

Quantitative in vivo absorption spectra of phytoplankton: Detrital absorption and comparison with fluorescence excitation spectra¹

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Abstract

Spectral absorption of phytoplankton from cultures and natural samples was measured on filters with various optical setups including collimated and diffuse irradiation and measurements of wetted filters within an integrating sphere. In suspensions within an integrating sphere, specific absorption coefficients for laboratory cultures varied by a factor of only two.

Measurements on filters yielded values dependent on filter load. Specific absorption coefficients derived from measurements of sample filters were considerably higher than values obtained from suspensions in an integrating sphere due to increased diffuseness of irradiance and to pathlength amplification by filter-particle and particle-particle interactions. Measured absorption of phytoplankton in the blue can be increased greatly by absorption of detritus, evident from absorption spectra of depigmented samples on filter. After subtracting detrital absorption, absorption spectra of phytoplankton are qualitatively similar to the corresponding quantum-corrected fluorescence excitation spectra. The detritus-corrected ratio of absorption at 440 vs. 675 nm shows average values between 1 and 1.5.

Comparison with published values shows that specific in vivo absorption coefficients of phytoplankton are mostly overestimated as a result of the methodology applied. In the blue region of the spectrum, overestimation of phytoplankton absorption in field samples is possible if detrital absorption is neglected.

The standard experimental approach to measuring absorption of scattering samples is to place the sample inside an Ulbricht (integrating) sphere (Grum and Becherer 1979), thus collecting all scattered light in addition to transmitted light. Measuring seawater at natural concentrations of chlorophyll inside an integrating sphere calls for extremely high resolution and stability of radiative quantities because of high background absorption by water and dissolved matter. Means of achieving such resolution are discussed elsewhere (Haardt and Maske 1987).

Shibata et al. (1954) proposed a method in which opal glass is placed between the sample and the detector, thus changing the radiance detector of the photometer to a detector of irradiance (cosine characteris-

tic). This method will therefore yield values similar to the diffuse attenuation coefficient k_d (Preisendorfer 1976, v. 5),

$$k_d = D \cdot a + b_b$$

where a is the absorption coefficient, b_b the backscattering coefficient, and D the distribution function. $D = 1$ for collimated light and $D = 2$ for uniform radiance distribution. Because $D \geq 1$ and in addition b_b occurs, data obtained by this method overestimate the real absorption coefficient. As judged by the spectra of total pigment extracts of plant material, the backscatter coefficient of phytoplankton is low enough to allow for satisfactory spectral signatures of the opal glass technique (Bricaud et al. 1983).

Yentsch (1960) found a convenient way to make a similar method usable for natural phytoplankton samples. The absorbing particles are concentrated on a membrane filter and placed in the sample compartment of a spectral photometer. Thus the filter serves at the same time as a diffuser plate, substi-

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tuting for the opal glass diffuser. The filter method allows attenuation measurements of dilute suspension because the sample is concentrated on the filter. The great sensitivity and simplicity of the method has led to its considerable popularity among researchers (Kiefer and SooHoo 1982; Dubinsky et al. 1984; Faust and Norris 1982; Gieskes pers. comm.).

The methods we investigated are intended to produce quantitative specific absorption data under collimated or diffuse states of illumination. For application in calculating and interpreting the quantum yield of fluorescence and of photosynthesis, as well as chromatic adaptation and the upwelling sea surface radiance, the spectra thus obtained must be weighted with the appropriate state of diffuseness within the water column.

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Methods

Absorption coefficients were measured as follows.

1. "Filter-collimated light" spectra were obtained with the procedure of Yentsch (1960) by sample filtration on an 0.8- μ m membrane or glass-fiber filter and placement of the filter next to the spectral photometer detector window (Zeiss PM6) with the sample toward the light source. The choice of filter material did not alter results. The filter was mounted between a glass on the detector side and a 2-mm-thick diaphragm covered by glass on the light source side thus preventing the filter from drying during measurement. Spectral bandwidth was 2 nm for all measurements, spectral increment was 5 nm between 670 and 680 nm and 10 nm otherwise. Wavelength accuracy was >1 nm. 100% transmission was set according to a clean, wetted membrane filter in the same position.

2. For the "filter-integrating sphere" measurements, the bare sample filter was held in the center of an integrating sphere by a glass rod without being covered. The 11-cm-diameter sphere (Spindler and Hoyer, Göttingen) was placed in the sample compartment of a spectrofluorometer (Shi-

madzu RF 540). The instrument was operated in the synchronous scan mode, where the transmission wavelength of light source and detector monochromator were set at the same wavelength. Spectral bandwidth for the light source was 2 nm and for the detector 30 nm. The sphere had three ports: toward light source, sample entry, and the detector. The latter had a baffle to shield the port from specular reflection. Sample filters were dried at 45°C under reduced atmospheric pressure over silica gel. The dried filters were wetted with immersion oil (index of refraction = 1.52, matching the one of glass fiber). The sample filter was suspended at about 10° between the normal of the filter plane and the optical axis to prevent specular reflection back into the excitation window. The loaded side of the filter faced the incoming light. Reference measurements were made with a clean filter or depigmented filter wetted with immersion oil. The effect of drying on the filtered sample was routinely controlled by chlorophyll analysis with the trichromatic method on both the original and dried filter. No significant change in chlorophyll concentration was measured.

Phytoplankton pigments were selectively extracted from sample filters to obtain the absorption of nonchlorophyllous particles. The depigmented filters were obtained by washing each twice with acetone and methanol (3 min) while the filters were still in the filter holder. Afterward the filters were treated like the sample filters. Dinoflagellate samples needed to be extracted with methanol four times. The degree of depigmentation was determined by chlorophyll measurements on depigmented and homogenized filters. Between only 0 and 5% of the original chlorophyll concentrations of the samples were found in depigmented filters.

3. "Suspension-collimated light" values were obtained by measuring phytoplankton suspensions in cuvettes 200 mm long within an integrating sphere of 450-mm diameter. Details of this method are described elsewhere (Haardt and Maske 1987). Reproducibility was at the 0.0002-OD level. Spectral bandwidth was 2 nm and wavelength accuracy within 0.2 nm. As a reference, fil-

Table 1. Dominant species in samples from Kiel harbor.

	(cells ml ⁻¹)	($\mu\text{g C liter}^{-1}$)
Sample A		
<i>Leptocylindrus danicus</i>	118	16
<i>Heterocapsa triquetra</i>	22	4
<i>Gyrodinium</i> sp.	0.7	4
Sample B		
<i>Heterocapsa triquetra</i>	648	127
<i>Leptocylindrus danicus</i>	635	84

tered samples (0.2- μm Nucleopore, differential pressure 0.2 bar) were used to exclude absorption of water and Gelbstoff.

Fluorescence excitation and emission spectra were measured in a spectral fluorometer (Shimadzu RF 540) in 1-cm² cuvettes equipped with a miniature magnetic stirrer. The fluorometer was equipped with red-sensitive photomultipliers (R928 SF) in both channels. Excitation (390–690 nm) was measured with a spectral bandwidth of 2 nm, wavelength accuracy within ± 1 nm, and emission (730 nm) with 30-nm spectral bandwidth. The excitation spectra were corrected for a constant quantum response of the fluorometer with 0.0114 M Oxazin 1 (Lambda Physik) measured in triangular cuvettes, excitation 360–720 nm, emission 730 nm (Kopf and Heinze 1984). Every day the Oxazin stock solution was remeasured and the new spectrum used for normalization. According to the peak height of the Oxazin spectra, the fluorometer response varied randomly during the 2-week measuring period 6% (SE). Constant quantum response of the Oxazin was verified with a thermopile in place of the sample. Excitation energy of the illumination was about 1 W m⁻² at 440 nm and 2-nm bandwidth. Excitation illuminated about 30% of the sample volume.

Chlorophyll concentration was measured by the trichromatic method (Jeffrey and Humphrey 1975). Samples were extracted in 90% acetone with glass beads in a homogenizer for 5 min. In the case of *Nannochloris* sp., chlorophyll was extracted with methanol and glass beads at 80°C in a homogenizer. In this case Chl *a* was calculated without subtraction of Chl *b* and Chl *c* absorption overlaps (Marker et al. 1980).

Cells were counted and their size determined visually with an inverted (Utermöhl) microscope (10-ml counting chamber).

Cultures were grown in natural seawater of 30‰ (*Nannochloris* sp., 15‰) with nutrients added at f/20 concentration (Guillard and Ryther 1962). Culture illumination by "cool-white" fluorescent bulbs was 1.5×10^{-4} mol quanta m⁻² s⁻¹ except for dark-adapted cultures where it was 5×10^{-6} . Cultures were diluted at least once a week. Culture strains were obtained from M. Elbrächter (BAH, List). They were originally isolated from the North Sea. The cell sizes were: *Thalassiosira eccentrica*, 36–50- μm diameter, 17–40 μm high; *Scrippsiella trochoidea*, 16- μm diameter, 23 μm long; *Cricosphaera carterae*, 10–12- μm diameter. *Nannochloris* sp. was obtained from the aquaculture project at Kiel/Bülk. Samples from Kiel harbor were taken from the laboratory pier. For these samples phytoplankton carbon of the dominant species was calculated (Edler 1979) for better comparison and is shown together with cell numbers in Table 1.

Results

Our various methods consistently showed an absorption peak at 675–677 nm for seawater samples (e.g. see Fig. 5). The absorption maximum in the blue was at 440 nm except with the *S. trochoidea* culture where it was at about 460 nm. Specific absorption coefficients, a^* [m² (mg Chl *a*)⁻¹] were obtained by dividing measured absorption coefficients by the in vitro chlorophyll concentrations of the samples. Data are presented in Tables 2 and 3 with those obtained on the same day listed on one line. Absorption spectra taken with the filter-collimated light method showed considerable absorption in culture samples at 730 nm where no phytoplankton absorption was present as shown by the culture sample values obtained with the suspension-integrating sphere value (Table 2). Samples from Kiel harbor showed considerable absorption at 730 nm with each of the methods. All data presented for the filter-collimated light method were corrected by subtracting a baseline value as measured at 730 nm. The suspension-integrating sphere values of Kiel

Table 2. The specific absorption coefficients [$\text{m}^2 (\text{mg Chl } a)^{-1}$] at wavelengths 675/440 nm.

Sample	Suspension-integrating sphere*	Filter-collimated light†	Filter-integrating sphere*‡
<i>Scrippsiella trochoidea</i> §	—	0.024/0.040	0.017/0.023
	0.013/0.018	0.022/0.036	0.017/0.016
	0.013/0.018	—	0.013/0.010
<i>Thalassiosira eccentrica</i>	0.010/0.015	0.017/0.024	0.012/0.018
	0.011/0.020	0.026/0.038	0.019/0.025
	—	0.018/0.025	0.013/0.017
<i>Cricosphaera carterae</i>	—	0.024/0.040	0.018/0.027
	0.011/0.013	0.022/0.032	0.020/0.026
	0.007/0.008	0.023/0.030	0.022/0.022
	0.009/0.011	—	0.010/0.014
	0.015/0.021	—	—
Nannochloris sp. Kiel harbor	0.017/0.045	0.033/0.080	0.030/0.042
	0.009/0.019†	0.022/0.045	0.017/0.017

* Collimated light.

† Value at 730 nm subtracted.

‡ Detritus excluded.

§ Maximum value at 460–470 nm used.

harbor samples also had the 730-nm absorption subtracted.

Calculated specific absorption coefficients at 675 nm of the filter-collimated light method plotted against filter loading (the amount of pigment per filter area) show that with increased filter loading the specific absorption decreases (Fig. 1). The continuous curve represents coefficients obtained from a series of dilutions obtained with one sample. The relationship of coefficients and filter load shows considerable variability if all

data points of this method are considered (Fig. 1). The absorption coefficients obtained with the filter-collimated light method and other filtered sample data presented in Table 2 were chosen from samples with higher filter loads. This selection resulted in average coefficients of specific absorption obtained with the filter-collimated light method of $0.023 \text{ m}^2 (\text{mg Chl } a)^{-1}$.

When the sample filter was placed inside the integrating sphere, measurements yielded average filter-integrating sphere values

Table 3. The ratio of absorption at 440 nm to that at 675 nm measured by different techniques and the ratio of fluorescence excitation at 440 and 675 nm.

Sample	Suspension-integrating sphere*	Filter-collimated light	Filter-integrating sphere*†	Fluorescence	Acetone extract‡
<i>Scrippsiella trochoidea</i> §	—	1.66	1.35	1.48	3.12
	1.38	1.60	0.96	1.38	3.03
	1.30	—	0.77	1.75	2.77
<i>Thalassiosira eccentrica</i>	1.37	1.42	1.50	1.30	2.52
	1.78	1.46	1.32	1.23	2.56
	—	1.39	1.28	0.97	2.17
<i>Cricosphaera carterae</i>	—	1.64	1.43	1.14	2.31
	1.23	1.44	1.34	1.21	1.97
	1.27	1.34	1.00	1.11	2.07
	1.15	—	1.04	1.07	2.13
	1.40	—	—	—	2.01
Nannochloris sp. Kiel harbor	2.65	2.56	1.40	1.16	2.89
	2.11	2.00	1.00	1.31	2.62

* Collimated light.

† Detritus excluded.

‡ Maximum value at 665 nm used.

§ Maximum value at 460–470 nm used.

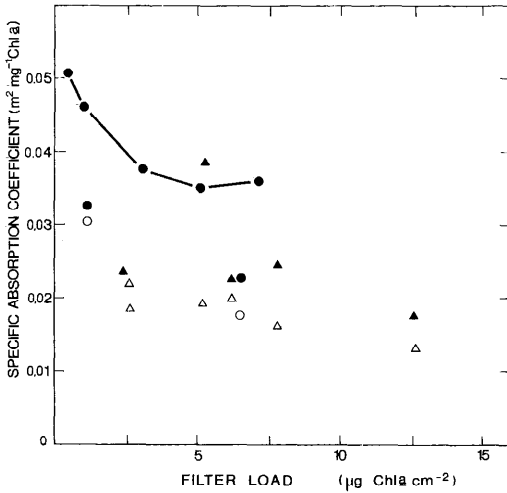


Fig. 1. Specific absorption coefficients vs. filter load. Filled symbols—values obtained with the filter-collimated light method; empty symbols—filter-integrating sphere method. Circles—samples from Kiel harbor; triangles—*Cricosphaera carterae*. Connected data points—dilutions from one sample.

at 675 nm of $0.019 \text{ m}^2 (\text{mg Chl } a)^{-1}$. Absorption spectra were calculated by subtracting the depigmented filter absorption from sample absorption. Similar to the filter-collimated light method, this method produces coefficients of filter load-dependent specific absorption as indicated in Fig. 1. Significant detrital absorption was measured in samples from Kiel harbor (Fig. 2), although no significant detrital absorption could be found in samples of cultures. The detritus spectra show a nearly featureless increase toward the blue (Fig. 2) of approximately logarithmic character (Fig. 3). To investigate whether treatment of depigmented filters might have altered the absorption spectra of the detritus, we measured detritus absorption with a sample of 4-week-old *S. trochoidea* culture. The culture had deteriorated to a point that no in vivo fluorescence was detectable and apparently it contained only detrituslike material. The sample filter and the depigmented filter of this culture showed no significant difference in absorption; we therefore concluded that the organic solvent treatment made no alteration in the spectral characteristics of the detritus.

The suspension-integrating sphere meth-

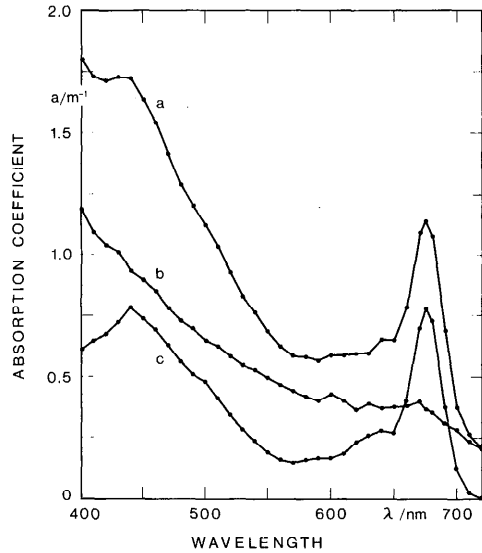


Fig. 2. Absorption spectra of sample B from Kiel harbor measured with the filter-integrating sphere method. Curve a—spectrum of particulate material; b—spectrum of detritus; c—spectrum of phytoplankton. To obtain absolute absorption coefficients these values still must be corrected for methodological absorption enhancement.

od permits determination of absorption spectra of three separate components in seawater: particles (a_{part}), dissolved matter (a_y), and water (a_w). Absorption of the sample suspension is given by

$$a = a_{\text{part}} + a_y + a_w.$$

The absorption coefficient of particles was calculated by subtracting the absorption spectra of suspension filtrate ($a_w + a_y$) from the sample suspension a . For Kiel harbor samples we subtracted the absorption measured at 730 nm. Culture samples showed zero absorption at 730 nm. The specific absorption coefficients obtained by this method were lower than those of other methods investigated. Values obtained at 675 nm were between 0.007 and $0.017 \text{ m}^2 (\text{mg Chl } a)^{-1}$ (Table 2). This range of values is the result of the optical property of the individual cells of the sample and not related to methodological noise or systematic errors such as concentration dependence (Haardt and Maske 1987). With methods other than the filter-integrating sphere, samples from Kiel harbor show relatively high blue:red

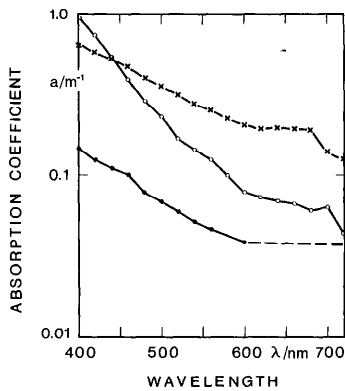


Fig. 3. Absorption coefficients of detritus including pigment-extracted algae and dissolved yellow substance from Kiel harbor samples. Sample A: Chl *a* in sample 7.21 mg m^{-3} , in depigmented sample 0.39 mg m^{-3} , detritus—●, yellow substance—○. Sample B: Chl *a* in sample 52 mg m^{-3} , in depigmented sample 6.3 mg m^{-3} , detritus absorption—×.

ratios of absorption (cf. Table 2 and Fig. 4)—roughly twice those in cultures (Table 3).

The absorption coefficient of yellow substance (a_y) was calculated from absorption of the filtrate ($a_w + a_y$) and water absorption (a_w). Dissolved yellow substance showed considerable difference in spectral character from detrital absorption (Fig. 3). The influence of pigment content per cell on specific absorption was investigated with dark-adapted cultures of *C. carterae* and *S. trochoidea*. Their specific absorption coefficients at 675 nm [0.009 and $0.013 \text{ m}^2 (\text{mg Chl } a)^{-1}$] were not significantly different from normally grown cultures (cf. Table 2).

Fluorescence excitation spectra were taken at natural concentrations in the visible part of the spectrum including the 675-nm absorption peak of Chl *a*. Detector wavelength was set at 730 nm. We excluded the possibility that the excitation spectra were distorted due to the measurement of photosystem I emission by comparing emission spectra (bandwidth, 2 nm) of a concentrated natural population from Kiel Bay. When the excitation wavelength was varied between 450 and 670 nm (bandwidth, 2 nm) the relative shape of the emission spectra did not vary. We concluded that there would be no major qualitative difference in excitation

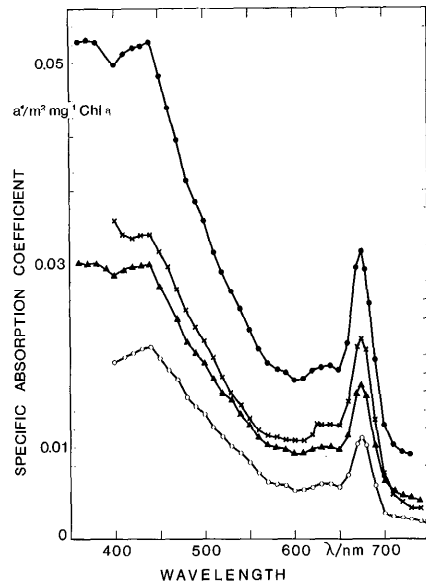


Fig. 4. Specific absorption spectra of sample A from Kiel harbor. Suspension-integrating sphere method—○; filter-diffuse light—▲; filter-integrating sphere—×; filter-collimated light—●. The filter-diffuse light values are calculated by dividing the measured absorption coefficient by two.

spectra with emission set at either 685 or 730 nm.

As a first approximation we expected that all absorbed quanta have the same probability of exciting fluorescence, i.e. a spectrally independent quantum yield. Consequently there should have been reasonable agreement between quantum-corrected excitation spectra and the spectra of absorbance. This assumption seems generally justified; Table 3 shows agreement of the peak-height ratio of excitation with the filter-integrating sphere method within the reproducibility of the latter method. Figure 5 presents spectra of normalized absorption coefficients and fluorescence excitation taken over a 2-week period from a culture of *C. carterae*. The observed variability in absorption is probably not due to changes in pigment composition if the spectra of the acetone extract are any indication. Variability of the fluorescence excitation spectra was kept to a minimum by normalizing to an Oxazin reference spectrum taken shortly before measurements were made. Fluorescence excitation and absorbance peak-height

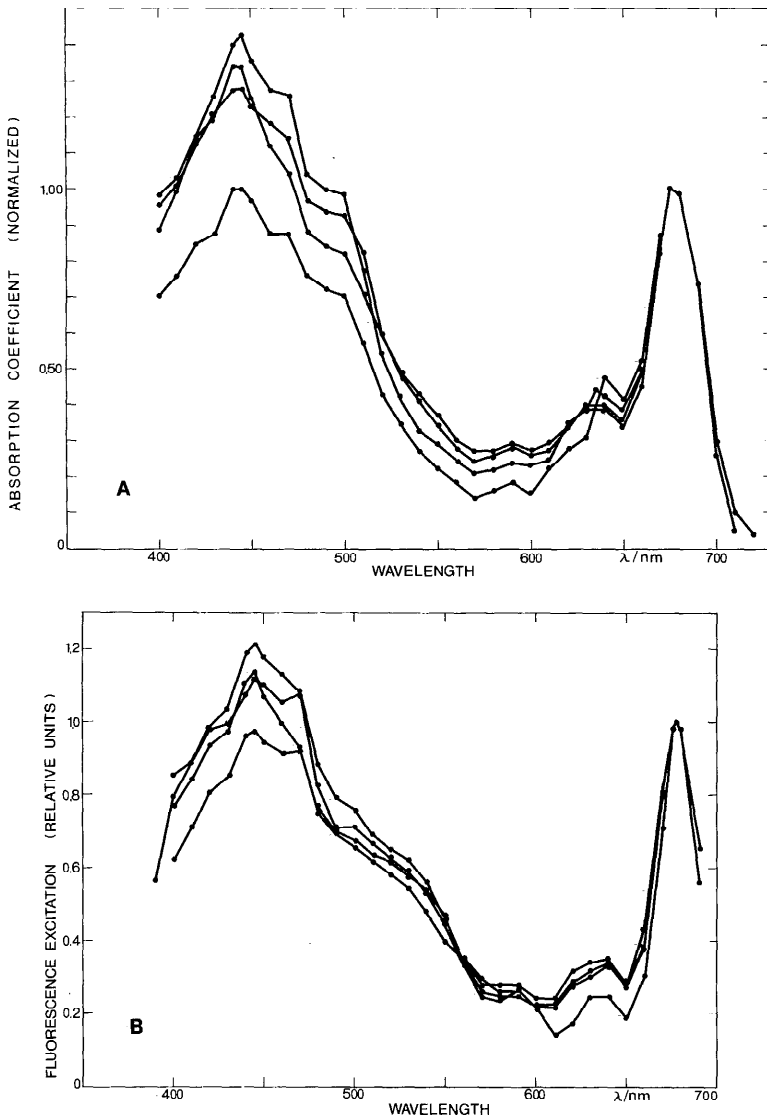


Fig. 5. A. Absorption spectrum of *Cricosphaera carterae* measured with the filter-integrating sphere method. Absorption is normalized at 675 nm. Separate samples were taken over a 2-week interval. B. Quantum-corrected fluorescence excitation spectrum of *C. carterae*. Excitation is normalized at 675 nm. Separate samples taken at the same time as in panel A.

ratios in the red and blue agree considerably better with culture samples (Table 3, Fig. 5) than with the Kiel harbor samples (Fig. 4).

Acetone extracts of the samples always presented a much higher absorption in the blue compared to any of the above-mentioned methods (Table 3). The absolute values at 665 nm are, as expected, close to $0.0202 \text{ m}^2 (\text{mg Chl } a)^{-1}$ —the specific *in vitro* absorption of Chl *a* in 90% acetone (Jef-

frey and Humphrey 1975). The absorption ratio 440:675 nm stayed rather constant within sample type, indicating no major change in the ratio of accessory pigments to Chl *a* during the sampling period.

Discussion

A knowledge of light absorption characteristics of phytoplankton is essential to understanding primary production in the sea.

Table 4. Measurements of chlorophyll-*a*-normalized in vivo absorption coefficients of phytoplankton for collimated light. (Not available—NA.)

Source	Method	Specific absorption coefficient [m ² (mg Chl <i>a</i>) ⁻¹]		Spectral bandwidth, resolution (nm)	Remarks
		440 nm	675 nm		
Morel and Prieur 1977	In situ irradiance	0.024	0.019	NA	Model calculations
Smith and Baker 1978	In situ irradiance	0.039–0.168		NA	Model calculations
Privoznik et al. 1978	Suspension close to photomultiplier	0.006–0.013	0.004–0.010	NA, 10	
Bannister 1979	Suspension/opal glass	0.030	0.020	NA, 5	Calculated from extract
Kiefer et al. 1979	Integrating spheres both sides of cuvette	0.050–0.050*	0.020–0.028	NA	Calculated from diffuse transmittance and reflectance
Yentsch 1980	NA	0.028	0.017	NA	
Morel and Bricaud 1981	Suspension/opal glass	0.036–0.092	0.020–0.031	NA	Culture suspension
Prieur and Sathyendranath 1981	In situ irradiance	0.036–0.092	0.020–0.031	NA, 5	Model calculations
Kiefer and SooHoo 1982	Filter and Butler photometer	0.022	0.012	1.0	Corrected by path-length amplification factor = 6
Yentsch and Phinney 1982	Filter/opal glass		0.026	0.5	
Bricaud et al. 1983	Suspension/opal glass		0.0125–0.022	NA	Same method as Morel and Bricaud 1981
Farmer et al. 1983	Filter/opal glass		0.034	NA	Subtract value at 720 nm
Kirk 1983	NA	0.015	0.009	NA	<i>Euglena</i> culture
Sathyendranath and Morel 1983	NA		0.010–0.026	NA	Laboratory measurements
Bannister and Weidemann 1984	Suspension/opal glass	0.036	0.019	NA	Scattering transmittance photometer

* Lowest values used to exclude detritus.

The wide range of values of specific absorption coefficients in the literature (Table 4) suggests that some of the variation might be due to methodological differences. Our own measurements produced method-specific absorption coefficients with progressively higher values for the suspension-integrating sphere, filter-integrating sphere, and filter-collimated light methods. Respective average values (Table 2) were 0.011, 0.019, and 0.023 m² (mg Chl *a*)⁻¹. For this limited data set, values varied between ±20 and 30% (SE) from the mean for the various methods. Some of this variability within the filter methods is systematic. In Fig. 1 the specific absorptions measured with the filter-collimated light and filter-integrating sphere methods are presented against filter

loading (μg Chl *a* cm⁻²). With both methods, specific absorption decreases with increasing filter load. This dependence has been observed by Clarke (1982*b*) for aerosol particles on dry membrane filters. We considered that the filter load will influence reflectivity or angular distribution of transmittance or both. For comparison we therefore measured the same wet sample filter in an integrating sphere. The resulting values were similar, however, to the filter-collimated light method. We conclude that absorption enhancement was not due to light scattered beyond the aperture of the receiver. Microscopic observation gave no indication that heterogeneous sample distribution on the filter could be a reason for the variation in specific absorption.

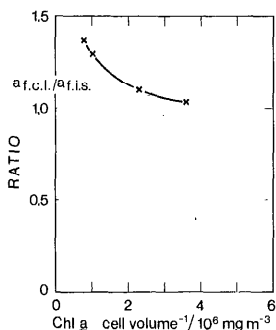


Fig. 6. Ordinate—ratio of specific absorption coefficients at 675 nm measured by the filter-collimated light method (f.c.l.) to that measured by the filter-integrating sphere method (f.i.s.); Abscissa—Chl *a* per cell volume.

The filter load dependence is generated through interaction of absorbing substances with nonabsorbing but scattering particles, leading to longer effective pathlength of light within samples. It leads to overestimation of absorption coefficients with amplification factors between 1 and >100 in the case of strong scattering and weak absorption (Butler 1962). In our case the filter takes the role of a nonabsorbing scatterer. Therefore, with less sample on the filter, specific absorption is enhanced more than with a higher filter load. We reduced filter scattering by drying and wetting the filter with immersion oil which has a refractive index close to glass (filter-integrating sphere method). The resulting absorption coefficients were lower than those obtained with the filter-collimated light method, probably as a result of reduced optical scattering compared to absorption (Table 2, Fig. 4). But the absorption coefficients were still well above absorption measured by the suspension-integrating sphere method.

The filter-integrating sphere absorption coefficients still show filter load dependence (Fig. 1). Both methods vary considerably in Fig. 1. The limited data set available does not indicate a dependence of specific absorption coefficients of either filtered-sample method on the optical density of the cells (cf. packaging effect: Duysens 1956). But the ratio of absorption coefficients obtained for the same samples and equivalent filter loads with the filter-collimated light and the filter-integrating sphere methods does show a

highly significant dependence on the chlorophyll concentration of the phytoplankton cells (Fig. 6). This dependence suggests increased amplification factors of the collimated-light method for decreasing Chl *a* per cell volume. The mechanism awaits further explanation.

The increased specific absorption generally measured for filtered samples is partly due to increased diffuseness within the sample layer because the refractive index of immersion oil (1.09) does not match that of phytoplankton (1.05). An indication of the state of diffuseness is given by the results we obtained by placing opal glass between the light source and the sample. We expected a doubling of absorption with diffusivity $D = 2$ (Kubelka 1947; Preisendorfer 1976, v. 5), but the actual increase was only about 20%. Apparently the light flux within the sample does not maintain a collimated state. We conclude that diffuseness of light within the particle layer is a major cause for overestimated absorption coefficients with filter methods. Particle-particle interaction may also lead to enhanced absorption in samples concentrated on filters, but this mechanism should be of minor importance because absorption coefficients at 675 nm (obtained with diffuse light and divided by two) did not significantly exceed those coefficients measured with the suspension-integrating sphere method.

In conditions of low chlorophyll concentrations, absorption is sometimes measured on sample-filter sandwiches. In this case, absorption is greatly enhanced due to high reflectivity of the filters. A detailed analysis of this method is given by Clarke (1982a). Under these conditions, Kiefer and SooHoo (1982) found that their absorption measurement yielded sixfold higher values than their reference method. In our case of a single filter layer, the filter-collimated light values are about two times the corresponding suspension-integrating sphere values. The calibration of methods utilizing filters is made more difficult by the sample-specific packaging effect (Haardt and Maske 1987). The values obtained with the various methods (Table 2) within our one study span nearly the entire literature range (Table 4). The suspension-integrating sphere method

with samples at close to natural concentrations seems to be the only one that yields absorption coefficients for collimated light with a minimum of scatter interaction. We conclude so from the fact that it consistently produced the lowest, and concentration-independent, specific absorption coefficients, and any kind of interaction would raise specific absorption.

Peak-height ratios 440:675 nm—Absorption measurements of healthy phytoplankton cultures show relative peak-height ratios (440:675 nm) between 1 and 2 (Kiefer et al. 1979). With age of culture or in natural samples (Fig. 4) absorption at 440 nm relative to 675 nm may increase considerably (cf. Table 3; Kiefer et al. 1979; Yentsch and Phinney 1982). Possible causes of this increase are accumulation of accessory pigments, changes in pigment content per cell or in cell morphology (packaging effect: Duysens 1956), buildup of dead phytoplankton cells with residual carotene and carotenoid content, accumulation of detritus (all of which would lead to absorption selectively in the blue), production of small light-scattering particles like bacteria or organics, or enhancement of water absorption by nonabsorbing scatterers. The first two factors are of physiological significance to primary producers; the others reflect relative abundance of primary producers to other particles.

We tried to differentiate between non-photosynthetic and photosynthetic absorption by subtraction of depigmented sample absorption (Fig. 2) from sample absorption. The results are apparent in Table 3 where the ratios of absorption 440:675 nm are presented. Samples from cultures show ratios between 1.15 and 1.78 with the suspension-integrating sphere method, indicating that the cultures were in a healthy state. Comparison between the suspension-integrating sphere and the filter-integrating sphere values suggests that in cultures of *T. eccentrica* and *C. carterae* nonbiological absorption was negligible. Samples from Kiel harbor show peak-height ratios above 2.0 for the suspension-integrating sphere and filter-collimated light methods. In these samples only the filter-integrating sphere method, where detrital absorption was sub-

tracted, yielded ratios similar to the fluorescence-excitation ratio. The latter can be used as a reference to indicate detrital contamination of the absorption spectra. Peak-height ratios of *S. trochoidea* obtained with the filter-integrating sphere method were significantly lower than those measurements normally including detritus. One interpretation would be that these samples actually contained detritus. Another explanation might be incomplete pigment extraction leading to overestimated detrital absorption and underestimated biological absorption. The retained pigments would have to be carotenoids or Chl *c* because the detritus absorption spectra showed no typical absorption signature near 675 nm.

Because there is the possibility of physiological adaptation through adjustment of accessory pigments, ratios of absorption in vitro are given in Table 3. As expected (Duysens 1956) the in vitro ratios are always between 1.5 and 2 times higher than the corresponding in vivo ratios. The in vitro values vary little within but show significant differences between sample types. The culture samples from one species thus seem to have reproducible states of adaptation with respect to relative pigment composition. This does not exclude variations in pigment concentration per cell volume with resulting changes in specific absorption. As a result of this packaging effect, variations of ratios of absorption at different wavelength are possible (Duysens 1956).

Detrital absorption—Detrital absorption coefficients were obtained from depigmented filters measured with the filter-integrating sphere method. Unconcentrated sample A from Kiel harbor showed detrital absorption comparable to phytoplankton absorption in the blue part of the spectrum (Fig. 2). Because the absorption coefficients in Fig. 2 were obtained with the filter-integrating sphere method, we assume they are exaggerated. Therefore we corrected the detrital absorption coefficients with a factor equal to the ratio of phytoplankton absorption coefficients at 675 nm in the suspension-integrating sphere and the filter-integrating sphere methods (Fig. 3). A wavelength dependence of the correction factor was not observed.

We assume that washing the filter with acetone and methanol will extract accessory pigments and Chl *a* but leave detritus absorption untouched. This assumption is difficult to verify. It seems that no detrital pigments are extracted by acetone because the absorption ratio (440:675 nm) for the acetone extract of Kiel harbor samples with their considerable detrital contamination is similar to that of culture samples (Table 3). We could show that no wavelength shift took place due to organic solvent treatment by processing a sample that contained only detritus (*see results*). Depigmentation of samples was not always complete. Residual Chl *a* in sample B from Kiel harbor was 12% because the phytoplankton biomass was dominated by the dinoflagellate *Heterocapsa triquetra*. As pointed out in the methods section, marine samples containing significant amounts of dinoflagellates need to be extracted by a modified routine. Absorption by the residual chlorophyll of sample B is clearly visible in Figs. 2 and 3.

Doucha and Kubin (1976) obtained depigmented phytoplankton cells by adding peracetic acid followed by strong UV-irradiation of the sample. The bleached cells were supposed to keep their cellular geometry with unaltered scattering characteristics. Because this method can be expected to bleach detritus as well, we chose the extraction method to obtain absorption spectra of detritus.

The detritus absorption spectrum in Kiel harbor (Figs. 2 and 3) is very similar to the absorption spectrum of "pigments that covary with pheopigments" obtained by statistical treatment by Kiefer and SooHoo (1982, figure 8) from oceanic samples. The spectrum of detritus differs considerably in quality from the absorption spectrum of dissolved yellow substance in an unconcentrated Kiel harbor sample (Fig. 3). At 400 nm, detritus absorption was about $\frac{1}{8}$ of the absorption by yellow substance.

Absorption and fluorescence excitation spectra—Assuming constant curves of spectral quantum efficiency of photosynthesis and fluorescence of in vivo phytoplankton pigments for most of the visible spectrum leads to comparable fluorescence-excitation spectra and absorbance spectra. In the spec-

tral range of carotenoid absorption, a decrease of photosynthetic quantum efficiency might be expected from literature data. The quantum response of fluorescence is sensitive to the spectral emission component because antennae and reaction centers show slightly different absorption spectra for each photosystem, due to heterogeneous distribution of accessory pigments and to the type of protein complexing. A review of this subject is given by Larkum and Barrett (1983).

Generally it might be expected that fluorescence excitation spectra obtained with emission of photosystem 1 (PS1) at 730 nm would be representative for the major spectral characteristics of the complete photosynthetic apparatus with some PS1-specific features. We looked for spectral characteristics in the excitation of PS1 and photosystem 2 (PS2) (685 nm) and found no significant difference (*see results*). Thus excitation spectra between 400 and 680 nm with emission at 730 nm can be expected to follow closely the spectra of absorbance of all light-harvesting pigments. The "red drop," a decrease of fluorescence yield at longer wavelength, is usually found to start at 680 nm, and therefore it should be of little concern with respect to comparison between absorption and excitation spectra below this wavelength. The advantages of emission detection at 730 nm are that the excitation peak at 675 nm can be detected and that there will be no significant reabsorption of fluorescence.

For a comparison of biologically active absorbance and fluorescence-excitation spectra we subtracted detritus absorption as measured by depigmented filters. The resulting absorption spectra of the filter-integrating sphere method and fluorescence-excitation spectra of *C. carterae* in general show good agreement (Fig. 5). Compared to the fluorescence yield at 675 nm, the yield is higher in the wavelength range 510–560 nm and lower at wavelengths <510 nm.

One aim of this research was to arrive at absorption spectra of phytoplankton from coastal samples heavily contaminated by detritus. Therefore we combined information from the suspension-integrating sphere method and the filter-integrating sphere method to yield a spectrum of biologically

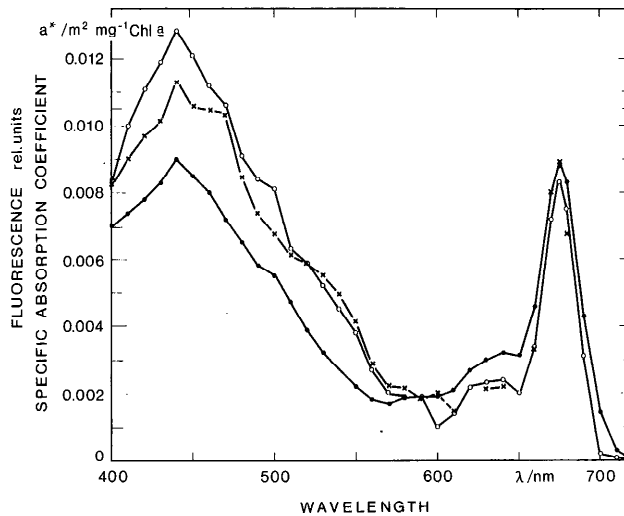


Fig. 7. Comparison of calculated biologically active absorption coefficients (O, ●) and fluorescence excitation (x) in sample B from Kiel harbor.

active absorption from a sample of Kiel harbor. We calculated the difference spectrum of the specific absorption (Fig. 7, O) obtained with the suspension-integrating sphere method and the corrected detritus spectrum of this sample (Fig. 3) obtained from the depigmented filter. Alternatively a spectrum was calculated (Fig. 7, ●) by correcting the specific absorption spectrum obtained with the filter-integrating sphere method with a factor derived from the ratio of specific absorption coefficients at 675 nm of the suspension-integrating sphere method and the filter-integrating sphere method, all from the same sample (Table 2). By definition, both spectra (Fig. 7) show a similar peak height at 675 nm, but the second spectrum shows lower absorption in the blue. This decrease would still persist if specific absorption coefficients of the filter-integrating sphere were corrected so that both spectra coincided at 675 nm. A comparison of particulate absorption (detritus and phytoplankton) measured with the filter-integrating sphere and the suspension-integrating sphere methods yielded only small variations in proportionality factors over the spectrum for another sample. In this particular case (Fig. 7) wavelength-dependent scattering might have led to some underestimation of absorption in the blue. Higher absorption coefficients of detritus would de-

crease the calculated absorption spectrum (Fig. 7, O), thus improving the correspondence of the absorption spectrum with the fluorescence-excitation spectrum. There is some qualitative difference noticeable between absorption spectra at 460, 490, and 580 nm.

For comparison of absorption spectra with fluorescence-excitation spectra, it was unnecessary to calculate the absorbance: because of the low Chl *a* concentration and the cuvette dimension of the fluorometer (1 cm square), the series expansion of $\exp(-al)$ can be truncated after the first term. The quantum-corrected fluorescence-excitation spectrum in Fig. 7 was scaled to suit the absorption spectra based on data points at 675 and 600 nm. On comparison with the first absorption spectrum, only small variations in the relative quantum yield of fluorescence (fluorescence divided by absorption coefficient) are apparent. The quantum yield is relatively high from 510–560 nm and low below 510 nm (cf. Figs. 5 and 7). Some species-specific differences in pigment composition and efficiency of exciton transfer leading to variations in relative quantum yield were to be expected (cf. Larkum and Barrett 1983). Such differences might represent adaptive properties of phytoplankton (Neori et al. 1984). A comparison of ratio of peak height of excitation and absorption

(Table 3) shows higher excitation in the blue in some cases. There are several explanations for this unexpected feature: as indicated by Table 3 there is some noise in the data (cf. both absorption spectra in Fig. 7); the depigmentation of the filter for the filter-integrating sphere methods left some residual accessory pigment on the filter, thus underestimating the amount of pigments; and the "red drop" of fluorescence excitation decreased fluorescence excitation at wavelengths <680 nm, although this is not found to be the case normally (Govindjee et al. 1968).

We would like to emphasize again that absorption spectra of natural samples are made up to a large extent of detrital absorption (cf. Fig. 2). Interpretation of biologically active absorption is not possible without subtracting detritus. Otherwise the ratio of absorption at 440:675 nm can increase considerably, leading to a seemingly decreased fluorescent quantum efficiency in the blue. Neori et al. (1984) presented spectra of relative fluorescence yield decreasing by about half from 540 to 400 nm. Part of this decrease may be due to high concentrations of detritus. Because of the strong increase in detrital absorption with shorter wavelength, seemingly low efficiencies of fluorescence result.

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