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# Problems in the assessment of the package effect in five small phytoplankters

**B. A. Osborne** [1] **and R. J. Geider** [2]

[1] **Department of Botany, University College Dublin, Belfield, Dublin 4, Ireland**
[2] **College of Marine Studies, University of Delaware, Lewes, Delaware 199958-1298, USA**

## Abstract

The magnitude of the package effect in five small phytoplankters [*Thalassiosira* sp., Clone 2601 (an unidentified eucaryote), *Nannochloris atomus, Synechococcus* 'Syn' and *Synechococcus* WH 7803] was assessed by comparison of the absorption spectra of intact and disrupted cells. The package effect was considerably reduced with reductions in cell size and this was broadly in agreement with theoretical predictions based on Mie theory. However, the quantitative assessment of the package effect is confounded by an inability to assign attenuation (apparent absorption) measurements at $\lambda = 750$ nm to either scattering *or* absorption. The magnitude of the apparent absorption at $\lambda = 750$ nm was greatest with the smallest picoplankton species examined, and was reduced, but not eliminated, after cell disruption. Whilst the apparent absorption at $\lambda = 750$ nm is commonly thought to be due to residual scattering losses, the available evidence does not exclude the possibility that this may be due in part to absorption by cells or cell constituents and this requires further examination. Although these difficulties are particularly evident with the small picoplankton species, there is no reason to expect that they will not complicate the assessment of the package effect in larger phytoplankton cells.

## Introduction

The packaging of light-absorbing pigments within organelles or cells reduces the amount of light that can be absorbed relative to the same quantity of pigment in solution. Absorption spectra of intact cells are normally reduced at the peaks of maximum absorption compared to the same quantity of extracted pigment (Duysens 1956, Kirk 1975a, b, 1976, Geider and Osborne 1987). Theoretical calculations based on in vivo spectra indicate that absorption can be reduced by as much as 70% at the absorption maxima of chlorophyll *a* (Morel and Bricaud 1981, Bricaud et al. 1983, Latimer 1983), although the magnitude of this effect has not been well characterized in a range of unicells of different sizes (but see Sathyendranath et al. 1987). The reduced absorption by pigments contained within particles is referred to as the package effect (≡ sieve effect). Theoretical predictions have indicated that the magnitude of the package effect is dependent on cell size and intracellular pigment concentration (Duysens 1956, Kirk 1975a, b, 1976, Morel and Bricaud 1981). However, these theoretical estimates of the package effect do not take into account inhomogeneities in intracellular pigment distribution caused by the localization of pigments within organelles, or give detailed explanations of the influence of variations in cell shape. The complexities and uncertainties encountered in extending theoretical treatments to complicated cell shapes and intracellular pigment distributions argues for an experimental approach to this problem. Recently, we have used a technique, initially proposed by Das et al. (1967), to assess the magnitude of the package effect by comparing the absorption spectra of intact and disrupted cells (Geider and Osborne 1987). In the present publication we have extended these investigations to examine the significance of the package effect in a number of small phytoplankton cells.

Recent evidence indicates that the photosynthetic picoplankton (< 2 μm diam) contribute significantly to phytoplankton biomass and primary production in many aquatic ecosystems (Li et al. 1983, Platt et al. 1983, Takahashi and Bienfang 1983, Fahnenstiel et al. 1986). The success of picoplankton may be related, in part, to energetic advantages associated with a small cell size, including a reduced package effect (Raven 1984, 1986). Chlorophyll *a*-specific absorption coefficients of these small unicells may be significantly greater than those of larger cells because of a reduction in the magnitude of the package effect, indicating a more efficient utilization of the light-harvesting pigments by picoplankton in comparison to the larger phytoplankton. In this paper we provide direct estimates of the extent of the package effect in five small phytoplankton cells, including two picoplankters. Complications in the assessment of the pack-

age effect are discussed in detail, including the significance of attenuation measurements and scattering corrections at wavelengths greater than $\lambda = 750$ nm.

## Materials and methods

The phytoplankton species examined in this study were the diatom *Thalassiosira* sp. (Strain M64 supplied by Professor F. E. Round, Bristol University), the chlorophyte *Nannochloris atomus* (supplied by Mr. M. Turner, Scottish Marine Biological Association), an unidentified eucaryote (Clone 2601 supplied by Professor P. J. Syrett, University of Swansea), and two *Synechococcus* clones ['Syn' and WH7803 (formerly DC2)] supplied by Professor Carr, University of Warwick). Cells were maintained in exponential growth in batch cultures as described previously for *Thalassiosira* sp. (Geider and Osborne 1987).

Cells of the three eucaryotic species were concentrated by centrifugation at $2\,000 \times g$ in a Sorval RT6000 refrigerated centrifuge operated at 10 °C, whereas the two *Synechococcus* strains were concentrated using a Sorval RC-5 refrigerated centrifuge at $5\,000 \times g$ for 10 min at 10 °C. Aliquots of the concentrated cell suspension were preserved in 2% glutaraldehyde for cell counts and measurements of cell dimensions. The concentrations of the larger, eucaryotic cells were determined from quadruplicate counts of about 200 cells each, using an improved Neubauer haemacytometer, whilst the concentrations of the two *Synechococcus* strains were determined from counts using epifluourescence microscopy of cells concentrated onto 0.2 $\mu$m pore-size Nuclepore filters and stained with a fluorescent compound (Sherr and Sherr 1983). Cell size-distributions (Table 1) for all species were determined from photographic enlargements of stained cells. Cell diameters and geometric cross-sections were determined assuming the cell shape to be that of an oblate spheroid. The size of *Thalassiosira* sp. obtained from epifluorescence microscopic examination compared favourably with that reported previously using transmitted light microscopy (Geider and Osborne 1987) (see present Table 1).

Cell disruption was achieved by passing a frozen suspension (−40 °C) through an X-press pressure cell (L.K.B.-Biotec) as described previously (Geider and Osborne 1987). Disrupted cell suspensions were stored on ice until optical measurements were made. A 15 s burst of sonication (Kerry Ultasonics Ltd.) was used to disperse aggregates in the thawed suspension immediately prior to optical measurements. Microscopic investigations indicated that there were no intact cells left after these treatments. Absorption measurements on intact and disrupted cells were made using a Pye-Unicam ultraviolet recording spectrophotometer (Pye-Unicam, SP8-100) equipped with an integrating sphere (Pye-Unicam, diffuse reflection accessory) as described previously (Geider and Osborne 1987). Absorbance spectra were digitized at 4 to 10 nm intervals between 400 and 750 nm for determination of absorption coefficients and related optical parameters. Absorbance spectra for extracted pigments were determined on aliquots of the disrupted cell suspension which had been extracted in 90% acetone (Geider and Osborne 1987). Chlorophyll *a* concentration was calculated from the equations of Jeffrey and Humphrey (1975).

Measurements of attenuation were made using a similar technique to that described by Bricaud et al. (1983), using the SP8-100 spectrophotometer with either narrow (half-angle of 0.4°) or wide (half-angle of 8°) acceptance angles for collecting attenuated light. Total scattering was determined from attenuation-absorption measurements.

### Calculation of optical parameters

Absorption coefficients were determined from:

$$a = 2.303\ A/0.002, \tag{1}$$

where $a$ is the absorption coefficient ($m^{-1}$), $A$ is the absorbance, and 0.002 is the pathlength (m). Specific-absorption coefficients were determined from the following:

$$a^* = a/C, \tag{2}$$

where $a^*$ is the chlorophyll *a*-specific absorption coefficient ($m^2\ mg^{-1}$) and $C$ is the chlorophyll *a* concentration ($mg\ m^{-3}$).

Intracellular chlorophyll *a* concentrations can be calculated from:

$$c_i = C/Nv, \tag{3}$$

where $c_i$ is the intracellular chlorophyll *a* concentration ($kg\ m^{-3}$), $N$ is the number of cells per unit volume, $v$ is the average cell volume ($m^3$) and $v = \pi d^3/6$, where $d$ is the cell diameter (m).

Absorption cross-sections were calculated from:

$$s Q_a = a/N, \tag{4}$$

where $s$ is the geometric cross-section ($m^2$), $s Q_a$ is the absorption cross-section ($m^2$). For a sphere $s = d2/4$. $Q_a$ is the efficiency factor for absorption (dimensionless).

## Results

Cell size-distributions for the five phytoplankters are given in Table 1. These cover a five-fold range in cell diameter, from the two small *Synechococcus* 'Syn' and WH7803 strains (<2 $\mu$m diam) to the larger *Thalassiosira* species (Table 1). Cell numbers varied by over two orders of magnitude (Table 2). Chlorophyll *a* content varied from 0.01 to $0.02 \times 10^{-7}$ $\mu$g cell$^{-1}$ in the *Synechococcus* clones to $4.0 \times 10^{-7}$ $\mu$g cell$^{-1}$ in *Thalassiosira* sp. (Table 2).

Although cell disruption resulted in an increase in absorption for the larger species (Fig. 1) after adjusting for the baseline correction at $\lambda = 750$ nm, similar observations were not found with the two smaller, *Synechococcus* clones (Fig. 2). With these, cell disruption resulted in a lower absorption throughout the visible spectrum (Fig. 2), if we assume that the apparent absorption at $\lambda = 750$ nm represents

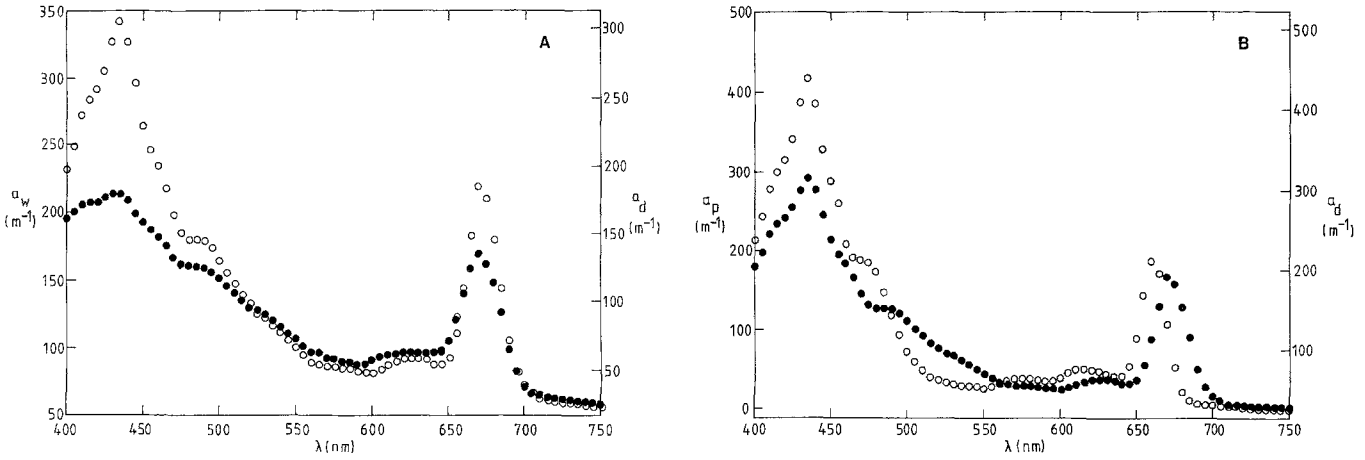


**Fig. 1.** *Thalassiosira* sp. Absorption spectra of (A) intact ($a_w$, •) and disrupted ($a_d$, ○) cells, and (B) disrupted cells ($a_d$, •) and acetone extracts ($a_p$, ○). Note difference in scales on left- and right-hand ordinates in this figure and in Figs. 2 and 4 is due to an offset accounting for the difference in measurements obtained at $\lambda = 750$ nm

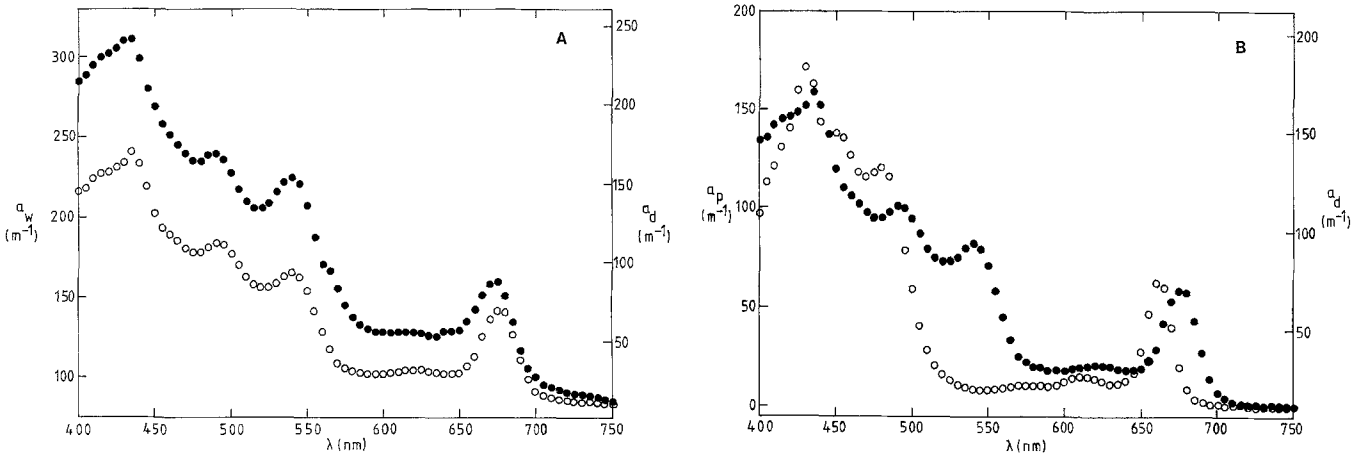


**Fig. 2.** *Synechococcus* sp. WH7803. Absorption spectra of (A) intact ($a_w$, •) and disrupted ($a_d$, ○) cells, and (B) disrupted cells ($a_d$, •) and acetone extracts ($a_p$, ○)

**Table 1.** Cell size-distributions of five phytoplankter cultures used for optical measurements. Asterisks (*) indicate data from transmitted light microscopy (see Geider and Osborne 1987); all other estimations determined using epifluorescence microscopy

| Species or clone | No. of cells | Cell diameters and statistical parameters (μm) | | | | |
|---|---|---|---|---|---|---|
| | | min. | max. | $\bar{x}$ | (SD) | median |
| *Thalassiosira* sp.* | 51* | 3.55* | 6.39* | 5.02* | (0.68*) | 5.15* |
| *Thalassiosira* sp. (Strain M64) (Bacillariophyta) | 83 | 3.96 | 6.58 | 5.14 | (0.49) | 5.18 |
| Clone 2601 (unidentified eucaryote) | 103 | 3.24 | 5.45 | 4.11 | (0.42) | 4.08 |
| *Nannochloris atomus* (Chlorophyta) | 100 | 2.70 | 4.62 | 3.66 | (0.43) | 3.65 |
| *Synechococcus* 'Syn' (Cyanobacteria) | 176 | 1.00 | 1.94 | 1.47 | (0.18) | 1.47 |
| *Synechococcus* WH7803 (Cyanobacteria) | 135 | 0.93 | 1.57 | 1.21 | (0.14) | 1.23 |

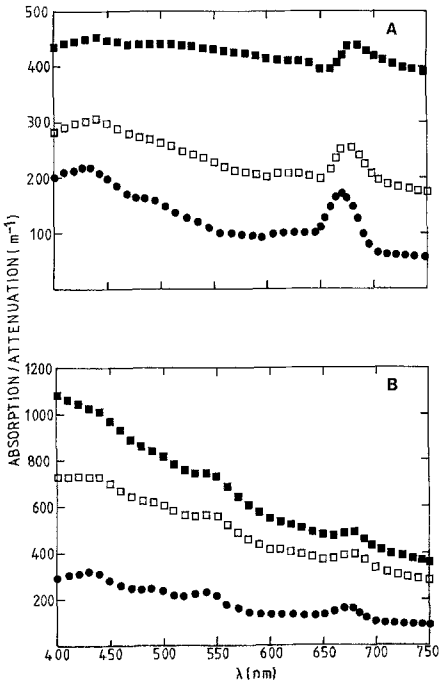


**Fig. 3.** *Thalassiosira* sp. (A) and *Synechococcus* WH7803 (B). Comparison of narrow (□)- or wide-angle (■) attenuation measurements with absorption spectra (●)

**Table 2.** Culture characteristics of the five phytoplankters. *N*: cell number; C: chlorophyll *a* concentration

| Species or clone | *N* ($10^8$ cm$^{-3}$) | C ($\mu$g cm$^{-3}$) | C:*N* ($10^{-7}$ $\mu$g cell$^{-1}$) |
|---|---|---|---|
| *Thalassiosira* sp. | 0.23 | 9.2 | 4.0 |
| Clone 2601 | 0.36 | 4.2 | 1.2 |
| *Nannochloris atomus* | 1.06 | 9.7 | 0.9 |
| *Synechococcus* 'Syn' | 14.0 | 2.1 | 0.02 |
| *Synechococcus* WH7803 | 32.0 | 3.1 | 0.01 |

a wavelength independent, constant "residual" scattering. The extent of the difference in absorption between whole cells and disrupted cells was wavelength-dependent, with larger differences at shorter wavelengths (Fig. 2).

In order to try to account for the differences in absorption between intact and disrupted cells, we assumed that the differences were due to scattering losses which increased with decreases in wavelength. We assumed also that the value at $\lambda = 750$ nm was due to residual scattering and that the increase in scattering from $\lambda = 700$ to 400 nm scaled with measurements of attenuation-absorption = scattering. For scattering, values qualitatively similar to those reported by Bricaud et al. (1983) and Morel and Bricaud (1986) were obtained. Quantitative agreement with the results of Bricaud et al. (1983) would not, however, be expected, as the smallest collection half-angle that we used (0.4°) was nearly two-fold greater than the acceptance angle used by these workers (0.25°). Our experimental system would still be expected to collect significant amounts of scattered light.

The larger *Thalassiosira* sp. had a flat attenuation spectra between $\lambda = 400$ to 700 nm, whilst the small *Synechococcus* clones showed a marked increase in attenuation as the wavelength decreased (Fig. 3; see also Bricaud et al. 1983). Correction of the in vivo absorption spectra of the *Synechococcus* clones using the wavelength-dependent scattering correction obtained from attenuation and absorption measurements and scaling this with either narrow (0.4°)- or wide (8°)-angle determinations are shown in Fig. 4. With both *Synechococcus* clones this wavelength-dependent correction improves the correspondence between absorption spectra of intact and disrupted cells. With the narrow-angle scattering correction a significant package effect is evident in the blue region of the spectrum, whilst there is little effect in the red $\lambda = 675$ nm (Fig. 4A, C). In contrast, with the wide-angle scattering correction there is little evidence for a significant package effect in the blue (Fig. 4B, D).

Optical parameters derived from spectral measurements of absorption ($\lambda = 400$ to 750 nm) of intact cells, disrupted, cells, or 90% acetone extracts are shown in Tables 3–5. Estimates of the optical parameters are based on either uncorrected or corrected spectra (Table 3), where uncorrected spectra refer to the raw data and corrected spectra refer to data in which the apparent absorption at $\lambda = 750$ nm has been subtracted from all spectral values between $\lambda = 400$ nm and 700 nm. This correction is commonly made to account for photons that have been scattered out of the collection angle of the detection system used for absorption measurements where the apparent absorption at $\lambda = 750$ nm is assumed to result solely from scattering. The magnitude of the correction at $\lambda = 750$ nm was variable. For the larger *Thalassiosira* sp., the value at $\lambda = 750$ nm was approximately 30% of that at $\lambda = 675$ nm, whilst for the smaller *Synechococcus* clones this was about 50% of that at $\lambda = 675$ nm (Table 3). After cell disruption, the value at $\lambda = 750$ nm was reduced by more than two-fold in the large cells and nearly seven-fold in the small cells (Table 3).

Uncorrected measurements show higher absorption at $\lambda = 675$ nm. The ratio between the absorption coefficients of intact cells ($a_w$) with those of disrupted cells ($a_d$) based on uncorrected measurements was greater than 1.0 for all but the larger *Thalassiosira* sp. (Table 3).

A size-dependency of the ratio of $a_w : a_d$ is evident in both uncorrected and corrected measurements. An increase in $a_w : a_d$ was found with a reduction in cell diameter (Table 3). After subtraction of the values found at $\lambda = 750$ nm from in vivo measurements, only the two *Synechococcus* clones had $a_w : a_d$ values $> 1.0$ (Table 3).

Comparison of measurements of $a_d$ with the absorption coefficients of cells extracted in acetone ($a_p$) indicates a lack of agreement between $a_d$ and $a_p$ (Table 3, see also Fig. 1). At $\lambda = 675$ nm, uncorrected measurements consistently exceed $a_p$, whilst corrected values are less than $a_p$ (Table 3). Corrected values for $a_d$ were 5 to 10% less than measurements of $a_p$. A similar pattern was evident in comparisons of chlorophyll *a*-specific absorption coefficients of disrupted cells

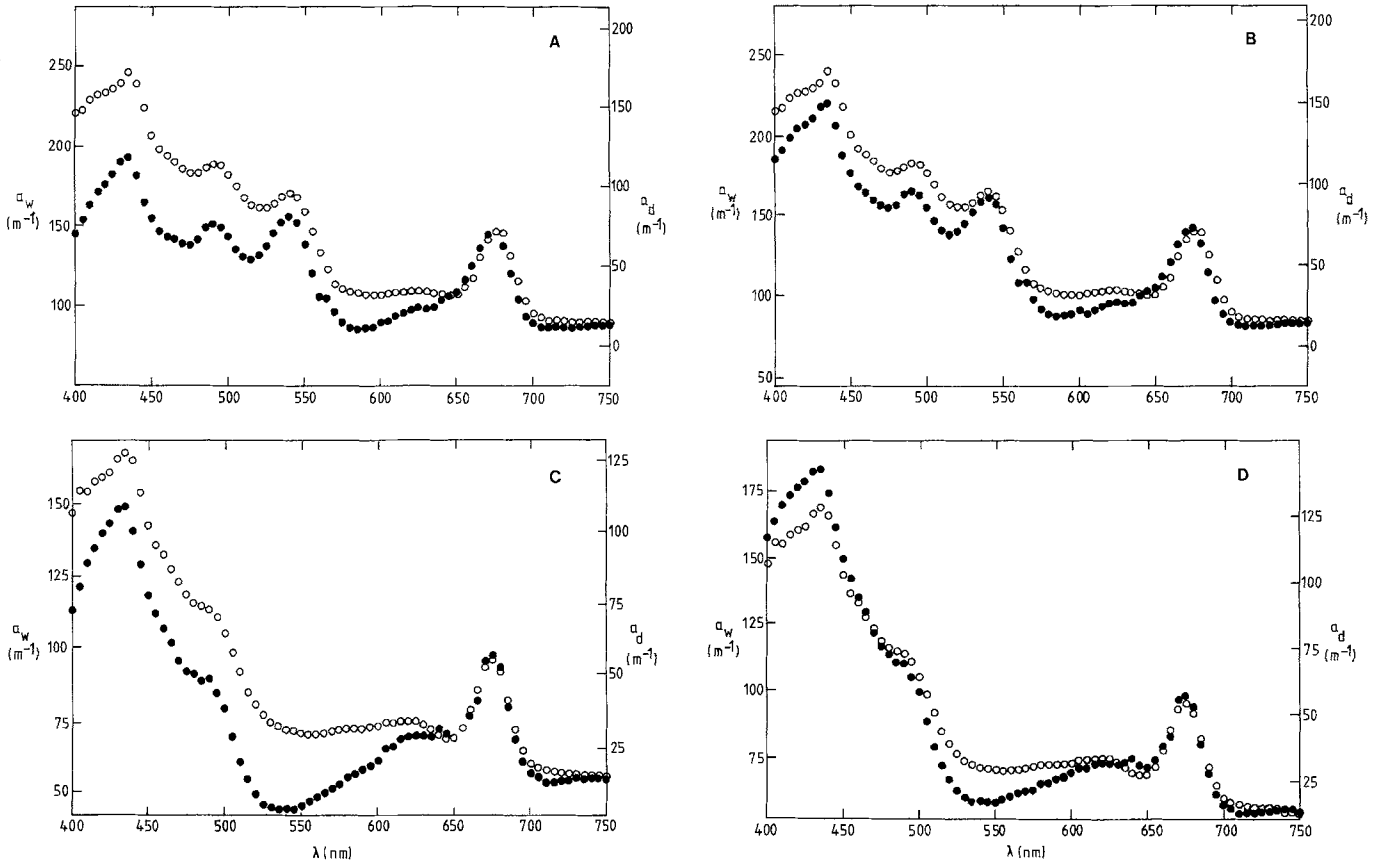


**Fig. 4.** *Synechococcus* WH7803 (A, B) and *Synechococcus* 'Syn' (C, D). Comparison of intact ($a_w$, •) and disrupted ($a_d$, ○) cell absorption spectra using either narrow (A, C)- or wide-angle (B, D) correction measurements

**Table 3.** Uncorrected and corrected absorption coefficients ($m^{-1}$) at $\lambda = 750$ or 675 nm for intact cells ($a_w$), disrupted cells ($a_d$) and acetone extracts ($a_p$) for the five phytoplankters. Corrected absorption coefficients were obtained by subtracting values obtained at $\lambda = 750$ nm from uncorrected measurements at $\lambda = 675$ nm. $d$: cell diameter ($\mu$m)

| Species or clone | $d$ | Uncorrected | | | | | Corrected | | | Acetone extracts |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 750 nm | | 675 nm | | 675 nm | 675 nm | | 675 nm | |
| | | $a_w$ | $a_d$ | $a_w$ | $a_d$ | $a_w$:$a_d$ | $a_w$ | $a_d$ | $a_w$:$a_d$ | $a_p$ |
| *Thalassiosira* sp. | 5.15 | 56.4 | 23.6 | 172.0 | 190.0 | 0.905 | 115.6 | 166.4 | 0.695 | 186.0 |
| Clone 2601 | 4.08 | 54.6 | 20.6 | 128.0 | 93.0 | 1.376 | 72.4 | 73.4 | 0.986 | 85.0 |
| *Nannochloris atomus* | 3.65 | 75.0 | 34.4 | 244.0 | 214.0 | 1.140 | 169.0 | 179.6 | 0.941 | 196.0 |
| *Synechococcus* 'Syn' | 1.47 | 54.0 | 13.1 | 109.0 | 53.0 | 2.06 | 55.0 | 39.9 | 1.378 | 42.5 |
| *Synechococcus* WH7803 | 1.23 | 88.0 | 11.5 | 165.0 | 71.0 | 2.32 | 77.0 | 59.5 | 1.294 | 62.6 |

($a_d^*$) and acetone extracts ($a_p^*$) (Table 4). An increase in $a_w^*$ and a reduction in the efficiency factor for absorption ($Q_a$) at $\lambda = 675$ nm (Tables 4 and 5) with a decrease in cell size is consistent with theoretical predictions (e.g., Duysens 1956, Morel and Bricaud 1981). A four-fold decrease in cell size (Table 1) was correlated with a two-fold increase in $a_w^*$ (Table 4) and a ten-fold decrease in $Q_a$ (Table 5).

## Discussion

An increase in the ratio $a_w : a_d$ or $a_w^* : a_d^*$ with decreases in cell diameter (Tables 1, 3, 4) provides experimental evidence consistent with previous theoretical predictions, which have indicated a reduced package effect in small phytoplankton cells. However, changes in $a_w^*$ and $Q_a$ can result from varia-

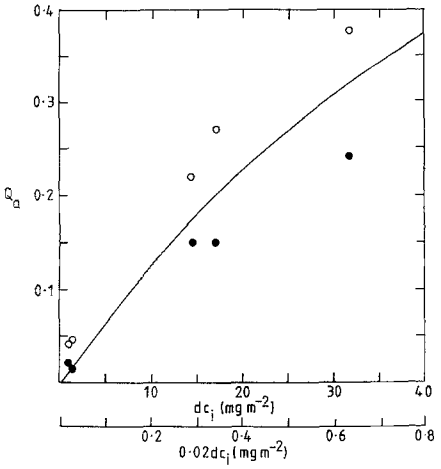


**Fig. 5.** Individual data points for all five species showing relationship between dimensionless efficiency factor for absorption ($Q_a$) and the product $dc_i$, where $d$=mean cell diameter and $c_i$=intracellular chlorophyll *a* concentration, using either corrected (•) or uncorrected (○) measurements ($\lambda$=675 nm). Also shown, as a continuous line, is the theoretical relationship between $Q_a$ and $dc_i$ given a constant value for the specific absorption coefficient of cellular pigment (see "Discussion" for details)

**Table 4.** Uncorrected and corrected chlorophyll *a*-specific absorption coefficient (m$^2$ mg$^{-1}$ chl *a*) at $\lambda$=675 nm in intact cells ($a_w^*$), disrupted cells ($a_d^*$) and acetone extracts ($a_p^*$) for the five phytoplankters

| Species or clone | Uncorrected | | Corrected | | $a_p^*$ |
|---|---|---|---|---|---|
| | $a_w^*$ | $a_d^*$ | $a_w^*$ | $a_d^*$ | |
| *Thalassiosira* sp. | 0.0187 | 0.0206 | 0.0125 | 0.0181 | 0.0202 |
| Clone 2601 | 0.0305 | 0.0221 | 0.0172 | 0.0175 | 0.0202 |
| *Nannochloris atomus* | 0.0252 | 0.0221 | 0.0174 | 0.0185 | 0.0202 |
| *Synechococcus* 'Syn' | 0.0519 | 0.0252 | 0.0262 | 0.0187 | 0.0202 |
| *Synechococcus* WH 7803 | 0.0532 | 0.0229 | 0.0248 | 0.0192 | 0.0202 |
| Mean $a_d^*$ | | 0.0226 | | 0.0184 | 0.0202 |
| Standard deviation | | 0.0017 | | 0.0006 | |

tions in both cell diameter ($d$) and intracellular pigment content ($c_i$). Morel and Bricaud (1986) have illustrated the consequences of variations in $d$ and $c_i$ on $Q_a$ (Eq. 6).

$$Q_a = (2/3)\, a^* dc_i. \tag{6}$$

If $Q_a$ is plotted as a function of $dc_i$ and $a^*$ is constant, a straight-line relationship should be obtained. If, as we have shown, $a^*$ in vivo is not constant, then the relationship should be non-linear. $Q_a$ is plotted against $dc_i$ in Fig. 5. Although there is some evidence of curvature in agreement with other measurements and theoretical predictions (see Morel and Bricaud 1986), more points are required to accurately assess the relationship between $Q_a$ and $dc_i$. There is also reasonable, quantitative agreement between our values and those published by Morel and Bricaud (1986). We can also convert the product $dc_i$ to values based on a constant specific absorption coefficient for chlorophyll in acetone extracts (0.020 mg$^2$ mg$^{-1}$). From this we can calculate a theoretical value for $Q_a$ based on the van de Hulst (1957) approximation. This is plotted as a continuous line in Fig. 5. It is evident that neither uncorrected nor corrected data points give exact agreement with theoretical predictions (Fig. 5). In the case of the data of Morel and Bricaud (1986), the scatter of the experimental values precludes an exact agreement between experimental results and theoretical predictions. However, the conversion of the product $dc_i$ to values based on a constant specific absorption coefficient for cell pigments in vivo of measurements obtained from cell pigments in vitro is problematic. For instance, the use of a constant value for the specific absorption coefficient of cellular material may be inappropriate if absorption enhancement by intracellular scattering occurs (see below). Differences in pigment composition could also account for some of the discrepancies between theory and experiment (see Sathyendranath et al. 1987).

In an earlier report (Geider and Osborne 1987) we used cell disruption to assess the magnitude of the package effect. In those experiments reasonable agreement was found between theory and measurement, although cell diameter was found to be an inappropriate measure of cell size for determining the magnitude of the package effect (Geider and Osborne 1987). There are also other problems, related to the use of ray-optics approximations (Latimer 1984) or due to

**Table 5.** Intracellular chlorophyll *a* concentrations, geometric cross-sections and efficiency factors for absorption at $\lambda$=675 nm for the five phytoplankters. $c_i$: intracellular chlorophyll *a* concentration; $sQ_a$: absorption cross-section, $s$: geometric cross-section; $Q_a$: dimensionless efficiency factor for absorption. Values in parentheses refer to absorption cross-sections and dimensionless efficiency factors for absorption ($Q_a$) based on disrupted cells

| Species or clone | $c_i$ (kg m$^{-3}$) | $sQ_a$ ($\mu$m$^2$ cell$^{-1}$) | $s$ ($\mu$m$^2$ cell$^{-1}$) | $Q_a$ |
|---|---|---|---|---|
| *Thalassiosira* sp. | 5.6 | 5.1 | 21.0 | 0.24 |
| Clone 2601 | 3.3 | 2.0 | 13.1 | 0.15 |
| *Nannochloris atomus* | 3.6 | 1.6 | 10.5 | 0.15 |
| *Synechococcus* 'Syn' | 0.63 | 0.039 (0.029) | 1.7 | 0.023 (0.017) |
| *Synechococcus* WH 7803 | 0.97 | 0.024 (0.019) | 1.2 | 0.020 (0.016) |

variations in the refractive index of cells or cellular contents, which could have led to a discrepancy between theory and measurement. It is clear also from the present work that there are additional complications in the experimental assessment of the package effect, which are particularly evident at small (<3 μm diam) cell sizes, and these are discussed below.

In the majority of experimental measurements of in vivo light absorption by microalgal cells, attenuation (apparent absorption) beyond $\lambda = 750$ nm is treated as being entirely due to scattering (e.g. Kirk 1983, Davies-Colley et al. 1986, Geider and Osborne 1987). The validity of this interpretation has been questioned, as the magnitude of apparent absorption at $\lambda = 750$ nm cannot be accounted for in Mie scattering calculations and exceeds direct measurements of backscattering (Bricaud et al. 1983). It does not, however, exceed Bryant et al.'s (1969) estimates of scattering at 700 nm for spinach chloroplasts and yeast cells. Bryant et al. concluded that residual scattering losses for spinach chloroplasts at angles greater than 35° can amount to 5% of total scattering. Considering the conservative case with a scattering efficiency ($Q_b^w$) of approximately 2.0 (see Table 6), an apparent absorption efficiency due to residual scattering would be $2.0 \times 0.05 = 0.01$, or 10%. Based on our measurements of $Q_a$ and values for $Q_b^w$ obtained from Morel and Bricaud (1986) at $\lambda = 750$ nm, we can estimate losses due to residual scattering. Estimated losses vary from 2 to 4% (Table 6), which are less than those found by Bryant et al. (1969) with spinach chloroplasts. Similar estimates can also be made with disrupted cells, where predicted losses amount to 0.5–1.5% of $Q_b^w$ (Table 6). The uncertainty is whether these predicted losses are unreasonably high and cannot be fully accounted for as residual scattering. If the values at $\lambda = 750$ nm are due to absorption by unidentified pigments or cell structures, these must be insoluble in organic solvents or are broken down in acetone extracts (Davies-Colley et al. 1986). In the present paper we have shown that the magnitude of the apparent absorption at $\lambda = 750$ nm could vary between species and within clones of the same species (Table 3), but was not related to variations in cell size or culture density (Tables 1 and 2). Our results also show that the magnitude of the attenuation at $\lambda = 750$ nm is reduced but not eliminated after 'unpackaging' by cell disruption (Table 3). Whether the "apparent absorption" at 750 nm after cell disruption represents true absorption by cell constituents or is due to scattering by membrane fragments is uncertain. Comparison of $a_w$ and $a_d$ (Table 3) shows that the magnitude of the difference between disrupted cell and intact cell measurements at $\lambda = 750$ nm is dependent on cell size. For the two *Synechococcus* clones there was a 75 to 88% difference between $a_w$ and $a_d$ at 750 nm, whereas the difference for the larger species was 54 to 62% (Table 3). Whether this represents a greater scattering loss with small cells or is related to a novel absorption phenomena which increases with decreases in cell size remains to be evaluated.

The improved matching between the blue and red absorption peaks of intact and disrupted cells for the small *Synechococcus* clones, when the apparent absorption at $\lambda = 750$ nm is scaled with wide-angle attenuation measurements, is indicative of scattering losses at large angles. The larger discrepancy between whole and disrupted cell absorption-spectra at shorter wavelengths is also consistent with this interpretation (Latimer et al. 1968). Whilst these small species may approach the Rayleigh domain where scattering is more isotropic, Latimer et al. have indicated that particle sizes would have to be less than $\lambda/10$ (= 0.04 μm diam at 400 nm) for them to behave as point sources (see also Osborne and Raven 1986). Subcellular structures or internal inhomogeneities could also lead to an increase in wide-angle scattering (Latimer et al. 1968), but this is inconsistent with the volume-scattering function of Cyanobacteria of the same size and pigment composition as used in the present experiments, where most of the light is scattered within a 10° cone of the forward direction (Morel and Bricaud 1986) and would be collected by our system (see Geider and Osborne 1987). Multiple scattering could increase wide-angle losses (Osborne and Raven 1986) and cannot entirely be ruled out. However, the values at $\lambda = 750$ nm are not related to variations in cell concentration (Tables 2 and 3) despite an expectation that increases in culture density would increase the possibility of multiple scattering. Additional measurements on the same culture at different cell concentrations are required to resolve this point (see Sathyendranath et al. 1987).

Backscattering, which is unaccounted for in these experiments, is generally low for microalgal cells (Morel and Bricaud 1986). Although backscattering may be more significant with picoplankton (see Carder et al. 1986), this is unlikely to account for more than 1% of the total attenuation (Kiefer et al. 1979, Morel and Bricaud 1986). Reflection losses at the walls of the cuvette could result in systematic errors of the order of 2 to 4% (Haardt and Maske 1987), but should largely be accounted for with our system because of the use of reference cuvettes.

**Table 6.** Estimation of residual scattering losses based on absorption coefficients in Table 3, efficiency factors for absorption ($Q_a^w$, $Q_a^d$) and scattering ($Q_b^w$) at $\lambda = 750$ nm. Values for $Q_a^w$ ($\lambda = 750$ nm) were calculated by assuming $Q_a$ values in Table 5 scaled with changes in the values of the absorption coefficients (Table 3). $Q_b^w$ estimates are based on data of Morel and Bricaud (1986) at $\lambda = 675$ nm, using cell size-distributions in Table 1. It was assumed that for the first three species $Q_b^w$ ($\lambda = 675$ nm) = $Q_b^w$ ($\lambda = 750$ nm) based on total scattering measurements, whilst the remaining values were corrected according to spectral changes in $Q_b^w$ (see Morel and Bricaud 1986)

| Species or clone | $Q_a^w$ | $Q_b^w$ | $Q_a^w : Q_b^w$ | $Q_a^d$ | $Q_a^d : Q_b^w$ |
|---|---|---|---|---|---|
| *Thalassiosira* sp. | 0.079 | 2.28 | 0.035 | 0.046 | 0.015 |
| Clone 2601 | 0.065 | 2.23 | 0.029 | 0.041 | 0.011 |
| *Nannochloris atomus* | 0.047 | 2.20 | 0.021 | 0.025 | 0.010 |
| *Synechococcus* 'Syn' | 0.012 | 0.29 | 0.041 | 0.009 | 0.010 |
| *Synechococcus* WH 7803 | 0.011 | 0.29 | 0.038 | 0.010 | 0.005 |

If the values obtained at $\lambda = 750$ nm are not entirely due to scattering, then part of the measured attenuation must be due to absorption. Support for this assertion comes from the theoretical analysis of Bricaud et al. (1983), who maintain that this cannot be accounted for as residual scattering. The poor agreement between uncorrected and corrected values for $a_d$ and $a_p$ (Table 3) and the absence of exact correspondence between theoretical and experimental measurements of $Q_a$ (Fig. 5) could be interpreted as indicating that part of the measured attenuation at $\lambda = 750$ nm was due to absorption. Exact agreement between $a_d$ and $a_p$ is, however, unlikely due to solvent effects. It is also uncertain whether the cell-disruption techniques used could significantly modify the absorption properties of cellular pigments.

Intact cells of both *Synechococcus* clones exhibit absorption enhancement compared to disrupted cells or acetone extracts if part of the measured attenuation at $\lambda = 750$ nm is due to absorption. It is difficult to envisage, however, how this can be achieved, particularly at wavelengths $> 700$ nm. The wave-optics effect described by Latimer (1984) could enhance in vivo absorption, possible due to total internal reflection and related surface phenomena (see van de Hulst 1957, Osborne and Raven 1986). Whilst the cell wall prevents light from entering the cell, captured photons are unlikely to escape if they are totally internally reflected, and the cell behaves as a light trap. A related phenomena has been proposed for multicellular plant leaves, where removal of the cuticle results in a decrease in absorption (Lin and Ehleringer 1983, Vogelmann and Björn 1986). However, the wave-optics effect described by Latimer (1984) can only account for a marginal (10 to 20%) increase in absorption, whereas we need to account for 2 to 7-fold increases. Therefore Latimer's wave-optics effect is unlikely to account for all of the difference between intact and disrupted cell absorption coefficients at $\lambda = 750$ nm. Absorption peaks at $\lambda = 750$ nm have been noted previously with *Anacystis nidulans*, and it has been suggested that they could be due to absorption by bile pigments (see Govindjee and Braun 1974). It is also clear that macromolecular interactions between chlorophyll and other molecules contribute significantly to the optical characteristics of chlorophyll in vivo. These properties would not be evident in in vitro absorption spectra of chlorophyll in organic solvents. Recent evidence that isolated light-harvesting pigments or synthetic chlorophyll-macromolecular complexes can have significant absorption at $\lambda = 700$ nm (Shibata et al. 1986, Inamura et al. 1988) is also relevant to the problem of assigning in vivo attenuation measurements at $\lambda = 750$ nm to either absorption or scattering. Of particular interest is the demonstration of a shift in the red absorption maxima of chlorophyll *a* to $\lambda = 750$ nm in synthetic chlorophyll-bovine-serum albumin compounds through increased hydration of the synthetic complexes (Inamura et al. 1983, Shibata et al. 1986). This would seem to have important implications for long-wave light absorption in vivo.

In view of the uncertainties regarding the significance of scattering losses, or the possibility of absorption enhancement, the magnitude of the package effect in small cells remains equivocal. Although the difficulties in relating disrupted cell absorption-spectra to those of intact cells are more obvious in the picoplankton, there is no reason to expect that these problems will be absent when larger cells are considered. This may be partly the reason for a failure to reconstruct exactly the in vivo absorption-spectra from the optical properties of disrupted cells (Geider and Osborne 1987). Additional studies are therefore required on the absorptive and scattering properties of microalgal cells before we can accurately assess the magnitude of the package effect.

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## Literature cited

Bricaud, A., Morel, A., Prieur, L. (1983). Optical efficiency factors of some phytoplankters. Limnol. Oceanogr. 28: 816–832

Bryant, F. D., Seiber, B. A. Latimer, P. (1969). Absolute absorption cross sections of cells and chloroplasts. Archs Biochem. Biophys. 135: 79–108

Carder, K. L., Steward, G., Paul, J. H., Vargo, G. A. (1986). Relationships between chlorophyll and ocean colour constitutents as they affect remote-sensing reflectance models. Limnol. Oceanogr. 31: 403–413

Das, M., Rabinowitch, E., Szalay, L., Papageorgiou, G. (1967). The "sieve effect" in *Chlorella* suspensions. J. phys. Chem., Wash. 71: 3543–3549

Davies-Colley, R. J., Pridmore, R. D., Hewitt, J. E. (1986). Optical properties of some freshwater phytoplanktonic algae. Hydrobiologia 133: 165–178

Duysens, L. N. M. (1956). The flattening of the absorption spectrum of suspensions as compared to that of solutions. Biochim. biophys. Acta 19: 1–12

Fahnenstiel, G. L., Sicko-Goad, L., Scavia, D., Stoermer, E. F. (1986). Importance of picoplankton in Lake Superior. Can. J. Fish. aquat. Sciences 43: 235–240

Geider, R. J., Osborne, B. A. (1987). Light absorption by a marine diatom: experimental observations and theoretical calculations of the package effect in a small *Thalassiosira* species. Mar. Biol. 96: 299–308

Govindjee, Braun B. Z. (1974). Light absorption, emission and photosynthesis. In: Stewart, W. D. P. (ed.) Algal physiology and biochemistry. Blackwell Scientific Publications, Oxford, p. 346–390

Haardt, H., Maske, H. (1987). Specific in vivo absorption coefficient of chlorophyll *a* at 675 nm. Limnol. Oceanogr. 32: 608–619

Hulst, van de, H. C. (1957). Light scattering by small particles. Wiley, New York

Inamura, I., Araki, T., Shei, M., Nishikawa, T., Shibata, H., Ochiai, H. (1988). Properties of the water-soluble chlorophyll-synthetic linear macromolecular complexes. Biochim. biophys. Acta 932: 335–344

Inamura, I., Ochiai, H., Toki, K., Watanabe, S., Hikino, S., Araki, T. (1983). Preparation and properties of chlorophyll/water-soluble macromolecular complexes in water. Stabilization of chlorophyll aggregates in the water-soluble macromolecule. Photochem. Photobiol. 38: 37–44

Jeffrey, S. W., Humphrey, G. F. (1975). New spectrophotometric equations for determining chlorophylls a, b, c, and $c_2$ in higher plants, algae and natural phytoplankton. Biochem. Physiol. Pfl. 167: 191–194

Kiefer, D. A., Olson, R. J., Wilson, W. H. (1979). Reflectance spectroscopy of marine phytoplankton. Part 1. Optical properties as related to age and growth rate. Limnol. Oceanogr. 24: 664–672

Kirk, J. T. O. (1975a). A theoretical analysis of the contribution of algal cells to the attenuation of light within natural waters. I. General treatment of pigmented cells. New Phytol. 75: 11–20

Kirk, J. T. O. (1975b). A theoretical analysis of the contribution of algal cells to the attenuation of light within natural waters. II. Spherical cells. New Phytol. 75: 21–36

Kirk, J. T. O. (1976). A theoretical analysis of the contribution of algal cells to the attenuation of light within natural waters. III. Cylindrical and spheroidal cells. New Phytol. 77: 341–358

Kirk, J. T. O. (1983). Light and photosynthesis in aquatic ecosystems. Cambridge University Press, Cambridge

Latimer, P. (1983). The deconvolution of absorption spectra of green plant materials – improved corrections for the sieve effect. Photochem. Photobiol. 38: 731–734

Latimer, P. (1984). A wave-optics effect which enhances light absorption by chlorophyll *in vivo*. Photochem. Photobiol. 40: 193–199 (1984)

Latimer, P., Moore, D. M., Bryant, F. D. (1968). Changes in total light scattering and absorption caused by changes in particle conformation. J. theor. Biol. 21: 348–367

Li, W. K. W., Subba-Rao, D. V., Harrison, W. G., Smith, J. C., Cullen, J. J., Irwin, B., Platt, T. (1983). Autotrophic picoplankton in the tropical ocean. Science, N.Y. 219: 292–295

Lin, Z. F., Ehleringer, J. (1983). Epidermis effects on spectral properties of leaves of four herbaceous species. Physiologia Pl. 59: 91–94

Morel, A., Bricaud, A. (1981). Theoretical results concerning light absorption in a discrete medium, and application to specific absorption of phytoplankton. Deep-Sea Res. 28A: 1375–1393

Morel, A., Bricaud, A. (1986). Inherent optical properties of algal cells including picoplankton: theoretical and experimental results. Can. Bull. Fish. aquat. Sciences 214: 521–559

Osborne, B. A., Raven, J. A. (1986). Light absorption by plants and its implications for photosynthesis. Biol. Rev. 61: 1–61

Platt, T., Subba-Rao, D. V., Irwin, B. (1983). Photosynthesis of picoplankton in the oligotrophic ocean. Nature, Lond. 301: 702–704

Raven, J. A. (1984). A cost-benefit analysis of photon absorption by photosynthetic unicells. New Phytol. 98: 593–625

Raven, J. A. (1986). Physiological consequences of extremely small size for autotrophic organisms in the sea. Can. Bull. Fish. aquat. Sciences 214: 1–70

Sathyendranath, S., Lazzara, L., Prieur, L. (1987). Variations in the spectral values of specific absorption of phytoplankton. Limnol. Oceanogr. 32: 403–415

Sherr, B., Sherr, E. (1983). Enumeration of heterotrophic microprotozoa by epifluorescence microscopy. Eastuar., cstl Shelf Sci. 16: 1–7

Shibata, H., Ochiai, H., Kawashima, T., Okamato, T., Inamura, I. (1986). Preparation and properties of the water-soluble chlorophyll – bovine serum albumin complexes. Biochim. biophys. Acta 852: 175–182

Takahashi, M., Bienfang, P. K. (1983). Size structure of phytoplankton biomass and photosynthesis in subtropical Hawaiian waters. Mar. Biol. 76: 203–211 (1983)

Vogelmann, T. C., Björn, L. O. (1986). Plants as light traps. Physiologia Pl. 68: 704–708

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