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Spectral differences and temporal stability of phycoerythrin fluorescence in estuarine and coastal waters due to the domination of labile cryptophytes and stabile cyanobacteria

Abstract—Laser fluorosensing and epifluorescence microscopy were used jointly to identify the origin of different spectral peaks of phycoerythrin in estuarine and coastal samples. The fluorescence of the samples was also examined as a function of the time elapsed after a water circulation system was turned on. Coastal samples were dominated by cyanobacteria and exhibited a constant phycoerythrin fluorescence with time. The phycoerythrin fluorescence of the Chesapeake Bay estuarine samples first increased strongly, reached a maximum, and then decreased to below the original level; these samples were dominated by cryptophytes which epifluorescence techniques revealed were being destroyed by the circulation system. A simple mathematical model was developed to describe the effects of cell disruption, the uncoupling of energy transfer between pigments, and the subsequent breakdown of the solubilized phycoerythrin.

Ocean and estuarine dynamics vary in a complex manner and require, for their understanding, that synoptic measurements be taken with a time resolution typically a fraction of a tidal cycle. There is also a need to resolve features on a much smaller spatial scale for such phenomena as plankton patchiness. Passive techniques for global monitoring with satellite-borne ocean color scanners now under development will need accurate oceanic data for interpreting and modeling the upwelled solar irradiance. To supplement the satellite information, we are developing airborne light detection and ranging (LIDAR) systems using lasers to provide such data on a smaller spatial and temporal scale.

As a first step toward understanding laser excitation of a water sample and the resulting backscattered spectrum, Exton

et al. (1983) analyzed spectra from samples in a laboratory tank with the laser, tank, and spectrometer arranged to simulate a remote sensing geometry and concluded that laser fluorosensor techniques could be used to quantify total suspended solids (TSS), dissolved organic matter (DOM), effective attenuation coefficient, chlorophyll *a*, and phycoerythrin. Houghton et al. (1983) moved the fluorosensor and tank to a pier to compare spectra obtained in situ with those from the tank. As a part of these tests, the airborne oceanographic lidar (AOL) from the NASA Wallops Flight Center flew a course from the mouth of the Chesapeake Bay, to the pier at Yorktown, Virginia. Samples were acquired by ships along the flight path and returned to the pier for analysis using the tank. Phycoerythrin in the "coastal" samples gathered near the mouth of the bay had different spectral characteristics from those in samples from the bay and its tributaries (Fig. 1). Variations in the intensity of phycoerythrin fluorescence with time for some natural samples seemed to be related to the use of a circulation system in the tank. This difficulty necessitated the use of uncirculated samples in the field experiments.

To identify the origin of the different phycoerythrin peaks in the tributary and coastal samples and to examine the effects of circulation of water samples on the fluorescence response, we supplemented laser fluorosensor techniques by epifluorescent microscopy so that we could identify and count the phytoplankton in each water sample.

We thank R. W. Gregory for his sup-

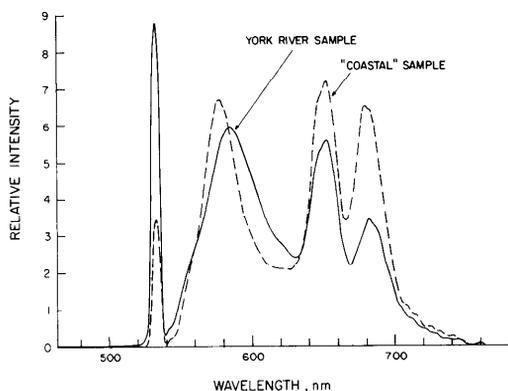


Fig. 1. Spectra obtained from a York River sample and a coastal sample illustrating different spectral characteristics for the phycoerythrin peak.

port in assembling the laser fluorosensor and performing the spectral measurements.

The laser fluorosensor system is fully described by Exton et al. (1983) and Houghton et al. (1983). A beam from a pulsed, frequency-doubled Nd:Yag laser is directed into an 80-cm-deep tank from above. The scattered light and fluorescence is collected in a 180° backscatter arrangement simulating a remote-sensing configuration. The collected light is analyzed with an optical multichannel analyzer (OMA). The OMA is gated and synchronized with the laser pulses to reject ambient light. The OMA acquires a complete spectrum for each laser pulse and averages a preset number of spectra to obtain a suitable signal-to-noise ratio. Circulation in the tank is provided by a rotary vane (flexible impeller) centrifugal pump with a flow rate of about 20 liters \cdot min⁻¹. The circulation system takes in water from a point 20 cm below the surface and resupplies the tank near the bottom.

The epifluorescence microscope (Zeiss Standard) was equipped with a 12-V, 100-W halogen light source, a 450–490-nm bandpass excitation filter, a 510-nm chromatic beam splitter, and a LP 520-nm barrier filter. The fluorochrome, proflavine, used to make the cells fluoresce, has the advantages over acridine orange that it eliminates background fluorescence

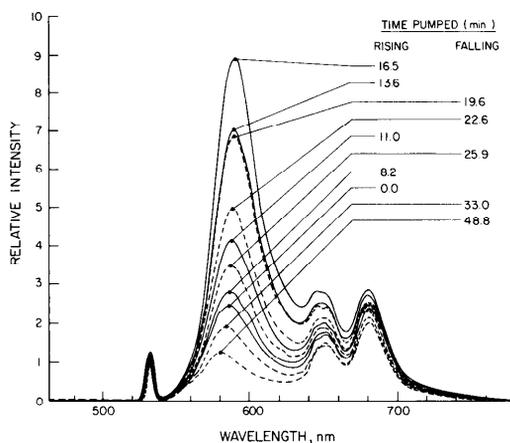


Fig. 2. An overlay of spectra obtained from a Potomac River sample showing an initial increase in the phycoerythrin fluorescence which reaches a maximum, then decreases in intensity to a level below the original level. The times shown are measured from the time that the circulation system is turned on. The water Raman peak intensity changes by about 100% as a result of the influence of the strong phycoerythrin fluorescence, but the strength of the *integrated* water Raman band remains constant. The Chl *a* peak intensity may also be influenced, to a lesser degree, by the phycoerythrin fluorescence.

and does not cause flagella to fall off the cells. With this system, autofluorescing photosynthetic pigments in general may be observed and phycoerythrin can be distinguished by a gold-orange autofluorescence. The technique and preparation of samples were described by Haas (1982).

Spectra from natural and cultured samples were recorded by the laser fluorosensor every few minutes after the circulation pump was turned on. Immediately after each reading, a 2-ml sample was drawn from the tank by pipette and fixed, refrigerated, and epifluorescent counts were done within 1 week.

The phycoerythrin fluorescence of samples from the Chesapeake Bay and its tributaries had a peak at 586 nm with a full width at half-maximum (FWHM) of 46 nm; samples from the mouth of the bay had a coastal character with a peak at 576 nm and a FWHM of 25 nm (Fig. 1). When samples from the bay and its tributaries are circulated in the tank, the phycoerythrin peak shows an unusual behavior (Fig.

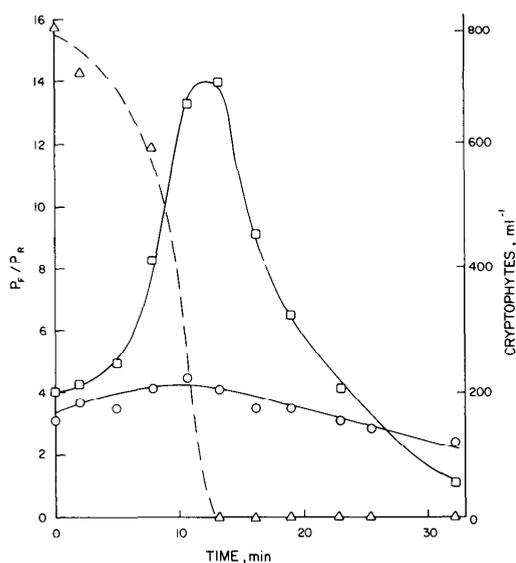


Fig. 3. Time histories of the (normalized) phycoerythrin (□) and Chl *a* (○) fluorescence intensities for a York River sample. The cell count for cryptophytes (△), obtained using epifluorescence microscopy, is also shown.

2). Although most features are relatively constant, the phycoerythrin fluorescence increases with time, reaches a maximum, and then decreases to a level below the initial level. Figure 3 shows a plot of the phycoerythrin:Raman and chlorophyll:Raman ratios for a sample from the York River. The fluorescence intensities in Fig. 3 (and Fig. 4) are normalized by the strength of the integrated water Raman band to account for changes in laser power, penetration, and instrumental parameters (Exton et al. 1983). Examination of the York River sample showed two classes of phycoerythrin-bearing microorganisms: a small (ca. 0.5 μm) coccoid cyanobacterium, *Synechococcus*, and larger (10–15 μm) flagellates belonging to the Cryptophyceae. The cyanobacterial numbers remained essentially constant at $1.7 \times 10^4 \cdot \text{ml}^{-1}$, whereas the cryptophytes decreased from $7 \times 10^2 \cdot \text{ml}^{-1}$ to zero in 15 min (also shown in Fig. 3). Without circulation, the cell counts and fluorescence spectra were unchanged for a similar period. The larger cryptophytes are completely destroyed by the circulation system while the small-

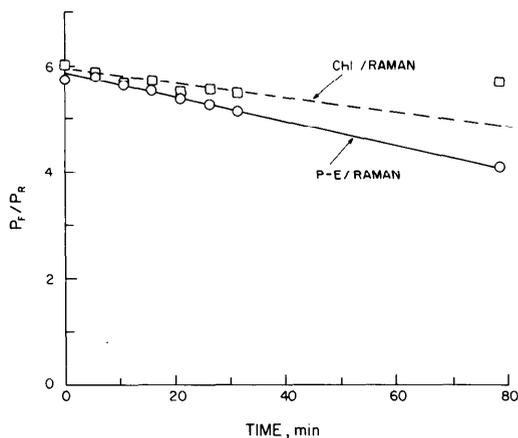


Fig. 4. Time histories of the (normalized) phycoerythrin and Chl *a* fluorescence intensities are shown for a culture of *Porphyridium purpureum*.

er cyanobacterial cells are relatively unaffected. The destruction of the cryptophytes is, therefore, responsible for the observed variation in phycoerythrin fluorescence; the phycoerythrin release into the water can be more efficiently excited momentarily before it is degraded. The peak in intensity for several samples occurred at about 12 ± 4 min for the present circulation system. The Chl *a* fluorescence may also show a similar but less obvious effect (Fig. 3).

Spectra from the coastal sample showed no perceptible changes during a 30-min observation period. Epifluorescent observation of this sample showed both cyanobacteria and cryptophytes but at concentrations much different from those in the York River sample. The cyanobacterial concentration is again constant, but is an order of magnitude higher ($1.3 \times 10^5 \cdot \text{ml}^{-1}$) while the initial cryptophyte concentration ($1.5 \times 10^2 \cdot \text{ml}^{-1}$) is about 20% of that in the York River sample. The cryptophytes in the coastal sample are also destroyed by the pump in about 13 min but the fluorescence is apparently dominated by the phycoerythrin in the cyanobacteria so that disruption of the cryptophytes is imperceptible.

The temporal stability of cyanobacterial phycoerythrin fluorescence is consistent with tests performed earlier (Exton et al. 1983), in which a culture of *Synecho-*

Table 1. ^{32}Si activities in waters and suspended matter of the Gironde estuary.

Sample	Collection period (Feb 76)	Quantity ($\times 10^3$ liters)	Chlorinity (‰)	Dissolved Si in water (ppm $\text{SiO}_2 \cdot \text{liter}^{-1}$)	Net ^{32}P act (cph)	^{32}Si concn*	
						Absolute (dpm $\cdot 10^{-3}$ liter)	Sp act (dpm $\cdot \text{kg}^{-1} \text{SiO}_2$)
GW1 (La Reole)	3	1.52	0.06	4.48	3.97	0.23 ± 0.03	51.3 ± 7
GW2 (Pauillac)	5	1.40	2.77	5.0	4.99	$\left\{ \begin{array}{l} 0.17 \pm 0.02 \dagger \\ 0.18 \pm 0.02 \dagger \end{array} \right.$	$\left\{ \begin{array}{l} 38.0 \pm 4 \dagger \\ 36.0 \pm 4 \dagger \end{array} \right.$
GW3 (LeVerdon)	7	3.28	13.85	2.0	2.70	0.08 ± 0.01	40.0 ± 5
GW4 (Ile d'Oleron)	11	4.60	17.73	0.66	4.07	0.035 ± 0.007	53 ± 11
Suspended matter GS2 (Pauillac)	5	300 g	3	$1.82 \text{ g} \ddagger$	1.10	—	$\left\{ \begin{array}{l} 33 \pm 5 \dagger \\ 42 \pm 4 \dagger \end{array} \right.$

* Errors quoted are due to counting statistics only.

† Sample remilked.

‡ Amorphous silica. The suspended matter was oven-dried at 110°C , then treated with 2.5 N NaOH.

silicon reaching the ocean is available as its behavior in the estuarine region is not well understood. Both conservative and nonconservative behavior of dissolved silicon have been observed in different estuaries (Burton et al. 1970b; Boyle et al. 1974; Borole et al. 1977; Bennekorn et al. 1978). Detailed accounts of earlier work on the behavior of dissolved silicon in estuaries by Boyle et al. (1974), Liss (1976), and Aston (1978) suggest that in some estuaries there is no significant removal of dissolved silicon and that wherever removal has been found its magnitude is $<30\%$. In a few estuaries such as the Gironde (Martin et al. 1973), the Hudson (Simpson et al. 1975), and the Charente (Salvadori 1976), an excess of dissolved silicon has been observed. We report here the first attempt to use ^{32}Si as a tracer for silicon geochemistry in estuaries, in particular to identify the source of excess stable silicon sometimes found in the low salinity zone; we have chosen the Gironde estuary with its unusual silicon behavior for this study.

Silicon-32 is a cosmic ray-produced isotope which has been used successfully in glaciology (Clausen et al. 1967; Clausen 1973), oceanography (Somayajulu 1969; Lal et al. 1976), and hydrology (Nijampurkar et al. 1966; Lal et al. 1970; Nijampurkar 1974). The half-life of ^{32}Si is not yet established; most recent estimates range from 108 ± 18 years (Elmore et al. 1980) to 276 ± 32 years (Demaster 1980).

Part of the work reported here was done during a stay of V. N. Nijampurkar as associate scientist of CNRS in the Laboratoire de Geologie, ENS.

We thank A. J. Thomas for assistance in collecting samples and B. L. K. Somayajulu and S. Krishnaswami for discussions.

Samples were taken in the Garonne River and its estuary, the Gironde (Allen 1973; Cauwet et al. 1980).

Because of the very low activities commonly encountered in surface waters (e.g. see Nijampurkar 1974), the ^{32}Si measurements require sampling and processing of very large volumes of water ranging from 1.4 to 4.8×10^3 liters. Therefore only four samples, over a chlorinity range of 0.06–17.73‰, were collected during this study in February 1976 (Table 1). The samples were centrifuged in the field by continuous flow centrifuge at 11,000 rpm to separate suspended matter from the water, which was processed in 2,000-liter capacity plastic pools. One sample of estuarine suspended matter was collected from a sample of chlorinity of 3‰ and also analyzed for ^{32}Si . Amorphous silica was extracted from suspended sediment (300 g) by treatment with warm 2.5 N NaOH.

When needed, a stable silicon carrier (5–10 g of SiO_2 per 10^3 liters of water) was added and dissolved silicon scavenged with $\text{Fe}(\text{OH})_3$ (Kharkar et al. 1966). Silicon extracted from $\text{Fe}(\text{OH})_3$ was purified and stored for about 3 months so that ^{32}P (half-

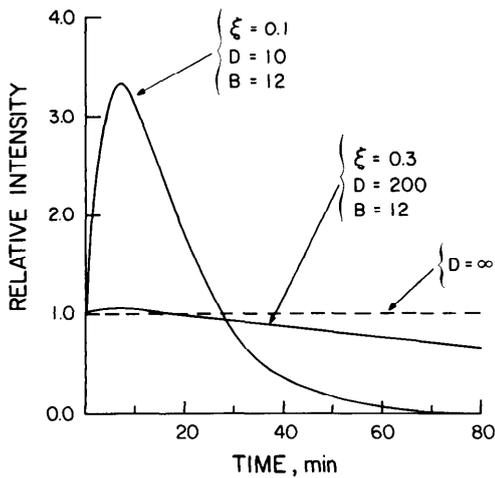


Fig. 5. Theoretical curves showing three applications of the mathematical model. For $(D, B, \xi) = (10, 12, 0.1)$, the model approximates the time-history characteristic of the phycoerythrin fluorescence from cryptophytes. Increasing the disruption period to 200 min renders an overall decrease in fluorescence intensity similar to that shown by *Porphyridium purpureum*. The use of an infinite disruption period describes the unvarying fluorescence exhibited by *Synechococcus*. The initial intensity for each curve is normalized to 1.0.

orescence exemplified by the cyanobacteria.

The Chesapeake Bay and river samples were dominated by cryptophytes that were destroyed by the circulation system. Cryptophytes are a ubiquitous and at times abundant component of the lower York River phytoplankton community and are generally observed in temperate estuarine phytoplankton communities. The Cryptophyceae have no true cell wall and consequently are often destroyed by traditional methods of collecting and counting (Sieburth 1979). In the Rhodophyceae and the Cyanophyceae phycoerythrin is contained in phycobilisomes on the thylakoid surfaces; however cryptophyte phycoerythrin is free within the thylakoids and is more likely to be released into solution when cells are disrupted. Accompanying this release is the loss of energy transfer between phycoerythrin and Chl *a* and an attendant increase in the fluorescence cross section for phycoerythrin.

The culture of *P. purpureum* showed

only a small decrease in phycoerythrin fluorescence in 80 min. *Porphyridium purpureum* is about the same size as the cryptophytes but is characterized by a thick mucilaginous sheath (Sheath et al. 1981). The congeneric *Porphyridium cruentum* from terrestrial habitats is resistant to desiccation (Sieburth 1979). These robust cells are probably less susceptible to disruption than the naked cryptophytes (Gantt 1971).

The cyanobacteria are ubiquitous in marine waters, reaching maximal numbers in temperate estuarine areas during summer and present in high concentration year-round in offshore waters (Johnson and Sieburth 1979; Waterbury et al. 1979; Krempin and Sullivan 1981; Perkins et al. 1981). Their characteristic multilayered cell wall (Perkins et al. 1981) and small size undoubtedly account for their resistance to destruction by pumping.

Similar variations in fluorescence from Chesapeake Bay waters have been reported in experiments with dye tracers (Pritchard 1979; Zertuche-Gonzalez 1981). The dye was detected with a shipboard fluorometer in which the sample was continuously pumped through the fluorometer. Excitation was provided by a Hg line at 546 nm and detection was optimized for the red fluorescence of the Rhodamine dyes centered at 590 nm. In the process of tracking dye, it was discovered that variations in the natural background fluorescence produced an unacceptable loss in sensitivity to the dye fluorescence. When the sample was continuously circulated through the system, the background fluorescence first increased, reached a maximum, and then decreased. This effect was believed to be due to extraneous ultraviolet light which also irradiated the sample. Further tests with samples pumped through a UV sterilizer reinforced this idea since the intensity of the UV light from the sterilizer influenced the peak intensity and temporal position of the fluorescence. The temperature of the circulating sample also caused a slight change in the fluorescence levels. The temporal characteristics of the fluorescence and the wavelengths used suggest

that these observations also involve fluorescence from phycoerythrin contained in cryptophytes. On the basis of our observations, we suggest that the disruption of cryptophytes by the pump represents the primary mechanism responsible for the temporal variation in fluorescence. Ultraviolet irradiation and temperature effects are probably of secondary importance. Solutions to the problem of dye tracking include the use of filters to remove the cryptophytes or to pump or irradiate the sample until the phycoerythrin fluorescence is stabilized (as was done by Pritchard 1979). The best solution, however, may be to excite the sample at a wavelength outside of the phycoerythrin absorption band; this may also require the use of a different dye.

In contrast to the negative impact of cell disruption on dye tracing, the disruption phenomenon may be of use in developing a more sensitive detection system for phycoerythrin in cryptophytes. This could be accomplished by standardizing the disruption (pumping) procedures and by using the integral of the curve of intensity vs. time (*see Fig. 3*) as an indicator of the phycoerythrin concentration. Our results suggest that this procedure may be as much as an order of magnitude more sensitive than the direct measurement of the static fluorescence intensity. By monitoring the residual phycoerythrin fluorescence, such a system would also help separate the contributions by different algae to the total amount of phycoerythrin.

The results of our circulation tests also prompted an analysis of the opposite situation of a static (undisturbed) sample environment. These tests were done in the laboratory in a separate experiment with 514.5-nm excitation provided by a C.W. argon ion laser (*see Exton et al. 1983* for details of the apparatus). A Chesapeake Bay sample was poured into the tank and allowed to come to rest. A laser beam was incident on the sample continuously and spectra were recorded every 5 min for 1 h; the phycoerythrin fluorescence during this period increased by about an order of magnitude. Upon the slightest stirring of

the sample, the fluorescence abruptly returned to its original value. This obviously cannot be attributed to cell disruption but is most likely due to the positive phototactic action of the motile cryptophytes which are able to swim toward the laser beam when the turbulence is minimal; the stirring redistributes them with a concomitant return of the fluorescence to its original, well mixed value. Laser fluorosensing may therefore be a good technique for quantifying phototactic action spectra. For example, "snapshot" measurements of the algal concentration could be made by occasional triggering of a pulsed laser. Controlling the duration and repetition rate of the laser would prevent the algae from responding to the sensing apparatus. Such a system may be an improvement on the systems currently used in which concentration measurements are made under red light to which the algae are insensitive (Watanabe and Furuya 1974).

The cell disruption effects shown here enforce the concerns of marine biologists with regard to sample handling. Biological analysis must be performed carefully and further investigations of handling techniques are warranted. The use of remote sensing is certainly advantageous since there is no disruption. Furthermore, if the remote fluorosensor can attain the spectral resolution and high signal-to-noise used here, the system could be used to distinguish differences between the phycoerythrin spectra from cyanobacteria (peak at 576 nm) and cryptophytes (peak at 586 nm), both of which can be major components of nearshore/estuarine nanophytoplankton communities. The spectral differences in phycoerythrin fluorescence for these two classes of algae also suggest that different fluorescence efficiencies are involved. This point warrants additional study along the lines of the research on chlorophyll.

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The time-course of uptake of inorganic and organic nitrogen compounds by phytoplankton from the Eastern Canadian Arctic: A comparison with temperate and tropical populations

Abstract—Uptake of inorganic (NO_3^- , NH_4^+) and organic (urea) nitrogen compounds by arctic phytoplankton was linear for at least 30 h of incubation under natural temperature and light conditions. Extrapolation of linear fits of the data showed positive ordinal intercepts for NH_4^+ , suggesting relatively more rapid uptake early in the incubation period. Short term uptake experiments confirmed this; rates computed from 20-min incubations were on the average 3-fold higher than 24-h uptake rates. The transient nature of these enhanced uptake rates resulted in their contributing little (<10%) to

the total mass flux observed over 24 h. Comparison of these experiments with similar measurements from temperate and tropical waters suggests that the often observed nonlinearity in nitrogen uptake in the field may be more a consequence of isotope dilution and recycling than substrate exhaustion.

Recent research on the relationship between nitrogen uptake and growth in marine algae has suggested that the long es-