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Light—Shade Adaptation[1]

TWO STRATEGIES IN MARINE PHYTOPLANKTON

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PAUL G. FALKOWSKI AND THOMAS G. OWENS
Oceanographic Sciences Division, Department of Energy and Environment, Brookhaven National Laboratory, Upton, New York 11973

ABSTRACT

Using chlorophyll/P700 ratios, the size and number of photosynthetic units were estimated, as a function of light-shade adaptation in two species of marine phytoplankton: *Skeletonema costatum*, a diatom, and *Dunaliella tertiolecta*, a chlorophyte. In the diatom, light-shade adaptation is characterized primarily by changes in the size and not the number of P700 units, whereas in the chlorophyte, overall changes in chlorophyll content are related to changes in the number and not the size of P700 units. A correlation between the characteristics of P700 units and photosynthetic responses was not established. Both strategies of light-shade adaptation effectively harvest and transfer light energy to reaction centers, however, the *Skeletonema* strategy is more effective at subsaturating intensities. The two strategies may represent an evolutionary divergence in photosynthetic adaptation to variations in light intensity.

In unicellular algae, light-shade adaptation is characterized by changes in intracellular pigment content (2, 6, 17), changes in photosynthetic response (4, 18), and is often accompanied by changes in chemical composition and cell volume (17). Previous studies from our laboratory (19, 21) and that of Grumbach *et al.* (9) indicate that Chl metabolism is highly dynamic in some species, implying that changes in pigment content can occur within a relatively short time. Changes in pigment content may partially compensate for changes in light intensity by optimizing the ability of the cell to harvest the available light. By themselves, such changes do not confer an adaptive advantage unless the light harvested is transferred to photosynthetic reaction centers, where it can be coupled to an electrochemical gradient. Reaction centers, in conjunction with antenna Chl molecules, accessory pigments, and electron carriers comprise PSU.[2] It is not clear whether the size or number of PSU changes in the course of light-shade adaptation (4), however, it has been suggested that changes in the characteristics of PSU are associated with changes in photosynthetic response (3, 5, 11, 20).

Chl/P700 ratios have been proposed as one method of estimating the average size of PSU (4, 22), although it has been shown that this ratio may differ (on an electron equivalent basis) from PSU sizes estimated from O_2 flash yields (14). Recognizing that the ratio of PSI/PSII reaction centers may not be unity, we measured Chl/P700 ratios (henceforth referred to as P700 units) and photosynthetic response to gain an understanding of: (*a*) the relationship between light-shade adaptation and the size and number of P700 units; (*b*) the relationship between changes in the characteristics of P700 units and photosynthetic response; and (*c*) the effect of light-shade adaptation on cell growth and division. We selected a common neritic diatom, *Skeletonema costatum* (Grev.) Cleve, and a motile chlorophyte. *Dunaliella tertiolecta* Butcher, because these two species markedly differ in pigment composition (15), chloroplast ultrastructure (8), and photosynthetic response (7).

MATERIALS AND METHODS

Culture Conditions. *S. costatum* (Woods Hole clone SKEL, Bacillariophyceae) and *D. tertiolecta* (Woods Hole clone DUN, Chlorophyceae) were cultured axenically at 15 C in natural seawater enriched with f/2 nutrients (10). Cultures were maintained in 4-liter aspirator bottles; the upper and lower surfaces were made opaque with black vinyl tape allowing light to enter only through the vertical sides. Light was provided from above by cool-white fluorescent tubes on a 14:10 h L/D cycle. In experiments with *S. costatum*, maximum incident light intensity (PAR), measured at the center of the culture bottles, was 130 μE m^{-2} s^{-1}. Light was increased up to 400 μE m^{-2} s^{-1} for *D. tertiolecta*. PAR was measured in the culture bottles with a Biospherical Instruments QSL-100 quantum meter equipped with a calibrated 4-π sensor. Neutral density screens (Perforated Products, Inc., Cambridge, Mass.) were wrapped around the bottles to attenuate the light to 50, 30, 15, 7, 2, and 0.5% I_0.

The cultures were constantly mixed by bubbling with sterile air and maintained at constant cell densities by dilution with fresh media for at least 72 h during log growth. For all analyses, cells were harvested during log growth at densities of 3.2×10^5 cells/ml for *S. costatum* and 1.2×10^5 cells/ml for *D. tertiolecta*. Steady-state cell densities could be maintained in a large number of culture vessels simultaneously by diluting periodically. Additional cultures were maintained at steady-state densities in a turbidostat under continuous illumination. Both culturing techniques provided a means of obtaining highly reproducible data on cellular chemical composition and characteristics of P700 units without artifacts caused by differential mutual shading.

Pigment Determinations. Chl *a*, *b*, and *c* were measured spectrophotometrically in 90% acetone extracts (13). Cells were filtered on Gelman type A-E glass fiber filters and immediately ground in spectral grade 90% acetone in a glass mortar with a Teflon pestle. The glass fibers were removed by filtration, reextracted with 90% acetone, and the acetone extracts pooled. The *A* of the acetone extracts was measured between 350 and 750 nm against 90% acetone.

P700 was measured in Triton X-100 extracts of whole cells by light-induced oxidation according to the general procedure of Marsho and Kok (16). Cells were harvested by filtration on 47-mm Gelman type A-E glass fiber filters and were disrupted by

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[2] Abbreviations: C_0, compensation light intensity for photosynthesis; I_0, intensity of incident light; PSU, photosynthetic unit(s); L/D, light to dark.

homogenization in ~2 ml of 50 mM Tris-HCl (pH 8.0) containing 0.01% (v/v) Triton X-100 at 0 to 4 C. The suspensions were clarified by centrifugation, and the extracts were adjusted to Chl *a* concentrations of 5 to 10 μM. Chl *a* concentrations were determined using an extinction coefficient of 60 mM^{-1} cm^{-1} at 677 nm (23).

The reversible, light-induced oxidation of P700 was measured using the dual wavelength mode of an Aminco DW-2a spectrophotometer. The Triton X-100 extract was placed in a 10 × 4 mm cuvette (Precision Cells, type 52) in the secondary sample position. Sodium ascorbate and methyl viologen were added to final concentrations of about 10 mM and 100 μM, respectively, and the sample was allowed to equilibrate in the dark for 2 min. Absorption changes (ΔA) at 697 nm (P700) were measured relative to an isosbestic wavelength of 720 nm. Actinic illumination of 5-s duration was provided by a focused 150-w tungsten-halogen source filtered through two Corning 5543 filters (λ max = 420 nm). The photomultiplier was protected by a single Corning 2030 blocking filter. The actinic illumination was sufficient to saturate the P700 signal at Chl *a* concentrations less than 12 μM.

ΔA was calculated as the difference between the baseline A (reduced P700) and the fully oxidized A measured after the rapid fluorescence decay at the end of actinic illumination. (Background fluorescence was minimized by placing the cuvette in the secondary sample position.) P700 concentrations were calculated using an A difference coefficient of 64 mM^{-1} cm^{-1} (12).

Photosynthetic O_2 evolution was measured as a function of light intensity in each of the cultures during log growth with a Radiometer O_2 polarographic electrode as previously described (7).

Cell counts were made with a hemocytometer for *S. costatum* and with a model TA II Coulter Counter for *D. tertiolecta*. Cell volumes were measured with a Coulter Counter, following a brief sonication period for *S. costatum* to break up cell chains (19). Cellular C and N were measured with a Carlo-Erba CHN analyzer interfaced with a digital integrator. Cells were filtered on precombusted glass fiber filters, washed with filtered seawater, and stored at −30 C for CHN analyses.

RESULTS AND DISCUSSION

Pigment Content. Both *S. costatum* and *D. tertiolecta* respond to decreased light intensity by increasing pigment content (Table I). In both species, maximum Chl *a* content was observed at about 20 μE m^{-2} s^{-1}; at lower light intensities, cells tended to become slightly bleached. Over the range of light intensities that the cells are capable of light-shade adapting (*i.e.* prior to bleaching), intracellular Chl *a* pools can be empirically fit to a logarithmic function of I_0 with correlation coefficients (r^2) >0.95. In addition to changes in Chl *a*, Chl *b* and *c* vary with I_0 in the chlorophyte and diatom, respectively (Table I). As cells become shade adapted, there is a disproportionate increase in either Chl *b* or *c* relative to Chl *a*; consequently, the ratios of Chl *a*/*b* and Chl *a*/*c* decrease with decreasing I_0.

There is a contrast between *S. costatum* and *D. tertiolecta* with respect to changes in the size and number of P700 units as the two species adapt to various light intensities. As *S. costatum* becomes shade adapted, the size of P700 units increases while the number of PSI reaction centers per cell decreases. In *D. tertiolecta*, the size of P700 units decreases as the cells become shade adapted while the number of PSI reaction centers per cell increases (Table I). At the lower light intensities, where Chl content decreases as a result of bleaching, there is a corresponding decrease in both the size and number of P700 units in both species.

These results suggest that there are at least two distinct strategies of light-shade adaptation in marine phytoplankton. In *S. costatum*, increased Chl content results from increases in the size, but not the number, of P700 units, whereas in *D. tertiolecta*, increased Chl content results from increases in the number, but not the size, of P700 units. Both strategies are macroscopically indistinguishable on the basis of Chl or accessory pigment content.

Table I. *Effects of Light Intensity on Photosynthetic Pigment Characteristics and Photosynthetic Response in S. costatum and D. tertiolecta during Steady-State Growth at 15 C*

S. costatum

I_o[a]	Chl *a*[b]	*a*/*c*[c]	Chl *a*/P700	P700[d]	C_o[e]	P_{max}[f]	P_{max}/R[g]
130	4.5	5.6	650	4.3	0.25	15.8	6.8
65	5.4	5.8	875	3.7	0.20	15.9	7.3
39	5.9	4.5	960	3.7	0.22	15.1	6.6
20	7.1	3.1	1,340	3.2	0.20	10.9	7.2
9	5.1	2.8	1,130	2.7	0.20	7.4	7.3
2.6	5.0	2.4	1,110	2.7	0.20	3.9	6.6
0.7	5.0	1.9	1,100	2.7	0.20	3.1	6.6

D. tertiolecta

I_o[a]	Chl *a*[b]	*a*/*b*[c]	Chl *a* + *b*/P700	P700[d]	C_o[e]	P_{max}[f]	P_{max}/R[g]
400	11.8	5.6	530	15.8	20	78	8.8
200	14.9	4.0	550	20.4	19	74	8.8
120	20.9	3.0	560	29.9	16	71	9.7
60	27.6	2.7	520	43.8	12	65	9.0
20	30.9	2.3	380	70.2	8	46	8.9
8	25.5	2.1	370	61.2	4	38	9.4
2	24.3	2.0	360	61.0	4	31	9.9

[a] Incident light in μE m^{-2} s^{-1}.
[b] Mol Chl/cell (×10^{-16}).
[c] Molar ratio.
[d] Numbers of PSI reaction centers/cell (×10^5).
[e] Compensation light intensity in μE m^{-2} s^{-1}.
[f] Light saturated rate in μmol O_2 $cell^{-1}$ min^{-1} × 10^{-10}.
[g] Gross photosynthesis to respiration ratios.

The average size of P700 units in *Dunaliella* (470 Chl *a* + *b*/P700) is considerably smaller than those found in *Skeletonema* (650–1340 Chl *a*/P700) and other diatoms (Falkowski, unpublished) but is similar to P700 unit sizes reported in higher plants (1, 3, 4). Despite smaller P700 units, the total Chl content in the chlorophyte is higher than in the diatom (Table I). This discrepancy is attributed to differences in the cellular density of reaction centers in the two species. Although *S. costatum* and *D. tertiolecta* have comparable cell volumes (Table II), the chlorophyte has more PSI reaction centers per cell than the diatom (Table I). This difference probably reflects increased thylakoid stacking and a generally greater membrane surface area in chlorophyte chloroplasts relative to those of diatoms (8).

Photosynthetic Characteristics. Light-saturated photosynthetic capacities (P_{max}) decrease in both species as they become shade adapted (Fig. 1). Expressed on a Chl *a* basis, P_{max} values obtained with *D. tertiolecta* (Fig. 1B) are greater than those obtained with *S. costatum* (Fig. 1A) when both species are adapted to similar light intensities. For example, P_{max} for the chlorophyte is 5.5 μmol O_2 μmol^{-1} Chl *a* min^{-1} for cells adapted to 120 μE m^{-2} s^{-1}, whereas in the diatom, P_{max} is 4.0 μmol^{-1} O_2 μmol^{-1} Chl *a* min^{-1} for cells adapted to 130 μE m^{-2} s^{-1}. Expressed on a per cell basis, P_{max} values are on the average about 5.6 times higher in the chlorophyte, whereas compensation light intensities for photosynthesis (C_0) are about 50-fold lower in *S. costatum* (Table I). In the diatom, C_0 remains relatively constant as the cells become shade adapted, whereas in the chlorophyte, C_0 increases with I_0.

The initial slopes of the P *versus* I curves (on a per Chl basis) do not significantly differ for *D. tertiolecta* adapted over the range of light intensities examined (Fig. 1). These P *versus* I curves for the

Table II. *Effect of Light Intensity on Division Rates, Cell Volume, and Carbon Content in S. costatum and D. tertiolecta during Steady-State Growth at 15 C*

I_o[a]	κ[b]	V[c]	C/cell[d]	C/N[e]
		S. costatum		
130	0.95	91 ± 7	14 ± 1.7	5.6 ± 1.0
65	0.88	92 ± 15	15 ± 1.9	5.0 ± 1.6
39	0.77	88 ± 16	16 ± 1.8	3.7 ± 0.8
20	0.62	84 ± 12	18 ± 2.5	3.4 ± 0.7
9	0.45	82 ± 11	19 ± 4.4	4.3 ± 1.8
2.6	0.28	79 ± 10	20 ± 3.3	3.8 ± 1.0
0.7	0.19	77 ± 14	20 ± 3.4	4.5 ± 1.8
		D. tertiolecta		
400	1.25	115 ± 36	29 ± 0.7	5.3 ± 0.5
200	0.87	112 ± 10	30 ± 0.6	4.1 ± 0.6
120	0.66	104 ± 20	28 ± 0.6	3.8 ± 0.7
60	0.42	90 ± 21	31 ± 0.9	3.4 ± 0.6
20	0.09	84 ± 14	37 ± 1.1	3.2 ± 0.7
8	0	73 ± 10	41 ± 1.3	3.0 ± 0.8
2	0	69 ± 9	40 ± 1.3	3.1 ± 0.9

[a] Incident light in $\mu E\ m^{-2}\ s^{-1}$.
[b] Daily cell division rate (d^{-1}).
[c] Cell volume in μm^3 (± SD).
[d] pg carbon $cell^{-1}$ (±SD).
[e] Carbon to nitrogen ratios (by atoms, ± SD).

chlorophyte are similar to those reported for *Atriplex* (4), which has similar P700 unit sizes (3). In *S. costatum*, however, the initial slopes of the P *versus* I curves decrease (on a per Chl basis) as the cells become shade adapted. Chl/P700 ratios theoretically represent the average cross-section of a PSU, including PSII reaction centers. The most obvious effect of a change in PSU size ought to be a corresponding change in light utilization efficiency (*i.e.* initial slope of a P *versus* I curve) (11). In *S. costatum*, light utilization efficiencies do not increase as Chl/P700 ratios increase. A change in the number (or cellular density) of PSU should theoretically result in a corresponding change in photosynthetic capacity (11). In *D. tertiolecta*, photosynthetic capacities (on a per cell or per Chl basis) decrease while the number of PSI reaction centers increases. The inconsistencies between the characteristics of P700 units and photosynthetic responses strongly suggest that Chl/P700 ratios do not correspond to PSU sizes as defined by more classical methods of O_2 flash yields (14).

Growth Rates, Cell Volumes and Carbon Content. Cellular division rates (κ) decrease with I_o (Table II). During log growth, the relationship between κ and I_o can be empirically fit by a relationship of the form $\kappa = a + b \log I_o$ with correlation coefficients (r^2) >0.97. Under the specified growth conditions, the calculated compensation light intensity for division is 0.32 $\mu E\ m^{-2}\ s^{-1}$ for *S. costatum* and 18 $\mu E\ m^{-2}\ s^{-1}$ for *D. tertiolecta*.

In both species, changes in κ, resulting from decreasing I_o, are accompanied by decreases in cell volume and increases in cellular C content (Table II). As cells shade adapt, however, there are significantly greater accumulations of cellular N which result in decreased C/N ratios. These relationships are especially pronounced in *D. tertiolecta*.

In both species, dark respiration rates decrease as the cells became shade adapted. The decrease in respiration is associated with decreased κ. Gross photosynthesis: respiration ratios remain relatively constant for each species over the range of light intensities examined (Table I). These ratios average 6.9 ± 0.3 is *S. costatum* and 9.4 ± 0.8 in *D. tertiolecta*.

The major physiological outcome of light-shade adaptation is modification of growth rates with variation in light intensity.

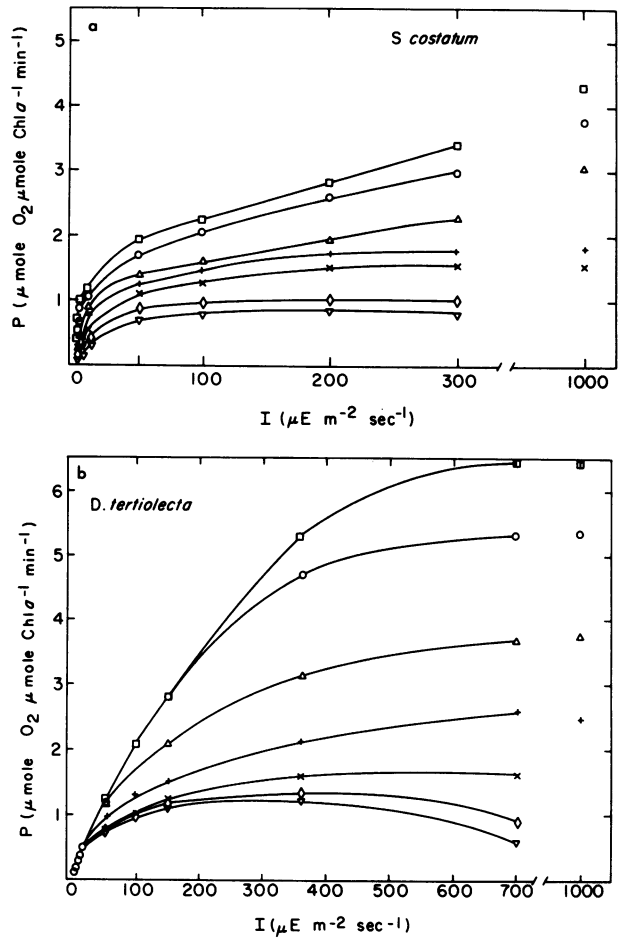

FIG. 1. Photosynthesis-irradiance curves normalized to Chl for *S. costatum* and *D. tertiolecta* during steady-state growth at 15 C. Cells were suspended in fresh f/2 media immediately prior to measurement. Cultures were adapted to 100% (□), 50% (○), 30% (△), 15% (+), 7% (×), 2% (◇), and 0.5% (▽) of the maximum incident light intensity. 100% I_o = 130 $\mu E\ m^{-2}\ s^{-1}$ for *S. costatum* and 400 $\mu E\ m^{-2}\ s^{-1}$ for *D. tertiolecta*. For clarity, only points of the initial slope of 50% I_o cultures of *D. tertiolecta* are shown; the slopes of the remaining cultures are identical.

Growth rate *versus* I curves are roughly analogous to P *versus* I curves, however, κ is a function of photosynthetic performance (*i.e.* the photosynthetic rate at the light intensity in which the cells are growing). Over the range of I_o examined, for each species the relationship between κ and I_o is logarithmic. If the cells did not light-shade adapt, and Chl content remained constant, the fraction of I_o absorbed by the cells would be expected to remain constant. If κ is a function of the rate of light absorbed (2), then the relationship between κ and I_o would be expected to be linear for a hypothetical nonadapting cell. As Chl increases exponentially with decreasing I_o, however, the fraction of light absorbed by the cells increases exponentially with decreasing I_o. The effects of light-shade adaptation are therefore reflected by a logarithmic relationship between κ and I_o.

These data (Table II) suggest that by light-shade adapting the effect of I_o on κ can be attenuated. The attenuation is not strictly a result of increased Chl content, however, but is achieved via reduction in cell volume, respiration, and modification of C content as well. Nevertheless, light-shade adaptation does not fully compensate with respect to κ at low I_o. Were this the case, κ would be predicted to remain virtually constant over a wide range of light intensities.

The data presented here suggest it is not feasible to relate

photosynthetic responses qualitatively (*i.e.* fluxes) to PSU characteristics based on Chl/P700 ratios (*i.e.* pools). A similar conclusion was reached by Armond *et al.* (1) who observed qualitative discrepancies between Chl/P700 ratios and photosynthetic characteristics in higher plants. The determination of PSU size based on the ratio of bulk Chl molecules to an electron transport component (*e.g.* P700) does not provide information about reaction center turnover. Myers and Graham (18) have presented data suggesting that photochemical turnover is not constant and decreases as cells become shade adapted. In addition to problems of estimating photochemical turnover, inconsistencies between PSU and sizes as indicated by Chl/P700 ratios and O_2 flash yields may arise if the ratio between PSI and PSII reaction centers is not 1:1 or changes as cells adapt to various light intensities (14).

A comparison of the two species used in this study indicates that the absolute ratios of Chl/P700 are invariably larger in *S. costatum* than in *D. tertiolecta*, whereas the absolute cellular density of reaction centers is invariably greater in the chlorophyte. These data can be qualitatively related to interspecific differences in photosynthetic responses; photosynthetic efficiency is higher in *S. costatum*, but P_{max} is lower. The fundamental differences between the two strategies of light-shade adaptation may be related to the ecological niches occupied by the two species. The evolution of a light harvesting system that is most effective at higher light intensities (*i.e. Dunaliella*) is not generally adaptive to aquatic environments, but is more compatible with terrestrial light regimes. *D. tertiolecta* is primarily found in shallow waters (such as tide pools) and at generally lower latitudes, whereas *S. costatum* is successful at lower light intensities in deeper waters of temperate continental shelves. The strategy of light-shade adaptation observed in *D. tertiolecta* is similar to that observed in *Chlorella* (18) and higher plants (3, 4) and may reflect an evolutionally conserved adaptation to generally higher light intensities.

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