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INTRODUCTION

In studies of both marine productivity and pollution, knowledge of the concentration and the taxonomic composition of phytoplankton populations is required. This knowledge provides both direct and indirect information about the biological and chemical conditions in the water column and can often be used to estimate the level of productivity or pollution.

Laboratory measurements of population concentration generally use concentration of chlorophyll a, cell count, cell volume, or cell weight (per unit volume) as indices. Of these, only chlorophyll a provides an indication of both concentration and biochemical activity, and it is therefore the most useful and convenient parameter to measure. The three primary laboratory and field spectrophotometric methods currently used to measure chlorophyll a are in vitro absorbance (ref. 1), in vitro fluorescence (ref. 2), and in vivo fluorescence (ref. 3). All have their limitations, errors which in mixed populations cannot always be corrected. A fourth method, in vivo absorbance, introduced by Yentsch in 1957 (refs. 4 and 5) and further developed by Konovalov and Bekasova in 1969 (ref. 6), is not currently in general use.

Population composition has traditionally been measured using differential cell-count techniques. These techniques involve the identification of the organisms in a sample by comparing against morphological standards and then grouping the species found by standard classification techniques. Although accurate, this type of measurement is very time-consuming and requires highly trained personnel. An alternative method, the use of unique spectral characteristics (known as signatures) of these organisms, has been discussed in the literature (refs. 7 and 8) but never tested.

It is proposed that a method based on the in vivo spectral absorption of phytoplankton can be developed to measure both concentration and composition of a phytoplankton population. Furthermore, this method would be well suited for passive remote-sensing applications following the lead provided by Gramms and Boyle (ref. 9). To provide a data base for testing assumptions related to the proposed method, 20 phytoplankton species were grown under laboratory conditions and their absorbance spectra over the visible light range were measured. Descriptive statistics were then obtained and used to compare the spectra. The primary questions to be answered were the following:

(1) Are there strong similarities in the absorbance spectra of members of major taxonomic groups?

(2) Are there strong differences between the absorbance spectra characteristic of these same groups?

The significance of these similarities and differences was then tested by classifying each culture as to "color" group (green, blue-green, golden-brown, and red) based only on its absorbance spectra. The validity of the concept of unique absorbance spectra for the color groups was tested further by using the spectra of pure cultures of species from three color groups to resolve the components of mixtures of the three species. In addition, this data base was used to test Yentsch's contention that the in vivo absorption of seawater samples at a wavelength of 670 nm can be used to

quantitate their chlorophyll content (ref. 4). Finally, since the quantity measured by passive optical remote sensors is reflectance rather than absorbance, mean absorbance spectra of the color groups of the algal species used by Gramms and Boyle were compared with their reflectance data.

METHODS AND MATERIALS

The quantity measured in these studies was percent transmission. The data are presented as absorbance because that quantity is more easily related to absorption, a very important component of the remote-sensing equation. The relationship between these quantities is presented in equation (1).

$$A = \log_{10} e^{d(a+s)} \tag{1}$$

where

A	absorbance
a	absorption coefficient
d	distance light traveled
s	scattering coefficient

Transmission of the algae cultures was determined over a wavelength range of 360 to 720 nm, essentially the visible light range, using a scanning transmission spectrophotometer (ref. 10). When operated in the absorbance mode, this spectrophotometer automatically computes absorbance from the transmission data and displays it on a strip-chart recorder. For these studies, the recorder was augmented by a magnetic tape recorder, which allowed the absorbance data to be recorded in a format compatible with subsequent computer reduction and analysis methods.

The spectrophotometer used for the measurements consists of a tungsten filament lamp as a light source and a rotating prism monochrometer. A motor-rotated, slotted mirror is used to alternately route the monochrometer light through a reference cell or a sample cell. In the absorbance range of 0 to 1, where approximately 90 percent of the measurements were made, the precision of absorbance data from this spectrophotometer is reported by the manufacturer to be ± 0.005 absorbance units.

The Shibata technique (ref. 11) was used to improve the spectral definition obtained in these measurements. Two identical pieces of opal glass were placed directly behind the sample and the reference cells. The opal glass produced random scattering of all light not absorbed by the sample and thereby increased the fraction of the light scattered by the sample which reached the sensor. Since the light scattered by the sample contained more spectral difference caused by light absorption than did the nonscattered light, an improved spectral definition resulted from the use of the opal glass (ref. 5).

Calibration of the spectrophotometer was performed with a 3:1 mixture of algae growth medium (nutrients plus seawater) and 10-percent dextran solution made with filtered seawater. This mixture was placed in both the reference and sample cells.

Absorbance readings were then set to 0 throughout the portion of the spectrum where measurements would be made. This procedure enabled the instrument to respond to changes in transmission caused only by the presence of the phytoplankton in the sample cell. This mixture was used in the reference cell for all subsequent measurements.

Absorbance data for the phytoplankton species were obtained with mixtures (3:1) of dense, pure cultures of the different species and 10-percent dextran solution made with filtered seawater. The purpose of the dextran was to immobilize the phytoplankton so they could neither migrate into nor settle out of the light path during the course of the measurement. In conjunction with the absorbance data, measurements were made on the same cultures for concentration of chlorophyll a (ref. 1), in vivo fluorescence, and cell count (ref. 12). Algae cell counts were performed with a Palmer cell, using a Whipple disc inserted in one of the microscope oculars. Three drops of formalin were added to a 5 mL sample from the culture flask, and 0.1 mL of the resultant mixture was introduced into the Palmer cell. The algae were allowed to settle for 5 minutes before counting. The mean of three field counts was used to calculate the cell concentration in cells per milliliter. Fluorescence excitation spectra obtained at the same time from all of these cultures are being prepared for publication. Selection of the phytoplankton species was dictated primarily by their availability. An effort was made to cover a diverse range but still work primarily with species of interest in marine productivity and/or pollution studies. Cultures were maintained and provided by the Algae Culture Division of the Virginia Institute of Marine Science (VIMS). These cultures were unialgal but not free of bacteria. They were grown on N_2M medium (ref. 13) at a temperature of 19°C under continuous lighting from a mixture of "warm white," "cool white," and "daylight" fluorescent bulbs. Measurements were made when the cultures reached early-senescent phase, when cell counts remained constant for 3 consecutive days. Early-senescent-phase cultures were selected for the spectral measurements because they were felt to be most representative of the physiological state of most phytoplankton in their natural habitat. Duplicate data sets were obtained for most cultures, and multiple cultures were used for those organisms of principal interest. Table 1 lists the marine phytoplankton species on which the initial set of absorbance data was obtained.

TABLE 1.- ALGAE COLOR GROUPS AND STANDARD CLASSIFICATION OF ABSORBANCE-TEST SPECIES

Color group	Division	Class	Genus	Species	VIMS code no.
Blue-green	Cyanophyta	Cyanophyceae	Anacystis	marinus	VA-9
Blue-green	Cyanophyta	Cyanophyceae	Synechococcus	elongatus	VA-54
Red	Cyanophyta	Cyanophyceae	Aphanocapsa	roberti-lami	VA-61
Red	Rhodophyta	Rhodophyceae	Porphyridium	purpureum	VA-70
Green	Chlorophyta	Chlorophyceae	Nannochloris	oculata	VA-19
↓	↓	↓	Chlorella	stigmatophora	VA-62
↓	↓	↓	Chlamydomonas	coccoides	VA-63
↓	↓	↓	Dunaliella	euchlora	VA-74
↓	↓	Prasinophyceae	Tetraselmis	suecica	VA-82
Golden-brown	Pyrrophyta	Cryptophyceae	Hemiselmis	(a)	VA-30
↓	↓	Dinophyceae	Prorocentrum	minimum	VA-13
↓	↓	Dinophyceae	Peridinium	triquetum	VA-14
↓	↓	Dinophyceae	Gonyaulax	tamarensis	VA-56
↓	Chrysophyta	Haptophyceae	Pseudoisochrysis	paradoxa[b]	VA-12
↓	↓	Haptophyceae	Coccolithus	(a)	VA-44
↓	↓	Bacillariophyceae	Skeletonema	costatum	VA-28
↓	↓	↓	Chaetoceros	simplex	VA-46
↓	↓	↓	Thalassiosera	fluviatilis	VA-59
↓	↓	↓	Thalassionema	nitzschoides	VA-64
↓	↓	↓	Phaeodactylum	tricornutum	VA-72

[a]Species unknown.
[b]Nom. prov.

Absorbance spectra were also obtained on mixtures of three species, one from each of three color groups. These species were Phaeodactylum tricornutum (golden-brown), Dunaliella euchlora (green), and Anacystis marinus (blue-green). Mixtures of these species were based on chlorophyll a content of each culture as determined by the trichromatic method. Eight different mixtures were made, with the content of each species varied from 10 to 80 percent of the total volume. Mixtures were either 1:1:1, 2:1:1(3), 8:1:1(2), or 5:4:1(2). Absorbance spectra were first obtained for the pure cultures; then the mixtures were made from these cultures and their absorbance spectra were determined. Procedures were the same as those used for the pure cultures.

All absorbance spectra were recorded with a 1/4-in. magnetic tape recorder. This approach allowed computer reduction of the data and more flexibility in the selection of data points for comparative studies.

DATA REDUCTION AND ANALYSIS

The primary aim of the data reduction was to convert the raw absorbance spectra into a data form which allowed valid comparisons between the spectra of algae from different taxonomic groups and between these data and those available in the literature. A second aim was to provide some standard means by which the variability of either species or group absorbance spectra could be compared. To achieve these goals, a data reduction program was written which incorporated the following steps:

1. All spectra were normalized to a 10-cm light path length. This step was based on the assumption of a linear relationship between absorbance and cell length (Beer's law).

2. All spectra from each color group were normalized at 680 nm to a chlorophyll a content of about 100 µg/L. The chlorophyll a value closest to 100 µg/L was selected as a normalization point.

3. Spectra in each color group were normalized to a common cell count. Different groups had different normalization points because senescent populations of different algae produce different cell concentrations.

4. Mean and standard deviation spectra were computed for each species having data from more than one culture and for each color group. Computation of these statistics was done at 0.5-nm intervals throughout the visible light spectrum. The mean spectra for species were computed from the mean spectra for cultures, and the mean spectra for groups were computed from the mean spectra for species. Neither was weighted to account for differences in the number of data points from these sources. The standard deviations of the color groups were based on the variation of both the cultures and the species using equation (2).

$$s^2 = \frac{\sum_{1-k} (n_i - 1)\left[\frac{\sum_{1-n_i} (x_{ij} - x_i)^2}{n_i - 1}\right]}{\sum_{1-k} (n_i - k)} + \frac{\sum_{1-k} (x_i - x)^2}{k - 1} \qquad (2)$$

where

x_{ij}	jth culture of ith species
x_i	mean of ith species
$\bar{x}$	mean of color group
n_i	number of cultures of ith species
k	number of species in color group
S	standard deviation of color group

Initially, data analysis was based largely on visual examination of absorbance spectra and comparisons between species or color groups. The coefficient of variation $S/\bar{X}$ was used to compare variation of spectra within species or color groups. To determine the statistical significance of differences observed between the mean spectra of the four color groups, a series of t-tests was performed at 0.5-nm intervals on each of the eight possible spectra pairs. This procedure was also helpful in determining the regions of maximum differences between these representative spectra.

A classification procedure was established based solely on the absorbance spectra of the 20 species to determine if the in vivo absorbance spectrum over the visible light range can function as a unique signature of the species of any color group. First, a multivariant normal-density function (MVNDF) was computed for each run of each culture of each species (ref. 14) using the absorbance obtained from that run and the mean absorbance of each of the three color groups (blue-green, golden-brown, and green) at three different wavelengths. The wavelengths selected on the basis of the t-test results were the points in the spectra where the most significant differences occurred. For example, one combination used was 535, 570, and 625 nm, which were the points of maximum difference between the mean absorbance spectra of the green and golden-brown, the red and golden-brown, and the blue-green and green color groups. The MVNDF is essentially a probability that the species represented by the tested absorbance spectrum belongs to a given color group. The second step in the classification procedure was to identify the color group of the species by assigning it to that group having the largest MVNDF. The results of this classification procedure were then tested for error against the known group for each species. The effectiveness of any three-wavelength combination as a basis for the classification procedure was rated on the basis of percent correct classifications for each color group.

The components of the three-species mixtures were determined from a least-squares solution of their absorbance spectra. At each wavelength, the absorbance of the mixture was assumed to be the sum of the absorbances of the components. Therefore, a least-squares solution could be obtained for a three-component mixture if the absorbances of the mixture and of the individual species were known at three or more wavelengths. Use of this solution also assumes that the spectral shape remains the same when a species is transferred from a pure-culture environment to a mixed-culture environment. Two types of data bases were used for these calculations. The minimum data base was the absorbance of the mixture at three wavelengths selected on the basis of the t-test results, whereas the maximum data base was the absorbance of the mixture at 0.5-nm intervals from 360 to 720 nm (720 data sets). The first made use

of only the points of maximum difference, whereas the second used all possible differences throughout the visible spectrum. Regression analysis was used to quantitate the degree of fit between the calculated and actual concentrations of the components in the mixtures.

Available data from both the pure- and mixed-culture absorbance spectra were used to test the hypothesis that in vivo absorbance in the red region of the spectrum is a good index of chlorophyll a content. Regression analysis was used to quantitate the degree of fit between the chlorophyll a content, determined by the trichromatic method of Strickland and Parsons (ref. 1), and the maximum absorbance in the region of 675 nm. This analysis was performed on several data sets, including all cultures of all 20 species, all species but blue-greens, and all mixtures. Modified hypotheses which made use of the difference in absorbance maximum (at 680 nm, or at the actual wavelength of maximum absorbance in this region) and minimum (minimum absorbance in the 600- to 660-nm region, or at 720 nm) were also tested.

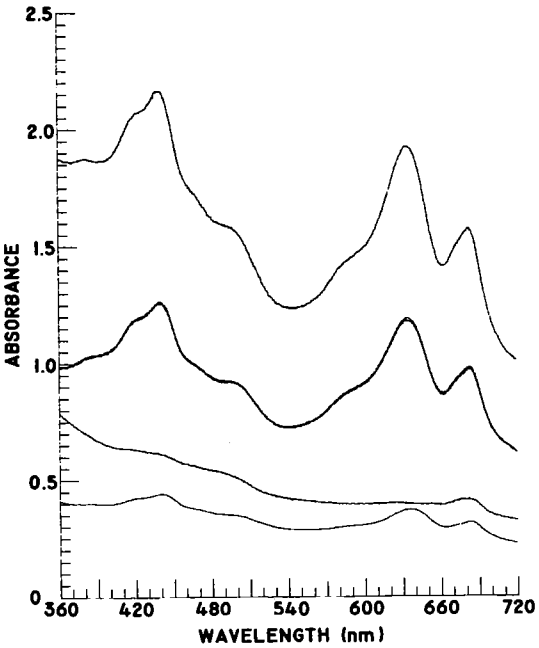
DISCUSSION OF RESULTS

Raw Data

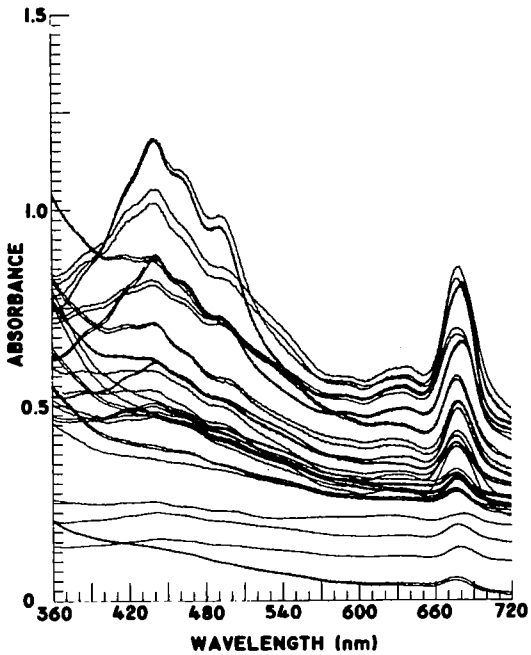
The raw absorbance spectra, normalized to a 10-cm cell length, are presented in figures 1(a) to (d). These data have been organized according to "color" group, an organization which is basically along conventional taxonomic lines. Only one of the species examined in this study had an absorbance spectrum which did not follow the classification scheme presented in table 1. This organism, Aphanocapsa roberti-lami, will be discussed in a later section.

The raw data are presented primarily to show the degree of agreement of repeat runs of the same cultures. This agreement was very good, as can be seen in spectra 2* and 3 in the blue-green color group and in spectra 1 and 2 in the green color group. An example of three repeat runs of one culture can be found in spectra 7 to 9 of figure 1(c). In most cases repeat runs were almost identical, indicating that the reproducibility of the instrument was excellent. In general, the shapes of the absorbance spectra are very similar within color groups, particularly in the 440- to 720-nm region. Differences in magnitude are primarily the result of variations in culture concentration. Although spectra obtained for cultures of low concentration appear to be quite flat, it should be remembered that absorbance is an exponential function, and thus the difference between an absorbance of 1.5 and 0.5 is attributable to a tenfold concentration difference. One point of interest that shows up best in the raw data is the differences in slopes between 360 and 440 nm in the golden-brown species. Examination of the organisms involved revealed that species with negative slopes in this region were dinoflagellates, whereas those with positive slopes were primarily diatoms. The best examples of this phenomenon are spectra 5 and 6 compared with 7 and 8 at 430 nm in figure 1(b).

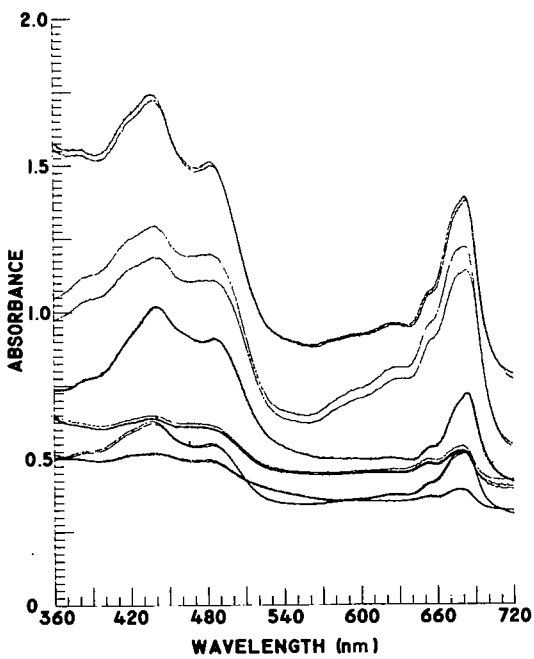
*Unless otherwise indicated, the number of a spectrum is counted down from the top spectrum at 360 nm.



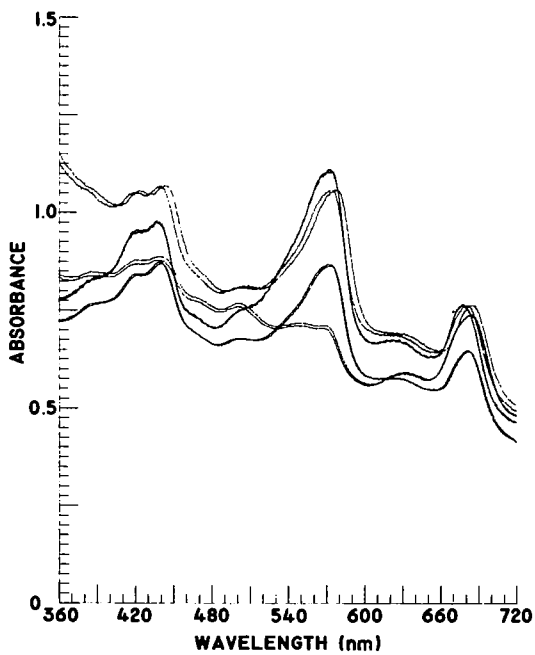
(a) Blue-green color group.



(b) Golden-brown color group.



(c) Green color group.



(d) Red color group.

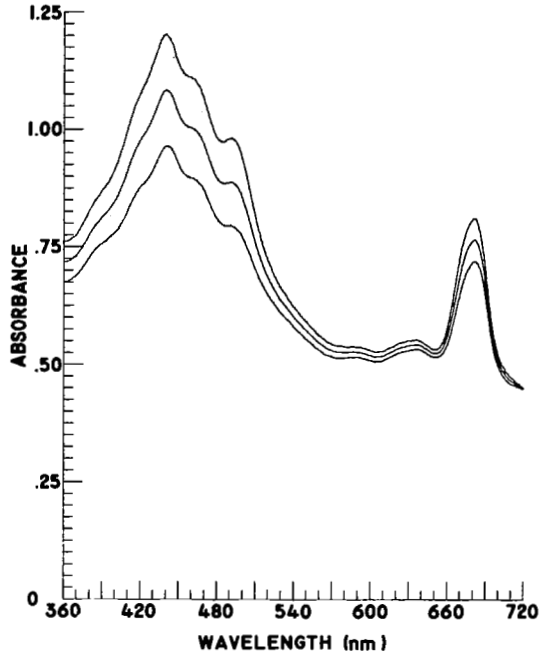
Figure 1.- Nonnormalized absorbance spectra of marine phytoplankton from the four major color groups.

Descriptive Statistics

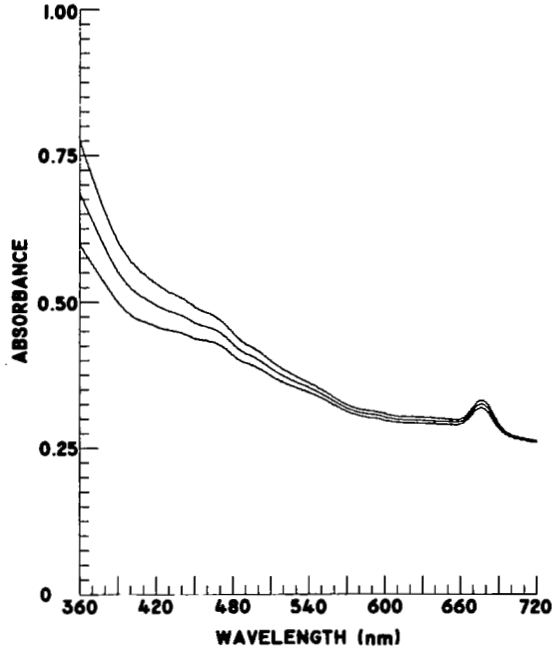
Figure 2 presents the mean absorbance spectra of several species for which multiple cultures were grown. These spectra have been normalized at 720 nm to a known value of chlorophyll *a* concentration to reduce the effect of light scattering (ref. 4); in most cases, a value near 100 μg/L was selected. The upper curve is the mean plus one standard deviation, and the bottom curve is the mean minus one standard deviation. Represented in this group are one species each from the blue-green, green, and red color groups and four species from the golden-brown color group. The last species represent four classes within the two divisions comprising this color group. (See table 1.) The primary purpose of this type of data presentation is to allow a comparison of the variability of cultures of the same species. In five of the seven species the standard deviation was small, varying between 1 and 10 percent of the mean. Two of the golden-brown species, however, had standard deviations that were considerably greater. The standard deviation of *Skeletonema costatum*, a diatom, was about 20 percent of the mean, and that of the *Hemiselmis* species, a cryptomonad, was slightly greater than 20 percent. The cause of this phenomenon is unknown, but considering that only three cultures were used as a data base for each organism, a significant measurement error in one would be sufficient to cause a change in standard deviation of this magnitude. This is one area in which additional data might be illuminating.

The mean absorbance spectra for each species are organized according to color group in figure 3. These mean spectra have been normalized at 680 nm to an absorbance value of 1.0 to remove the effect of variations in culture concentration among species. In the blue-green color group (fig. 3(a)), the two species have absorbance spectra that are similar but have a few recognizable differences. The most important difference is the relatively small peak at 640 nm in the *Synechococcus elongatus* (VA-54) spectrum. Another difference is in the slope between 360 and 440 nm, where one species has a negative slope and the other has a positive slope. This is a very similar phenomenon to that observed in the golden-brown color group (fig. 3(b)). In the golden-brown, another difference is the lack of structure in the 360- to 660-nm region for the dinoflagellates (VA-13, VA-14, and VA-56). In the green color group (fig. 3(c)), all species show very similar spectra, with the ratios of peak heights being very close. Finally, in the red species (fig. 3(d)), the absorbance peaks are at the same locations, but the peak magnitudes are considerably different. This is particularly noticeable at 570 nm, where absorbance is primarily due to the pigment phycoerythrin. The differences between the absorbance spectra of these two species are very likely because of the physiological differences between the organisms. *Porphyridium purpureum* (VA-70) is a true red algae (member of the division Rhodophyta), whereas *Aphanocapsa roberti-lami* (VA-61) is a blue-green species which is pigmented like the red algae.

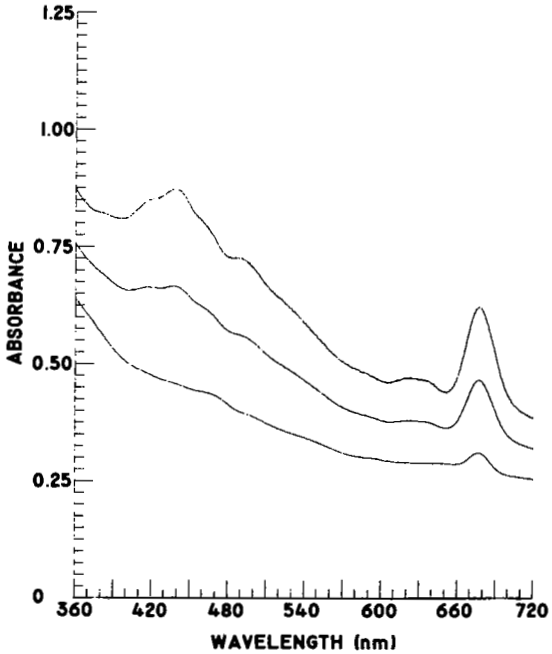
The mean absorbance spectra for each color group are presented in figure 4. These mean spectra have been normalized at 680 nm to an absorbance of 1.0 and plotted plus and minus one standard deviation. This type of data presentation allows comparisons of the variation of the absorbance spectra of species within each color group. The blue-green, red, and green color groups show only slightly greater variation than found in single species, whereas the golden-brown color group shows a much greater variation in some portions of the spectra. In the first three color groups, the variation is greatest where the absorbance is the greatest, which indicates that the variation in the amount of pigments per cell may be the primary source of the differences. The variation found in the golden-brown group, however, is much greater, particularly in the off-peak area below 660 nm, and has a different source. In order to provide more insight into the source of this variation, the mean



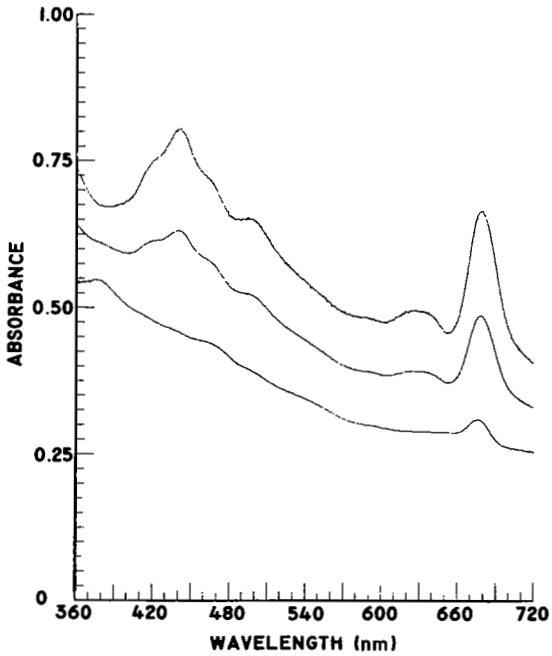
(a) Pseudoisochrysis paradoxa nom. prov. (VA-12); golden-brown/haptophyte; 450 µg/L chlorophyll a.



(b) Procentrum minimum (VA-13); golden-brown/dinoflagellate; 100 µg/L chlorophyll a.

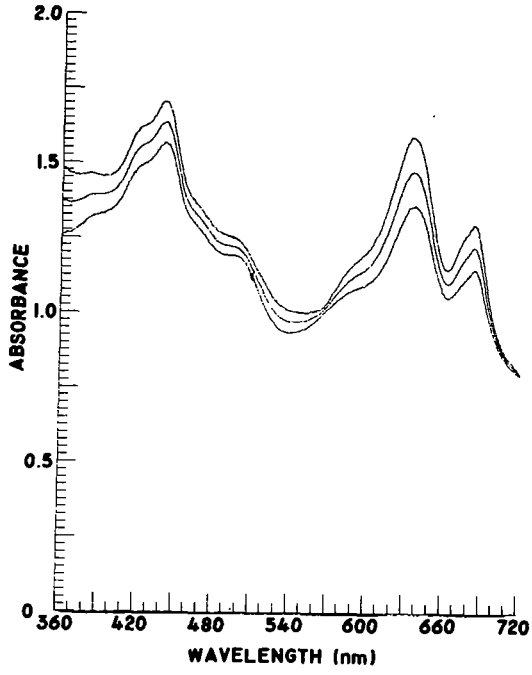


(c) Skeletonema costatum (VA-28); golden-brown/diatom; 100 µg/L chlorophyll a.

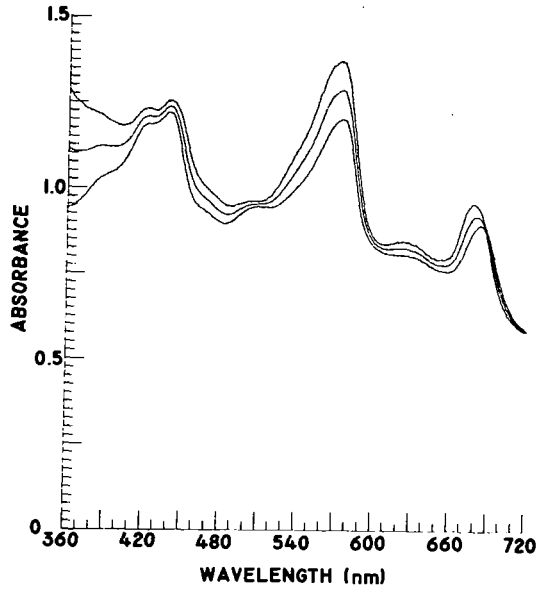


(d) Hemiselmis sp. (VA-30); golden-brown/cryptophyte; 99 µg/L chlorophyll a.

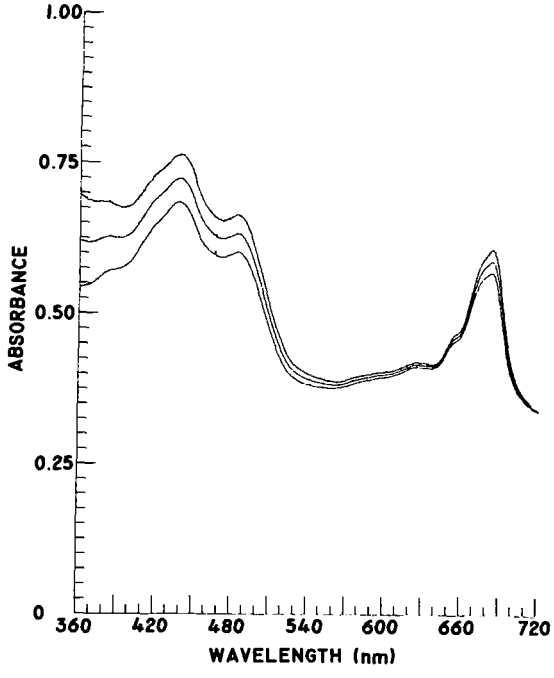
Figure 2.- Mean absorbance spectra, and the mean plus and minus one standard deviation, of several species representative of the four major phytoplankton color groups, normalized to chlorophyll a concentration given.



(e) Anacystis marinus (VA-9); Blue-green; 100 µg/L chlorophyll a.

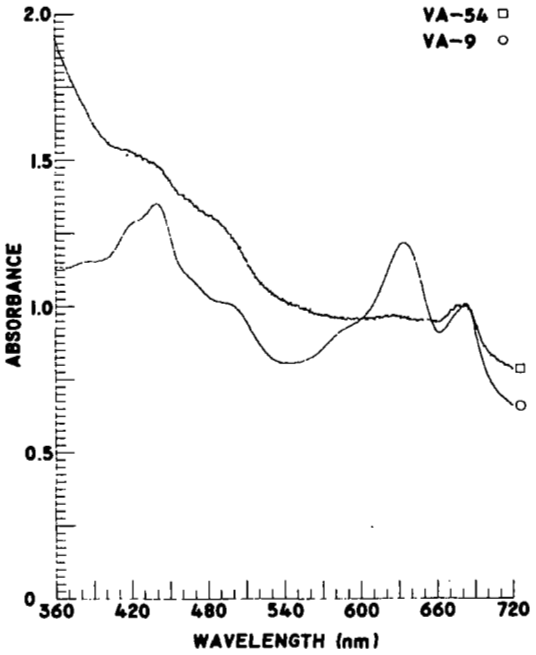


(f) Aphanocapsa roberti-lami (VA-61); Red; 90 µg/L chlorophyll a.

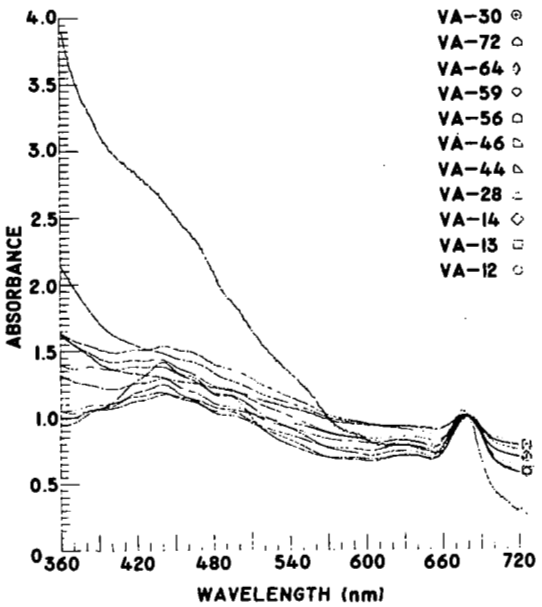


(g) Chlorella stigmatophora (VA-62); Green; 90 µg/L chlorophyll a.

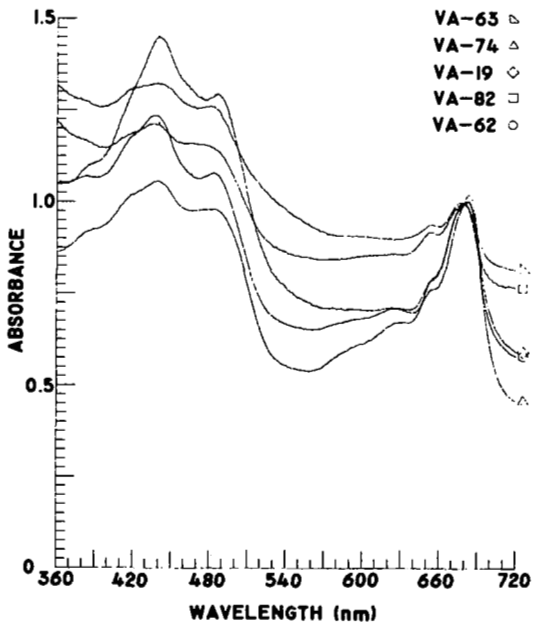
Figure 2.- Concluded.



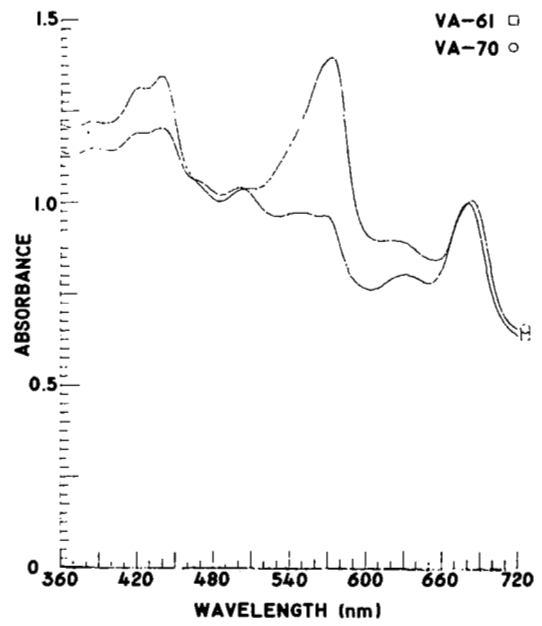
(a) Blue-green color group.



(b) Golden-brown color group.

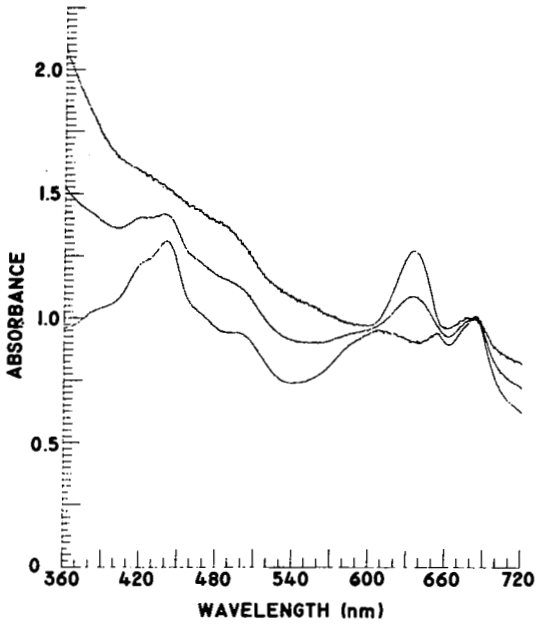


(c) Green color group.

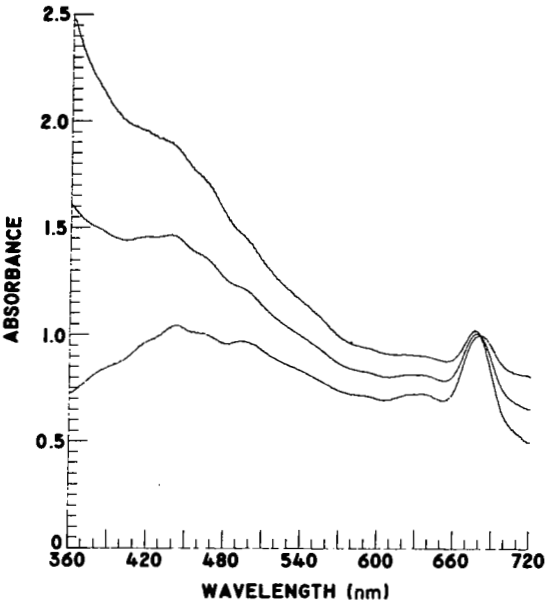


(d) Red color group.

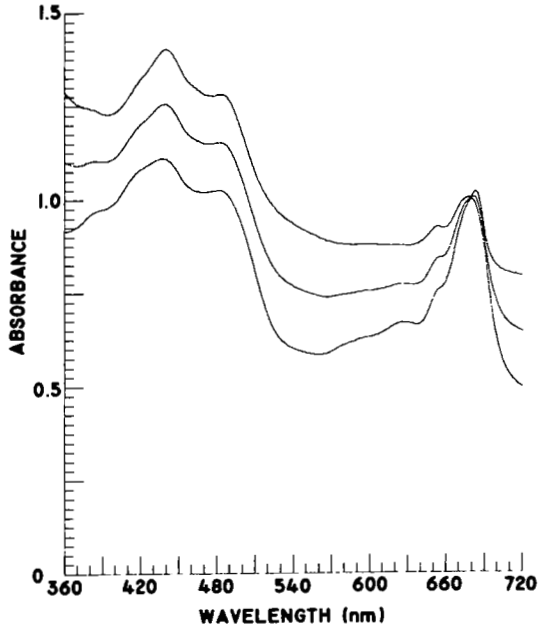
Figure 3.- Mean absorbance spectra for all phytoplankton species by color group normalized to an absorbance of 1.0 at 680 nm. (Species names are given in table 1.)



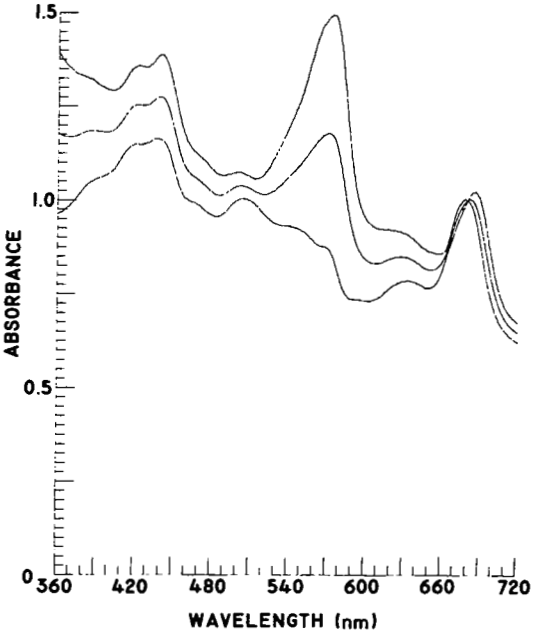
(a) Blue-green color group.



(b) Golden-brown color group.



(c) Green color group.

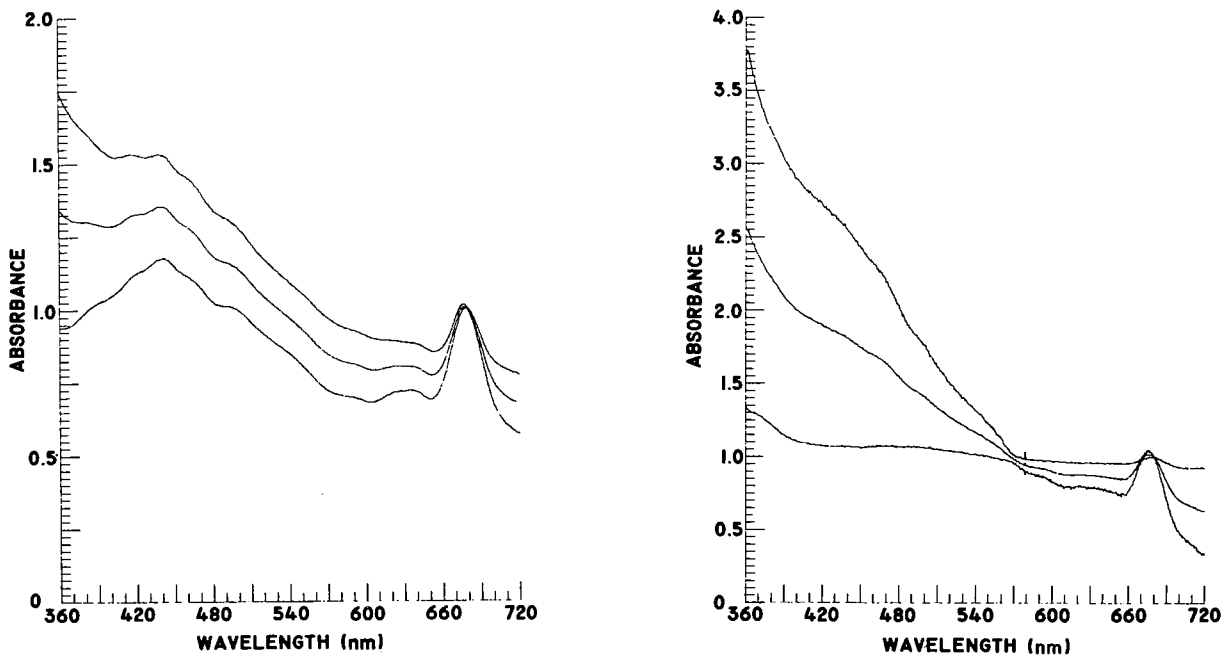


(d) Red color group.

Figure 4.- Mean absorbance spectra, and the mean plus and minus one standard deviation, for the four major phytoplankton color groups normalized to an absorbance of 1.0 at 680 nm.

absorbance spectra for the two major golden-brown subgroups and the mean plus and minus one standard deviation were computed and are presented in figure 5. In addition, the mean spectra for all species in these subgroups are presented in figure 6. Note that the variation in the spectra of the diatoms is approximately that noted in the other color groups, but the variation in the spectra of the dinoflagellates is 50 to 100 percent of the mean, very similar to that noted in the golden-brown species as a whole. Also note that while the mean absorbance of the two subgroups at 680 nm is the same, at 440 nm it differs by a factor of 1.4. Since these two wavelengths are the locations of the two major chlorophyll a absorbance peaks, it was expected that the ratios would be the same. This observation, combined with the observation of the absorbance spectra slope differences between 360 and 440 nm, is interpreted as indicative of a predominance of light scattering by dinoflagellates in this region. Although efforts were made to minimize effects due to scattering, some appreciable particle scattering could have reduced transmission in this area of the spectra and resulted in larger "absorbance" values.

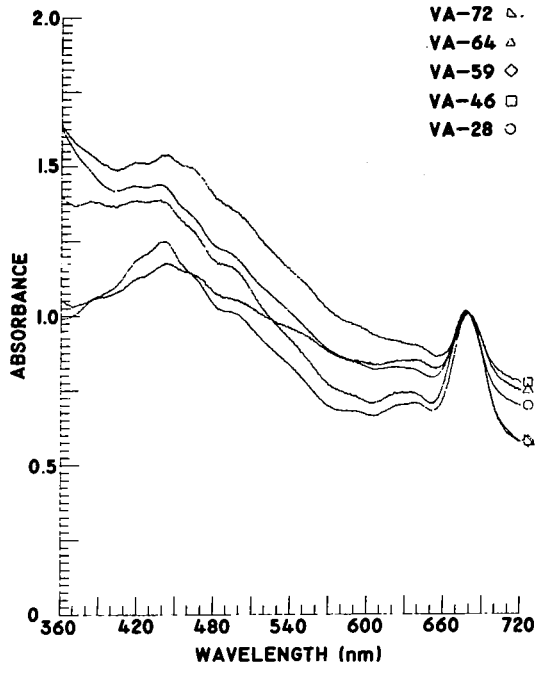
To further investigate this unexpected behavior of the dinoflagellate absorbance spectra, an additional series of absorbance spectra were obtained from six species instead of the original three. The measurement procedure was modified to further reduce the contribution made to the absorbance measurement of light losses due to scattering. A 1-mm path-length cuvette of the same cross section was used for these measurements. It was placed as close to the detector as possible so small-angle scattered light would be collected. The use of the very short light-path cuvette also minimized the number of multiple scattering events. In addition to two of the original species, spectra were obtained for Prorocentrum micans (VA-5), Gymnodinium splendens (VA-6), Massartia rotundata (VA-11), and Glenodinium foliaceum (VA-43).



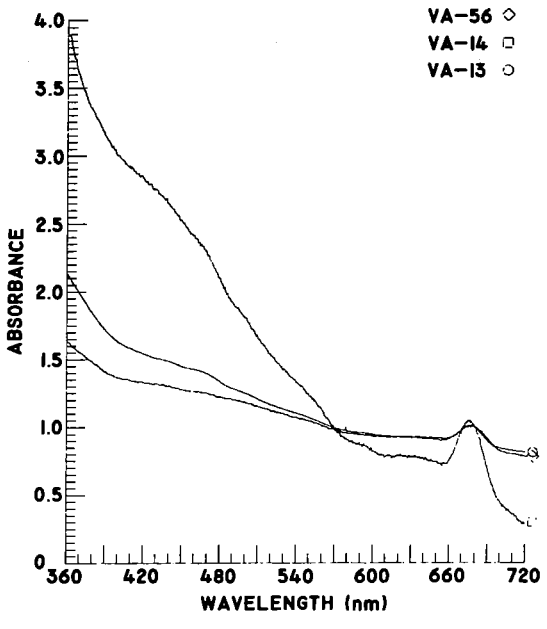
(a) Diatoms.

(b) Dinoflagellates.

Figure 5.- Mean absorbance spectra, and the mean plus and minus one standard deviation, for two major subgroups of the golden-brown color group normalized to an absorbance of 1.0 at 680 nm.

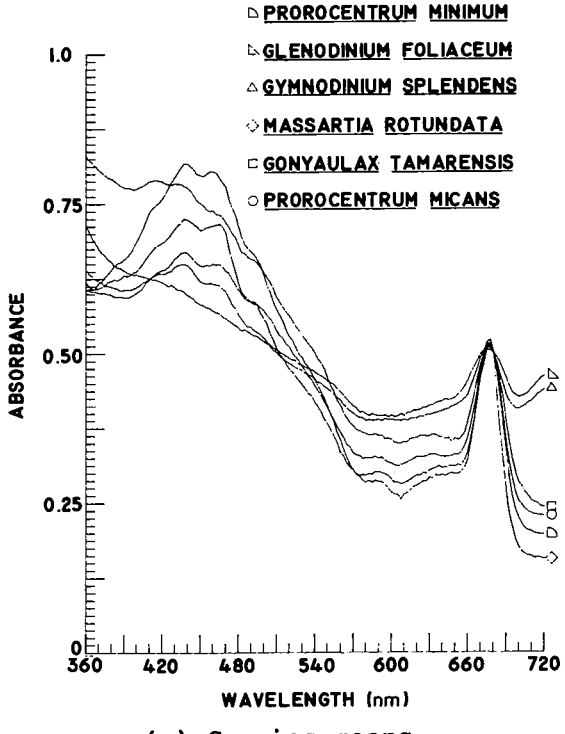


(a) Diatoms.

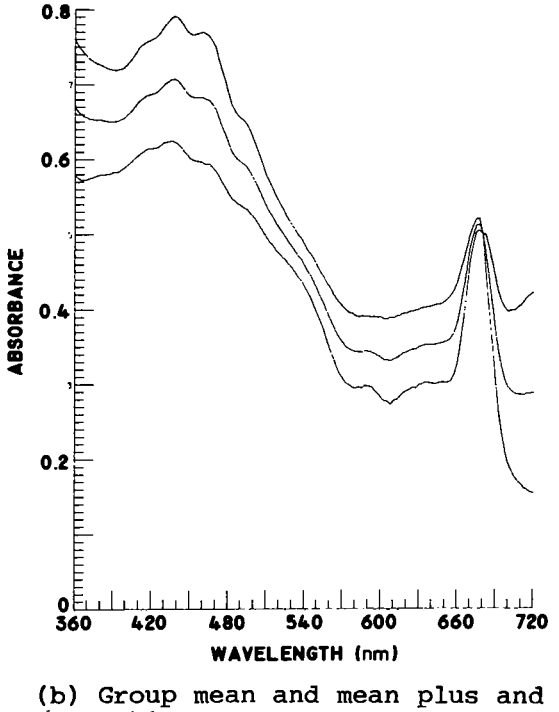


(b) Dinoflagellates.

Figure 6.- Mean absorbance spectra for phytoplankton species belonging to the two major subgroups of the golden-brown color group normalized to an absorbance of 1.0 at 680 nm.



(a) Species means.



(b) Group mean and mean plus and minus one standard deviation.

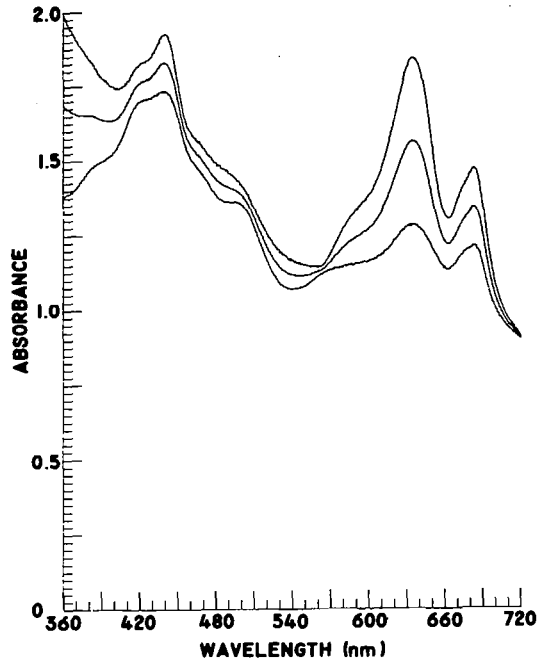
Figure 7.- Absorbance spectra of dinoflagellate species obtained with the 1-mm cuvette normalized at 680 nm to an absorbance of 0.50.

The mean absorbance spectra obtained are presented in figure 7(a). These are the mean spectra for each species, as duplicate cultures were used. Note that four of the species now exhibit spectral absorbance characteristics very similar to the diatoms (see fig. 6(a) for comparison), and only one species continues to show characteristics similar to those observed before for the dinoflagellates. The species showing the most "absorbance" in the 360- to 400-nm region relative to its absorbance at 440 nm, G. foliaceum, is also the largest of this group of dinoflagellates.* Figure 7(b) presents the mean absorbance spectrum for all species examined in the additional data set and the mean plus and minus one standard deviation. Notice the much greater similarity between these mean spectra and those of the diatoms (fig. 5(a)) as compared with the original data (fig. 5(b)).

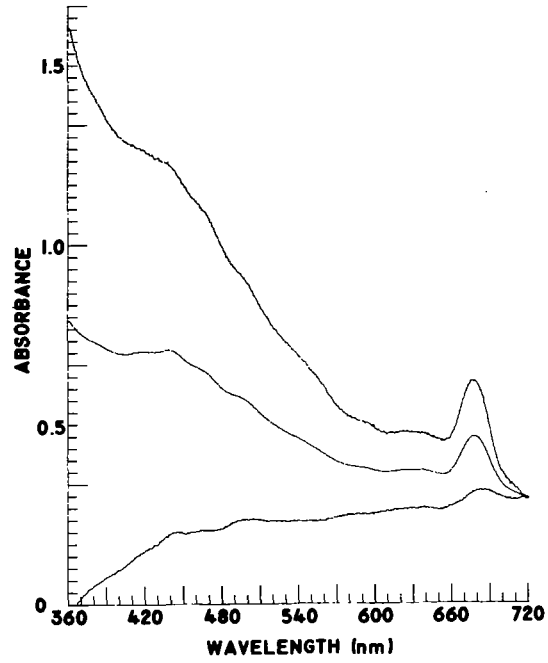
Figure 8 presents the mean absorbance spectra for the four major color groups and the mean plus and minus one standard deviation normalized at 720 nm to cell count. This type of data presentation allows a comparison of absorbance as a function of cell number, a measurement often used in population composition studies. Although a lack of overlapping cell-count data somewhat restricts direct comparisons, absorbance of the red species is considerably less than the same concentration of blue-green species. This relationship was surprising, as the blue-green species are much smaller and would thus be expected to have less chlorophyll and other pigments per cell. However, this effect may be at least partially due to the reduction in the "sieve effect" (ref. 15) noted by Das et al. (ref. 16) for the blue-green algae Anacystis nidulans. This reduction is due to the relatively small size and uniform pigment content of these organisms, which increases large-angle scattering and decreases small-angle scattering, respectively. These effects decrease light transmission and thus cause an apparent increase in absorbance relative to that of larger organisms whose pigments are discretely distributed.

Figure 9 presents the mean absorbance spectra for the four major color groups normalized to cell count. This manner of data presentation allows comparison of general shape characteristics among the four groups. With the exception of the two major peaks at 570 and 635 nm, the mean absorbance spectra of the red and blue-green color groups are very similar. These groups are characterized, therefore, almost solely by the absorbance peaks produced by their water-soluble pigments, phycoerythrin for the red (centered at 570 nm) and phycocyanin for the blue-green (centered at 635 nm). The green and golden-brown color groups differ primarily in the slight effects produced by the absorbance of chlorophyll b (655 nm) in the green species and chlorophyll c (470 nm) in the golden-brown species, as well as some difference in the region of carotenoid absorbance (540 nm). Although the species of these two color groups have unique predominant carotenoid pigments, their in vivo absorbances overlap to such a degree that no unique absorbance peak appears in the carotenoid region of either group.

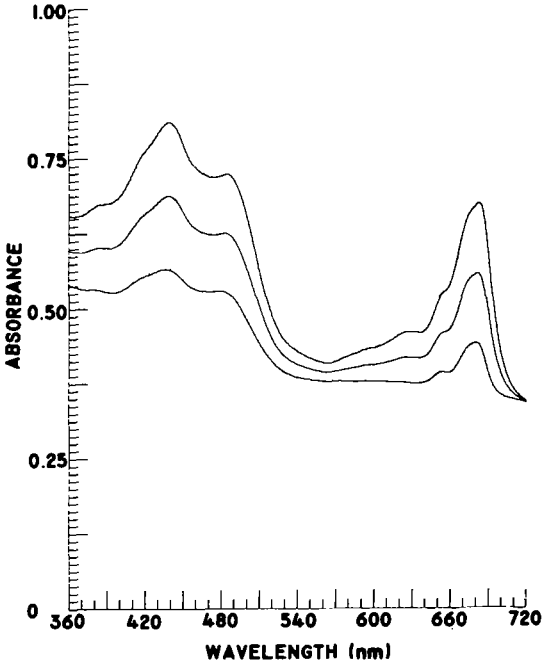
*The reduction in "absorbance" in the short-wavelength region achieved by placing the shorter cuvette nearer the light sensor indicates the original observed effect was caused primarily by small-angle scattering of light. Similar effects were noted by Yentsch (ref. 5).



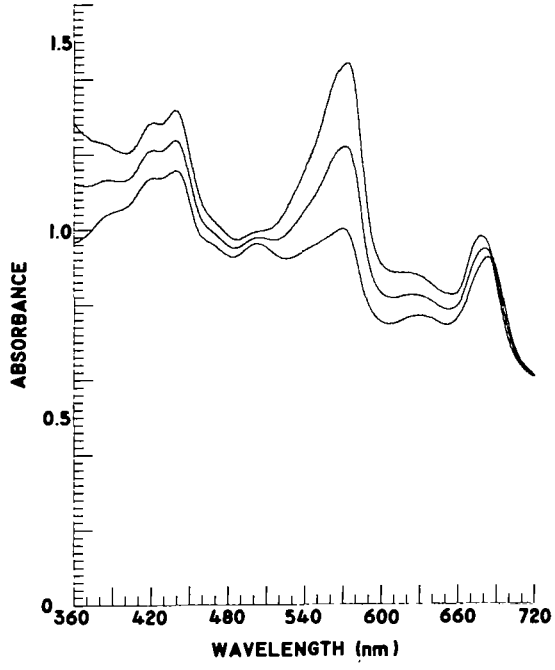
(a) Blue-green color group normalized to 1×10^7 cells/mL.



(b) Golden-brown color group normalized to 1×10^6 cells/mL.



(c) Green color group normalized to 3×10^6 cells/mL.



(d) Red color group normalized to 1×10^7 cells/mL.

Figure 8.- Mean absorbance spectra, and mean plus and minus one standard deviation, for the four major phytoplankton color groups normalized at 720 nm to indicated cell count.

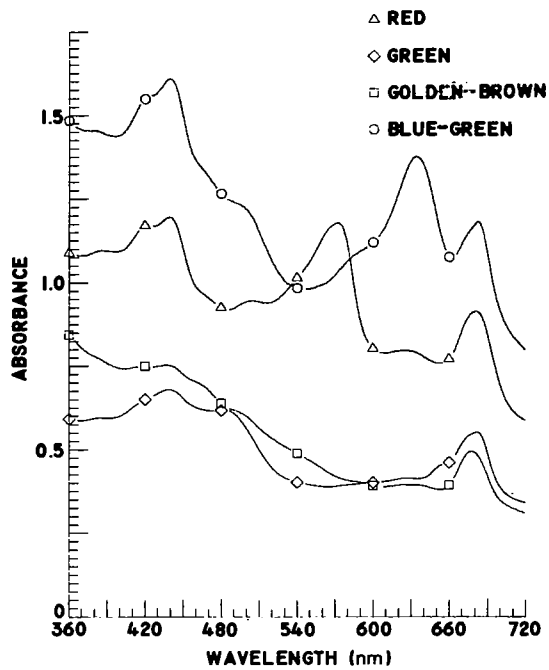
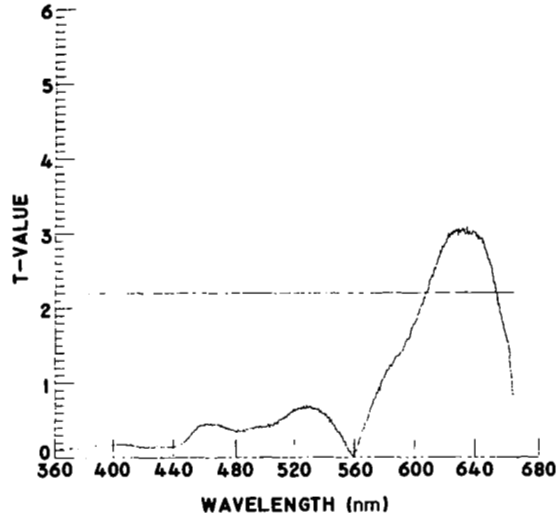


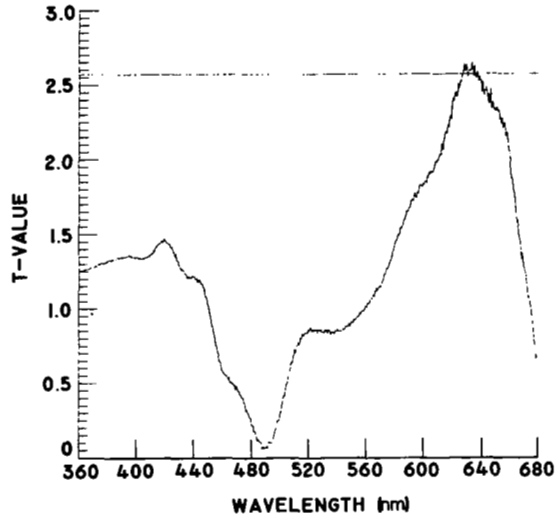
Figure 9.- Mean absorbance spectra of the four major phytoplankton color groups normalized to cell count.

Analytical Statistics

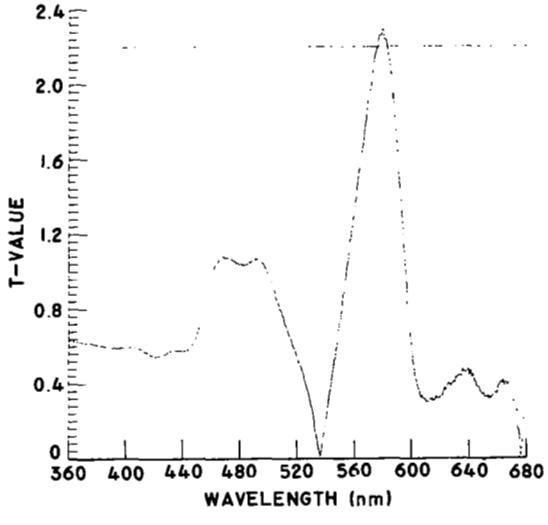
The results of the t-test analysis of differences between the mean absorbance spectra of the color groups are shown in figure 10. Plotted on each graph is a line representing the t-value equivalent to a level of significance α of 0.05 (95-percent confidence level). In four of the six possible comparisons, t-values in excess of this value were computed for some portion of the spectrum, indicating a significant difference between the mean spectra of those color groups in that region of the light spectrum. Only these four comparisons are shown in figure 10. Table 2 presents the key statistical data and the resulting range of significant difference for all possible combinations. The spectral range of the significant, or maximum, difference is usually associated with a region of maximum absorbance by a single photopigment or group of photopigments. For example, the comparisons of mean absorbance spectra of the blue-green and golden-brown color groups and of the blue-green and green color groups are dominated by the absorbance of phycocyanin in the 610- to 660-nm region, whereas the comparisons of absorbance spectra of the red and green color groups and of the red and golden-brown color groups are dominated by absorbance of phycoerythrin in the 550- to 590-nm region. Of particular interest is that the spectral region around 535 nm, where the significant difference between the mean spectra of the golden-brown and green color groups occurs, is a region associated with in vivo absorbance by the carotenoid pigments. This difference is associated with the long-recognized high carotenoid content of the golden-brown algae. More specifically, it is very likely a result of the presence of the carotenoids fucoxanthin or peridinin



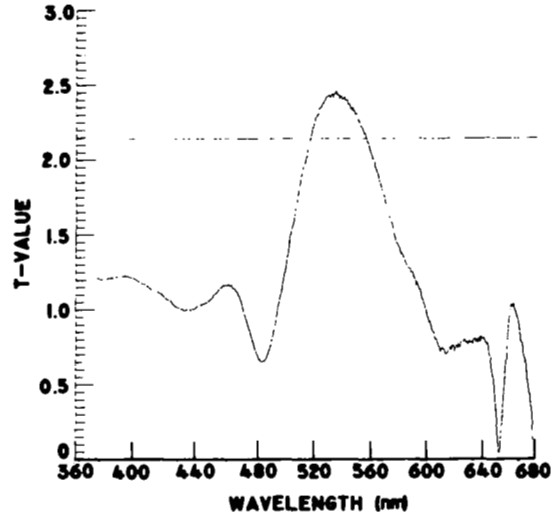
(a) Blue-green versus golden-brown.



(b) Blue-green versus green.



(c) Golden-brown versus red.



(d) Golden-brown versus green.

Figure 10.- Results of t-tests for differences between the mean absorbance spectra of the major phytoplankton color groups. Significant differences for $\alpha = 0.05$ occur where curve extends above the line.

TABLE 2.- T-TEST DATA AND RESULTS OF ANALYSIS OF SPECTRAL DIFFERENCES

Color group pair	Degrees of freedom	$t_{0.95}$	t_{max}	Wavelength of t_{max}, nm	Range of significant difference, nm
Blue-green/golden-brown	11	2.20	3.09	638	610 to 657
Blue-green/green	5	2.57	2.65	625	619 to 627
Blue-green/red	2	4.30	3.02	655	None*
Golden-brown/green	14	2.14	2.45	535	517 to 555
Golden-brown/red	11	2.20	-2.30	572	569 to 576
Green/red	5	2.57	-2.18	568	None*
Diatoms/dinoflagellates	6	2.45	1.89	557	None*
			1.77	360	None*

*No significant difference at $\alpha = 0.05$; data are for t_{max}.

in the golden-brown species and their absence in green species. Fucoxanthin has been reported to have in vivo absorption extending to 590 nm (ref. 7).

The results of the classification experiments were very interesting and encouraging. An example of some of the data obtained is presented in table 3.

TABLE 3.- CLASSIFICATION OF GREEN ALGAE SPECIES BASED ON THEIR IN VIVO ABSORBANCE

[Wavelengths of 535, 570, and 625 nm]

VIMS code no.	Culture	Run	LLF of being -			Classification error
			Blue-green	Golden-brown	Green	
VA-19	1	7	(a)	4.46	6.78	No
VA-19	↓	8	(a)	2.93	6.63	No
VA-62		63	(a)	3.27	7.27	No
↓		69	(a)	1.61	6.95	No
	2	79	(a)	3.03	7.09	No
↓	2	80	(a)	3.29	7.16	No
VA-63	1	31	-198	7.13	6.92	Yes
VA-63	↓	32	-194	7.04	6.77	Yes
VA-74		44	(a)	3.11	6.64	No
VA-74		45	(a)	3.36	6.49	No
VA-82		4	-272	5.80	7.02	No
VA-82	↓	5	(a)	5.88	7.11	No
VA-82		6	-156	4.94	6.65	No

[a]LLF was less than -999.9.

These are the tests for the green species for wavelengths of 535, 570, and 625 nm. In this example, the log of the multivariant normal density function (MVNDF), known as the log likelihood function (LLF), is presented. Note that 11 of the 13 runs (84.6 percent) are correctly classified. In the case of the two incorrectly classified runs (same culture and species), the color group is identified as golden-brown when it is actually green. This error is the most likely because the mean absorbance spectra of the two color groups are the most similar. However, the difference in the LLF in these cases is 0.02 out of a total range of about 5.0×10^4, certainly well within the error of either measuring the absorbance (0.5 percent) or computing the LLF. Obviously these absorbance spectra could belong to either color group, but definitely not to the blue-green color group. The same might also be said for runs 4 and 5, which are correctly classified although their difference is only 0.12 out of a possible range of about 7.0×10^4. In spite of these errors, this type of classification procedure is accurate and demonstrates the feasibility of classifying algae as to color group based on their absorbance spectrum.

The effect of selecting absorbance values at different combinations of wavelengths on the effectiveness of the classification procedure is demonstrated in table 4. One of the most effective combinations of wavelengths is 535, 570, and 625 nm. Note that this combination includes a wavelength (570 nm), which was

TABLE 4.- EFFECTIVENESS OF VARIOUS WAVELENGTH COMBINATIONS FOR CLASSIFICATION OF ALGAE AS TO COLOR GROUP

Absorbance wavelengths nm	Effectiveness of classification, percent correct, for -			
	Blue-green	Golden-brown	Green	Total
535, 570, 625	100.0	100.0	84.6	96.1
535, 620, 647	100.0	75.8	100.0	84.3
535, 625, 680	100.0	84.8	100.0	90.2
440, 535, 625	100.0	81.8	100.0	88.2
535, 625, 655	80.0	97.0	100.0	96.1

identified in the t-tests as the locus of the most significant difference between the red and golden-brown and the red and green color groups, although the red species were not included in the classification tests. However, for some unknown reason, this wavelength is much more effective than 440, 647, or 680 nm, indicating that the third wavelength has an important impact on the effectiveness of the classification procedure. An equally effective wavelength combination was 535, 625, and 655 nm, a combination which uses the location of a secondary peak (655 nm) identified in the t-test series between the mean absorbance spectra of the golden-brown and green color groups, giving two points of differentiation between these two color groups and one point of differentiation between them and the blue-green color group. The percent of correct classifications for the best wavelength combinations (96.1 percent, or 17 of 18 species) is a further indication of the feasibility of the classification technique and the uniqueness of the absorbance spectra of species belonging to the same color group.

Analysis of Mixtures

Results of the two types of mixed-species data analysis are presented in tables 5 and 6. The estimated percentage for each component of each mixture is given opposite the actual percentage. Both are based on chlorophyll a content. The

TABLE 5.- COMPONENTS OF MIXTURES OF THREE GROUPS OF ALGAL SPECIES FOR THREE-WAVELENGTH DATA SET

[535, 620, and 647 nm]

Mixture code letter	Blue-green species, percent		Golden-brown species, percent		Green species, percent	
	Actual	Computed	Actual	Computed	Actual	Computed
A	0.35	0.23	0.35	0.42	0.30	0.35
B	.26	.21	.52	.49	.22	.30
C	.27	.21	.27	.45	.46	.34
D	.52	.26	.26	.38	.22	.36
E	.81	.32	.10	.24	.09	.44
F	.12	.23	.11	.35	.77	.42
G	.10	.17	.81	.56	.09	.27
H	.11	.19	.53	.51	.36	.30
Correlation coefficient	0.93		0.91		0.32	

TABLE 6.- COMPONENTS OF MIXTURES OF THREE GROUPS OF ALGAL SPECIES FOR 720-WAVELENGTH DATA SET

[360 to 720 nm at 0.5-nm intervals]

Mixture code letter	Blue-green species, percent		Golden-brown species, percent		Green species, percent	
	Actual	Computed	Actual	Computed	Actual	Computed
A	0.32	0.28	0.37	0.44	0.31	0.28
B	.47	.46	.28	.34	.25	.20
C	.23	.20	.54	.63	.23	.17
D	.23	.20	.29	.45	.48	.35
E	.38	.32	.57	.68	.05	.00
F	.08	.08	.83	.90	.09	.02
G	.10	.13	.12	.12	.78	.75
H	.09	.11	.43	.37	.48	.52
Correlation coefficient	0.99		0.96		0.98	

correlation coefficient R determined by linear regression analysis of the actual versus the computed percentage is also given for each component. Table 5 presents the results of analysis using the best three wavelengths whereas table 6 presents the results of analysis using the 720 wavelengths from 360 to 720 nm at 0.5-nm intervals. Note that even the best three wavelengths (535, 620, and 647 nm) give a good correlation for only two of the three components. In all cases (see table 4 for other wavelength combinations used), the blue-green component gives the best correlation between actual and computed percentage, whereas the green component gives the poorest correlation. This effect is attributed to the strong, unique absorbance spectrum of the blue-green algae and, conversely, to the lack of unique characteristics in the absorbance spectrum of the green algae. The golden-brown algae are intermediate in the uniqueness of their absorbance spectrum and are also intermediate in the correlation obtained between their actual and computed percentages in the mixtures.

The results of the analysis of the three-wavelength data set indicate that absorbance at more than three wavelengths is needed to obtain good correlations between the actual and computed percentages for all three components. The 720-wavelength data set analysis indicated that excellent correlations could be obtained if enough absorbance data sets (i.e., wavelengths) were used. The ideal number of data sets lies somewhere between 3 and 720 and can be determined once the desired level of correlation between actual and computed percentages is established.

The success of the 720-wavelength data set computation for estimating the components of the 8 mixtures gives further support to the concept of unique absorbance spectra for algal color groups. In addition, it provides another potential use of these spectral "signatures," which can be related to remote sensing of phytoplankton population composition.

Variability of Phytoplankton Absorbance/Reflectance Spectra in Nature

Although the data reported herein indicate significant differences among the absorbance spectra of algae from the four color groups, there is some question as to the practicality of any technique for differentiating among these groups on this basis because of known variations in the spectral response of algae caused by changes in their environment. Environmental factors which have been reported to affect the pigment content/spectral response of algae include light intensity and spectral quality, nutritional stress, and extremes of temperature and salinity (Halldal, ref. 17). In examining these reported effects to determine their potential for influencing the absorbance/reflectance of algae in their natural habitats, two criteria were applied. First, were the environmental factors used in the laboratory studies representative of those that the algae would normally encounter in these habitats? And second, were the spectral effects observed in these laboratory studies of a sufficient magnitude to affect significantly any analytical technique which might be developed to differentiate and quantitate these algal groups based on their absorbance/reflectance spectral characteristics? We have found that, in general, the environmental parameters were chosen for these studies on the basis of their capacity to cause the desired environmental effects, particularly those which would help illucidate photosynthetic-energy transfer patterns, and not because they represented natural habitat conditions. This is particularly true when the effects of intensity or spectral quality of light have been studied (refs. 18 and 19). Also, many of these studies relate to changes in the efficiency of light utilization, which do not affect the absorption or reflectance of light. In many of those cases in which the environmental effects studied were similar to those noted in natural habitats (mostly

nutritional studies), changes noted in the absorbance spectrum were not large in relation to the differences noted between color groups in this study. Thus, it does not seem that most of the environmentally induced effects are significant. A possible exception to this observation may be the effect on phycobilin pigment content and related absorbance characteristics caused by some nutritional stresses (Allen and Smith, ref. 20).

In Vivo Absorbance as an Indicator of Chlorophyll *a* Concentration

A comparison of five different approaches for handling the absorbance data showed that the best correlation with chlorophyll *a* was obtained by either of two methods used to correct for residual scattering effects and that, in all cases, the correlation with chlorophyll *a* was equal to or better than that calculated from Yentsch's data (ref. 4). Correction for scattered-light losses was provided by subtracting either the absorbance at 720 nm or the minimum absorbance in the 620- to 660-nm region from the maximum absorbance in the region of 680 nm. Since both of these methods appeared equally effective, the first was selected for further analyses because it more closely parallels the method used by Yentsch (ref. 4). It was also found that even though the absorptivity maximum in the region of 680 nm was greater for the blue-green species than for any others, the correlation of absorbance to chlorophyll *a* concentration was not significantly improved when these species were deleted from the data base.

It is interesting to note that the maximum absorbance in the red region of the spectrum occurred at 679 nm with a standard deviation of 2.3 nm. This, surprisingly, is considerably different from the 670 nm proposed by Yentsch (ref. 4). The source of this shift of the absorbance maximum to longer wavelengths appears to be spectrally selective light scattering. Latimer (ref. 21) found that this shift can be as great as 16 nm and as little as 8 nm, depending upon the growth phase of the organisms. The maximum shift was obtained with cells in the log phase of growth. His data indicated that the technique of Shibata et al. (ref. 11) significantly reduced spectrally selective scattering. Based upon our data obtained using their technique compared with the data of Yentsch (ref. 4), it appears that Yentsch's technique is even more effective in achieving that reduction.

When the absorbance maximum in the region of 680 nm minus the absorbance at 720 nm is plotted against chlorophyll *a* concentration (fig. 11), a pattern is observed which appears to correlate with color group membership. Linear regression analysis shows high correlations ($R = 0.716$ to 0.976) within color groups, with characteristic slopes and intercepts for golden-brown (diatoms and dinoflagellates), green, and blue-green color groups. However, further examination of the data shows the slope differences are more likely the result of errors in estimation of chlorophyll *a* concentration caused by incomplete extraction of the chlorophyll with 90-percent acetone (Strickland and Parsons procedure, ref. 1). Those color groups and subgroups exhibiting the highest slopes are those which we have found to be the most difficult to completely extract with that solvent (unpublished data of Powell and Winstead) (i.e., the diatoms and the blue-greens), whereas those showing lower slopes are the greens and dinoflagellates, which are completely or almost completely extracted by the Strickland and Parsons procedure. If all the data are therefore treated as a single population, a least-squares relationship such as presented in figure 11 is obtained. However, because of the bias in the data caused by the extraction error, an artificially high intercept is obtained with this approach. The hypothetical relationship should be more closely approximated by the line drawn

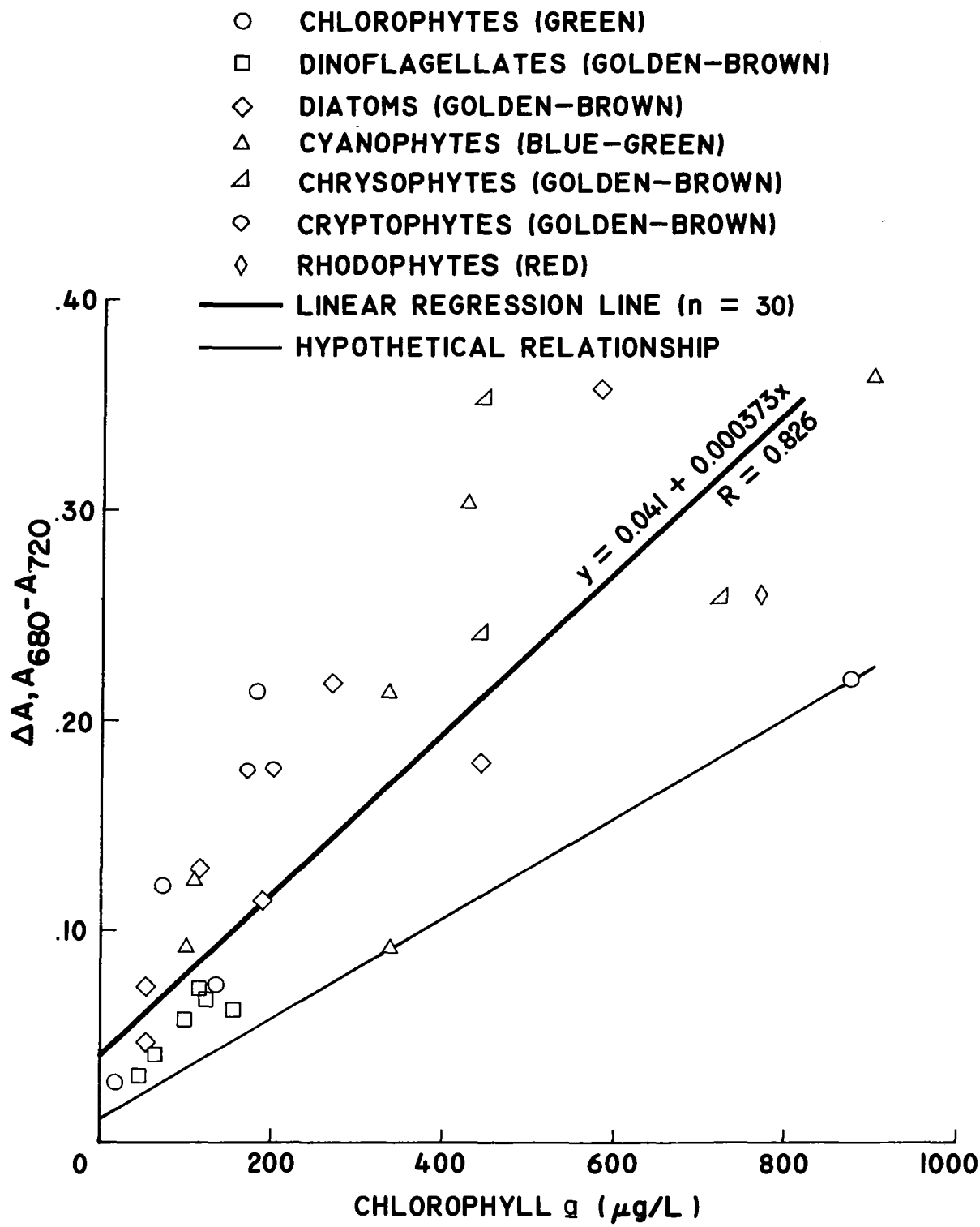


Figure 11.- Relationship of absorbance at 680 nm A_{680} corrected for scattering by subtraction of absorbance at 720 nm A_{720} and chlorophyll a concentration of 30 algae cultures. Linear regression coefficients are for all data. The "hypothetical relationship" assumes complete extraction of chlorophyll a as indicated by two species known to be completely extracted by 90-percent acetone.

through the points representing Synechococcus elongatus and Dunaliella euchlora, two species which have repeatedly been shown to be completely extracted with 90-percent acetone (unpublished data of Powell and Winstead). This relationship is supported by a further analysis which corrects the estimated chlorophyll a values by multiplying by an extraction efficiency ratio E for each species:

$$E = \frac{\text{Chlorophyll a extracted with dimethyl-sulfoxide/methanol (4/3)}}{\text{Chlorophyll a extracted with 90-percent acetone}}$$

This ratio is available for six species from previous unpublished work in our laboratory. The regression line obtained when the corrected chlorophyll a values are substituted for the original estimates is very close to the two-species line mentioned above and plotted in figure 11 as the hypothetical relationship. With this latter relationship, an in vivo absorptivity value of 24.6 L/g-cm can be calculated. This value is about half the values calculated from cultures (ref. 9) and ocean samples (ref. 22) for which acetone was used and is about one-third to one-quarter of the absorptivity of chlorophyll a in other organic solvents (ref. 7).

The absorbance data from the mixed-species measurements largely supports the conclusions based on the single-species data. The correlation between the absorbance increment (at about 680 to 720 nm) and chlorophyll a content is fair to good for all data sets. (See table 7.) In the cases of mixtures I and IV, in vivo quantitation

TABLE 7.- EFFECT OF PHYTOPLANKTON MIXTURE COMPONENT SPECIES ON RELATIONSHIP OF ABSORBANCE AND CHLOROPHYLL a CONTENT AS REVEALED BY LINEAR REGRESSION ANALYSIS

[680-nm to 720-nm wavelength]

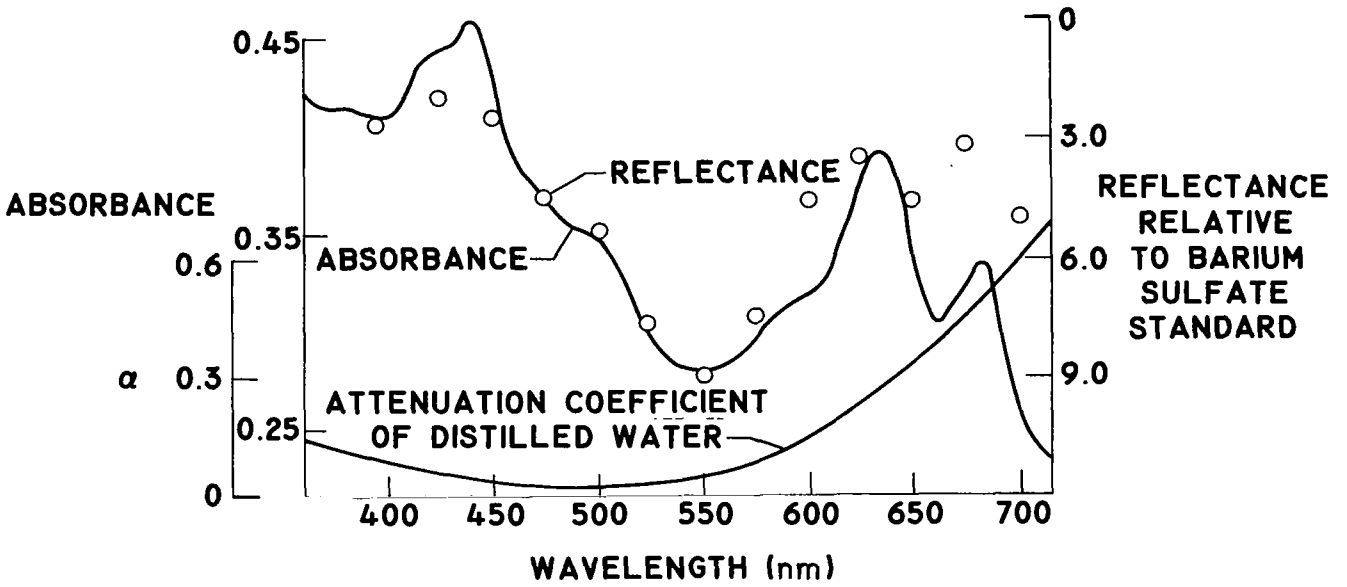
Mixture	Species	Linear regression data		
		Slope	R	n
IV	P. tricornutum Chlorella sp. A. marinus	0.000235	0.957	8
I	P. tricornutum D. euchlora A. marinus	0.000246	0.820	8
II	P. minimum D. euchlora A. marinus	0.000620	0.707	8
III	P. paradoxa D. euchlora A. marinus	0.000436	0.701	8

of chlorophyll a based on the value for absorptivity of 24.6 L/g-cm gives values almost identical to those obtained by extraction with dimethyl-sulfoxide/methanol (4/3) and quantitation by in vitro absorbance (ref. 1). However, in the two other mixtures, the chlorophyll a content is overestimated with the in vivo technique. In these cases, extraction errors should not contribute significantly, and it must be concluded that the species (color group) composition of a mixture has an inherent effect on the in vivo absorptivity. However, calibration of the in vivo technique using an in vitro method which gives complete extraction would make the approach more attractive. This could be done in a manner similar to that used for the estimation of chlorophyll a content by in vivo fluorescence (ref. 3).

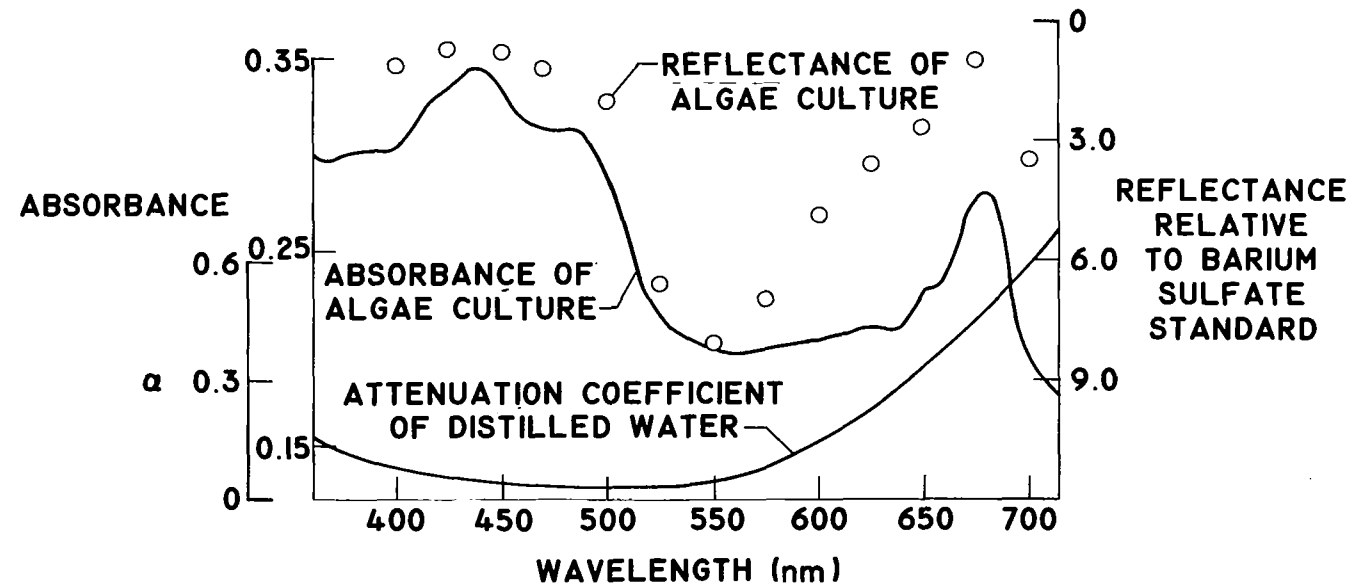
Comparison of Spectral Absorbance and Reflectance

A comparison of the mean absorbance spectra of the green and blue-green color groups with the mean reflectance obtained by Gramms and Boyle (ref. 9) for one species from each of these color groups is presented in figures 12(a) and (b). Reflectance relative to a barium sulfate standard is plotted reciprocally so that a direct comparison can be made between the two quantities. It is interesting to note how close the relationship is, particularly in the blue and green portion of the spectrum. Above 550 nm, however, a distinct difference begins to appear, which is particularly noticeable in the green algae. This disparity is largely due to the reduced reflectance (actually radiance) caused by the absorption of water in this region of the spectrum. This effect was not recorded in our absorbance data because of the use of a reference sample. The attenuation data on distilled water obtained by Duntley (ref. 23) are presented for comparison. When absorbance and reflectance data for the same color group are plotted against each other, a correlation to the linear regression line of -0.982 and -0.990 is obtained for the green and blue-green color groups for the portion of the spectrum from 400 to 550 nm. For the region from 400 to 700 nm, the respective correlations are -0.833 and -0.800. Note that in spite of the water effect above 550 nm, the absorption of the algae predominates in the reflectance "signature." This predominance results in the easily recognized features produced by the absorption of phycocyanin and chlorophyll a. This indicates that, at the very least, this technique should allow remote differentiation between green/golden-brown species and blue-green species if these organisms are the predominant light absorbers.

Further support for the hypothesis that the light absorption characteristics of algae are mirrored by reflectance is provided by the recent unpublished data of Farmer, Collins, and Lewis, who have measured the radiance of sunlight-irradiated cultures of algae in a 100-L tank. A comparison between their data for a culture of Phaeodactylon tricornutum mixed with a light-scattering sediment (concentration of 10 mg/L) and the absorbance of the same organism measured in this study (fig. 13) shows that all major absorbance peaks are reflected by minima in the radiance spectrum. In addition, the effect is proportional, except at longer wavelengths where the absorption of light by water enhances the radiance reduction caused by chlorophyll a absorption. From linear regression analysis, it was determined that the correlation between the radiance and absorbance spectra is -0.82 for this data set. An improved correlation would result if the absorbance of water is added to the algae absorbance and this sum is used in the regression analysis. This same relationship between light absorption/absorbance and reflectance/radiance was noted by Kiefer et al. (ref. 24) in continuous cultures of Pavlova (Monochrysis) lutheri and, to a lesser extent, in batch cultures of the diatom Thalassiosira psuedonana.



(a) Blue-green algae.



(b) Green algae.

Figure 12.- Comparison of mean absorbance of two color groups and reflectance of a representative species from each color group (ref. 9). Note inverted reflectance scale.

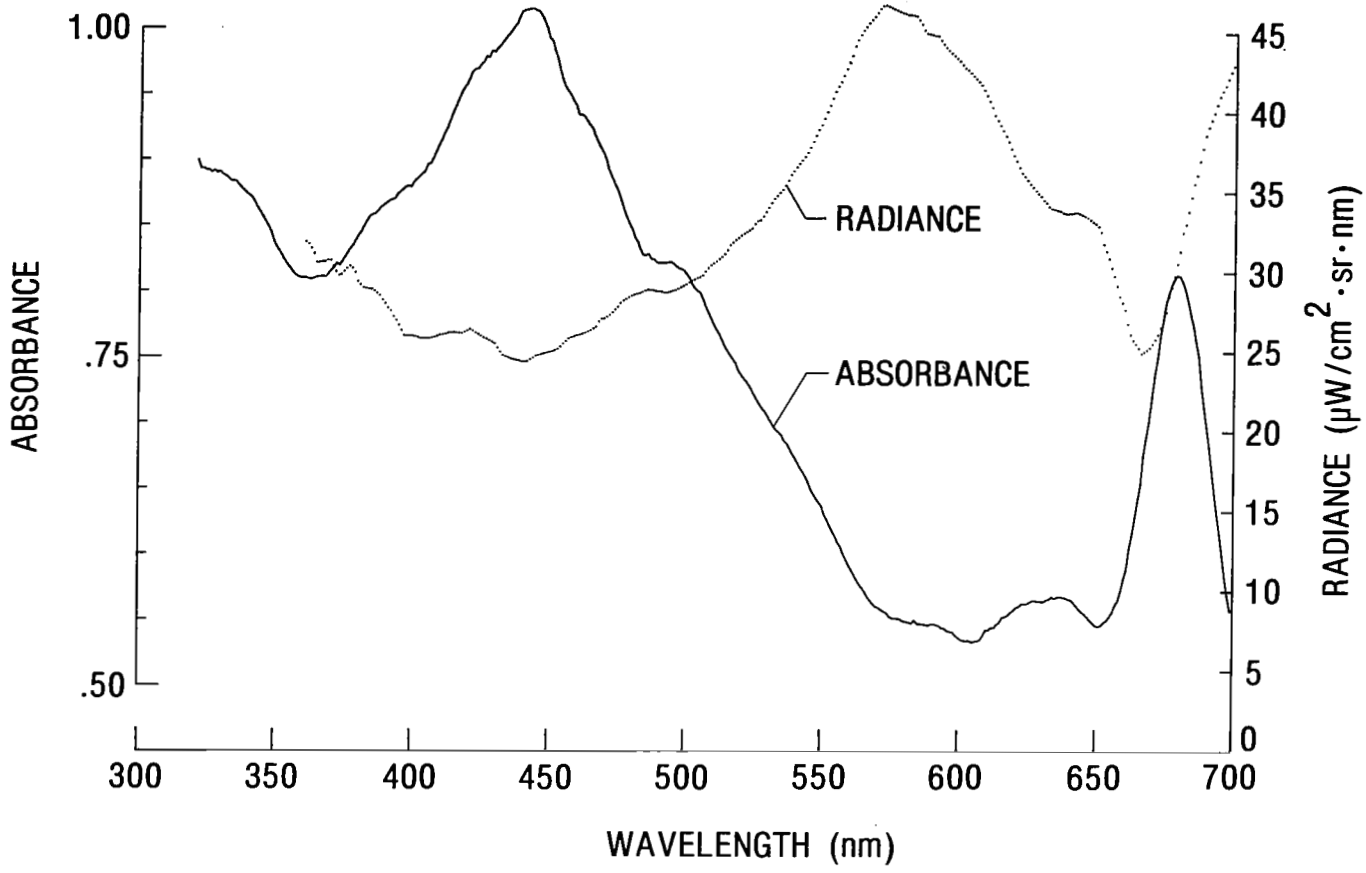


Figure 13.- Comparison of absorbance and radiance (unpublished data of Farmer, Collins, and Lewis) of cultures of Phaeodactylon tricornutum.

CONCLUSIONS

Examination of the absorbance spectra of 20 species of phytoplankton from the 4 major color groups has led to the following conclusions about their variation, similarities, and differences:

1. The variation of the absorbance spectra of different cultures of the same species (grown under the same conditions) is usually small, the standard deviation being generally less than 10 percent of the mean.

2. The variation of the absorbance spectra of different species of the same color group (grown under the same conditions) is also small (except in the golden-brown color group).

3. The major sources of spectral variation in the golden-brown color group are the dinoflagellates, whose large size and low ratio of pigment to volume evidently cause more variability in light scattering and structural absorbance.

4. The mean absorbance spectra for the red and blue-green color groups are quite unique and can be easily differentiated from each other and from those of the golden-brown and green color groups.

5. The mean absorbance spectra for the golden-brown and green color groups are similar but have some detectable differences.

6. Regions of significant difference between color group mean absorbance spectra are centered at 535, 570, and 625 nm and are characteristic of the species of the golden-brown, red, and blue-green color groups, respectively. The green color group species have no significant, unique absorbance peaks, just the absence of the above features.

Further analysis of the mean absorbance spectra for both species and color groups has led to the following conclusions about the significance of both their spectral characteristics and relative magnitudes:

7. The absorbance spectra of algae color groups are sufficiently unique in that a classification procedure was developed which correctly classified 17 of 18 species as to color group based only on their visible absorbance spectra. This classification procedure appears to have limited usage in the laboratory identification of algae, but the results do support the feasibility of the algae color group "signature" concept.

8. The components of a mixture of three species, each representative of a different color group, can be quantitated from the absorbance spectrum of the mixture if the absorbance spectrum of each species is known. The number of points on the spectra required to obtain quantitation is dependent upon the accuracy required. The same results can be obtained using mean color group absorbance spectra, but more spectral points are required.

9. In pure phytoplankton cultures, absorbance at the maximum in the region of 680 nm is strongly indicative of the chlorophyll _a_ concentration, provided the absorbance peak is adjusted for residual scattering effects by subtracting the absorbance at 720 nm. The in vivo absorptivity value of 24.6 L/g-cm calculated from this data base should provide a more accurate estimate of total chlorophyll _a_ than previously published values because it is based on in vitro chlorophyll _a_ concentrations determined from more completely extracted samples.

10. In mixed cultures containing representative species from three color groups, the adjusted absorbance maximum in the region of 680 nm is also strongly indicative of the chlorophyll _a_ concentration, but the absorptivity value varies significantly with species composition. Calibration of the in vivo technique by occasional in vitro analyses is possible and makes the technique more attractive for remote-sensing application.

11. The mean absorbance spectra for the green and blue-green color groups correlate well with the inverse of reflectance for two species from these groups, and the mean absorbance spectrum for the diatom _Phaeodactylum tricornutum_ is inversely correlated with radiance of the same organism in sunlight. Since reflectance and radiance are quantities measured by passive remote sensors, the differences in absorbance spectra noted among the algal color groups should form a basis for passive remote sensing of phytoplankton population composition at this level. However, these

observations are probably only applicable to those waters in which light absorption by the phytoplankton is a major component of the total absorption.

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16. Abstract

An optical method is proposed which could be used either in situ or remotely to measure both the concentration and composition of phytoplankton populations. This method is based upon the in vivo light absorption characteristics of phytoplankton. To provide a data base for testing assumptions relative to the proposed method, visible absorbance spectra of pure cultures of 20 marine phytoplankton were obtained under laboratory conditions. Descriptive and analytical statistics were computed for the absorbance spectra and were used to make comparisons between members of major taxonomic groups and between groups. Spectral variation between the members of the major taxonomic groups was observed to be considerably less than the spectral variation between these groups. It was concluded that in several cases the differences between the mean absorbance spectra of major taxonomic groups may be significant enough to be detected with passive remote-sensing techniques.

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