1050
		2566	1083
	0600	3290	3090
		2880	2420

Table II. The ratio of maximum to minimum photosynthetic rates during a day (Pmax/min) for phytoplankton grown under different photoperiods.

Photoperiod (hours light per day)	Pmax/min *S. costatum*	Pmax/min *A. carterae*
8	1.37	1.41
10	1.38	1.92
12	2.44	2.98
14	1.67	6.67
16	2.01	2.77

Table III. Analysis of variance for ratios of maximum to minimum photosynthetic rate during a day (Pmax/min). Species S. *costatum* and *A. carterae* grown under different photoperiods. Photosynthetic rates measured under constant light.

Source of Variation	SS	df	MS	F	P
Species	9.4	1	9.4	156.6	$<.01$
Photoperiod	19.3	4	4.8	80.3	$<.01$
Species X Photoperiod	17.0	4	4.2	70.8	$<.01$
Error	0.6	10	0.06		

Table IV. Nutrient Solution (N_2M, Virginia Institute of Marine Science

Sodium silicate solution ($Na_2SiO_3 \cdot 9H_20$, 4.66 g/100 ml)	100
Soil extract	200
Arnon's micronutrient solution	50
Ketchum and Redfield's solution "A"	200
Ketchum and Redfield's solution "B"	100
Sodium molybdate solution ($Na_2MoO_4 \cdot 2H_2O$, 0.0119 g/100 ml).	50

In preparing the culture medium, 3 ml of the above were added per liter of marine salt solution.

Formulae for Ketchum and Redfield, and Arnon's solutions can be found in Bold, H.C., 1942. The cultivation of algae. Bot. Rev. 8:69-138.

Table V. Counting efficiency correction factors and liquid scintillation fluor.

Counting Efficiency Correction Factors

External Standard Count Rate (counts per 10 min)	Correction
464967	1.30
418087	1.32
306685	1.46
259624	1.54
210565	1.67
182681	1.78
167415	1.88
149225	2.00
139001	2.06
121741	2.21
111207	2.32
94592	2.46
70500	2.80
57694	3.12
49751	3.28
33471	3.84
20860	4.60

Liquid Scintillation Fluor

Liquifluor (New England Nuclear Corp.)	90 ml
Triton X 100 (Packard Instrument Co.)	346 ml
Toluene	564 ml
	1000 ml

Figure 1. Relative photosynthetic rates during a day for S. costatum grown under different photoperiods. Relative rates taken as the difference between uptake of C^{14} in light and dark bottles. Dark period indicated by shaded areas.

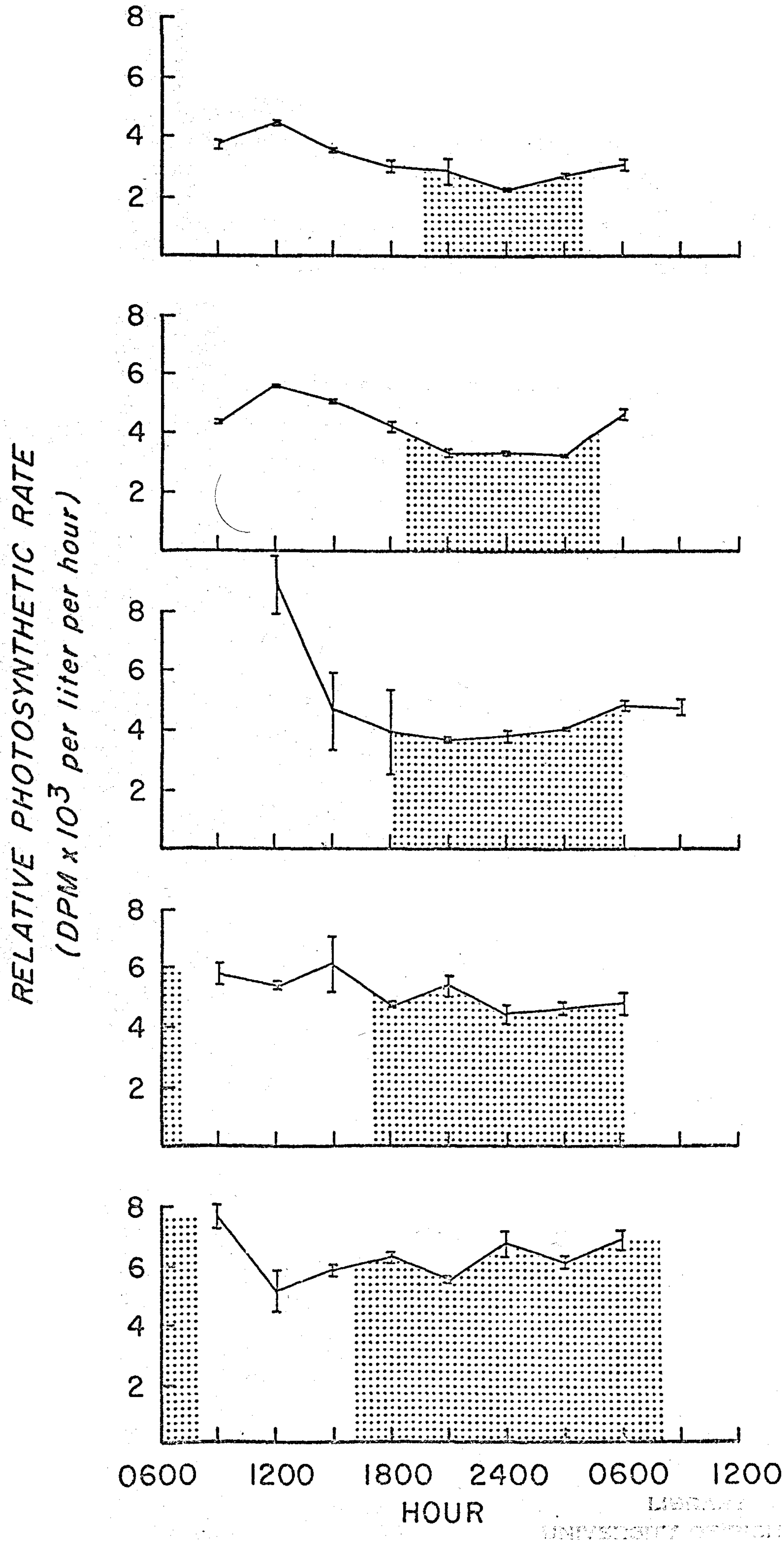
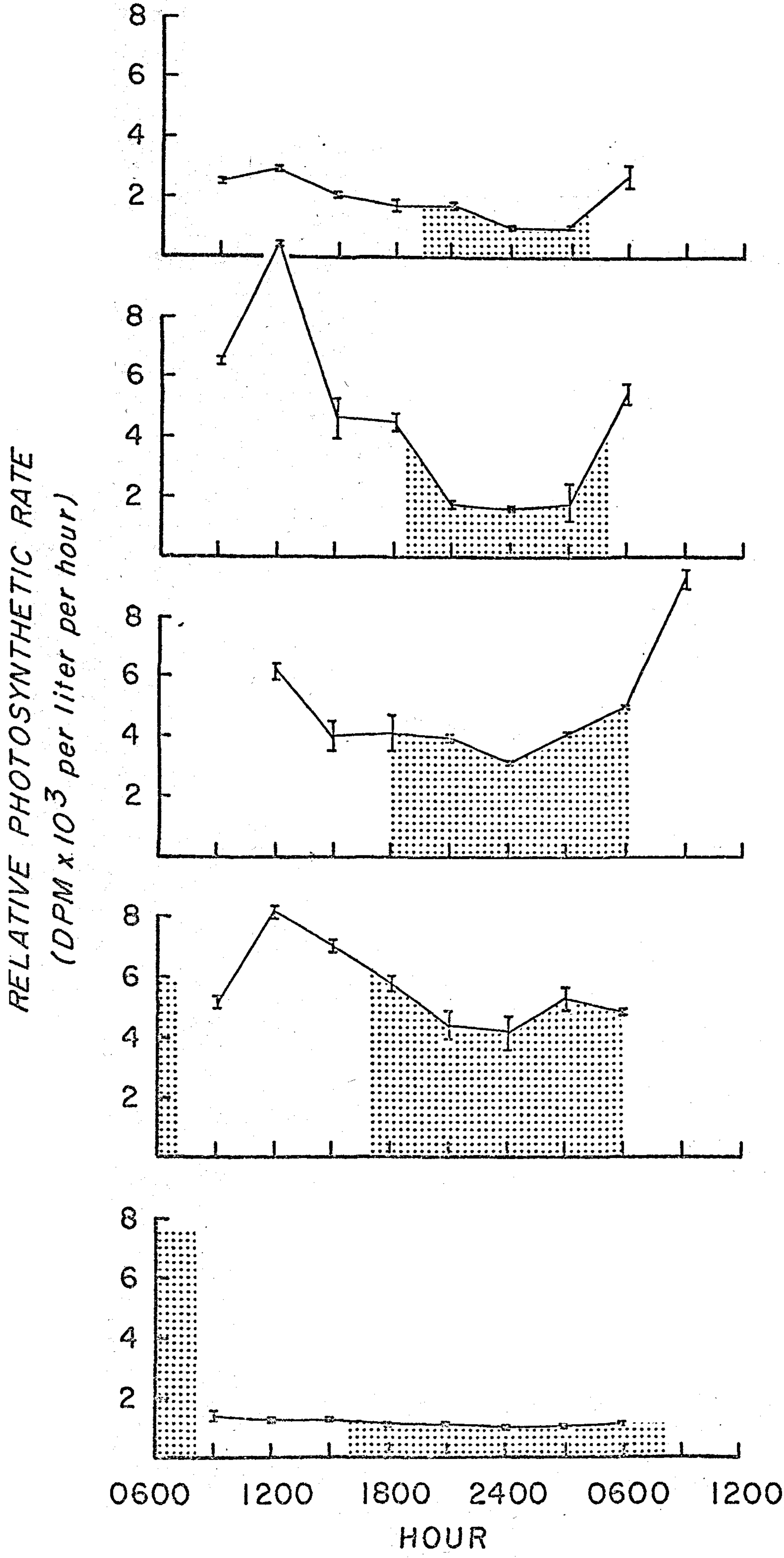
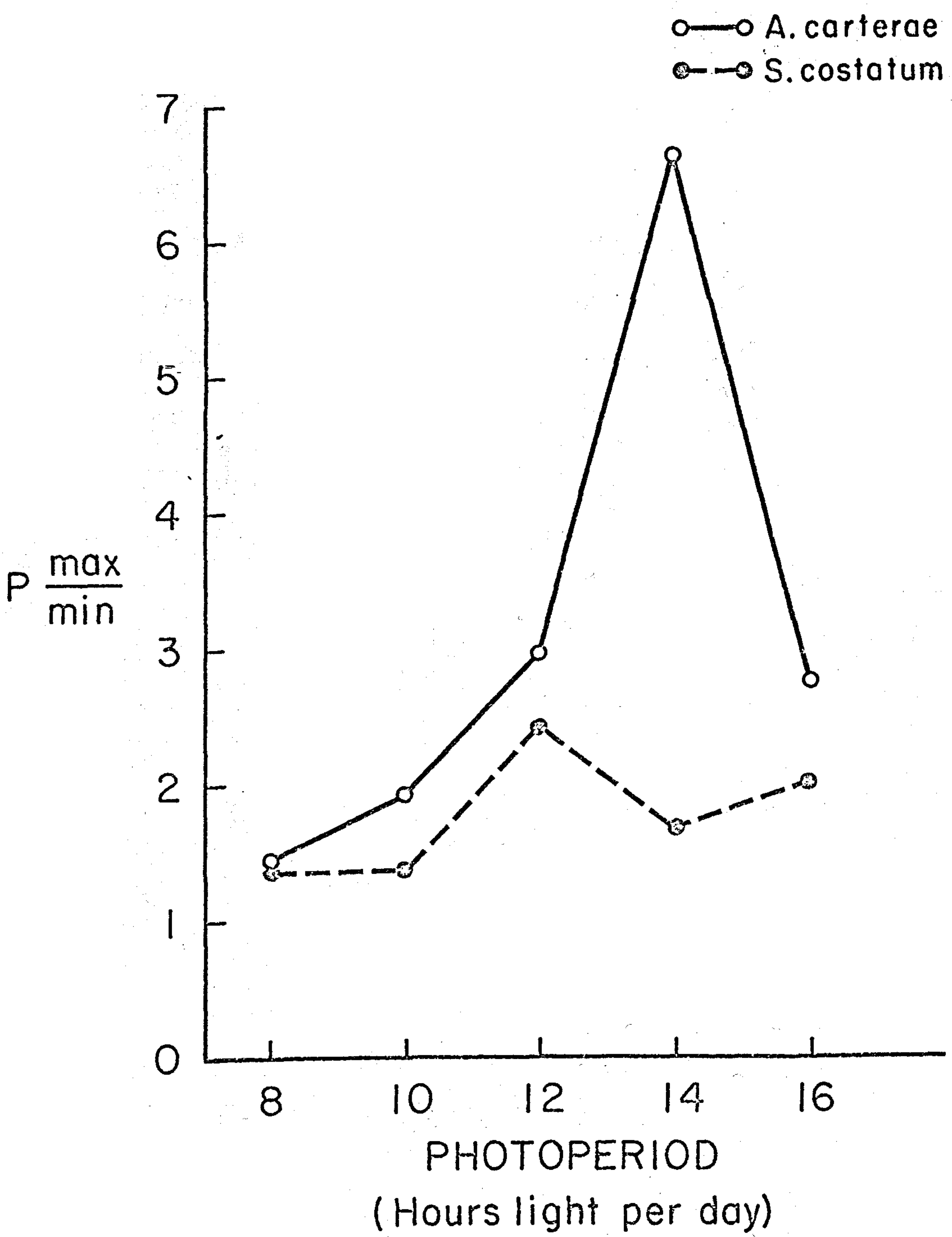

RELATIVE PHOTOSYNTHETIC RATE
(DPM x 10³ per liter per hour)
8
6
4
2
0600
1200
1800
2400
0600
1200
HOUR

Figure 2. Relative photosynthetic rates during a day for *A. carterae* grown under different photoperiods. Relative rates taken as the difference between uptake of C^{14} in light and dark bottles. Dark period indicated by shaded areas.



RELATIVE PHOTOSYNTHETIC RATE
(DPM x 10^3 per liter per hour)
8
6
4
2
0600
1200
1800
2400
0600
1200
HOUR

Figure 3. The ratio of maximum to minimum photosynthetic rates during a day (Pmax/min) for phytoplankton grown under different photoperiods.



A. carterae
S. costatum
7
6
5
4
3
2
1
0
P max/min
8
10
12
14
16
PHOTOPERIOD
(Hours light per day)

VITA

Richard Allmon Smith was born on June 19, 1945, in Baltimore, Maryland. He completed primary and secondary education in the public school system, graduating from the Baltimore Polytechnic Institute in June, 1963. He entered the University of Richmond the following September and received a B.S. in Biology in June, 1967. As an undergraduate he was elected to membership in Beta Beta Beta Honorary Biological Fraternity. He entered the Graduate School of the University of Richmond in September, 1967 and completed requirements for the Masters of Science in Biology in August, 1969. He is a member of the Atlantic Estuarine Research Society and the American Association of Limnology and Oceanography.