

Phycoerythrin Signatures in the Littoral Zone

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LONG-TERM GOALS

My long-term goal is to contribute to our understanding of factors that determine the distribution and productivity of individual phytoplankton taxa. I am particularly interested in the way that evolutionary diversification in the architecture and pigment organization of different phytoplankton groups influences their distribution among different optical environments. To this end, I have been working with experts in remote sensing and ocean optics toward the development of an “optical biogeography” for marine picophytoplankton, focusing on different spectral forms of marine *Synechococcus* and *Prochlorococcus*.

OBJECTIVES

Phycoerythrin (PE) is the principal light harvesting pigment of marine *Synechococcus*, and *Trichodesmium*, and a minor component of the photosynthetic apparatus of some strains of *Prochlorococcus*. The wavelengths of light which can be used for photosynthesis by organisms which depend on PE for light-harvesting are determined by the spectral form(s) of PE the organisms synthesize. This, in turn, depends on the relative concentration of two different chromophores that can be incorporated in the PE heterodimer. These are phycoerythroblin (PEB, $\lambda_{AbsMax} \sim 550$ nm), found in all PEs, and phycourobilin (PUB, $\lambda_{AbsMax} \sim 500$ nm) found in varying concentration in some forms of PE. PEB provides for efficient utilization of green wavelengths of light; as the relative abundance of PUB increases, the spectral signature of the PE becomes more complex (i.e. additional peaks and shoulders in the fluorescence excitation spectrum). The ecological effect of increasing the PUB:PEB ratio is that the cells' ability to use shorter wavelengths that penetrate more transparent water masses greatly increases.

Recent work (Wood et al., 1998) suggests that phycoerythrin (PE) fluorescence signatures can be used to discriminate between water of neritic water and “green” water of oceanic origin along the continental shelf of North America. This hypothesis was tested in the Arabian Sea where upwelling conditions led to dramatic shifts in the PE spectral signature of water as the phytoplankton bloom associated with the Southwestern Monsoon progressed (Wood et al. 1999) These combined results led me to propose the following optical biogeography for dominance by organisms synthesizing different spectral forms of PE:

PEB- lacking spectral form of PE

Case II Waters

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Low PUB spectral form of PE
High PUB spectral form of PE

“Green” [$K_d(440) > K_d(550)$] Case I Waters
“Blue” [$K_d(440) < K_d(550)$] Case II Waters

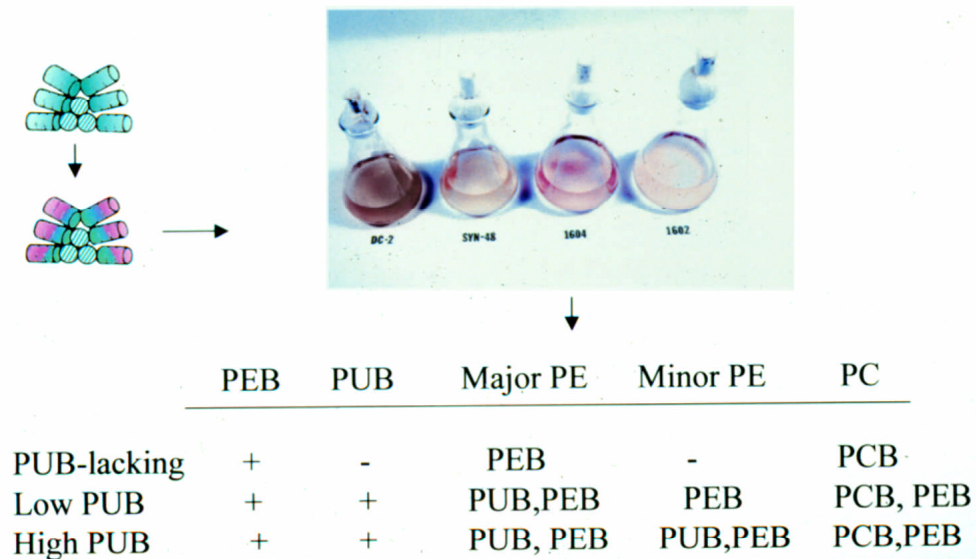
This research is designed to test the hypothesis that this is a robust optical biogeography for the distribution of organisms synthesizing spectrally different PEs. A further objective of the research is to evaluate the mechanisms by which spectral adaptation to changing optical environments occurs. Historically, I had viewed the spectral phenotype of a particular strain of marine *Synechococcus* to be genetically fixed; this was supported by early work on the PE and fluorescence signature of eight strains grown in a range of environmental conditions (Wood et al., 1985). The discovery that three strains of *Synechococcus* with PUB-containing PEs actually synthesize at least two spectrally different PEs led me to hypothesize that some strains might adapt to changing optical environments by altering the relative expression of these two forms of PE. Initially, this research was intended to determine whether or not any of the strains of *Synechococcus* which synthesized more than one type of PE were capable of chromatic adaptation by culturing the strains in blue and green light. However, early in the grant period, I learned that Brian Palenik at Scripps Inst. Oceanography had found that WH8020 could chromatically adapt (Palenik, unpublished) so, while both Palenik and I continue to screen new strains for their ability to chromatically adapt, I have also begun looking at the molecular basis for the evolution and expression of different PE spectral phenotypes in marine *Synechococcus*. Questions to be addressed are: how common is the ability to change PUB:PEB ratios among strains of different spectral phenotype? Do all cells with the same spectral phenotype achieve this by the same molecular mechanisms? Is there any aspect of the molecular architecture of the PE operon that could be exploited for *in situ* hybridization to determine which cells in a population are capable of chromatically adapting?

APPROACH

The project has a laboratory and a field component. In the field, the PE fluorescence signature of bulk water is being determined across a wide range of water mass types found in continental shelf environments. Sampling locations include the Gulf of Mexico, the Gulf of California, and the continental shelf off of Oregon. The correlation of PE spectral type and concentration of PE-containing organisms is being determined using measurements of inherent optical properties, radiometric properties, hyperspectral surface radiance measurements, and basic hydrographic properties. The research is coordinated with that of collaborators who are funded independently to optical properties: Gulf of Mexico: A., Wedieman, R. Arnone R. Gould, D. Johnson, and C. Davis, Gulf of California: Jim Mueller and Chuck Trees at CHORS (UCSD), Helmut Maske (CICESE, B.C. Mexico), and Ron Zaneveld and S. Pegau, (OSU); Oregon Coast: R. Zaneveld, S. Pegau, T. Cowles (OSU). Flow cytometric analysis of picocyanobacterial abundance is being done with Dr. W.K.W. Li (BIO, Halifax).

The laboratory component of the work involves physiological experiments with cultured strains of marine *Synechococcus*; I have a collection of approximately 60 strains of PE-containing *Synechococcus*. These are being grown under conditions of comparable PFD, but with different wavelengths of available light. A simple *in vivo* fluorescence assay is used to detect changes in the relative proportion of PUB:PEB in the PEs synthesized under different conditions. I am approaching the question of multiple forms of PE in different strains by developing PCR primers for PE in order to sequence the PE operon(s) in multiple strains from each of the major spectral groups. Since the prediction that some strains of marine *Synechococcus* might be able to change their PUB:PEB ratio by

differential expression of two spectrally different forms of PE, it is important to determine how similar wild strains of *Synechococcus* are to the four genotypes that have been well-characterized in terms of their pigment complement (Ong and Glazer, 1987, 1991). Figure 1 shows the predicted pattern of distribution of multiple PEs among different spectral phenotypes of marine *Synechococcus*.



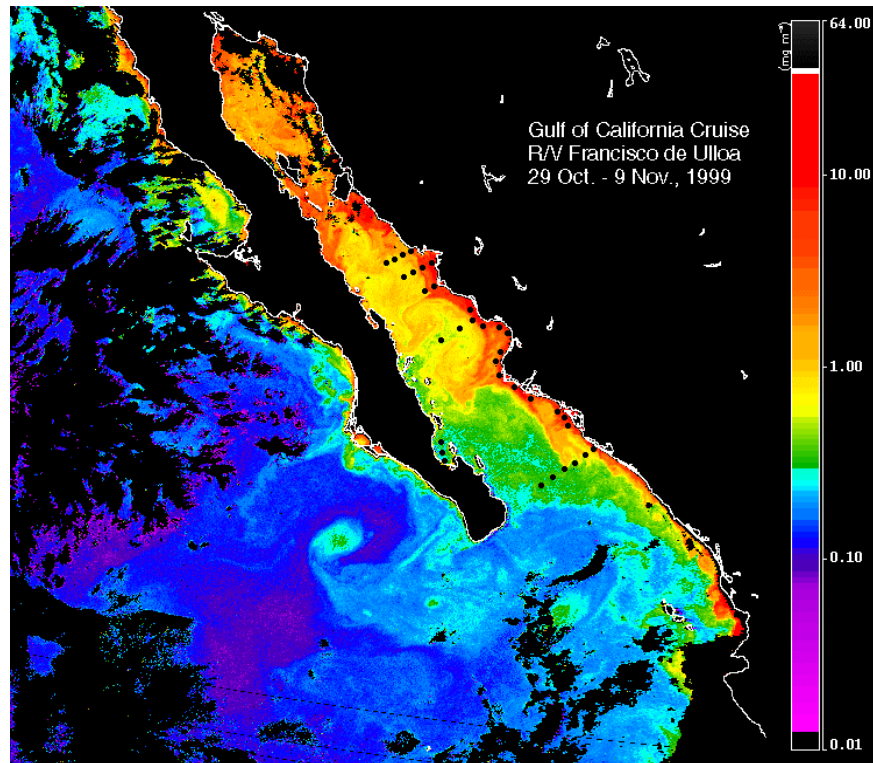
1. Complexity of light-harvesting apparatus in various spectral forms of marine *Synechococcus*. Cartoons in the upper left corner represent models of phycobilisomes with the grey-green pigment allophycocyanin always present in the core of the PBS and the blue-green pigment phycocyanin (PC) always present in the proximal ends of the PBS rods (PCB=phycocyanobilin). Phycoerythrin, when it is present, occupies the distal ends of the rods and can contain one or two types of chromophores (see text). Note that all strains which synthesize a PUB-containing PE tested to date (WH7803, WH8020, WH8103) appear to have two sets of genes for phycoerythrin (Wilbanks et al, 1991, Wilbanks and Glazer 1993 a,b).

WORK COMPLETED

We have completed two cruises, one in the Gulf of California, and one in the Gulf of Mexico. A cruise off the Oregon coast will be conducted this fall. Most of the optical data from both cruises has been processed and we have completed most of the analysis of PE spectral signature and flow cytometric analysis for *Synechococcus* and *Prochlorococcus*. We are proceeding to grow and screen our strains of *Synechococcus* for the ability to change PE spectral phenotype in response to changes in the wavelengths of available irradiance. While a major focus of our effort is manuscript preparation from the fieldwork, we are beginning work on primer development for PCR of PE genes based on data for cyanobacteria in Genbank and, in particular, the sequences encoding the a and b subunits of the major and minor PEs from marine *Synechococcus* WH8020 and WH8103 (cf. Wilbanks et al, 1991; Wilbanks and Glazer, 1993a,b, Glazer, 1999).

RESULTS

The two cruises were both very successful with a wide range of optical environments sampled between the two regions. Of great interest is the fact that the PE spectral signature at all stations in the Gulf of California were associated with relatively low PUB:PEB ratios. The range of PE spectral types was comparable to that found in the Arabian Sea in upwelling influenced waters (Wood et al., 1999). Since the stations included a transect near the southern end of the Gulf expected to be very oligotrophic and dominated by high-PUB PEs, this was somewhat surprising until it became clear that coastal upwelling was strong enough during our cruise to generate “green” Case I water at nearly all stations (Fig. 2).



2. SeaWiifs remapped chlorophyll a image for 1 Nov., 1999, processed using SeaDAS from the level 1A H RTP (HMBR: Moss Landing, CA) data. (Image courtesy of OSU Optics group). Station locations are noted with black dots

In the Gulf of Mexico, we sampled a wider range of optical environments in the sense that there were some very oligotrophic stations; at these stations *Prochlorococcus* was much more abundant than *Synechococcus*, but *Synechococcus* dominated the picocyanobacterial community in all nearshore stations and at outer shelf stations as conditions became more turbid and productive during an upwelling event. Both high-PUB and low PUB- PE fluorescence signatures were recorded on this cruise, with the transition from low PUB-PE to high PUB-PE occurring coincidentally with changes associated with a decreasing availability of blue wavelengths.

We were fortunate to be able to examine the optical aspects of this kind of shift in dominance in the Gulf of California because our cruise was preceded by the MOCE 5 cruise during which the conditions

were more oligotrophic in the Gulf of California than they were on our cruise. At present, optical data from that cruise have been more thoroughly analyzed than those from our cruise at this point in time; they indicate that these very small cells may produce changes in IOP signatures that can influence the reflectance signal. *Prochlorococcus* and *Synechococcus* have remarkably low package effects and large fractional backscattering relative to other taxonomic groups (Morel et al., 1993); the results from these two consecutive cruises will help us evaluate how much information about the distribution of *Prochlorococcus* and different spectral forms of *Synechococcus* can be obtained from optical data, particularly hyperspectral data.

IMPACT/APPLICATION

It is commonly believed that most marine *Synechococcus* show a high PUB spectral phenotype. This work, combined with our Arabian Sea work indicates that the most common form of PE synthesized by marine *Synechococcus* in Case I waters under bloom conditions is the low PUB spectral form. *Synechococcus* blooms appear to be common in Case I environments when there is an input of nutrients (i.e. coastal upwelling environments and during strong mixing events) and these are conditions where algorithms for deducing chlorophyll *a* concentration and rates of primary production are most thoroughly developed. However, because cyanobacteria which use phycobiliproteins as their major light harvesting pigments (e.g. *Synechococcus* and *Trichodesmium*) have very little antennae chlorophyll *a* in Reaction Center II but a great deal of zeaxanthin ($I_{\text{AbsMax}} \sim 463,491$) as a photoprotectant, typical algorithms could significantly overestimate chlorophyll concentration. Further, because of the substitution of phycobiliproteins for antennae chlorophyll *a* in reaction center II of *Synechococcus* and *Trichodesmium*, it is likely that assimilation efficiency (e.g. productivity per unit chlorophyll) will be much higher in surface blooms of these organisms than in blooms of *Prochlorococcus* or eukaryotic phytoplankton. Thus, the combined data sets these cruises are producing will be very helpful in providing information about parameters that can identify conditions when these organisms are responsible for a major component of the variation in remote sensing reflectance. This research is also significant for its basic contribution to our understanding of the ecology of *Synechococcus* in coastal waters. Most data on the distribution and abundance of *Synechococcus* has been collected in offshore waters. These organisms, however, are very abundant in nearshore waters and little is known about their ecology in sediment and organic-rich waters on continental margins.

RELATED PROJECTS

Funding for collection of optical data for the Gulf of California and the Oregon Coast is through NASA in SIMBIOS projects to Ron Zaneveld and Scott Pegau (OSU), and separately to Jim Mueller and Chuck Trees (CHORS/SDSU). Ship-time for the Sea of Cortez cruise was funded through Conacyt (to Helmut Maske, CICESE, B.C., Mexico). In the Gulf of Mexico, optical data collected by the NRL/SSC optics groups was funded through two projects, "Spectral Signatures of Optical Processes" (NRL 6.1 core funding) and "Applications of the SeaWiFS for coastal monitoring of harmful algal blooms" (EPA). Together, these groups of specialists in ocean optics and I developed a NASA proposal to develop methods for identification of phytoplankton functional groups from hyperspectral data. This work has also benefited from the results of Palenik's NSF-funded project on *Synechococcus* diversity in the California Current (SIO).

REFERENCES

- Glazer, A.N. 1999. Cyanobacterial photosynthetic apparatus: an overview. IN, L. Charpy and A. W. D. Larkum. Marine Cyanobacteria. Bul. Inst. Oceanographique. Sp. Number 19. Pp. 419-434.
- Morel, A. 1997. Consequences of a *Synechococcus* bloom upon the optical properties of oceanic (Case I) waters. Limnol. Oceanogr. 42:1746-1754.
- Ong, L.J. and A. N. Glazer. 1998. R-Phycocyanin II, a new phycocyanin occurring in marine *Synechococcus* species. J. Biol. Chem. 262:6323-6327.
- Ong, L.J. and A. N. Glazer. 1991. Phycoerythrins of marine unicellular cyanobacteria. J. Biol. Chem. 266:9515-9527.
- Wilbanks, S.M., R. de Lorimer, and A. N. Glazer. 1991. Phycoerythrins of a marine unicellular cyanobacteria. J. Biol. Chem. 266:9535-9539.
- Wilbanks S. M. and A. N. Glazer. 1993. Rod structure of a Phycoerythrin II-containing phycobilisome. I. Organization and sequence of the gene cluster encoding the major phycobiliprotein rod components of the genome of marine *Synechococcus* sp. WH8020. J. Biol. Chem. 268:1226-1235.
- Wilbanks S. M. and A. N. Glazer. 1993. Rod structure of a Phycoerythrin II-containing phycobilisome. II. Complete sequence and bilin attachment site of a phycoerythrin gsubunit. J. Biol. Chem. 268:1236-1241.
- Wood, A.M., D.A. Phinney, and C.S. Yentsch. 1998. Water column transparency and the distribution of spectrally distinct forms of phycoerythrin-containing organisms. Mar. Ecol. Prog. Ser.. **162**: 25-31.
- Wood, A.M., P.K. Horan, K. Muirhead, D.A. Phinney, C.M. Yentsch, and J.B. Waterbury. 1985. Discrimination between pigment types of *marine Synechococcus* spp. By scanning spectroscopy, epifluorescence microscopy, and flow cytometry. Limnol. Oceanogr. 30:1303-15.
- Wood, A.M. M. Lipsen and P. Coble, 1999. Fluorescence-based characterization of phycoerythrin-containing cyanobacterial communities in the Arabian Sea during the Northeast and early Southwest Monsoon (1994-1995). Deep-Sea Research II, **46**: 1769-1790.

PUBLICATIONS

- Sherry, N.S. and A.M. Wood. 2000. Phycoerythrin-containing picocyanobacteria in the Arabian Sea in February, 1995: diel patterns, spatial variability, and growth rates. Deep-Sea Research II. In Press.