Phycoerythrin (PE) refers to a family of highly fluorescent photosynthetic pigments found in some marine phytoplankton. In subtropical and temperate waters, PE-producing organisms are common enough that a spectral signature for PE can be obtained from bulk seawater, regardless of the concentration or type of other colored materials in the water. This project had two goals: to determine if the PE spectral signature of a water mass carries oceanographically useful information and to determine the biological mechanism by which the PE spectral signature changes in a water mass. The project showed that the nutrient status and color of oceanic waters can be inferred from PE spectral signature and that there is a distinctive PE spectral signature associated with turbid coastal water. We discovered several new types of PE-containing marine picocyanobacteria, described a totally new biochemical mechanism by which PE-containing phytoplankton can adapt to changes in the spectral quality of available light, and showed that lateral gene transfer is a key mechanism of evolution for PE and other genes in picocyanobacteria.
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Phycoerythrin pigments (PEs) are highly fluorescent, water-soluble pigments that are the primary light harvesting pigments of many marine picocyanobacteria, *Trichodesmium*, red algae, and cryptomonads. Each PE is composed of a colorless protein heterodimer to which four or five chromophores are attached; all PEs contain the chromophore phycoerythrobilin (PEB, $\lambda_{\text{Abs}}$ max $\sim 550$ nm), but some extend their absorption into the bluegreen region of the spectrum by substituting a second chromophore, phycourobilin (PUB, $\lambda_{\text{Abs}}$ $\sim 500$ nm), at one or more binding sites. The relative proportion of PEB and PUB associated with the dominant PE in a bulk water sample or in a suspension of cells can be assessed by fluorescence spectroscopy (c.f. Wood et al., 1999). In a preliminary study, Wood et al. (1998) showed that PUB-containing PEs were dominant in water masses where blue wavelengths predominated over green wavelengths and that PUB-lacking PEs (e.g. all binding sites occupied by PEB chromophores) were dominant in water masses where green wavelengths predominated. While these results are consistent with the idea that light quality is a strong selective force on PE-containing marine picocyanobacteria, they do not actually provide any information on the mechanisms by which the predominant PE in a water mass complement the available wavelengths, nor do they provide any information about the optical properties of water masses in which different types of PUB-containing PEs occurred.

The pigment may also occur in the picobiliphytes, a newly described group of picoeucaryotes whose distribution is unknown, and for which the function of the PE is as yet undefined (Not et al., 2007). While the name of this new group suggests that they are true picoplankton, the actual cellular dimensions of the cells are much larger than essentially all picocyanobacteria that contain PE (compare average cell dimensions of $\sim 3 \mu m \times 5 \mu m$ for picobiliphytes to $<1 \mu m \times 3 \mu m$ for marine *Synechococcus* and other picocyanobacteria). In this project, water samples for spectroscopy were examined using epifluorescence microscopy as well as by flow cytometry, so the presence of abundant PE-containing cells that are either larger (or smaller) than typical PE-containing picocyanobacteria would have been noticed. All evidence indicates all spectral data collected in this study were from environments in which the PE spectral signature was produced by PEs in picocyanobacteria, either marine *Synechococcus* or other groups of PE-containing unicellular prokaryotic picoplankton $<2 \mu m$ in diameter. *Trichodesmium* was present at some sampling stations but never at concentrations that suggest it was exclusively responsible for the PE spectral signature measured by fluorescence spectroscopy.
At least three forms of PE-containing picocyanobacteria have been identified in cultures: those which lack PUB, those with small amounts of PUB (low PUB), and those with enough PUB that the fluorescence excitation peak at 500 nm is higher than that at 550 nm (high PUB) (Wood et al. 1985, Glazer 1999, Wood and Everroad 2006, and unpublished). In this project, we sought to test the hypothesis that there is an optical biogeography associated with cells producing these three types of PEs as their dominant PE. Specifically,

<table>
<thead>
<tr>
<th>PUB-lacking PEs</th>
<th>“Green” Case II Water</th>
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<tbody>
<tr>
<td>Low PUB PEs</td>
<td>“Green” Case I Water</td>
</tr>
<tr>
<td>High PUB PEs</td>
<td>“Blue” Case I Water</td>
</tr>
</tbody>
</table>

where Case I waters are defined as those where the only factors influencing ocean color were plant pigments and the inherent optical properties of water. Case II waters were, at the time of the initiation of this project, defined as other water masses in which ocean color is also influenced by suspended and dissolved material not directly of phytoplankton origin. This simple dichotomous classification system continues to evolve but, for purposes of this report, environments where green wavelengths have come to dominate previously transparent blue water as a result of phytoplankton growth (e.g. boundary current upwelling systems) are classified as “green” Case I waters.

This hypothetical “optical biogeography” was tested by surveying the PE spectral signature in a wide range of optical environments on cruises where ancillary measurements of the inherent and apparent optical properties of the water were being made by other investigators. Over the course of the project, samples were collected off the California Coast, the Gulf of California, in the Gulf of Mexico, at the LEO 15 site of the continental shelf of North America, and in the Florida Keys and Gulf Stream; data from the Arabian Sea collected in an earlier ONR project add to the total data set although they are not supported by as much optical information. Thus, a range of temperate and subtropical optical environments were studied, including transparent and nutrient-enriched Case I environments and a variety of Case II environments. The publication of results from these highly collaborative projects has been very slow, but the most important finding of the research is that the low PUB-form of PE is a dependable diagnostic marker for blooms of PE-containing picocyanobacteria like marine *Synechococcus* when they occur in Case I waters. This appears to happen routinely when upwelling or storms increase nutrient concentration in the water column, with correlated “greening” of the light field. Additionally, the PUB-lacking form of PE dominates only in water masses where there is substantial influence of suspended sediments and terriginous (river or marsh) sources of CDOM. The high PUB-form of PE only dominates in very transparent blue water. A summary of the relationship between different spectral forms of PE and different optical environments was published as a “box” in Coble et al. (2004) and some of the data from the spectral surveys was published in an extended abstract in *Ocean Optics* (Wood et al., 2003). Additional manuscripts relating to this portion of the project are still planned. The optical data sets, collected by either Scott Pegau or the optical oceanography group at NRL/SSC (Robert Arnone and Rick Gould) are archived and all investigators still retain interest in
publishing a more detailed analysis of the value of PE spectral signature as an oceanographically informative parameter. AMW has obtained some salary support from the University of Oregon to help ensure that this happens.

This project also addressed questions relating to the mechanism by which the concordance between the spectral signature for PE and the in situ light field was achieved. As noted previously, cell counts confirmed that the main sources of PE in our samples were chroococloid picocyanobacteria and, occasionally, *Trichodesmium*. In these forms of picocyanobacteria, PE is responsible for essentially all photosynthetically active light harvesting. Thus, changes that enable cells to harvest more light (such as a shift from high PUB to low PUB forms of PE as the light field becomes more green) can be assumed to be advantageous. Since this work observed changes in the spectral properties of PE in bulk water between samples collected in water masses of differing color, it is possible to imagine several mechanisms by which the differences in spectral signature could have been achieved:

1) genetic adaptation by the community, i.e. individual genotypes of chroococloid cyanobacteria are thought to have a fixed spectral phenotype (i.e. described as constitutively either PUB-lacking, high PUB, or low PUB spectral types). Each would be favored in a different optical environment and changes in the spectral composition of the light field would lead to changes in the relative abundance of genotypes with different spectral phenotypes.

2) different picophytoplankton have the capacity to alter the spectral form of PE that they synthesize, and the “match” between dominant spectral form of PE and available wavelengths observed in field samples is a result of physiological adaptation by the picoplankton that are present. In other words, the cells physiologically adapt by synthesizing a high PUB PE in bluer light fields and a low PUB PE or low PUB in greener light fields. All marine *Synechococcus* studied to date that have a PUB-containing PE actually make two PEs, each with different ratios of PUB:PEB so that, when this project commenced, it was hypothesized that there might be strains of marine *Synechococcus* that could physiologically adapt to changing light fields by differential regulation of the synthesis of the two different PEs. Such a mechanism would be functionally analogous to forms of chromatic adaptation known in some freshwater cyanobacteria that differentially regulate the synthesis of PE and phycocyanin (a blue green pigment that absorbs red light) when shifted between red and green light fields.

Most of this work was conducted by R. Craig Everroad as part of his dissertation research. Everroad found that natural populations of marine *Synechococcus* are spectrally diverse and can include representatives of all spectral types, including physiologically adapting forms that change their spectral signature (Everroad, 2007; Everroad and Wood, In Prep). Since strains representing all spectral types were isolated from a single 50 ml sample of water, it appears that either mechanism suggested above (or both acting together) may account for the concordance between spectral signature of
PE from bulk water and the relative availability of blue and green wavelengths of light as indicated from such measures as hyperspectral remote sensing reflectance, $R_{Rs}(\lambda)$, or $k_d(\lambda)$. The mechanism for physiological adaptation in the chromatically adapting strains was found to be a novel mechanism, never before described in cyanobacteria. It does not involve changes in the relative synthesis of the two forms of PE in white or green light (relative to blue light) but, rather, changes in the composition of chromophores bound to the proteins (Everroad et al., 2006).

Early in this project Lauren Wingard, an M.S. student who was partially supported by this grant, discovered a new type of PE-containing picocyanobacteria that is unrelated to the widely studied phylogenetic cluster of marine *Synechococcus* thought to dominate the oceans (Wingard et al. 2002). This strain was isolated from oceanic regions of the Arabian Sea and would be classified as “marine *Synechococcus*” by standard methods based on flow cytometry. However, the strain makes cyanophycin, a nitrogen storage compound that does not occur in members of subcluster 5.1 of *Synechococcus* (typically referred to as “marine *Synechococcus*”) and it is more closely related to several taxa of filamentous cyanobacteria than any genus of unicellular cyanobacteria. In an effort to understand the overall diversity and sources of PE in marine picocyanobacteria, Everroad undertook a phylogenetic analysis of other strains from the Arabian Sea that shared the same spectral phenotype as the new strain described by Wingard et al. (2002). The six strains included in Everroad’s study were found to represent yet another new phylogenetic lineage of marine picocyanobacteria, most closely related to *Cyanobium*, a genus from which PE has not been described before (Everroad and Wood, 2006).

Portions of this project required development of a capacity for molecular research in the PI’s lab. While she published the first molecular phylogeny of marine *Synechococcus* (Wood and Townsend, 1990), it was a multilocus study using restriction fragment length polymorphisms (RFLPs). Since that research was done, developments in both the theory of phylogenetic reconstruction and the use of SSU markers for bacterial taxonomy meant that new methods needed to be mastered for this project. In the course of learning PCR-based methods for sequencing small subunit (SSU, 16SrRNA) genes with cyanobacterial specific primers, additional examples of picocyanobacteria from saline environments that were more closely related to filamentous cyanobacteria than the marine *Synechococcus* were found (Wood et al. 2002). More important, a picocyanobacterium in our culture collection was found to have a hybrid cyanobacterial-proteobacterial ribosome (Miller et al., 2005). Structural reconstruction of the spur of the ribosome encoded by the proteobacterial sequence and anomalies on nucleotide usage combined to produce a convincing demonstration that lateral gene transfer was responsible for the novel ribosomal sequence in this unique organism (Miller et al., 2005). This demonstration that genes like 16SrRNA are not immune to lateral gene transfer is an important contribution to debate over the stability of the bacterial genome.

Recent work by Everroad (Everroad 2007) has shown that the phylogeny of the PE apoprotein from marine *Synechococcus* with different spectral phenotypes does not match
the phylogeny of the organisms as inferred from the sequences of housekeeping genes like 16S rRNA or rpoC1 (RNA polymerase). Instead, apoproteins for PEs that have the same spectral phenotype share the same phylogeny, suggesting that the each PE spectral type evolved once, and now spread through the marine Synechococcus radiation by lateral gene transfer between different clades (Everroad 2007; Everroad and Wood, In Prep.).

Overall, this project made major contributions to our knowledge of the distribution, diversity, and evolution of different spectral forms of PE and the role of the natural light field in defining the optical niche of marine picocyanobacteria. Publication of results has proceeded fastest in the areas where there were dedicated personnel for the project, but all collaborators and participants remain committed to publication of all key findings. The project also provided evidence that marine Synechococcus may be available as food for larvae from deep-sea invertebrates. This led to a new project on picocyanobacteria currently funded by the National Science Foundation.

Development of a method for estimating the abundance of PUB and PEB chromophores in seawater, initially proposed as part of this research, requires new funding to solve problems relating to extraction and concentration of the pigment from seawater. While the basic quantitative estimate of chromophore number by spectroscopic analysis of the denatured protein still seems like a good approach, efficient recovery of the pigment from cells that are initially present at natural concentrations remains difficult. Recent advances in trace detection and concentration of proteins in the biomedical community offer several promising technologies that could be pursued with additional funding.

REFERENCES NOT IN THE FOLLOWING LIST OF PUBLICATIONS:

PUBLICATIONS FROM THIS PROJECT:

THESES AND DISSERTATIONS:


Wingard, Lauren. 1999. Cyanophycin production in the Arabian Sea Synechococcus Strain G2.1 M.S. Thesis


PUBLICATIONS:


PUBLISHED ABSTRACTS:


Everroad, R. and A. M. Wood. 2003c. Diversity of phycoerythrin-containing picocyanobacteria that share the same spectral phenotype. Published online at www.psaalgae.org.
