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EFFECTS OF TEMPERATURE ON GROWTH, LIGHT ABSORPTION, AND QUANTUM YIELD IN *DUNALIELLA TERTIOLECTA* (CHLOROPHYCEAE)[1]

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ABSTRACT

The effects of growth temperature on the marine chlorophyte Dunaliella tertiolecta *Butcher were studied to provide a more mechanistic understanding of the role of environmental factors in regulating bio-optical properties of phytoplankton. Specific attention was focused on quantities that are relevant for modeling of growth and photosynthesis. Characteristics including chlorophyll* a *(chl* a*)-specific light absorption ($a^*_{ph}(\lambda)$), C:chl* a *ratio, and quantum yield for growth (ϕ_μ) varied as functions of temperature under conditions of excess light and nutrients. As temperature increased over the range examined (12°–28° C), intracellular concentrations of chl* a *increased by a factor of 2 and $a^*_{ph}(\lambda)$ values decreased by more than 50% at blue to green wavelengths. The lower values of $a^*_{ph}(\lambda)$ were due to both a decrease in the abundance of accessory pigments relative to chl* a *and an increase in pigment package effects arising from higher intracellular pigment concentrations. Intracellular pigment concentration increased as a consequence of higher cellular pigment quotas combined with lower cell volume. At high growth temperatures, slightly more light was absorbed on a per-cell-C basis, but the dramatic increases in growth rate from $\mu = 0.5\ d^{-1}$ at 12° C to $\mu = 2.2\ d^{-1}$ at 28° C were primarily due to an increase in ϕ_μ (0.015–0.041 mol C (mol quanta)$^{-1}$). By comparison with previous work on this species, we conclude the effects of temperature on $a^*_{ph}(\lambda)$ and ϕ_μ are comparable to those observed for light and nutrient limitation. Patterns of variability in $a^*_{ph}(\lambda)$ and ϕ_μ as a function of growth rate at different temperatures are similar to those previously documented for this species grown at the same irradiance but under a range of nitrogen-limited conditions. These results are discussed in the context of implications for bio-optical modeling of aquatic primary production by phytoplankton.*

Key index words: absorption; Chlorophyta; Dunaliella tertiolecta; *modeling primary production; phytoplankton optics; quantum yield; temperature limitation*

Accurate modeling of aquatic primary production is a goal that currently has broad implications not only for many ecological questions posed by limnologists and biological oceanographers but also for geochemistry, climate change research, and other earth sciences. As algorithms for this purpose are being developed and evaluated (e.g. Kiefer and Mitchell 1983, Platt and Sathyendranath 1988, Balch et al. 1989, Sakshaug et al. 1989, Smith et al. 1989, Morel 1991), the need to adequately describe the effects of environmental factors such as light, nutrients, and temperature on phytoplankton physiology is becoming more apparent. Specifically, the magnitude and variability of optical and photosynthetic properties must be understood and quantified so that they can be accurately incorporated into models and algorithms.

Temperature has been shown to affect the rates of phytoplankton growth and photosynthesis in laboratory studies (see Raven and Geider 1988, Davison 1991) and has been implicated to explain patterns of chlorophyll *a* (chl *a*)-specific photosynthetic rates in the ocean (Eppley 1972). Phytoplankton cultures fully adapted to lower temperatures exhibit decreases in the chl *a*-specific photosynthetic rate at light saturation (P_{max}), whereas little variability is

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observed in the light-limited rate (α) (Steemann Nielsen and Jørgensen 1968, Li 1980, Li and Morris 1982, Mortain-Bertrand et al. 1988). In addition, decreasing growth temperature has been shown to result in lower chl $a \cdot \text{cell}^{-1}$ (reviewed by Li 1980), higher C:chl a (reviewed by Geider 1987), and higher cell volume (Eppley and Sloan 1966, Jørgensen 1968, Olson et al. 1986).

Despite numerous studies on temperature effects in phytoplankton, important growth model parameters such as chl a-specific spectral absorption ($a^*_{ph}(\lambda)$) and quantum yield (ϕ) have received much less attention. Using a spectral version of the model proposed by Kiefer and Mitchell (1983), phytoplankton growth can be described as the result of light absorption by pigments and subsequent net C fixation specified by the growth quantum yield (ϕ_μ):

$$\mu = \text{chl:C} \int \phi_\mu(\lambda) a^*_{ph}(\lambda)\, E_o(\lambda)\, d\lambda, \qquad (1)$$

where μ is the specific growth rate, chl:C is the ratio of chl a to C, $a^*_{ph}(\lambda)$ is the chl a- specific spectral absorption coefficient, $E_o(\lambda)$ is spectral scalar irradiance, and $\phi_\mu(\lambda)$ is the net amount of C fixed per photon of light absorbed. In this representation of phytoplankton growth, the importance of the physiological parameters $a^*_{ph}(\lambda)$ and $\phi_\mu(\lambda)$ is evident.

Recent laboratory investigations have shown that light and nutrient limitation of growth can be important sources of variability in the parameters of Eq. 1. Under these conditions, pigmentation changes and pigment package effects are responsible for the changes in $a^*_{ph}(\lambda)$ (Dubinsky et al. 1986, Morel and Bricaud 1986, Bricaud et al. 1988, Mitchell and Kiefer 1988, Berner et al. 1989, Sosik and Mitchell 1991). Since growth temperature is known to affect pigmentation and cell size, these same mechanisms are expected to result in $a^*_{ph}(\lambda)$ also being a function of temperature. Although the mechanisms behind variability in ϕ are less well understood, ϕ has been shown to vary widely under previously examined growth conditions (light and nutrients) when it is estimated using both light absorption and growth or photosynthesis measurements (Falkowski et al. 1985, Herzig and Falkowski 1989, Chalup and Laws 1990, Sosik and Mitchell 1991). Given this evidence, we expected that temperature limitation of growth should also be associated with changes in ϕ.

To test our hypothesis that growth temperature should affect both specific absorption and quantum yield, we grew semicontinuous cultures of *Dunaliella tertiolecta*, a species that has been well described with respect to optical and photosynthetic responses to other factors (Falkowski et al. 1981, Osborne and Raven 1986, SooHoo et al. 1986, Kolber et al. 1988, Mitchell and Kiefer 1988, Berner et al. 1989, Sukenik et al. 1990, Sosik and Mitchell 1991, Greene et al. 1992). Cells were grown at each of four temperatures under the same irradiance and with excess nutrients. Growth and cellular characteristics including optical properties, pigmentation, and elemental composition were assayed and will be described and discussed in the context of optical modeling of primary production.

MATERIALS AND METHODS

Duplicate cultures of the marine chlorophyte *Dunaliella tertiolecta* Butcher (FCRG culture collection formerly at Scripps Institution of Oceanography) were grown at 12°, 16°, 18°, and 28° C. The cultures were kept under constant light provided by cool-white fluorescent lamps. Irradiance was adjusted to 165 μmol quanta $\cdot m^{-2} \cdot s^{-1}$ as measured with a Biospherical Instruments QSL-100 quantum scalar irradiance meter. Absolute spectral irradiance was determined based on these measured values of irradiance integrated from 400 to 700 nm and the spectral quality of the lamp output provided by General Electric. All cultures were maintained in exponential growth by periodic dilution with nutrient-replete GPM media (Loeblich 1975), and specific growth rate (μ) was monitored by measuring *in vivo* fluorescence of cell suspensions as a function of time using a Turner Designs model 10 fluorometer.

After at least 30 generations of growth at a given temperature, cultures were sampled for triplicate analyses to determine spectral absorption coefficients, cell counts, cell size, and concentrations of pigment, C and N. Cell counts and spherical equivalent cell sizing were performed using a Coulter Electronics model Z_{BI} 64-channel particle counter with a 100-μm aperture calibrated with polystyrene microspheres. Samples filtered onto Whatman GF/C glass-fiber filters for spectrophotometric pigment determinations were immediately extracted in 90% acetone and refrigerated for 24 h before analysis. Chlorophyll a and chl b concentrations were determined from absorbance measurements on the 90% acetone extracts using the trichromatic equations of Jeffrey and Humphrey (1975). Organic C and N samples were filtered onto precombusted GF/C filters, stored at −20° C, and quantified with a Perkin Elmer PE 2400 CHN elemental analyzer.

Absorption of cell suspensions and extracts was measured on a Perkin Elmer Lambda 6 UV/VIS dual beam spectrophotometer equipped with an integrating sphere. The details of the methods are the same as those reported in Sosik and Mitchell (1991). Briefly, spectral absorption of cell suspensions concentrated approximately fivefold was measured in 1-cm cuvettes. Absorption properties of these same suspensions were also measured after treatment with Triton X-100 (0.5% final concentration) and sonication (Berner et al. 1989). In contrast to extraction in organic solvents, this procedure disrupts the cells, effectively "unpackaging" the pigments without dramatically changing the absorption by constituent pigment–protein complexes. Triton X treatment does result, however, in some spectral shifts (Sosik and Mitchell 1991). To compensate for these shifts when comparing changes in absorption with and without Triton X, the region of the unpackaged spectrum below 558 nm was shifted 1 nm while values above 550 nm were shifted 8 nm toward longer wavelengths.

RESULTS AND DISCUSSION

Specific growth rates of semicontinuous cultures of *D. tertiolecta* increased linearly from 0.5 d^{-1} at 12° C to 2.2 d^{-1} at 28° C (Table 1). The cells grown at the highest temperature had the highest chl a quota while changes in chl $a \cdot \text{cell}^{-1}$ for temperatures less than 20° C had no overall trend (Table 1). This pattern of variability in chl $a \cdot \text{cell}^{-1}$ is similar to the result observed for this species by Eppley and Sloan (1966). Cell volume, $C \cdot \text{cell}^{-1}$, and $N \cdot \text{cell}^{-1}$ decreased with increasing growth temperature (Table 1). While $C \cdot \text{cell}^{-1}$ and cell size were similar to those

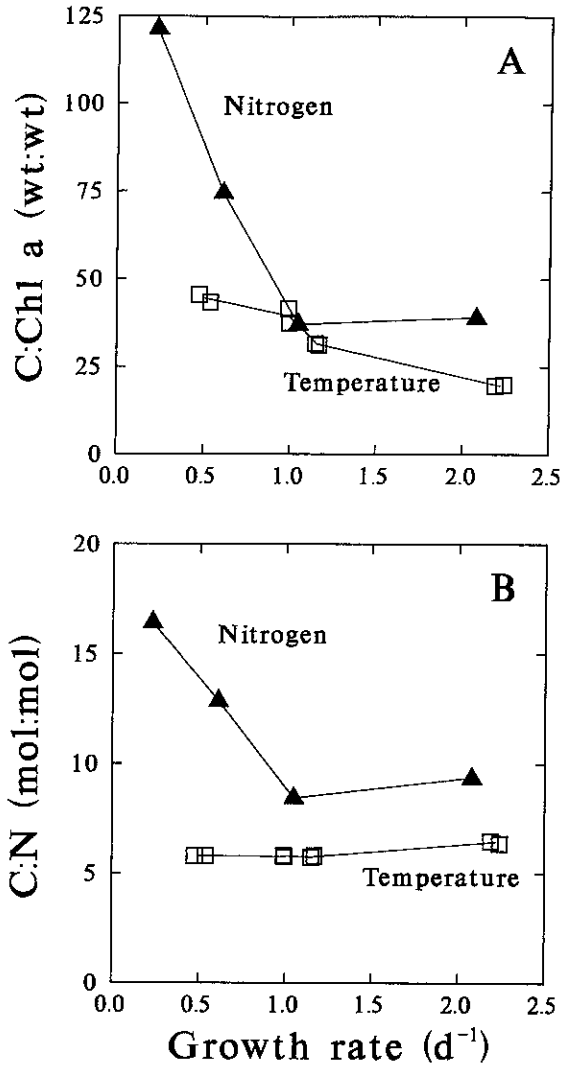

FIG. 1. Comparison of cellular characteristics for cultures of *D. tertiolecta* grown under nutrient-replete conditions at different temperatures (this study) with data from Sosik and Mitchell (1991) for growth at 22° C in nitrogen-limited continuous culture. A) Ratios of C to chl *a*. B) Ratios of C to N. Symbol positions represent average determinations for a single culture with replicate cultures shown for the case of the temperature treatments.

reported for this species grown under nitrogen limitation with the same range in μ (Sosik and Mitchell 1991), C:N and C:chl *a* ratios were consistently lower for temperature-limited conditions (Fig. 1). Lower chl *a*·cell^{-1} and increased cell volume at lower temperatures produced a systematic decrease in intracellular concentration of chl *a* (c_i; see Table 2), which is expected to have consequences for light absorption efficiency.

Absorption. Changes in cellular characteristics were accompanied by changes in the absorption properties of the cells. As growth temperature increased, chl *a*-specific absorption ($a^*_{ph}(\lambda)$) decreased (Fig. 2A). Changes in specific absorption can be caused by shifts in the relative abundance of chl *a* and accessory pigments and by changes in the absorption efficiency

TABLE 1. *Observed characteristics of duplicate* D. tertiolecta *cultures grown under light- and nutrient-replete conditions at different experimental temperatures. Values represent the average of triplicate determinations with SD given in parentheses, except for growth rate where the regression slope and its SE are given.*

	Temperature (° C)							
	12		16		18		28	
	A	B	A	B	A	B	A	B
Growth rate[a]	0.477 (0.010)	0.538 (0.009)	0.991 (0.017)	0.996 (0.008)	1.15 (0.006)	1.17 (0.013)	2.19 (0.016)	2.24 (0.006)
Chl *a*[b]	0.910 (0.015)	0.953 (0.047)	0.750 (0.018)	0.858 (0.017)	1.060 (0.028)	1.093 (0.070)	1.548 (0.062)	1.435 (0.024)
Chl *b*[b]	0.239 (0.004)	0.237 (0.010)	0.157 (0.003)	0.171 (0.003)	0.229 (0.008)	0.220 (0.018)	0.391 (0.016)	0.367 (0.008)
C[b]	41.36 (0.83)	41.16 (0.98)	31.08 (0.49)	32.15 (0.93)	33.38 (0.90)	33.99 (0.79)	30.50 (1.84)	28.47 (1.11)
N[b]	8.32 (0.30)	8.28 (0.25)	6.24 (0.10)	6.53 (0.12)	6.81 (0.21)	6.84 (0.16)	5.50 (0.42)	5.22 (0.23)
Cell volume[c]	213 (8)	212 (11)	164 (7)	172 (5)	159 (4)	165 (3)	155 (1)	181 (<1)
$a^*_{ph}(435)$[d]	0.026 (0.001)	0.024 (0.001)	0.025 (0.001)	0.022 (0.001)	0.021 (0.001)	0.022 (0.001)	0.016 (0.001)	0.016 (0.001)
$a^*_{ph}(677)$[d]	0.017 (0.001)	0.015 (<0.001)	0.016 (0.001)	0.015 (0.001)	0.014 (0.001)	0.015 (0.001)	0.013 (<0.001)	0.012 (0.001)
$a^*_{Tx}(434)$[d]	0.041 (0.002)	0.040 (0.001)	0.036 (0.002)	0.037 (0.001)	0.037 (0.001)	0.038 (0.002)	0.034 (<0.001)	0.034 (0.002)
$a^*_{Tx}(668)$[d]	0.018 (0.001)	0.019 (<0.001)	0.017 (0.001)	0.018 (<0.001)	0.020 (0.001)	0.020 (0.001)	0.018 (<0.001)	0.017 (0.001)

[a] d^{-1}.
[b] pg·cell^{-1}.
[c] μm^3.
[d] m^2 (mg·chl *a*)$^{-1}$.

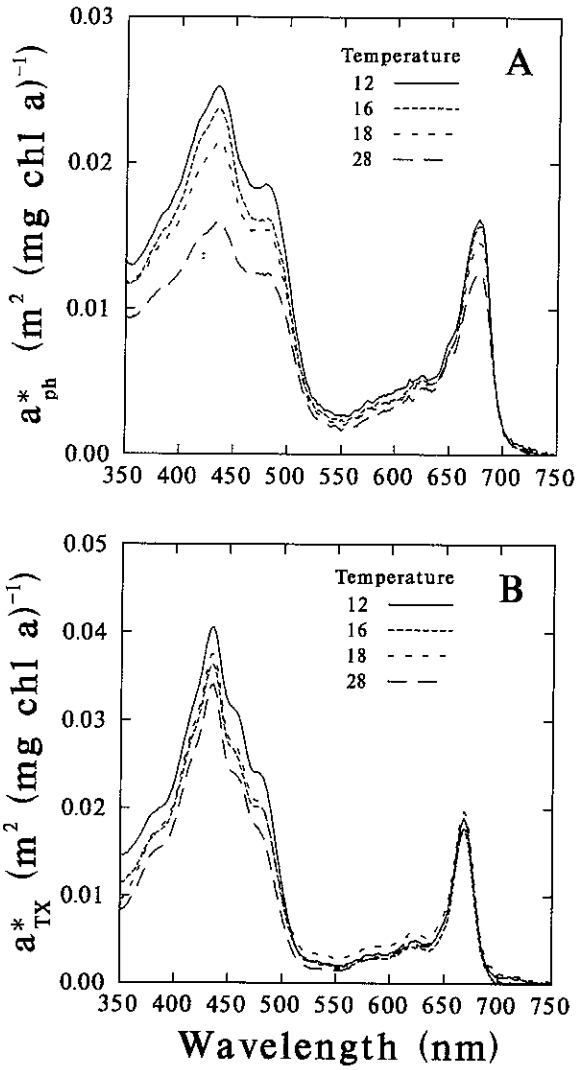

FIG. 2. Chlorophyll *a*-specific absorption spectra for *D. tertiolecta* showing spectral flattening and a decrease in magnitude as growth temperature increases. A) *In vivo* whole cell specific absorption with variability due to both package effects and changes in the relative abundance of pigments. B) Absorption after disruption of cells with Triton X-100 indicating variability due to changes in pigment ratios only.

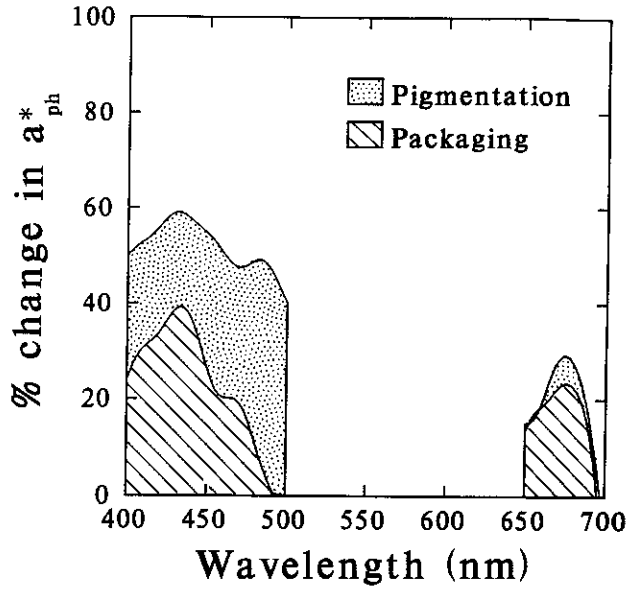

FIG. 3. Contribution to percentage of change in $a^*_{ph}(\lambda)$ for cells grown at 12° C compared to those at 28° C caused by package effects and pigmentation changes. As expected, package effects dominate in the blue and red regions of the spectrum, while pigment changes are important at blue-green and green wavelengths. Data between 500 and 650 nm have been omitted from this presentation because low absorption values in this region make the difference spectra unreliable.

associated with variations in cell size and pigment·cell^{-1}. To determine the extent to which pigment ratio changes contributed to the observed variability in $a^*_{ph}(\lambda)$ independent of package effects, we examined changes in specific absorption after solubilizing the cells using Triton X-100 and sonication ($a^*_{TX}(\lambda)$). Differences in $a^*_{TX}(\lambda)$ reflect only changes in the relative abundance of pigments since package effects have been removed.

As growth temperature increased, values of $a^*_{TX}(\lambda)$ fell in the blue-green portion of the spectrum, which is evidence for a systematic decrease in relative absorption by accessory pigments (Fig. 2B). This interpretation was supported by a similar trend in absorption in 90% acetone extracts (not shown). For the average of wavelengths between 400 and 500 nm, the spectrally shifted $a^*_{TX}(\lambda)$ values were 27% higher for the cells grown at 12° C compared to those at 28° C. The comparable difference in $a^*_{ph}(\lambda)$ was 53%, suggesting, on average, 27/53 or 51% of the decrease in $a^*_{ph}(\lambda)$ was due to changes in pigment composition alone, with the remainder due to increased package effects. Since each pigment has a characteristic absorption spectrum with different shape in the 400–500-nm region, the importance of pigmentation changes compared to package effects is expected to depend on wavelength. For the comparison of cells grown at the two extreme temperatures (12° and 28° C), package effects accounted for more than half of the change in $a^*_{ph}(\lambda)$ at wavelengths between 400 and 450 nm and nearly all the change in the red (650–700 nm), where absorption by accessory carotenoid pigments is minimal (Fig. 3). Changes in pigment composition are most important between 450 and 500 nm, where absorption is highest for accessory pigments such as chl *b*, lutein, and *β*- carotene. Although chl *b* has an absorption peak near 650 nm, the chl *a*-to-chl *b* ratio did not vary between the 12° and 28° C cultures, which is consistent with the weak pigmentation effects observed in this region of the spectrum. We have not interpreted changes for the wavelength range 500–650 nm, where absorption values are low (see Fig. 2) and difference spectra are unreliable. Given the low absorption at these wavelengths, package effects are not expected to be large.

TABLE 2. *Calculated values including absorption efficiency (Q_a) and optical thickness (ρ') for the blue and red absorption peaks and quantum yield for growth in* D. tertiolecta. *Results presented are the average of the duplicate cultures grown at each temperature.*

	Temperature (° C)			
	12	16	18	28
Growth rate	0.51	0.99	1.16	2.22
c_i[a]	4.38	4.78	6.65	8.97
$Q_a(435)$	0.54	0.52	0.64	0.65
$Q_a(677)$	0.35	0.34	0.44	0.51
$\rho'(435)$	1.31	1.19	1.69	2.07
$\rho'(677)$	0.61	0.57	0.88	1.08
ϕ_μ[b]	0.015	0.028	0.028	0.041

[a] $pg \cdot \mu m^{-3}$.
[b] mol C (mol quanta)$^{-1}$.

The observation that package effects played a role in the observed variability in $a^*_{ph}(\lambda)$ is also supported by estimation of the cellular absorption efficiency (Q_a) and optical thickness (ρ'). For homogeneous spheres, Q_a, or the fraction of incident photons that are absorbed, can be shown to be a function of cell size and the absorption coefficient of the intracellular material, a_{cm} (Morel and Bricaud 1981):

$$Q_a(\lambda) = 1 + \frac{2\exp(-\rho'(\lambda))}{\rho'(\lambda)} + \frac{2(\exp(-\rho'(\lambda)) - 1)}{\rho'(\lambda)^2}, \quad (2)$$

where

$$\rho'(\lambda) = a_{cm}(\lambda)d \quad (3)$$

and d is particle diameter. Following Sosik and Mitchell (1991), $Q_a(\lambda)$ and $\rho'(\lambda)$ can be estimated from measured absorption and cell size:

$$Q_a(\lambda) = \frac{a_{ph}(\lambda)}{(\pi d^2/4)} \cdot \frac{V}{N} \quad (4)$$

and

$$\rho'(\lambda) = a^*_{TX}(\lambda)c_i d, \quad (5)$$

where N represents the number of cells in a suspension of volume V and c_i is the intracellular concentration of chl *a*. The results of these calculations showed that $Q_a(\lambda)$ and $\rho'(\lambda)$ are highest at the two fastest growth rates, conditions where $a^*_{ph}(\lambda)$ values were lowest (Table 2, Fig. 2A). As previously observed (Sosik and Mitchell 1991), we found good agreement between observations and the theoretical relationship between $Q_a(\lambda)$ and $\rho'(\lambda)$ (Eq. 2) despite the obvious violation of the theoretical assumption of homogeneous spheres. At the absorption peaks, observed values of Q_a (Eq. 4) were within 10% of those predicted by Eq. 2 given the ρ' values estimated from the a^*_{TX} observations (Eq. 5).

Since *D. tertiolecta* has been comparatively well studied, it is possible to compare the range of variability in $a^*_{ph}(\lambda)$ induced by different sources of growth limitation. Work on the effects of steady-state nitrogen limitation has shown that specific absorption values at the blue peak range from 0.025 to 0.040 m^2 (mg chl *a*)$^{-1}$ for growth rates between 0.2 and 2.0 d^{-1} (Sosik and Mitchell 1991). This represents a similar amount of variability; however, the values are consistently higher than we observed in this study of temperature-limited cells growing at similar rates (0.016–0.025 m^2 (mg chl *a*)$^{-1}$). The combined range of values for temperature and nutrient limitation is similar to that observed for adaptation to different irradiances in studies by Mitchell and Kiefer (1988) (0.021–0.065 m^2 (mg chl *a*)$^{-1}$), Berner et al. (1989) (0.018–0.040 m^2 (mg chl *a*)$^{-1}$), and Mitchell et al. (unpubl. data) (0.025–0.053 m^2 (mg chl *a*)$^{-1}$). From this information, we conclude that temperature and nutrient effects are at least as important as photoadaptation in causing variations in $a^*_{ph}(\lambda)$.

It is also possible to unify our explanation of variability in $a^*_{ph}(\lambda)$ for all the sources of growth limitation that have been examined in *D. tertiolecta*. By combining Eqs. 2, 3, and 4, $a^*_{ph}(\lambda)$ can be expressed in terms of the product $c_i d$ and the specific absorption coefficient of intracellular material, $a^*_{cm}(\lambda)$:

$$a^*_{ph}(\lambda) = \frac{3}{2c_i d}\left(1 + \frac{2e^{-a^*_{cm}(\lambda)c_i d}}{a^*_{cm}(\lambda)c_i d} + \frac{2(e^{-a^*_{cm}(\lambda)c_i d}}{(a^*_{cm}(\lambda)c_i d)^2}\right). \quad (6)$$

Using the data for temperature limitation from this study, nitrogen limitation from Sosik and Mitchell (1991), and light limitation from unpublished data of Mitchell et al., we can show that much of the observed variability in $a^*_{ph}(\lambda)$ can be explained by changes in c_i and d. As predicted by Eq. 6, we observe that, for a given wavelength, the lowest values of $a^*_{ph}(\lambda)$ correspond to conditions that result in the highest product $c_i d$ (Fig. 4). Using the measured values of $a^*_{ph}(\lambda)$ and the product $c_i d$, the least-squares best fit for Eq. 6 results in values of $a^*_{cm}(\lambda) = 0.052$ and 0.024 m^2 (mg chl *a*)$^{-1}$ for the blue and red absorption peaks, respectively. For the wide range of $a^*_{ph}(\lambda)$ values and experimental conditions examined, we find good agreement of the data with Eq. 6 for these constant values of $a^*_{cm}(\lambda)$ (Fig. 4). We expect $a^*_{cm}(\lambda)$ to vary with changes in pigment ratios, so to the extent that we can describe the data with a constant value of a^*_{cm} for a given wavelength, package effects dominate the variability in a^*_{ph}. Particularly for the blue peak, some of the scatter about the curve for constant a^*_{cm} in Figure 4 is certainly due to changes in pigment ratios both between and within different sources of growth limitation (see Fig. 3 for example).

Quantum yield. As classically defined, the photosynthetic quantum yield is based on total photons absorbed by the cell (Rabinowitch and Govindjee 1969), including those that are not efficiently transferred to the photosynthetic reaction centers. We have chosen to follow this definition, although it should be noted that this results in a somewhat lower and more variable yield than would be obtained if

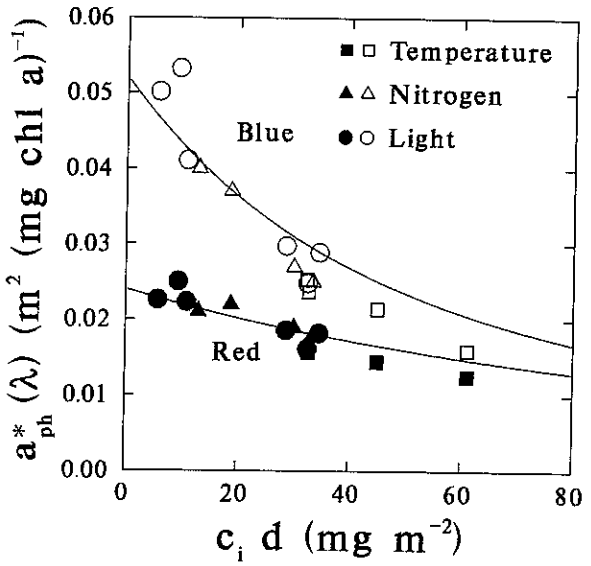

FIG. 4. Variations in $a^*_{ph}(\lambda)$ for the blue (open symbols) and red (filled symbols) peaks as a function of the product of intracellular pigment concentration and cell diameter (c_id) in *D. tertiolecta* under different forms of growth limitation. The curves represent solutions of Eq. 6 with single values of a^*_{cm} for the blue and red peaks as detailed in the text. Square symbols indicate data from this study on temperature effects; triangles are for nitrogen-limited conditions from Sosik and Mitchell (1991); and circles represent data for *D. tertiolecta* grown under light limitation from Mitchell et al. (unpubl. data).

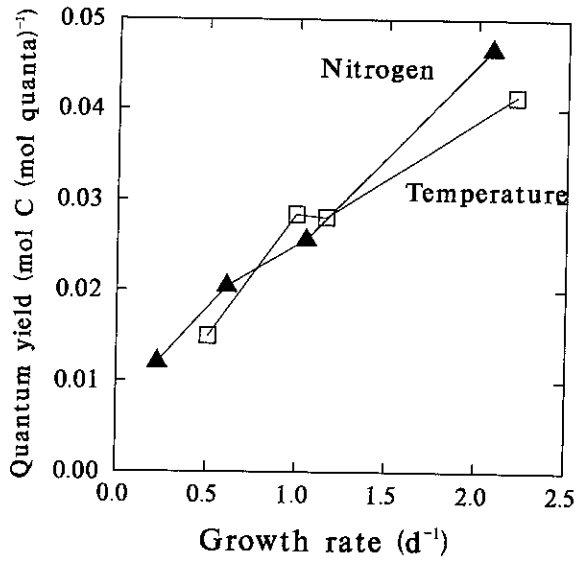

FIG. 5. Variations in quantum yield for growth as a function of growth rate for *D. tertiolecta* maintained under different environmental conditions. Both temperature (this study) and nitrogen limitation (data from Sosik and Mitchell 1991) cause ϕ_μ to decrease in a similar manner.

it were calculated based only on photosynthetically active absorbed photons (Sakshaug et al. 1991, Johnsen and Sakshaug 1993, Sosik 1993). The quantum yield for growth based on the integrated light absorption from 400 to 700 nm was calculated by rearranging Eq. 1:

$$\phi_\mu = \frac{\mu}{\text{chl} : \text{C} \int a^*_{ph}(\lambda) E_o(\lambda)\, d\lambda}. \quad (7)$$

As previously reported for nutrient-limited growth (Herzig and Falkowski 1989, Chalup and Laws 1990, Sosik and Mitchell 1991), we found ϕ_μ increased with growth rate under conditions where temperature was limiting. This is in contrast to light-limited growth, where ϕ_μ decreases with growth rate (Kiefer and Mitchell 1983, Falkowski et al. 1985, Morel et al. 1987). For this species, the same relationship between μ and ϕ_μ was observed for both nitrogen and temperature limitation of growth (Fig. 5). Over the range of conditions we examined, the relationship appears linear. Presumably asymptotic approach to a maximum quantum yield would be observed if sufficiently high growth rate could be obtained.

Changes in the quantum yield for growth of *D. tertiolecta* with temperature are important. Despite lower $a^*_{ph}(\lambda)$, cells grown at 28° C absorbed 55% more light per C than did cells at 12° C because the faster growing cells had more than twice as much chl *a* per C. While increased light absorption per C contributed to the faster growth at 28° C, the increase was small compared to the observed difference in growth rate (more than four times greater in the cells growing at 28° C). The nearly threefold increase in ϕ_μ was the main mechanism by which the cells maintained at higher temperatures were able to grow rapidly.

These results showing an increase in ϕ_μ with increasing growth temperature are consistent with evidence for variability in the photosynthesis–irradiance curve for cells grown at different temperature. It has been repeatedly shown that the saturation parameter ($I_k = P_{max}/\alpha$) decreases with decreasing growth temperature (e.g. Steemann Nielsen and Jørgensen 1968, Li and Morris 1982, Mortain-Bertrand et al. 1988, Levasseur et al. 1990). Our results probably reflect that the growth irradiance is close to I_k for the low temperatures we examined and below I_k at the highest temperature. Values of ϕ_μ are expected to be higher for cells adapted to an irradiance that is low relative to the "instantaneous" I_k, provided that acclimation is not also accompanied by large decreases in $a^*_{ph}(\lambda)$.

Although we did not measure characteristics necessary to determine the cause of the observed changes in ϕ_μ, previous studies point to the importance of factors such as changes in the number of functional reaction centers and in energy transfer efficiency between the light-harvesting pigments and the photosynthetic reaction centers. Levasseur et al. (1990) reported a study of nutrient-replete growth of *D. tertiolecta* at 12° and 20° C but at somewhat higher irradiance than our study (200 compared to 165 μmol quanta·m^{-2}·s^{-1}). These authors observed a decrease in the light-limited rate of photosynthesis per chl *a* during short-term photosynthesis–irradiance incubations of cultures previously adapted to

growth at 12° C compared to 20° C. For the two temperatures examined in their study, no significant differences were found in chl $a \cdot \text{cell}^{-1}$ or in spectrally averaged chl a- specific absorption, suggesting that changes in light absorption did not contribute to this decrease in light-limited chl a-specific photosynthesis at low temperature. Based on fluorescence induction curves, the authors reported that this decrease was primarily due to a loss of electron transfer efficiency to photosystem II reaction centers. Kolber et al. (1988) documented changes in thylakoid protein abundance in response to increasing nitrogen limitation in *D. tertiolecta,* which are consistent with a loss of functional reaction centers and a reduction in energy transfer between harvesting pigments and the reaction centers. Although we have considered the quantum yield for growth rather than the maximum photosynthetic quantum yield, it is probable that these same factors also played an important role in the decrease in ϕ_μ, which we observed at low temperature.

Changes in the importance of respiration and excretion relative to growth are another potentially important source of variability in ϕ_μ. If at higher temperatures metabolic costs are elevated, respiration may be a larger fraction of gross photosynthesis, resulting in decreased energy available for growth. In contrast, excretion may be a larger fraction of gross carbon fixation under conditions of temperature stress, resulting in low values of ϕ_μ at low temperatures. Additionally, for the purposes of monitoring total photosynthetic carbon fixation, the relevant quantum yield is the photosynthetic quantum yield, ϕ_{ps}, not ϕ_μ. Since respiration and excretion are sinks for fixed carbon, ϕ_{ps} should be greater than ϕ_μ. The extent to which variability we have observed in ϕ_μ will be manifest in ϕ_{ps} depends on how variable respiration and excretion rates are with respect to growth. In other studies examining light and nutrient effects, respiration estimated based on carbon loss in the dark has been shown to be a linear function of growth rate, and excretion of cellular carbon has generally been shown to be a small fraction of total carbon uptake for a variety of species (reviewed by Langdon 1988). These results suggest that respiration and excretion may not be a large source of variability in ϕ_μ; however, issues such as increased respiration in the light relative to the dark (e.g. Weger et al. 1989) and the specific responses to growth temperature deserve further attention.

CONCLUSIONS

This study confirms that if accurate results are to be obtained from bio-optical algorithms for phytoplankton production and growth, the models must incorporate effects of growth temperature in addition to light and nutrient effects. We have shown that, even for a single temperate phytoplankton species, dramatic changes in steady-state photophysiology can be induced by changes in growth temperature under light- and nutrient-replete conditions. Variability in $a^*_{ph}(\lambda)$ and ϕ_μ due to the effects of temperature has been shown to be comparable to that previously observed due to changes in light or nutrient availability. In addition, evidence for this well-studied species suggests that similar parameterizations for nutrient and temperature effects may be suitable. For the irradiance we have used in our nutrient- and temperature-limited experiments, the relationship between ϕ_μ and μ can be described by a single linear function. For $a^*_{ph}(\lambda)$, variability due to temperature, nutrient, and light limitation can be well described using a theoretical relationship with cell size and pigment concentration. Many questions remain, however, before these effects can be adequately parameterized for model application in the ocean. The responses of other phytoplankton taxa to temperature and the potential interactive effect of growth temperature on the photophysiology of cells growing under suboptimal light or nutrient conditions must still be addressed. An important initial goal should be to determine the extent to which, in natural phytoplankton populations, the variability in specific absorption and quantum yield is dominated by differing responses between species or by acclimative responses within a species or group. If the interspecific differences are larger, perhaps attention should be focused on general characterization of the optical properties of different groups and then turned to understanding the dynamics of community structure in response to environmental forcing. In contrast, if physiological acclimation results in greater variability, more complete studies of the matrix of interacting environmental variables should be conducted for representative species.

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