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Spectral observations of pigment fluorescence in intermediate depth waters of the North Pacific

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ABSTRACT

Vertical profiles at three stations off the California coast showed spectral differences between fluorescence at the primary fluorescence maxima (near 100 m) and the deeper maximum (near 800 m). Two broad-band excitation and several narrow or high-pass emission filters were used with an *in situ* fluorometer to 1500 m. Interpretation of these data suggests: (1) chlorophyll *a* was found throughout the water column, with intermediate depth fluorescence about one-third the intensity of that in the primary maximum; (2) phycobilin fluorescence was stronger at intermediate depths than near the surface; and (3) a pigment which fluoresces beyond 700 nm was also found in the primary fluorescence maximum.

1. Introduction

In earlier work we observed intermediate depth fluorescent maxima in the North Pacific that we believed to be associated with microbial populations in the oxygen minimum (Broenkow *et al.,* 1983b; Lewitus and Broenkow, 1985). During the VERTEX 2 and 3 studies in the oxygen minimum zone west of central Mexico (Broenkow *et al.,* 1983b), the primary fluorescence maximum at 60 m was associated with primary producers in the photic zone, a sharp secondary fluorescence maxima was found at about 125 m near the top of the intense oxygen minimum, and a broad tertiary fluorescence maximum was observed between 200 and 700 m. The secondary maximum was found only between 14 and 19N latitude in waters having oxygen concentrations below 3 μM/kg (about 1% saturation). The tertiary maximum was observed at all stations in the VERTEX 2 and 3 studies and additionally at all stations between the coast of central California and Hawaii during the VERTEX 4 cruise (Lewitus and Broenkow, 1985). The center of the tertiary fluorescence maximum varied in depth from 300 m at 24N latitude, 116W longitude off Baja California to 1000 m at 28N latitude, 155W longitude north of Hawaii. The core of the tertiary fluorescence maximum appears to be found in the center of the oxygen minimum zone.

The object of our previous work was to determine the geographic extent of these features, and we made some speculations on their probable cause. The fluorescence

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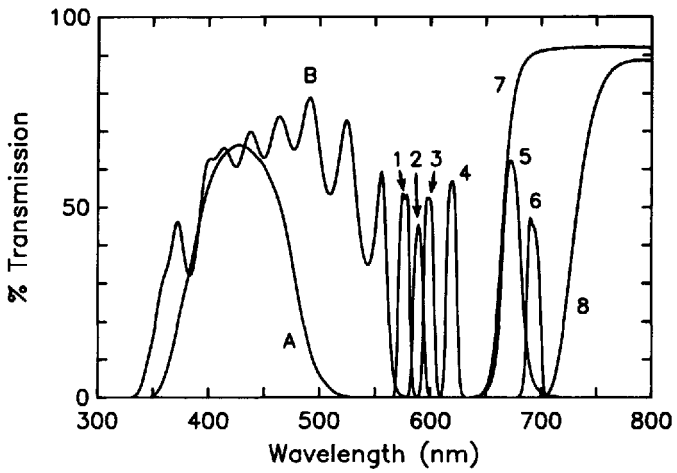

Figure 1. Emission and excitation filter transmission spectra of filters used with the Variosens fluorometer. See Table 1.

signals were "noisy"; that is, they showed high variability in the vertical with occasional large positive spikes. This suggests at least a partially particulate origin. The general broad tertiary fluorescence maximum could likewise indicate the presence of dissolved materials. The origin of the fluorescent materials was attributed to a potentially large array of pigment containing organisms or aggregates (Lewitus, 1984; Lewitus and Broenkow, 1985). These included: marine snow (Silver and Alldredge, 1981); fecal pellets (Urrere and Knauer, 1981); cyanobacteria (Yentsch and Yentsch, 1979; M. Silver, personal communication); nitrifying bacteria; purple nonsulfur bacteria (Olson and Stanton, 1966); and chlorophyll *a* and its degradation products (Goedheer, 1966).

The purpose of this report is to examine the *in situ* spectral characteristics of the intermediate depth tertiary fluorescence maximum off the California coast in a further attempt to understand its origin.

2. Methods

Continuous vertical profiles of conductivity, temperature, dissolved oxygen, fluorescence and beam attenuation were made with a CTOD profiling system described by Broenkow *et al.* (1983a). In all previous observations during the VERTEX 2, 3, and 4 cruises, the Variosens II fluorometer employed a Corning 5–60 excitation filter and a Corning 2–64 emission filter. Observations during the present work were made with a variety of filter combinations by adapting a filter holder to the Variosens silicon diode detector. Two excitation and eight emission filters were used (Fig. 1, Table 1). Broad-band Corning 5–60 (348 to 515 nm) and Balzer (336 to 572 nm) filters were used for excitation. Emission filters included Ditric 20 nm band-pass interference

Table 1. Filter characteristics and relative excitation and emission responses (Eq. 1) of filters used with the Variosens fluorometer.

Color	Filter	Wave length (nm)	Chlorophyll *a*		Filter responses			
			−Carot	+Carot	Phycoerythrin	Phycocyanin	Hematoporphorin	B. Chlorophyll
Blue	A	348–515	67	100	35	2	?	58
Blue	B	336–572	51		4	<1	?	19
Yellow	1	567–587	0		100	0	8	0
Yellow	2	578–598	0		95	<1	14	0
Orange	3	589–609	0		93	2	41	0
Orange	4	610–630	<1		43	23	44	0
Red	5	653–693	100		2	100	100	0
Red	6	678–698	52		0	16	29	0
Red + Far-red	7	667 hp-	345		0	209	256	*
Far-red	8	733 hp-	18		0	3	16	*

−Carot = without carotinoid excitation
+Carot = including carotinoid excitation
? = unknown
hp = half-power wavelength of high pass filter
* = emission spectrum unknown, but peak fluorescence is greater than half-power wave length of these filters

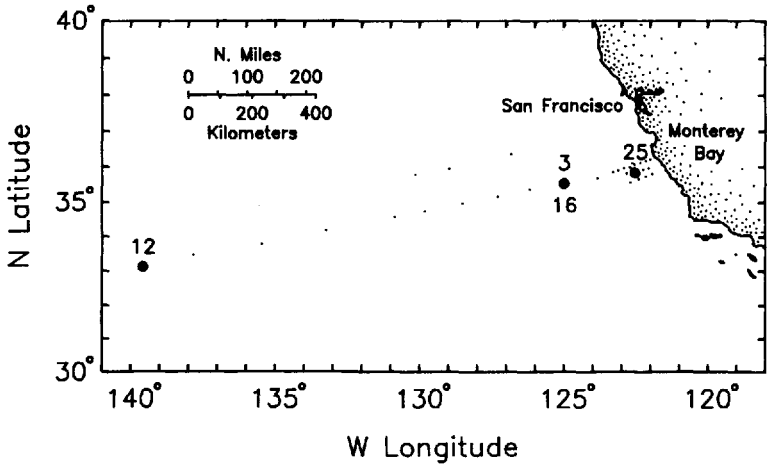

Figure 2. Station locations for the VERTEX 5 cruise. Stations 3 through 12 are from leg 1 (21 and 26 May 1984). Stations 16 and 25 are from leg 2 (13 and 16 June 1984).

filters with peak transmissivities of 577, 588, 599, 620, and 688 nm and a Schott 673 nm 40 nm band-pass interference filter. Corning 2–64 (667 nm half power) and Hoya RT-830 (733 nm half power) high-pass emission filters were also used.

At three stations off the central California coast (Fig. 2), different filter combinations were used on successive fluorescence casts. Throughout the text, excitation/emission filter combinations will be referred to as A6, for example, for the Corning 5–60 with the Ditric 688 nm filter. A Martek 1-m path beam transmissometer with either a Wratten 61 (520 nm peak transmission) or Wratten 45 (480 nm) filter was used to determine the total attenuation coefficient.

3. Results

We were able to test for a few hypothetical fluorescent pigments (Table 2) using the available filter combinations (Table 1): (1) yellowish fluorescent phycoerythrin (filters 1, 2, 3); (2) orange fluorescing cytochromes (filters 3, 4, 5); (3) red fluorescent chlorophylls (filters 5, 6, 7); and (4) far-red fluorescence from bacteriochlorophyll *a* (filter 8).

At our most offshore location (Station 12; Fig. 2) the primary red and far-red fluorescence (A7) maximum was at 122 m, and a broad tertiary maximum was at 900 m in the oxygen minimum (Fig. 3). Chlorophyll *a* specific fluorescence (B5) showed a smaller primary maximum at 128 m and the deep maximum was not well formed. Orange fluorescence (B2) at this station showed minimum values in the upper 300 m and a deep maximum that generally paralleled far-red fluorescence.

At Station 3 from Leg 1 and at the same location two weeks later, (Station 16), similar results were observed (Fig. 4). Red fluorescence profiles using the different

Table 2. Some potential microbial fluorescence sources.

Organism	Pigment	Excitation spectra (nm)	Emission* (nm)
Green Algae	chlorophyll *a*	300–500	685
Diatoms and	chlorophyll *a*	300–500	685
Dinoflagellates	carotenoids	450–580	non-fluorescent
Purple non-sulfur	bacterio-		
bacteria	chlorophyll *a*	350–630	900
Cyanobacteria and	chlorophyll *a*	300–500	685
Cryptomonads	phycoerythrin	420–610	570–580
	phycocyanin	520–720	650
	allophycocyanin	500–700**	660
Nitrifying and			
other bacteria	cytochromes	365–440	605–620

*Wavelength of fluorescence maximum
**Absorption spectrum

excitation spectra (A7 and B7) showed sharp primary maximum at almost precisely the same depth (89 m). The higher energy, broader excitation spectrum with the B7 filter pair caused a two fold increase in fluorescence relative to A7 throughout the water column. Far-red fluorescence (B8) showed a somewhat deeper (99 m) primary fluorescence peak with higher curvature than that observed 6.5 hours earlier using the

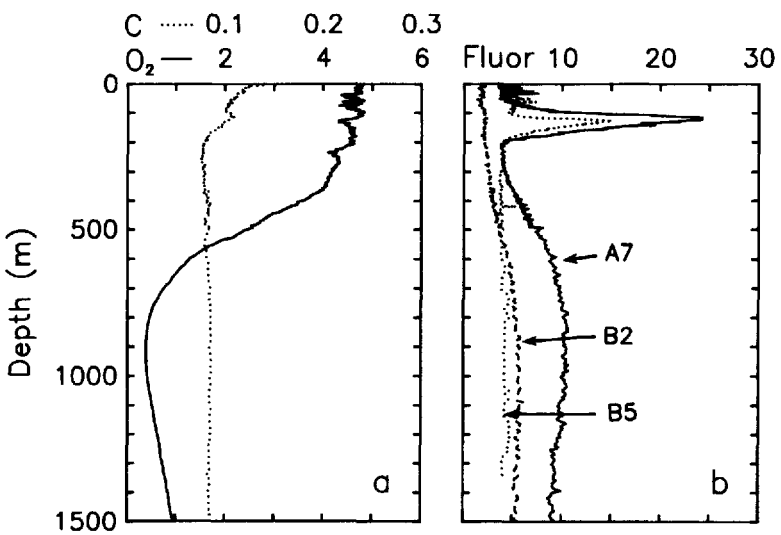

Figure 3. Vertical profiles of (a) dissolved oxygen (ml/liter) and total attenuation coefficient (/m) at 520 nm; and (b) *in situ* fluorescence (relative units) at Station 12. Excitation-emission filter pairs are indicated in Table 1.

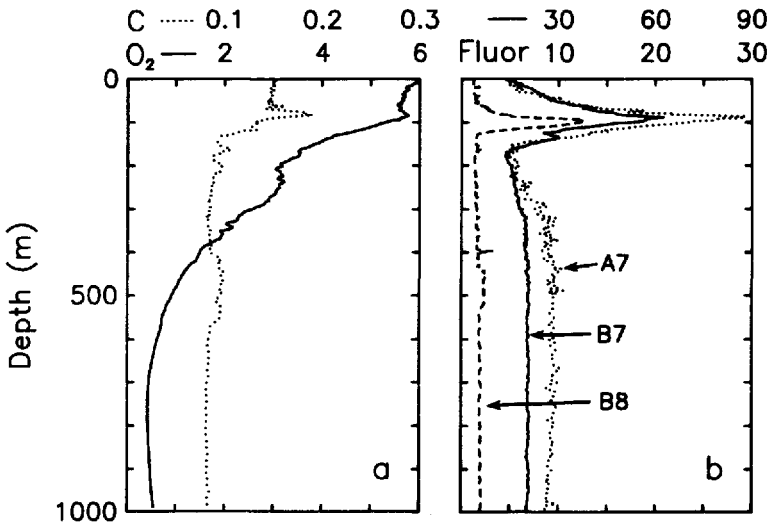

Figure 4. Vertical profiles of (a) dissolved oxygen (ml/liter) and total attenuation coefficient (/m) at 480 nm; and (b) *in situ* red fluorescence (relative units) at Station 16. Excitation-emission filter pairs are indicated in Table 1. (Note B7 scale is 3× others.)

B7 filters. A layer of increased far-red (B8) fluorescence between 420 and 520 m was also noted.

Results using excitation filter, B, with 6 different emission interference filters at Station 16 are shown in Figure 5. Yellow (filters 1 and 2) and red (filters 5 and 6) fluorescence maxima were observed. However, orange fluorescence (filters 3 and 4) was small and nearly featureless.

At nearshore Station 25 the B7 filter pair showed quite a different profile shape compared to offshore waters (Fig. 6). The primary red fluorescence maximum was at 15 m with another at 60 m. Below that, the fluorescence decreased continually with depth to 1500 m. Chlorophyll *a* specific fluorescence (filter B5) exhibited a maximum at 19 m, a much weaker maximum at 89 m, and small but nearly constant fluorescence at greater depths. Yellow fluorescence (B2) showed low but sporadic fluorescence from the surface to 250 m. Below that, yellow fluorescence increased gradually, similarly to that observed with the same filters at Station 12.

4. Discussion

The main intent of this work was to investigate the origin of intermediate depth fluorescence, which we suspected to be caused in part by fluorescence from senescent algae (e.g., diatoms, dinoflagellates, green algae) and cyanobacteria (Table 2). We will discuss the fluorescence profiles with regard to each of these possible sources.

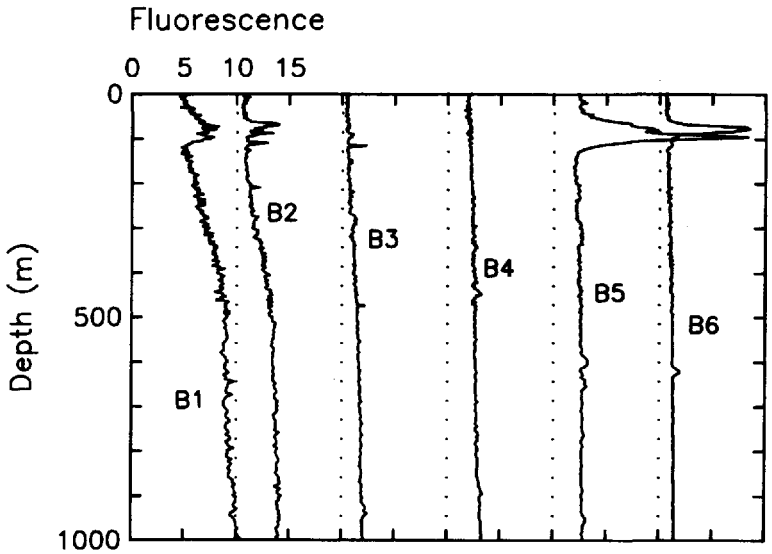

Figure 5. Vertical profiles of *in situ* fluorescence (relative units) at Station 16. Filter B was used in combination with the following emission filters. Each profile is offset by 10 units.

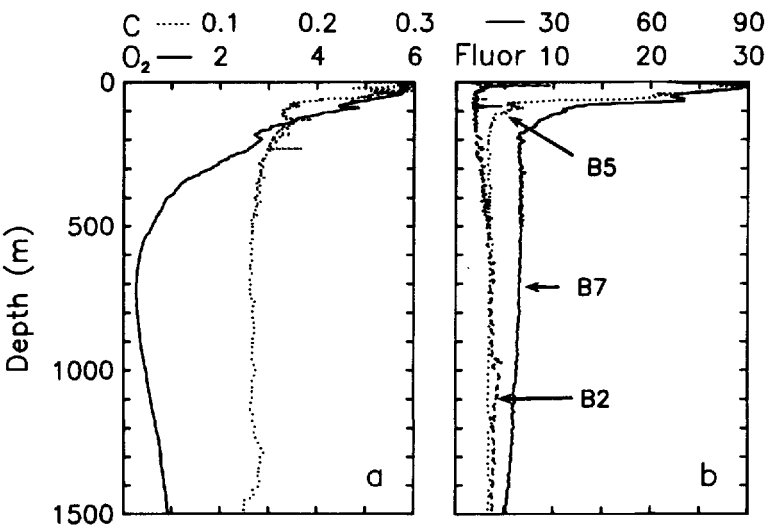

Figure 6. Vertical profiles of (a) dissolved oxygen (ml/liter) and total attenuation coefficient (/m) at 480 nm; and (b) *in situ* fluorescence (relative units) at Station 25. (Note B7 scale is 3× others.)

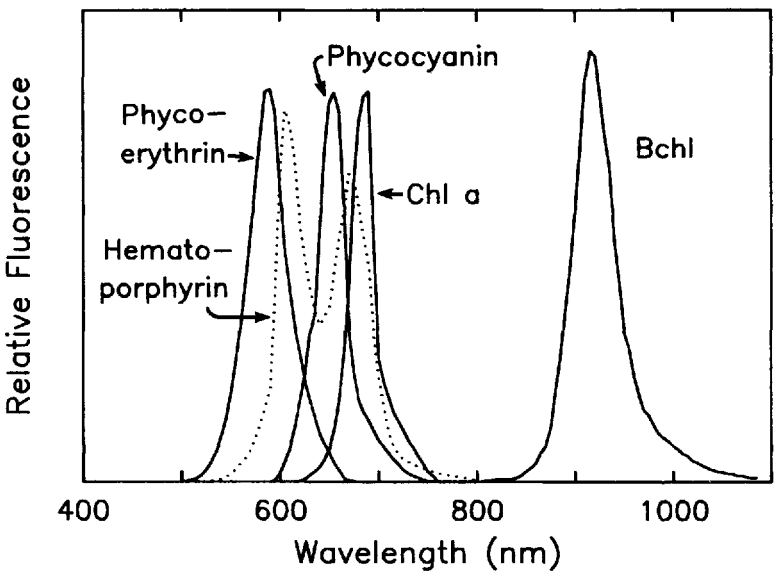

Figure 7. Relative emission spectra of fluorescent pigments (from Yentsch and Phinney, 1981).

a. Presence of accessory pigments. Our initial observations of intermediate depth fluorescence in the ocean were made with the A7 pair. Filter A is well-suited for chlorophyll *a* excitation but does not provide excitation energy for carotenoid accessory pigments (see Yentsch and Phinney, 1981). Filter B provides excitation energy almost precisely over the excitation spectrum of chlorophyll *a* with carotenoids. The relative efficiencies of these filters to excite fluorescence were estimated by integrating the scalar product of the filter transmission spectra $T(\lambda)$ and the excitation spectra $Ex(\lambda)$ or emission spectra $Em(\lambda)$ (Fig. 7). The integrals,

$$R_{ex} = \int_{300}^{800} T(\lambda)Ex(\lambda)d\lambda\,,$$

and

$$R_{em} = \int_{300}^{800} T(\lambda)Em(\lambda)d\lambda\,, \tag{1}$$

provide only a rough approximation of the relative excitation response, R_{ex}, or emission response, R_{em}, of the filter combinations, because we have not accounted for the spectral responses of the xenon flash lamp and silicon diode detector, and because the excitation spectra provided by Yentsch and Phinney (1981) for chlorophyll *a* and accessory pigments and by Nishimura and Takamiya (1966) for bacteriochlorophyll *a* were derived from pigment extracts, not from *in vivo* cells. The ratio $R_{ex}(B)/R_{ex}(A)$ for chlorophyll plus carotenoids excitation is 1.9. For chlorophyll *a* excitation only, the

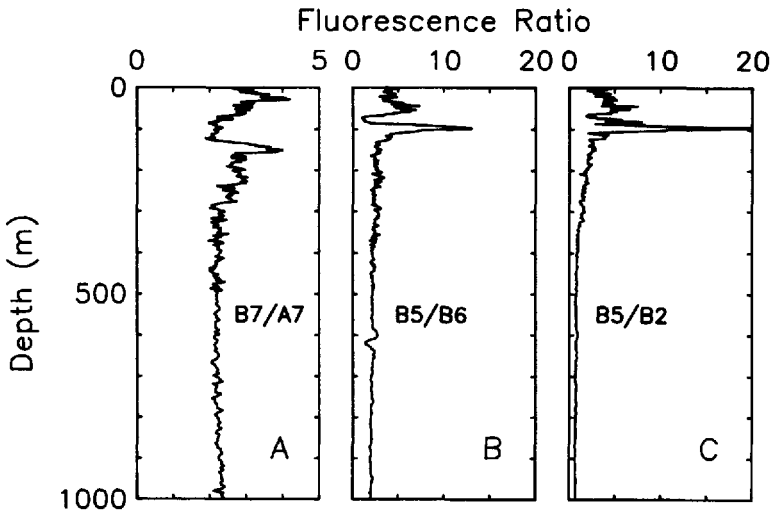

Figure 8. Water column fluorescence ratios from Stations 3 and 16. Filters are described in Figure 1, Table 1.

response ratio for these two filters is 1.3. The observed fluorescence ratio using these filters with emission filter 7 at Stations 3 and 16 (the same location 2 weeks later) decreased from about 3.5 near the surface to 2.0 at the depth of the primary fluorescence maximum, increased just below that maximum to 3.5, and gradually decreased to 2.2 at 300 m and below (Fig. 8a). The 2.0 ratio at the primary maximum suggests that fluorescence there is primarily from diatom and/or dinoflagellate chlorophyll *a*, in which carotenoid accessory pigments are important in the energy transfer mechanism (Rabinowitch and Govindjee, 1969). The fact that $R_{ex}(B)/R_{ex}(A)$ outside the primary maximum was greater than two suggests that inhanced red fluorescence is caused by additional light harvesting pigments or that large changes in the water column during the two weeks between these observations precludes this comparison.

Enhanced red fluorescence could be from organisms containing phycoerythrin (i.e. some cyanobacteria and all cryptomonads), because filter B is more efficient than filter A in stimulating light absorption by phycoerythrin (Table 1). In these organisms the phycobilins are the primary light harvesting pigments in the chlorophyll *a* fluorescence action spectrum (Haxo and Blinks, 1950; French and Young, 1952; Goedheer, 1972), and blue light absorption by chlorophyll *a* and carotenoids is irrelevant (Goedheer, 1965; Randy Alberte, personal communication). In phycoerythrin-containing cyanobacteria (see Chapman, 1973), the sequence of light energy transfer is from phycoerythrin to phycocyanin to allophycocyanin to chlorophyll *a* (Gantt, 1975; Harnischfeger and Cod, 1977), while in cryptomonads the sequence is from phycoerythrin to phycocyanin to chlorophyll *c* to chlorophyll *a* (MacColl and Berns, 1978; Jeffrey,

1980). Thus, the enhancement of phycobilin (primarily phycoerythrin) light absorption could explain the increased red fluorescence observed with excitation filter, B.

Alternatively, non-chlorophyll pigments that emit red fluorescence under blue to green light excitation may be important (e.g., phycoerythrin, phycocyanin, allophycocyanin, chlorophyll degradation products, bacteriochlorophyll *a* and its degradation product, and cytochromes).

b. Presence of chlorophyll a. Use of the red-transmitting filter 7 is not very selective in determining the source of deep fluorescence. Its bandpass (Table 1) accepts the entire emission spectrum from phycocyanin and about one-third of the emission spectrum of hematoporphyrin (Figs. 1 and 7). According to the emission spectra of Yentsch and Phinney (1981), filter 5 (Fig. 1, Table 1) accepts some phycoerythrin fluorescence, the lower half of the chlorophyll *a* spectrum, and the upper third of the phycocyanin spectrum. It is also centered on the high wavelength lobe of hematoporphyrin emission (Fig. 7). Filter 6 should be more specific toward chlorophyll *a* fluorescence, but because of its smaller bandwidth, it is about one-half as sensitive.

The water column fluorescence ratio of the B5 and B6 filter pairs (Fig. 8b) shows that near the primary fluorescence maximum and below 200 m the ratio is about 2.0, as predicted by these filter responses toward chlorophyll *a* fluorescence (Table 1). This would suggest that in the deeper waters little fluorescence at these wavelengths is caused by the phycobilins or hematoporphyrin. However, it is interesting to note that intact phycobilisomes emit *in vitro* fluorescence similar to chlorophyll *a* emission. Upon excitation of phycoerythrin, maximum fluorescence from the emitting species (allophycocyanin) is at 670 to 675 nm (Gantt, 1975).

A variety of microorganisms contain hematoporphyrins (cytochromes) that are assayed using fluorescence techniques (Udenfriend, 1969). Hematoporphyrin fluorescence is excited at wavelengths between 365 and 440 nm, and emission occurs between 590 and 700 nm with a peak between 600 and 650 nm, depending on the solvent (Vannotti, 1954; Udenfriend, 1962). Both of these authors agree that hematoporphyrin fluorescence in nature is unlikely because the porphyrin must be separated from the iron complex for fluorescence to occur (also see Vanderkooi *et al.*, 1976).

Chlorophyll degradation products, such as chlorophyllide, phaeophorbide, and phaeophytin, have fluorescence spectra similar to chlorophyll *a* (Gowen, 1981). However, these pigments are only weakly fluorescent *in vivo* (Yentsch, 1974; Kiefer, personal communication). Thus, we conclude that the deep fluorescence detected by the filters 5 and 6 is most likely from chlorophyll *a*.

c. Far-red fluorescence. To further characterize red fluorescence, filter 8 was used to look for fluorescence beyond 700 nm (Fig. 1). This filter nearly excludes the detection of fluorescence from the chlorophyll *a* form that normally dominates marine algal

fluorescence (Fig. 7). According to Eq. 1, this filter should produce only an 18% response to chlorophyll fluorescence relative to the filter 5 (Table 1). At Station 16, far-red fluorescence readings of 12 at 98 m and 2.2 at 900 m (Fig. 4) were about 80% of that indicated by chlorophyll *a* (filter 5) fluorescence at the same depths (Fig. 5). Therefore, other sources of far-red fluorescence may have been present here. Three possible sources are bacteriochlorophyll *a* from purple non-sulfur bacteria (Goedheer, 1966); long wavelength forms of chlorophyll *a* present in many algal species (Goedheer, 1972); and the phycobilins, phycocyanin and allophycocyanin, which fluoresce above 700 nm (French *et al.*, 1956).

Carotenoid absorption can contribute significantly to bacteriochlorophyll *a* fluorescence. The efficiency of light energy transfer from carotenoids to bacteriochlorophyll fluorescence has been reported to vary between 20 and 90% for purple non-sulfur bacteria (Goedheer, 1959; Nishimura and Takamiya, 1966, Goedheer, 1972). Excitation peaks for bacteriochlorophyll *a* fluorescence in one species occur at 375 and 588 nm (both from bacteriochlorophyll *a* absorption) as well as 420, 500, and 540 nm (absorption by carotenoids) (Nishimura and Takamiya, 1966). Fluorescence is emitted from 825 to 1050 nm (Olson and Stanton, 1966; Zankel and Clayton, 1969) with a peak at 895 nm (Udenfriend, 1969) or 900 nm (Goedheer, 1972). Thus, the B8 combination is capable of detecting bacteriochlorophyll *a* fluorescence.

Chlorophyll *a* in photosynthesizing cells is present in two or more forms (Goedheer, 1964; Goedheer, 1972): a strongly fluorescent photosystem 2 form with an emission peak near 685 nm, and one or more weakly fluorescent photosystem 1 forms with emission peaks near 725 nm. Fluorescence from the latter form(s) (Vredenberg and Slooten, 1967; Goedheer, 1972) could contribute to far-red emission we have observed. In addition, various algal species are characterized by chlorophyll *a* fluorescence peaks at wavelengths greater than 700 nm. These include some green algae species, which have fluorescence maxima near 740 nm (Goedheer, 1972) and 710 nm (Giraud, 1964; Brown, 1966) and the diatom *Pheodactylum tricornutum,* which has a fluorescence peak near 710 nm (Brown, 1967).

d. Presence of phycoerythrin. The use of fluorescence techniques to study distributions of cyanobacteria and cryptomonads in ocean surface waters has been described by Moreth and Yentsch (1970) and Yentsch and Yentsch (1979). Cyanobacteria may contain up to four classes of fluorescent pigments: chlorophyll *a*, the phycoerythrins (which may be absent), the phycocyanins, and the allophycocyanins (Kirk, 1983). Phycoerythrin fluorescence is exicted by radiant energy between 420 and 600 nm, and its emission occurs from 510 to 665 nm with a maximum at 570 or 580 nm (Yentsch and Yentsch, 1979; Yentsch and Phinney, 1981). Phycocyanin fluorescence is excited by light between 520 and 720 nm, and emission occurs from 600 to 750 nm with its peak near 650 nm (Yentsch and Phinney, 1981). Allophycocyanin, which occurs almost always in small amounts (Kirk, 1983), absorbs between 500 and 700 nm

(French *et al.*, 1956; Chapman, 1973; Gantt, 1975) and emits between 625 and 750 nm (660 nm peak) (French *et al.*, 1956; O'h Eocha *et al.*, 1964).

Three filter pairs (B1, B2, B3) were used in an attempt to identify phycoerythrin fluorescence. Pair B1 produced a larger signal than B2 or B3 (Fig. 5), but because the emission filter 1 bandpass slightly overlaps that of the excitation filter B, some of this signal is contaminated by light scattering from non-fluorescent particles. The B1 fluorescence maximum at 90 m correlates positively with the attenuation coefficient (Fig. 4a). Filter 1 theoretically should be the most sensitive of the three filters for detecting phycoerythrin fluorescence, but its use would require a broad-band excitation filter with a sharp cut-off at 530 nm.

The presence of phycoerythrin-containing organisms in the water column is most strongly suggested by the B2 filter pair (Figs. 3, 5, and 6). The presumed phycoerythrin fluorescence was weakest near the surface, and greatest between 900 and 1000 m at the offshore stations. At all three stations phycoerythrin fluorescence increased in the near-surface chlorophyll *a* maximum. Because filter 2 has zero response toward chlorophyll *a* fluorescence and filter 5 has only a very small response toward phycoerythrin fluorescence, the B5/B2 fluorescence ratio (Fig. 8c) suggests that in the oxygen minimum phycoerythrin fluorescence is about 1.4 times the chlorophyll *a* fluorescence. Phycoerythrin fluorescence in the oxygen minimum is about three times that in surface layers, while chlorophyll *a* fluorescence in the oxygen minimum is about one-third that in the primary fluorescence maximum. Because of unknown quantum efficiencies, these values do not indicate concentration ratios.

The predicted B3 filter response toward phycoerythrin is 98% of the B2 fluorescence (Table 2), but the observed response was only 40% (Fig. 5). Filter pair B4 should have some sensitivity toward both phycoerythrin and phycocyanin (Table 2); however, the correlation coefficient between B4 and B2 fluorescence indicated about half the predicted fluorescence. Hence, results from filter pairs B3 and B4 are ambiguous: they neither agree with the predicted phycoerythrin response nor show an obvious presence of phycocyanin or allophycocyanin. We did not expect to observe phycocyanin or allophycocyanin fluorescence since their excitation occurs primarily above the bandpass of filter B.

e. Particulate or dissolved fluorescent sources? Though the profiles obtained with filters 1–4 can be best explained by phycobilin fluorescence, other fluorescent sources should be examined. Hematoporphyrin (cytochrome) fluorescence at 599 and 620 nm is possible, but only remotely so, as discussed above. On the other hand, a variety of dissolved organic materials present in the ocean fluoresce, though weakly in the 565 to 630 nm region. These include the so-called "yellow substance" (Kalle, 1966; Kirk, 1976) fluorescein (or pyoverdine), and various aromatic amines, pyridine nucleotides, and vitamins.

Studies on the fluorescence of natural surface waters (assumed to be due to yellow

humic substances) show very little emission at wavelengths above 550 nm (e.g., Traganza, 1969; Smart *et al.*, 1976; Gienapp, 1979), and we feel yellow substance is an unlikely source of detected fluorescence. Fluorescence above 550 nm is also relatively low for fluorescein, a soluble pigment produced by certain pseudomonads, (Elliot, 1958). To the authors' knowledge, soluble fluorescence in the ocean above 650 nm has not been satisfactorily shown. The so-called "red soluble fluorescence" that Herbland (1978) observed in seawater is disputable in terms of technical misinterpretations (Parker, 1981; Lewitus, 1984). The possibility exists that photosynthetic pigments (i.e. chlorophyll and the phycobilins) may be present in a dissolved state, but high dilution in seawater would likely quench any detectable fluorescence (John Waterbury, personal communication). Therefore, dissolved sources of fluorescence can, at best, explain only part of the observed intermediate depth fluorescence between 565 and 630 nm.

At the most offshore station (12), the general "noisiness" of the fluorescence signal below 300 m (Fig. 3b) indicates the particulate nature of the fluorescence. Dissolved materials in the deep sea have much less pronounced curvature due to eddy diffusion. For example, the dissolved oxygen profile (Fig. 3a) shows high curvature in the upper 300 m where current shear and mesoscale mixing processes are relatively vigorous, but below that depth oxygen curvature is much reduced.

A direct correlation between the total attenuation coefficient and fluorescence argues for a particulate source of fluorescence. At Station 12, using the A7 filters, the primary fluorescence maximum (120 m) corresponded to a secondary maximum in total attenuation (Fig. 3a, b). A minimum in the attenuation coefficient between 200 and 300 m coincided with the depth of minimum fluorescence while a slight attenuation maximum at 900 m coincided with the deep fluorescence maximum. Thus, the association between *red* fluorescence and particles is strong.

The deep fluorescence maximum from B2 at Station 12 also coincided with a maximum in total attenuation, but above 200 m the profiles showed a lack of correspondence. Therefore, the structure in the attenuation profile may be wholly or partially related to red fluorescence. In the former case, dissolved materials could contribute this yellow fluorescence.

The attenuation maximum at the offshore station (12) was at the surface (Fig. 3a), though fluorescence was low here (Fig. 3b). This resulted from photo-inhibition in the near-surface waters (Kiefer, 1973a; Setser *et al.*, 1982) and demonstrated that *in vivo* fluorescence does not quantitatively represent pigment concentrations (Kiefer, 1973b; Loftus and Seliger, 1975).

At the nearshore station (25), the maximum fluorescence and total attenuation coefficient were found near the surface, though there is some indication that in the upper-most few meters fluorescence decreased somewhat due to photo-inhibition (Fig. 6a, b). The fluorescence profile at Station 25 was made during the late afternoon when photo-inhibition effects were presumably greatest. Evidently, in these productive

coastal waters, irradiance attenuation must have been sufficiently rapid compared with the more oligotrophic waters at Stations 12 and 16, so that photo-inhibition affected only the topmost layer of the water column.

5. Conclusions

These observations show that *in situ* spectral fluorescence measurements should be extended to intermediate and deeper depths in the sea. Depths below the photic zone have not yet been plumbed by microbiologists using these relatively simple survey techniques that we feel are potentially powerful tools for the study of deep-sea ecology. Our data suggest the presence of:

(1) organisms with chlorophyll *a* and carotenoids at the primary fluorescence maximum (i.e. diatoms and/or dinoflagellates);

(2) chlorophyll *a* throughout the water column, with a three-fold increase in near-surface waters compared to intermediate depths;

(3) phycobilins (especially phycoerythrin) at intermediate depths and possibly in near-surface waters (though in reduced amounts);

(4) a far-red (>700 nm) fluorescing pigment, found primarily near the primary fluorescence maximum, which could be bacteriochlorophyll *a*, chlorophyll *a*, or phycobilins (phycocyanin or allophycocyanin);

(5) particulate fluorescence in the red region and either particulate or dissolved fluorescence at lower wavelengths (565 to 630 nm).

The observations discussed here are in their infancy. Though fluorometric techniques have been used for decades in the photic zone for continuous monitoring of chlorophyll-containing organisms and methods to spectrally extend the method to phycoerythrin-containing organisms have been developed recently, we believe ours is the first attempt to extend the spectral fluorometric measurements to intermediate depths. The instrument available for our work is not ideally suited to the task because simultaneous measurements using different excitation or emission filters could not be made. The interpretation of these profiles requires an act of faith that between-measurement water column changes were negligible and that diurnal effects on fluorescence efficiencies were not large. Future studies will require not only concomitant microbiological identifications but more sophisticated instrumentation.

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