

Variations in the spectral values of specific absorption of phytoplankton

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Abstract

Absorption spectra of laboratory cultures of eight species of phytoplankton were studied. These spectra, normalized per unit of chlorophyll *a* concentration (specific absorption) show variability in magnitude and spectral form. Specific absorption at 440 nm varied over a factor of three. Spectra of phytoplankton belonging to the same taxonomic group tended to have similar forms. Over 95% of the variability in specific absorption could be explained in terms of changes in cell size, intracellular pigment concentration, and the relative importance of auxiliary pigments. Shape was not an important factor for this sample set.

The spectral characteristics of absorption by intact cells of phytoplankton have been the subject of various studies because of their importance in primary production, radiative transfer in seawater, and in passive remote-sensing of phytoplankton. At sufficiently low concentrations, the total absorption coefficient *a* of a given substance at a given wavelength λ can be expressed as a product of a specific absorption coefficient a^*_c and the concentration of the substance *C*. That is,

$$a(\lambda) = Ca^*_c(\lambda). \quad (1)$$

The specific absorption a^*_c is the absorption per unit of concentration. In the case of phytoplankton, *C* is generally taken to be the concentration of the main pigment chlorophyll *a* or the sum of concentrations of Chl *a* and its degradation products, the pheopigments. Prieur and Sathyendranath (1981) showed that the in situ values of $a^*_c(\lambda)$ of phytoplankton are highly variable from one region to another and pointed out that similar variability is also implicit in the existing literature on in vivo absorption of phytoplankton.

Changes in pigment composition could account for some of the variation. It is well known that pigments other than Chl *a* (mainly Chl *b*, Chl *c*, carotenoids, biliproteins, and pheopigments) are present in phytoplankton in substantial quantities and that

their proportions (i.e. the pigment composition) can vary with species and with the physiological state of the cells. These auxiliary pigments and degradation products have distinctive, well-defined absorption bands in the visible region. Therefore, it appears reasonable to assume that variations in pigment composition could account for some of the observed variations in phytoplankton absorption efficiency.

Another cause of variability could be the flattening of absorption spectra due to a particle effect. Duysens (1956) demonstrated theoretically that the absorption spectrum of particles in suspension appears flattened when compared with absorption by a solution of the same substance in the same concentration. He showed that the flattening due to the particle effect varies according to the size, shape, and optical density of the particles. He pointed out that, in the case of phytoplankton, variations in the particle effect from species to species could lead to differences in absorption spectra, not only in absolute values but also in the form of the spectra. Using a similar approach, Kirk (1975*a,b*, 1976) examined in detail the probable consequences of this effect on the distribution of underwater irradiance. Morel and Bricaud (1981) have pointed out that the same effect can be deduced through the Mie theory of scattering.

Many laboratory studies have been carried out on the in vivo absorption by monospecific cultures of phytoplankton (e.g. Latimer and Rabinovitch 1959; Yentsch 1960; Duntley et al. 1974; Privoznik et al. 1978; Faust and Norris 1982). It is difficult to compare results, however, because of the

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differences in techniques. Furthermore, interpretation in terms of available theory is usually not possible due to lack of data on the size, shape, and concentration of particles.

The aim of our study was to examine the specific absorption curves of eight monospecific cultures of phytoplankton and to attempt to explain the observed variations in terms of changes in pigment composition and the theory of spectral flattening in the case of spherical, homogeneous particles.

We thank A. Morel for encouragement and help. A. Bricaud is thanked for making available experimental data on *Tetraselmis maculata* and *Platymonas* sp. We also thank J. T. O. Kirk for commenting on the original manuscript.

The particle effect

The flattening of phytoplankton absorption spectra due to the particle effect has been treated theoretically by Duysens (1956), Kirk (1975*a,b*, 1976), and Morel and Bricaud (1981). Particles of various shapes have been considered by Duysens and Kirk. Here, only the principal results for the case of spherical, homogeneously distributed particles are recalled.

Let us consider a narrow, collimated beam of flux ϕ_0 incident on a point on the surface of a spherical particle of diameter d , and passing through the center of the particle. If ϕ is the flux leaving the particle (scattered or transmitted), the absorption coefficient of the cell material, a_{cm} , is given by

$$a_{cm} = \frac{1}{d} \ln \frac{\phi}{\phi_0}. \quad (2)$$

Let N be the number of such particles per unit of volume of the medium. If the substance which forms the particles were uniformly distributed in the medium, the absorption coefficient of the solution would be the same as a_{cm} , but modified by the dilution factor, $\pi d^3 N/6$. This calculation leads to the expression for the absorption coefficient of the solution, a_{sol} :

$$a_{sol} = \frac{\pi}{6} d^3 N a_{cm}. \quad (3)$$

The efficiency factor for absorption by one particle, Q_a , is defined as the ratio of the absorbed flux to the total flux incident on the particle. Duysens (1956) and van de Hulst (1957) have derived the following expression for the case of a spherical particle of diameter d :

$$Q_a = 1 + \frac{2 \exp(-\rho')}{\rho'} + \frac{2[\exp(-\rho') - 1]}{\rho'^2} \quad (4)$$

where

$$\rho' = da_{cm}. \quad (5)$$

Duysens (1956) has shown that the applicability of Beer's law to suspensions depends on two ratios: that of the total volume occupied by the particles to the volume of the medium, and that of the fraction of incident light that is absorbed by a particle (Q_a). If the product of these two ratios is small, then Beer's law may be assumed to hold (see also Stokes 1975). If the experimental setup satisfies this condition, then the measured absorption coefficient of a suspension of N particles is related to the efficiency factor Q_a by the equation:

$$a = \frac{\pi}{4} d^2 N Q_a. \quad (6)$$

We can now define the factor of spectral flattening, F , due to the particle effect, as the ratio of the absorption coefficient of the suspension to the absorption coefficient of an equivalent solution. F is therefore given by

$$F = \frac{a}{a_{sol}} = \frac{3Q_a}{2da_{cm}}. \quad (7)$$

It can be shown that F is always < 1 . It tends toward 1 when ρ' is small (i.e. the particles are small, or a_{cm} is small). Figure 1 presents the variations of Q_a and F as functions of ρ' .

These expressions are obtained for a given wavelength. As F decreases with increasing a_{cm} , absorption maxima for a suspension will be much smaller than for the equivalent solution, but the minima will be

little modified. Consequently, the absorption spectra of suspensions will appear flatter than those of an equivalent solution.

In the case of a polydisperse suspension, the estimated parameters are the mean values of the populations concerned.

Each phytoplankton cell is composed of a cell wall and cytoplasm with chromatophores containing the pigments. The shape and size of the cell, the shape, size, and number of chromatophores per cell, and the pigment composition can vary with the species and the physiological state of the cells. Therefore, the theory of spectral flattening, when applied to phytoplankton, can only be considered as a highly simplified model. Here we examine the extent to which this simplification explains variations in specific absorption among several species of phytoplankton.

Experimental setup

The eight species studied were *Hymenomonas elongata*, *Platymonas suecica*, *Platymonas* sp., *Dunaliella marina*, *Tetraselmis maculata*, *Chaetoceros didymum*, *Chaetoceros protuberans*, and *Skeletonema costatum*. They were chosen to cover wide variations in the shape and size of the cells, pigment composition, structure of the cell wall, and intracellular pigment concentration.

Batch cultures of the first six species were grown in Provasoli medium (Provasoli 1966) at 18°C and under continuous lighting of 400 $\mu\text{Einst m}^{-2} \text{s}^{-1}$ intensity. Cultures of the other two species (*C. protuberans* and *S. costatum*) were maintained at almost constant concentration by partially renewing the medium every 24 h. The volume of the medium replaced each time was proportional to the growth rate of the cells, so that the biomass concentration of the cultures at the beginning of every 24-h cycle was about the same. These cultures were grown in medium "f" (Guillard and Ryther 1962) at concentration f/2 and subjected to blue, green, or white light of varying intensities (Table 1).

Measurement of in vivo absorption—The instrument used was a Perkin-Elmer 571 spectrophotometer with a scattered transmission accessory. It was observed that the

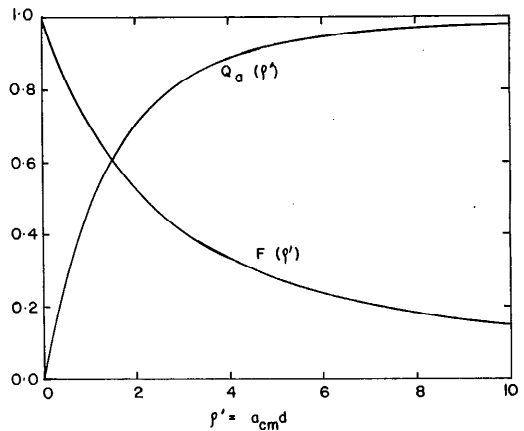


Fig. 1. The parameters Q_a and F as functions of ρ' .

diffusing plate in front of the photomultiplier was not efficient enough to eliminate the inequality effect of the optical response of different areas of the photocathode. A second diffusing plate was therefore added to eliminate this effect and to increase the reception angle. The resulting half-angle of reception of the instrument was 43°. Using angular scattering functions representative of phytoplankton, Bricaud et al. (1983) calculated that this setup captures >99.5% of the scattered light. We used sample cells of 1-cm path length and the absorption spectra measured from 340 to 760 nm with filtered water from the same culture as our blank.

Measurement of absorption by acetone extracts and calculation of pigment concentrations—Pigments were extracted in 90% acetone (Strickland and Parsons 1968). Absorption spectra of acetone extracts were also recorded from 340 to 760 nm, and the equations of Lorenzen (1967) were used to calculate the concentrations of Chl *a* and pheopigments; SCOR/UNESCO (1966) equations were used to calculate Chl *b* and *c* concentrations, and the Richards and Thompson (1952) method was used for carotenoids.

Cell size—The distribution of cell volume was determined for each culture. For spherical and ellipsoidal cells, the measurements were made with a Coulter Counter (model ZB for *P. suecica* and *H. elongata*, and model TA for *D. marina*, *Platymonas* sp., and *T. maculata*). The cell volumes of *C. di-*

Table 1. Some characteristics of the cultures used. PAR is photosynthetically available radiation ($\mu\text{Einst m}^{-2} \text{s}^{-1}$) (see Morel, 1978). Color notations are: W—white; B—blue; G—green. Data on *Tetraselmis maculata* and *Platymonas* sp. were made available by A. Bricaud.

Sample No.	Age (d)	PAR	Color	Pigment concn (mg m^{-3})					Chl c	Crmd*	No. cells (10^{10}m^{-3})	Diam d (μm)	a(440) (m^{-1})	a(737) (m^{-1})
				Chl a	Pheo	Chl b	Chl c	Crmd*						
1	3	400	W	138	5.1	42.3	0	114	26	5.8	4.61	0.55		
2	7	400	W	245	46.5	131	0	261	49	5.6	10.1	1.24		
3	7	400	W	225	27.2	88.8	0	219	16	8.5	8.89	1.79		
4	4	400	W	467	0	231	0	435	90	8.5	23.76	4.03		
5	7	400	W	624	25.3	298	0	662	207	6.8	35.5	7.67		
6	7	400	W	247	23.6	0	49.1	233	5.8	13.4	6.26	0.65		
7	9	400	W	384	7.5	0	79.0	333	8.1	13.5	8.03	0.24		
8	14	400	W	781	10.3	0	166	640	17	14.0	16.16	0.88		
9	17	400	W	1,019	22.7	0	300	769	23	15.1	20.89	1.38		
10	5	400	W	143	6.1	0	81.5	73.4	13	26.5	7.63	0.87		
11	1	150	W	270	18.3	0	159	109	6.4	13.3	6.58	0.62		
12	1	35	W	176	6.2	0	95.9	65.8	4.3	12.6	3.98	0.38		
13	1	35	B	159	9.9	0	89.1	59.4	4.0	11.9	3.69	0.37		
14	1	150	W	356	19.8	0	199	156	7.1	14.4	7.94	0.48		
15	1	300	W	235	21.0	0	139	127	4.9	14.9	6.61	0.63		
16	1	150	B	159	11.8	0	91.0	78.0	3.7	14.0	4.42	0.48		
17	1	75	B	181	0	0	90.1	75.0	8.3	9.1	4.64	0.32		
18	2	35	W	142	32.5	0	61.0	64.1	33	4.8	5.18	0.57		
19	2	35	G	174	27.0	0	66.9	78.3	41	4.8	5.90	0.63		
20	1	35	G	148	0	0	66.7	58.7	3.9	11.1	3.23	0.46		
21	21	400	W	234	23.7	93.0	0	280	29	5.9	8.56	1.11		
22	5	400	W	236	33.0	106	0	216	36.2	5.6	9.36	1.08		
23	25	400	W	822	86.8	332	0	1,063	102	6.2	33.57	5.82		
24	11	400	W	500	79.4	285	0	534	80	5.4	18.91	2.47		
25	1	35	W	222	0	0	109	86.5	4.5	11.0	4.24	0.57		
26	1	150	W	172	14.9	0	95.6	73.4	7.5	9.5	5.00	0.48		
27	1	150	W	227	0	0	113	97.2	11	9.1	6.63	0.51		
28	1	450	W	122	18.0	0	78.7	31.1	10	9.1	5.67	0.38		

* Carotenoid (mSPU m^{-3}).

dymum, *C. protuberans*, and *S. costatum* were estimated by microscopic measurements of about 80 cells in each case. The cell volume is used to calculate the diameter d of the equivalent sphere. Since the distribution of diameter is very narrow in most cases (e.g. half-bandwidth = $3.85 \mu\text{m}$ with peak at $13.52 \mu\text{m}$ for a sample of *H. elongata*; half-bandwidth = $0.79 \mu\text{m}$ with peak at $5.82 \mu\text{m}$ for a sample of *P. suecica*), the diameter corresponding to the mode is taken as characteristic of the culture. The samples were fixed in Lugol's solution until the size measurements were carried out (within a few weeks).

Concentration of cells—The number of cells per unit volume N was counted with a Fusch-Rosenthal or a Lemaur hemacytometer.

Hymenomonas elongata and *P. suecica* were cultured in relatively large volumes (4 liters). By taking out small samples periodically, we followed their optical properties during about 3 weeks of growth. Only single measurements were made on batch cultures of *D. marina*, *Platymonas* sp., *T. maculata*, and *C. didymum*. In the case of *C. protuberans* and *S. costatum*, we carried out several measurements for different conditions of illumination.

We subtracted the values of absorption at 737 nm from absorption at all wavelengths, assuming that phytoplankton pigments do not absorb at this wavelength and that the absorption observed at this wavelength represents absorption by detritus and constituents of the phytoplankton cells other than photosynthetic pigments. Subtracting these values over the entire wavelength implies that their absorption is neutral; this assumption is untested but is used as a first approximation. Duysens (1956) and Bannister (1979) have applied similar corrections to their data, though they attributed the observed absorption at this wavelength range to residual scattering. Neither our experimental setup nor theoretical considerations, however, support such an interpretation.

Results and discussion

Spectra of specific absorption in vivo (in suspension)—Typical spectral values of spe-

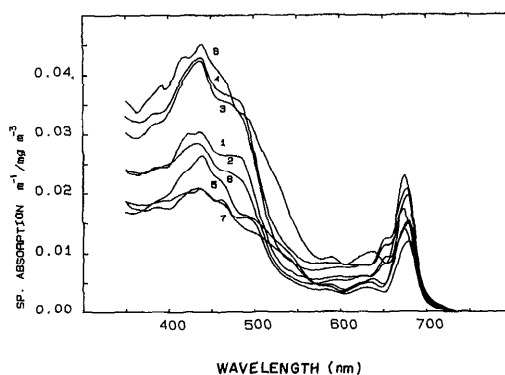


Fig. 2. Examples of in vivo absorption by eight phytoplankton species. 1—*Platymonas suecica*; 2—*Dunaliella marina*; 3—*Tetraselmis maculata*; 4—*Platymonas* sp.; 5—*Hymenomonas elongata*; 6—*Chaetoceros didymum*; 7—*Chaetoceros protuberans*; 8—*Skeletonema costatum*. Spectra are normalized by concentration C (=Chl a + pheopigments) (mg m^{-3}). The value of absorption at 737 nm is subtracted from absorption at all wavelengths.

cific absorption of the eight species studied are shown in Fig. 2. Absorption spectra were studied per unit concentration of C (=Chl a + pheopigments). The value of the specific absorption a^*_c at 440 nm varied between 0.0187 and $0.0473 \text{ m}^{-1} (\text{mg m}^{-3})^{-1}$, within the range from 0.013 to 0.077 estimated for in situ conditions by Prieur and Sathyendranath (1981).

Concentration effect—Before examining the importance of either the particle effect or pigment composition, we have to make sure that the measurements are carried out under conditions wherein Beer's law holds. As stated earlier, the validity of Beer's law in the case of suspensions depends on two parameters. Of them, Q_a is an intrinsic property of a particle and cannot be changed. Therefore, when making absorption measurements on particles, the concentration has to be kept sufficiently low to ensure applicability of Beer's law. At higher concentrations, the specified linear relationship between absorption and concentration is invalidated.

Figure 3 shows $a(440)$ as a function of concentration C for *P. suecica* and *H. elongata*. The plotted points include measurements on the same culture on different days, as well as several measurements on the same culture on the same day at different dilu-

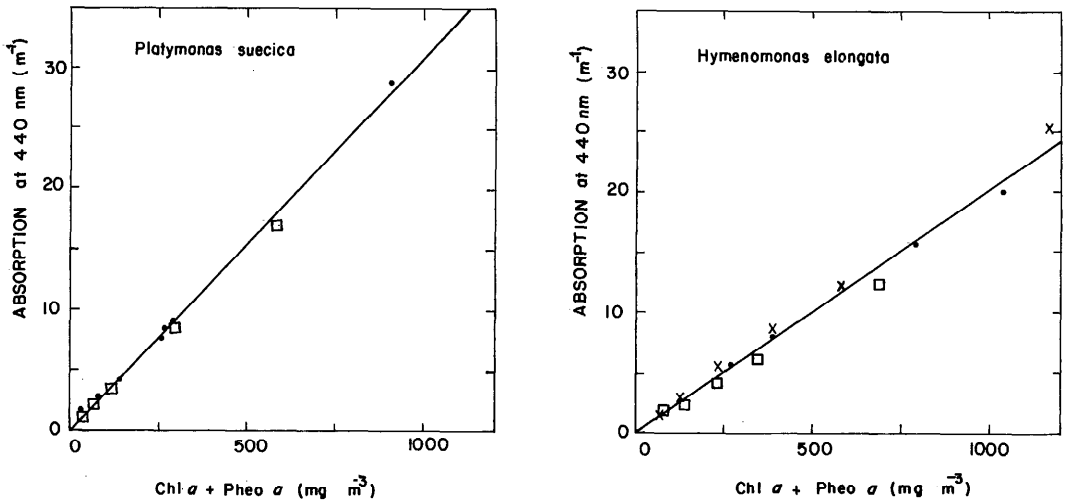


Fig. 3. Absorption at 440 nm as a function of concentration C in *P. suecica* and *H. elongata*. Measurements when the culture was 11 d old—□; when the culture was 21 d old—×; on other days when culture < 25 d old—●. The regression lines are given by $a(440) = 0.0146 + 0.0310C$ with $r^2 = 0.996$ and $n = 12$ for *P. suecica* and $a(440) = 0.0037 + 0.0210C$ with $r^2 = 0.988$ and $n = 15$ for *H. elongata*. Measurements of attenuation (not presented here) on the same cultures show that specific attenuation is also independent of C .

tions. The figure shows that the relationship remains linear, even at concentrations as high as 10^3 mg m^{-3} . Estimation of specific absorption at 440 nm, $a^*_c(440)$, by linear regression of a on C yields: $a^*_c(440) = 0.0310 \text{ m}^{-1} (\text{mg m}^{-3})^{-1}$ for *P. suecica* and 0.0201 for *H. elongata*. Linearity shows that Beer's law is valid under the experimental conditions.

We have checked that the relationship between attenuation coefficient and concentration is also linear. Consequently, the relationship is linear for the scattering coefficient too. According to van de Hulst (1957), it is then safe to assume that only single scattering is of importance in the measurements. This conclusion is important, since the theory used here does not account for multiple scattering.

These specific absorption coefficients are therefore inherent properties of the cultures studied, independent of concentration. This fact is established at 440 nm (which corresponds to the absorption maximum); it may be reasonably assumed that the same holds at other wavelengths. In fact, the spectra of specific absorption obtained on different days are very similar (Fig. 4A, B). However, in the case of *P. suecica*, in the

spectral range from about 460 to 490 nm, the variability is higher than elsewhere. This variation apparently is related to changes in the ratio of carotenoid to Chl a in the culture. In the case of a culture of *Thalassiosira pseudonana*, Kiefer et al. (1979) observed a progressive deformation of specific absorption spectra with the age of the culture (5–17 d). Such a deformation, probably due to changes in the physiological state of the cells or to relatively high accumulation of detritus, was not observed in our cultures. However, the specific absorption spectra of *C. protuberans* grown under differing illumination show notable scatter (Fig. 4C). Lazara (1983) linked this variability to changes in the intensity of light supplied to the cultures, though not to the wavelength of light. It therefore follows that the specific absorption spectrum of an individual species can be considered as an intrinsic property only if changes in the environment have not produced alterations in the light-harvesting mechanisms of the organism. The problem of chromatic adaptation of phytoplankton has been discussed elsewhere (Lazara 1981, 1983). Here, we stress that the observed spectra are not influenced by conditions of measurement—notably the high pigment

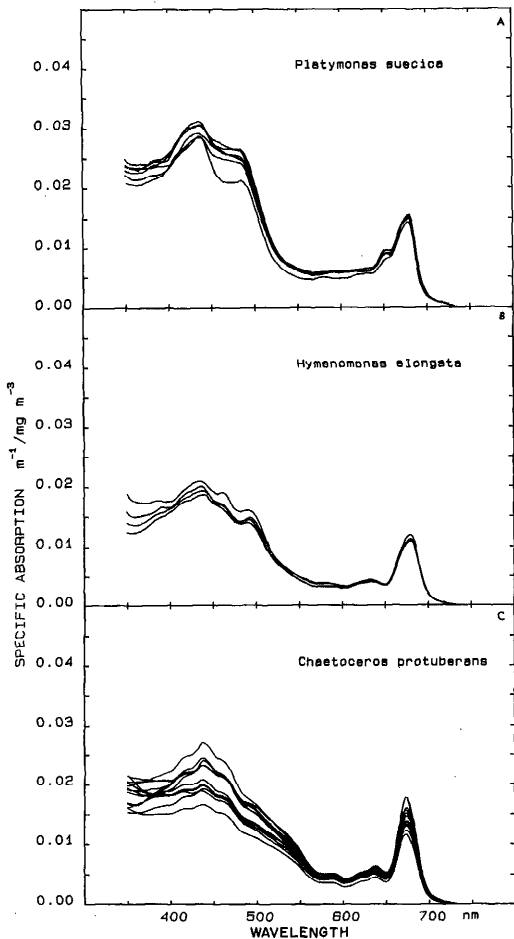


Fig. 4. Spectra of in vivo specific absorption of *P. suecica*, *H. elongata*, and *C. protuberans*.

concentrations of the cultures. These experiments, however, do not permit isolation and estimation of any nonlinear interactions within the algal cells.

Effect of variability in pigment composition—Figure 5 shows the spectra of acetone extracts, corresponding to the eight spectra of in vivo specific absorption presented in Fig. 2. The spectrum of pure Chl *a* in 90% acetone is also shown for comparison. The figure clearly brings out the importance of absorption by pigments other than Chl *a*, notably in the 350–550-nm region. At 440 nm, the total pigment absorption in various cultures is 2–3 times that of Chl *a* alone. Some variability is also seen near 650 nm. These spectra represent a case where pos-

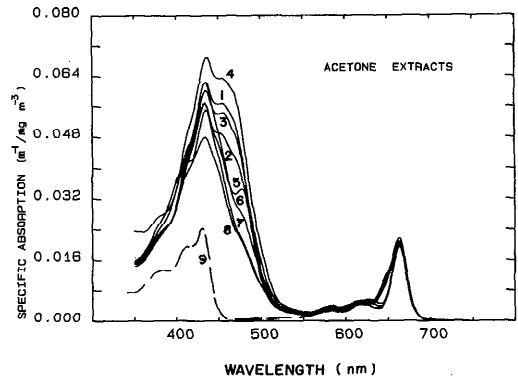


Fig. 5. Spectra of specific absorption of acetone extracts of the eight species studied. The spectra are normalized by $C(\text{mg m}^{-3})$ and correspond to the in vivo spectra presented in Fig. 2. The spectrum of specific absorption of pure Chl *a* in 90% acetone is also shown for comparison (curve No. 9).

sible causes of variability other than pigment composition (such as the particle effect or any eventual absorption by cell material other than pigments) have been eliminated. However, it is well known that when phytoplankton pigments are extracted from the cells with a solvent such as acetone, their absorption properties undergo changes due to the solvent. Acetone extracts thus cannot be used for quantitative evaluation of variability of in vivo specific absorption due to changes in pigment composition.

In Fig. 6, the eight in vivo absorption spectra are shown after normalization based on absorption at 440 nm. It appears from the figure that variations in the shape of the absorption spectra are less important than changes in the absolute values of absorption efficiency. It is seen that spectra of phytoplankton belonging to the same class and having similar pigment composition tend to group together. Diversity in spectral form is maximal where the auxiliary pigments are known to have absorption bands (e.g. Chl *b* at 650 nm, Chl *c* at 620 and 630 nm, carotenoids in a broad band from about 460 to 500 nm). Auxiliary pigments are also known to absorb light at 440 nm, as does Chl *a*, while at 675 nm, the absorption is principally due to Chl *a*. Disregarding any possible particle effect, the variability of the 675-nm band height in the normalized spectra can be taken to reflect variability in ab-

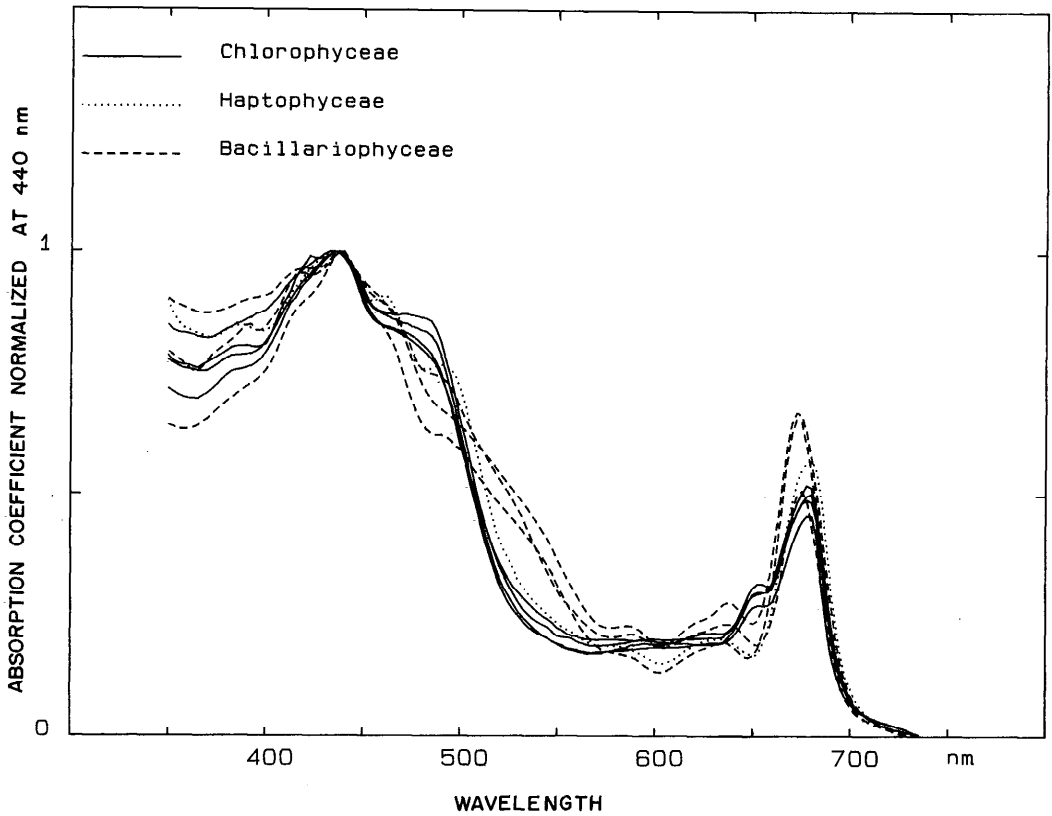


Fig. 6. The specific absorption curves of the eight species normalized at 440 nm. (The same curves are presented in absolute values in Fig. 2.)

sorption by auxiliary pigments at 440 nm. These arguments also indicate that pigment composition could be an important factor in explaining the variability of in vivo absorption efficiency.

Equation 1 is valid in the case of a single absorbing material with concentration C . Since the total absorption coefficient obeys the principle of superposition, the equation can be rewritten for the case of n substances with concentration C_i , and specific absorption coefficients a^*_{ci} ,

$$a(\lambda) = \sum_{i=1}^n C_i a^*_{ci}(\lambda). \quad (8)$$

If the hypothesis that all the variability in the in vivo specific absorption can be explained by variations in pigment composition is valid, it follows that, for our sample set,

$$a^*(\lambda) = a^*_{c1}(\lambda) + \sum_{i=2}^5 \frac{C_i}{C_1} a^*_{ci}(\lambda). \quad (9)$$

Here, $a^*(\lambda)$ is defined as the observed in vivo absorption per unit of concentration of Chl a . C_1 is the concentration of chlorophyll a , and a^*_{c1} is the in vivo absorption per unit of Chl a in the absence of all other pigments. Corresponding parameters for Chl b , Chl c , carotenoids, and the pheopigments are represented by subscripts 2–5.

This hypothesis was tested by multiple regression at 440 nm for 28 samples. Here, $a^*(440)$ was taken as the dependent variable and the concentrations of Chl b , Chl c , carotenoids, and pheopigment per unit of concentration of Chl a as the independent variables. An overall regression coefficient of 0.66 was obtained, implying that the changes in absorption efficiency are related to those in pigment composition. However, the re-

lation explains only 44% of the observed variability.

Particle effect—According to Eq. 4–7, the factor F is computed from the parameter ρ' ($=a_{cm}d$), if the particles are spherical and homogeneous. Our measurements of a , N , and d permit us to estimate ρ' for any wavelength λ with Eq. 4 and 6. Equation 4 being a monotonic relationship between Q_a and ρ' , we estimated the parameter ρ' by successive approximation. The values of ρ' (440) for the 28 samples were found to vary from 0.15 to 5.87, which means that the factor of spectral flattening F varies from 0.95 to 0.24. The particle effect is therefore far from negligible for this sample set.

We now define

$$a_{sol}^* = \frac{a_{sol}}{C_1} = \frac{Nv}{C_1} a_{cm} = \frac{Nv\rho'}{C_1 d}. \quad (10)$$

If the hypothesis is true that the particle effect is the only significant cause of variability in phytoplankton absorption efficiency, then a_{sol}^* would be a constant, as it represents the in vivo absorption efficiency in the absence of any particle effect.

It should be pointed out that ρ' estimated in the manner described above may be highly erroneous for high values of ρ' (Fig. 7). The relationship between Q_a and ρ' being nonlinear, a given error in Q_a does not always introduce the same error in ρ' . Figure 7 shows that, for a given error in Q_a , the lack of precision in the value of ρ' estimated from Q_a increases with the actual value of ρ' (the error in Q_a is the cumulative effect of the errors in a , N , and d). For our hypothesis testing, we therefore selected only 20 of the 28 samples, with estimated ρ' (440) values below 2.9. For these 20 samples, a_{sol}^* (440) was found to vary from 0.027 to 0.073 $m^{-1} (mg\ m^{-3})^{-1}$. It thus appears that the flattening effect in itself cannot account for all the variations in phytoplankton absorption efficiency, even though it is an important factor.

Combined effect of flattening and variations in pigment composition—We now examine the possibility that the variations in absorption efficiency, which persist even after eliminating the particle effect, are due to

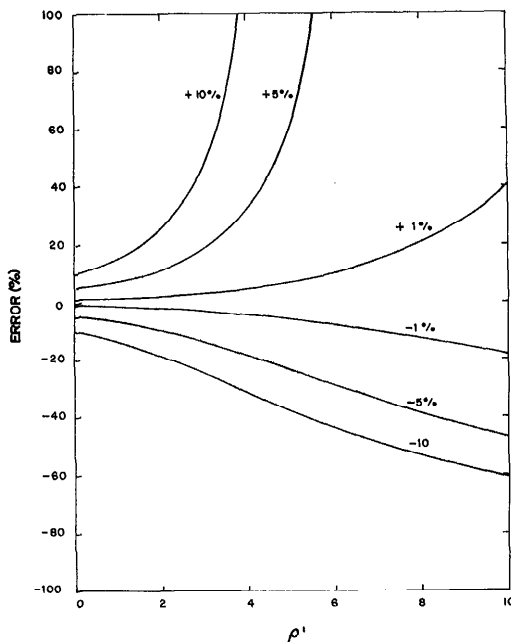


Fig. 7. Error in ρ' as a function of ρ' and for a given error in Q_a ($\pm 1\%$, $\pm 5\%$, or $\pm 10\%$). The asymmetry between positive and negative errors is very high. Error amplification observed here does not occur when Q_a is estimated from ρ' (error in Q_a < error in ρ' evident from Fig. 1).

changes in pigment composition. Equations 9 and 10 are combined as follows:

$$a_{sol}^* = a_{sol\ 1}^* + \sum_{i=2}^5 \frac{C_i}{C_1} a_{sol\ i}^*. \quad (11)$$

Here, the specific absorptions are analogous to those in Eq. 9 but represent cases wherein the flattening effect has been eliminated. The validity of Eq. 11 was tested as before, by multiple regression (a_{sol}^* as the dependent variable and C_i/C_1 , $i = 2-5$ as the independent variables). For the subset of 20 samples at 440 nm, we obtained a correlation coefficient of 0.88. Equation 11 can be rewritten in a slightly different manner, as

$$a_{sol} = a_{sol\ 0}^* + \sum_{i=1}^5 C_i a_{sol\ i}^*. \quad (12)$$

Equation 12 admits a constant background absorption $a_{sol\ 0}^*$ in addition to absorption by the five pigments. Multiple regression of the 20 samples at 440 nm yielded a coeffi-

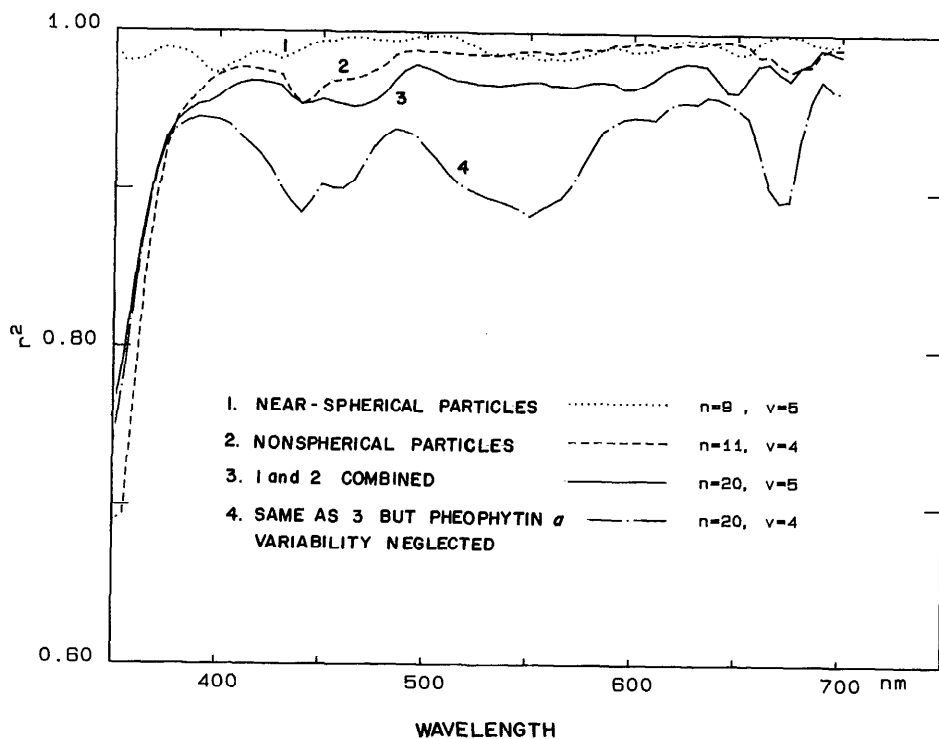


Fig. 8. Spectral values of the coefficient of determination, r^2 , for multiple regression between intracellular absorption coefficient and pigment concentrations (Eq. 12). Curve 1—the case of near-spherical particles (the first nine samples from Table 1) with five pigments. Curve 2—the case of nonspherical particles (samples 10–20 of Table 1) with four pigments (Chl *b* is absent in this group). Curve 3—cases 1 and 2 combined. Curve 4—the case wherein the samples are the same as in the previous case, but variations in pheopigments have been neglected. (Number of observations— n ; number of variables— v .)

cient of determination (r^2) of 0.96 (with a_{sol} as the dependent variable and C_i , $i = 1-5$ as the independent variables). It thus appears that, at 440 nm, the combined effects of spectral flattening and variation in pigment composition account for about 96% of the variation in absorption efficiency.

The coefficient of determination, which is a measure of the percentage of variance explained by the hypothesis, was then calculated for every 10 nm across the spectrum (Fig. 8). It is seen that r^2 is generally higher than 95%, except for wavelengths <390 nm. Figure 8 also shows the spectral values of r^2 when pheopigment variability is neglected. It is seen that there is a marked decrease in r^2 at almost all wavelengths, even though pheopigment concentrations are generally low in the samples.

The particle effect considered here assumes that the particles are spherical and

that the absorbing material is uniformly distributed within the particles. Neither of these assumptions is true in the case of phytoplankton. Eleven samples of the subset of 20 represent nearly spherical phytoplankton. The remaining nine samples of diatoms, with their nonspherical shapes and vacuoles within cells, depart most from the initial assumptions. The 20 samples were subdivided into two groups of near-spherical and nonspherical particles, and the coefficient of determination recalculated for the two groups separately. From Fig. 8 we see that there is a systematic increase in r^2 at almost all wavelengths for both groups, as expected if the degree of correlation remains the same and sample size is decreased. In the case of the near-spherical particles, the value of r^2 remains very high even for wavelengths <390 nm. It thus appears that the predictive precision of the model can be

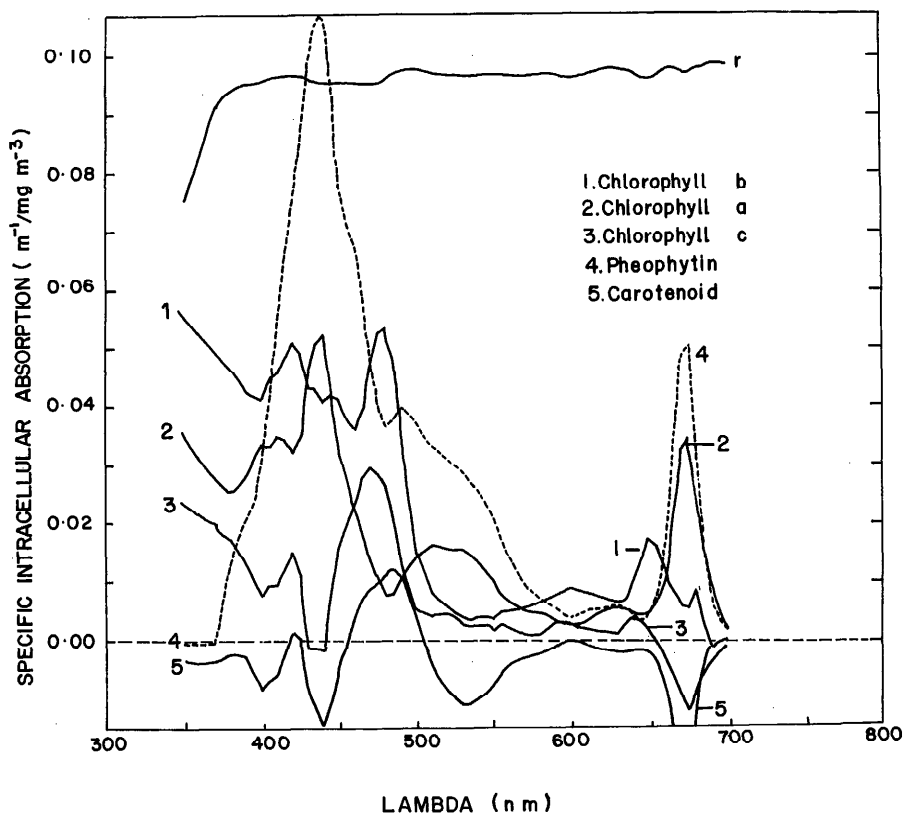


Fig. 9. The specific intracellular absorption for the five pigments from multiple regression (corresponding to curve 3 of Fig. 8). The spectral values of the regression coefficient r are also given.

improved very little above 390 nm by considering variations in shape.

Spectral values of specific intracellular absorption—The spectral values of the coefficients $a^*_{sol i}$ given by the multiple regression (Eq. 12) represent the in vivo intracellular specific absorption of the five pigments in the absence of any particle effect. These curves are shown in Fig. 9.

It is seen that some of the spectral values drop below zero, particularly in the case of carotenoids. The negative values occur at wavelengths where no absorption bands are expected for the respective pigments. When multiple regression is applied to a sample set wherein the independent variables do not satisfy the condition of orthogonality, the coefficients are unstable and may have incorrect signs and magnitudes (Hoerl and Kennard 1970*a,b*; Marquardt 1970; Jones 1972). There is additional evidence that the

effects of the different pigments have not been separated correctly, for example the presence of the peak near 420 nm in the curves of three pigments with a corresponding depression in another curve. The details of curves therefore have to be interpreted with caution. Only major features are resolved reliably.

Chlorophyll *a* has a peak value of about $0.05 \text{ m}^{-1} (\text{mg m}^{-3})^{-1}$ in the blue and a secondary peak of around 0.035 in the red, which are comparable with the curve obtained by Kiefer and SooHoo (1982). However, their pheopigment absorption curve does not match ours. The results of Kiefer and SooHoo showed that pheopigment-specific absorption in the blue was about four times that of Chl *a*. Their curve does not show any pronounced peak in this region; instead, the absorption values increase progressively toward shorter wavelengths. Their

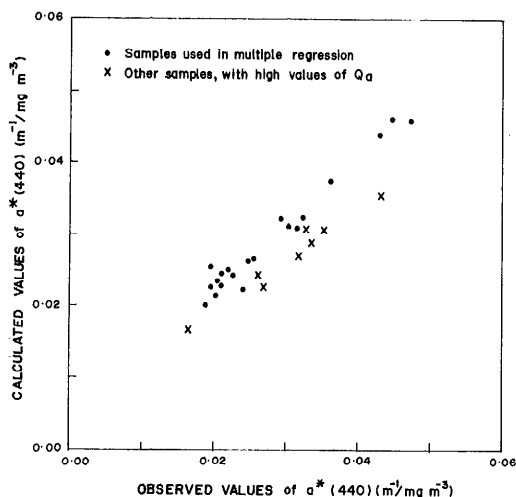


Fig. 10. Comparison between observed and calculated values of the specific absorption at 440 nm for the 28 samples. Here, the values are normalized with respect to the concentration of Chl *a* only. Calculated values of specific absorption are obtained from the concentrations of pigments, the particle size, the number of particles per unit of volume of the medium, and the intracellular specific absorption coefficients given in Fig. 9. Samples not used in the multiple regression because of their high Q_a values— \times .

peak in the red is very weak, around $0.02 \text{ m}^{-1} (\text{mg m}^{-3})^{-1}$. In our results, the curve associated with pheopigments shows a well-defined absorption peak at 440 nm (and not at 410 nm as in the case of acetone extracts). The peak value is about twice that of Chl *a*. The secondary peak in the red is also higher than the Chl *a* peak, with a value of around $0.05 \text{ m}^{-1} (\text{mg m}^{-3})^{-1}$. These differences are probably because Kiefer and SooHoo applied multiple regression directly to observed *in vivo* absorption of samples, disregarding the particle effect, and because only two pigments were considered (Chl *a* and pheopigments). Besides, they worked on natural seawater samples (from coastal waters of Baja California), with possible contamination of the absorption signal from materials other than phytoplankton. In Fig. 9, the Chl *a* and pheopigment curves show absorption in the 490–540-nm region, which is totally absent in the extracted pigments. This absorption may be linked with the proteins associated with the pigments.

Reconstruction of in vivo absorption from

pigment concentrations, cell size, and cell number density—The values of a_{sol} at 440 nm were calculated from the concentrations of the five pigments with the corresponding five $a_{\text{sol},i}^*$ values (Eq. 12). $a_{\text{sol},0}$ was neglected as a regression parameter having no physical significance. *In vivo* absorption was then calculated with data on the size and number of cells (through Eq. 3–6). In Fig. 10, these values are plotted against the observed values of $a(440)$. The eight samples with high values of Q_a (which consequently were not used for the multiple regression) are identified by a symbol different from those used in the analysis. The calculated values compare well with the observed ($r^2 = 86\%$), although there is a slight tendency for the samples with high Q_a to align below the others.

Conclusion

Laboratory studies on eight phytoplankton species show that variations in specific absorption coefficient (from 400 to 700 nm) can be satisfactorily explained in terms of a particle effect and pigment composition. Neglect of either factor leads to a marked decrease in the correlation. For our sample set, consideration of shape as an additional factor yields no significant increase in the explained variance for wavelengths above 390 nm. The 20 samples used in multiple regression were insufficient to separate satisfactorily the intracellular absorption spectra of the five major pigments considered here. Better separation requires a larger data set selected in a manner that minimizes covariance between pigments.

The model presented here offers a tool for calculating the *in vivo* absorption coefficient of phytoplankton in the sea by using information on pigment concentrations and size spectra of cells in the absence of complicated optical measurements.

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Submitted: 3 March 1986
Accepted: 29 August 1986