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Fluorescence-based characterization of phycoerythrin-containing cyanobacterial communities in the Arabian Sea during the Northeast and early Southwest Monsoon (1994–1995)

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Abstract

Scanning fluorescence spectroscopy was used to investigate the spatial and temporal variability in the fluorescence signature of phycoerythrin-containing organisms in the Arabian Sea during the early Northeast and early Southwest Monsoon (1994–1995). Phycoerythrin (PE) emission spectra were relatively invariant among all the samples collected on either cruise; the relatively symmetrical PE emission peaks showed maxima at wavelengths ranging from 563–572 nm. PE excitation spectra always showed either a strong shoulder or a peak at wavelengths absorbed maximally by phycourobilin (PUB) chromophores as well as a peak at wavelengths absorbed maximally by phycoerythrobilin (PEB) chromophores. Thus, the Arabian Sea appears to be different from the Black Sea or Gulf of Maine in that PUB-lacking forms of PE rarely, if ever, dominate the PE signal. Fluorescence excitation signatures differed in the relative excitation of PE emission by wavelengths absorbed by PUB ($\sim$ 495 nm, Ex_{PUB}) and by wavelengths absorbed by PEB ($\sim$ 550 nm, Ex_{PEB}); these were distinguished by having either very low ($\sim$ 0.6), very high ($\sim$ 1.8), or intermediate Ex_{PUB}:Ex_{PEB} ratios. The distribution of samples with different PE fluorescence signatures was investigated extensively during the early Southwest Monsoon, and communities characterized by the low Ex_{PUB}:Ex_{PEB} ratios were closely associated with cooler (24–27°C), fresher (35.7–36.25 psu) water influenced by coastal upwelling. In general, "ambient" surface water of the Arabian Sea during the early Southwest

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Monsoon was of intermediate temperature (27–29°C) and salinity (36.15–36.4 psu) and showed intermediate or high values for Ex_{PUB}:Ex_{PEB}. This suggests that the PE fluorescence signature can be used to follow the fate of upwelling-influenced water masses and the populations they transport.

1. Introduction

Most planktonic marine *Synechococcus* contain phycoerythrin (PE) as their principal light-harvesting pigment (Waterbury et al., 1976). The characteristic orange/yellow fluorescence from phycoerythrin that dominates the fluorescence emission of marine *Synechococcus* is routinely used to distinguish chroococcoid cyanobacteria from small eukaryotes and prochlorophytes using flow cytometry and epifluorescence microscopy (Li and Wood, 1988; Booth, 1987; Olson et al., 1988,1990; Campbell and Vaulot, 1993). In the Arabian Sea, HPLC and flow cytometric studies show marine *Synechoccocus* to be an important component of the phytoplankton biomass (Burkill et al., 1993; Pollehne et al., 1993; Veldhuis and Kraay, 1993; Jochem, 1995; Bidigare et al., 1997; Veldhuis et al., 1997; Campbell et al., 1999). Veldhuis and Kraay (1993) identified two distinct populations of cells based on flow cytometric signature: cells with very bright fluorescence in both green (PE) and red (chlorophyll) emission when excited at 488 nm, and cells with very dim fluorescence under the same conditions. In general, they found that the "bright" or "Type A" cells were a greater proportion of the total population in samples collected from deeper depths, and the "dim" or "Type B" cells were a greater proportion of the total population in samples collected from shallower depths where irradiance was at least 5% of surface values (Veldhuis and Kraay, 1993).

"Bright" and "dim" *Synechococcus* populations have been seen in flow cytograms obtained in the Atlantic and Pacific Oceans by other workers. Olson et al. (1988) sorted "bright" and "dim" populations from the Sargasso Sea and found that they retained their "bright" and "dim" phenotype when maintained in cultures. Based on this result, they concluded that the "bright" and "dim" populations in the original sample were genetically distinct. However, as discussed by Olson et al. (1988), mixtures of "brights" and "dims" in natural samples cannot always be assumed to represent genetically distinct populations because mixtures of "bright" and "dim" cells would be expected if cells that had been growing in low light were mixed with cells growing in high light. In this case, "brights" would represent cells with greater phycoerythrin (and hence greater phycoerythrin fluorescence) per cell and "dims" would represent cells with lower amounts of PE per cell.

Genetically determined differences in the form(s) of PE synthesized by the cells are the primary cause of genetic differences in the fluorescence yield and fluorescence signature of different strains of marine *Synechococcus* (Alberte et al., 1984; Wood et al., 1985; Ong and Glazer, 1991). While the basic structure of all PEs is highly conserved, there is considerable variation in the relative abundance of phycoerythrobilin and phycourobilin chromophores that may be attached to the protein. Phycoerythrobilin

(PEB, $\lambda_{AbsMax} \cong 540–570$ nm) is found in all known forms of PE (Glazer, 1985; Sidler, 1994). In cryptomonads, red algae, *Trichodesmium*, and many marine *Synechoccocus*, the green absorbance of PEB is complemented with shorter wavelength absorbance by the presence of phycourobilin chromophores (PUB; $\lambda_{AbsMax} \cong 495–500$ nm, Glazer, 1985; Sidler, 1994).

Maximum PE emission tends to occur at longer wavelengths in strains containing only PUB-lacking PEs than in strains that synthesize PEs composed of both PUB and PEB (Alberte et al., 1984; Wood et al., 1985; Shalapenok and Shalapenok, 1997). This feature of in vivo PE emission has been used in the Atlantic Ocean and Black Sea to identify patterns in the distribution of *Synechococcus* communities dominated by organisms with either PUB-lacking or PUB-containing PEs (Shalapenok and Shalapenok, 1997; Wood et al., 1998; Hoge et al., 1999). In both ecosystems, communities characterized by the predominance of PUB-lacking PEs occurred in more turbid waters, particularly over the continental shelf in the northwest Atlantic. Phycourobilin-containing PEs predominated in highly transparent waters. None of these studies were able to discriminate among water masses dominated by organisms synthesizing different types of PUB-containing PEs because there appears to be little difference in λ_{max} of in vivo PE emission among the different forms of PUB-containing PEs. There are a wide range of PUB-containing PEs synthesized by marine *Synechococcus*, and they differ in the relative abundance of PUB and PEB chromophores (Ong and Glazer, 1991); *Synechocystis*, another chroococcoid marine cyanobacterium, synthesizes a PE with the highest known PUB content (Swanson et al., 1991). While it is difficult to infer the relative abundance of PUB and PEB chromophores from the $\lambda_{max}$of PE emission, fluorescence excitation spectra provide information about both the chromophore content and relative abundance of PUB and PEB.

Campbell and Iturriaga (1988) used single-cell fluorescence excitation spectroscopy to demonstrate that most marine *Synechococcus* in the central north Atlantic produce a PE very high in PUB. Two flow cytometric studies that used dual lasers to excite cells at 488 and 514 nm obtained similar results from both the North Atlantic and Pacific Oceans (Olson et al., 1988,1990). These three studies serve as the principal basis for the widespread assumption that the "high PUB" type of marine *Synechococcus* dominate most open-water habitats. Recent work by Campbell et al. (1998), and the "brights" and "dims" described by Veldhuis and Kraay (1993), however, suggest that at least two PE types occur in offshore waters of the Arabian Sea.

In fall 1994 we developed a protocol for sampling the fluorescence excitation and emission signature of photosynthetic pigments of PE-containing phytoplankton in the Arabian Sea. Samples were found to be stable with respect to the wavelength and relative height of peaks and shoulders in the PE fluorescence excitation and emission spectra for extended periods (e.g., weeks) when stored at low temperature ($\sim 4^{\circ}C$) in the dark. In this paper, we describe the development of the method and present results that show that a low PUB-form of PE, spectrally similar to the major PE of *Synechococcus* strain WH7803, tended to dominate upwelling-influenced surface waters during the early Southwest Monsoon in 1995. To our knowledge, this is the first time PE-excitation spectra have been used to evaluate the distribution of spectrally distinct forms of PE in the sea.

2. Methods

2.1. Study area and cruise descriptions

Data reported here are from cruises TN042 (Nov. 28–Dec. 17, 1994) and TN048 (June 21–July 13,1997) aboard the R.V. *Thomas G. Thompson*. These cruises occurred during the early Northeast Monsoon (TN042) and early Southwest Monsoon (TN048). The cruise tracks for both cruises were similar (Fig. 1), and included most of the western and southern portions of the standard U.S. JGOFS sampling area (e.g., the regions covered by U.S. JGOFS Process Study Stations N1 and S1-S11, compare Fig. 1 with Fig. 1 in Smith et al. (1998). Each cruise consisted of two approximately 10-day sampling periods. During the first 10-day period, the ship traveled at a constant speed, towing a SeaSoar instrument package used to measure hydrographic and optical properties of the upper water column. During the second 10-day period, the ship stopped for a variety of operations at representative stations along the SeaSoar

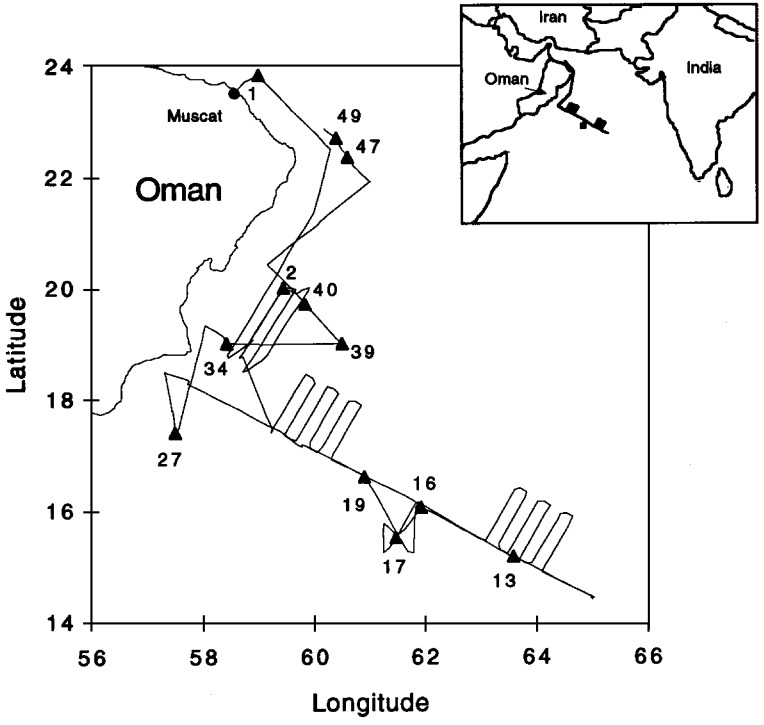

Fig. 1. Cruise track for TN048 (early Southwest Monsoon). Surface samples were collected from the ship's flow-through sampling system every 2 h while the ship towed a SeaSoar instrument package as described in the text; these samples were collected between 21 June and 4 July, 1995 beginning at 23.13°N, 59.68°E and ending at 14.46°N, 64.98°E. Station locations and sampling dates are given in Table 2. Inset shows SeaSoar track during the Northeast Monsoon (TN042).

track. TN048 occurred during the beginning of the Southwest Monsoon (Weller et al., 1998), and the cruise track included regions heavily influenced by coastal upwelling (Fig. 2).

2.2. Sample collection

When the ship was towing the SeaSoar, samples were collected from the ship's continuous seawater system which had an intake at the bow of the ship, $\sim$ 3 m below

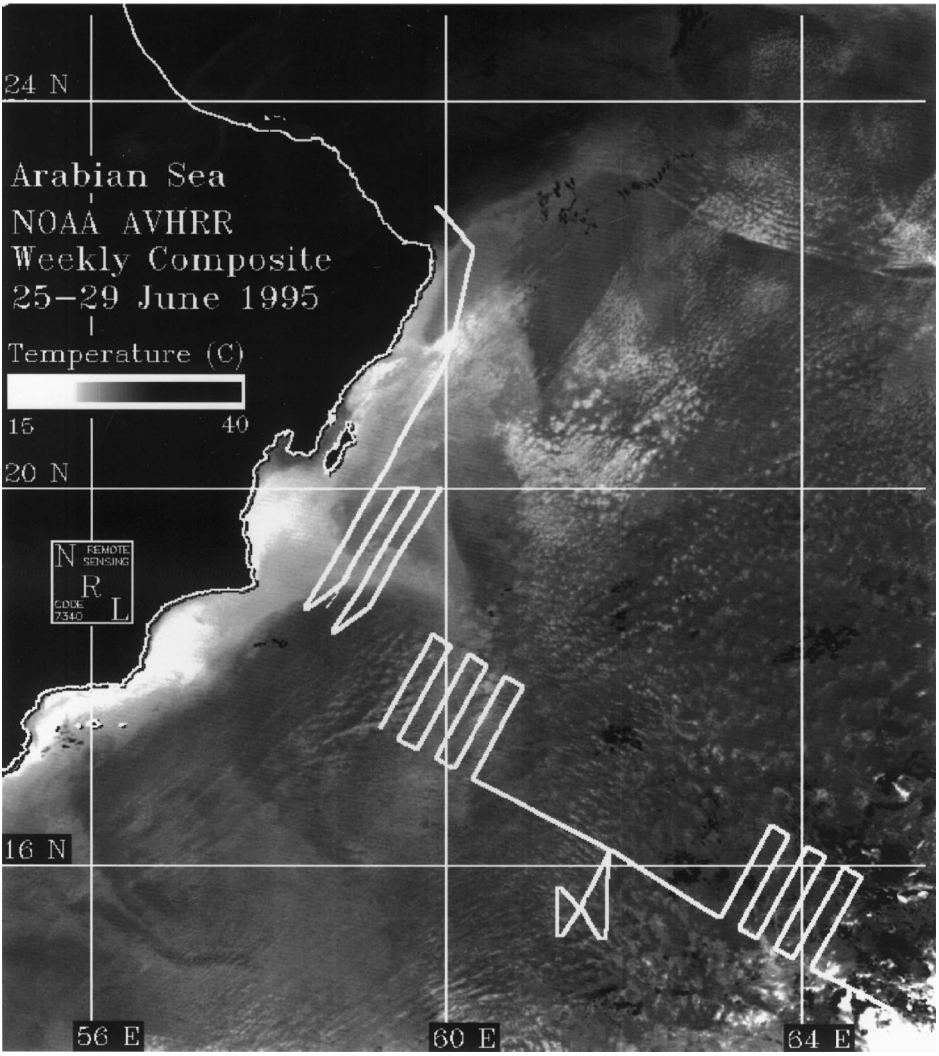

Fig. 2. Sea surface temperature composite image for early June, 1995. The SeaSoar sampling track for TN048 is shown as a solid white line; lighter gray shades represent the cooler water.

the sea surface. During the Southwest Monsoon, 500 ml samples were drawn from the ship's seawater system every two hours. When the ship occupied stations, samples were collected from 10 l General Oceanics Niskin bottles on the CTD rosette (Seabird Electronics, Seattle, Washington). Samples were processed for estimation of *Synechoccocus* abundance and characterization by fluorescence spectroscopy. Triplicate slides were prepared for estimation of *Synechoccocus* abundance using procedures described in Li and Wood (1988); one set was counted at sea and the other two were transported on dry ice to the University of Oregon where they were stored at $-80^{\circ}C$ until they were counted in the lab. Samples were concentrated for fluorescence analysis; seawater (usually 50 ml) was filtered onto a 0.22 μm Nuclepore filter and resuspended into 4 ml of unfiltered sample water in a 4 ml cryovial. Samples for fluorescence were stored on ice as they were prepared, and then kept at $4^{\circ}C$ in the dark if they were not run immediately. Samples were placed on ice to inhibit grazing activities in the concentrate, and examination of "before-and after" spectra showed no detectable effects of short-term storage on ice. The effects of longer-term storage at $4^{\circ}C$ in the dark are discussed below. Samples were transported from Oman to Eugene, Oregon, in containers packed with frozen blocks of "Blue Ice", and refrigerated upon arrival. When removed for analysis at sea or in the laboratory, the samples were kept on ice except during the brief period required to bring a sample to room temperature before it was placed in the sampling cuvette.

2.3. Shipboard fluorescence analysis

Excitation scans (Chl_{Ex}) were run to identify the wavelengths stimulating chlorophyll a emission at 680 nm (Photosystem II). Phycoerythrin emission and emission spectra (PE_{Em}, and PE_{Ex}, respectively) were obtained on a SPEX Fluorolog II equipped with the manufacturer's quantum counting accessory. Water blanks were run immediately before samples were processed on any given day, and subtracted from excitation spectra. Spectra were collected in ratio mode (signal/reference), which corrects for fluctuations in the intensity of the light source. Spectra were corrected further for spectral bias in the instrument configuration, according to the manufacturer's instructions. Correction factors for excitation spectra were obtained several times during the cruise using a saturated rhodamine solution in both sample and reference cuvettes. Instrument settings for each type of spectrum are given in Table 1.

2.4. Laboratory fluorescence analysis

Samples were analyzed in Oregon using an Aminco/Bowman Series II scanning spectrofluorometer. All samples for the Southwest Monsoon cruise were run within two weeks of the end of the cruise although, as described below, samples appear to be stable for longer periods. Water blanks were obtained for excitation and emission spectra at the beginning and end of each day's analysis using freshly filtered Arabian Sea water, and the average of the two spectra used as a sample blank. In general, both spectra were nearly identical, but we found that cuvettes had to be acid washed (5% HCl) after each day's activities to avoid build-up of weakly fluorescent material on the

Table 1
Instrument settings for acquisition of spectra. Units in nanometers

Instrument and spectum type	Scan range	Excitation monochrometer	Bandwidth (Ex)	Emission monochrometer	Bandwidth (Em)	Scan speed
SPEX (Ship)						
Chl_{EX}	400–650	Variable	5	680	5	4 nm s^{-1}
PE_{Ex}	400–560	Variable	5	564	5	4 nm s^{-1}
PE_{Em}	510–800	500	5	Variable	5	4 nm s^{-1}
AMINCO (Lab)						
Chl_{EX}	400–660	Variable	8	680	8	2 nm s^{-1}
PE_{Ex}	400–560	Variable	4	568	4	2 nm s^{-1}
PE_{Em}	510–800	500	4	Variable	4	2 nm s^{-1}

cuvette walls. Spectra were collected in ratio mode (signal/reference) and corrected for spectral bias in the instrument configuration using factory-installed correction algorithms. The correction algorithm for excitation spectra was obtained using rhodamine as the quantum counter. Instrument settings for each type of spectrum are given in Table 1.

2.5. Characterization of PE types using PE excitation spectra

As noted above, fluorescence emission spectroscopy distinguishes between PEs composed only of PEB chromophores and PEs composed of both PUB and PEB chromophores, but cannot distinguish among PUB-containing PEs which differ in the relative abundance of the two chromophores. Thus, we relied on fluorescence excitation spectroscopy to distinguish among PE types because it is sensitive to both the presence or absence of PUB, and to the relative abundance of PUB and PEB chromophores. PEB and PUB chromophores are revealed as separate peaks and/or shoulders in the in vivo excitation spectra for both PE and chlorophyll *a* emission, and the relative height of these peaks and shoulders is indicative of the relative concentration of the two chromophores (cf Wood et al., 1985; Olson et al., 1988; Ong and Glazer, 1991). These peaks or shoulders occur near the wavelengths of maximum absorption of each chromophore ($\sim$ 495 nm for PUB and $\sim$ 550 nm for PEB), with the precise wavelength determined by protein–chromophore interactions that vary among PEs. Essentially all the PE_{Ex} spectra we collected showed either a shoulder or a peak at about 500 nm, presumably due to the presence of PUB, and a peak at about 550 nm, presumably due to the presence of PEB (e.g., Fig. 3C, F, I). In order to compare differences among excitation spectra quantitatively, we calculated the value "Ex_{PUB}:Ex_{PEB}", which is the ratio of peak (or shoulder) heights at $\approx$ 500 nm and $\approx$ 550 nm; this ratio is always obtained from the PE excitation spectra (PE_{Ex}). For the fresh samples (solid lines) in Fig. 3C, F, and I, the values for Ex_{PUB}:Ex_{PEB} are 0.76, 1.0, and 1.5, respectively. The notation "Ex_{PUB}:Ex_{PEB}" rather than "PUB:PEB" was used to emphasize the fact that we are inferring the relative abundance of PUB and PEB

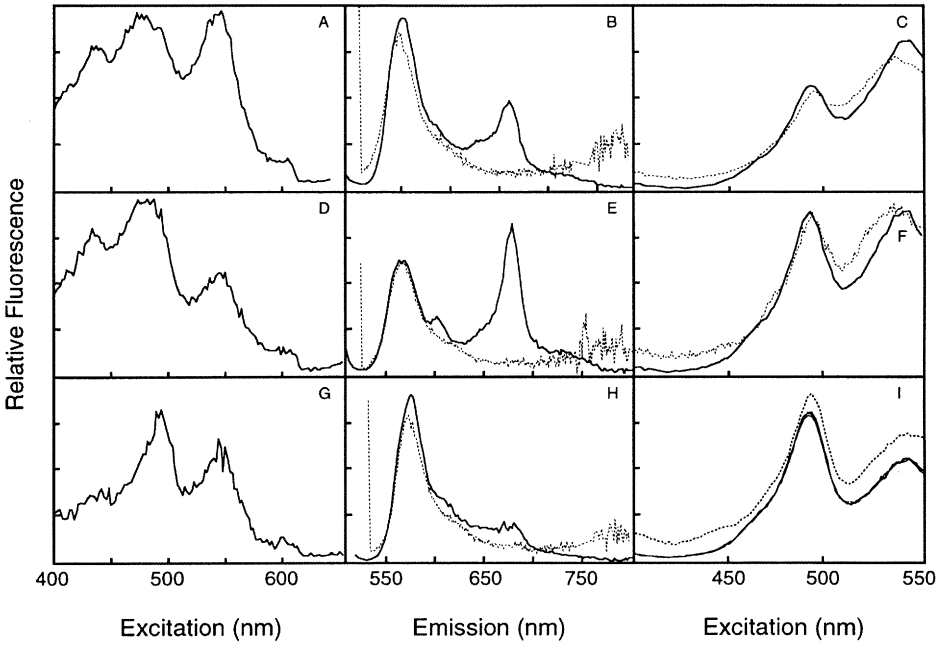

Fig. 3. Representative spectra obtained on board the ship during TN042; Chl_{Ex} spectra are in the left-hand column , PE_{Em} spectra are in the center column, and PE_{Ex} are on the right. Each series of three panels across (A–C; D–F, and G–I) were obtained from the same sample. In panels B,C,E,F,H, and I, solid lines show data collected on board the ship, and dotted lines show data collected from the same samples more than two months later in the laboratory. Results from triplicate runs of the same PE excitation spectra, run within minutes of one another, are plotted in panel I to show the high degree of repeatability in the PE_{Ex} spectra; it is difficult to see individual replicates, but the overall shipboard spectrum is slightly darker in Panel I than other panels because all three replicates are plotted. Maximum PE emission in panels B, E, and H was at 567, 567, and 570 nm, respectively.

chromophores from excitation spectra; we are not measuring chromophore abundance directly.

In general, we relied on visual inspection to determine whether or not peaks could be distinguished from instrument noise in the fluorescence spectra. However, during the SW Monsoon, when there were very few PE-containing cells in some samples, peak heights in the PE_{Ex} spectra were sometimes hard to distinguish from the instrument noise because there was so little overall signal. In these cases, we discarded data from the samples if the signal-to-noise ratio in the PE_{Ex} spectrum was < 10.0. For these purposes, the "signal" in the PE excitation spectrum was the maximum value measured between 500 and 550 nm, and the "noise" in the PE excitation spectrum was the average value measured between 420 and 440 nm. The region 420–440 nm was selected for quantification of instrument noise since these wavelengths do not excite PE emission in vivo; examination of water blanks indicated that instrument noise in the range 420–440 nm was comparable to that between 500 and 550 nm.

2.6. Sample storage and intercomparison of instruments

Three of the first samples collected on TN042 were run repeatedly throughout the cruise as a measure of sample stability. In two of the samples, the intensity of PE fluorescence decreased after 48 h, and then remained relatively constant for more than a week; in the third sample, fluorescence yield was essentially constant throughout the study (Fig. 4A). Despite repeated sample handling, the Ex_{PUB}:Ex_{PEB} ratio of three samples with differing fluorescence properties was essentially the same at the end of an 11-day measurement period as it was at the beginning, although one sample did show more day-to-day variation than the other two (Fig. 4B).

During the Southwest Monsoon, time constraints did not permit analysis of all the samples on board the ship, so a selected set of twelve samples were run on the ship and then again in the laboratory to provide an assessment of the effects of sample storage and/or systematic differences associated with spectral data obtained using the two different instruments. Both fluorescence yield and the Ex_{PUB}:Ex_{PEB} ratio measured on fresh samples at sea were highly correlated with the same measurements made on the same samples in the lab (Fig. 5). Over a period of a four to eight weeks, no major changes were observed in the wavelength of maximum PE emission or in the Ex_{PUB}:Ex_{PEB} ratios. In some samples, these parameters appeared to remain stable for many months (Fig. 3). The great difference between storage temperature ($\sim 4^{\circ}C$) and ambient water temperature ($> 24^{\circ}C$ in surface waters) may have facilitated successful sample storage; the effectiveness of this approach needs to be carefully re-established if applied to samples collected from colder environments.

Chlorophyll *a* emission in the PE_{Em} spectra deteriorated more rapidly with time than emission from PE (Fig. 3B, E, H). Since the wavelength used to excite fluorescence emission in these spectra (500 nm) also excites in vivo chlorophyll *a* fluorescence in many taxa of phytoplankton, this differential degradation of emission by chlorophyll *a* may have resulted from faster degradation of the spectral properties of diatoms, dinoflagellates, or other non-cyanobacterial taxa. This interpretation seems reasonable since the chlorophyll *a* emission peak and its degradation are much more

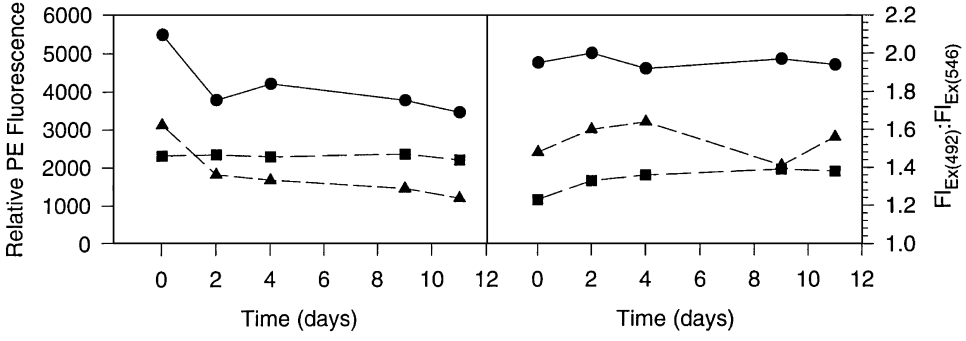

Fig. 4. Time course of change in the spectral properties for three samples run repeatedly over a period of 12 days during the Northeast Monsoon.

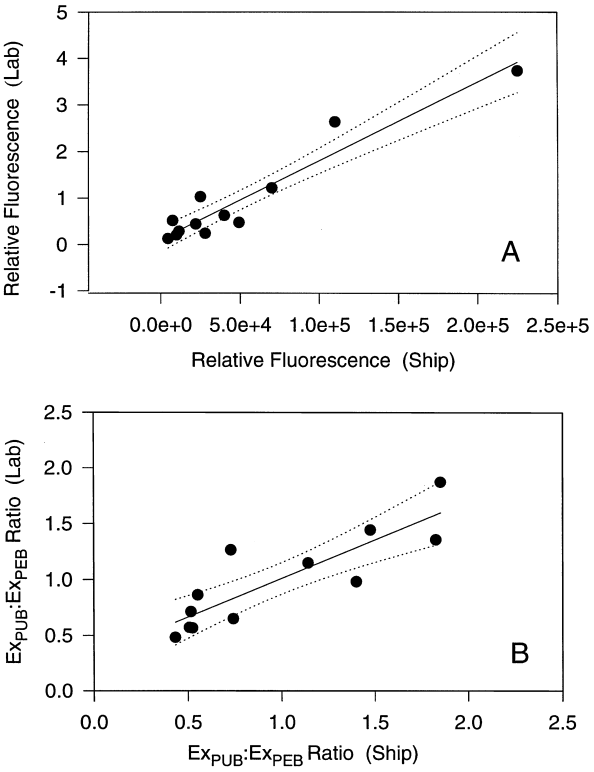

Fig. 5. Relationship between spectral properties of 12 intercalibration samples measured at sea and in the lab. Solid line shows linear regression, and the dotted lines delimit 95% confidence intervals for the regressions; R^2 for the regressions are 0.98 (A) and 0.71 (B).

pronounced in Fig. 3B and 3E than in Fig. 3H, and the Chl_{Ex} spectra from these same samples indicates that Fig. 3B and 3E were obtained from samples with relatively high concentrations of eukaryotes or prochlorophytes whereas Fig. 3H was obtained from a sample composed primarily of PE-containing cyanobacteria (see below).

3. Results

3.1. Excitation spectra for chlorophyll a emission

Chl_{Ex} spectra consistently showed excitation peaks or strong shoulders between 500 and 550 nm (Fig. 3A, D, G), and usually showed strong peaks in the blue region of the spectrum (400–450 nm). The Chl_{Ex} spectrum shown in Fig. 3G is a contrast to this pattern, in that there is relatively little participation of blue light in the excitation of chlorophyll *a* emission at 680 nm; although there are strong peaks at 495 and 500 nm.

Blue wavelengths (i.e., 400–450 nm) do not excite Photosystem II in cyanobacteria or red algae (Haxo and Blinks, 1950; Wood, 1985; Neori et al., 1986), which indicates there were more eukaryotic phytoplankters or prochlorophytes in the first two samples, and a predominance of PE-containing picocyanobacteria in the third.

Chl_{Ex} spectra will not be discussed in further detail since they cannot be used to distinguish the type of phycoerythrin in the samples. This is because, in bulk water samples, the accessory pigments of diatoms and other phytoplankton taxa also contribute to the excitation of chlorophyll *a* emission in the green and blue-green region of the spectrum (cf Wood, 1985; Neori et al., 1986; Lazzara et al., 1996). Thus, while Chl_{Ex} spectra were run for each sample, their significance in this study lies in the fact that they never suggested the presence of cyanobacteria, which use phycocyanin as the principal light-harvesting pigment or the presence of highly unusual phycobiliproteins that might not be excited at conventional wavelengths.

3.2. Spectral forms of PE identified during the early Northeast Monsoon

While peak heights varied among samples, there was very little variation in λ_{max} of PE emission. PE_{Em} spectra usually showed a symmetrical peak between 560 and 570 nm ($\bar{x} = 567 \pm 3$ nm, $n = 28$), and often showed an additional relatively symmetrical peak at 680 nm, the wavelength of maxium emission by chlorophyll *a* (e.g., Fig. 3B, E, H). The longer wavelength peak is particularly noteworthy in Fig. 3B and 3E where the chlorophyll *a* emission is probably due to fluorescence by both PE-containing picocyanobacteria and other photosynthetic plankton (see discussion above). In the sample that contained relatively few of these other phytoplankton taxa, the PE emission at 570 nm is much greater than the peak at 680 nm (Fig. 3H). We can assume that emission at 570 nm is due to PE since it is the only photosynthetic pigment that fluoresces in this region of the spectrum.

As illustrated in Fig. 3, the overall shape of PE_{Ex} spectra was more variable than that of PE_{Em} spectra. PE_{Ex} spectra obtained during the early Northeast Monsoon could be divided into three major types based the Ex_{PUB}:Ex_{PEB} ratio: those where the excitation at $\sim$ 500 nm (Ex_{PUB}) is much less than the Ex_{PEB} at $\sim$ 550 nm (Fig. 3C), those where Ex_{PUB} is greater than Ex_{PEB} (Fig. 3I), and those where the Ex_{PUB}:Ex_{PEB} ratio is about equal (Fig. 3F). When replicate spectra from the same sample were collected within minutes of one another, they were nearly identical from replicate to replicate (Fig. 3I).

Trichodesmium, a filamentous cyanobacterium, is often abundant in tropical waters and is a potential source of PE fluorescence in bulk water samples. We did not observe *Trichodesmium* blooms on our cruises, and it was only rarely found in the small-volume samples we filtered for epifluorescence microscopy. Samples for routine fluorescence measurements were always examined for the presence of *Trichodesmium* filaments and, on the rare occasion when they were present, they were removed. Filaments of *Trichodesmium* were, however, occasionally abundant in plankton net tows. Since there are only a few published fluorescence spectra for the PEs of *Trichodesmium*, we picked several filaments by hand from a net tow and suspended them in filtered seawater to produced the spectra shown in Fig. 6. Peak emission from

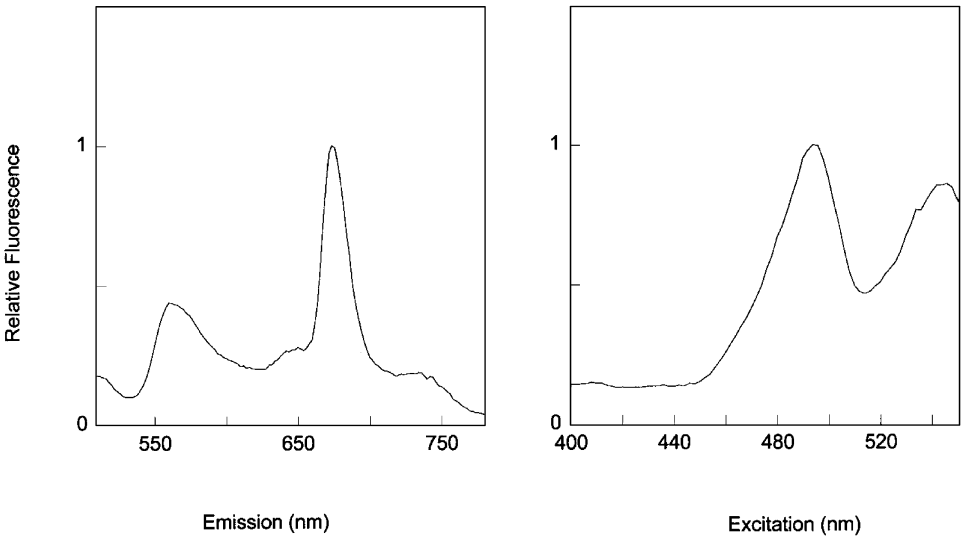

Fig. 6. PE_{Em} (left) and PE_{Ex} (right) spectra for filaments of *Trichodesmium* obtained from surface waters in December, 1994 (TN042).

PE in this sample occurred at 567 nm, and there is a strong emission signal from chlorophyll *a*. This peak is not always observed in PE emission spectra from *Trichodesmium* (A. Subramian, National Oceanographic and Atmospheric Administration, personal communication). The Ex_{PUB}:Ex_{PEB} ratio ranged from 1.11 to 1.18 in triplicate runs of the sample, indicating that the unidentified species of *Trichodesmium* we collected has relatively high amounts of PUB. Variability among replicate scans of the *Trichodesmium* sample was slightly higher than variability among triplicate runs of picocyanobacteria samples, which may reflect an optical effect of the packaging of the pigment in a few larger cells, or it may mean that the different filaments in the sample contained slightly different forms of PE. Sinking or stirring could have changed the relative intensity of excitation of different filaments as measurements were made, increasing the variability between replicates.

3.3. Spectral variation in PE from surface waters

3.3.1. Early Southwest Monsoon

As noted below, most samples collected from Niskin bottles were subsurface samples (c.f. Table 2). In this section, we report data collected from ~ 3 m using the ship's flow-through system while the ship towed the SeaSoar (Fig. 2). Fluorescence samples were collected at a total of 145 positions. This covered an area over which *Synechococcus* cell numbers ranged from 10^3 to nearly 10^6 cells ml^{-1} in surface waters. Cell numbers were especially low at sampling locations east of 61.5°E. Because of the very low cell abundances at some locations, PE concentration was also very low

Table 2
Spectral data from samples collected at hydrographic stations

Station	GMT date	Lat/long	Sample depth	PE emission	PE excitation		
				λ_{Max}	$\lambda_{max(PUB)}$	$\lambda_{max(PEB)}$	Ex_{PEB}:Ex_{PUB} ratio
1	6/25/95	23.84°N/58.99°E	16	564	498	547	0.66
			26	568	500	545	0.75
			46	563	498	546	0.84
			66	566	496	548	1.08
2	6/25/95	20.0°N/59.44°E	18	571	503	545	0.78
13	7/5/95	15.17°N/63.58°E	86	571	494	545	1.69
16	7/5/95	16.06°N/61.92°E	46	586	494	549	1.49
			67	568	493	550	1.52
17	7/6/95	15.51°N/61.47°E	65	567	492	545	1.10
19	7/6/95	16.60°N/60.91°E	24	564	494	549	1.23
27	7/8/95	17.40°N/57.50°E	16	572	492	545	0.70
			26	570	497	543	0.62
			46	570	496	543	0.66
34	7/9/95	19.0°N/58.42°E	11	569	497	545	0.58
			16	572	499	548	0.61
39	7/10/95	19.00°N/60.50°E	25	575	497	544	0.81
40	7/10/95	19.71°N/59.83°E	9	575	497	545	0.67
			15	571	493	545	0.68
47	7/12/95	22.35°N/60.60°E	5	569	496	547	0.60
49	7/12/95	22.70°N/60.40°E	16 m	568 nm	495 nm	544 nm	0.55

and the PE_{Ex} spectra were, therefore, very difficult to interpret. Approximately half of the samples were discarded on the basis of a combination of low overall signal and low signal-to-noise ratio (see methods). A total of 65 acceptable spectra were obtained from the surface samples collected while we towed the SeaSoar, and they provide data from surface water along the coast of Oman and along the western half of the onshore-to-offshore transect covered by SeaSoar.

Phycoerythrin spectral properties showed variations comparable to those observed on TN042 during the Northeast Monsoon. All PE_{Em} spectra were relatively similar, with a single peak at about 567 nm ($\pm$ 2.72 nm s.d., $n = 65$; Fig. 7). The Ex_{PUB}:Ex_{PEB} ratio observed in PE_{Ex} spectra ranged from 0.41 to 1.60, and there was always a shoulder or peak at $\sim$ 495 nm as well as a peak at $\sim$ 550 nm (Fig. 7). As described earlier, the shorter wavelength shoulder or peak was defined as the Ex_{PUB} peak, and the longer wavelength peak defined as the Ex_{PEB} peak. On average, the Ex_{PUB} peak occurred at 497 nm ($\pm$ 4.14 s.d., $n = 65$) and the Ex_{PEB} peak occurred at 546 nm ($\pm$ 4.06 nm, s.d., $n = 65$). As on TN042, spectra were observed with low, high, and intermediate Ex_{PUB}:Ex_{PEB} ratios, with a distinctly trimodal distribution frequency for phycoerythrin spectral types based on this ratio (Fig. 8). Samples in which the PE_{Ex} spectra were characterized by relatively low PUB excitation (Ex_{PUB}:$Ex_{PEB} \cong 0.7$) were

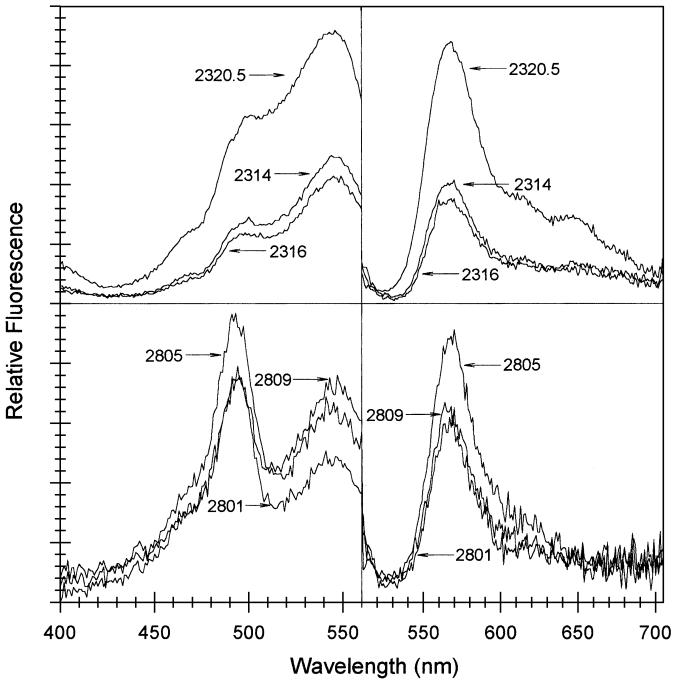

Fig. 7. Representative PE_{Ex} spectra (left) and PE_{Em} spectra (right) from TN048 (early Southwest Monsoon). Samples were obtained from the following locations: 2314–19.22°N, 58.94°E; 2316–19.45°N, 59.08°E; 2320.5–20.0°N, 59.45°E; 2801–17.42°N, 59.76°E; 2805–17.83°N, 60.0°E; 2809–18.24°N, 60.24°E.

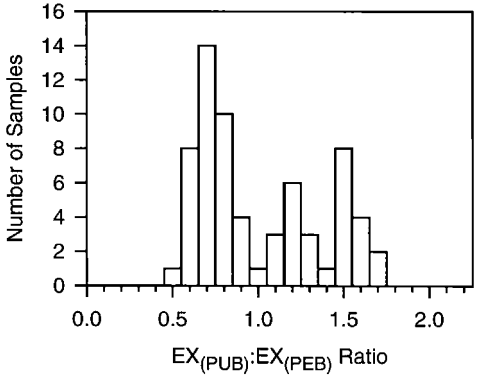

Fig. 8. Frequency distribution for Ex_{PUB}:Ex_{PEB} ratios obtained in samples collected from surface waters during the early Southwest Monsoon.

most common, but about 20% of the samples had Ex_{PUB}:Ex_{PEB} values ≥ 1.5, and another 20% showed intermediate Ex_{PUB}:Ex_{PEB} ratios (Fig. 8). Examples of the PE_{Ex} spectra obtained from samples with different Ex_{PUB}:Ex_{PEB} ratios are shown in Fig. 7. Samples with low Ex_{PUB}:Ex_{PEB} ratios generally were found along the coast (Fig. 9) and

in relatively cooler, less saline waters (Fig. 10). One notable exception to these trends was observed at 16°N and 63.5°W (Fig. 9). This exception (marked 'B' on Fig. 10) was collected in water that was exceptionally warm and salty by comparison to most of the offshore water masses we sampled. It should be noted that the concentration of

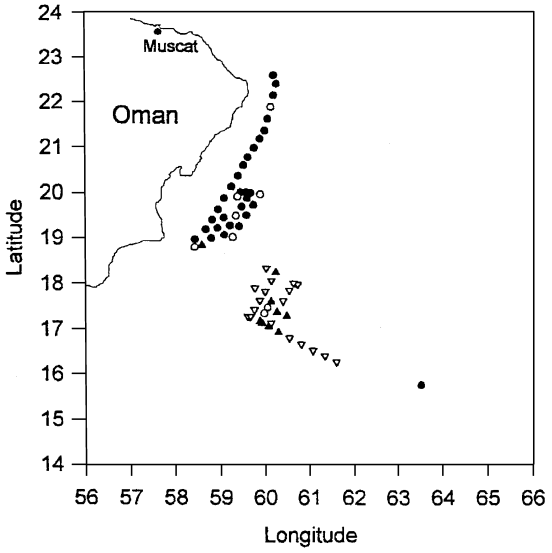

Fig. 9. Geographic distribution of samples with differing Ex_{PUB}:Ex_{PEB} ratios during the early Southwest Monsoon. Ex_{PUB}:Ex_{PEB} ratios < 0.75 (closed circles), $\geqslant 0.75 \cap < 1.0$ (open circles); $\geqslant 1.0 \cap < 1.25$ (closed triangles); $\geqslant 1.25$ (open triangles). Samples were collected between 21 June and 29 June, 1995, while towing the SeaSoar instrument array (cf. Fig. 2).

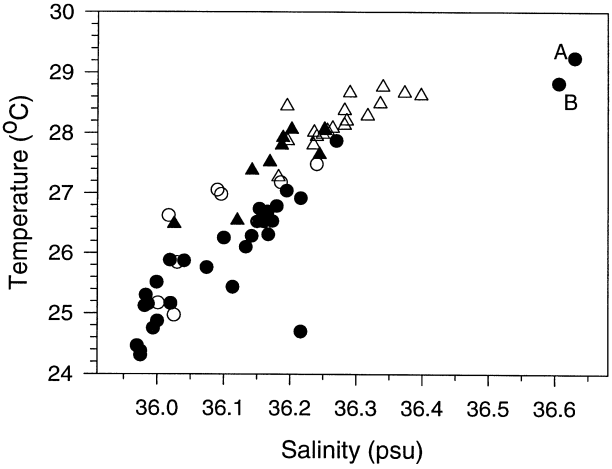

Fig. 10. Distribution of samples with differing Ex_{PUB}:Ex_{PEB} ratios plotted against T/S properties. Symbols as in Fig. 10; points labeled 'A' and 'B' are discussed in the text.

chroococcoid cyanobacteria was very low ($\sim 10^3$ ml^{-1}) at this location; while the spectra were easily interpreted, the cells may not have been very robust. The only other samples obtained in water with salinity and temperature similar to that of sample 'B' were surface samples collected at Station 1 (see below) and a sample collected near the entrance to the Gulf of Oman while we towed the SeaSoar (Sample marked 'A' on Fig. 10). All three samples from warm, unusually salty water had a low Ex_{PUB}:Ex_{PEB} ratio (~ 0.75).

3.4. Subsurface and vertical variation in PE spectral properties

3.4.1. Early Southwest Monsoon

Sampling at the stations was limited in scope, and often targeted the deep chlorophyll maximum. Thus, the concentration of PE-containing organisms in many of these samples was too low to provide PE_{Ex} and PE_{Em} spectra with acceptable signal-to-noise properties; the stations and sampling depths from which acceptable spectra were obtained are shown in Table 2, and the station locations are plotted in Fig. 1. As with the underway samples, little variation in λ_{max} of PE emission was observed among the station samples (Table 2). In general, samples collected from different depths at the same station also had similar Ex_{PUB}:Ex_{PEB} ratios, and these ratios were comparable to those observed in surface samples collected when the ship was in the same geographical position during SeaSoar operations. Station 1 (Table 2, Fig. 11) is a notable exception. While the low Ex_{PUB}:Ex_{PEB} ratio in surface samples was similar to that measured by underway sampling in the same region, samples from deeper depths showed a progressive increase in the Ex_{PUB}:Ex_{PEB} from a low of 0.66 at 16 m to a high of 1.08 at 66 m. While a depth-dependent increase in PE fluorescence per cell is commonly noted below the mixed layer, differences in the type of PE found

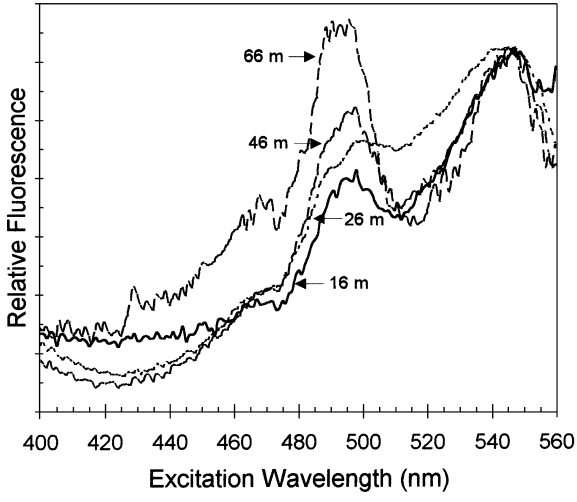

Fig. 11. PE_{Ex} spectra for samples collected at four depths at Station 1 (23.83°N, 58.98°E) on 21 June, 1995.

at different depths have rarely been observed (Olson et al., 1988; Vernet et al., 1990; Wyman, 1992; Wood et al., 1998). This station, near the entrance to the Gulf of Oman, is a region of complex hydrography, and the evidence for different PE types at different depths may result from an interleaving of water masses of different origin. The mixed layer depth at Station 1 was approximately 15 m, and the surface water was very warm and salty, whereas the water below the mixed layer was much cooler and fresher (Fig. 12A).

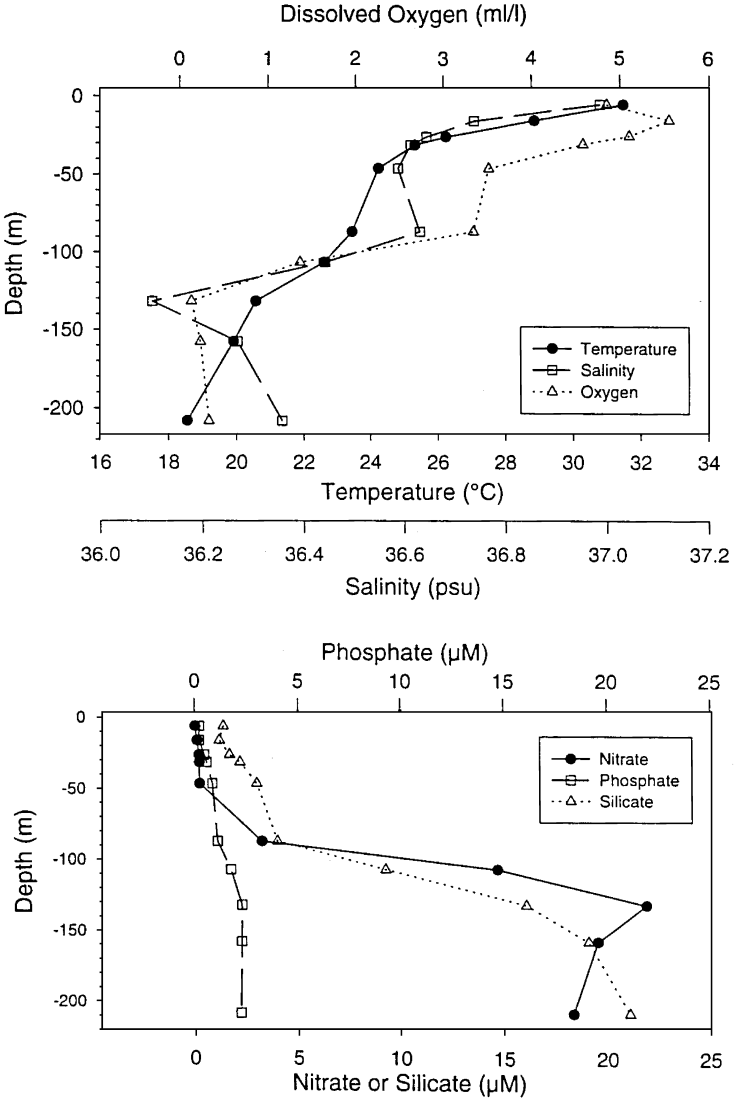

Fig. 12. Vertical profiles for hydrographic variables (A) and nutrients (B) at Station 1.

4. Discussion

PE excitation spectra in our samples always showed either a strong shoulder or a peak at wavelengths absorbed maximally by PUB as well as a peak at wavelengths absorbed maximally by PEB; this, combined with the relatively narrow range in λ_{max} for PE emission (567 ± 2.72 nm s.d., $n = 65$ on TN048), indicate that PUB-containing PEs were responsible for the PE fluorescence signature. The absence of any water masses dominated by organisms that make a PUB-lacking PE is intriguing since this pigment type is common on the continental shelf off North America (Wood et al., 1998), and strains of picocyanobacteria that synthesize a PUB-lacking PE have been isolated from the Arabian Sea, (A.M. Wood, unpublished data). Campbell et al. (1998) report high abundances of cells which appear to contain a PUB-lacking PE at only one station from their four cruises (N1, TN054).

Aircraft-operated lidar (AOL) can be used to evaluate the amount of PE in surface waters of the ocean and to distinguish between water masses dominated by PUB-lacking PEs and water masses dominated by PUB-containing PEs (Hoge et al., 1999). AOL data were collected repeatedly along a flight line which corresponds to the southern JGOFS line during TN048. The lidar data confirm the low abundance of PE-containing organisms offshore when we sampled that region, and also indicate that water masses dominated by PUB-lacking PEs may occur on the continental shelf of Oman, in regions that we did not sample (F. Hoge and R. Swift, Wallops Flight Center, personal communication). Wood et al. (1998) suggest that organisms synthesizing PUB-lacking PEs as the predominant light-harvesting pigment occur primarily in Case 2 waters; this could easily restrict such organisms to continental shelf waters in the Arabian Sea. Our sampling locations, while relatively close to the continental margin, were mostly seaward of the shelf-break where Case 1 optical conditions generally prevailed (R. Arnone and R. Gould, Naval Research Laboratory, personal communication).

The demonstration that PUB-lacking PEs were associated with Case 2 waters in the Northwest Atlantic Ocean was based on a method that did not distinguish among different PUB-containing PEs (Wood et al., 1998). Thus, while that study showed that PUB-containing PEs occurred almost exclusively in Case 1 waters, it was not possible to determine whether or not there was any pattern in the distribution of different PUB-containing PEs. In the current study, PE excitation spectra provide a basis for distinguishing among different PUB-containing PEs, and differences in the Ex_{PUB}:Ex_{PEB} ratio show clear association of low-PUB PEs and high PUB-PEs with different water masses.

Many samples had fluorescence excitation spectra for PE emission that were very similar to the excitation spectra of the predominant PE synthesized by the commonly studied laboratory strain WH7803, (= DC2; compare top panels of Fig. 7 with Fig. 4 in Wood et al., 1985 and Fig. 11 in Ong and Glazer, 1991). While the distribution of chroococcoid cyanobacteria that synthesize high-PUB PEs is relatively well described, little is known about the biogeography of organisms that primarily synthesize this low PUB-form of PE.

The results of our survey of the distribution of different spectral forms of PE at the beginning of the Southwest Monsoon are presented in Figs. 9 and 10; data reported in

these figures are from samples collected over a nine-day period and are nearly synoptic. PE_{Ex} spectra from sampling locations marked with a solid circle (Ex_{PUB}:Ex_{PEB} < 0.75) were similar to those shown in the top panels of Fig. 7. As noted above, they are very similar to that of strain WH7803 and other isolates with a similar PE (cf Alberte et al., 1983; Wood et al., 1985). Samples collected at locations noted with a triangle in Figs. 9 and 10 yielded PE_{Ex} spectra that were similar to those shown in the bottom panels of Fig. 7, indicative of a predominance of PE-containing organisms that synthesize PEs more similar to those of the "high urobilin" *Synechococcus* strain WH8103 than the PEs of strain WH7803 (Ong et al., 1984).

Samples characterized by low Ex_{PUB}:Ex_{PEB} ratios occurred almost exclusively along the shelf-break where AVHRR imagery reveals significant upwelling of cool water (compare Figs. 2 and 9). This explains the association of low Ex_{PUB}:Ex_{PEB} ratios with low-temperature, low-salinity water shown in Fig. 10. The cluster of samples with low Ex_{PUB}:Ex_{PEB} ratios collected slightly offshore between 18 and 20° N coincide with the presence of a filament of cool, upwelled water in the upper levels of the water column. This filament is believed to represent the offshore deflection of currents that flow north along the shelf break (Brink et al., 1998). Nitrate levels are high (~ 10 μM) in the filament and much lower (< 1 μM) in the warmer (~ 28°C), saltier (36.15–36.4 psu) surface water outside the filament (Brink et al., 1998). As the filament moved offshore, it subducted below this low-nutrient "ambient" water and, as indicated by the cluster of samples with high Ex_{PUB}:Ex_{PEB} ratios seen near 17.5°N and 60°E, the community of PE-containing organisms in the "ambient" surface water was dominated by organisms with high PUB PEs (Figs. 2 and 10; Brink et al., 1998).

Nearly all the samples we collected at the beginning of the Southwest Monsoon had T/S properties that linked them to either water influenced by coastal upwelling, or to warmer water with a salinity < 36.4 psu (Fig. 10); as indicated above, each of these water-mass types was dominated by PE-containing cells with different PE excitation spectra. On this basis we propose that there are at least two physiological types of PE-containing chroococcoid cyanobacteria in the region we sampled: those that predominate in "ambient" oligotrophic surface waters and contain PUB-rich PEs, and those that predominate in upwelling-influenced waters and synthesize PEs that contains PUB, but in proportions similar to those of *Synechococcus* strain WH7803.

Two underway samples were collected from a third water-mass type, which is about the same temperature as the "ambient" water described above but is much saltier. These samples, noted 'A' and 'B' in Fig. 10, are both characterized by a low Ex_{PUB}:Ex_{PEB} ratio similar to that of the cells found in the upwelling region. Sample 'A' was collected at the entrance to the Gulf of Oman, and Sample 'B' is the low Ex_{PUB}:Ex_{PEB} sample collected at 15.76°N, 63.52°E (cf Fig. 9). Station 1, located near the entrance to the Gulf of Oman, provides a third example of a sample in which a low Ex_{PUB}:Ex_{PEB} ratio characterized the PE-containing organisms in a warm, very salty water mass.

This study represents the first systematic effort to characterize the distribution of different PEs in seawater using fluorescence excitation spectroscopy. Most other methods that have been applied to bulk seawater rely on differences in fluorescence emission properties (cf Shalapenok and Shalapenok, 1997; Wood et al., 1998; Hoge

et al., 1999). These methods would have been insensitive to the close association of different forms of PUB-containing PEs with different water masses discovered in this study. The distinctive Ex_{PUB}:Ex_{PEB} ratios of the PEs dominating different water masses may be useful tracers of the fate of particular water masses. In particular, the close association between picocyanobacteria with low PUB-PEs and recently upwelled water in the Arabian Sea raises the interesting possibility that their distinctive fluorescence signature can be used to follow the fate of upwelling-influenced water masses and the organisms they contain.

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