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Absorption, fluorescence, and quantum yield for growth in nitrogen-limited *Dunaliella tertiolecta*

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Abstract

The effects of steady state nitrogen limitation on the optical properties of *Dunaliella tertiolecta* were investigated. Growth rate was varied in a continuous culture under constant irradiance and temperature with absorption, fluorescence, and cellular characteristics including pigment contents determined at each steady state. The cellular concentration of Chl *a* increased with growth rate while Chl-*a*-specific absorption and fluorescence both decreased. In addition, the quantum yield for growth varied by more than a factor of 3.5 over the growth conditions examined, with the highest yield in the most rapidly growing cells. The decrease in magnitude of Chl-*a*-specific optical properties is caused by pigment package effects and changes in the abundance of accessory pigments relative to Chl *a*. Changes in absorption and fluorescence properties are consistent with theoretical predictions for discrete packages. In addition, pigment-based reconstruction techniques overestimate the magnitude of both in vivo and unpackaged absorption by *D. tertiolecta* under these growth conditions. The observed variability in Chl-*a*-specific absorption and quantum yield is a fundamental aspect of phytoplankton physiology that should be incorporated into models of oceanic primary production.

Optical measurements from in situ profiling instruments, moorings, aircraft, and satellites provide information at temporal and spatial scales not possible with traditional biological oceanographic techniques. The potential for this information to enhance understanding of primary productivity in the ocean depends on models that relate optical properties to photosynthesis. Although bio-optical models for primary production and phytoplankton growth have been developed and are being evaluated (Bannister 1979; Kiefer and Mitchell 1983; Smith et al. 1989), an increased knowledge of the sources and magnitude of variability in the parameters of these models is critical for accurate assessment of modeling capabilities.

An important component of these models involves prediction of photosynthetic production or growth from light and chlorophyll data. Variability in this relationship may be caused by changes in Chl *a*-specific absorption or changes in the quantum yield of photosynthesis. The spectral Chl-specific absorption coefficient, $a_{ph}^*(\lambda)$ (units given in list of notation), is an important parameter of the models; it provides an estimate of the amount of light absorbed by phytoplankton cells from knowledge of ambient irradiance and Chl *a* concentration. The photosynthetic quantum yield, ϕ_{ph}, is the efficiency with which the absorbed light is used for C fixation. Knowledge of the variability in these physiological parameters with growth conditions is important in accurately applying bio-optical models to diverse oceanographic regions and conditions.

Modeling primary production over large scales also relies on estimates of pigment data from remote sensing. The accuracy of current algorithms for retrieving phytoplankton pigment concentrations from optical data is limited by lack of knowledge concerning the variability in pigment-specific light absorption. This variability includes both changes in $a_{ph}^*(\lambda)$ and changes in the relative importance of phytoplankton, detritus, and dissolved material as absorbers in the water column (e.g. Morel 1988; Mitchell and Holm-Hansen 1991). Thus physiological variability in pigment-specific absorption can potentially be important in estimating the biomass of primary produc-

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Notation

$a_{ph}(\lambda)$, $a_{TX}(\lambda)$, $a_{cm}(\lambda)$	Absorption coefficient for phytoplankton in vivo, after solubilization in 0.5% Triton X-100, and for intracellular material, m^{-1}
$a_{ph}^*(\lambda)$, $a_{TX}^*(\lambda)$, $a_{sol}^*(\lambda)$, $a_{cm}^*(\lambda)$	Chl *a*-specific absorption coefficient for phytoplankton in vivo, after solubilization in 0.5% Triton X-100, for a solution of cell material, and for intracellular material, m^2 (mg Chl *a*)$^{-1}$
$a_i^*(\lambda)$	Specific absorption coefficient for pigment *i*, m^2 (mg pigment *i*)$^{-1}$
$b_b(\lambda)$	Backscatter coefficient, m^{-1}
c	Intracellular concentration of Chl *a*, mg m^{-3}
C_i	Concentration of pigment *i* in a cell suspension, mg m^{-3}
d	Cell diameter, μm
$D(\lambda)$, G	Detection or geometric constant, dimensionless
$E_0(\lambda)$	Growth irradiance, mol quanta m^{-2} d^{-1} nm^{-1}
$E(\lambda)$	Fluorescence excitation irradiance, mol quanta m^{-2} s^{-1} nm^{-1}
F	Fluorescence from a single cell, quanta s^{-1}
$\bar{F}$	Broadband fluorescence per cell, relative quanta s^{-1} $cell^{-1}$
$\bar{F}^*$	Broadband fluorescence per Chl *a*, relative quanta s^{-1} (mg Chl *a*)$^{-1}$
$Q_a(\lambda)$	Absorption efficiency, dimensionless
$Q_a^*(\lambda)$	Packaging parameter, dimensionless
λ_e, λ_f	Wavelength of excitation light and fluoresced light, nm
μ	Specific growth rate (base *e*), d^{-1}
ϕ_{ph}, $\phi\mu$	Quantum yield for photosynthesis and growth, mol C (mol quanta)$^{-1}$
ϕ_f	Quantum yield for fluorescence, mol quanta fluoresced (mol quanta absorbed)$^{-1}$
ρ'	Optical thickness along the particle diameter, dimensionless

ers as well as the production realized by that biomass.

Chl *a*-specific absorption at the blue peak and ϕ_{ph} have been shown to vary in laboratory cultures by at least fourfold and tenfold, respectively. Differences in $a_{ph}^*(\lambda)$ among species have been observed, and variability within species caused by changes in growth irradiance has been well documented. This variability in $a_{ph}^*(\lambda)$ is attributed in part to pigment packaging effects that vary with cell size and pigment content per cell (Morel and Bricaud 1981; Mitchell and Kiefer 1988) as well as changes in the abundance of accessory pigments relative to Chl *a* (Bricaud et al. 1983; Sathyendranath et al. 1987; Berner et al. 1989). In addition to variability in $a_{ph}^*(\lambda)$, there are differences in ϕ_{ph} among species and as a function of growth irradiance for a single species (Kiefer and Mitchell 1983; Falkowski et al. 1985; Sakshaug et al. 1989).

Other factors besides light can lead to physiological adaptations that result in variability in the optical properties of phytoplankton cells. Chl *a*-specific absorption and quantum yield have been shown to be affected by N depletion in batch cultures (Welschmeyer and Lorenzen 1981; Cleveland and Perry 1987). The physiological effects of starvation may be quite different, however, from those observed under steady state nutrient limitation in continuous culture. Although $a_{ph}^*(\lambda)$ can vary under steady state N-limited growth, Kiefer and Mitchell (1983) hypothesized that there should be no effect on ϕ_{ph}. Recent studies of phytoplankton in N-limited continuous culture have shown significant variability in both $a_{ph}^*(\lambda)$ and ϕ_{ph} (Chalup and Laws 1990; Herzig and Falkowski 1989). Before the effects of physiological variability on bio-optical modeling of primary production can be evaluated, a more complete characterization of this variability and its sources is necessary.

In vivo fluorescence is another optical property widely used to characterize primary producers in the ocean. Measurements can be made rapidly over a range of scales. Accurate assessment of phytoplankton biomass is limited, however, by variability in the relationship between in vivo fluorescence and other variables like Chl *a* concentration. Previous studies of laboratory cultures have documented that for a single species fluorescence per Chl *a* varies with growth conditions such as light and nutrient availability (e.g. Kiefer 1973; Mitchell and Kiefer 1988; Sosik et al. 1989), but further investigations into the sources of this variability are necessary to reliably interpret fluorescence signals from natural samples.

In this work, we have examined the effects of steady state NO_3^--limited growth on pigmentation, absorption, and fluorescence in the marine chlorophyte *Dunaliella terti-*

olecta. The roles of pigment packaging and pigment composition in determining light utilization efficiency have been considered and the results placed in the context of theoretical work on absorption and fluorescence by single cells.

Materials and methods

Dunaliella tertiolecta Butcher (FCRG culture collection) was maintained in NO_3^--limited continuous culture at four dilution rates. The vessel was kept at 22°C; sterile GPM media (Loeblich 1975) at full strength except with 40 μM NO_3^- was used for dilution, and the cultures were stirred continuously and bubbled with sterile, water-saturated air. Constant light was provided with "cool-white" fluorescent lamps at an irradiance of 165 μmol quanta m^{-2} s^{-1} for all dilution rates. Light intensity was measured with a Biospherical Instruments QSL-100 quantum scalar irradiance meter. Absolute spectral irradiance was determined from these measurements and the relative spectrum for "cool-white" lamps provided by General Electric. In each case, analysis was conducted when fluorescence of the cell suspension remained constant for at least 2 d and always after at least 10 d at a constant dilution rate. Specific growth rate μ (base e) was determined as the dilution rate of the culture (flow rate divided by the culture volume).

Subsamples of the cell suspensions were filtered onto Whatman GF/C glass-fiber filters and stored immediately at −70°C in cryotubes flushed with N_2 gas. These samples were later analyzed for pigments by reverse-phase, high-performance liquid chromatography (HPLC) following the methods described by Bidigare (1989). Additional samples were filtered onto previously combusted GF/C filters and frozen in combusted Pyrex test tubes. After desiccation of these samples, carbon and nitrogen contents were measured with a Perkin Elmer P-E 2400 CHN elemental analyzer. Cell density and equivalent spherical cell volume were determined on a Coulter Electronics 64-channel, model ZH electronic particle counter. After 15 min of dark incubation, fluorescence of the cell suspension was measured on a Turner Designs model 10 fluorometer. Bulk fluorescence was also measured with a Sea Tech in situ fluorometer after a dilution of ~1 : 40 with filtered seawater.

Absorption properties were determined with a dual-grating Perkin Elmer lambda 6 UV/VIS spectrophotometer equipped with an integrating sphere. Conventional spectroscopic techniques do not provide accurate measurements of absorption for particles in suspension (Shibata 1958), but the integrating sphere allows collection of light scattered by particles in the forward direction thus improving absorption estimates. Cell suspensions were concentrated about fivefold by centrifugation. In vivo spectral absorption, $a_{ph}(\lambda)$, was measured on the concentrated samples in a 1-cm cuvette. Fresh culture medium was used in the reference beam. Backscattered light is not collected by the integrating sphere, so our estimates are potentially greater than the true $a_{ph}(\lambda)$. Based on the results of Bricaud et al. (1983), however, we do not expect that the backscatter coefficient, $b_b(\lambda)$, was ever >1% of $a_{ph}(\lambda)$. Absorption was also measured on unconcentrated cell suspensions, but spectra were found to be noisy for optical densities <0.02. Comparison of the magnitude of peak absorption before and after concentration showed a linear increase with concentration factor, which was calculated from cell densities determined with the Coulter counter.

In addition to determining in vivo absorption, we treated a subsample of the concentrated suspension with the detergent Triton X-100 at a final concentration of 0.5%, with subsequent sonication. This treatment disrupts the cells and thylakoid membranes, removing pigment package effects without extraction by organic solvents (Berner et al. 1989). Spectral absorption, $a_{TX}(\lambda)$, was determined on these solubilized cells with 0.5% Triton X-100 in filtered seawater as a reference. This treatment to remove package effects should not be considered a general one as we have worked with other species and found variable success with disruption. A further limitation of the Triton X method involves spectral shifts in the wavelengths of peak absorption when compared to in vivo absorption. We have observed shifts toward shorter wavelengths of 1 nm at the

Table 1. Cellular characteristics of *Dunaliella tertiolecta* at several growth rates (base *e*) in N-limited continuous culture. Values in parentheses are 1 SD ($n = 2$ for pigments and $n = 3$ for C and N determinations). Pigments, C, and N are given in pg cell^{-1}.

	Growth rate (d^{-1})			
	0.23	0.61	1.05	2.08
Chl *a*	0.327(0.026)	0.470(0.017)	0.730(0.033)	0.763(0.025)
Chl *b*	0.095(0.010)	0.153(0.003)	0.213(0.010)	0.241(0.004)
Lutein	0.149(0.008)	0.212(0.007)	0.227(0.017)	0.281(0.006)
α-carotene	0.014(0.002)	0.013(<0.001)	0.005(0.002)	0.017(0.004)
β-carotene	0.096(0.009)	0.079(0.001)	0.075(0.018)	0.105(0.006)
Neoxanthin	0.020(0.003)	0.032(0.001)	0.042(0.003)	0.051(0.001)
Violaxanthin	0.028(0.001)	0.037(0.001)	0.057(0.004)	0.065(0.001)
Antheraxanthin	0.010(0.001)	0.010(0.001)	0.012(0.001)	0.014(0.001)
Carbon	39.68(1.41)	34.98(0.63)	26.97(0.42)	29.68(0.77)
Nitrogen	2.07(0.13)	2.33(0.09)	2.74(0.16)	2.72(0.27)
Cell diam (μm)	6.94(0.06)	6.91(n.d.)	6.45(0.15)	6.96(0.20)

blue peak and 8 nm at the red peak. To compensate for these shifts when using $a_{TX}^*(\lambda)$ in calculations or comparison with $a_{ph}^*(\lambda)$, we have shifted the spectra by 1 nm for wavelengths 350–557 nm and 8 nm for 551–750 nm. The region between 551 and 557 nm was repeated to prevent introducing a gap in the spectra; values are relatively low and uniform at these wavelengths, so this does not distort the spectra. The spectral values of $a_{TX}^*(\lambda)$, however, are presented without adjustment (*see Fig. 2B and Table 2*).

A subsample of the cell suspension was also concentrated on Whatman GF/C filters and extracted for 24 h in 90% acetone at 4°C in the dark. Spectral absorption of the extract was measured as described above except that 90% acetone was used as the reference.

Results and discussion

Cellular characteristics—As previously documented for N-limited growth of phytoplankton (e.g. Caperon and Meyer 1972; Kolber et al. 1988), we observed an increase in cellular Chl *a* as the growth rate of *D. tertiolecta* increased in the chemostat (Table 1). This more than twofold change in Chl *a* was accompanied by a corresponding increase in Chl *b* per cell (Table 1), resulting in no trend in the ratio of Chl *b* to Chl *a* (Fig. 1A). Increases were also observed in the absolute cellular concentrations of the carotenoids (Table 1). As found for Chl *b*, the amounts of the minor yellow pigments relative to Chl *a* changed very little (Fig. 1B). This constant stoichiometry is not true, however, for lutein, which decreases relative to Chl *a* at higher μ (Fig. 1A). Variability in the ratios of α- and β-carotene to Chl *a* was also observed (Fig. 1C).

The increase in Chl *a* per cell at higher dilution rate was accompanied by a parallel increase in cellular N and a decrease in cellular C (Table 1). As previously observed for this species grown under N limitation, molar C : N ratios were high and varied with growth rate (Caperon and Meyer 1972), ranging from 22 for μ of 0.23 d^{-1} to 11 at μ of 2.08 d^{-1}. In contrast to cell C, cell size did not vary systematically with μ (Table 1).

Absorption changes—Chl *a*-specific absorption in vivo, $a_{ph}^*(\lambda)$, was highest at low growth rates (Fig. 2A) that, as described above, corresponded to decreased amounts of pigment per cell. This trend also held for absorption after treatment with Triton X (Fig. 2B) and after extraction in acetone (not shown). Changes in $a_{ph}^*(\lambda)$ may be due to changes in the pigment package effect or to changes in the relative abundance of accessory pigments compared to Chl *a* (Morel and Bricaud 1981; Berner et al. 1989). Because the effect of packaging pigments in the cells and chloroplasts has been removed in the Triton X-treated samples, any changes in $a_{TX}^*(\lambda)$ should be due only to changes in the pigment composition of the cells. At the

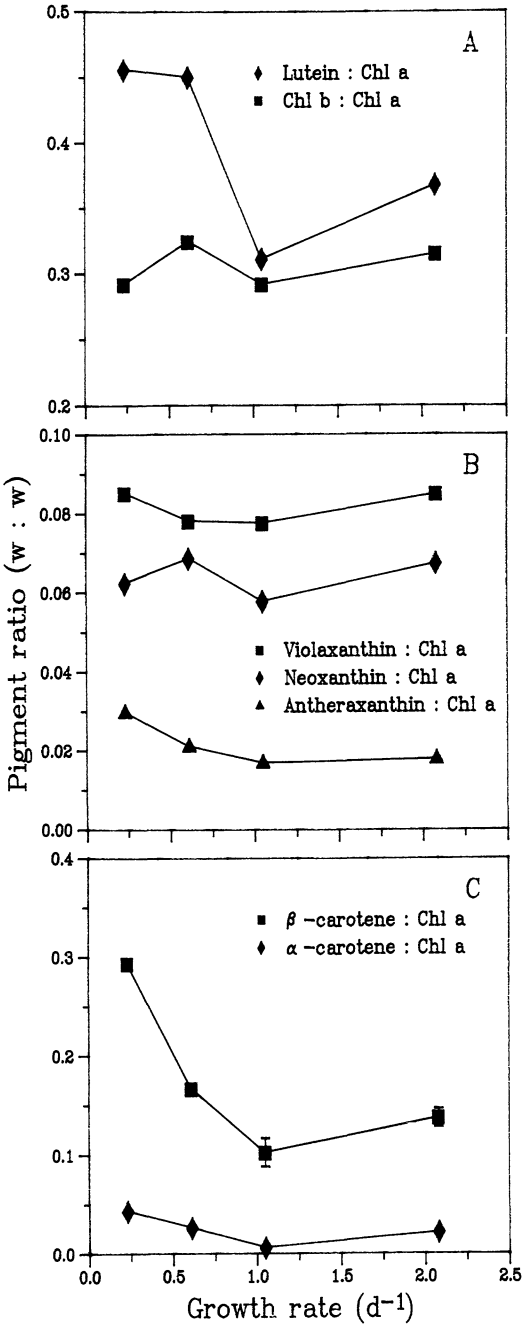

Fig. 1. Abundances of accessory pigments relative to Chl *a* as a function of growth rate in N-limited *Dunaliella tertiolecta*. Ratios for each pigment are shown with symbols identified in the panels. Standard deviations are smaller than the symbol size except where error bars are present.

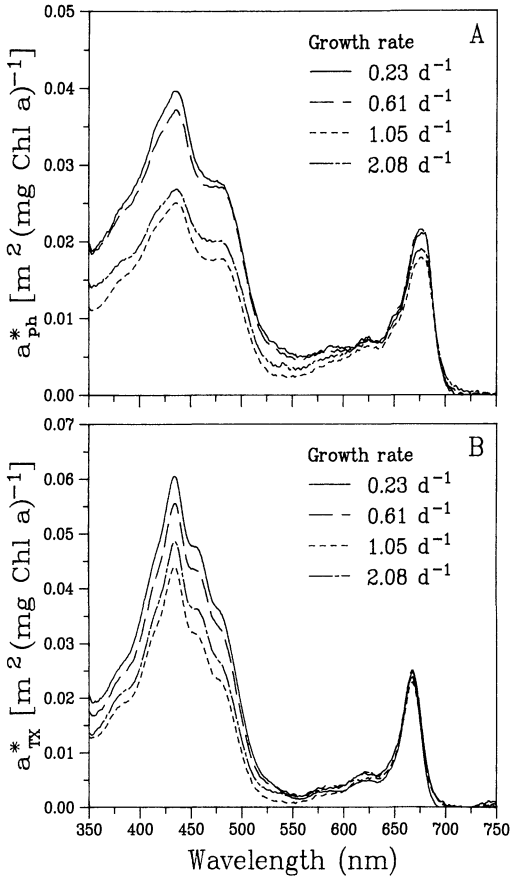

Fig. 2. Chl-*a*-specific absorption in N-limited *Dunaliella tertiolecta* at several growth rates. A. Absorption determined on whole-cell suspensions showing variability due to changes in pigmentation and pigment packaging. B. Absorption of cells disrupted by treatment with Triton X-100 so that variability is due only to changes in the abundance of accessory pigments relative to Chl *a*.

blue absorption peak, $a_{ph}^*(\lambda)$ is 48% higher at the lowest growth rate compared with the highest. In contrast, the corresponding difference for the Triton X absorption is only 24%. Because the relative variation in specific absorption without package effects is smaller than the difference in vivo, package effects and changes in pigment ratios must both contribute to variations in $a_{ph}^*(\lambda)$ with growth rate. Our results suggest that, for absorption of light at the blue peak, about half of the observed variability in $a_{ph}^*(\lambda)$ is due to package effects with the remainder caused by changes in pigment composition.

Changes in pigment composition are expected to affect specific absorption at the blue Chl *a* peak because absorption spectra for accessory pigments overlap the Chl *a* spectrum in this region. Examination of the pigment ratio data (Fig. 1) shows that response of the various accessory pigments to increased N limitation differs. It is apparent that the most important changes leading to increased $a_{TX}^*(\lambda)$ at low μ are in lutein and β-carotene relative to Chl *a*. Although the other pigments remain in fairly constant proportions to Chl *a* or are present at relatively low levels, lutein and β-carotene ratios are higher particularly at the two lowest μ. These growth rates also correspond to the highest $a_{TX}^*(\lambda)$ (Fig. 2B). In addition, at a μ of 1.05 d^{-1} the ratios are the lowest and $a_{TX}^*(\lambda)$ is also lowest.

Although changes in pigment composition play a significant role in determining in vivo absorptive properties, as the comparison of absorption for whole cells and solubilized cells indicates, the packaging of pigments into discrete particles is also important. A theoretical framework for understanding this package effect has been developed (Duysens 1956; Morel and Bricaud 1981), and its applicability for describing changes in absorption properties of light-limited algal cultures has been verified (Sathyendranath et al. 1987; Bricaud et al. 1988). Variability in $a_{ph}^*(\lambda)$ observed for nutrient-limited growth has not previously been interpreted with respect to the model of pigment packaging. In addition, an important parameter of the model, absorption by intracellular matter, $a_{cm}(\lambda)$, has remained largely uncharacterized. It is possible to estimate the magnitude and variability in $a_{cm}(\lambda)$ from Triton X absorption measurements and cellular pigment concentrations.

Theoretical work on absorption by discrete particles allows absorption efficiency as a function of wavelength, $Q_a(\lambda)$, to be predicted based on cell size and $a_{cm}(\lambda)$ (Morel and Bricaud 1981). The product of $a_{TX}^*(\lambda)$ and the intracellular concentration of Chl *a* (c) is an estimate of $a_{cm}(\lambda)$,

$$a_{cm}(\lambda) = a_{TX}^*(\lambda)\, c. \tag{1}$$

Whereas Morel and Bricaud (1981) assumed a constant value of a_{cm} ($= 2 \times 10^5$ m^{-1}) at the blue peak for calculation of Q_a, we found that values varied from 1.1 to 2.3 $\times$ 10^5 m^{-1} mainly due to changes in c. At the red absorption peak, values were as low as 0.4 $\times$ 10^5 m^{-1} due to the decreased Chl *a*-specific absorption at red wavelengths compared to blue and the lack of absorption by accessory pigments at red wavelengths.

Morel and Bricaud (1981) have described absorption efficiency as a theoretical function of the dimensionless parameter, $\rho'(\lambda)$. This parameter represents the optical thickness of a particle along its diameter and is defined as

$$\rho'(\lambda) = a_{cm}(\lambda)\, d \tag{2}$$

where d is the particle diameter. The absorption efficiency, $Q_a(\lambda)$, is the fraction of the photons incident on the geometric cross-section of the cell that is absorbed by the cell. From the absorption coefficient for phytoplankton in a suspension $a_{ph}(\lambda)$, $Q_a(\lambda)$ can be calculated as

$$Q_a(\lambda) = \frac{a_{ph}(\lambda)\, V}{(\pi d^2/4)\, N}. \tag{3}$$

Here N is the number of cells in a volume V of the suspension, and cells are assumed to be spherical and homogenous (Morel and Bricaud 1981). The measurements that we have made allow independent calculations of both $\rho'(\lambda)$ and $Q_a(\lambda)$ for *D. tertiolecta* at each μ (Fig. 3). An index of the package effect, $Q_a^*(\lambda)$, which is the ratio of absorption by discrete packages to the absorption by the same material dispersed in solution, has also been presented by Morel and Bricaud (1981):

$$Q_a^*(\lambda) = a_{ph}^*(\lambda)/a_{sol}^*(\lambda). \tag{4}$$

We applied this equation with $a_{TX}^*(\lambda)$ for $a_{sol}^*(\lambda)$ in order to calculate values of $Q_a^*(\lambda)$. For these growth conditions and wavelengths, Q_a ranges from 0.2 to nearly 0.6, while Q_a^* varies from almost 0.9 to as low as 0.5 and agreement with theory is excellent (Fig. 3). This variability suggests that less severely N-limited cells can absorb more light and thus grow more rapidly. This increase in overall absorption by the cell is accompanied, however, by a decrease in the

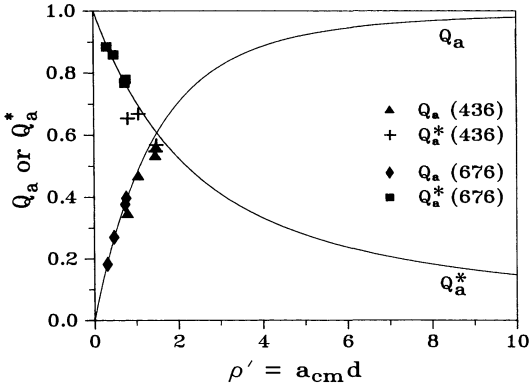

Fig. 3. In vivo absorption efficiency (Q_a) and the packaging parameter (Q_a^*) at the red and blue absorption peaks as a function of cellular optical thickness (ρ') for each of the growth conditions examined. Q_a, Q_a^*, and ρ' are estimated from measurements with Eq. 1–4 as described in the text; the theoretical relationships of Morel and Bricaud (1981) are shown for comparison (solid lines).

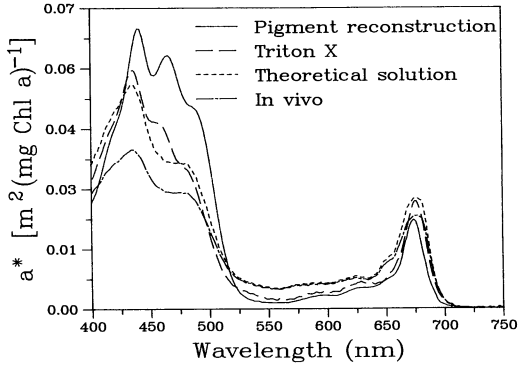

Fig. 4. A comparison of several methods for estimating unpackaged Chl-*a*-specific absorption for *Dunaliella tertiolecta* grown at 0.61 d^{-1}. Absorption by cells solubilized in Triton X is similar to that calculated for a theoretical solution of cell material, but the pigment reconstruction technique significantly overestimates unpackaged absoprtion (details of these methods given in text). The magnitude of in vivo absorption, which includes the manifestation of package effects, is shown for comparison. These results are typical of all the growth rates.

efficiency with which each molecule of pigment absorbs.

Pigment reconstruction of absorption—Because of the need for accurate estimates of $a_{ph}^*(\lambda)$ in bio-optical models, a great deal of effort is currently being focused on the determination of this parameter for natural samples. Because other absorbing particles are present in these samples, direct measurements of particulate absorption are not sufficient to specify $a_{ph}^*(\lambda)$. This problem has led to the development of alternative methods for deriving $a_{ph}^*(\lambda)$, including reconstruction of $a_{ph}^*(\lambda)$ from HPLC data on individual pigment abundances (Bidigare et al. 1987). By summing the contributions of each of the individual pigments, $a_{ph}^*(\lambda)$ can be estimated:

$$a_{ph}^*(\lambda) = \sum a_i^*(\lambda)\, C_i/\text{Chl } a. \qquad (5)$$

Here $a_i^*(\lambda)$ is the specific absorption coefficient of pigment i at wavelength λ, C_i the concentration of pigment i in the suspension, and Chl a the concentration of Chl a in the suspension.

We have made this calculation for each of the growth conditions studied using the spectral specific absorption coefficients for Chl *a*, Chl *b*, and photoprotective carotenoids from Bidigare et al. (1990). Because these coefficients are derived from absorption of individual pigments in solvents, any in vivo package effects will not be reflected in $a_{ph}^*(\lambda)$ estimated from Eq. 5. For this reason we expect this estimate of $a_{ph}^*(\lambda)$ to be comparable to the measured $a_{TX}^*(\lambda)$. In the blue-to-green region of the spectrum, reconstructed $a_{ph}^*(\lambda)$ values are up to 2.5 times the true $a_{ph}^*(\lambda)$ and as much as 2 times $a_{TX}^*(\lambda)$, with the largest differences at the highest growth rates (Fig. 4 shows results at $\mu = 0.61$ d^{-1}). For the various growth rates, the mean value from 400 to 500 nm of the reconstructed $a_{ph}^*(\lambda)$ ranges from 23 to 31% higher than $a_{TX}^*(\lambda)$.

As an additional comparison, we have also calculated the theoretical absorption in solution $[a_{sol}^*(\lambda)]$ following the method of Morel and Bricaud (1981). Inputs to this calculation are the measured in vivo absorption and the cell diameter, with the theory then allowing extrapolation to absorption at zero particle diameter (i.e. for the same material in solution). For the average absorption from 400 to 500 nm, $a_{sol}^*(\lambda)$ is consistently lower than the measured $a_{TX}^*(\lambda)$, but in contrast to the reconstructed estimate the difference ranges from 2% to at most 13%.

The highest values of $a_{ph}^*(\lambda)$ we estimated

with the pigment reconstruction technique correspond to values of $Q_a(\lambda)$ that are higher than the theoretical maximum of 1. Undoubtedly package effects must be included before this method can provide realistic estimates. The reason for the discrepancy between $a_{TX}^*(\lambda)$ and the estimate of $a_{ph}^*(\lambda)$ from pigment reconstruction, however, remains elusive. The overestimate seems to be due mainly to the contribution of lutein, although the other carotenoids also play a role. It is possible that the magnitude of absorption by these carotenoids in vivo is significantly different than in organic solvents due to more than package effects. Recent application of the pigment reconstruction technique in a dinoflagellate includes a correction for package effects (Nelson and Prézelin 1990), but our observations suggest that even this modification would not give accurate results for *D. tertiolecta*. We speculate that the molar extinction coefficient for some of these pigments in organic solvents is greater than the corresponding coefficient for the same pigment in theoretical aqueous solution. Whether this type of problem exists for other taxa with different pigmentation must be tested with further study of laboratory cultures.

Fluorescence changes—Fluorescence from a single cell has been described by a theoretical model that incorporates the role of pigment packaging in modifying light absorption efficiency and intracellular reabsorption of fluorescence (Collins et al. 1985; Mitchell and Kiefer 1988). This model can be applied to conditions of broadband excitation and emission and can be modified to estimate the relative fluorescence measured by a specific detector, thus allowing the model to be evaluated with our data set. Collins et al. (1985) presented a functional form for fluorescence emission from a cell which is generalized here to reflect the effects of both excitation and emission wavelengths:

$$F(\lambda_e, \lambda_f) = \phi_f(\lambda_e, \lambda_f)\, E(\lambda_e)\, Q_a(\lambda_e) \cdot (\pi d^2/4)\, Q_a^*(\lambda_f). \quad (6)$$

Here ϕ_f is the quantum yield for fluorescence, E is the incident irradiance, and λ_e and λ_f are the excitation and emission wavelengths. This generalized equation should be applicable in cases where multiple chromaphores with different quantum yields such as chlorophyll and phycoerythrin may be contributing to the fluoresced light and is valid for both excitation and emission spectra. It is also possible to express fluorescence in terms of $a_{ph}^*(\lambda_e)$, $a_{ph}^*(\lambda_f)$, and $a_{cm}(\lambda_f)$ if the available information is not sufficient to determine absorption efficiencies (Mitchell and Kiefer 1988).

For spectrally specified excitation irradiance and absorption properties, $Q_a(\lambda)$ and $Q_a^*(\lambda)$, Eq. 6 can be integrated over the ranges of λ_e and λ_f to give the total flux of fluoresced quanta in a broad emission band as a result of polychromatic excitation. In order to calculate the flourescence from a single cell measured by a specific instrument, it is necessary to scale the total flux to include the detection characteristics $D(\lambda_f)$ and the geometric constant G that indicates the fraction of the emitted light intercepted by the detector. The resulting expression is

$$\bar{F} = G \int_{\lambda_f} \int_{\lambda_e} D(\lambda_f)\, F(\lambda_e, \lambda_f)\, d\lambda_e\, d\lambda_f \quad (7)$$

where $\bar{F}$ is the fluorescence from a single cell for broadband excitation and emission, and $F(\lambda_e, \lambda_f)$ is as defined in Eq. 6 with $E(\lambda_e)$ the product of the spectral irradiance emitted by the excitation source and the transmission properties of any filters or optical components between the source and the sample. Similarly, the detection function $D(\lambda_f)$ is the product of the transmission of any emission filters or optical components and the response of the detector at each wavelength λ_f. This fluorescence can also be expressed per unit of Chl as $\bar{F}^*$ which is simply $\bar{F}$ divided by the cellular concentration of Chl *a*.

Consistent with the foregoing theory, variability with growth rate documented for specific absorption is also evident in the fluorescence data. Despite differences in spectral characteristics, results for the Sea Tech fluorometer (Table 2) and trends observed with the Turner Designs fluorometer were similar. For comparison of trends, all fluorescence data have been expressed in rel-

Table 2. Quantum yield for growth and optical properties of N-limited *Dunaliella tertiolecta*. Quantum yield has units of mol C (mol quanta)$^{-1}$; specific absorption coefficients are given for the red and blue peaks in m^2 (mg Chl *a*)$^{-1}$; absorption efficiencies are dimensionless and fluorescence is expressed as relative flux per cell ($\bar{F}$) or per unit of Chl *a* ($\bar{F}$*).

	Growth rate (d^{-1})			
	0.23	0.61	1.05	2.08
$\phi\mu$	0.013	0.021	0.027	0.048
a_{ph}*(436)	0.040	0.037	0.025	0.027
a_{ph}*(676)	0.021	0.022	0.018	0.019
a_{TX}*(435)	0.061	0.056	0.044	0.049
a_{TX}*(668)	0.024	0.025	0.023	0.025
Q_a(436)	0.35	0.47	0.56	0.54
Q_a(676)	0.18	0.27	0.40	0.38
Q_a*(436)	0.66	0.67	0.57	0.55
Q_a*(676)	0.88	0.86	0.78	0.77
Sea Tech fluorometer				
Measured				
$\bar{F}$	1.00	1.50	1.20	1.40
$\bar{F}$*	1.00	1.04	0.53	0.60
Modeled				
$\bar{F}$	1.00	1.35	1.32	1.54
$\bar{F}$*	1.00	0.94	0.59	0.66

ative numbers scaled to be 1 at the lowest μ. At the highest μ, although pigment per cell more than doubled relative to the lowest μ, Q_a is higher by only 60% at the blue peak and a correspondingly small increase is observed in $\bar{F}$. As pigment per cell increases, changes in the pigment composition and in the severity of package effects are acting as described above to decrease a_{ph}*(λ).

A similar decline in $\bar{F}$* is also evident. Because cell suspensions were kept optically thin [OD(675) $\leq$ 0.01] for all fluorescence measurements, reabsorption of fluoresced light within the suspension cannot account for the observed variability in fluorescence. For the Turner Designs measurements, red absorption by the cells in suspension was about equal to absorption by the water medium and an order of magnitude lower than absorption by the water for measurements with the Sea Tech fluorometer. Although it is possible that variability in the quantum yield of fluorescence (number of photons fluoresced per number absorbed) could occur for cells from different growth conditions, the trends we observed suggest that variability in the efficiency with which light is absorbed is an important factor.

From observed values of absorption and cell size and the known characteristics of the Sea Tech fluorometer, we have also predicted the relative fluorescence with Eq. 6 and 7. Because we do not have information concerning variability in ϕ_f, as a first approximation we have assumed that it is constant for the excitation and emission conditions used and that it does not vary over the growth conditions examined. Because we do not know the absolute values of ϕ_f and G, fluorescence values have been presented as relative numbers scaled to maximum of 1 at μ of 0.23 d^{-1}. As indicated above, these calculations involve not only spectral values of the absorption factors for the cells but also the spectral output of the lamp and the spectral transmission of the blue excitation filter, the acrylic window, the red emission filter, and the gold mirror, as well as the spectral sensitivity of the detector (*see* Bartz et al. 1988).

Trends in values of $\bar{F}$* predicted with Eq. 6 and 7 agree well with actual measurements (Table 2, r^2 = 0.96), which supports our hypothesis that changes in absorption efficiency are an important source of variability in fluorescence per Chl *a* and demonstrates the validity of the model for broadband excitation and emission of fluorescence. Regression with the measured and modeled values as independent and dependent variables, respectively, reveals an intercept of 0.20. With so few points it is not reasonable to attach much significance to this intercept, but it is possible that it reflects a change in ϕ_f with growth conditions. Only a larger range of experimental conditions and more detailed work on variability in $\bar{F}$* can test this hypothesis. Further, the broadband approach presented here is somewhat crude, and spectral fluorescence methods such as those of Mitchell and Kiefer (1988) will be necessary to resolve the details in model verification.

The variability in fluorescence properties observed must be considered in the context of the increasing use of in situ fluorometers to estimate phytoplankton biomass. Although with proper calibration this method may provide useful information, no simple relationship can be expected to exist between fluorescence and biomass estimates

such as Chl *a* concentration. Both variability with growth conditions for a single species and the potential for interspecific differences must be considered. In addition, the lack of knowledge concerning variability in the quantum yield for fluorescence continues to limit applications such as the use of natural fluorescence to estimate productivity.

Quantum yield for growth—The quantum yield at the irradiance under which cells have been grown, ϕ_μ, can be determined from a spectral version of the model of Kiefer and Mitchell (1983):

$$\mu = \phi_\mu \, \text{Chl:C} \int_\lambda a_{ph}^*(\lambda) \, E_0(\lambda) \, d\lambda. \quad (8)$$

Here μ is the specific growth rate of the cells, $E_0(\lambda)$ is the spectral growth irradiance, and Chl : C is the cellular Chl *a* : C ratio. Solving this equation for ϕ_μ at each of the growth rates examined results in nearly fourfold variability in ϕ_μ, with values decreasing as N limitation increases (Table 2).

A potential source of variability in ϕ_μ or ϕ_{ph} is changes in $a_{ph}^*(\lambda)$ due to pigment-ratio shifts. If the abundance of photoprotective pigments increases relative to photosynthetic pigments, $a_{ph}^*(\lambda)$ is expected to increase but the energy available for C fixation would decrease. The result is a decrease in ϕ_μ with higher $a_{ph}^*(\lambda)$, consistent with our observations of increased abundance of lutein and β-carotene relative to Chl *a* at low μ. This increase in $a_{ph}^*(\lambda)$ can only account for ~25% of the change we oberved in ϕ_μ, however, because ϕ_μ varied by more than threefold and pigment-ratio-induced changes in $a_{ph}^*(\lambda)$ were much smaller (*see Table 2*).

Many factors besides $a_{ph}^*(\lambda)$ may contribute to a decrease in ϕ_μ, including changes in respiration relative to photosynthesis with growth rate. Although we have not measured respiration in this study, it is unlikely that increased respiration relative to growth could account for all of the decrease in ϕ_μ observed at low μ. For N-limited growth of *Pavlova lutheri,* Chalup and Laws (1990) found a more than twofold increase in ϕ_{ph} with μ and also demonstrated an increase in ϕ_{ph} with decreased irradiance at the same dilution rate. For N limitation of *Isochrysis galbana* at a single irradiance, Herzig and Falkowski (1989) observed a range in ϕ_μ similar to our observations and additionally found that variability in ϕ_{ph} was 85% of that for ϕ_μ.

Additional sources of variability in quantum yield were not investigated in this study. Changes in energy transfer efficiency, in the reaction center turnover time, or in the abundance of pigment in photosystem 1 relative to photosystem 2 could all contribute to changes in ϕ_μ. Although Cleveland and Perry (1987) have implicated these mechanisms to explain quantum yield changes in transition from nutrient-replete to nutrient-starved conditions, results may differ for cultures allowed to adapt to a steady state N-limited growth condition. Using pump and probe fluorescence techniques and immunochemistry, Kolber et al. (1988) suggested that N limitation in several species, including *D. tertiolecta,* leads to a decrease in the energy conversion efficiency of photosystem 2 caused by a loss of functional reaction centers or inefficient energy transfer to the reaction centers.

All of these results suggest that studies considering photosynthetic efficiency as independent of nutrient availability must be viewed with caution. Kiefer and Mitchell (1983) hypothesized that ϕ_{ph} is a function only of irradiance, consistent with their model for growth of *Thalassiosira fluviatilis* in which spectrally averaged a_{ph}^* was assumed to be constant. Sakshaug et al. (1989) used a similar fitting technique to model growth rate under a large number of light- and nutrient-limited conditions and found a constant value of the product $a_{ph}^* \cdot \phi_{ph}$ to be adequate for a given irradiance. The conclusion that ϕ_{ph} is only a function of growth irradiance alone and not of nutrient limitation, however, has not been substantiated by studies in which light absorption and photosynthetic efficiency have been measured (e.g. this study; Kolber et al. 1988; Herzig and Falkowski 1989).

Conclusion

It is evident that variability in the physiological responses of phytoplankton to growth conditions cannot be ignored in bio-optical characterization of ocean waters both

with respect to productivity and optical properties. Models for the remote estimation of pigments or for propagation of light through the water column rely on accurate knowledge of the relationships between pigments and in situ optical properties. For example, recent work suggests that predictions with current algorithms for pigment estimation may lead to significant errors for the Antarctic Ocean (Mitchell and Holm-Hansen 1991). These errors result from variability in $a_{ph}^*(\lambda)$ and in the relative abundance of phytoplankton and other absorbing material in the water column. Estimating photosynthetic biomass from in situ fluorescence measurements is equally complex and can be significantly affected by the physiological state of the phytoplankton.

Bio-optical models of phytoplankton growth and productivity must also incorporate physiological variability if light absorption is to be accurately related to C fixation. Working with a model similar to that of Kiefer and Mitchell (1983), Sakshaug et al. (1989) found that, compared to other model parameters, changes in the value of a_{ph}^* caused relatively large errors in modeled growth rate. Increasing evidence of large variability in the optical properties of phytoplankton with growth conditions suggests that modification of existing models to incorporate physiology and environmental conditions besides light intensity must be a high priority. A major obstacle to the achievement of this goal continues to be a lack of comprehensive physiological studies that include simultaneous measurement of pigments and optical properties for a wide range of species and growth conditions.

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