

## Remote sensing of ocean colour: Towards algorithms for retrieval of pigment composition

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Ocean colour varies as an inverse function of the absorption coefficient. In Case 1 waters, phytoplankton are known to be the principal agents responsible for variations in the total absorption coefficient. The concentration and composition of pigments present have a strong influence on phytoplankton absorption spectra. Empirical (regression) algorithms exist to recover the wavelength-specific absorption coefficient of phytoplankton from the concentration of the main pigment, chlorophyll-*a*. However, to explain the residuals about such regressions remains a major challenge. We have analysed a set of over 1,600 absorption spectra of phytoplankton collected from various oceanographic provinces. In parallel, we examined the corresponding pigment complexes, as revealed by High Performance Liquid Chromatography (HPLC). We have uncovered broad trends in the shapes of the absorption spectra and in the pigment complexes, consequent upon changes in the pigment biomass, with clear implications for the remote sensing of ocean colour.

[**Key words:** Ocean colour, phytoplankton pigments, absorption spectra, remote sensing]

### Introduction

It is well known that ocean colour reflectance varies directly with the back-scattering coefficient, and inversely with the absorption coefficient. In principle, the total absorption coefficients at specific wavelengths can be retrieved from ocean-colour data, and separated into its components. Provided that the retrieval can be achieved with sufficient accuracy and precision, and with high spectral resolution, the possibility exists that the retrieved absorption spectrum can be used to derive some information on the taxonomic composition of phytoplankton. For example, Subramaniam *et al.*<sup>1</sup> were able to distinguish blooms of *Trichodesmium* from other phytoplankton in the upper ocean using remotely-sensed information, and Sathyendranath *et al.*<sup>2</sup> were able to map the distribution of diatom blooms in the North West Atlantic.

Generally, oceanic waters are classified as Case 1 if phytoplankton is the primary agent responsible for controlling the optical properties, and as Case 2 otherwise<sup>3-5</sup>. For Case 1 waters, which are optically more simple than Case 2 waters, methods are

available to compute phytoplankton absorption and scattering as functions of just the chlorophyll concentration<sup>6-11</sup>. However, chlorophyll concentration is not the only biological property that changes when the abundance of phytoplankton changes. Usually, there are corresponding changes in the relative concentrations of auxiliary pigments, and in the mean size of the cells: both of these influence the optical properties of the phytoplankton and the water in which they are suspended. Because size and pigment composition change with community structure, the optical properties of the water will also vary with the seasonal succession of species.

That the empirical regression algorithms (to recover phytoplankton absorption from chlorophyll concentration) have any success at all is an indication that at least some of the variation in optical properties with species composition follows definite trends with changes in concentration of chlorophyll. Here, using data on optical properties and pigment complexes from some 1,600 samples of marine phytoplankton collected from different oceanographic provinces, we report general trends in community composition (and pigment complex) with chlorophyll concentration.

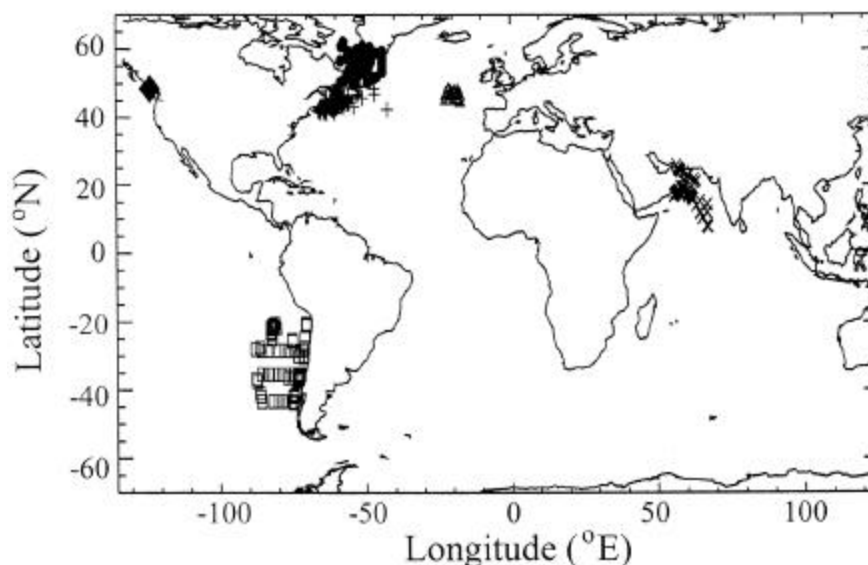


Fig. 1—Map showing the location of sampling stations. The various oceanographic cruises are represented by symbols (◆ = Off Vancouver Island, r = Cruises off Chile, ● = Labrador Sea cruises, + = Scotian Shelf cruises, △ = Off Portugal, + = Arabian Sea cruises)

We also show that there is some variability around these trends, and discuss their implications for remote sensing of ocean colour.

#### Data and Methods

Data were collected during a number of oceanographic cruises off the coast of eastern Canada, as well as in various other oceanographic regimes, ranging from eutrophic upwelling areas off the coast of Chile, to stratified, oligotrophic areas in the Arabian Sea (Table 1, Fig. 1). Reverse-phase, high-performance liquid chromatography (HPLC) was used to determine pigment concentrations as described in Head & Horne<sup>12</sup>. Filters were homogenised in 1.5 ml of 90% acetone, centrifuged and diluted with 0.5 M ammonium acetate buffer at a ratio of 2:1 before injection. The samples were run on a Beckman C18, reverse-phase, 3 $\mu$ m Ultrasphere column (70  $\times$  4.6 mm), using the solvents methanol/0.5 M ammonium acetate (80/20) and methanol/ethyl acetate (70/30). Chromatographic peaks were detected by a UV-VIS photodiode array detector (Beckman 168) and identified by retention time and comparison of absorbance spectra with spectra of known pigment standards [Sigma Chemical Company, and DHI Water and Environment (Denmark)]. Divinyl chlorophyll-*a* and divinyl chlorophyll-*b*, characteristic of *Prochlorococcus* sp., were quantified by acidifying samples with 1 N HCL and recording the peak heights of divinyl phaeophytin-like pigments. Note that HPLC determined chlorophyll-*a* represents a total of

chlorophyllide-*a*, mono- and divinyl chlorophyll-*a*, plus the chlorophyll-*a* epimer and allomer.

Phytoplankton absorption samples were processed using the filter method of Yentsch<sup>13</sup> as modified by Kishino *et al.*<sup>14</sup>, using a dual-beam Shimadzu UV-2101 PC scanning spectrophotometer equipped with an integrating sphere. Optical density measurements were divided by the geometrical path length (volume filtered divided by clearance area of the filter) and multiplied by a factor of 2.3 (conversion factor for transforming decimal logarithms to natural logarithms) to obtain the absorption coefficient. The value of the absorption coefficient at 750 nm was subtracted from the values at all other wavelengths, as a rudimentary correction for errors due to scattering by the phytoplankton cells. Measurements were corrected for path-length amplification due to scattering by the filter, using the  $\beta$ -correction algorithms of Hoepffner & Sathyendranath<sup>15</sup> and Moore *et al.*<sup>16</sup>, weighted according to phytoplankton species composition as described in Kyewalyanga *et al.*<sup>17</sup> and Stuart *et al.*<sup>18</sup>. Pigments were extracted from the filters using 20 ml hot methanol, and the extracted filters were scanned again to measure the detrital component. Absorption by the phytoplankton component,  $a_{ph}(\lambda)$ , was obtained by subtracting the detrital component,  $a_d(\lambda)$ , from the total particulate absorption spectrum. Pigment specific absorption coefficients of phytoplankton [ $a_{ph}^*(\lambda)$ ] were calculated through division of absorption by HPLC-

Table 1— Summary of datasets used for phytoplankton absorption and HPLC pigment analyses, including cruise names, dates and number of samples collected. The symbols correspond to those used in Fig. 1 showing the geographic location of the various cruises

Area [Cruise]	Dates	<i>n</i>	Symbol
Arabian Sea (Arabesque 1 Cruise)	28 Aug – 30 Sep 1994	109	×
Arabian Sea (Arabesque 2 Cruise)	17 Nov – 15 Dec 1994	95	×
Off Chile	11 May – 26 Jun 1995	211	□
Labrador Sea (JGOFS Cruise)	15 May – 30 May 1996	45	●
Labrador Sea (JGOFS Cruise)	24 Oct – 17 Nov 1996	28	●
Off Portugal	10 Sep – 3 Oct 1996	33	△
Off Vancouver Island	5 Mar – 14 Mar 1996	33	◆
Labrador Sea (JGOFS Cruise)	12 May – 9 Jun 1997	52	●
Scotian Shelf	26 Oct – 8 Nov 1997	24	+
Arabian Sea	15 Jun – 17 Jul 1997	85	×
Scotian Shelf	18 Apr – 28 Apr 1997	20	+
Labrador Sea	24 Jun – 8 Jul 1998	18	●
Scotian Shelf	8 Apr – 21 Apr 1998	62	+
Off Chile (MIRC Cruise)	16 Oct – 27 Oct 1998	89	□
Scotian Shelf	3 Oct – 20 Oct 1998	11	+
Off Chile (Coquimbo–Iquique Cruise)	10 Feb – 16 Feb 1999	16	□
Scotian Shelf	9 Apr – 17 Apr 1999	22	+
Labrador Sea	27 Jun – 12 Jul 1999	61	●
Scotian Shelf	24 Oct – 12 Nov 1999	51	+
Off Chile (MinOx Cruise)	19 Mar – 27 Mar 2000	11	□
Scotian Shelf	7 Apr – 30 Apr 2000	112	+
Scotian Shelf	13 Jun – 14 Jun 2000	6	+
Scotian Shelf	1 Oct – 15 Oct 2000	105	+
Labrador Sea	21 May – 6 Jun 2000	49	●
Labrador Sea	31 May – 13 Jun 2001	50	●
Scotian Shelf	2 May – 16 May 2001	126	+
Scotian Shelf	24 Oct – 7 Nov 2001	109	+

determined chlorophyll-*a* concentration. All phytoplankton absorption and pigment samples were processed using consistent methodology by the same team of analysts.

## Results and Discussion

### *Absorption spectra and community composition*

The HPLC results indicate that in very oligotrophic conditions, small *Prochlorococcus* sp. (as indicated by the presence of DV-chlorophyll-*a*) tended to dominate (Fig. 2A). As chlorophyll concentration increases, the representation of the slightly larger cyanophytes (as indicated by the relative proportion of the pigment zeaxanthin to total chlorophyll-*a*) also

increases (Fig. 2B). Note that zeaxanthin is also present in *Prochlorococcus* sp. At the highest chlorophyll concentrations, diatoms (containing high relative proportions of fucoxanthin and chlorophyll-*c*) are usually the dominant group (diatoms are generally large-celled species). This pattern of increasing cell size with increasing phytoplankton biomass has also been noted by Yentsch & Phinney<sup>19</sup>, Chisholm<sup>20</sup>, and Irigoien *et al.*<sup>21</sup>. Similarly, Babin *et al.*<sup>22</sup> and Vidussi *et al.*<sup>23</sup> noted that oligotrophic waters were generally dominated by pico- and nanophytoplankton. Prymnesiophytes, which contain the pigment 19'-heaxnoyloxyfucoxanthin, can occur in high abundances in a variety of conditions. Similarly, chlorophytes, which contain the pigment chlorophyll-*b*,

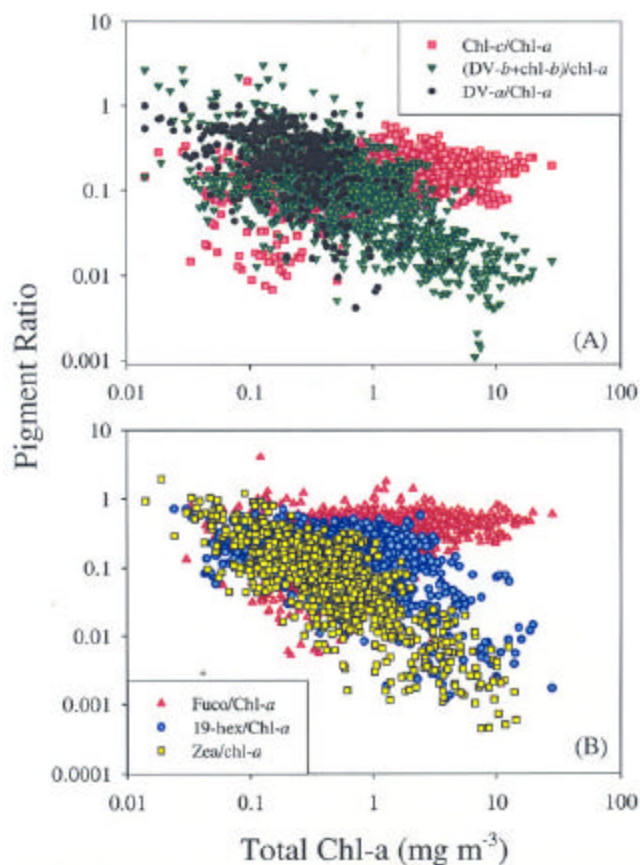


Fig. 2— Ratio of various phytoplankton pigments relative total chlorophyll-*a* (chlorophyll-*a* plus divinyl chlorophyll-*a*) plotted against total chlorophyll-*a* concentration.

are also ubiquitous, but note that in very oligotrophic waters it is the divinyl form of chlorophyll-*b* which is present (found in *Prochlorococcus* sp.).

The specific absorption coefficient at 440 nm was highest for the communities dominated by smaller cells such as cyanophytes and *Prochlorococcus* sp. and lowest for the diatom-dominated communities. Figure 3 shows the specific absorption coefficients for samples dominated by cyanophytes (zeaxanthin/*chl-a* > 0.2) and those dominated by diatoms (fucoxanthin/*chl-a* > 0.4). As well as the absolute magnitude of absorption, the shapes of the absorption spectra changed with community composition. For example, the magnitude of the ratio of absorption at 490 nm to that at 555 nm (two of the wavebands used in the SeaWiFS OC4 algorithm) varies according to pigment composition (Fig. 4). For populations dominated by cyanophytes (containing the pigment zeaxanthin), the  $a(490)/a(555)$  ratio is very high, whereas the opposite is true of populations dominated by diatoms (containing the pigment fucoxanthin).

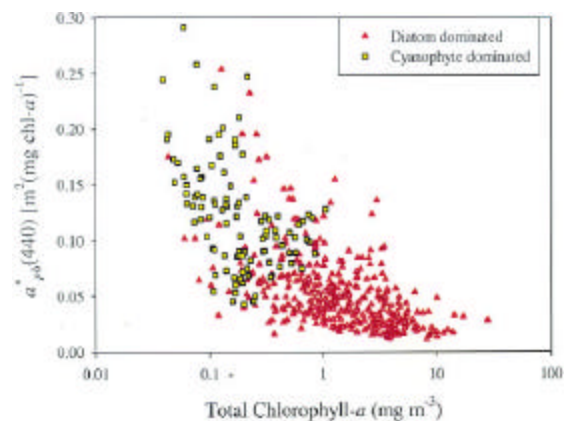


Fig. 3— Phytoplankton specific absorption coefficient at 440 nm versus total chlorophyll-*a* concentration (chlorophyll-*a* plus divinyl chlorophyll-*a*), for samples dominated by diatoms (fucoxanthin/chlorophyll-*a* > 0.4) and cyanophytes (zeaxanthin/chlorophyll-*a* > 0.2).

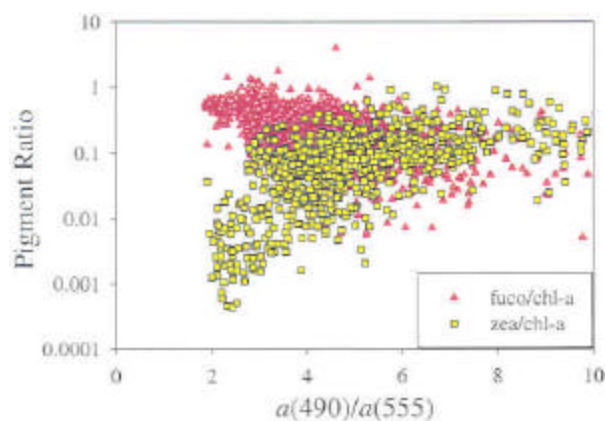


Fig. 4— Ratio of phytoplankton pigments relative to total chlorophyll-*a* (chlorophyll-*a* plus divinyl chlorophyll-*a*) plotted against the ratio of phytoplankton absorption at 490 nm versus that at 555 nm. Pigments shown are fucoxanthin/*chl-a* and zeaxanthin/*chl-a*.

Variations in community composition can thus have a significant influence on the spectral quality of the submarine light field.

#### Auxiliary pigments and chlorophyll concentration

We consider the concentrations of auxiliary pigments relative to that of chlorophyll-*a*. As phytoplankton abundance increases, the concentration of chlorophyll-*b* relative to chlorophyll-*a* declines, whereas that of chlorophyll-*c* relative to chlorophyll-*a* increases (see Fig. 2A). The carotenoid pigments were divided into two groups, the photosynthetic carotenoids (PC) and the non-photosynthetic carotenoids (NPC), as in Bricaud *et al.*<sup>5</sup>. The photosynthetic carotenoids consisted of fucoxanthin, peridinin, 19'-

butanoyloxyfucoxanthin, 19'-hexanoyloxyfucoxanthin, prasinoxanthin and  $\alpha$ -carotene; and the non-photosynthetic carotenoids consisted of zeaxanthin, alloxanthin,  $\beta\epsilon$ -carotene, diadinoxanthin and diatoxanthin in this partition. We found that the highest proportions of non-photosynthetic carotenoids occurred at low latitudes (up to 20° N and S), predominantly in the surface layers (0-10 m), although there was considerable spread in the data, probably caused by seasonal differences as well as by depth-dependent

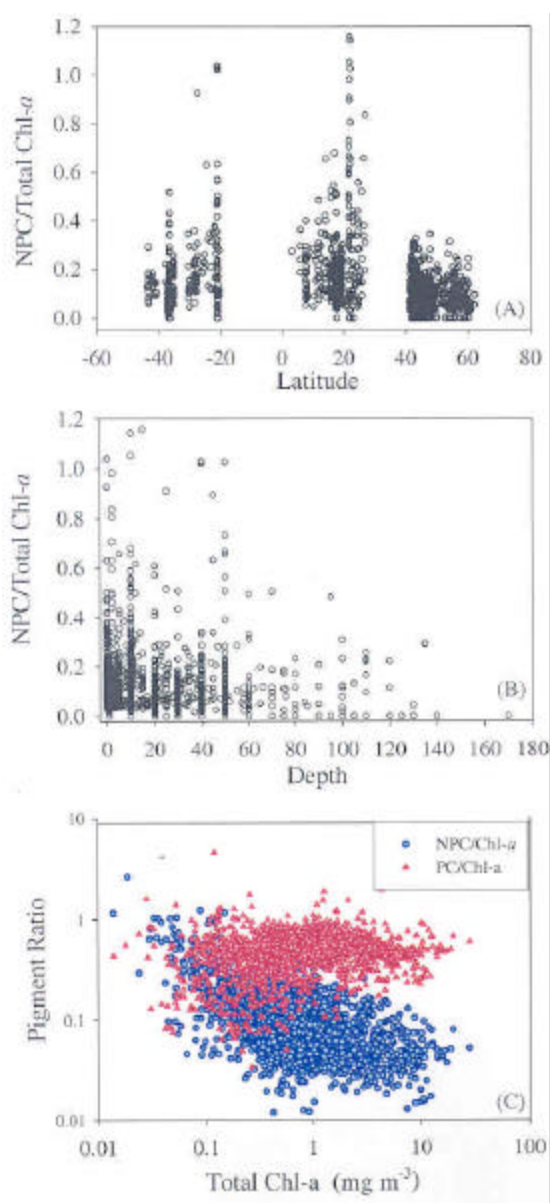


Fig. 5— Ratio of non-photosynthetic carotenoids (NPC) relative to total chlorophyll-*a* (chlorophyll-*a* plus divinyl chlorophyll-*a*) plotted as a function of (a) latitude, (b) depth and (c) total chlorophyll-*a* concentration. Fig. 5 c also shows the ratio of photosynthetic carotenoids (PC) to chlorophyll-*a*.

variations (Fig. 5A, B). For example, the pigment zeaxanthin was especially prominent at the surface in tropical waters. Non-photosynthetic carotenoids such as zeaxanthin are known to play a photoprotective role in the cell, preventing photo-oxidation at high light intensities, which are often encountered in the surface layers of oligotrophic waters<sup>24-26</sup>. NPCs tended to decrease with increasing concentration of chlorophyll-*a* (Fig. 5C). On the other hand, the concentration of photosynthetic carotenoids (predominantly fucoxanthin) relative to chlorophyll-*a* increased with increasing abundance of phytoplankton. The latitudinal variation in non-photosynthetic carotenoids seen in Fig. 5A suggests that the performance of global algorithms for chlorophyll retrieval could probably be improved by tuning them to allow for such large-scale trends in optical properties.

#### Pigment composition and magnitude of absorption

Because the size structure and the pigment composition of the communities change with changing phytoplankton abundance, the relation between the magnitude of absorption at 440 nm and chlorophyll-*a* concentration is non-linear. Empirical functions of chlorophyll-*a* concentration using three parameters, such as that used by Sathyendranath *et al.*<sup>10</sup>, could account for only 72% of the variation in magnitude of absorption (Fig. 6). A linear relation could account for some 68% of the variance. If the concentrations of chlorophyll-*b* and chlorophyll-*c* and

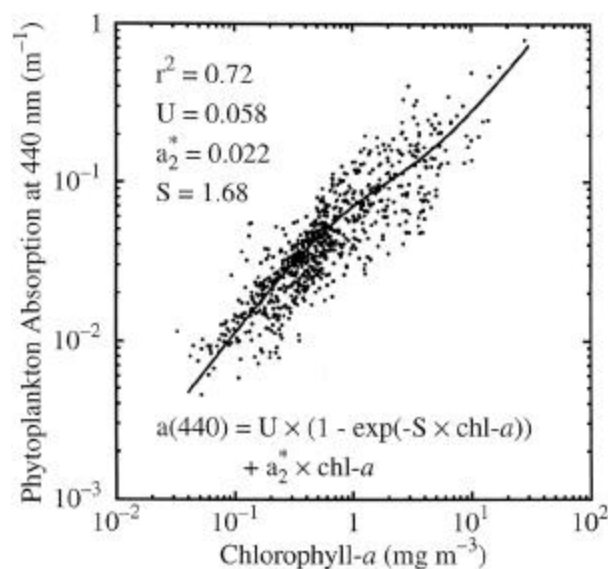


Fig. 6—Phytoplankton absorption at 440 nm plotted as a function of chlorophyll-*a* concentration, and fitted using the two-population, three parameter model of Sathyendranath *et al.* 2001.

the two groups of carotenoids were included as independent variables in a multiple linear regression, some 79% of the variance in magnitude of absorption could be accounted for, suggesting that the variability in accessory pigments is responsible for some of the scatter we see in the relationship between phytoplankton absorption and chlorophyll-*a* concentration (Fig. 6).

#### Implications

What causes variations in ocean colour in Case 1 waters? As shown schematically in Fig. 7, the optical properties of phytoplankton, and hence of the waters,

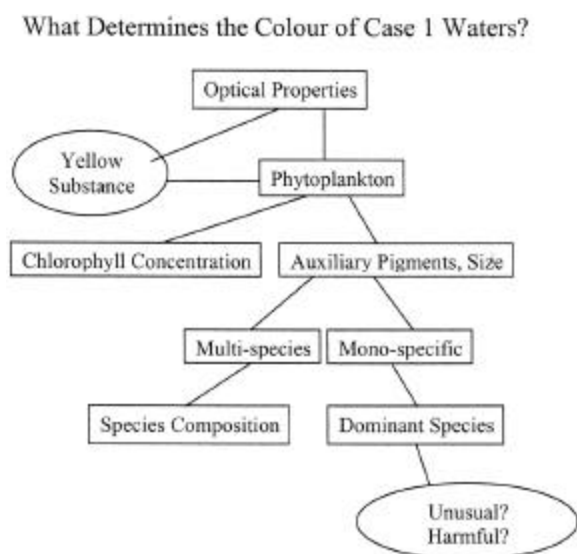


Fig. 7— Schematic diagram showing sources of variations in phytoplankton absorption characteristics in the aquatic environment. These in turn influence ocean colour.

will of course depend on the concentration of chlorophyll-*a*, which is present in all phytoplankton, either in its regular form, or as the variant divinyl chlorophyll-*a*. However, it must not be overlooked that changes in chlorophyll-*a* concentration are typically accompanied by variations in the relative concentrations of auxiliary pigments, and in the size structure of the phytoplankton population, both of which can affect the optical properties of phytoplankton. Pigment composition and size structure vary with the species composition of the phytoplankton population. Therefore, one may expect the optical properties to differ depending on whether the population is dominated by a single species or class, or by many species belonging to many classes. In the case of multi-species populations, the optical properties would depend on the species composition, whereas in the case of mono-specific blooms, they would depend on the dominant species present. A certain amount of yellow substance or coloured dissolved organic matter is also expected to be present in Case 1 waters, and to modify ocean colour by its presence (Fig. 7).

If indeed so many changes accompany variations in phytoplankton concentration, how is it that we have been able to develop successful models that employ a single variable, chlorophyll-*a*, to parameterise the optical properties of phytoplankton in Case 1 waters? The success of these models implies that many of the changes listed above follow certain predictable trends, which follow changes in chlorophyll-*a*. In this paper we have analysed a data base of phytoplankton absorption spectra and pigment composition, to

Table 2 - Results of multiple linear regression analysis of HPLC-derived pigment concentration on phytoplankton absorption at SeaWiFS wavelengths in the visible region of the spectrum, using the entire database ( $n = 1633$ ). Coefficients for the wavelengths used in each multiple linear regression are given, as well as the constant. Only the significant ( $p = 0.0000$ ) coefficients and constants associated with these wavelengths are indicated. Extending the multiple linear regression to include all six SeaWiFS wavelengths in the visible did not result in any marked increase in the reported  $r^2$  values. 19-Hex = 19'-Hexanoyloxyfucoxanthin; PC = photosynthetic carotenoids; NPC = non-photosynthetic carotenoids (see text for list of carotenoids in each group).

Pigment	412 nm	443 nm	490 nm	510 nm	555 nm	670 nm	Constant	$r^2$
Chlorophyll- <i>a</i>	-12.27		-74.13	77.09		93.86	0.274	0.83
Chlorophyll- <i>b</i>	-1.04	-1.70	15.91	-21.44	11.49	2.07	0.022	0.49
Chlorophyll- <i>c</i>	5.67	-10.10		7.49	-17.03	19.87		0.84
Fucoxanthin			-74.64	108.24	-28.68	30.60		0.89
19-Hex	-4.67	8.35				-4.64		0.75
PC	-6.10		-62.13	122.90	-55.04	-30.78		0.89
NPC			-4.02	11.49	-9.64	2.45		0.82

Table 3 - Results of multiple linear regression analysis of HPLC-derived pigment concentration on phytoplankton absorption at SeaWiFS wavelengths in the visible region of the spectrum, similar to the analyses presented in Table 2 in all respects, except that only samples with fucoxanthin/chlorophyll-*a* ratios < 0.4 ( $n = 1194$ ) are used here.

Pigment	412 nm	443 nm	490 nm	510 nm	555 nm	670 nm	Constant	$r^2$
Chlorophyll- <i>a</i>		-29.07	-39.94	54.82		116.75	0.140	0.76
Chlorophyll- <i>b</i>		-2.90	21.29	-28.35	15.88		0.014	0.59
Chlorophyll- <i>c</i>	7.40	-11.82	3.85		-12.07	18.91		0.89
Fucoxanthin	10.61	-27.08		14.66	-15.11	46.80		0.93
19-Hex			16.72	-18.40	11.81	-5.94		0.78
PC		-10.28	-21.45	62.41	-32.09	30.34		0.89
NPC				5.18	-8.32	1.88		0.78

highlight general trends in species succession that accompany changes in phytoplankton concentration (as indexed by chlorophyll-*a* concentration). The data show that changes in phytoplankton cell size and in pigment composition follow some broad trends with change in chlorophyll concentration, illustrating why the Case-1 approach to modelling open-ocean waters has been so successful. But there is variability around these general trends as well, indicating that chlorophyll retrieval algorithms could be improved if such deviations from the general trends could be accounted for. In fact, the multiple linear regression between pigments and phytoplankton absorption at 440 nm suggests that more variability in the absorption coefficient can be explained if the effects of auxiliary pigments are also taken into account.

Furthermore, in the context of ocean-colour remote sensing, we might consider the possibility that information on pigment complexes of phytoplankton might be recovered from the shape of the absorption spectrum or from the magnitude of absorption at a number of discrete wavelengths. In particular, from the SeaWiFS visible wavelengths, we found that information on pigments other than chlorophyll-*a* could indeed be extracted from the absorption data. The results were least good for chlorophyll-*b* (Table 2). We have noted that the relative concentration of chlorophyll-*b* decreased with increasing abundance of phytoplankton, and it is possible that the recovery of chlorophyll-*b* would be improved in those waters where chlorophyll-*b* pigment concentrations are relatively high. To test this, we divided the data into two blocs according to the concentration of fucoxanthin relative to chlorophyll-*a*, since high proportions of fucoxanthin (relative to chlorophyll-*a*) are generally associated with diatoms, which frequently occur in eutrophic waters (see

Sathyendranath *et al.*<sup>2</sup>). In the bloc with the ratio of fucoxanthin/chl-*a* < 0.4, the recovery of chlorophyll-*b* was somewhat improved (Table 3), although the recovery of chlorophyll-*a* itself was less good.

### Conclusion

Many existing empirical models of phytoplankton absorption in waters of the Case 1 type benefit from the (fortuitous) changes in cell size and pigment complexes of phytoplankton corresponding to changes in phytoplankton abundance. Nevertheless, the accompanying changes in community composition are noisy, and the noise tends to degrade the utility of the empirical algorithms as shown by Sathyendranath *et al.*<sup>10</sup> for samples collected during diatom and prymnesiophyte blooms in the Labrador Sea.

Provided that the absorption spectra of phytoplankton can be retrieved accurately from ocean-colour data, existing algorithms might be improved by exploiting multiple wavebands to discriminate major pigments other than chlorophyll-*a*. The improvement in variance explained when the data were divided into blocs according to the pigment composition, suggests that branching algorithms might offer more promise than universal ones. The branching decisions could be made using criteria that exploit information in the remotely-sensed radiances. Our results are preliminary, and ignore any complications arising from the non-linearity in the relationship between absorption and ocean colour, and the possible effects of coloured dissolved organic matter. Nevertheless, the fact that multiple linear regression analyses of pigment concentration versus phytoplankton absorption were significantly improved by including various SeaWiFS wavebands, implies that it is indeed possible to extract information on these pigment complexes from absorption data.

Recently, Sathyendranath *et al.*<sup>2</sup> successfully used the signals from the 490, 510, 555 and 670 nm SeaWiFS wavebands to determine whether a given pixel in a satellite image was dominated by diatoms or mixed phytoplankton populations. Further studies will no doubt lead to the development of new algorithms for mapping other groups of phytoplankton.

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