Chlorophyll a specific absorption and fluorescence excitation spectra for light-limited phytoplankton

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(Received 10 July 1987; in revised form 12 November 1987; accepted 19 November 1987)

Abstract—Methods are described for the measurement of spectral absorption coefficients, fluorescence excitation, and fluorescence yields for pigmented particles retained on filters. The corrections required for absorption coefficients include determining increased optical pathlength while corrections for fluorescence include determining system spectral variability, mean light level and reabsorption. The empirical technique is consistent with and validated by theoretical relationships for light transmission and fluorescence of absorbing particulate material embedded in a medium with intense scattering.

These methods were applied to a study of photoadaptation in several phytoplankton species and revealed variations in the blue for chlorophyll a specific absorption \[a_{ch}^{\alpha}(\lambda)\] and fluorescence excitation \[F^*(\lambda)\] of greater than 3- and 10-fold, respectively. Variations in the spectral shapes and the magnitude of \[a_{ch}^{\alpha}(\lambda)\] and \[F^*(\lambda)\] with photoadaptation are determined largely by the effect of pigment absorption in discrete particles, sometimes referred to as the sieve or package effect. A model is presented expressing \[F^*(\lambda)\] in terms of \[a^\alpha(\lambda)\] which predicts large variability in \[F^*(\lambda)\] due to cell size and cellular pigmentation and which may help reconcile the previously reported, but unexplained variations in \[F^*(\lambda)\]. Spectral variations in the fluorescence yield appear to be caused by variations in the fraction of light absorbed by photosystem II which fluoresces as compared to photosystem I or photoprotective pigments which do not fluoresce. The techniques presented provide a rapid, reproducible, and simple approach for routine analysis, particularly for field applications where particle densities are too low for direct analysis of absorption spectra.

INTRODUCTION

Analysis of optical properties of particles retained on filters has proven to be a convenient, though generally qualitative, approach for the study of dilute suspensions found in aquatic environments. Improved understanding of primary production and light absorption in aquatic environments requires quantitative techniques. For example, an improved understanding of the utilization of radiant energy by the phytoplankton crop could be expected if quantitative techniques were available for studying the relationship between light absorption, photochemistry and fluorescence. Aquatic ecologists have relied on simple methods for fluorescence and photosynthetic measurements in studying natural populations. The radiocarbon tracer technique (STEEMANN NIELSEN, 1952) has allowed description of the temporal, regional and vertical distribution of primary production. Fluorescence methods applied to the assessment of chlorophyll concentrations \textit{in vitro} (YENTSCH and MENZEL, 1963; HOLM-HANSEN \textit{et al.}, 1965) and \textit{in vivo} (LORENZEN, 1966) have provided the means to easily study phytoplankton biomass. In

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addition many studies have combined these techniques together with variable light incubations to define the photosynthesis-irradiance response of algae (Ryther, 1956; Dunstan, 1973; Platt and Jassby, 1976). However, relatively few studies have dealt with the problem of light absorption and utilization (Tyler, 1975; Dubinsky and Berman, 1976; Morel, 1978; Bannister and Weidemann, 1984; Lewis et al., 1985; Kishino et al., 1986). This is due, in large part, to the lack of simple quantitative techniques for directly measuring light absorption by the dilute suspension of cells in situ. Furthermore, most studies of fluorescence or absorption of field samples have not used techniques that provide spectral information and therefore are limited in information content. Since the spectral composition, as well as the intensity of photosynthetically available radiation, varies for phytoplankton in situ, techniques that provide information on spectral absorption and fluorescence response would be valuable tools for routine studies of phytoplankton ecology.

Development of capabilities to estimate primary production from either remote or in situ optical sensors will require the application of physiological models of phytoplankton growth like that proposed by Kiefer and Mitchell (1983). An initial effort has been presented by Collins et al. (1986). The lack of a method to measure directly the absorption coefficient of marine particulates has limited the capability to validate such models in the field.

Determinations of the absorption coefficient of laboratory cultures have been obtained with greater accuracy and spectral resolution than field measurements. The opal glass technique (Shibata et al., 1954; Shibata, 1958) helps to minimize distortions caused by light scattering within the sample (Duysens, 1956). Because cell densities are low for field samples, the particles must be concentrated for direct absorption measurements. Kirk (1980) first concentrated samples by filtration and then measured the absorption coefficient of resuspended material using a spectrophotometer with an integrating sphere attachment. Weidemann and Bannister (1986) concentrated samples by filtration or centrifugation and measured the re-suspensions using the Shibata technique. Yentsch (1957) directly measured qualitative absorption spectra for cultures and field samples collected on filters (Yentsch, 1962). Faust and Norris (1982) have applied derivative spectroscopy to the study of phytoplankton spectra determined on glass fiber filters. A significant problem for analysis on filters arises from large modifications of the light transmission by the filters: signal amplification due to increased optical pathlength (Butler, 1962) must be considered for quantitative determinations. Kiefer and Soohoo (1982) proposed a constant factor to correct for the amplification by scattering of the glass fiber filters. Mitchell and Kiefer (1984) demonstrated that the correction factor is not constant, but varies with sample density. A more accurate technique for correcting the amplification by several different filter types is described here. The technique provides a method for the determination of volume absorption coefficients ($a_p$) for field particles and cultures.

Qualitative measurements of chlorophyll $a$ fluorescence excitation provide insight to the taxonomic and photosynthetic characteristics of populations. Using uncorrected fluorescence excitation and emission spectra, Yentsch and Yentsch (1979) proposed that phytoplankton taxonomic groups could be defined according to their major accessory pigment characteristics. Recent field and laboratory studies have also demonstrated that the shape of fluorescence excitation spectra can be interpreted as features of photoadaptation (Mitchell and Kiefer, 1984, 1988; Neori et al., 1984; Soohoo et al.,
1986; Mitchell, 1987). Furthermore, Neori et al. (1986) has shown that the fluorescence excitation for Chl a in phytoplankton is representative of the action spectrum for oxygen evolution. Methods are presented for quantitative correction of fluorescence excitation spectra which compensate for variations in excitation energy due to optical components of the system, sample density, and extracellular reabsorption of the fluoresced light by the red Chl a absorption band. A simple model indicates that intracellular reabsorption of fluoresced light is an important cause of variations in Chl a specific fluorescence in vivo. Both the absorption and fluorescence techniques are easily applied for either cultures or field samples, offering aquatic ecologists an improved ability to explore the conversion of solar energy into biomass.

**MATERIALS AND METHODS**

**Sample preparation**

Cultures of Pavlova (Monochrysis) lutheri, Dunaliella tertiolecta, and Chaetoceros gracilis were grown in f/2 enriched sterile seawater (Guillard, 1975) with continuous illumination from cool white fluorescent lamps. Variable growth irradiances for light-limited experiments were attained with neutral density screens, or by placement of culture vessels at different distances from the light source. All samples were filtered with low vacuum pressure (<5 mm Hg). Filtered samples were kept dark at 0.5–3°C before analysis of fluorescence spectra, which was done as soon as possible after filtration. Tests of the stability of the fluorescence signal under these conditions revealed no significant changes for samples stored up to 8 h. However, freezing of the samples results in a large drop in the fluorescence signal, and in some cases changes in the spectral shape, even if the samples are analysed while still frozen. Absorption spectra appear to be stable if frozen at -20°C.

For absorption analysis, sample and blank filters were mounted on a clear plexiglass sheet at the back of the sample compartment to minimize the distance between the sample and detector. Before measurement the filters were moistened with filtered seawater until saturated, because the reflectance and transmittance of the filters varies with the degree of saturation. Saturation of both the sample and blank was verified if a drop of water remained after lifting the filter from the mounting plexiglass plate. The sample may be mounted between two plexiglass plates to minimize dehydration, but this was not essential when the spectra were run within 2–3 min after saturation.

Algorithms have been developed for three Whatman glass filter types (GF/F, GF/C, 934AH) and Millipore cellulose acetate type HA. For the analysis of photoadaptive responses described here, the cultures were collected on GF/C filters.

**Instrumentation**

All analog optical signals were digitized, stored, and processed with a Digital Equipment corporation PDP 1123 computer. Spectra of diffuse transmission of sample filters relative to a blank filter were measured with a Bausch and Lomb Spectronic 2000 scanning, dual-beam spectrophotometer. The filter medium itself acted as a diffuser of the measurement beam providing the benefits of the Shibata (1958) technique. No additional diffuser was used. We wish to stress that the corrections we present are specific to analyses made with the Spectronic 2000 spectrophotometer with sample positioning as described. Acceptance angles and measurement geometry of different
instruments may be important. Some instruments we have tested do not have sufficient
dynamic range to resolve the absorbance since, for our configuration, the transmittances
of a blank filter relative to air was only a few percent. Recent analysis indicates that the
correction factors for pathlength amplification will depend on sample position, possibly
due to changes in the acceptance angle (A. Morel, personal communication).

In order to derive empirical corrections of absorption measurements for filters,
experiments were conducted comparing the absorption for cells in suspension to the
same material on filters. Absorption coefficients of the suspensions for these experiments
were measured with a vertical path (Butler) transmissometer (Butler, 1962; Kiefer and
SooHoo, 1982; Mitchell and Kiefer, 1984), which consisted of a Bausch and Lomb high
intensity monochromator (33-86-76) coupled to a Bausch and Lomb 45 W tungsten-
halide light source. Transmitted light intensities of suspensions and blanks were mea-
sured with a Gamma 20-20-10 photomultiplier and 2900 autophotometer. For measure-
ments of suspensions with the Butler spectrophotometer, a diffuser was placed between
the sample and detector in accordance with the Shibata technique. The instrument
optical geometry has the design advantage of maximizing the angle of acceptance of the
measurement beam and minimizing effects due to settling of the cells in the suspension.

Measurements of fluorescence excitation spectra were made with the same instrument
used for absorption spectra of suspensions but reconfigured in the following way. The
mirror used to reflect the monochromatic excitation beam onto the sample was replaced
by a long wavelength transmitting dichroic filter (OCLI CSF-A). This filter reflected
wavelengths <550 nm onto the sample but allowed the Chl a fluorescence to pass to the
photomultiplier which was mounted above the dichroic mirror. A 680 nm bandpass filter
(Melles-Griot 03-FIV-065) placed in front of the photomultiplier prevented excitation
light reflected off the sample from reaching the photomultiplier while allowing the Chl a
fluorescence to pass (Fig. 1). This optical configuration facilitated calibration of the
relative spectral excitation quantum flux at the sample using the fluorescent dye,
rhodamine-B, which was dissolved in ethylene glycol at a concentration of 3 g l⁻¹. Under
these conditions, rhodamine-B has been found to be a quantum counter which absorbs all
photons and emits them with a constant fluorescence quantum yield in a broad band
above 600 nm (Melhuish, 1962). A smoothed average of five rhodamine-B spectra was
normalized to unity at the maximum (545 nm for this system) and used in the algorithm
to correct for the system spectral response. Because of spectral limitations of the dichroic
filter, spectra presented here are restricted to excitation wavelengths <525 nm. Signal
stability was tested with a fluorescent glass (Corning no. 3486) which has excitation and

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Fig. 1. Schematic diagram of the vertical path spectrophotometer configured for fluorescence
excitation measurements.
Chlorophyll \( \textit{a} \) specific absorption and fluorescence

emission characteristics similar to Chl \( \textit{a} \) and is stable through time (PARKER and VAUDREY, 1980). The signal at 512 nm was tested before and after sample measurements, and system drift was compensated by varying the high voltage to the photomultiplier. Spectral variations due to lamp aging were tested by comparing the shape of the rhodamine-B spectra through time. If signals for the fluorescent glass or system spectral shapes deviated significantly from nominal conditions, the lamp was replaced and the system recalibrated.

CORRECTION ALGORITHMS

Volume absorption coefficient algorithm

To maximize the spectral absorption signals for field samples when water sample volume is limited, measurements are sometimes made on double layers of a sample filter which has been folded or cut in half and stacked. Therefore, a two-step procedure has been developed to derive the phytoplankton volume absorption coefficient, \( a_{\text{ph}}(\lambda) \) (m\(^{-1}\)), from these measurements. Beer–Lambert law for absorbing materials in pure solution predicts a doubling of absorption when the geometric pathlength is doubled. Since the filters diffuse the light in the measurement beam making the actual optical pathlength of the sample greater than the geometric pathlength, theories for non-scattering solutions are not applicable (SHIBATA, 1958). BUTLER (1962) has defined the term \( \beta \) as the ratio of the optical to geometric pathlength.

The problem of assessing \( \beta \) has been approached as follows. For two-layer samples one must first correct for \( \beta^*(\lambda,2) \) for a second layer relative to a single layer and then for \( \beta(\lambda,1) \) for a single layer relative to a non-scattering suspension. The definition of \( \beta \), according to BUTLER (1962) specifically referred to the absorption amplification induced by diffusing media relative to non-diffusing media. The parameter \( \beta^* \) is defined as the increased optical pathlength observed for multiple filter layers relative to a single layer, where both are the same diffusing medium.

The optical density, the signal generated by most commercial spectrophotometers, is defined as

\[ \text{OD} = -\log_{10} \left( \frac{T_s}{T_b} \right), \]

where \( T_s \) and \( T_b \) are the transmitted irradiance of the sample and blank, respectively. Defining \( \text{OD}_s \) to be the sample optical density, \( X \) to be the ratio of the volume of sample filtered to the clearance area of the filter, \( \lambda \) to be the wavelength, and \( n \) to be the number of stacks, the phytoplankton volume absorption coefficient can then be determined by equation (2):

\[ a_{\text{ph}}(\lambda) = \frac{2.3 \ \text{OD}_s(\lambda,n)}{X\beta(\lambda,n)} \]

\( \text{OD}_s(\lambda,n) \) in all cases has been corrected by subtracting the measured value at 750 nm. We thus assume that phytoplankton absorption at 750 nm is negligible.

When measurements are made on two filters, \( \beta^*(\lambda,2) \) required to convert the value to an appropriate value for a single layer is defined by the OD for two layers divided by two times the OD for one layer:

\[ \beta^*(\lambda,2) = \frac{\text{OD}_s(\lambda,2)}{2 \ \text{OD}_s(\lambda,1)}. \]
This relationship is a function of the sample OD and the specific diffusing medium containing the sample. Empirical studies were conducted to develop algorithms for estimates of $\beta^*(\lambda,2)$. The results of this analysis for Whatman GF/C filters are illustrated in Fig. 2, which is a composite of measurements on two different volumes of culture filtered so that there was a broad range of ODs. In principle $\beta^*$ cannot be less than 1. The data show the dependence of $\beta^*$ on OD, and agree well with the prediction of DUNTLLEY'S (1942) two flow radiative transfer model.

The parameter $\beta$ in equation (2) is defined to be the ratio of the absorption of cells measured on a single filter layer to the same equivalent pathlength of cells suspended in a solution of bovine serum albumin (BSA) which closely matched the refractive index of the cells. By matching the refractive index of the medium to the cells, scattering was minimized but not eliminated. The BSA technique, combined with the favorable geometry (wide acceptance angle) of our Butler vertical path spectrophotometer and use of the Shibata technique allowed accurate determination of the volume absorption coefficient for phytoplankton suspensions used to develop the correction algorithm for filters. Inspection of Figs 2 and 3 reveals that both $\beta$ and $\beta^*$ show strong dependence on the sample OD. Thus, these two parameters of optical pathlength will vary with the amount of material filtered, and will vary spectrally for individual samples as the OD varies. We assume that scattering within the sample is dominated by the filter itself, and that this scattering has no spectral dependence.

The detailed study of $\beta$ and $\beta^*$ for GF/C filters provides a convenient algorithm, which is easily implemented on a digital computer. However, much evidence now indicates that
the filtration efficiency of GF/C filters may not be sufficient to retain the picoplankton, including cyanobacteria which are now recognized as being important components of the phytoplankton (Li et al., 1983; Platt et al., 1983; Iturriaga and Mitchell, 1986). Therefore, additional techniques have been developed which allow the use of three filter types with finer particle retention capabilities than the GF/C. These include Whatman glass fiber types 934AH and GF/F, and Millipore cellulose acetate type HA. Empirical relationships between $\beta(\lambda)$ and OD($\lambda$) were assessed by filtering five different volumes of a culture of *D. tertiolecta* onto GF/C filters and each of the other three filter types. A simple plot of the OD($\lambda$) observed for the finer retention filters against the OD($\lambda$) for the GF/C revealed linear relationships with the slopes >1.0 and intercepts of 0.0. This was true for double or single layer comparisons. Figure 4 shows the results of this analysis for a single GF/F sample plotted against a single GF/C sample. Since the correlation coefficients ($r^2$) for least-squares linear fits of these data were >0.98 for 300 points in each comparison, OD data collected for various filter types can reliably be converted to the equivalent OD on a GF/C filter. Once this is done, the detailed algorithm developed for GF/C filters can be used to correct the spectra. The slopes of these relationships are higher for the finer retention filters as shown in Table 1. This is apparently due to denser weave and thicker glass fiber mats in the 934AH and G/FF, and more refractive material in the HA.

The agreement between the corrections for the different filter types is illustrated in Fig. 5. The 10-fold range of maximum OD for the uncorrected spectra is a much broader
Fig. 4. Demonstration of the linearity between the ODₜ(λ) measured for various filter types and that observed for GF/C filters. Presented here are the ODₜ(λ,1) for single layers of GF/F vs GF/C. The regression equation for 300 data points (400-700 nm) is ODGFF(λ,1) = 0.0 + 1.3 ODGFC(λ,1) r² = 0.998.

Table 1. Regression analysis of several filter types relative to Whatman GF/C. Optical density of Millipore HA, Whatman 934 AH and GF/F are the dependent variables; optical density of Whatman GF/C is the independent variable. For analyses, the intercept is 0.0

<table>
<thead>
<tr>
<th>Filter type</th>
<th>Mean slope</th>
<th>S.D.</th>
<th>Number of volumes</th>
</tr>
</thead>
<tbody>
<tr>
<td>GF/C</td>
<td>1.0</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>934 AH</td>
<td>1.2</td>
<td>0.10</td>
<td>5</td>
</tr>
<tr>
<td>GF/F</td>
<td>1.3</td>
<td>0.09</td>
<td>5</td>
</tr>
<tr>
<td>HA</td>
<td>1.5</td>
<td>0.07</td>
<td>5</td>
</tr>
</tbody>
</table>

range in sample density than one would encounter in routine analyses. The spectra are statistically indistinguishable, except in the Soret peak where the maximum and minimum values at 435 nm (for the HA and GF/C types, respectively) are significantly different (P < 0.005, n = 5). The flattest peaks were obtained with the GF/C and 934AH types which are the least diffusing, judging from the slopes in Table 1. If the measurement and reference beams are not adequately diffused by light scattering within the filter, then the benefits of the SHIBATA (1958) technique will not be fully realized unless an additional diffuser is used.

Chlorophyll a fluorescence excitation algorithm

In order to quantitatively compare fluorescence excitation spectra between different samples, several corrections are necessary. The minimum requirements include correction for variability of the spectral quantum flux of the excitation beam, the reflectivity
Chlorophyll \( a \) specific absorption and fluorescence

Fig. 5. A comparison of the correction algorithm applied to samples of \( D. \) tertiolecta filtered onto the four filter types studied. Each curve represents the mean value of the derived volume absorption coefficient \( [\alpha_{ph}(m^{-1})] \) for five different volumes filtered representing a 10-fold range in raw OD for each filter type. The curves for different filters are symbolized as follows: Millipore HA (-----); Whatman glass fiber types GF/F (-----); 934AH (---); and GF/C (-----).

and absorption of excitation energy by the sample, and reabsorption of emitted light in the fluorescent waveband. Correction for these factors provides what is defined to be the relative fluorescence excitation spectrum. Further corrections for system geometry, and absolute calibration would yield a measure of the absolute fluorescence excitation spectrum. Our system geometry and calibration procedure ensures that the latter factors remain constant for the measurements, but an absolute calibration has not been performed, so the techniques described here provide relative fluorescence excitation spectra. The fluorescence, \( F \), measured by any system can be expressed in simplest terms as

\[
F = GR\Phi_f A_e A_f.
\]  

(4)

G and R represent geometric and reflectivity factors, which are assumed to be constant. The reflectivity is defined as the fraction of quanta reflected by the sample filter in the backward direction divided by the total incident quanta. The measuring geometry \( (G) \) is constant, but \( R \) is probably not constant over all possible sample densities. Non-constancy in \( R \) is due to the fact that as OD increases, the probability for escape in the backward direction decreases. Limitations imposed by this constraint are discussed below. The fluorescent quantum yield is represented by \( \Phi_f \) while \( A_e \) and \( A_f \) are parameters for the absorption of excitation quanta and reabsorption of fluoresced quanta. General expressions which allow analysis of equation (4) have been derived by Förster (1951) who presented phenomenological equations for correction of luminescence data for both turbid and strongly absorbing samples. Many authors have applied these concepts to a variety of problems (Weber and Teale, 1957; Melhuish, 1961;
Demas and Crosby, 1971; Parker and Rees, 1962). A general summary can be found in Lipsett (1967). The product \([A_e A_f]\) in equation (4) has been stated more explicitly by Winefordner et al. (1972):

\[
A_e A_f = \left[ \int L(\lambda_e) (1 - 10^{-OD_s(\lambda_e)}) \, d\lambda_e \right] \frac{\int (1 - 10^{-OD_s(\lambda_f)}) \, d\lambda_f}{\int OD_s(\lambda_f) \, d\lambda_f},
\]

(5)

where \(L(\lambda_e)\) is the spectral irradiance quantum flux of the excitation energy and \(OD_s(\lambda_e)\) and \(OD_s(\lambda_f)\) are the optical density for the sample at the excitation and fluorescence wavelengths, respectively. The first integral in equation (5) is the absorbed irradiance, integrated over the bandwidth of the excitation source. The final ratio of integrals in equation (5) accounts for reabsorption of fluorescence within the sample and is integrated over the emission bandwidth. The values for \(F\), \(OD_s(\lambda_e)\) and \(OD_s(\lambda_f)\) are measured directly for each sample. \(L(\lambda_e)\) is constant for all samples through normalization with the rhodamine-B spectrum and the fluorescent glass standard. To compare spectra quantitatively, the measurement and correction techniques must provide linearity with variable sample volume over a practical range expected for samples. A comparison of corrections at 485 nm for \(L(485)\) only, and a full correction utilizing an analytical solution of equation (5) is provided in Fig. 6. One can see that over a narrow range corresponding to very low OD, linearity exists for a simple correction involving the excitation energy only. However, satisfactory absorption measurements at such low OD are impractical due to instrumentation noise, especially in the low absorbing yellow–green (550–650 nm) spectral region for phytoplankton. Linearity can be achieved over a broad range of

![Graph](image)

Fig. 6. A comparison of the Chl a fluorescence excitation at 485 nm corrected for the excitation energy only (x) and a full correction applying an analytical solution of equation (5) (●). The experiment was conducted over a 100-fold range in sample density determined by the volume filtered. Note the non-linearity at relatively low sample densities if the appropriate corrections for reabsorption and mean excitation quantum flux in the sample are not considered. The corrections presented here linearize the response over a broad range of sample densities.
sample density by using the fluorescence corrections presented here. For the data presented in Fig. 6, the ODs for the chlorophyll peaks at 435 and 675 ranged from 0.008 to 0.48 and 0.005 to 0.29, respectively. The non-linearity after correction which occurs at higher sample volume [OD(435) > 0.3] is believed to be caused by changes in the term R in equation (4). We did not measure R, but have used our empirical data (Fig. 6) to estimate the ODs where it becomes a significant concern. To ensure high quality

Fig. 7. Mean spectra for determinations of three different volumes bracketed by the standard error of the mean for (a) the Chl a specific absorption $a_{ph}(\lambda)$ and (b) the Chl a specific fluorescence $F^*(\lambda)$. The spectra are for Pavlova lutheri grown at 25 μEin m$^{-2}$ s$^{-1}$. 
absorption spectra, and linearity in the fluorescence correction which assumes $R$ to be constant, a working range for OD$_5$ between 0.1 and 0.3 is recommended for the peak phytoplankton absorption in the Soret band. This corresponds approximately to 1–3 μg Chl $a$ on a 25 mm filter or 0.3–1.0 μg Chl cm$^{-2}$.

Figure 7a and b illustrate the mean spectra for Chl $a$ specific absorption and fluorescence excitation [a$_a^m(\lambda)$ and $F^*(\lambda)$], respectively, bracketed by the standard error of the mean for _Pavlova lutheri_ grown at 25 μEin m$^{-2}$ s$^{-1}$. The data represent results from three separate volumes filtered corresponding to a 5-fold range of sample density. The results indicate rather good reproducibility of the absorption and fluorescence techniques described.

**RESULTS**

*Spectral changes: the effects of discreteness*

The techniques described here were applied to a study of the response of nutrient saturated batch cultures to light limitation. Absorption, fluorescence excitation and fluorescence efficiency spectra showed spectral shifts associated with increases in the relative contribution in the accessory pigment bands compared to the Chl $a$ peak in the Soret at 435 nm. Spectral summaries of the data for _P. lutheri_ and _D. tertiolecta_ are presented in Figs 8–10. Table 2 summarizes data for these species and for _Chaetoceros gracilis_. Neori et al. (1984) and Mitchell and Kiefer (1984) proposed that the ratio of the fluorescence signal in the accessory pigment bands to the signal at 435 nm can be used as an indicator of photoadaptation for field populations. For this study the F-ratio is defined to be $[F(465)+F(550)]/F(435)$, where $F(\lambda)$ is the corrected fluorescence excitation at wavelength $\lambda$. An analogous parameter, the A-ratio can be defined for absorption spectra. Inspection of Figs 8 and 9 reveals that low-light-adapted cultures have higher A- and F-ratios which are summarized in Table 2.

Analysis of the summary data in Table 2 indicates that spectral shifts are not due exclusively to increases in accessory photosynthetic pigments relative to Chl $a$ or changes in $\Phi_{r}$. The consequences of packaging the pigment molecules into discrete particles are hypothesized to be of equal or greater significance in determining the spectral shifts which are observed. Comparing the _P. lutheri_ culture growing at the highest light level to the intermediate light level, Chl $a$ and $c$ per cell increase 2.5- and 6.6-fold, respectively. The increases in the F- and A-ratios for this adaptive transition are presumed to be caused primarily by increases in accessory pigments relative to Chl $a$. However, adaptation for growth at the lowest irradiance induces a further two-fold increase in Chl $a$ per cell, while Chl $c$ per cell remains constant. In spite of this increase in the Chl $a$:Chl $c$ ratio, both the F- and A-ratios increase further. This result may be caused by increases in other accessory photosynthetic pigments which do not covary with Chl $c$. However, we hypothesize that the enhanced spectral ratios in this photoadaptive range, for this species, are caused largely by the packaging or sieve effect which governs *in vivo* absorption properties of discrete particles (Duyvends, 1956; Morel and Bricaud, 1981; Bricaud et al., 1983; Latimer, 1983; Falkowski et al., 1985; Collins et al., 1985; Dubinsky et al., 1986). This effect is expected to be more pronounced in the strongly absorbing regions of the spectrum altering the spectral ratios for fluorescence and absorption. Our hypothesis is supported by the data of Gallagher et al. (1984) who found that the ratios among Chl $a$, Chl $c$, and fucoxanthin for three clones of
Fig. 8. The photoadaptive response of the Chl $a$ specific spectral absorption coefficient $a_{ph}^s(\lambda)$ (m$^2$ mg Chl $a^{-1}$) for $P. lutheri$ (a) and $D. tertiolecta$ (b). Curves for relative light levels for each culture are symbolized as: high (---); moderate (-----); low (----). Each curve is the mean of 2 or 3 separate determinations for different volumes. The significant drop in $a_{ph}^s$ and increases in the $A$-ratio and ratios of $a_{ph}(675)/a_{ph}(435)$ at lower growth irradiance are attributable primarily to the effects of absorption in discrete particles.
Fig. 9. The photoadaptive response of the Chl a specific fluorescence excitation spectra for 683 nm emission ($F^*$) for the same samples presented in Fig. 8: P. lutheri (a), D. tertiolecta (b). The line symbolism is consistent with Fig. 8. Changes in shape and magnitude of the spectra are attributable to the effects of discreteness and intracellular reabsorption of fluorescence.

*Skeletonema costatum* behave differently for identical growth conditions with no consistent trend in ratios for high- and low-light cultures. Even though the pigment ratios did not show a clear pattern, they did observe that both total pigment per cell and fluorescence excitation by accessory pigments relative to Chl a increased at low light for each clone.
Chlorophyll a specific absorption and fluorescence excitation spectra

The hypothesized effects of discreteness are consistent with the trends in the quantitative Chl a specific absorption and fluorescence spectra in Figs 8 and 9. Fig. 8 indicates that the Chl a specific absorption in the blue [e.g. $a_{pd}^*(435) \text{m}^2 \text{mg Chl a}^{-1}$] is significantly lower at low growth irradiance compared to the higher growth irradiances. This effect is
Table 2. Pigment and optical parameters for three species during light-limited exponential growth. Units are (m⁻¹) for τₚₐₜ, (m² mg⁻¹ Chl a) for aₚₐₜ, and fluorescence (Chl a) for F*.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pavlova lutheri</th>
<th>Chaetoceros gracilis</th>
<th>Dunaliella tertiolecta</th>
</tr>
</thead>
<tbody>
<tr>
<td>μEin m⁻² s⁻¹</td>
<td>640</td>
<td>300</td>
<td>200</td>
</tr>
<tr>
<td>Chl a/c or alb</td>
<td>6.0</td>
<td>3.3</td>
<td>2.8</td>
</tr>
<tr>
<td>A-ratio</td>
<td>0.64</td>
<td>0.75</td>
<td>0.81</td>
</tr>
<tr>
<td>F-ratio</td>
<td>0.85</td>
<td>1.20</td>
<td>0.84</td>
</tr>
<tr>
<td>E-ratio</td>
<td>3.20</td>
<td>2.90</td>
<td>2.09</td>
</tr>
<tr>
<td>aₚₐₜ(675)/aₚₐₜ(435)</td>
<td>0.433</td>
<td>0.480</td>
<td>0.415</td>
</tr>
<tr>
<td>aₚₐₜ(400-700)</td>
<td>0.011</td>
<td>0.011</td>
<td>0.011</td>
</tr>
<tr>
<td>aₚₐₜ(435)</td>
<td>0.030</td>
<td>0.027</td>
<td>0.027</td>
</tr>
<tr>
<td>aₚₐₜ(675)</td>
<td>0.013</td>
<td>0.013</td>
<td>0.013</td>
</tr>
<tr>
<td>F*(400-525)</td>
<td>1.34</td>
<td>0.74</td>
<td>4.50</td>
</tr>
<tr>
<td>F*(435)</td>
<td>2.05</td>
<td>1.05</td>
<td>6.80</td>
</tr>
<tr>
<td>Relative Φ(435)</td>
<td>0.53</td>
<td>0.30</td>
<td>0.58</td>
</tr>
</tbody>
</table>

...much more pronounced in *D. tertiolecta* than in *P. lutheri*. Changes in aₚₐₜ(675) are not as large as in the blue, which is expected since the red peak is less strongly absorbing than the blue peak. Since the magnitude of absorption in the accessory pigment region from 460 to 530 nm is intermediate between the blue and red peaks, one would expect that this region will not be affected as much as the 435 nm peak. Thus, without any changes in the relative concentration of accessory pigments, increased pigment concentration per cell will lead to spectral shifts in the absorption and fluorescence excitation spectra. Indeed, for *D. tertiolecta*, no significant change in the Chl a:Chl b ratios were observed, although spectral shifts comparable to those observed for *P. lutheri* were evident.

The changes in the fluorescence excitation spectra presented in Fig. 9 are more complicated than the observations for absorption. For all species, the intermediate light level has slightly enhanced Chl a specific fluorescence excitation [F*(λ)] throughout the spectral range studied as compared to the highest light level. Two possibilities must be considered in explaining this phenomenon: increases in the ratio of PSII/PSI antennae Chl a, or increases in Φₐ. By increasing Chl a in PSII relative to PSI, a larger fraction of the chlorophyll present will be fluorescent, since PSI Chl a is considered to be non-fluorescing (BUTLER, 1978). An increase in Φₐ(λ) may also play a role in increased F*(λ); however these data are not adequate to distinguish between these two hypotheses.

At the lowest growth irradiance, a significant drop in F*(λ) is observed for all species. Again, two probable explanations exist for this observation. First, a decrease in PSII/PSI Chl a ratios could reverse the trend observed between the highest and intermediate light levels. PSI absorption may be enhanced more as cells undergo a transition from moderate to low irradiance in order to keep the flux of excitons balanced between the two photosystems. A second factor leading to the observed decrease in the specific fluorescence may be attributable to increasing intracellular reabsorption of the fluoresced light. This phenomenon, which has been shown to cause spectral shifts in fluorescence emission spectra (COLLINS et al., 1985) is expected to be increasingly important with increased Chl a per cell. Although these data cannot be used to test the significance of the first hypothesis, they are sufficient to test the second.

A model of specific fluorescence based on specific absorption

The 3-fold drop in F*(435) for *D. tertiolecta* and photoadaptive variability in spectral ratios associated with minimal changes in Chl a:Chl b ratios can be reconciled by...
examining absorption and fluorescence at the cellular level. COLLINS et al. (1985) examined the role of intracellular reabsorption of fluoresced light in modifying the spectral shape of the emission spectrum. They presented a general description for the volume integral of fluorescent flux from a cell:

\[ F(\lambda_c) = \Phi_t(\lambda_c) L(\lambda_c) Q_a(\lambda_c) \pi r^2 Q^*_a(\lambda_t) \]  

(6)

\( L(\lambda_c) \) is the excitation irradiance, \( \Phi_t(\lambda_c) \) is the fluorescence efficiency for absorbed light, and \( \pi r^2 \) is the cell’s geometric cross-section. The efficiency factor for absorption, \( Q_a \) and intracellular reabsorption \( Q^*_a \) have been defined by MOREL and BRICAUD (1981):

\[ Q_a(\lambda) = 4/3 Q^*_a(\lambda) a_{cm}(\lambda) r \]  

(7) \[ Q^*_a(\lambda) = (c_i/a_{cm}(\lambda)) a^*_m(\lambda), \]  

(8)

where \( c_i \) is the intracellular Chl a concentration and \( a_{cm}(\lambda) \) is the spectral absorption of cellular material dispersed into a theoretical suspension. By substituting equations (7) and (8) into equation (6) an expression for the chlorophyll specific fluorescence \( F^* \) at the cellular level can be represented by

\[ F^*(\lambda_c) = \left[ \Phi_t(\lambda_c)/a^*_m(\lambda_t) \right] a^*_m(\lambda_t) a^*_m(\lambda_t). \]  

(9)

where \( F^*(\lambda_c) \) is defined to be the emergent cellular fluorescence corrected for excitation energy divided by the intracellular Chl a and \( a^*_m \) is the Chl a specific absorption coefficient for cellular material dispersed into a theoretical solution:

\[ F^*(\lambda_c) = F(\lambda_c)/[L(\lambda_c)4/3\pi r^2 c_i] \]  

(10) \[ a^*_m(\lambda_t) = a_{cm}(\lambda_t)/c_i. \]  

(11)

Since absorption by cultures in the red peak centered at 675 nm is dominated by Chl a, \( a^*_m(\lambda_t) \) is assumed to be a constant. Thus equation (9) can be utilized to assess the relative importance of \( \Phi_t(\lambda_c) \) vs the product \( a^*_m(\lambda_t) \times a^*_m(\lambda_t) \) in determining the magnitude of the chlorophyll specific cellular fluorescence. Examination of Table 2 reveals that for the species studied, the apparent variability in \( \Phi_t(435) \) is a factor of 4 while the product \( a^*_m(435) \times a^*_m(675) \) varies by 10-fold. Although variations in \( \Phi_t \) are significant, in determining the magnitude of \( F^*(\lambda_c) \), one may conclude that the product of \( a^*_m(\lambda_t) \times a^*_m(\lambda_t) \) is of greater significance. Figure 11 is a plot of the relationship between the product \( a^*_m(435) \times a^*_m(675) \) and \( F^*(435) \) for the three species studied and the predicted variability based on \( a^*_m(435) \) estimated using the model embodied in equation (9). The modeled predictions assume that \( a_{cm}(435) \) is equal to \( 5 \times 10^5 \text{ m}^{-1} \) and that the ratio of \( a_{cm}(435)/a_{cm}(675) \) is 2.3 (see also BRICAUD et al., 1983). For the three species studied under light-limited growth, a significant positive trend was observed for this relationship and the model predictions provide a good fit to the observations. These results support the hypothesis that the magnitude of \( F^*(\lambda) \) is determined largely by the physical consequences of absorption in discrete particles.

As predicted by MOREL and BRICAUD (1981) and BRICAUD et al. (1983), if the \( a_{cm}(\lambda) \) and \( c_i \) for a theoretical cell suspension are held constant, while cellular diameters vary, then \( a^*_m(\lambda) \) will decrease for large cells as a consequence of the discreteness effect which effectively results in self-shading of pigment molecules on the interior of plastids, thereby decreasing pigment specific absorption efficiencies. One predicts, therefore, that \( F^* \) for
larger cells, for constant \( a_{cm}(\lambda) \) and \( c_i \), will vary inversely with the absolute size of the cell, and the relative absorption of the excitation band. The latter prediction results from a larger drop in \( a_{ph}^*(\lambda) \) in the more strongly absorbing region of the spectrum (Morel and Bricaud, 1981).

Analysis of Chl a fluorescence for size-fractionated field populations (Alpine and Cloern, 1985) and variable-sized phytoplankton in culture (Loftus et al., 1972) agree with these predictions. Using the nominal fraction or cell sizes reported by these authors, and the same assumptions regarding \( a_{cm}(\lambda) \) used to model the response of photoadaptation in Fig. 11, the response of \( F^* \) to variations in cellular diameter was investigated. The relative predictions of this model with the relative observations of Alpine and Cloern (1985) and Loftus et al. (1972) are presented in Table 3. The predicted range of variability in \( F^* \) agrees well with observations. Although one must assume that \( a_{cm}(435) \) is constant for lack of the appropriate data, it certainly is not constant. The fact that \( F^*(435) \) varies 6-fold for \( D. \) tertiolecta with presumably little change in cell diameter is attributable directly to large variability in \( a_{cm}(435) \) and \( a_{cm}(675) \). Nevertheless, the overall agreement is very encouraging. Based on theory, the observed variability induced by photoadaptation, and the observed variability attributed to size, we believe that the concepts embodied in equation (9) should be carefully considered in efforts to interpret the wide-ranging values for \( F^* \) reported in the literature (Loftus et al., 1972; Kiefer, 1973a; Alpine and Cloern, 1985). More detailed laboratory studies should be conducted in order to test the hypotheses presented here.

Fluorescence efficiency spectra

Compared to the absorption and fluorescence excitation spectra, the fluorescence efficiency spectra show smaller and inconsistent changes in shape depending upon

![Fig. 11. The relationship between \( F^*(435) \) and the product \( a_{ph}^*(435) \times a_{ph}^*(675) \) for the three species studied. The experimental observations are symbolized as (□). The solid line is the prediction of the model embodied in equation (9).](image-url)
Table 3. Comparison of model prediction of Chl a specific fluorescence using equation (9) with the field data of ALPINE and CLOERN (1985), and the laboratory data of LOFTUS et al. (1972). Estimates of $p'$ assume spherical cells and $a_m(435)$ of $2 \times 10^{-5}$ as approximated by MOREL and BRICAUD (1981)

<table>
<thead>
<tr>
<th>Size fractionation data of ALPINE and CLOERN (1985)</th>
<th>Assumed diameter (µm)</th>
<th>Estimated $p'(435)$</th>
<th>Relative fluorescence per Chl a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nominal size fraction</td>
<td></td>
<td></td>
<td>Model prediction</td>
</tr>
<tr>
<td>&lt;5</td>
<td>4</td>
<td>1</td>
<td>6.0</td>
</tr>
<tr>
<td>5-22</td>
<td>14</td>
<td>3</td>
<td>2.0</td>
</tr>
<tr>
<td>&gt;22</td>
<td>30</td>
<td>6</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Species studied by LOFTUS et al. (1972)

| Unidentified nanoplankter                     | 2                      | 0.4                 | 15.0              | 11.0     |
| Monochrysis sp.                              | 6                      | 1.2                 | 10.0              | 5.0      |
| Dunaliella tertiolecta                        | 6                      | 1.2                 | 10.0              | 4.0      |
| Fragillaria sp.                               | 10                     | 2.0                 | 7.2               | 5.0      |
| Phaeodactylum tricornutum                     | 10                     | 2.0                 | 7.2               | 4.7      |
| Gymnodymium splendens                         | 35                     | 7.5                 | 2.0               | 2.3      |
| Gymnodymium nelsoni                           | 48                     | 9.5                 | 1.0               | 1.0      |

photoadaptation. Whereas the $F$- and $A$-ratios exhibit increases for low-light-adapted cells, the analogous fluorescence efficiency ratio ($E$-ratio) does not show a similar pattern (Fig. 10, Table 2). For the three species studied, the $A$- and $F$-ratios increase between the highest and lowest light levels. The $E$-ratio decreases by 25% for $P. lutheri$ and by 10% for $C. gracilis$, while no pattern was observed for $D. tertiolecta$. The opposing trends between the $E$-ratio and both the $A$- and $F$-ratios may be attributed to the fraction of light absorbed which is coupled to the fluorescing mechanism of photosystem II (PSII). The observations for all ratios may be explained by a simple hypothesis where changes in the $A$- and $F$-ratios are determined primarily by the packaging effect and spectral augmentation of the relative magnitude at longer wavelengths by accessory pigments. These two effects will increase these ratios for low-light-adapted cells. The $E$-ratio is proposed to be determined primarily by the ratio of absorption by pigments coupled to PSII to pigments which are either coupled to PSI or are not coupled to the photosynthetic light harvesting system. Various carotenoid pigments have been proposed as photoprotective pigments which absorb light energy but do not transfer the energy to the photosystems (KRINSKY, 1968; MATHEWS-ROTH and KRINSKY, 1970). These pigments have absorption maxima below 500 nm (RABINOWITCH and GODVINDJEE, 1969).

If changes in the $A$- and $F$-ratios are both due primarily to the package effect, and the pigment complement remains constant while intracellular concentrations vary, then the changes will cancel each other in the calculation of the fluorescence efficiency, resulting in a flat efficiency spectrum. Two hypotheses, which may act simultaneously, are proposed which would be consistent with a drop in the $E$-ratio at lower growth irradiance. First, if the ratio of photosynthetic to non-photosynthetic or protective pigments increased at lower irradiance, then one would predict that the $E$-ratio would decrease, since absorption by photoprotective pigments occurs below 500 nm. Thus as
the fraction of light absorbed by non-photosynthetic pigments decreases at lower growth irradiance, one expects an increased probability that quanta absorbed in the blue will be coupled to the fluorescence system. Since the ratios reflect blue–green and green relative to the blue, the $E$-ratio would drop. Second, if low-light adaptation is achieved primarily by augmentation of PSII, then the fraction of blue light absorbed by Chl $a$ in PSII and coupled to the fluorescing mechanism will increase. In either case, the increased probability of quanta absorbed being coupled to fluorescence will be spectrally biased, and inversely proportional to the absorption spectra of either non-photosynthetic pigments in the case of the first hypothesis, or Chl $a$ in the second.

These two explanations are not mutually exclusive, and may occur simultaneously, or in stages. For example, photoprotective pigments might be lost in a transition from high to moderate irradiance, while significant augmentation of photosynthetic light harvesting pigments might not occur until lower growth irradiances are attained. The data of the current study are not sufficient to resolve which alternative hypothesis is valid for the species studied, since measurement of PSI and PSII in addition to a more detailed pigment study coupled with a complete optical analysis would be required.

**DISCUSSION**

A critical question regarding these techniques is whether this empirical approach to correction of complex optical phenomenon can truly be effective in providing quantitative results. This is of particular concern for measurement of marine particulate absorption coefficients since no simple alternative is currently available for such measurements in field studies. Furthermore, the original technique of KIEFER and SOOHOO (1982) has come to be applied to field studies recently (LEWIS et al., 1985; CARDER and STEWARD, 1985; KISHINO et al., 1985, 1986), often without consideration for the non-constancy of $\beta$ as discussed by MITCHELL and KIEFER (1984), or for the significant effects upon $\beta$ that result from different filter types as discussed here. The empirical corrections for measurements made on filters have been developed by direct comparison with measurements using the SHIBATA (1958) technique on concentrated suspensions. A comparative survey of reported literature values for $a_{ph}^*(\lambda)$ and the data presented here for cultures are compiled in Table 4. Unfortunately, there is no extensive literature on $a_{ph}^*(\lambda)$, since careful consideration of measurement geometry and technique is required. Nevertheless, the data of MOREL and BRICAUD (1981), BRICAUD et al. (1983) and DUBINSKY et al. (1986) provide the most detailed analyses using traditional techniques for comparison with the results presented here. Cell sizes, intracellular Chl $a$ and estimates of $Q_a$ were also tabulated, where possible.

Of obvious significance is the fact that $a_{ph}^*(\lambda)$ in the Soret peak (~435–440 nm) varies by at least a factor of 4 for cultures. Thus, it is not justified, as is common practice, to assume $a_{ph}^*(\lambda)$ to be constant for such applications as calculation of photosynthetic quantum yields (BANNISTER, 1974, 1979; TYLER, 1975; DUBINSKY and BERMAN, 1976; MOREL, 1978). The sources of variability in $a_{ph}^*(\lambda)$ are many, in theory. KIEFER et al. (1979) have reported that senescence of batch cultures, or nutrient limitation in continuous culture, are important physiological considerations. Starting with the optical theory of absorption by particles in non-absorbing media, MOREL and BRICAUD (1981) proposed that the effect of “discreteness” explains why the Beer–Lambert law is not appropriate for absorption by suspensions of unicellular algae. They studied a variety of
species grown at relatively high light levels and concluded that inter-specific variables including cell size and cellular pigmentation cause significant changes in $a_m^a(\lambda)$. The data of Dubinsky et al. (1986) and this report illustrate that growth irradiance for nutrient-saturated cells caused significant changes in $a_m^a(\lambda)$ which are manifestations of discreteness caused by variations in intracellular pigmentation.

This phenomenon is general since it is well documented that light limitation results in increased cellular pigmentation (Laws and Bannister, 1980; Falkowski et al., 1985).

Table 4. Summary of chlorophyll specific and cellular absorption for various species in unialgal culture from data reported in the literature, and from the present report

<table>
<thead>
<tr>
<th>Citation and species</th>
<th>Ein m$^{-2}$ day$^{-1}$</th>
<th>$a_m^a(435-440)$ m$^{-2}$ mg$^{-1}$ Chl a</th>
<th>Cellular diameter m $\times$ 10$^{-6}$</th>
<th>pg Chl a cell$^{-1}$</th>
<th>Intracellular mg Chl a m$^{-3}$ $\times$ 10$^6$</th>
<th>$Q_a(435)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bannister (1979)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorella pyrenoidosa</td>
<td>33</td>
<td>0.031</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bricaud et al. (1983)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hymenomonas elongata</td>
<td>28</td>
<td>0.03</td>
<td>13.5</td>
<td>3.8</td>
<td>2.94</td>
<td>0.79</td>
</tr>
<tr>
<td>Tetraselmis maculata</td>
<td>28</td>
<td>0.04</td>
<td>8.5</td>
<td>0.52</td>
<td>1.62</td>
<td>0.36</td>
</tr>
<tr>
<td>Platymonas sp.</td>
<td>28</td>
<td>0.045</td>
<td>6.7</td>
<td>0.27</td>
<td>1.87</td>
<td>0.37</td>
</tr>
<tr>
<td>Coccolithus huxleyi</td>
<td>28</td>
<td>0.085</td>
<td>3.4</td>
<td>0.045</td>
<td>1.14</td>
<td>0.22</td>
</tr>
<tr>
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<td></td>
<td></td>
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<tr>
<td>Proocentrum micans</td>
<td>53</td>
<td>0.062</td>
<td>27.0</td>
<td>2.8</td>
<td>0.27</td>
<td>0.3</td>
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<tr>
<td>Proocentrum micans</td>
<td>6</td>
<td>0.028</td>
<td>27.0</td>
<td>6.6</td>
<td>0.64</td>
<td>0.32</td>
</tr>
<tr>
<td>Thalassiosira weisflogii</td>
<td>52</td>
<td>0.025</td>
<td>12.0</td>
<td>5.1</td>
<td>5.6</td>
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<tr>
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<td>0.014</td>
<td>12.0</td>
<td>13.7</td>
<td>15.1</td>
<td>1.7</td>
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<td>52</td>
<td>0.048</td>
<td>6.0</td>
<td>0.12</td>
<td>1.1</td>
<td>0.21</td>
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<td>Isochrysis galbana</td>
<td>2.8</td>
<td>0.03</td>
<td>6.0</td>
<td>0.25</td>
<td>2.2</td>
<td>0.26</td>
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<td>Kiefer et al. (1979)</td>
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<tr>
<td>Thalassiosira pseudonana</td>
<td>17</td>
<td>0.04-0.10</td>
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<td></td>
<td></td>
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<tr>
<td>Pavlova lutheri</td>
<td>–</td>
<td>0.04-0.07</td>
<td></td>
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<td>Kirk (1975)</td>
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<td>0.78</td>
<td>2.9</td>
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<tr>
<td>Mitchell et al. (1984)</td>
<td></td>
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<tr>
<td>Pavlova lutheri</td>
<td>35</td>
<td>0.04</td>
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<td>Morel and Bricaud (1981)</td>
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<tr>
<td>Platymonas suecia</td>
<td>35</td>
<td>0.03</td>
<td>6.0</td>
<td>0.54</td>
<td>4.5</td>
<td>0.65</td>
</tr>
<tr>
<td>Coccolithus huxleyi</td>
<td>35</td>
<td>0.09</td>
<td>3.8</td>
<td>0.031</td>
<td>1.1</td>
<td>0.25</td>
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<td>26.5</td>
<td>1.05</td>
<td>0.11</td>
<td>0.10</td>
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<td>Present report</td>
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<td></td>
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</tr>
<tr>
<td>Pavlova lutheri</td>
<td>55</td>
<td>0.03</td>
<td>6.0</td>
<td>0.14</td>
<td>1.25</td>
<td>0.15</td>
</tr>
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<td>6.0</td>
<td>0.35</td>
<td>3.15</td>
<td>0.31</td>
</tr>
<tr>
<td>Pavlova lutheri</td>
<td>5</td>
<td>0.023</td>
<td>6.0</td>
<td>0.39</td>
<td>3.5</td>
<td>0.32</td>
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<tr>
<td>Pavlova lutheri</td>
<td>3</td>
<td>0.017</td>
<td>6.0</td>
<td>0.7</td>
<td>6.25</td>
<td>0.64</td>
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<td>21</td>
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<td>0.63</td>
<td>8.5</td>
<td>0.67</td>
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<tr>
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<td>5.7</td>
<td>1.7</td>
<td>17.5</td>
<td>1.2</td>
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<td>9</td>
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<td></td>
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<td>Chaetoceros gracilis</td>
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<td>0.019</td>
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<td>2</td>
<td>0.021</td>
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</tbody>
</table>
The consequences for phytoplankton ecology, particularly in deep chlorophyll maxima, may be significant. Since much evidence now indicates that the deep chlorophyll maxima in the oceans results from increased cellular pigmentation rather than increased cell concentrations (Kiefer et al., 1976; Beers et al., 1982), one can expect that the actual light harvesting capability of the cells in these maxima is not directly proportional to the observed increases in the Chl $a$ concentrations of vertical profiles. The data presented here indicate that fully shade-adapted cells may have chlorophyll specific absorption coefficients 4 times lower than their high-light-adapted counterparts. This phenomenon will be of less significance for small cells, including picoplankton. There may be an evolutionary selective advantage for small phytoplankton in the persistent deep chlorophyll maxima of open oceans.

The results presented for $a_{ph}$ based on quantitative corrections for analysis on filters fall in the range of values obtained by the Shibata (1958) technique. This validation for cultures justifies the application of these corrections to particle absorption in the field. Although the non-constancy of $\beta$ with sample density and filter type poses analytical problems, the general availability of digital computers makes the additional computational needs relatively insignificant. The techniques, properly applied, still offer the easiest and most accurate methods for direct determination of the particulate absorption coefficient $a_p(\lambda)$ for dilute suspensions of marine particles. One must be aware, however, that $a_{ph}(\lambda)$ will contribute a variable fraction to total particulate absorption, since significant detrital absorption is present in field samples (Kiefer and Soohoo, 1982; Mitchell, 1987; Mitchell and Kiefer, 1988).

The increasing application of in vivo fluorescence in biological oceanography requires a continuing effort to interpret the results. Kiefer (1973a,b) has discussed a variety of problems related to the direct extrapolation of in vivo fluorescence to estimates of Chl $a$ concentrations. Loftus et al. (1972) and Alpine and Cloern (1985) have presented evidence that cell size may influence $F^*$ in vivo. The strong dependence of $F^*$ on intracellular reabsorption by Chl $a$ concentrations which is demonstrated here must be considered as well. The extent of intracellular reabsorption is dependent on cell size which determines the pathlength for escape of fluoresced quanta and intracellular Chl $a$ concentration. Use of Chl $a$ fluorescence to study phytoplankton dynamics requires that the data be interpreted in the context of the physical as well as the physiological factors which control and modify the signals.

Acknowledgements—We wish to express our gratitude to D. K. Clark of the National Oceanic and Atmospheric Administration and M. Blizard of the Office of Naval Research who sponsored this work.

REFERENCES


Duyssens L. N. (1956) The flattening of the absorption spectrum of suspensions, as compared to that of solutions. *Biochimica et Biophysica Acta*, 19, 1–12.


