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RESPONSE OF *PROROCENTRUM MARIAE-LEBOURIAE* (DINOPHYCEAE) TO LIGHT OF DIFFERENT SPECTRAL QUALITIES AND IRRADIANCES: GROWTH AND PIGMENTATION[1]

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ABSTRACT

Growth and pigment concentrations of the estuarine dinoflagellate, Prorocentrum mariae-lebouriae *(Parke and Ballantine) comb. nov., were measured in cultures grown in white, blue, green and red radiation at three different irradiances. White irradiances (400–800 nm) were 13.4, 4.0 and 1.8 $W \cdot m^{-2}$ with photon flux densities of 58.7 ± 3.5, 17.4 ± 0.6 and 7.8 ± 0.3 μM quanta $\cdot m^{-2} \cdot s^{-1}$, respectively. All other spectral qualities had the same photon flux densities. Concentrations of chlorophyll* a *and chlorophyll* c *were inversely related to irradiance. A decrease of 7- to 8-fold in photon flux density resulted in a 2-fold increase in chlorophyll* a *and* c *and a 1.6- to 2.4-fold increase in both peridinin and total carotenoid concentrations. Cells grown in green light contained 22 to 32% more peridinin per cell and exhibited 10 to 16% higher peridinin to chlorophyll* a *ratios than cells grown in white light. Growth decreased as a function of irradiance in white, green and red light grown cells but was the same at all blue light irradiances. Maximum growth rates occurred at 8 μM quanta $\cdot m^{-2} \cdot s^{-1}$ in blue light, while in red and white light maximum growth rates occurred at considerably higher photon flux densities (24 to 32 μM quanta $\cdot m^{-2} \cdot s^{-1}$). The fastest growth rates occurred in blue and red radiation. White radiation producing maximum growth was only as effective as red and blue light when the photon flux density in either the red or blue portion of the white light spectrum was equivalent to that of a red or of blue light treatment which produced maximum growth rates. These differences in growth and pigmentation indicate that* P. mariae-lebouriae *responds to the spectral quality under which it is grown.*

Key index words: spectral quality; blue, green, red and white; irradiances; pigments; chlorophyll a *and* c*, peridinin, carotenoids; dinoflagellates; photoadaptation; radiation; growth; productivity*

Phytoplankton contain a variety of photosynthetic pigments including chlorophylls, phycobiliproteins and carotenoids. These pigments define the spectral radiation which a species can use for photosynthesis and hence, growth (Halldal 1970, Bogorad 1975). In aquatic environments the light which penetrates a water column is highly variable in both irradiance and spectral quality. The red region of the visible spectrum is absent at depth in clear waters because the water and dissolved salts absorb at these wavelengths. Consequently, the primary spectral quality at these depths is blue-green. However, in turbid waters, where suspended particles absorb most of the blue radiation, green and yellow-orange wavelengths predominate. Since blue-green radiation is the primary spectral irradiance in marine waters, it is thought to be important in regulating the photosynthetic capacity of the phytoplankton which live there (Atlas and Bannister 1980, Vesk and Jeffrey 1977, Haxo 1960). Yet, in highly sedimented estuaries such as the Rhode River and Chesapeake Bay, downwelling radiation is primarily green to orange (500 to 650 nm). Its importance in regulating photosynthetic rates is probably of equal importance to that of blue-green radiation in clear oceanic waters. In either case, the ecological significance of spectral quality has not been demonstrated.

White light of different irradiances can induce changes in algal growth and respiration (Brown and Richardson 1968), pigment composition (Vesk and Jeffrey 1974, Mandelli 1972), pigment ratios (Jones and Myers 1965, Brody and Emerson 1959, Fujita and Hattori 1959), ultrastructure (Jeffrey and Vesk 1977) and the peridinin-chlorophyll *a*-protein complex (PCP) (Prézelin et al. 1976, Prézelin and Sweeney 1978, Meeson and Sweeney 1981). However, light of other spectral qualities can result in faster growth rates and a change in the major accessory pigments for photosynthesis. Growth rates are generally higher in blue than in white light grown cells with the magnitude of the response mediated by the species as well as the ambient irradiance (Jeffrey and Vesk 1977, Hess and Tolbert 1967). In algae with phycobilins, green light can enhance the synthesis

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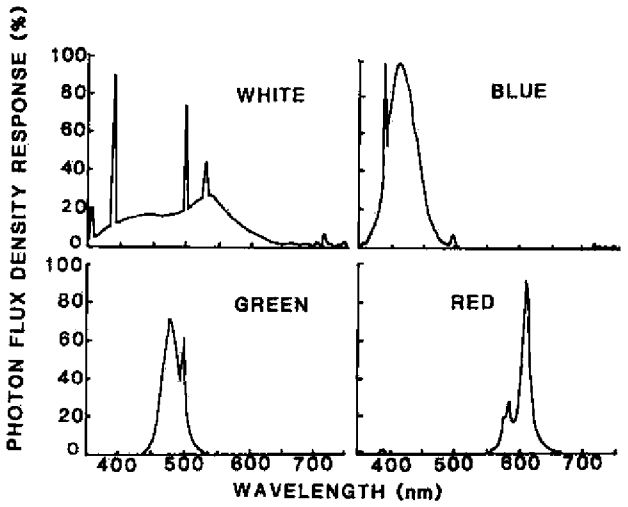

FIG. 1. Spectral quality of the white, red, blue and green light environments of the growth chamber.

of phycoerythrin and reduce phycocyanin synthesis (Fujita and Hattori 1959, Bennett and Bogorad 1973, Tandeau de Marsac 1977), while red light causes the opposite effect (Mann and Myers 1968, Bogorad 1975). This shift in synthesis of pigments in response to a change in spectral quality has been termed chromatic adaptation. Chromatic adaptation, thus is a response of some algal groups to alterations in the energy distribution in the visible light environment. As a consequence of this phenomenon, the pigments which absorb in the incident wavelength of spectral quality become predominant (Bennett and Bogorad 1973).

Our knowledge about chromatic adaptation in dinoflagellates is very limited. A large number of dinoflagellate species have notable differences in their response to irradiance (Vesk and Jeffrey 1977, Wall and Briand 1979), but little information is available on the growth and pigmentation of these organisms when grown in light of different spectral qualities (Halldal 1974). However, it is known that dinoflagellates possess three major photosynthetic pigments, chlorophyll *a*, chlorophyll *c* and peridinin (Jeffrey et al. 1975). The chromoproteins, PCP and the chlorophyll *a*—chlorophyll *c*—protein are known major light harvesting components in this class (Boczar et al. 1980). Because of their absorption spectrum, these two chromoproteins allow dinoflagellates to utilize radiation in the blue-green (458–553 nm) and orange (585–647 nm) regions of the visible spectrum.

Prorocentrum micans is responsible for red tides off the coast of southern California in the summer months (Sweeney 1975), *P. mariae-lebouriae* forms red tides in the Chesapeake Bay during late spring and summer (Faust 1974, Tyler and Seliger 1978). In these systems, spectral attenuation is significant over the year. Our aim was to explore the light requirement of *P. mariae-lebouriae*, which must adapt to be

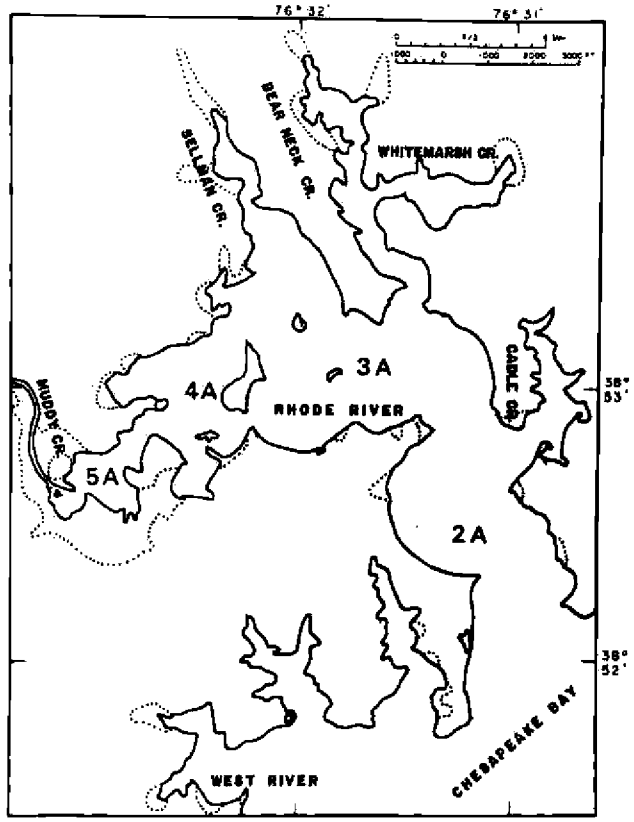

FIG. 2. Map illustrating the Rhode River tidal estuary and station 2A.

a successful estuarine species. Little is known about the factors affecting the penetration of solar radiation in turbid waters, or the effect of these changes upon phytoplankton growth and pigmentation (Seliger and Loftus 1974).

In the present study, we explored the possibility of using *P. mariae-lebouriae* as an experimental system to determine growth and photosynthetic pigment responses. Experiments in growth chambers were set up to identify effects on growth of white, blue, green and red spectral quality radiation adjusted to the same photon flux densities. In addition, photosynthetic pigment composition as a function of spectral response was determined.

MATERIALS AND METHODS

Batch experiments were designed to measure a change in cell numbers and pigmentation of *P. mariae-lebouriae*. Prior to the experiments, unialgal cultures of *P. mariae-lebouriae* (obtained from M. A. Tyler, University of Delaware, Lewes, DE) were grown in f/2 medium (Guillard and Ryther 1962) at 15‰ salinity and 20° C in Erlenmeyer flasks which were illuminated from above with daylight fluorescent (Westinghouse F40D) lamps. All cultures prior to treatment were grown on a 12:12 h LD cycle with an irradiance of 2.5 $W \cdot m^{-2}$. Twenty-five mL of the *P. mariae-lebouriae* culture described above were added to 175 mL of fresh medium to give an initial inoculum density of 1.68×10^4 $cell \cdot ml^{-1}$. During the experiment the cultures were maintained on a 16:8 h LD cycle at 20 ± 1° C. The cells were grown at three photon flux densities (PFD's) at each of four spectral qualities, white, blue, green and

TABLE 1. *Incident and solar radiation at the surface and 1 and 2 m depths at station 2A in the Rhode River.*

Date 1980	Depth	Spectral quality			
		White	Blue	Green	Red
		400–750 nm $W \cdot m^{-2}$ (%)	400–550 nm $W \cdot m^{-2}$ (%)	550–600 nm $W \cdot m^{-2}$ (%)	600–750 nm $W \cdot m^{-2}$ (%)
Clear day					
June 17	Surface	281.5 (100)	86.0 (100)	54.0 (100)	141.4 (100)
	1 m	38.6 (13.7)	9.1 (10.6)	10.0 (18.5)	19.4 (13.7)
	2 m	10.8 (3.8)	1.7 (2.0)	3.4 (6.3)	5.1 (3.6)
Overcast day					
May 13	Surface	128.9 (100)	41.0 (100)	26.0 (100)	61.8 (100)
	1 m	11.9 (9.2)	1.8 (4.4)	3.8 (14.6)	6.4 (10.4)
	2 m	2.7 (2.1)	0.3 (0.7)	1.0 (3.8)	1.4 (2.3)

red, as measured by a C-3 Spectral Scanning System (Gamma Scientific, San Diego, CA). The PFD's were adjusted with fiberglass screening, so that they were equivalent for all four spectral qualities. In white light, the irradiances were approximately 13.4, 4.0 and 1.8 $W \cdot m^2$ and were designated as high, medium and low. The corresponding PFD's were 58.7 ± 3.5, 17.4 ± 0.6 and 7.8 ± 0.3 μM quanta $\cdot m^{-2} \cdot s^{-1}$ between 400–800 nm. The experimental cultures were irradiated from above using spectral phosphor of daylight fluorescent lamps, Sylvania FY48T12/VH0 blue No. 246, green No. 2282, and red No. 236 in combination with plastic Rosolene filters blue No. 863, green No. 874 and red No. 832, respectively (Kliegl Brothers, Universal Electric Stage Lighting Co., Long Island, NY). The plastic filters were used to narrow the spectral quality and reduce the irradiance from the mercury bands inherent in the fluorescent lamps (Fig. 1). Eight replicates were used at each PFD and spectral quality. The entire experiment was repeated at least once.

After 12 days of treatment, aliquots were taken for pigment analysis and cell counts. Mean growth was estimated from changes in cell numbers between day 1 and day 12 of the treatment. Duplicate samples from each flask were counted in a Sedgwick-Rafter counting chamber and cell density was expressed as number of cells per mL (Stein 1973). Division rates were estimated from cell counts (Guillard 1973). Only two growth chambers were available at a given time so the experiments were conducted in pairs with the white light treatment repeated each time. Since the experiments were separated in time, mean growth rates and pigment composition were expressed relative to the white light treatment.

The photosynthetic pigments, chlorophylls *a* and *c* were extracted and estimated according to Jeffrey and Humphrey (1975) and total carotenoids as proposed by Strickland and Parsons (1972). Quantitative thin layer chromatography (TLC) according to the method of Jeffrey (1968, 1981) was used to estimate the peridinin content on each culture at the end of the 12 day treatment. Small amounts of concentrated pigments were spotted onto Baker-flux cellulose plates (Baker Chemical Co., Phildelphia, PA), pigments separated and concentrations determined.

Incident radiation and downwelling irradiances were surveyed (400–800 nm at 50 nm band widths) for eight spectral bands at station 2A in the Rhode River (Fig. 2). A water tight version of the scanning radiometer built by the Smithsonian's Radiation Biology Laboratory was used to measure this radiation (Goldberg and Klein 1974). The unit was calibrated with a National Bureau of Standard spectral reference standard and compared to an Eppley precision pyrometer. Incident surface radiation as well as the radiation at one and 2 m depths was measured. Light penetration was calculated as the percent of total surface radiation.

An in vivo absorption spectrum of *P. mariae-lebouriae* grown in white light was determined with a computerized recording spectrophotometer according to the method of Massie and Norris (1976).

Spectral quality and radiation parameters are expressed as recommended by Tibbitts and Kozlowski (1979). The radiation parameters used to describe the propagation of electromagnetic waves are: 1) spectral quality, the wavelength distribution or predominant spectrum of light energies available; 2) irradiance, the amount of radiant energy per unit area per unit time; and 3) the photon flux density (PFD), the moles of radiant quanta per unit area per unit time.

RESULTS

The spectral distribution of radiation at the surface and at one and 2 m depths at station 2A in the Rhode River are shown on a clear and overcast day (Table 1). A significant population of *P. mariae-lebouriae* was present in the water column on these two days. Although incident radiation was much lower on a cloudy day (when most of the radiation was highly scattered), the spectral distributions were similar. On both clear and overcast days, the incident and downwelling radiation were highest in the orange-red to red region (600–750 nm) of the spectrum. At all depths, however, a greater proportion of green and yellow-orange light (500–600 nm) penetrated the water column, than either the blue (400–550 nm) or red (600–750 nm). On a cloudy day proportionally less radiation in each spectral range penetrated the water column.

Growth occurred in all treatments over the 12 day period (Table 2). Mean cell division rates ($\bar{k}$) of *P. mariae-lebouriae* at high photon flux densities were similar in all spectral qualities ($\bar{k} = 0.24 \pm 0.02$ divisions d^{-1}). In the white and green treatments, the lowest PFD had one-sixth the number of cells as the higher treatment, while in the red radiation it was about one-half. Cell division rates declined with PFD (Fig. 3). However, in blue light, mean growth rates were about the same ($\bar{k} = 0.27 \pm 0.01$ d^{-1}) at all photon flux densities (Fig. 3).

Prorocentrum grown in white light reflected the presence of the three major photosynthetic pigments (Fig. 4). By comparing the in vivo and acetone extracted absorption spectra of cells grown in white radiation with that of purified peridinin in acetone, it appeared that peridinin contributed to the whole cell absorption in the range of 470 to 530 nm. This

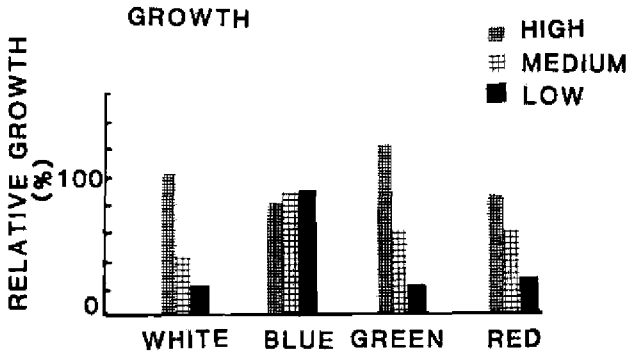

FIG. 3. Relative growth of *P. mariae-lebouriae* irradiated at three different photon flux densities and four spectral qualities (white, blue, green, and red). The PFDs were 58.7 ± 3.5 μM quanta·m^{-2}·s^{-1} for high, 17.4 ± 0.6 μM quanta·m^{-2}·s^{-1} for medium and 7.8 ± 0.3 μM quanta·m^{-2}·s^{-1} for low irradiances.

pigment accounted for about 52% of the total carotenoid content of whole cells.

The pigment content of *P. mariae-lebouriae* changed markedly with changes in radiation (Fig. 5). In all spectral qualities chlorophylls *a* and *c*, peridinin and total carotenoids were about two-fold greater in cells grown at low PFD, than in those grown at the higher ones. Cells grown in blue light contained 2 or 3-fold lower chlorophylls *a* and *c*, peridinin and total carotenoids at all irradiances than the other spectral qualities. The highest concentration of each pigment occurred in different light qualities, chlorophyll *a* was the highest when grown in red light while chlorophyll *c*, peridinin and total carotenoid were greatest when grown in green light.

The ratios chlorophylls *a* : *c*, peridinin : total carotenoid were also examined (Table 3). Chlorophyll *a* content per cell was approximately twice the chlorophyll *c* and did not vary with photon flux density i.e. the ratios were approximately 2.0 (Table 3). The ratio of chlorophylls *a* and *c* : total carotenoid ranged from 0.75 to 0.93 in the white, 0.74 to 0.95 in the green, 0.79 to 1.04 in the red and 0.59 to 0.70 in the blue radiation grown cells. In addition, total carotenoid concentrations were about the same at a single PFD for all spectral qualities except blue. Under the blue radiation the carotenoid content was notably lower than under another spectral quality at the same PFD. Peridinin as a proportion of total carotenoid also varied with PFD. Cells grown in the green radiation appeared to have proportionally the highest 52–61%, of total carotenoid content per cell; medium levels occurred in the white, 48–55%; and in the red, 36–49%; and the lowest amount in the blue, 32–43% radiation, respectively.

DISCUSSION

Measurements in the estuarine waters of the Rhode River showed green to red radiation (550–650 nm) as the principal component in the water column. The total irradiance was generally very low.

TABLE 2. *Photon flux density (PFD), final cell density and growth rate of* Prorocentrum mariae-lebouriae *after 12 days.*

Photon flux density	Final cell density	Growth rates (k)
μM quanta·m^{-2}·s^{-1} ± R[a]	Cells × 10^4·ml^{-1} ± SE[b]	Divisions d^{-1}
White irradiance		
58.7 ± 3.5	12.20 ± 0.06	0.25
17.4 ± 0.6	4.36 ± 0.06	0.13
7.8 ± 0.3	2.46 ± 0.04	0.06
Blue irradiance		
58.7 ± 3.5	8.80 ± 0.04	0.26
17.4 ± 0.6	9.40 ± 0.06	0.27
7.8 ± 0.3	10.00 ± 0.10	0.27
Green irradiance		
58.7 ± 3.5	13.00 ± 0.05	0.25
17.4 ± 0.6	6.40 ± 0.04	0.16
7.8 ± 0.3	2.20 ± 0.01	0.04
Red irradiance		
58.7 ± 3.5	12.40 ± 0.10	0.21
17.4 ± 0.6	8.50 ± 0.06	0.17
7.8 ± 0.3	5.00 ± 0.09	0.10

[a] R = ranges of PFD.
[b] SE = values are standard error means of 6 to 12 replicate samples. Initial cell density was 1.68 × 10^4 cells·ml^{-1}.

In order for phytoplankton to survive there they must be able to adapt to this low radiation and particular spectral environment. Dinflagellates are capable of adapting to very low irradiances (Prézelin 1976, Meeson and Sweeney 1981), but little is known about their ability to adapt to radiation of different spectral qualities. In the present work, radiation measured as photon flux density of four spectral qualities, identified differences between responses to irradiance and spectral quality. All spectral qualities had approximately the same PFD (measured as μM quanta·m^{-2}·s^{-1}) as those existing in situ. The data presented here indicated that *P. mariae-lebouriae* which occurs in the turbid waters of the Rhode River and Chesapeake Bay utilized estuarine radiation levels of each treatment to maintain cell division and synthesize pigments. Mean growth rates in blue spectral quality were similar at low, medium and high radiation levels, whereas white, red and green spectral qualities allowed only suboptimal growth.

The principal result was to establish that the dinoflagellate *P. mariae-lebouriae* has the potential to adapt to different spectral radiations. Cells grown in a green spectral quality contained the highest cellular concentration of peridinin and peridinin to chlorophyll *a* ratios. Peridinin is the principal photoreceptor for photosynthesis for *P. mariae-lebouriae*, since none of the other pigment components present in dinoflagellates including chlorophylls *a* and *c* contribute to the absorption maxima at 500–560 nm wavelengths (Prézelin and Haxo 1976). Of particular interest is the increase of peridinin—chlorophyll *a*—protein complex (PCP) of *P. mariae-lebouriae* in low green radiation (500–560 nm), indicating that it probably is required to provide additional energy

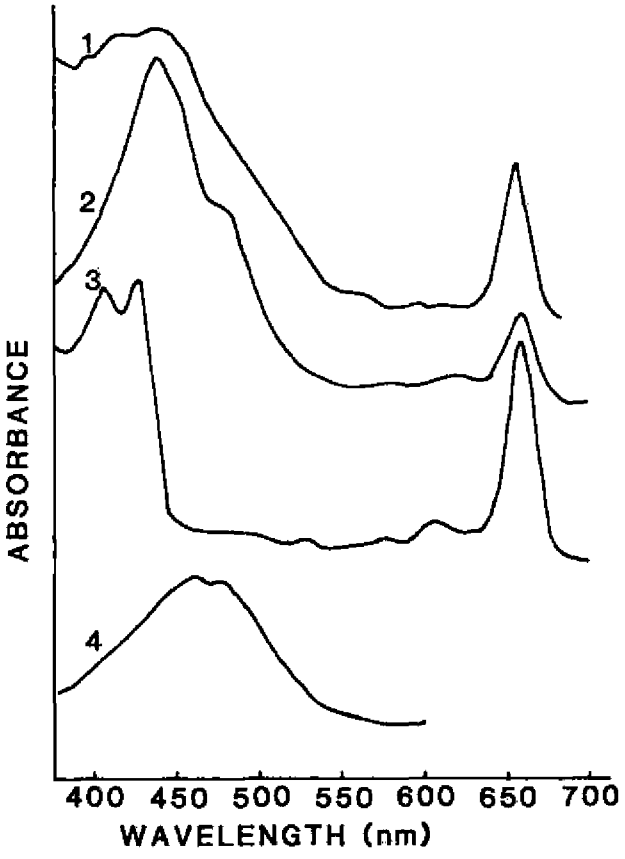

FIG. 4. *Prorocentrum mariae-lebouriae* absorption spectra: 1) In vivo absorption spectrum of whole cells; 2) absorption spectrum of total pigments in 90% acetone; 3) and 4) component pigment absorption spectra in 100% acetone separated on TLC plates for chlorophyll *a* (3) and peridinin (4), respectively.

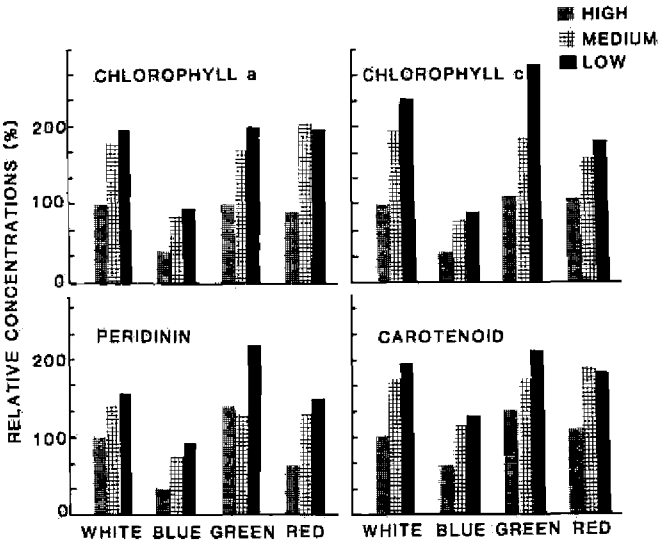

FIG. 5. Relative photosynthetic pigment concentrations for cells of *P. mariae-lebouriae* after 12 days of growth at three photon flux densities (PFD) and four spectral qualities (white, blue, green and red). The treatment PFD were 58.7 ± 3.5 μM quanta·m^{-2}·s^{-1} for high, 17.4 ± 0.6 μM quanta·m^{-2}·s^{-1} for medium and 7.8 ± 0.3 μM quanta·m^{-2}·s^{-1} for low irradiance.

for photosynthesis under very low irradiance level as in *Glenodinium* sp. (Prézelin, 1976). This is exactly what might be expected since PCP absorbs in the blue-green region of the visible spectrum. The enhancement appears as a shoulder and is seen in the photosynthetic action spectra of *Glenodinium* sp. grown in white radiation (Prézelin et al. 1976).

The spectral quality of estuarine radiation fits the absorption spectrum of accessory pigments of this dinoflagellate species, in which radiation is effectively absorbed over a wide spectral region. Radiation in the orange to red region was efficiently captured by *P. mariae-lebouriae* for chlorophylls *a* and *c* synthesis. Whether chlorophyll *c* (at 630 nm) was an effective accessory pigment or not for absorbing the orange wavelength of radiation for the moment is unanswered. Similarly, among dominant estuarine phytoplankton groups, diatoms contain accessory pigments chlorophyll *c* and the carotenoid fucoxanthin, and dinoflagellates contain the accessory pigments chlorophyll *c* and the carotenoid peridinin, with related absorption characteristics of fucoxanthin. In addition, cryptophyte algae are the next most numerous phytoplankton to dinoflagellates in the Rhode River (Faust and Correll 1976) and also have chlorophylls *a* and *c* and phycocyanin (Faust and Gantt 1973) pigments. Thus, they are abundant probably because they effectively absorb estuarine radiation as an energy source in the presence of essential nutrients.

Our knowledge regarding physiological responses to spectral qualities has shown that in addition to chlorophyll *a* the accessory pigments present within an organism define the radiation spectrum which is potentially available for photosynthesis (Bogorad 1975, Prézelin 1976). The best understood systems are in certain blue-green algae (Tandeau de Marsac 1977) that adapt to changes in spectral quality by altering the relative composition of the accessory pigments, phycoerythrin and phycocyanin (Bogorad 1975). In green radiation, the synthesis of phycocyanin was greatly reduced and that of phycoerythrin accelerated. Consequently, most of the flexibility in the pigment system of *P. mariae-lebouriae* to changes in spectral quality and irradiance is attributable to the fluctuating concentrations of chlorophyll *a* and accessory pigments, chlorophyll *c*, peridinin, and minor carotenoids. This type of photoadaptive-response enabled this species to increase the radiation absorbing capabilities of pigments in its potential spectral environments and to maintain growth comparable to that in a white spectrum with equal PFD.

The growth of *P. mariae-lebouriae* in various radiation conditions followed different patterns. Growth at low PFD was less in white, green and red light and decreased with decreasing radiations. However, similar growth rates at all irradiances in

TABLE 3. *Mean concentrations chlorophylls a and c, total carotenoids and peridinin in* Prorocentrum mariae-lebouriae *cells grown at different photon flux densities after 12 days of growth.*

Photo flux density μM quanta $\cdot m^{-2} \cdot s^{-1} \pm R^a$	μg chl *a* 10^6 cells	μg Chl *c* 10^6 cells	Chl *a* Chl *c*	μg total carotenoids 10^6 cells	μg Peridinin 10^6 cells	Peridinin Chl *a*	μg Chl *a* + *c* μg total carotenoids
White irradiance							
58.7 ± 3.5	1.98[b]	0.95	2.08	3.90	1.86	0.94	0.75
17.4 ± 0.6	3.41	1.83	1.86	5.77	3.18	0.93	0.91
7.8 ± 0.3	3.99	2.21	1.80	6.67	3.70	0.93	0.93
Blue irradiance							
58.7 ± 3.5	0.91	0.42	2.16	2.26	0.73	0.90	0.59
17.4 ± 0.6	1.89	0.87	2.17	4.26	1.55	0.82	0.65
7.8 ± 0.3	2.19	0.98	2.52	4.52	1.94	0.88	0.70
Green irradiance							
5.87 ± 3.5	2.25	1.24	1.81	4.73	2.45	1.09	0.74
17.4 ± 0.6	3.80	2.02	1.88	6.38	3.89	1.02	0.91
7.8 ± 0.3	4.55	2.77	1.64	7.69	4.68	1.03	0.95
Red irradiance							
58.7 ± 3.5	2.00	0.95	2.10	3.73	1.33	0.66	0.79
17.4 ± 0.6	4.67	2.40	1.94	6.90	2.85	0.61	1.02
7.8 ± 0.3	4.52	2.36	1.91	6.60	3.23	0.71	1.01

[a] R = ranges of PFD.

[b] Values are means with standard error of pigment concentrations SE ± 0.20 and SE ± 0.03 of pigment ratios for 6 to 12 measurements.

the blue region indicate that growth was not light limited. This organism has a variety of photosynthetic pigments most of which absorb radiation in the 400 to 550 nm range of wavelengths and which could account for the efficient use of blue radiation even at low PFD levels. Since, the PFD was equivalent for white and narrow spectral irradiances, the effectiveness of the blue irradiance appears to be due to the absorbing ability of the cells at low PFD. Other studies also showed that algae generally grow faster in a blue than in a white spectral quality and that the magnitude of the response is mediated by the species and the PFD (Wallen and Geen 1971, Jeffrey and Vesk 1977, Jones and Galloway 1979).

Jeffrey and Vesk (1977) reported better growth in blue and blue-green radiation for *Stephanopyxis turis* than in white at 4 $W \cdot m^{-2}$. Since PFD is lower in blue-green than in white for equal irradiance, the higher efficiency of fewer quanta in the blue-green spectral region in producing a higher rate of photosynthesis can be postulated. This was indeed the case. Carbon fixation, as they measured it (μm mole CO_2 uptake $\cdot mg^{-1}$ chl *a* and *c* $\cdot h^{-1}$) was 41% higher in blue-green grown cultures than in white light grown cultures at high irradiance level (25 $W \cdot m^{-2}$). The blue-green radiation showed no advantage over white light treatment (Jeffrey and Vesk 1977).

It appears that reactions of algae to low light irradiances (below 12.5 $W \cdot m^{-2}$) are two types. *Gonyaulax polyedra* exhibits a photostress response. At low irradiance levels, cells of this species are smaller, divide more slowly, and contain much less pigment (Prézelin and Sweeney 1978). In contrast, *Glenodinium* sp. remains unaltered at low irradiance levels, but simply does not receive sufficient radiant energy to photosynthesize at rates which support maximal growth (Prézelin et al. 1976).

Prorocentrum mariae-lebouriae appears to respond similarly to *Glenodinium* sp. at low levels of irradiance. The quanta received should have sufficiently high energy for effective photosynthesis, but they can be at various wavelengths of the photosynthetically active spectrum. Photosynthesis was maintained at the high level of the white spectral quality where approximately 30% of the total PFD (18 μM quanta $\cdot m^{-2} \cdot s^{-1}$) was in the blue spectral region. When the white radiation was decreased to medium and low PFD levels, PFD in the blue spectrum correspondingly decreased (5.2 and 2.3 μM quanta $\cdot m^{-2} \cdot s^{-1}$, respectively). If this is compared with the PFD received in the blue spectral region, it is evident that the lowest blue PFD level (7.8 ± 0.3 μM quanta $\cdot m^{-2} \cdot s^{-1}$) was higher than the PFD of the blue spectral region of low and medium PFD white light treatments. Thus, it appears that the maximal photosynthetic rate of *P. mariae-lebouriae* was supported by a PFD of about 8 μM quanta $\cdot m^{-2} \cdot s^{-1}$ in the blue region and when it was not received, photosynthetic activity was not saturated and cell division decreased.

The maximum photosynthetic rate for growth of *P. mariae-lebouriae* appears to be supported by a red radiation at a PFD level of 58.7 ± 3.5 μM quanta $\cdot m^{-2} \cdot s^{-1}$, but it definitely required more than 17.4 ± 0.6 μM quanta $\cdot m^{-2} \cdot s^{-1}$. The broad white radiation received at low irradiance appears not to be effective unless one of the photoreceptors receives the critical irradiance to drive the system. The critical PFD to

drive the photoreceptor system in *P. mariae-lebouriae* appears to be at least three times higher in the red than in the blue spectral region. Nevertheless, a very low PFD is able to drive the photosynthetic system in this organism. Consequently, *P. mariae-lebouriae* seems to maintain metabolic activities at low radiation conditions of the estuary. It is able to utilize very low levels of blue radiation and somewhat higher levels of red radiation separately or in combination for growth. It also appears that more red radiation is available in the estuary and red radiation levels are high enough to drive the photosynthetic system at depths below 2 m. At the same time, *P. mariae-lebouriae* probably can utilize the blue portion of the spectrum for growth only when close to the surface.

The success of *P. mariae-lebouriae* in a forming a red tide is probably due to the complement of pigments which enable cells to absorb a wide range of wavelengths in the visible spectrum. Consequently, an understanding of the environmental control of the photosynthetic processes is paramount to the understanding of aquatic primary production. There has been speculation that adaptations to changes in spectral quality can explain the existence of algal populations which exist in blue-green spectra of deep downwelling regions of low total irradiance (Jeffrey and Vesk 1977). The changes observed that were mediated by spectral quality in whole cell pigmentation and growth of *P. mariae-lebouriae* confirms this speculation. Further work is underway to assess the photosynthetic responses of this species when adapted to radiation of different spectral distribution.

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PHAGOTROPHY IN *GYMNODINIUM FUNGIFORME* (PYRRHOPHYTA): THE PEDUNCLE AS AN ORGANELLE OF INGESTION[1]

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ABSTRACT

The non-photosynthetic phagotrophic dinoflagellate, Gymnodinium fungiforme *Anissimova, ingests prey cytoplasm through a highly extensible structure called the peduncle. Although the peduncle is not observable when* G. fungiforme *is swimming, it protrudes 8–12 μm from the sulcal-cingular vicinity of the cell during feeding, and is approximately 3.3 μm wide when the cytoplasm of its prey is flowing through it. A circular-oval ring of overlapping microtubules, the 'microtubular basket' may be seen in transmission electron microscope sections of* G. fungiforme *and it is inferred that this structure is a cross section of a retracted peduncle. The microtubular basket-peduncle complex is discussed in relation to similar structures in other dinoflagellates and to the tentacle of the suctorian ciliates which have a homologous ingestion system.*

Key index words: dinoflagellate; Gymnodium fungiforme; *microtubular basket; peduncle; phagotrophy; suctorian ciliates*

Interest in the non-photosynthetic dinoflagellates has increased recently because they may be important links in estuarine and planktonic food webs (16,24). Although some of these non-photosynthetic species have been cultured heterotrophically, for example, *Gyrodinium lebouriae* (13), *Crypthecodinium cohnii* (18,27) and *Oxyrrhis marina* (10), many are phagotrophic, capable of ingesting a variety of protozoan and metazoan prey (24, for review). Three basic feeding types have been described within the phagotrophic group; (i) Prey is 'stunned' and held near the sulcal region as in *Gyrodinium pavillardi* (4) or brought near the cytostome via water currents set up by the transverse flagella of *Kofoidinium* (6), and subsequently engulfed; (ii) prey is captured by a tentacle in which case it is either engulfed immediately as in *Noctiluca* (22) and *Peridinium gargantua* (4) or digested extracellularly and subsequently ingested as in *Erythropsis pavillardi* (11), and (iii) the dinoflagellate attaches to its prey and ingests the cytoplasm or body fluids through a peduncle as described in *Gymnodinium fungiforme* (4,23,24) and *Gyrodinium vorax* (4).

The details of the life cycle and feeding behavior of the obligate heterotroph *Gymnodinium fungiforme* Anissimova, was described recently (23,24). Large numbers of *G. fungiforme* were reported to form dynamic aggregations around a prey organism and subsequently attach to it, ingesting the prey cytoplasm through a peduncle. The present paper further examines the phagotrophic feeding behavior of *G. fungiforme* and discusses a possible mechanism for the functioning of the peduncle.

MATERIALS AND METHODS

The general methods for culture and the techniques for electron microscopy have been described (24). *Gymnodinium fungiforme* was grown phagotrophically in mixed cultures with *Dunaliella salina* (UTEX 1644). Light micrographs were taken with a Zeiss ICM-405 inverted microscope using Nomarski interference

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