INTERPRETATION OF AN INDEX OF PHYTOPLANKTON POPULATION COMPOSITION CALCULATED FROM REMOTE AIRBORNE FLUOROSENSOR (RAF) DATA

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INTRODUCTION

Chlorophyll a fluorescence at 685nm excited by narrow band light at 454 and 539nm can be used to calculate a simple index of phytoplankton population composition. The ratio of the fluorescence excited by light of these two wavelengths is a function of the distribution of the phytoplankton between two "color" groups, designated the "golden-brown" and the "green". The "golden-brown" group consists of those species which have the highly photosynthetically active carotenoid-chlorophyll-a-protein complexes, i.e. members of the classes Bacillariophyceae, i.e. diatoms (ref. 1) Dinophyceae, i.e. dinoflagellates (ref. 2, 3 and 4), and evidently some members of the class Prymnesiophyceae (formerly Haptophyceae). The "green" color group consists of those species of phytoplankton which apparently lack those complexes, i.e. members of the classes Chlorophyceae, Euglenophyceae, Prasinophyceae, Eustigmatophyceae, Xanthophyceae, and a few members of the Prymnesiophyceae. A few species of phytoplankton appear to have intermediate characteristics, and would apparently belong to neither group. Most of these species are members of the class Cryptophyceae. The composition index varies from about 1.0, when the members of the "golden brown" color group are 100 percent of the phytoplankton population, to about 0.33, when the members of the "green" color group are 100 percent. Thus, an even distribution between the two color groups should produce an index of about 0.67.

This index of composition is similar to but not the same as a diversity index (ref. 5 and 6). The main difference between these two indexes is that the latter relates the number of phytoplankton species to the number of individuals, while the former indicates the relative concentration of two major multi-class components of the phytoplankton population. Also, the diversity index is directly tied to classical taxonomy, while the composition index is only indirectly related to it.

RECENT LABORATORY DATA

Figure 1 presents the fluorescence excitation spectra of some marine species of phytoplankton which are representative of both "color" groups, and of the intermediate species. Note that the "golden-brown" species all fluoresce strongly upon excitation with green light, while the "green" species do not. It is this characteristic, i.e. the absorption or non-absorption of green light, which produces the difference in color between members of the two groups. Since the primary difference in pigment content of these two groups is the presence or absence of fucoxanthin or peridinin based chlorophyll-a-protein complexes, it has been concluded that these complexes are responsible for the absorption/fluorescence excitation characteristics of the "golden-brown" species in the green region of the light spectrum.

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The composition index is also effected by the presence or absence of chlorophyllide c. Even though this compound is usually present in much lower concentrations than is chlorophyll a, there is evidently sufficient overlap of their in vivo spectra to produce a cumulative excitation effect at 454nm. However, the effect of chlorophyll b on the composition index is evidently negligible, as can be seen by comparing the index for the chlorophyll b containing Chlorophyceae (0.33) with that for the Eustigmatophyceae (0.33), which contain neither chlorophyll b nor chlorophyllide c.

The intermediate position of the cryptophytes is primarily due to their phycoerythrin content. This phycobilin pigment has a significant amount of absorption/fluorescence excitation in the region of 539nm, although its maximum is at a longer wavelength (570 vs. 555nm) than the fucoxanthin and peridinin complexes. Also, these organisms have alloxanthin, an xanthophyll very similar to fucoxanthin but not known to form complexes with chlorophyll a and protein. If the longer wavelength selected to compute the index were changed from 539nm to 525nm or even 530nm, the effect of the phycoerythrin would be considerably reduced and the cryptophytes would be closely aligned with the "green" species. However, since the cryptophytes are usually a minor component of the marine phytoplankton population, their effect on the composition index is usually ignored.

While there appears to be good coherence of composition index values within the "green" color group there is considerably more divergence of the index within the "golden-brown" color group. This variance is more by class than by species, and appears to be related to the relative concentrations of chlorophyll a and fucoxanthin or peridinin (possibly representative of the concentration of their respective complexes). For example, the three diatoms, two chrysophytes, and one "brown" benthic alga examined by Hagar and Stransky (ref. 7) exhibited a range of total fucoxanthin to chlorophyll a ratio (by weight) from 0.31 to 0.74. All diatoms gave essentially the same ratio (0.73/0.74), but the remaining species showed a wide variation in values. The ratio for an Isochrysis species (0.68), now included in the Prymnesiophyceae, was close to the diatoms, but the Ochromonas species (0.31), a sensu strictu chrysophyte, and the laminarian (0.40) were found to have only about half the fucoxanthin per unit chlorophyll a as did the diatoms. However, this variance should only impact the composition index when the non-diatom/dinoflagellate members of the "golden-brown" group are numerous, as occasionally happens in the coastal waters of the northwestern Atlantic during the winter months. When more spectral data is available, it may be possible to separate this group and quantitate its effect in cases when historical data indicates that these organisms may be present in large numbers.

REMOTE DATA

The ratio of fluorescence obtained by the Remote Airborne Fluorosensor (RAF) during the Chesapeake Bay Plume Study in 1980 has been presented by Jarrett (ref. 8) in the previous paper. He has also reviewed the operation of the RAF and the calculation used to obtain the fluorescence excitation ratios he presented. In this paper the primary focus will be on the data acquired on 17 March on Flight Legs 7, 9, and 11. Figure 2 presents the composition index, i.e. the ratio of fluorescence excited by light of a 539nm wavelength

(green) to that excited by light of a 454nm wavelength (blue) versus distance along the flight path starting at Jamestown Island in the James River and ending about 10 km east of the shelf break. This set of almost 1000 data points spread over 205 km reveals a number of interesting features. First, there is a general trend in the index which ranges from a 100% "golden-brown" population (1.0) in the lower James River and Hampton Roads to an equivalence, and possibly the dominance of "green" species (0.55) in the mid-shelf and east of the shelf break. Superimposed upon this general trend are two peaks of "golden-brown" dominance, at the eastern "front" of the Chesapeake Bay Plume and at the shelf break. It appears from this data that the "golden-brown" species predominate in the traditionally nutrient rich areas, while the "green" species dominate, or at least attain equivalence, in the traditionally nutrient poor regions. In the areas between the regions of dominance a gradual "linear" change in composition occurs which could simply be due to tidal mixing of the two components, or of the nutrients which support them. The variance in the index for any one area which would be expected to have constant composition seems to be uniform along the entire flight line, except in the upper region of the James River, where very high optical attenuation evidently increased the variance.

COMPARISON WITH IN SITU DATA

On the morning of the March 17th remote sensing overflight, five research vessels were positioned along the flight lines. Most of these vessels took water samples at three stations which were overflown by the aircraft, one station about an hour before overflight, one at the time of overflight, and one about an hour after the overflight. Thus a total of 16 stations were sampled in conjunction with this portion of the overflight, of which five (#2, 5, 8, 11, and 15) were at the time or very near the time of overflight. The locations of these stations are indicated in Figure 2. Surface (depth of one meter or less) samples were taken at all stations, and sub-samples for phytoplankton counts and identification were preserved with formalin at half of those. These samples were examined by Dr. Harold Marshall of Old Dominion University, who presents a detailed report of his findings in the next paper (ref. 9). His data has been summarized in Table I using a format suitable for making comparisons between the counts and the fluorescence ratio (539/454)/composition index. Note that the same general trend exists in the two data sets, i.e. a trend from highest "green" species content and lowest composition index at station #1, located just east of the shelf break, to the lowest "green" species content and highest composition index at stations #11 and 15, located at the entrance to Hampton Roads and well up the James River, respectively. Upon first examination this relationship was not obvious, because the fluorescence excitation characteristics of the coccolithophores were assumed to be the same as the other prymnesiophytes. It was later found that some of these organisms, the most predominant group of deep sea phytoplankton, have fluorescence excitation spectra very similar to the cryptophytes, but without the phycoerythrin effect. Their composition indexes range from 0.40 to 0.44. Since the only species in this group examined so far has been found to have 19'-hexanoyloxyfucoxanthin, a structural variant of fucoxanthin, as its primary carotenoid (ref. 10), it may be that these organisms do not have the

complexed fucoxanthin of the other prymnesiophytes. This makes them respond to the two wavelengths of excitation light in a manner similar to the "green" species. Exceptions to the general trend are most noticeable at stations #3 and #8 where substantially more "green" species were found than would be expected from the composition index. However, these two stations were also the only ones at which unidentified spherical shaped cells, called "small green spheres" by Dr. Marshall, dominated the "green" species component. These algae were assumed, in the absence of any further identification, to belong in the "green" component, simply on the basis of their color. If this assumption was incorrect and these organisms are actually "golden-brown" species, then the revised "green" species component would show no obvious exceptions to the general trend predicted from the remote data.

In some cases the composition index has apparently been affected by the presence of blue-green algae. Examples of this effect can be seen by a comparison of the data from station 4 with 5, and 11 with 15. The presence of phycoerythrin in some of the blue-green algae can cause a substantial increase in the fluorescence excited by green light (539nm) and thus result in a higher composition index when they are present, even though the distribution of the major components is the same. This effect could be countered by adding a third excitation wavelength in the yellow/orange region of the spectrum (570nm) which would primarily excite the phycoerythrin. This modification to the RAF could easily be made when blue-green algae are known from historical data to comprise a significant portion of the phytoplankton population.

In addition to the above points, it should be noted that the "in situ" data from Dr. Marshall supports the decision to ignore the effects of the cryptophytes, chrysophytes and prymnesiophytes in the computation of the composition index. At only one station (#3) were the cryptophytes a significant portion (5.6%) of the phytoplankton population, and species from the other two classes were not important at any of the stations. In fact, with a few minor exceptions, the phytoplankton population of the entire area could be characterized in terms of five major components (diatoms, dinoflagellates, coccolithophores, "small green spheres", and blue-green algae), with only 2-4 of these components occurring at any one station. Minor components which were occasionally important were the silicoflagellates and the "true" green species, such as members of the genera Scenedesmus and Euglena.

As an additional aid in the interpretation of the composition index for this experiment, the pigment content of the particulates in some of the water samples was determined. Separation of extracted pigments was accomplished by high pressure liquid chromatography and identification was based on location of absorption maxima. Emphasis was placed on the major pigments, i.e. the chlorophylls, fucoxanthin and peridinin. These pigment identifications were made on surface samples from four stations along the flight line, i.e. #3, 5, 6 and 10. No detectable amounts of chlorophyll b were found at any of these stations, which is not surprising as significant numbers of chlorophytes were not found at any of them. The other major pigments are presented as the amount found per unit chlorophyll a (Table II). The variation among these stations of both chlorophyll c and total primary xanthophyll (fucoxanthin + peridinin) relative to chlorophyll a was similar, as both showed highest

values in the plume area (stations #5 and 6) and lower values at the shelf break. However, at station #10, near the entrance to Hampton Roads, the normalized chlorophyll c level was considerably lower than elsewhere, and lower than would be expected from the xanthophyll level. The composition index for this station was also significantly higher than at the other three stations. A comparison of the composition index and the ratio of total primary xanthophyll to chlorophyll c revealed that neither varied much among stations #3, 5 and 6, but both were definitely higher at station #10. This agreement should be expected since the fluorescence of chlorophyll a when the organism which contains it is illuminated by blue (454nm) light is primarily a function of its total chlorophyll content, chlorophyll c being the strongest absorber at that wavelength, and since the same fluorescence when the organism is illuminated by green (539nm) light is evidently primarily a function of its complexed xanthophyll content. Thus, measurement of the pigment content of the particulates in the surface water at several stations was helpful in interpreting the variation of the composition index. However, the relationship between these two parameters was not constant and further investigation will be pursued utilizing a larger data base.

CONCLUDING REMARKS

Even though there are a number of unknowns still involved in the interpretation of the composition index, these are being resolved and hopefully within the next year or so it will have evolved into a technically and scientifically sound approach. So, the question is: How can this measurement be utilized; what is it good for? Although there are several potential areas of application, the primary use seems to be in studies of marine productivity. "Color" group and "size" group seem to be quite synonymous. The "golden-brown" species are physically larger than the "green" species, which are mostly nonoplankton. Feeding or grazing of zooplankton on the phytoplankton population is primarily keyed to size, i.e. certain zooplankton are only equipped to collect phytoplankton within a specific size range. The presence or absence of the right size of phytoplankton can mean the difference between a high and low grazing efficiency. Models of marine productivity usually take this factor into consideration, but the conventional methods of obtaining the data are extremely time consuming and labor intensive, even for the samples from a few stations. The availability of an index based on remote data which could be rapidly computed at dozens of points per square kilometer would help make the models much more spatially realistic, while reducing considerably the labor involved. If this index were to be keyed or calibrated to a few in situ stations, its accuracy would be increased to the level of other trophic measurements. In addition, the combination of the composition and "standing stock" measurements of phytoplankton, both of which can be made by the RAF or any similar remote fluorosensor, greatly increases the power of this type of tool.

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TABLE I - Summary of Phytoplankton Composition

Station #	"Golden-brown" Species (%)			"Green" Species (%)	F_{539}/F_{454}
	Diatoms	Dinoflag.	Others	Total	
1	60.2	6.2	0.4	33.2	0.54
2	75.0	15.1	-	9.9	0.54
3	50.9	3.0	5.6	38.8	0.69
5	34.4	64.5	0.8	0.3	0.73
4	84.9	0.8	10.9*	3.1	0.77
8	28.5	43.3	7.1*	21.1	0.71
11	98.9	1.1	-	-	0.84
15	56.7	41.5	1.8*	-	~1.0

* Significant content of blue-green algae.

TABLE II - Pigment Content of Particulates in Water Samples

Pigment* or Pigment Ratio	Station #			
	3	6	5	10
Chlorophyll *a*	55.1	28.8	70.1	69.9
Chlorophyll *b*	<5.0	<5.0	<5.0	<5.0
Chlorophyllide *c*	12.4	10.7	22.1	12.4
Peridinin	<2.0	13.5	27.6	21.0
Fucoxanthin	30.5	11.6	26.2	35.0
Chl *c*/Chl *a*	0.23	0.37	0.32	0.18
(Per.+Fuco.)/Chl *a*	0.55	0.87	0.77	0.80
F_{539}/F_{454} (Composition Index)	0.69	0.70	0.73	0.85

*µg/ml of extract.

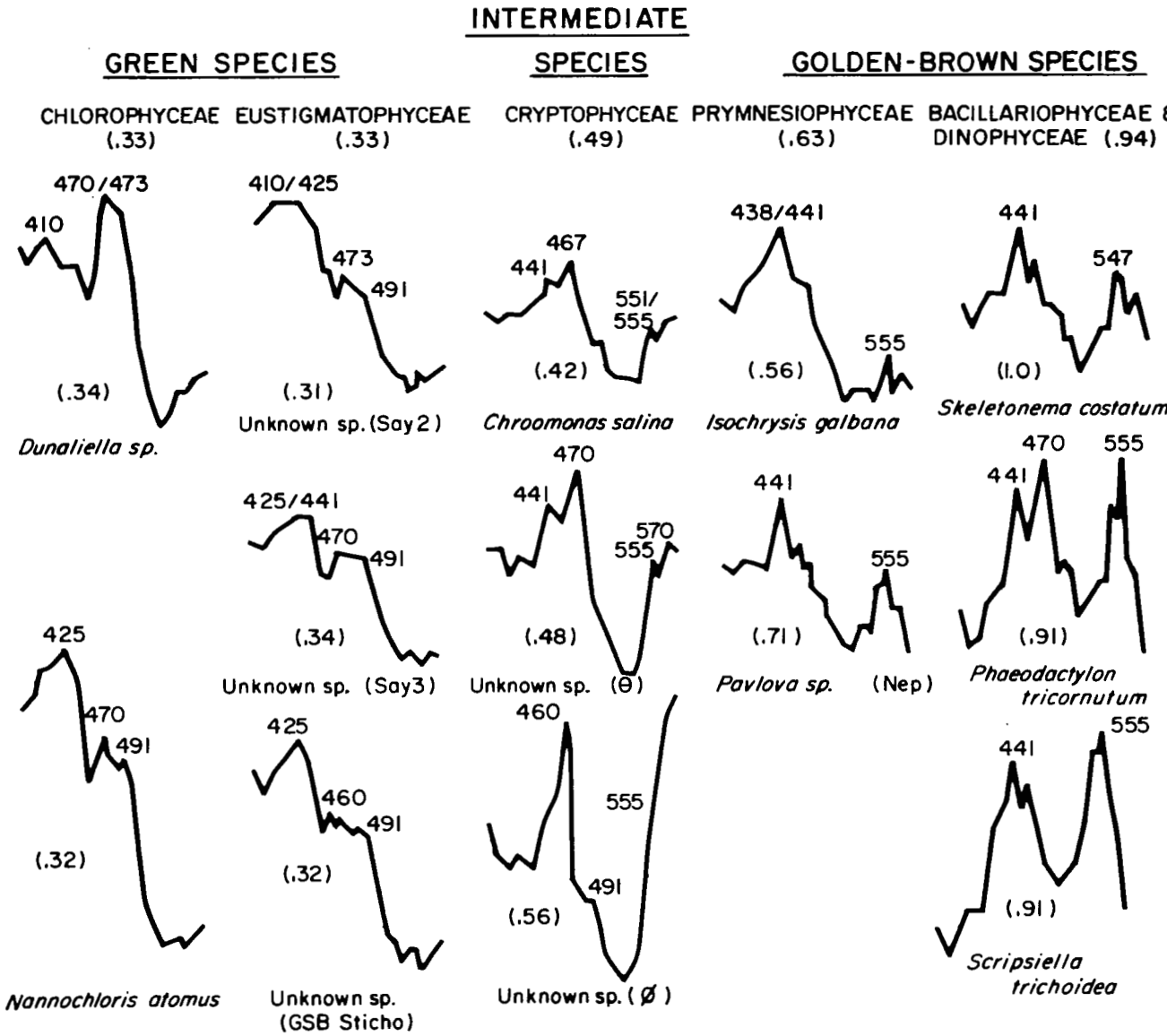

Figure 1.- Fluorescence (685 nm) excitation spectra of phytoplankton species from green, golden-brown, and intermediate color groups. Numbers in parentheses are composition indexes. Names and Greek letters in parentheses are clone names.

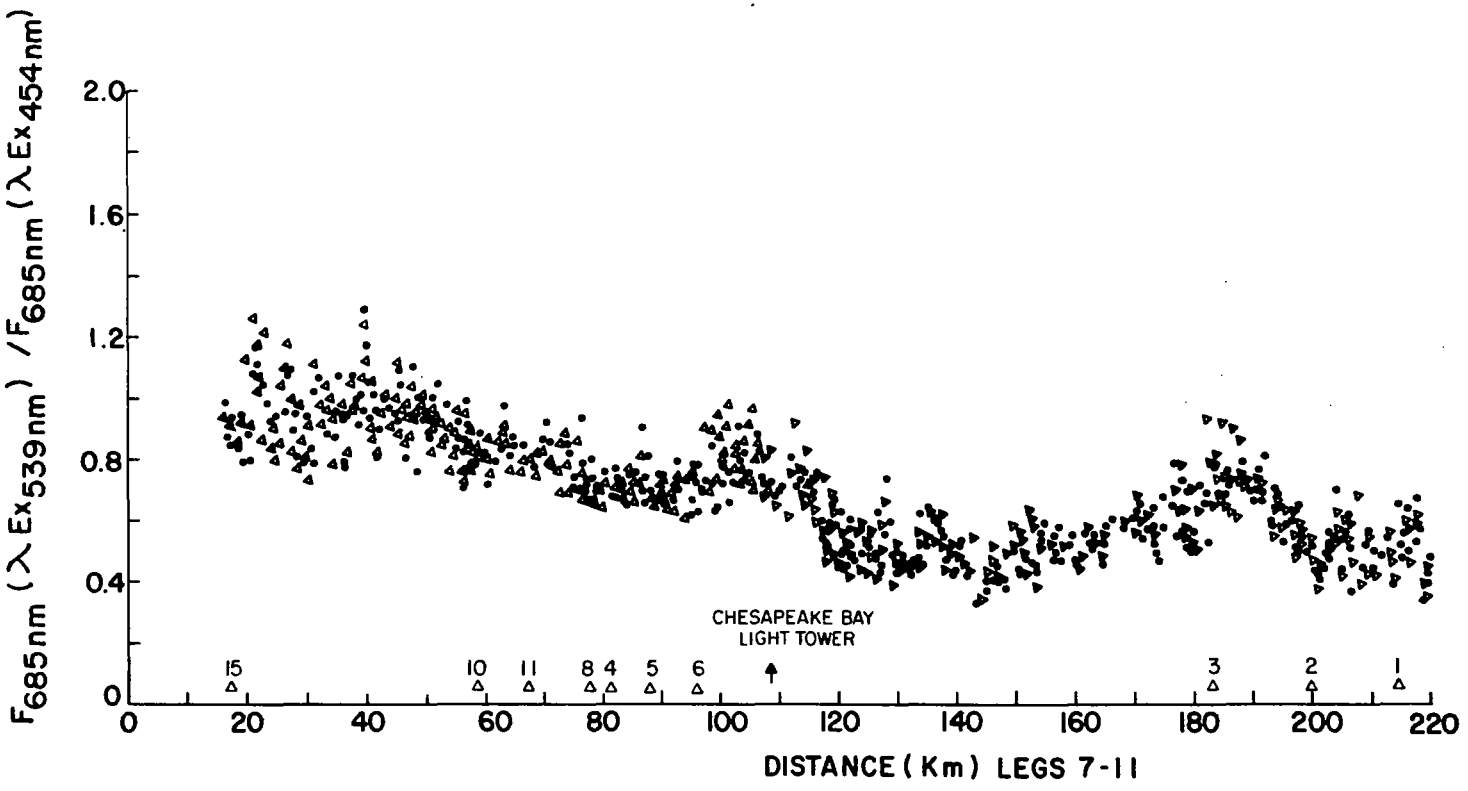

Figure 2.- Composition index (fluorescence excitation ratio) values along flight legs 7, 9 and 11. Numbers over triangles indicate sample station locations.