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REFLECTANCE SPECTROSCOPY OF MARINE PHYTOPLANKTON

Part 1. Optical Properties As Related To Age And Growth Rate

Part 2. A Simple Model Of Ocean Color

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ABSTRACT

Drawing upon principles of reflectance spectroscopy, we present a model of ocean color which consists of two optical components, sea water and phytoplankton. In this model diffuse spectral reflectance immediately below the sea surface is calculated from the diffuse absorption and diffuse backscattering coefficients for water and phytoplankton. Coefficients for phytoplankton were determined from measurements with a spectrophotometer of cells grown in the laboratory, while coefficients for water were calculated from optical measurements made in clear, blue water. Broad spectral changes in diffuse reflectance of the model ocean were affected by increases in the absorption and backscattering coefficients of the phytoplankton crop. Calculations with the model also demonstrated that diffuse reflectance is a nonlinear function of crop size, and that the age or physiological state of the cells can have important spectral effects. The study concludes with a derivation of a dichromatic function which allows calculation of the diffuse absorption and the diffuse backscattering coefficients for the phytoplankton crop from measured values of reflectance.

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REFLECTANCE SPECTROSCOPY OF MARINE PHYTOPLANKTON

Part 1. Optical Properties As Related To Age And Growth Rate

INTRODUCTION

Our ability to use ocean color spectra to determine the type and concentration of suspended and dissolved material in the surface waters of the ocean will depend upon a knowledge of the optical properties of such material. Covariation of both inherent and apparent optical properties with chlorophyll *a* concentration in surface waters (eg. Kiefer and Austin, 1974; Morel and Smith, 1974) suggests that variations in the crop of phytoplankton affect ocean color changes. Since phytoplankton are the supporting base of the planktonic food chain, we have studied in the laboratory their optical properties with the aim of calculating spectral changes affected by the type and size of the crop in surface waters.

In order to quantify the scattering and absorbing properties of the cells, we have based the measurements upon principles of reflectance spectroscopy and, in particular, upon the phenomenological model of radiative transfer introduced by Duntley (1942). This model, which is an extension of the work of Schuster (1905) and Kubelka and Munk (1931) among others, characterizes the optical properties of material with absorption and scattering coefficients for diffuse and collimated light. Such a model is useful not only because the coefficients define the efficiency with which the cells scatter and absorb, but also because they may be introduced into functions describing diffuse transmittance and reflectance for media of varying thicknesses and composition.

In our study we have measured the diffuse absorption coefficient and diffuse backscattering coefficient for two cultures of phytoplankton: one a batch culture of the diatom, *Thalassiosira pseudonana,* and the other a continuous culture of the chrysophyte, *Monochrysis lutheri.* In both experiments we observed large, predictable changes in the coefficients with changes in the age or growth rate of the culture. In considering the results, we attempt to explain why the relationship between growth rate and optical properties is expected, and we also examine the causes and spectral nature of such changes. The results indicate that in a well-defined suspension of phytoplankton such as a laboratory culture, spectral measurements which allow for solution of the absorption and scattering coefficients can be applied to estimates of both cell concentration and cell age or growth rate. In the accompanying paper we will introduce the absorption and backscattering coefficients into an equation for diffuse spectral reflectance, $R(0,\lambda)$ immediately below the air-water interface. An understanding of $R(0,\lambda)$ in natural systems is crucial to the understanding of ocean color spectra.

METHODS

The experimental design was quite simple. Cells were sampled from the culture, concentrated by centrifugation, and placed in a spectrophotometer, equipped for measurements of diffuse transmittance and diffuse reflectance. The optical measurements were supplemented by determinations of chlorophyll *a* concentration and particle size distribution for the concentrated suspension.

CULTURES

Thalassiosira pseudonana was grown in batch culture by inoculating four liters of sterile, nutrient-enriched seawater with an axenic strain. The medium was identical to F/2 medium described by Eppley, *et al.* (1967) except that the concentration of KNO_3 was reduced to 75μMolar, it being the limiting nutrient to crop size. The culture vessels were aerated and incubated in a water bath (20°C) before a bank of fluorescent lights. The irradiance was 1.2×10^{16} quanta $-cm^{-2}-sec^{-1}$ and continuous. Five to seventeen days after inoculation one liter samples of cell suspension were removed and concentrated by centrifugation (10^3 gravities x 8 min.) to a final volume of 50 mls. Microscopic examination of the concentrated suspension showed that the cells sampled from young cultures were healthy and intact. However, older cultures contained a considerable fraction of broken and clumped cells. In most concentrates bacteria were not seen, and in those older cultures where they were present, they were in relatively low numbers.

Monochrysis lutheri was grown in a nitrate-limited chemostat. The medium and culture conditions were identical to those of *Thalassiosira pseudonana*, except that greater care was taken to maintain the pH of the chemostat at 8.1. This was done with a pH controller system which consisted of a glass pH electrode partly submerged in the culture, a controller which activated a solenoid allowing a 5 percent (V/V) mixture of CO_2 in air to bubble through the culture. The condition of steady state growth within the chemostat was determined by measurements of chlorophyll and cell concentration; the specific growth rate μ being equal to the dilution rate of the culture (Herbert, Elsworth, and Telling, 1956). Preparation of the concentrated cell suspension was identical to that of *Thalassiosira pseudonana.* Microscopic examination of the concentrated cell suspension revealed that cells in slow growth were frequently clumped and a small fraction of these cells were fractured.

MEASUREMENTS OF CHLOROPHYLL *a* AND TOTAL CELL CROSS SECTION

The concentration of chlorophyll *a* in the concentrated suspension was determined by extraction in acetone and measurement with a fluorometer (Holm-Hansen, *et al.*. 1965). The concentration of total geometric cross section contributed by the cells A_T was estimated with an electronic particle counter, Coulter, Model T_A equipped with 100 μm orifice. A_T was calculated from the size distribution of particle volumes by assuming that the cells were spherical. The aggregation of older or slower-growing cells resulted in an increase in the fraction of particles falling within larger channels.

MEASUREMENTS OF OPTICAL PROPERTIES

After centrifugation and resuspension, the concentrated suspension of cells (roughly 10^7 cells $\cdot cm^{-3}$) was placed in a Hardy spectrophotometer (Hardy, 1935) equipped with integrating sphere. The cuvettes were placed either immediately in front of the integrating sphere so that all forward flowing light was collected (diffuse transmittance) or immediately behind the integrating sphere so that all backward flowing light was collected (diffuse reflectance). Three measurements of spectral diffuse transmittance were made using cuvettes with pathlengths of 1.0, 1.5, and 2.0 centimeters. Two measurements of spectral diffuse reflectance were made using cuvettes with pathlengths of 1.0 and 2.0 centimeters.

Following the arguments of Duntley (1942), the five measurements allow calculations of the spectral

diffuse absorption and backscattering coefficients, $a_p^*(\lambda)$, $b_p^*(\lambda)$, as well as three other coefficients describing the attenuation of the collimated light fluxes. We are concerned only with $a_p^*(\lambda)$ and $b_p^*(\lambda)$ for phytoplankton, since the spectral diffuse reflectance of irradiance, $R(0,\lambda)$, for natural waters may be considered a function of $a_p^*(\lambda)$ and $b_p^*(\lambda)$ (Duntley, *et al.*, (1974).

NORMALIZATION OF $a_p^*(\lambda)$ AND $b_p^*(\lambda)$

According to the theory of single particle scattering of collimated light, the volume scattering $s(\lambda)$ or the volume absorption coefficient $a(\lambda)$ for a dilute suspension of particles is equal to the product of the number of particles within the volume element of the light beam, the cross sectional area of the particle relative to the beam, and the optical efficiency factor of the particle (Van de Hulst, 1957). In our study we have assumed that the $a_p^*(\lambda)$ and $b_p^*(\lambda)$ are proportional to the concentration of particle cross sectional area in the cuvette, and thus we will present our results in terms of a normalized diffuse absorption $Q_{a_p}^*(\lambda)$ and diffuse backscattering $Q_{b_p}^*(\lambda)$. Since both $a_p^*(\lambda)$ and $b_p^*(\lambda)$ and the concentration of particle cross sectional area have units of reciprocal length, $Q_{a_p}^*(\lambda)$ and $Q_{a_p}^*(\lambda)$ are dimensionless and independent of particle concentration. Thus

$$Q_{a_p}^*(\lambda) = a_p^*(\lambda)/A_T$$

and $$Q_{b_p}^*(\lambda) = b_p^*(\lambda)/A_T \quad .$$

In a few instances we present $a_p^*(\lambda)$ and $b_p^*(\lambda)$ in terms of unit chlorophyll *a* concentration rather than in terms of unit concentration of particle cross section.

$$^{\circ}a_p^*(\lambda) = a_p^*(\lambda)/Ca$$

$$^{\circ}b_p^*(\lambda) = b_p^*(\lambda)/Ca$$

where Ca is the concentration of chlorophyll *a* (in mg – cm^{-3}). Finally, the data were corrected so that for a given species, all values for $a_p^*(675\ nm)$ normalized to unit chlorophyll *a* concentration have identical values.

RESULTS

BATCH CULTURES

As mentioned earlier samples of the marine diatom, *Thalassiosira pseudonana*, were taken from nitrate-limited batch cultures, and samples of the marine chrysophyte, *Monochrysis lutheri*, were taken from a nitrate-limited chemostat. In Table 1 we have summarized biological and optical parameters. In the nine measurements for *Thalassiosira pseudonana* the cultures ranged in age from 5 to 17 days. Such a range represents cultures in rapid exponential growth as well as cultures in which most of the cells

were dead. The range of chlorophyll *a* concentration of the corresponding concentrated samples was large, 0.050 to 1.7 $\mu g \cdot cm^{-3}$, relative to the range in the concentration of cell cross section, A_T, 0.86 to 3.9 cm^{-1}. As can be seen in column 4 of the table, there was a large systematic change in the ratio of cell cross section to cell chlorophyll *a*, Ca / A_T; the ratio decreasing over sixty-fold with age.

Since in collimated light, A_T is proportional to the volume attenuation or volume scattering coefficient and Ca is proportional to that fraction of the volume absorption coefficient contributed by this pigment, one would expect changes in the optical properties of the cells to parallel changes in Ca / A_T. Such changes in optical properties are shown in the remaining columns of the table. As previously mentioned we chose to present the results of the optical measurements in terms of the ratio $a_p^*(\lambda)/b_p^*(\lambda)$ because to a first approximation the ratio should be proportional to Ca / A_T. We present wavelengths of 450 and 675 nanometers because these wavelengths corresponded to both maximum blue and red absorption by the cells. We see from the data that changes in $a_p^*(\lambda) / b_{pp}^*(\lambda)$ do parallel changes in Ca/A_T, and that in the red region of chlorophyll *a* absorption the magnitude of both changes are comparable.

A graph such as Fig. 1 of the ratio of the absorption coefficient to backscattering coefficient versus the age of the culture, suggests a monotonic decrease in relative absorption with age. Such a dramatic decrease in $a_p^*(\lambda) / b_p^*(\lambda)$ is probably due to the degradation of pigments by metabolic as well as such irreversible processes as photooxidative.

Table 1

Biological and optical parameters for concentrated cell suspensions of *Thalassiosira pseudonana* grown in batch culture. The first column contains the age of the culture in terms of days after inoculation while the other parameters are explained in text. The culture is limited by nitrogen.

Days After Inoculation	Ca Chlorophyll *a* ($\mu g \cdot cm^{-3}$)	A_T Concentration of Cell Cross Section (cm^{-1})	Ca / A_T ($\mu g \cdot cm^{-2}$)	$\frac{a_p^*(450)}{b_p^*(450)}$	$\frac{a_p^*(675)}{b_p^*(675)}$
5	0.61	0.86	0.71	–	154
5	1.51	1.7	0.89	100	–
6	1.7	2.8	0.59	110	74
7	1.6	2.8	0.57	97	66
7	1.3	1.5	0.84	94	82
8	1.4	3.9	0.37	88	49
9	0.63	1.6	0.38	53	25
11	0.15	2.4	0.064	40	6.3
17	0.050	3.4	0.015	14	1.5

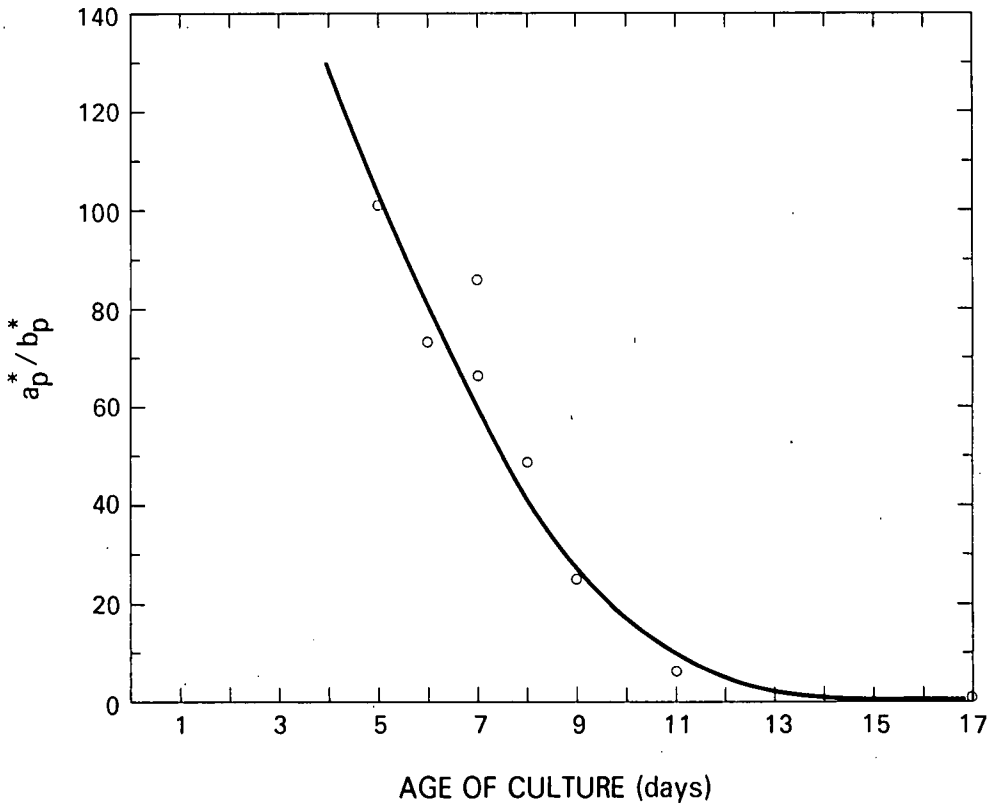

Fig. 1. Ratio of diffuse absorption coefficient to diffuse backscattering coefficient versus age of the batch culture of *Thalassiosira pseudonana*.

In Fig. 2a we show representative spectra of $Q^*_{a_p}(\lambda)$ and $Q^*_{b_p}(\lambda)$ for cultures of 7, 9, 11, and 17 days. Three features are noteworthy. First, the efficiency with which *Thalassiosira pseudonana* scatters diffuse light in the backward direction, $Q^*_{b_p}(\lambda)$, is relatively free of spectral structure and shows a general trend of decreasing efficiency with increasing wavelength. The efficiency with which the cells absorb diffuse light, $Q^*_{a_p}(\lambda)$, is characterized by the red absorption maximum of chlorophyll *a* and a broader blue absorption maximum of both chlorophyll *a* and carotenoid accessory pigments. Second, changes in $Q^*_{b_p}(\lambda)$ with age are much smaller than changes in $Q^*_{a_p}(\lambda)$. This is to be expected if the aged cells are unchanged in their size and relative index of refraction but have decreased amounts of pigments. Third, changes in $Q^*_{a_p}(\lambda)$ are much larger in the red absorption band than in the blue; $Q^*_{a_p}(\lambda)$ ranges from 0.0035 to 0.18 at 675 nanometers and from .042 to 0.28 at 450 nanometers. This suggests that losses of chlorophyll *a* with age are greater than the losses of the carotenoids. In Fig. 2b we show the same data except here the coefficients of absorption and backscattering have been normalized to 1 $\mu g \cdot cm^{-3}$ chlorophyll *a*. We see that for suspensions of equivalent chlorophyll *a* concentration blue absorption changed ten-fold and backscattering increased almost one hundred-fold.

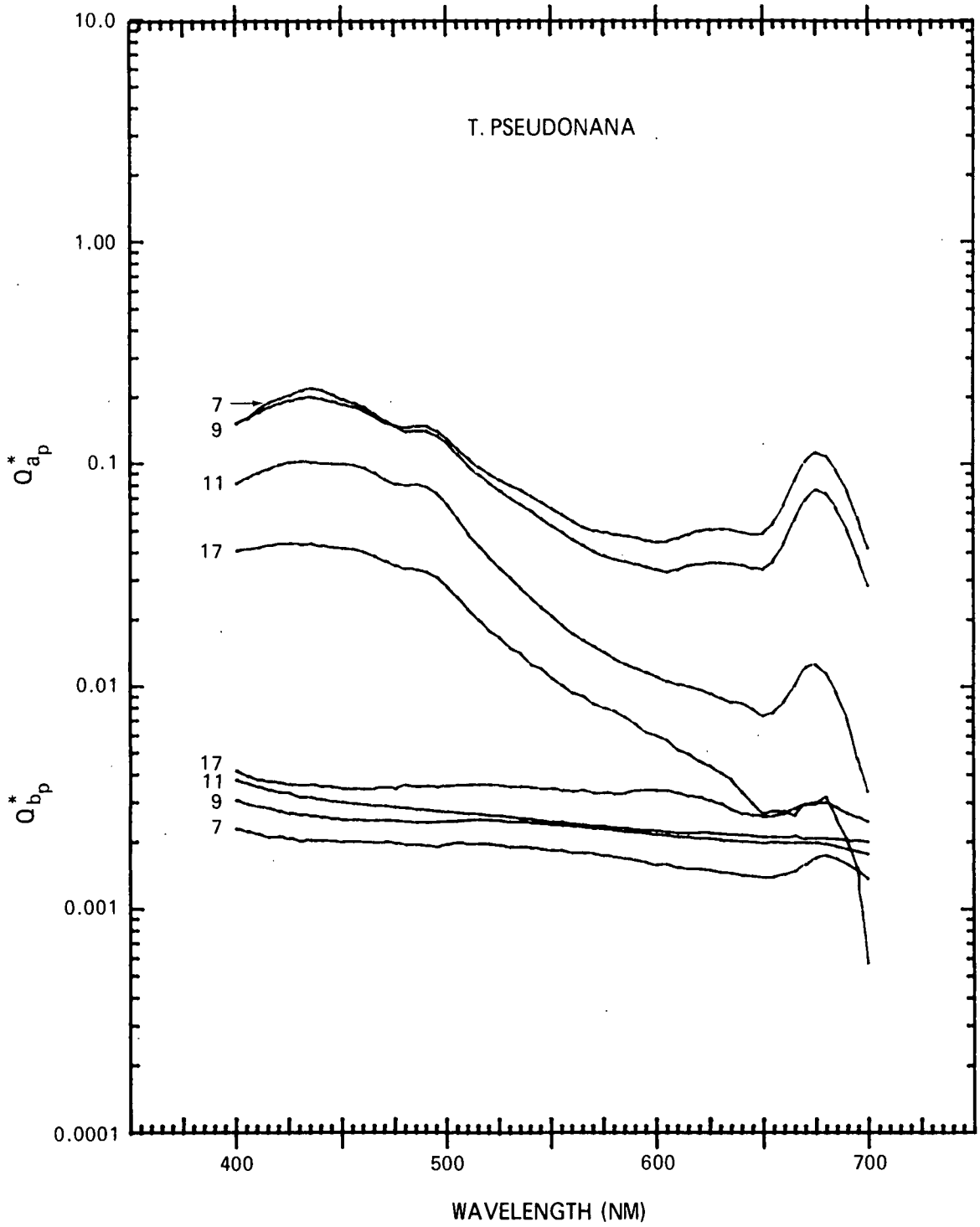

Fig. 2a. Diffuse absorption and backscattering coefficients for cultures of *Thalassiosira pseudonana* 7, 9, 11, and 17 days old. Top four curves are absorption coefficients. Bottom four curves are backscattering coefficients, normalized to unit concentration of cell cross section.

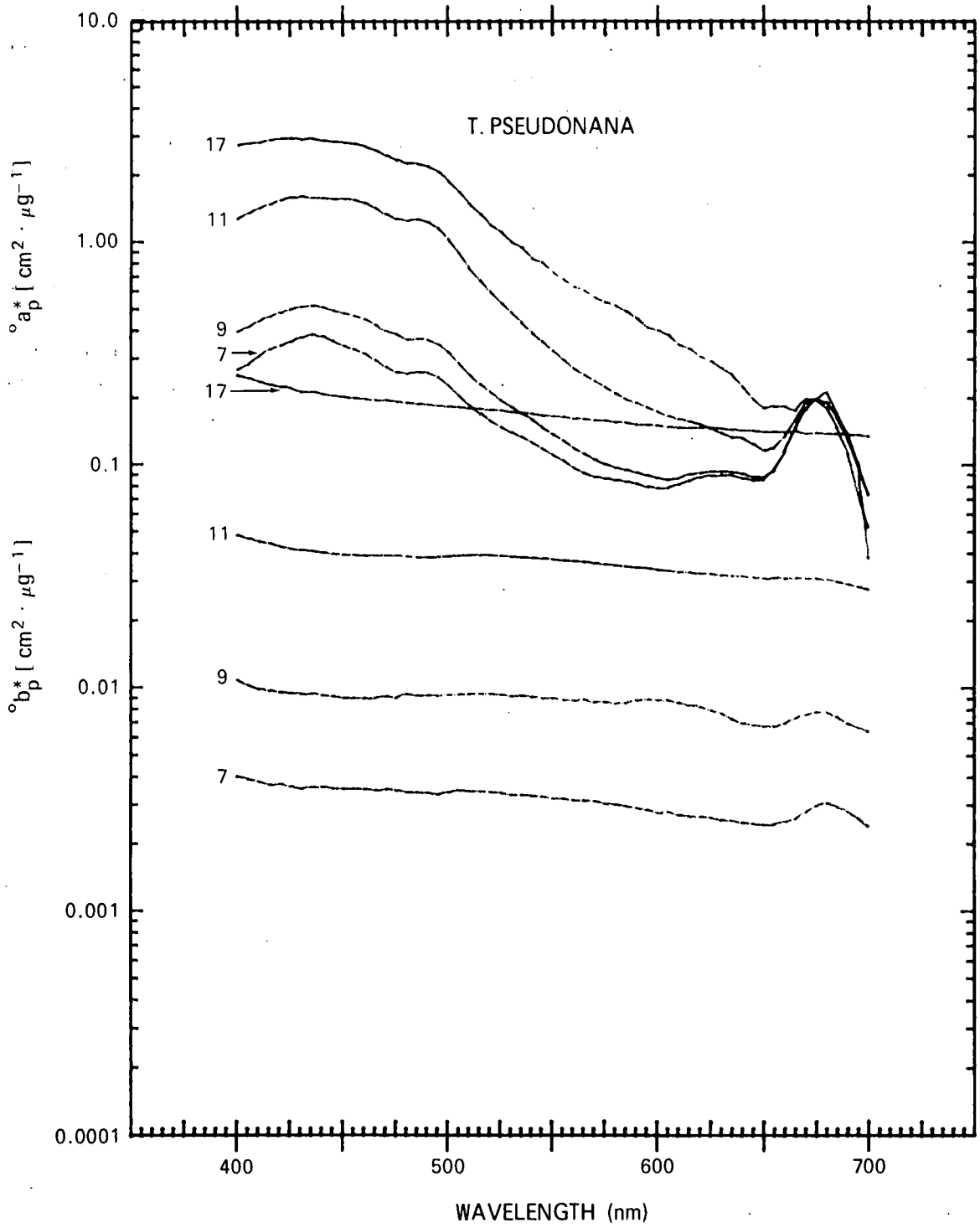

Fig. 2b. Diffuse absorption and backscattering coefficients for cultures of *Thalassiosira pseudonana* 7, 9, 11, and 17 days old. Top four curves are absorption coefficients. Bottom four curves are backscattering coefficients, normalized to unit concentration of chlorophyll *a*.

We summarize the spectral changes related to the aging and bleaching of *Thalassiosira pseudonana* in Fig. 3. Figure 3a contains mean values for $Q^*_{a_p}(\lambda)$ and $Q^*_{b_p}(\lambda)$ for the 9 measurements. The diffuse backscattering coefficient displays small structure in regions of strong absorption. Absorption centered at 450 nanometers decreases the efficiency of diffuse backscattering while absorption centered at 675 nanometers increases the efficiency. These spectra of $Q^*_{b_p}(\lambda)$ are in general agreement with the spectral nature of scattering by phytoplankton calculated by Mueller (1973). Mueller's calculations were based upon the solution of Mie theory for a polydisperse suspension of idealized phytoplankton cells.

Figure 3b is a statistical treatment of the 9 measurements. The figure contains the standard deviation of $Q^*_{a_p}(\lambda)$ and $Q^*_{b_p}(\lambda)$, and is comparable to a difference spectrum often used to compare two spectra. Comparison of Figs. 3a and 3b indicates that maximum changes in absorption occur at the wavelengths of maximum absorption and that decreases in absorption caused by aging do not greatly alter the spectral character.

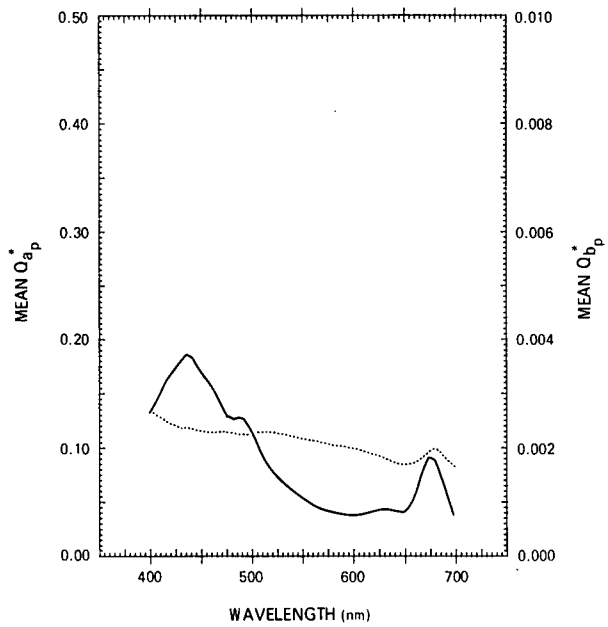

Fig. 3a. Mean values of $Q^*_{a_p}$ and $Q^*_{b_p}$ for the nine measurements of *Thalassiosira pseudonana*. $Q^*_{a_p}$ is represented by the solid line and uses the scale on the left. $Q^*_{b_p}$ is represented by the dotted line and uses the scale on the right.

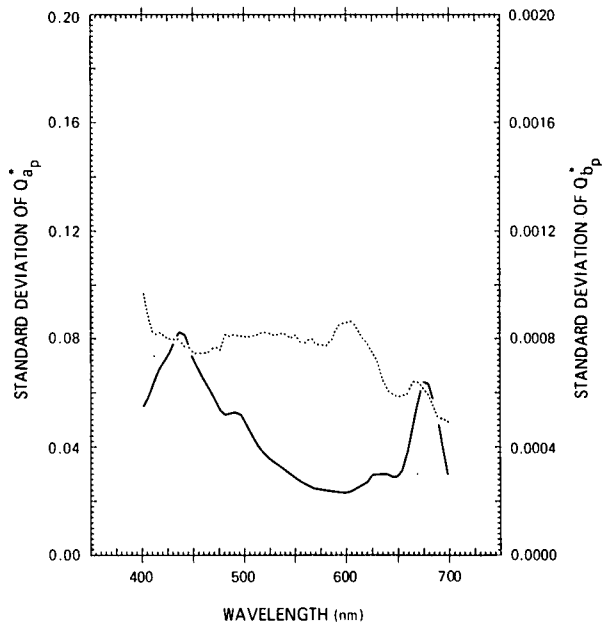

Fig. 3b. Standard deviations of $Q^*_{a_p}$ and $Q^*_{b_p}$ for the nine measurements of *Thalassiosira pseudonana* from the means of Fig. 3a. Standard deviations of $Q^*_{a_p}$ is represented by the solid line and uses the scale on the left. Standard deviations of $Q^*_{b_p}$ is represented by the dotted line and uses the scale on the right.

CONTINUOUS CULTURE

Table 2 shows similar data for six measurements of *Monochrysis lutheri* growing in a nitrogen-limited, steady-state condition. The specific growth rates of the cells, μ, ranged from 0.046 to 0.0016 hr^{-1}; this range corresponded to doubling times between 14 and 420 hours, which was an unusually large range for most laboratory cultures of phytoplankton. As in the case of batch cultures of *Thalassiosira pseudonana*, changes in chlorophyll *a* concentration of the concentrated suspension of *Monochrysis lutheri* were considerably larger than changes in the concentration of cell cross section. This is seen in column 4 where Ca / A_T ranged from a high of 1.0 for the cells which were growing rapidly to a low of 0.35 for cells which were growing very slowly.

Table 2

Biological and optical parameters for concentrated cell suspensions of *Monochrysis lutheri* grown in nitrogen-limited, continuous culture. The first column contains the steady-state growth rate of the cells.

μ Growth Rate (hr^{-1})	Ca Chlorophyll *a* ($\mu g \cdot cm^{-3}$)	A_T Concentration of Cell Cross Section (cm^{-1})	Ca / A_T ($\mu g \cdot cm^{-2}$)	$\frac{a_p^*(450)}{b_p^*(450)}$	$\frac{a_p^*(675)}{b_p^*(675)}$
.046	2.2	1.8	1.1	120	110
.021	1.8	–	–	89	66
.017	1.9	1.9	1.0	105	74
.0035	1.1	1.5	0.76	62	40
.0020	1.0	2.9	0.35	46	23
.0016	0.69	1.7	0.40	34	19

Such changes in the ratio of pigment concentration to particle cross section were paralleled by changes in the optical properties of the cells. At 450 and 675 nanometers $a_p^*(\lambda) / b_p^*(\lambda)$ increased roughly three-fold from the slower growing cells to the faster, Fig. 4. A graph of the ratio of the absorption coefficient to the backscattering coefficient versus the specific growth rate indicates a satisfactory linear relationship. The regression is significant within 95 per cent confidence. Thus, in steady-state systems, the absorption and scattering properties of phytoplankton appear to be a fairly accurate index of the growth rate or nutritional state of a given species. In the following discussion, we will argue that a linear relationship between μ and $a_p^*(\lambda) / b_p^*(\lambda)$ is to be expected, and that the relationship might be independent of the species.

Figures 5a and 5b are representative spectra of *Monochrysis lutheri* for the full range of growth rates. $Q_{a_p}^*(\lambda)$ decreased systematically with growth rate but the changes were much smaller than those seen with *Thalassiosira pseudonana*. This decrease was paralleled by an increase in $Q_{b_p}^*(\lambda)$. Figures 6a

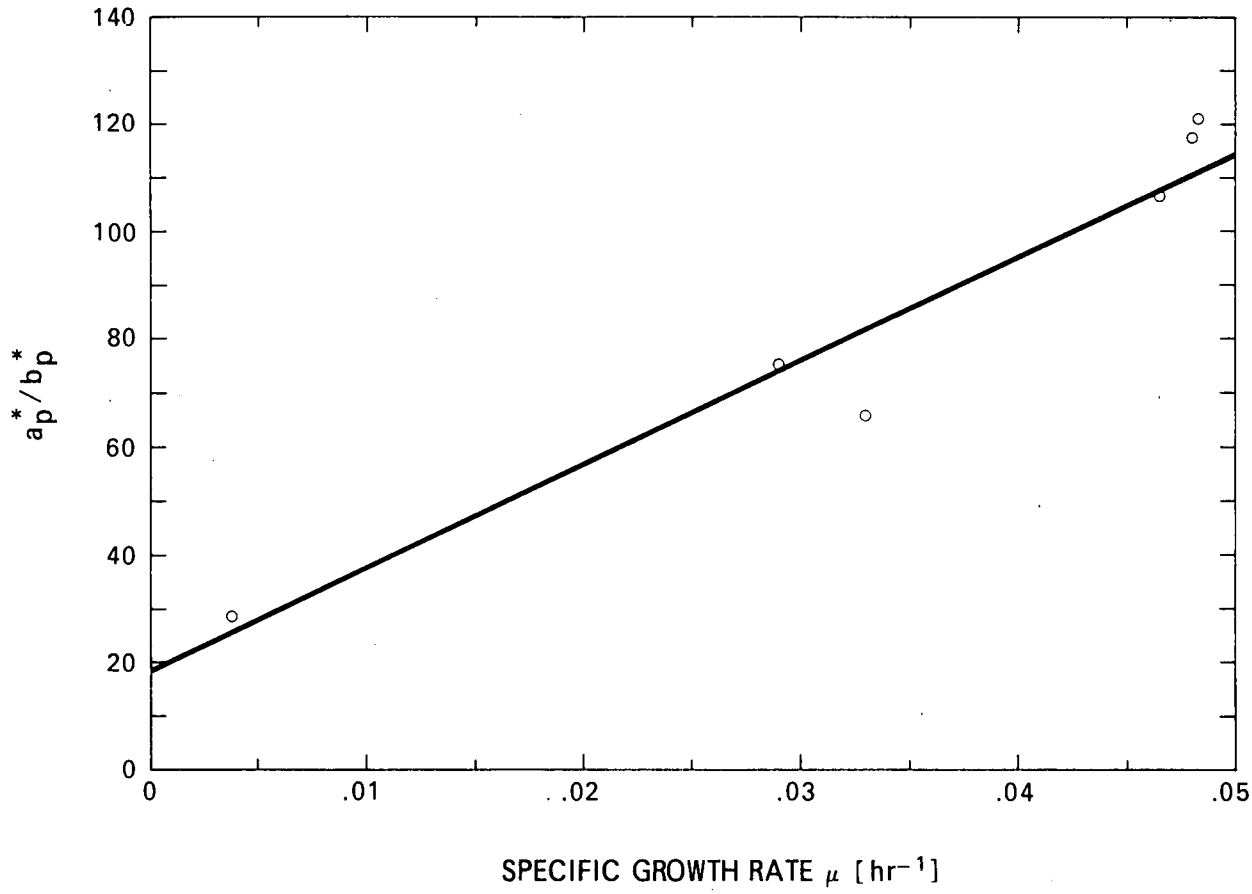

Fig. 4. Ratio of diffuse absorption coefficient to diffuse backscattering coefficient versus the specific growth rate for the continuous culture of *Monochyrsis lutheri*.

and 6b summarize all measurements, presenting the mean and standard deviation. A comparison of Figs. 3a and 6a indicate that while $Q^*_{a_p}(\lambda)$ and $Q^*_{b_p}(\lambda)$ were quantitatively different for the two species, the general spectral characters of both absorption and backscattering were similar. A comparison of Figs. 6a and 6b again demonstrates that the changes in $Q^*_{a_p}(\lambda)$ with growth rate paralleled the spectra for $Q^*_{a_p}(\lambda)$.

DISCUSSION

When considering the results of this study, we wish to look first at the relationship between growth rate and optical properties for the continuous culture, and then we wish to consider the relationship between age and optical properties for the batch culture. The relationship between steady-state growth rate and the ratio $a^*_p(\lambda)/b^*_p(\lambda)$ shown in Fig. 4 is to be expected if two conditions are satisfied: (1) the diffuse backscattering coefficient $b^*_p(\lambda)$ must be proportional to cellular biomass, and (2) $a^*_p(\lambda)$ must be proportional to the rate of production of cellular biomass. The argument is as follows.

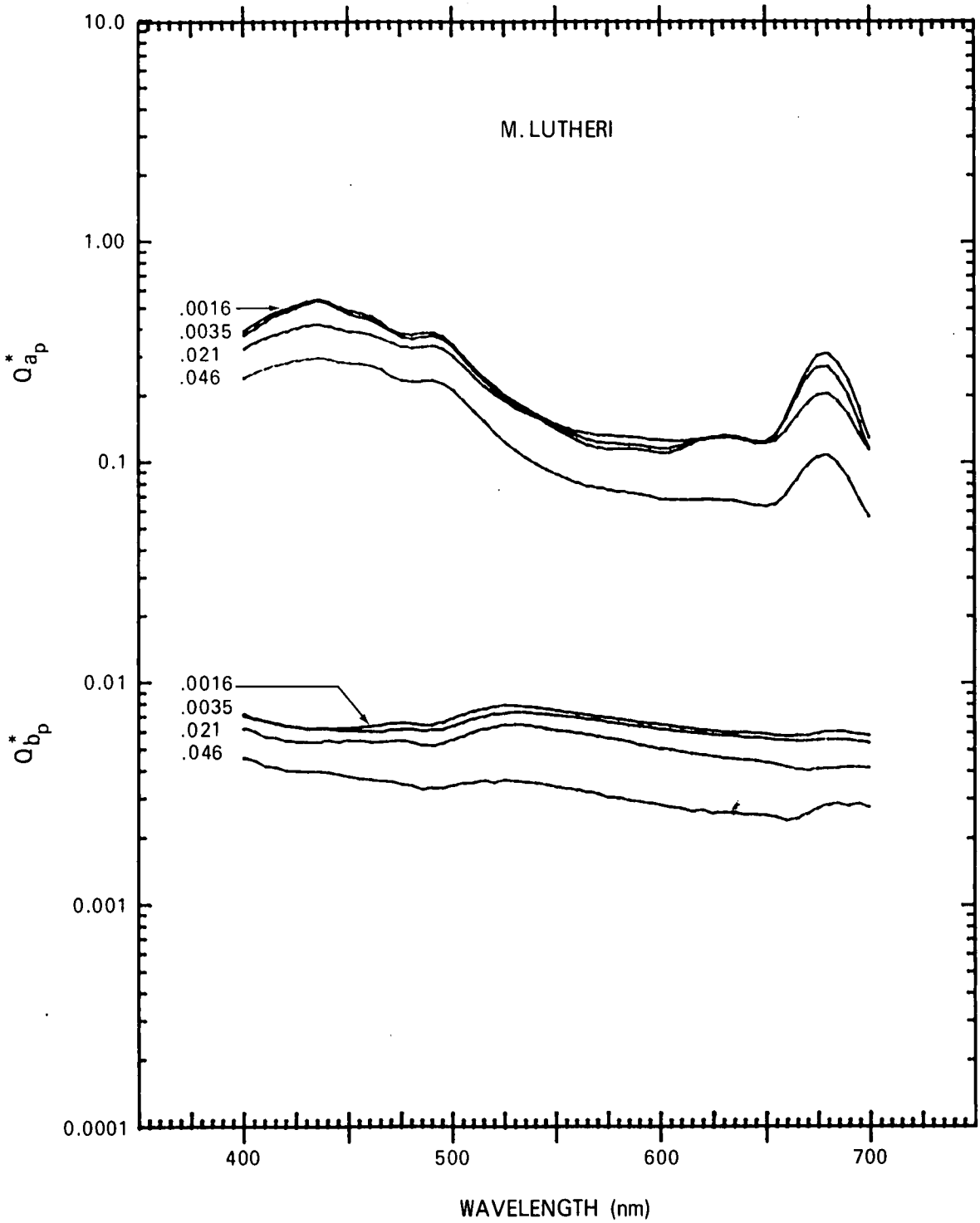

Fig. 5a. Diffuse absorption and backscattering coefficients for the continuous culture of *Monochyrsis lutheri* for growth rates of .046, .021, .0035, and .0016 hr^{-1}. Top four curves are absorption coefficients. Bottom four curves are backscattering coefficients, normalized to unit concentration of cell cross section.

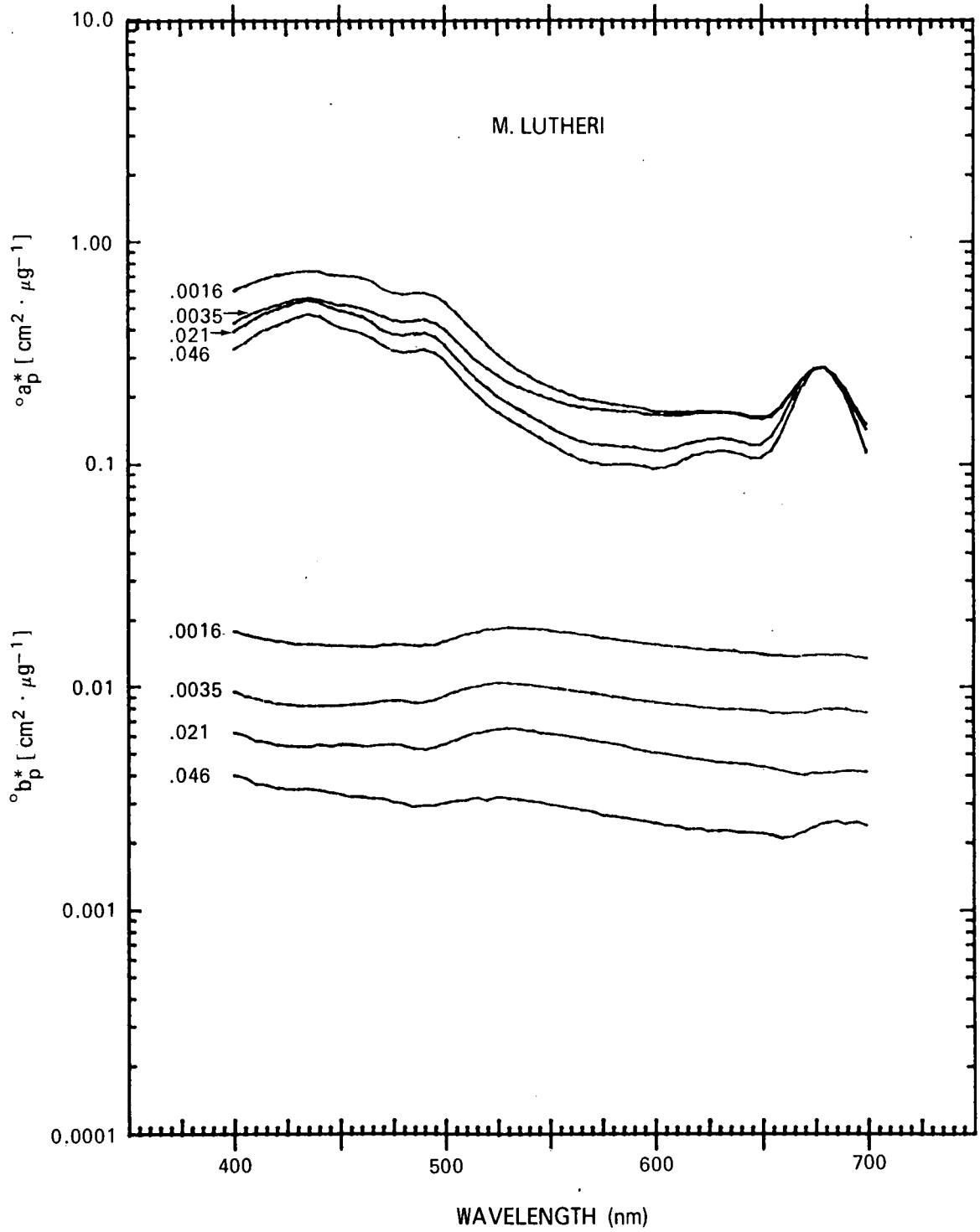

Fig. 5b. Diffuse absorption and backscattering coefficients for the continuous culture of *Monochyrsis lutheri* for growth rates of .046, .021, .0035, and .0016 hr^{-1}. Top four curves are absorption coefficients. Bottom four curves are backscattering coefficients, normalized to unit concentration of chlorophyll *a*.

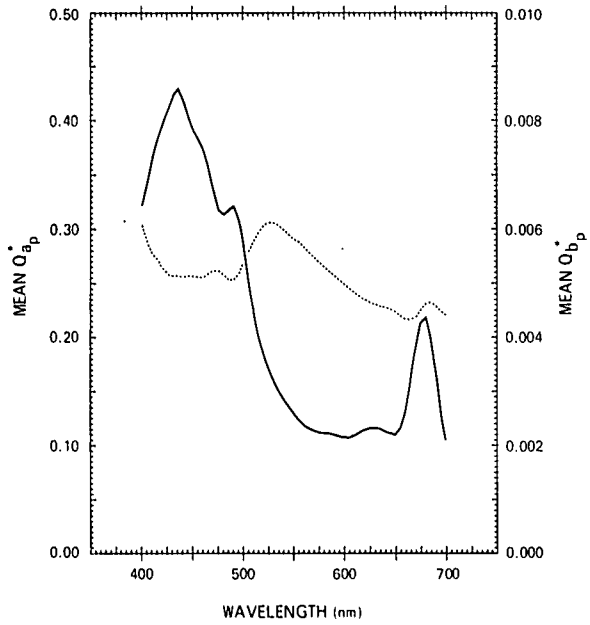

Fig. 6a. Mean values of $Q^*_{a_p}$ and $Q^*_{b_p}$ for the six measurements of *Monochyrsis lutheri*. $Q^*_{a_p}$ is represented by the solid line and uses the scale on the left. $Q^*_{b_p}$ is represented by the dotted line and uses the scale on the right.

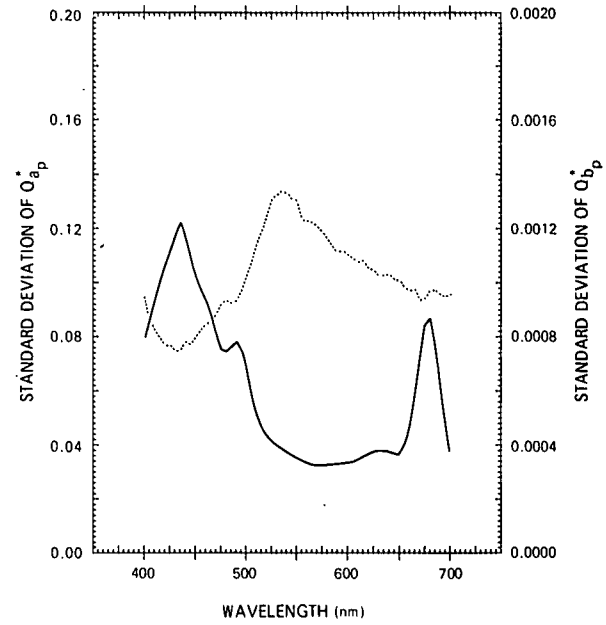

Fig. 6b. Standard deviations of $Q^*_{a_p}$ and $Q^*_{b_p}$ for the six measurements of *Monochyrsis lutheri* from the means of Fig. 5a. Standard deviations of $Q^*_{a_p}$ is represented by the solid line and uses the scale on the left. Standard deviations of $Q^*_{b_p}$ is represented by the dotted line and uses the scale on the right.

At steady-state, the specific growth rate of a crop may be defined in terms of the rate of carbon assimilation dc/dt and the carbon content of the crop c:

$$\mu = \frac{dc/dt}{c} \quad . \tag{1}$$

Given equation 1 we must show that at a given temperature and level of irradiance, $dc/dt \propto a^*_p(\lambda)$ and $c \propto b^*_p(\lambda)$. Proportionality between the diffuse absorption coefficient and rate of carbon assimilation will exist if the steady-state quantum efficiency of photosynthesis $\psi(\lambda)$ is constant at different growth rates. If this is true then for a thin suspension of cells exposed to incident irradiance, $H(\lambda)$,

$$\frac{dc}{dt} = \int_{400}^{700} \psi(\lambda)\, H(\lambda)\, a^*_p(\lambda)\, d\lambda \quad , \tag{2}$$

and the proportionality is proven.

Although there is no experimental proof that $\psi(\lambda)$ is independent of growth rate, a steady-state model of phytoplankton growth proposed by Kiefer and Enns (1976) offers a theoretical basis for such constancy. It is noteworthy that this steady-state model also predicts a constant quantum efficiency for phytoplankton whose growth rate is limited by temperature or light, as well as nutrient supply.

Proportionality of $b_p^*(\lambda)$ and the concentration of cellular carbon c for the crop may be argued on the basis that both $b_p^*(\lambda)$ and C are proportional to A_T, and thus proportional to each other. Although single particle scattering theory (eg. Van de Hulst, 1957) applies most rigorously to the attenuation of collimated light through a dilute solution, it is likely that to a first approximation in diffuse light, $b_p^*(\lambda)$ is also proportional to A_T. The second relationship is supported by Strathman (1967) who measured both cell size and cell carbon for a number of species of phytoplankton. If one assumes that the cells are spherical, his measurements indicate that cells cross section is roughly proportional to cell carbon.

While our study of steady-state growth has been limited to a single species, studies by Caperon and Meyer (1973) of steady-state growth with a number of marine phytoplankton indicated that there will be similar changes in cellular optical properties with growth rate. As an example, the cellular content of chlorophyll *a* in *Thalassiosira pseudonana* decreased five-fold, paralleling a ten-fold decrease in growth rate.

In contrast to the defined relationship between cellular optical properties and growth rate, changes in $a_p^*(\lambda)/b_p^*(\lambda)$ with age for batch cultures (Fig. 1) did not have general, quantitative applicability. It is likely that at some time between 5 and 17 days after inoculation, most of the cells are no longer viable. Thus, in a sense the time course of optical changes shown in this figure represents stages in the formation of dead or organic detrital material. Since the loss of photosynthetic pigments during the formation of detritus is caused predominantly by a physical process of photooxidation (eg. Moreth and Yentsch, 1970) rather than a process of metabolic regulation, the time course of bleaching is dependent upon the light regime within the culture. If irradiance were higher or lower, changes in $a_p^*(\lambda)/b_p^*(\lambda)$ would be faster or slower.

Despite the lack of kinetic significance, the batch culture study is of importance if the changes in optical properties for aging cells are similar to the changes occurring in the formation of natural detritus. In coastal and open ocean waters detritus is more abundant than living phytoplankton (eg. Zeitzschel, 1970), and a significant fraction of this detritus originates from the primary production. If these aged cultures bear similarity to detritus, one may point to several important optical properties of such material as shown in Fig. 3. First, the diffuse backscattering coefficient for such material contained little spectral structure (Fig. 3a). Second, depending upon the extent of bleaching, the material may be an important source of backscattered light, particularly in the red where $a_p^*(\lambda)/b_p^*(\lambda)$ approaches zero for the 17-day old culture (Fig. 1). Third, as is most apparent in Fig. 3b, there were increased losses of pigments absorbing toward the red end of the spectrum relative to losses toward the blue end of the spectrum. In this figure $^{\circ}a_p^*(450\,\text{nm})/\text{Ca}$ increases about eight-fold with age. Stated in another fashion, chlorophyll *a* and chlorophyll *c* bleach more rapidly than the carotenoids, a feature noted by earlier studies. Such spectral changes would severely limit the precision with which one could estimate chlorophyll *a* concentration from optical measurements made within the blue region of the spectrum. Similar but less dramatic changes in spectral absorption were seen in the continuous culture of *Monochrysis lutheri* (Figs. 5a and 5b).

In the accompanying paper, we will apply values of $a_p^*(\lambda)$ and $b_p^*(\lambda)$ measured for phytoplankton to a simple model of ocean color. This model, which assumes a homogeneous, optically deep body of water, consists of just two components, water and phytoplankton. Using the equations of reflectance spectroscopy, we will attempt to demonstrate how measurements of diffuse spectral reflectance may be analyzed to obtain estimates of phytoplankton crop size, despite the variability in cellular optical properties demonstrated here.

REFLECTANCE SPECTROSCOPY OF MARINE PHYTOPLANKTON

Part 2. A Simple Model Of Ocean Color

INTRODUCTION

The effect of phytoplankton crop upon ocean color spectra has been studied recently because of possible application to the remote sensing of the oceans. Research into the spectral properties of natural phytoplankton crops may be divided into two basic approaches. One approach has been a statistical analysis of concurrent field measurements of optical properties and chlorophyll *a*. An early example of the first approach was Tyler's (1961, 1964) testing of a spacelight spectrometer in an artificial pond containing a culture of the phytoplankton, *Scenedesmus*. The visible spectra for the ratio of spacelight radiance to the irradiance incident on the water surface, which is primarily influenced by spectral absorption within the water, contained features characteristic of the absorption spectrum of *Scenedesmus*. More recently Smith and Baker (1976a,b) have applied linear regression analysis for the visible spectrum of downwelling spectral irradiance in coastal and open ocean water. They found that the attenuation coefficient for downwelling irradiance covaried with chlorophyll *a* concentration and that the component of the coefficient which covaried with chlorophyll *a* was characterized by a spectrum which approximates the absorption expected of phytoplankton. Also Mueller (1976), applied principle component analysis to spectral measurements from an aircraft flown in coastal waters. Measurements of chlorophyll *a* concentration at sea indicated that estimates of chlorophyll *a* concentration were possible by linear combination of the principal components of the first three characteristic spectra.

A second approach in investigating ocean color spectra has been the development of models of radiative transfer and the comparison of model predictions with optical measurements in the field. These models include two basic types, those in which calculations of diffuse spectral reflectance $R(0,\lambda)$ at the sea surface is calculated from inherent optical properties, and those in which $R(0,\lambda)$ is calculated from hybrid optical properties. (See Preisendorfer (1961) for definitions.) Included in the former are the early single scattering model of Jerlov (1968) and the quasisingle scattering model of Gordon, Brown, and Jacobs (1975), Gordon (1976), and McCluney (1974). In these models $R(0,\lambda)$ is, for example, calculated from the volume scattering coefficient, the volume attenuation coefficient, and the volume scattering function. McNeil, Thomson, and Jerome (1976) have recently simplified these models and applied them to estimates of surface chlorophyll *a* concentration from color spectra over the Great Lakes.

The model introduced here is of the latter type in which $R(0,\lambda)$ is described by a function of two hybrid optical properties, a diffuse absorption coefficient $a^*(\lambda)$ and a diffuse backscattering coefficient $b^*(\lambda)$, each coefficient determined by two optical components, water and phytoplankton. The general approach used in the development of this simple model belongs to the field of reflectance spectroscopy, an approach with a considerable history of application (Kortum, 1969). The model will be applied to three problems associated with the interpretation of ocean color spectra, (1) calculations of changes in spectral diffuse reflectance with changes in crop size and crop age, (2) determinations of spectral regions which are most sensitive to such changes in crop size, and (3) derivation of a dichromatic function relating $R(0,\lambda)$ to the diffuse absorption contributed by the phytoplankton crop. The study will be concluded with a discussion of the limitations inherent in this two component laboratory model of ocean color.

EQUATIONS OF MODEL

In our model we define ocean color spectra by the diffuse spectral reflectance immediately below the sea surface $R(0,\lambda)$, merely the ratio of upwelling spectral irradiance $H(0+,\lambda)$ to downwelling spectral irradiance $H(0-,\lambda)$:

$$R(0,\lambda) = \frac{H(0+,\lambda)}{H(0-,\lambda)} .$$

As noted by Brown (1976), $R(0,\lambda)$ is an appropriate parameter of ocean spectra since it is both theoretically retrievable from remotely sensed radiometric data and is defined by the absorption and scattering properties of the water. The derivation of the general equations describing radiative transfer in this phenomenological model as well as the experimental techniques used in determining absorption and scattering coefficients are found in Duntley (1942). Our simple model contains all the assumptions regarding the radiance distribution as found in Duntley's general treatment. In addition, we describe our ocean as a homogeneous, unbounded body of water in which the radiance distribution of the diffuse light field is similar to that of the concentrated cell suspension measured with a spectrophotometer in the laboratory. Under such conditions $R(0,\lambda)$ becomes a function of the ratio of the diffuse backscattering coefficient to the diffuse absorption coefficient of the medium,

$$R(0,\lambda) = \frac{\dfrac{b^*(\lambda)}{a^*(\lambda)}}{1 + \dfrac{b^*(\lambda)}{a^*(\lambda)} + \left(1 + 2\,\dfrac{b^*(\lambda)}{a^*(\lambda)}\right)^{1/2}} . \qquad (1)$$

The coefficients are themselves the sum of the coefficients for water and phytoplankton cells,

$$b^*(\lambda) = b_p^*(\lambda) + b_w^*(\lambda)$$

and (2)

$$a^*(\lambda) = a_p^*(\lambda) + a_w^*(\lambda) .$$

As shown in Part I (Kiefer, Wilson, Olson, 1976), the coefficients for phytoplankton are a function of the concentration and age or growth rate γ of the cells. If the absorption and backscattering coefficients for phytoplankton are normalized to unit chlorophyll *a* concentration, $^{\circ}a_p^*$ and $^{\circ}b_p^*$, we write

$$b_p^*(\lambda,\gamma) = {}^{\circ}b_p^*(\lambda,\gamma)\,Ca$$

and (3)

$$a_p^*(\lambda,\gamma) = {}^{\circ}a_p^*(\lambda,\gamma)\,Ca \quad ,$$

where Ca is the concentration of chlorophyll *a* (here $\mu g \cdot cm^{-3}$). Measurements of the normalized coefficients for diffuse absorption and backscattering in Eq. 3 for batch cultures of the marine diatom, *Thalassiosira pseudonana* and for a continuous culture of the marine chrysophyte, *Monochrysis lutheri* are also described in Part I. The coefficients for clear natural water were calculated from measurements in the Sargasso Sea of $R(z,\lambda)$ and $K(-,z,\lambda)$, the attenuation coefficient for $H(-,z,\lambda)$. The chlorophyll *a* concentration of this water was roughly 0.01 $\mu g \cdot \ell^{-1}$, a lower limit for natural waters. Figure 1 contains values for $a_w^*(\lambda)$ and $b_w^*(\lambda)$ which were used in all calculations of diffuse spectral reflectance.

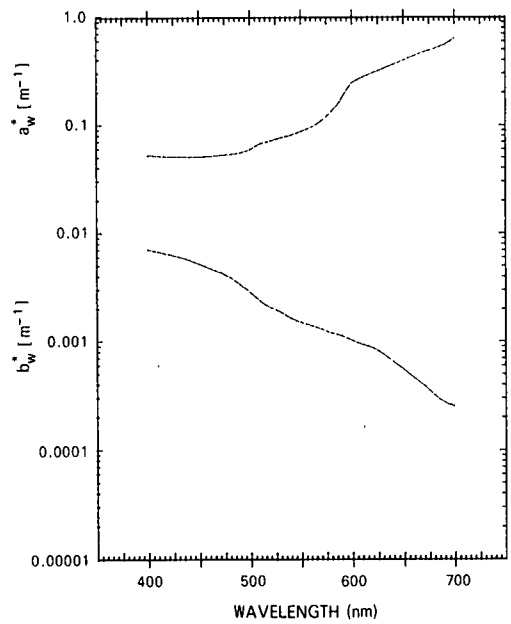

Fig. 1. Values of diffuse absorption and backscattering for clear natural water.

In most instances $a^*(\lambda)$ is between ten and one hundred times larger than $b^*(\lambda)$. (See Fig. 1 in this section and Figs. 2 and 5 of Part I.) By assuming $a^*(\lambda) >> b^*(\lambda)$ and combining Eqs. 1 and 2 we may write:

$$R(0,\lambda,\gamma) = \frac{1}{2} \frac{b_w^*(\lambda) + b_p^*(\lambda,Ca,\gamma)}{a_w^*(\lambda) + a_p^*(\lambda,Ca,\gamma)} \quad (4)$$

or from Eq. 3

$$R(0,\lambda,\gamma) = \frac{1}{2} \frac{b_w^*(\lambda) + {}^{o}b_p^*(\lambda,\gamma)\, Ca}{a_w^*(\lambda) + {}^{o}a_p^*(\lambda,\gamma)\, Ca} \quad . \quad (5)$$

This approximation is inadequate for model waters containing high concentrations of aged cells since these cells have a ratio of $b_w^*(\lambda,\gamma)/a_p^*(\lambda,\gamma)$ approaching one at long wavelengths. This approximation will be used later, not as a necessary condition, but as a means of keeping the functions simple.

RESULTS

Spectral Properties of Model.

Equations 1, 2, and 3 allow one to calculate diffuse spectral reflectance immediately below the sea surface as a function of crop size. $a_w^*(\lambda)$, $b_w^*(\lambda)$, ${}^{o}a^*(\lambda,\gamma)$, and ${}^{o}b^*(\lambda,\gamma)$ are all constants, and Ca is the independent variable of $R(0,\lambda,\gamma)$. In Fig. 2 we show the results of such calculations for a model ocean containing the marine diatom, *Thalassiosira pseudonana,* in concentrations equivalent to 0.01, 0.1, 1, and 10, $\mu g \cdot \ell^{-1}$ chlorophyll *a*. Figure 2a shows spectral changes for actively growing cells from a culture which was 7 days old, Fig. 2c shows changes for dead, detritus-like cells from a culture which was 17 days old, and Fig. 2b shows changes for intermediate cells which are severely chlorotic. For all cultures, changes in diffuse reflectance are large in the blue, and for the older cells there are also large changes in the green. In order to analyze the spectral features of such change we have calculated difference spectra for each change of an order of magnitude. Thus, Fig. 3 presents $R(0,\lambda,0.1) - R(0,\lambda,0.01)$, $R(0,\lambda,1) - R(0,\lambda,0.1)$, and $R(0,\lambda,10) - R(0,\lambda,1)$, for the three cultures of *Thalassiosira pseudonana.*

Figures 4 and 5 show reflectance spectra and difference spectra for varying concentrations of the marine chrysophyte, *Monochrysis lutheri,* at three rates of steady-state growth. The spectra for *Monochrysis lutheri* are similar in general features to those for *Thalassiosira pseudonana.*

Three features of the difference spectra are noteworthy. First, the spectral changes between water containing 0.01 and 0.1 $\mu g \cdot \ell^{-1}$ are quite small and only in the blue region of the spectrum. Thus, at these low concentrations it appears difficult to estimate chlorophyll *a* concentration with any precision. Second, because of the strong absorption of long wavelengths by water itself (Fig. 1) the absorption maximum at 670 to 680 nanometers by chlorophyll *a* in phytoplankton is not seen in diffuse reflectance even at concentrations of 10 $\mu g \cdot \ell^{-1}$. Third, the age or growth rates of cells within the crop appears to affect both the magnitude and spectral character of diffuse reflectance. In the following section we derive a function which relates spectral diffuse reflectance to the crop size. This function is chosen to minimize effects of age, growth rate, or species upon estimates of crop size.

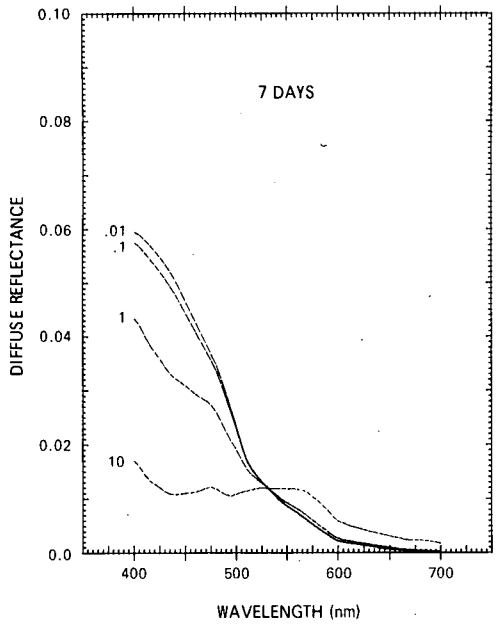

Fig. 2a. Actively growing cells from culture 7 days old.

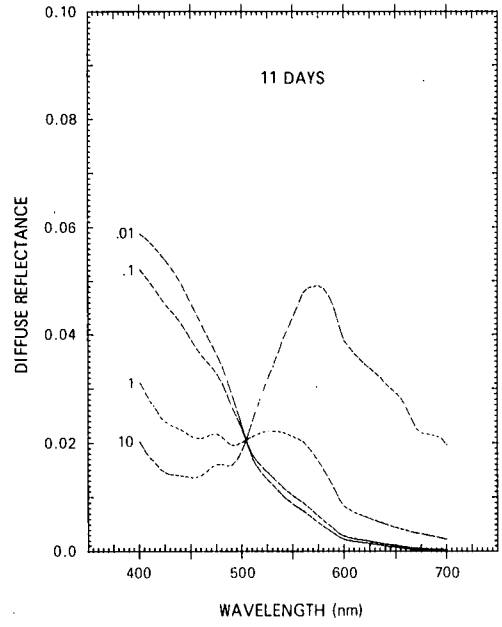

Fig. 2b. Intermediate aged cells from culture 11 days old.

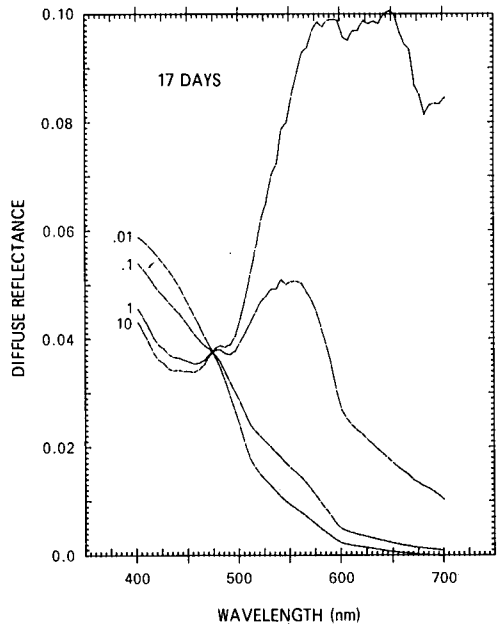

Fig. 2c. Dead detritus-like cells from culture 17 days old.

Fig. 2. Computed values of diffuse reflectance for model ocean containing *Thalassiosira pseudonana* cells in concentrations equivalent to 0.01, 0.1, 1.0, and 10 $\mu g \cdot \ell^{-1}$ chlorophyll *a*.

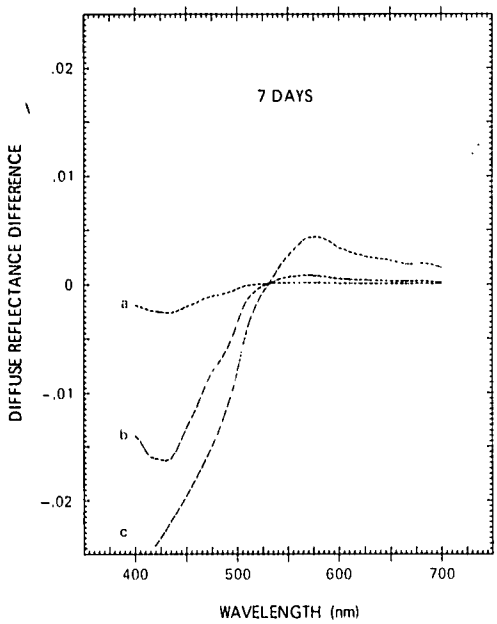

Fig. 3a. Actively growing cells from culture 7 days old.

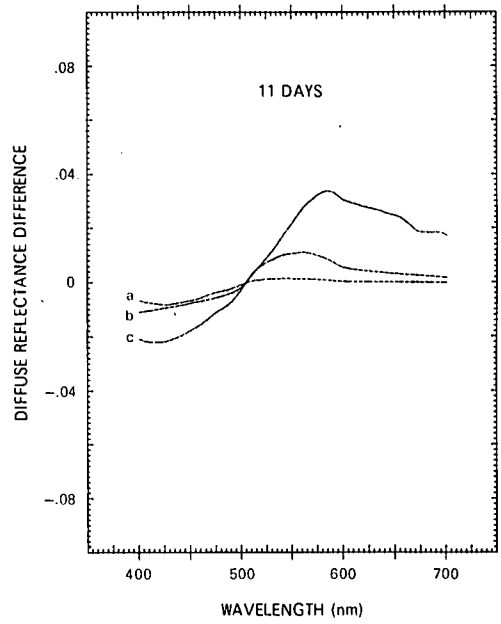

Fig. 3b. Intermediate aged cells from culture 11 days old.

Fig. 3. Difference spectra computed from diffuse reflectance of *Thalassiosira pseudonana* from Fig. 2.

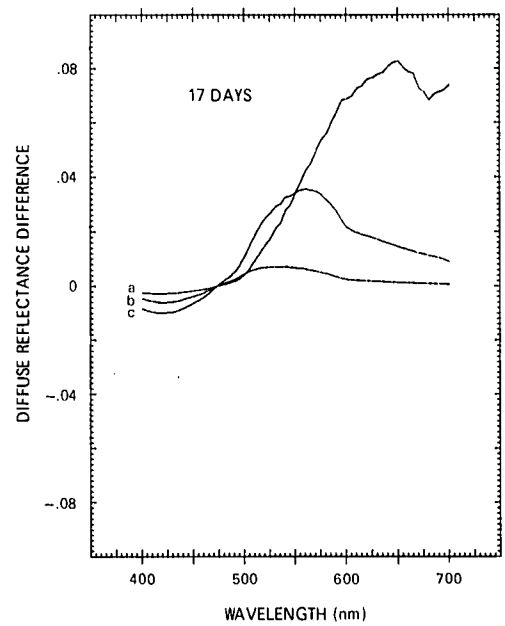

Fig. 3c. Dead detritus-like cells from culture 17 days old.

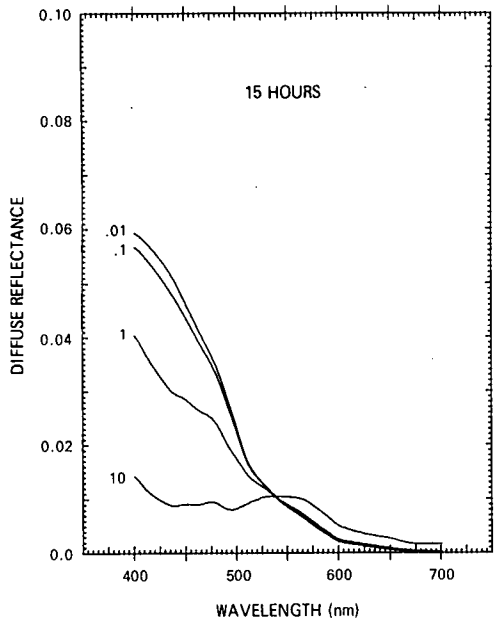

Fig. 4a. Cells from steady-state growth with doubling time of 15 hours.

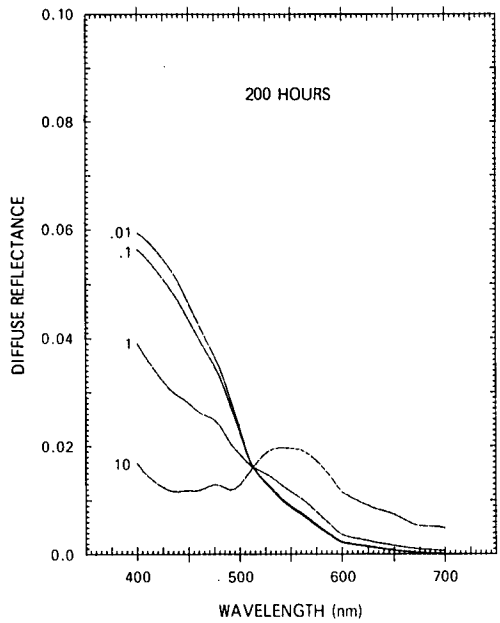

Fig. 4b. Cells from steady-state growth with doubling time of 200 hours.

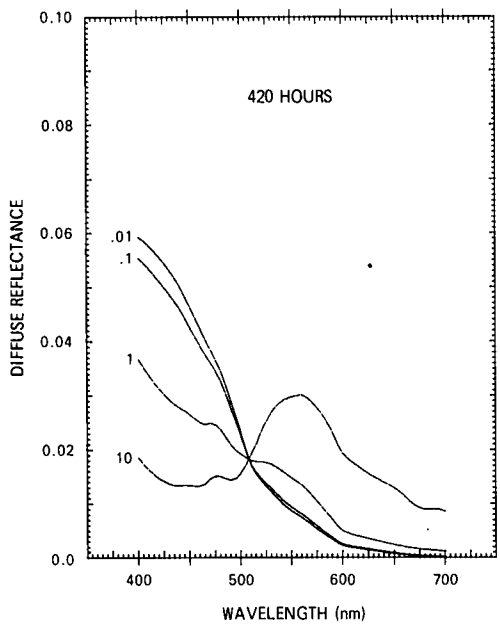

Fig. 4c. Cells from steady-state growth with doubling time of 420 hours.

Fig. 4. Computed values of diffuse reflectance for model ocean containing *Monochrysis lutheri* cells in concentrations equivalent to 0.01, 0.1, 1.0, and 10 $\mu g \cdot \ell^{-1}$ chlorophyll *a*.

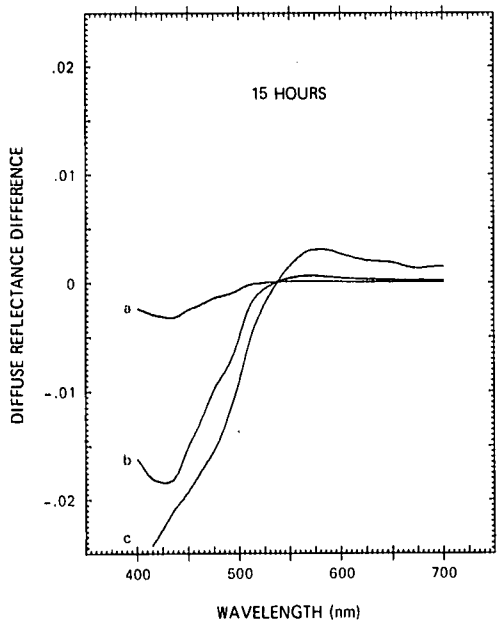

Fig. 5a. Cells from steady-state growth with doubling time of 15 hours.

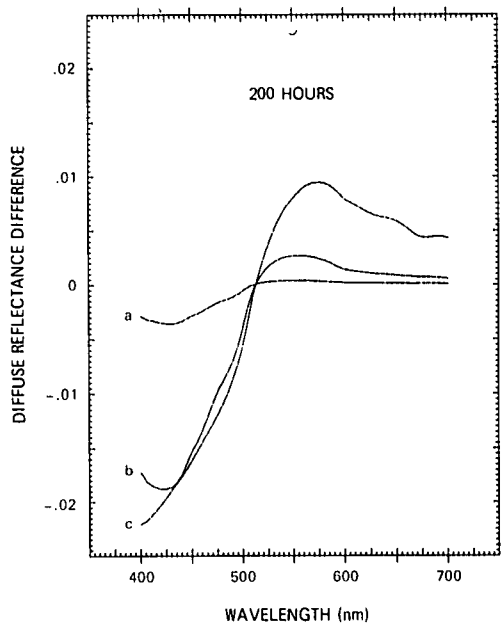

Fig. 5b. Cells from steady-state growth with doubling time of 200 hours.

Fig. 5. Difference spectra computed from diffuse reflectance of *Monochrysis lutheri* from Fig. 4.

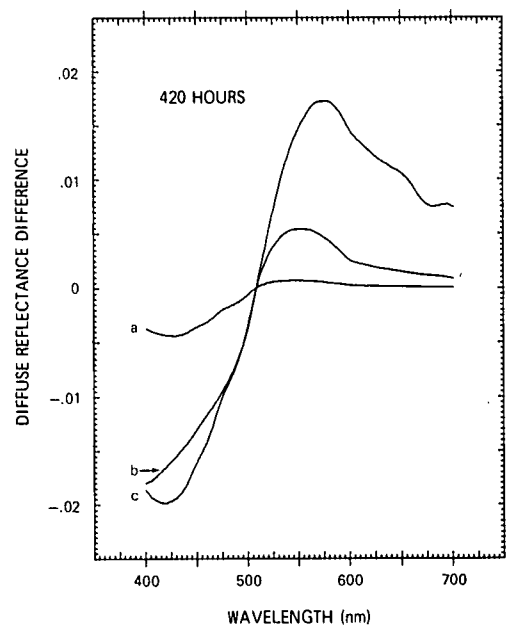

Fig. 5c. Cells from steady-state growth with doubling time of 420 hours.

Determination of $a_p^*(\lambda)$ and $b_p^*(\lambda)$ From Measured $R(0,\lambda)$.

Here we will derive an expression relating measured values of diffuse reflectance at two wavelengths to the absorption and scattering coefficients of phytoplankton. According to Eq. 5, two measurements of reflectance at λ_1 and λ_2 can be represented:

$$R(0,\lambda_1,Ca,\gamma) = \frac{1}{2} \frac{b_w^*(\lambda_1) + b_p^*(\lambda_1,Ca,\gamma)}{a_w^*(\lambda_1) + a_p^*(\lambda_1,Ca,\gamma)} \tag{6}$$

and

$$R(0,\lambda_2,Ca,\gamma) = \frac{1}{2} \frac{b_w^*(\lambda_2) + b_p^*(\lambda_2,Ca,\gamma)}{a_w^*(\lambda_2) + a_p^*(\lambda_2,Ca,\gamma)} . \tag{7}$$

Since $R(0,\lambda_1,Ca,\gamma)$ and $R(0,\lambda_2,Ca,\gamma)$ are measured and the absorption and backscattering coefficients for water are known constants, the two equations contain four unknowns. In order to solve we must find two additional relationships between these unknowns. An examination of spectral values of ${}^{o}a_p^*(\lambda,\gamma)$ and ${}^{o}b_p^*(\lambda,\gamma)$ for cultures of phytoplankton (Part I) indicate that within broad spectral regions the ratio ${}^{o}a_p^*(\lambda_1,\gamma)/{}^{o}a_p^*(\lambda_2\gamma)$ is relatively constant. The same is true for ratios of ${}^{o}b_p^*(\lambda,\gamma)$. Thus, the two relationships which lead to a solution of Eqs. 6 and 7 are:

$$a_p^*(\lambda_1,Ca,\gamma) = K_1 a_p^*(\lambda_2,Ca,\gamma) \tag{8}$$

and

$$b_p^*(\lambda_1,Ca,\gamma) = K_2 b_p^*(\lambda_2,Ca,\gamma) . \tag{9}$$

By combining Eqs. 7 through 9 and solving for $a_p^*(\lambda_1,Ca,\gamma)$, we obtain

$$a_p^*(\lambda_1,Ca,\gamma) = \frac{2K_1K_2a_w^*(\lambda_2)\,R(0,\lambda_2) - 2K_1a_w^*(\lambda_1)\,R(0,\lambda_1) + K_1b_w^*(\lambda_1) - K_1K_2b_w^*(\lambda_2)}{2[K_1R(0,\lambda_1) - K_2R(0,\lambda_2)]} . \tag{10}$$

One can also solve for $b_p^*(\lambda_1)$.

Values for the constants in Eq. 10 are shown in Table 1. The two wavelengths, 420 and 560 nanometers, were picked from the difference spectra of Figs. 3 and 5, and are regions of maximal change in

reflectance. Median values for K_1 and K_2 come from the range in values shown in parentheses. These ranges represent cultures of differing steady-state growth rates *(M. lutheri)* or batch cultures of differing age *(T. pseudonana)*. (See Part I.) Taking the median for both cultures we obtain a K_1 value of 3.5 and a K_2 value of 1.0.

Table 1

Values for constants in Eq. 10. K_1 and K_2 are median ratios of diffuse absorption and backscattering of *M. lutheri* and *T. pseudonana* for wavelengths 420 and 560 nanometers. Next four constants are values of diffuse absorption and backscattering for clear water at the two wavelengths. The final four are the median values for normalized diffuse absorption and backscattering for cultures of the two species. Values within parentheses are the ranges.

Constant	Dimension	Water	*M. lutheri*	*T. pseudonana*
K_1	Dimensionless		3.4(2.8 – 3.8)	3.7(2.0 – 5.7)
K_2	Dimensionless		0.91(0.87 – 1.2)	1.2(1.1 – 1.8)
$a_w^*(420)$	cm^{-1}	5.1×10^{-4}		
$a_w^*(560)$	cm^{-1}	9.5×10^{-4}		
$b_w^*(420)$	cm^{-1}	6.4×10^{-5}		
$b_w^*(560)$	cm^{-1}	1.4×10^{-5}		
$^{\circ}a_p^*(420)$	$cm^2 \cdot \mu g^{-1}$		0.50(0.42 – 0.7)	0.36(0.25 – 2.9)
$^{\circ}a_p^*(560)$	$cm^2 \cdot \mu g^{-1}$		0.16(0.11 – 0.21)	0.10(0.081 – 0.63)
$^{\circ}b_p^*(420)$	$cm^2 \cdot \mu g^{-1}$		$6.9 \times 10^{-3}(3.5 \times 10^{-3} - 1.6 \times 10^{-2})$	$4.1 \times 10^{-3}(2.5 \times 10^{-3} - 0.22)$
$^{\circ}b_p^*(560)$	$cm^w \cdot \mu g^{-1}$		$7.8 \times 10^{-3}(2.9 \times 10^{-3} - 1.8 \times 10^{-2})$	$3.5 \times 10^{-3}(1.4 \times 10^{-3} - 0.16)$

Chlorophyll *a* and Diffuse Absorption.

Chlorophyll *a* and not $a_p^*(\lambda)$ is of course the most commonly measured parameter of phytoplankton crop. Since $a_p^*(675)$ is due almost entirely to absorption by chlorophyll *a* within the cell, one expects relatively precise estimates of chlorophyll *a* from a determination of diffuse absorption in the red absorption maximum. Our model indicates, however, that at concentrations below 10 $\mu g \cdot \ell^{-1}$ the red absorption band of chlorophyll *a* is masked by the absorption of water. In the blue, absorption by carotenoid accessory pigments is superimposed upon the Soret bands of chlorophyll *a* and *c*. Thus, one would expect precise estimates of chlorophyll *a* from a determination of $a_p^*(\lambda_1)$ in the blue only if the cellular concentrations of accessory pigments covary closely with chlorophyll *a* concentration. As shown in the

accompanying paper such covariation is not close, chlorophyll *a* being more labile than carotenoids in aging or nutrient-starved cells.

The lower half of Table 1 contains values for normalized coefficients of diffuse absorption and backscattering for cultures of the two species. For batch cultures of *T. pseudonana* $^{\circ}a_p^*(420)$ ranged from 0.25 $cm^2 \cdot \mu g^{-1}$ for rapidly growing cells to a high of 2.9 $cm^2 \cdot \mu g^{-1}$ for dead, detritus-like cells. In the case of the continuous, nitrogen-limited culture of *M. lutheri* $^{\circ}a_p^*(420)$ ranged from 0.42 to 0.70. The median value for the two cultures is 0.43. Although the large variations in $^{\circ}a_p^*(420)$ for batch cultures may not be representative of the size of variations in the field, it is likely that estimates of chlorophyll *a* concentration from determinations of diffuse absorption by phytoplankton in the blue region of the spectrum will be limited in precision by variations in the concentration of pigmented detritus.

Based upon the values of Table 1 we can estimate the concentration of chlorophyll *a* in the model ocean from Eq. 10:

$$Ca = \frac{7.7R(0,560) - 4.2R(0,420) + 0.20}{3.5R(0,420) - R(0,560)} \quad \left[\mu g \cdot \ell^{-1}\right] \qquad (11)$$

We have examined the accuracy of Eq. 11 by introducing values of R(0,420) and R(0,560) from model oceans such as those shown in Figs. 2 and 4 into the equation. We then compared the estimates with known values of chlorophyll *a* for the models. Such a comparison indicated that the application of Eq. 11 is limited to waters containing at least 0.1 $\mu g \cdot \ell^{-1}$. In waters with 0.01 $\mu g \cdot \ell^{-1}$ for example, we estimate that phytoplankton contribute less than 1 percent of the total diffuse absorption and less than 0.1 percent of the diffuse backscattering. In model oceans containing more than 0.1 $\mu g \cdot \ell^{-1}$ chlorophyll, Eq. 11 generally allows estimates within a three-fold range of known values.

DISCUSSION

Physical Limitations of Model.

Although in the discussion we wish to consider primarily the assumptions regarding the kinds of dissolved and suspended material in sea water, we will first briefly discuss the assumptions regarding radiative transfer. As mentioned in the introduction a* and b* are hybrid optical properties whose values unlike the corresponding inherent properties of absorption and scattering for collimated light are dependent upon the radiance distribution of the light field. In our model, values for $a_p^*(\lambda)$ and $b_p^*(\lambda)$ are characteristic of the radiance distribution of the diffuse light field within the cuvette, this light field resulting from scattering and absorption by phytoplankton of a monochromatic beam of light normal to the sample. This sample is very turbid and contains cells in concentrations which are at least one hundred times greater than natural concentrations. Given this limitation in our model one wishes to know the sensitivity of $R(0,\lambda)$ to variations in the radiance distribution of the light field near the surface. Although we have not investigated this problem, studies using Monte Carlo Technique, indicate such variations have relatively small effects. Gordon (1976) calculated that the upwelling irradiance at the sea

surface is diffuse and can be adequately characterized by a lambertian reflector. Gordon, Brown, and Jacobs (1974) also found that $R(0,\lambda)$ varied little with solar zenith angle (less than 15 percent change for angles $< 40°$). These calculations indicate that spectral reflectance at the sea surface is diffuse and suggest that our model does not violate the basic features of the natural light field.

Biological Limitations of Model.

The application of reflectance spectroscopy to ocean color analysis is more severely limited by our knowledge of the type and distribution of optically important material in surface water. The model presented here describes a homogeneous body of water containing only phytoplankton. According to the derivation of Eq. 10, the diffuse absorption and diffuse backscattering coefficients for phytoplankton, $a_p^*(\lambda)$ and $b_p^*(\lambda)$, can be calculated from measurements of reflectance at two wavelengths. If other material in surface sea water contribute to ocean color spectra, our ability to determine the effects of such material upon reflectance will depend upon the extent to which $a_m^*(\lambda)$ and $b_m^*(\lambda)$ for a natural optical component differ from $a_p^*(\lambda)$ and $b_p^*(\lambda)$.

Four organic components of sea water which along with phytoplankton might contribute to ocean color are particulate and colloidal detritus, bacteria, and gelbstof. Surprisingly little is known about the optical effects of such material; however, since phytoplankton are a local source of organic material, it is likely that detritus, bacteria, and gelbstof will covary with crop size. Once the spectral properties of this material are known, one may apply such information to a more complex and precise estimate of $a_p^*(\lambda)$ or $b_p^*(\lambda)$ than that represented by Eq. 10. Generally speaking gelbstof will contribute to absorption in the blue and ultraviolet. Bacteria, which are generally smaller than 1 μm and colorless, will contribute to a wavelength-depending backscattering (Bryant, Seiber, and Latimer, 1969), and colloidal detritus will probably contribute to both absorption and wavelength-dependent backscattering. We will conclude the discussion with a more detailed consideration of the optical properties of particulate detritus, since this material is often more abundant than phytoplankton in nature waters (e.g. Zeitzschel, 1970).

Particulate Detritus.

We feel that the spectral distribution of $a_m^*(\lambda)$ and $b_m^*(\lambda)$ for particulate detritus is similar to that of $a_p^*(\lambda)$ and $b_p^*(\lambda)$ for dead phytoplankton cells from our oldest culture of *Thalassiosira pseudonana* for the following reasons. Particulate detritus in offshore waters derives from primary production either directly from dead phytoplankton or indirectly from dead animal tissue. Since a significant fraction of this material is as large or larger than the smallest phytoplankton and is of roughly comparable index of refraction, one would expect the diffuse backscattering for detritus to be weakly dependent upon wavelength as we have found for *Monochrysis lutheri* and *Thalassiosira pseudonana*.

Microscopic examination of sea water indicates that much particulate detritus is colorless or brown. If the brown color is due to carotenoids, then one might expect the spectral distribution of $a_m^*(\lambda)$ for detritus to be similar to that of aged cells found in batch culture of *Thalassiosira pseudonana*. As mentioned in Part I, these dead cells lack chlorophyll *a* and absorb almost exclusively in the blue. Thus,

according to our two component model increases in both pigmented and colorless particulate detritus will increase calculated values for $b_p^*(\lambda)$ at all wavelengths. Increases in pigmented detritus alone will, according to Eq. 10, increase calculated values of $a_p^*(\lambda)$ in the blue end of the spectrum. Since estimates of chlorophyll *a* concentration are based upon calculated values of $a_p^*(\lambda)$, variations in $b_p^*(\lambda)$ affected by particulate detritus will not in theory diminish the precision of such an estimate. However, estimates of chlorophyll *a* concentration based upon calculated values of $a_p^*(\lambda)$ in the blue absorption maximum will be limited in precision if there are variations in the relative concentrations of pigmented detritus.

In conclusion we feel that the application of the principles of reflectance spectroscopy helps in our understanding of ocean color spectra. In particular the solution of simultaneous equations which represent $R(0,\lambda)$ as a function of components $a^*(\lambda)$ and $b^*(\lambda)$ at selected wavelengths (Eqs. 6 thru 10) offers a relatively simple and adaptable means of analyzing ocean color spectra. By discriminating between those spectral effects caused by variations in backscattering and those affected by variations in absorption, one obtains a crude thermodynamic description of radiative transfer in upper waters. Values for $b^*(\lambda)$ index the redirection of downwelling radiant energy by suspended and dissolved material while values for $a^*(\lambda)$ index the loss of radiant energy. This lost radiant energy is available for photochemical reactions which in turn may alter the stock of dissolved and suspended material determining the magnitude of $b^*(\lambda)$.

ACKNOWLEDGEMENTS

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