Global Distribution of Prochlorophytes from HPLC Measurements of Divinyl Chl *a*. A Pigment Algorithm Maintenance & Refinement Application

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ABSTRACT

It has only recently been recognized that the distribution and trophic dynamics of marine photosynthetic picoplankton are important factors in understanding ocean productivity within the context of global carbon cycles. In tropical and temperate oligotrophic areas, picoplankton dominate the phytoplankton community with *Prochlorococcus* being the most abundant. This organism has unique optical characteristics, because of its small size (~0.6 μm), pigment composition (divinyl chls *a* and *b*, and concentric organization of pigments within the thylakoid layers. With the development of high-performance liquid chromatography (HPLC) to measure these pigment biomarkers, the distribution and contribution to total pigment biomass of *Prochlorococcus* can now be readily assessed. Over the past several decades, many pigment samples have been collected and analyzed by HPLC methods to develop algorithms relating pigment concentration to remotely sensed ocean color observations (SeaWiFS, SIMBIOS and MODIS). This extensive pigment database (>20,000) will be used to investigate the ubiquitous nature of *Prochlorococcus* at regional to global scales.

INTRODUCTION

For most of the world's oceans, it is recognized that phytoplankton signatures characterize hydrographic conditions. For example, large species of microplankton (mostly diatoms) dominate in upwelling environments, while picoplankton are a common feature of the oligotrophic subtropical and tropical open ocean. Phytoplankton size structure, species composition, and temporal dynamics are important determinants of trophic interactions in marine ecosystems. Phytoplankton identification and enumeration are typically obtained through microscopic examination, which requires a high level of taxonomic skill and may take considerable time. Chlorophylls and carotenoid pigments, either singly or in combination, have been used successfully for chemotaxonomic identification of phytoplankton in oceanic and coastal waters. Although pigments may vary among cells within a taxon or between taxa, the abundances of the diagnostic pigments generally reflect the major distributions of phytoplankton to the division or class level. Many of the classes represented in the picoplankton and nanoplankton have distinctive suites of pigment markers that can be used to indicate their presence and abundance in a mixed population (e.g. peridinin for dinophyceae; prasinoxanthin for some prasynophyceae; alloxanthin for cryptophyceae; and chl *b* for chlorophyceae. This poster will focus on divinyl chl *a* for prochlorophytes.

It has been over a decade since the discovery of the photosynthetic prokaryote *Prochlorococcus* (Chisholm *et al*., 1988) and that these organisms possess divinyl chls *a* and *b*, which are HPLC measurable chemotaxonomic markers (Goericke and Repeta, 1992). *Prochlorococcus* has unique optical properties because of their small size, pigment composition and concentric organization of pigments within the thylakoid layers (Partensky *et al*., 1999), making them a potential taxonomic group for remote sensing from space, or airborne, ocean colors sensors. For purposes of understanding and interpreting spectral measurements of optical properties in coastal and oceanic areas, it is important to determine the absorption spectrum of the mixture of pigment compounds actually present. This composite absorption spectrum, and resulting reflectance spectrum, will inevitably be different for various algal color groups and any hope of determining accurate and precise sets of remote sensing algorithms depends absolutely on the level of pigment specification provided by HPLC.

An extensive HPLC pigment database has been assembled that extends over two decades of sampling and analyses, and includes a variety of environments ranging from freshwater to marine, oligotrophic to eutrophic, and tropical to polar. Over 20,200 samples have been analyzed (Trees *et al*., 2000, SIMBIOS, MODIS, and from other research programs) using a variety of instruments and methods. A significant portion of this pigment data has been archived at NASA Goddard Space Flight Center in the SeaWiFS Bio-optical Archive and Storage System (SeaBASS). Fig. 1 shows a global map of the SeaBASS pigment data archive for samples collected from 1985 to the present (0-200 m). A subset of this data, from 1994 to the present, will be used to investigate the global distribution of *Prochlorococcus* as inferred from divinyl chl *a* concentrations.

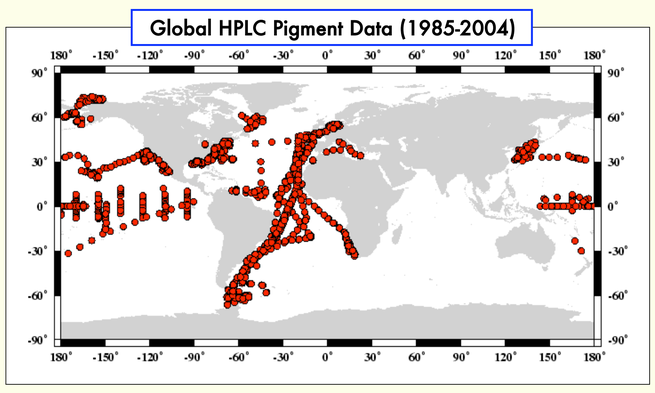

Fig. 1. Global distribution of HPLC measured pigment samples as archived in NASA's SeaBASS database (0 - 200 m) as of 28 July 2004.

METHODS

The Wright *et al*. (1991) method was used to analyze the pigment samples for divinyl chl *a*. An internal pigment standard, canthaxanthin, was added to the 90% acetone to correct for volume changes during the extraction process. Pigment peaks were detected by a 2-channel absorption detector (436 and 450 nm), a scanning diode array absorption detector (190 to 800 nm at 1 nm resolution) and a fluorescence detector for chl *a* degradation products. A sample chromatogram (absorbance at 450 nm) of a mixture of pigment standards is shown in Fig. 2. Although the absorption peaks for the monovinyl and divinyl chl *a* co-elute with the Wright *et al*. (1991) method, each compound absorbs differently at 436 nm and 450 nm and it is therefore possible to correct the divinyl chl *a* contamination by monitoring changes in this ratio as a function of changes in the divinyl percentage (Latasa *et al*., 1996). Calibration standards were purchased from Sigma Chemical Company and from DHI, Institute for Water and Environment, Denmark.

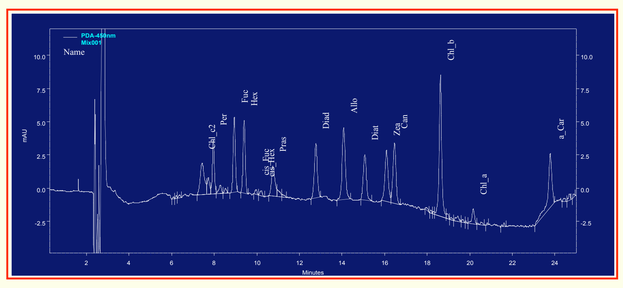

Fig. 2. HPLC absorbance (λ = 450 nm) chromatogram of a mixture of pigment standards. Note that there is a single chl *a* peak (mono- and divinyl chl *a* co-elute).

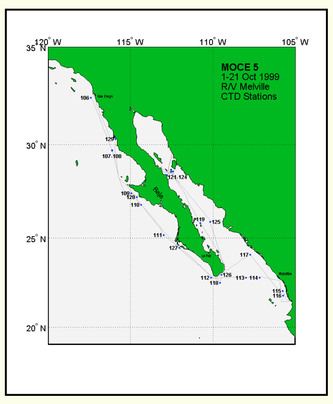

Fig. 4. Ship track and CTD stations during the October 1-21, 1999, MOCE 5 Cruise in the Gulf of California and around Baja California.

RESULTS

Prochlorococcus has a high absorption efficiency which significantly reduces its scattering and produces very low ratios of $b(\lambda)/a(\lambda)$ (Morel *et al*., 1993). Therefore, the remote sensing reflectance $[R_{RS}(\lambda)]$ is anomalously low in water-masses dominated by *Prochlorococcus*. Morel (1997) has shown for a *Synechococcus* bloom that ocean color algorithms (e.g. SeaWiFS) would overestimate chl concentrations by a factor of 3 and can be applied to *Prochlorococcus*. With the ability to routinely quantify divinyl chls *a* and *b* by HPLC, regional to global distributions of prochlorophytes can now be studied. The only limiting factor is the spatial and temporal distribution of our oceanographic sampling strategies. As a first attempt at generating a global map of the distribution of prochlorophytes, the pigment data in NASA's SeaBASS was merged with additional data collected from a variety of other cruises, increasing the SeaBASS database by 58% for near surface samples. Fig. 3 shows the distribution of the percent contribution by divinyl chl *a* to the total chl *a* concentration.

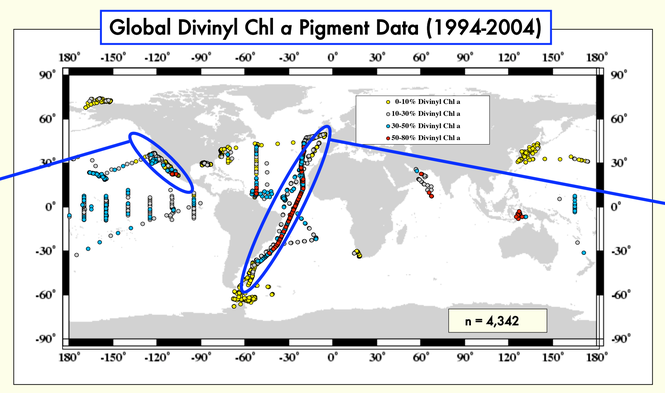

Fig. 3. Global distribution of the percent divinyl chl *a* to total chl *a* (mono- and divinyl chls *a*, chl *a* allomer and epimer, and chlorophyllide *a*) in near surface samples (0-10 m). The blue ellipses highlight regional areas that will discussed.

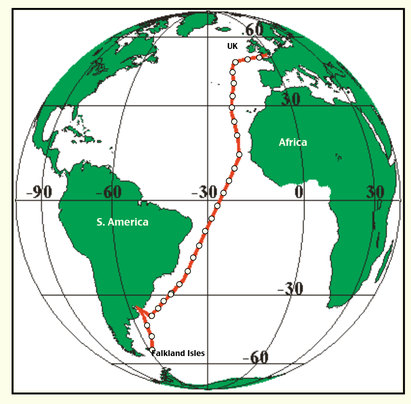

Fig. 8. A map showing the cruise track with CTD stations during the Atlantic Meridional Transect Cruise 3 (Sep - Oct 1996).

Marine Optical Characterization Experiment (MOCE) Cruise 5

MOCE was a series of field experiments, since 1992, collecting a comprehensive sets of bio-optical, physical and chemical measurements. MOCE 5 cruise sampled waters around Baja California and up into the Gulf of California. The cruise track with station positions are shown in Fig. 4 with the surface chl *a* distribution, as viewed by SeaWiFS, shown in Fig. 5. This image was acquired two-thirds of the way into the cruise, on the 14th of October. This temporal offset becomes important when comparing satellite measurements to those collected from the continuous flow through and CTD during the cruise. Chl *a* distributions within the Gulf of California were characterized by high values (~11 mg m^{-3}) to the north of the Mid-Rift Islands, while low values were observed (~0.3 mg m^{-3}) near the entrance to the Gulf as determined from the SeaWiFS image. The presence of *Prochlorococcus* in the surface waters for the southwestern region of the Gulf of California is confirmed by the high ratio (>30%) of divinyl chl *a* to total chl *a* concentration (Fig. 6; red filled circles).

The inherent optical properties of the surface waters were continuously monitored using a Wet LABS AC-9 instrument. The distributions of the single scattering albedo (b/c) at 440 nm were lowest (<0.6) in the southwest region of the Gulf of California and were elevated in the regions of high chl *a* (mesotrophic to eutrophic). The low b/c values are consistent with the dominance by *Prochlorococcus*, which are known to exhibit much lower single scattering albedos than other phytoplankton species (Morel *et al*., 1993). In addition, the steep slopes of the particle attenuation coefficients $c_p(\lambda)$ in the southwest region of the Gulf indicated smaller particle diameters, again suggest a dominant presence of prochlorophytes.

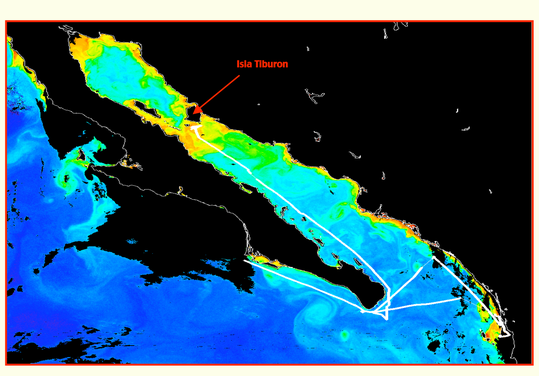

Fig. 5. SeaWiFS chl image collected on 14 October 1999. The ship was sampling at CTD Station 15 in the Mid-Rift islands area, just south of the Isla Tiburon.

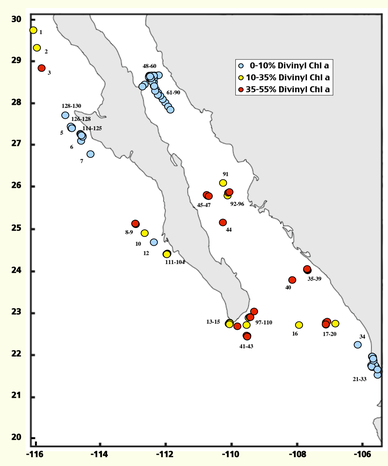

Fig. 6. Percent contribution of divinyl chl *a* to total chl *a* for surface samples (4 m).

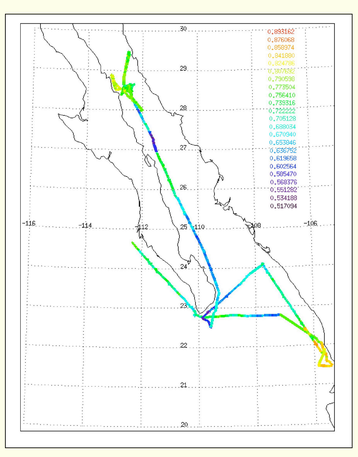

Fig. 7. Single scattering albedo (b/c) at 440 nm determined using an AC-9 attached to the ship's flow through system (4 m). The color scale is shown to the right with low b/c ratios (< 0.6) being dark blue to violet.

CONCLUSIONS

1. The distribution of *Prochlorococcus* as measured by divinyl chl *a* is ubiquitous, although there is substantial spatial and temporal variability.
2. In tropical and subtropical waters the contribution of *Prochlorococcus* to the total pigment biomass is high and can exceed that of other phytoplankton groups.
3. The optical properties of *Prochlorococcus* show differences that should be useful in developing remote sensing algorithms to estimate their concentrations.
4. Cellular concentrations of divinyl chl *a* and zeaxanthin (not shown) are similar to those found in cultures, although there are latitudinal and vertical trends.
5. The percent contribution of divinyl chl *a* to total chl *a* could be used to estimate percent composition and carbon biomass of *Prochlorococcus*.
6. This extensive pigment database was used to evaluate the contamination by divinyl chl *a* at estimating total accessory pigment concentrations from total chl *a*. There seems to be no significant difference (Fig. 13).
7. For the MODIS Program, 3,000 HPLC pigment samples will be processed per year, following the Bidigare *et al*. (2003) NASA protocol.

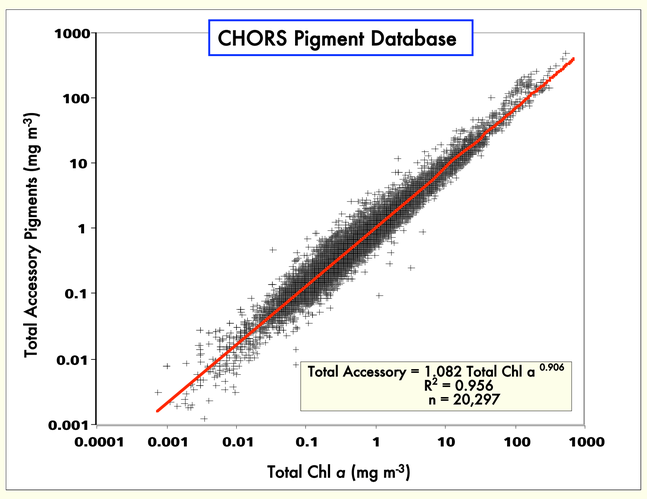

Fig. 13. Log-linear relationship between accessory pigments (chls *b* and *c*, phaeopigments and carotenoids) and total chl *a* (mono- and divinyl chls *a*, chl *a* allomer and epimer, and chlorophyllide *a*).

Atlantic Meridional Transect (AMT) Cruises 2 and 3

Phytoplankton pigment samples were collected daily from vertical CTD casts and from the surface underway supply (every 2 hrs at 6 m; Fig. 8). Total chl *a* concentrations showed high values in three areas; north latitude (45°N to 50°N), south latitude (35°S to 50°S) and the upwelling area (15°N to 22°N). The ratio of divinyl chl *a* to total chl *a* (mono + divinyl chl *a*, chl *a* allomer and epimer, and chlorophyllide *a*) was elevated in the oligotrophic tropical and subtropical areas, reaching values up to 70% (Fig. 9). Low ratios were found in the Mauritanian Upwelling and at high latitudes. Latitudinal distributions of percent divinyl chl *a* (Fig. 9) for AMT 3 and 4 are very similar, except for 35°N to 45°N, where a subtropical water mass was advected northward. High surface concentrations of photosynthetic carotenoids were found in the eutrophic cold waters of the high latitudes and the Mauritanian Upwelling, where as the photoprotectant carotenoid concentrations had a reverse trend (Fig. 10).

Plankton samples were collected from the CTD and the underway system for analysis by flow cytometry to discriminate between the following picoplankton; *Synechococcus* spp., *Prochlorococcus* spp., eukaryotic picoplankton and heterotrophic bacteria (Zubkov *et al*., 1998). Cellular divinyl chl *a* concentrations where calculated for surface and deep chl max samples and are shown in Fig. 12. Using this flow cytometry data, the latitudinal distributions of carbon biomass (%) for *Prochlorococcus* and *Synechococcus* are plotted in Fig. 13. The percent carbon biomass for *Prochlorococcus* (30°S to 10°N) equals those values found for the percent divinyl chl *a* in Fig. 9.

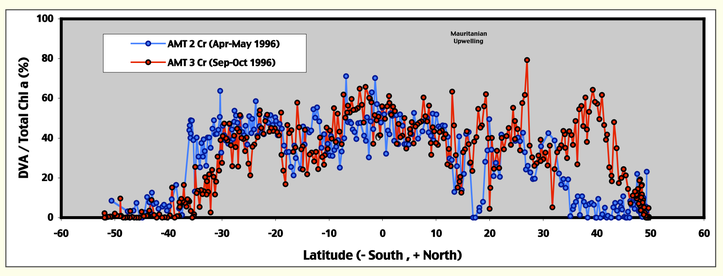

Fig. 9. Latitudinal distributions of the percent contribution of divinyl chl *a* to total chl *a* for AMT 2 and 3. Samples were collected from the flow through system (6 m).

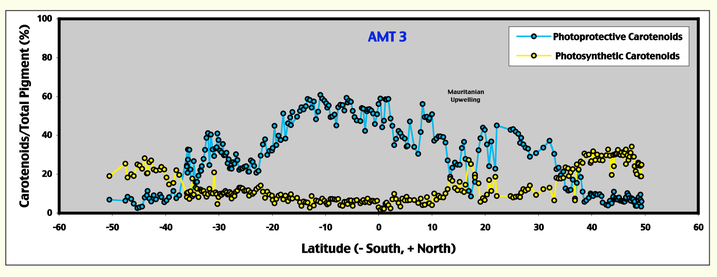

Fig. 10. Latitudinal distributions of the percent contribution of photoprotective and photosynthetic carotenoids to total pigment for AMT 3. Samples were collected from the flow through system (6 m).

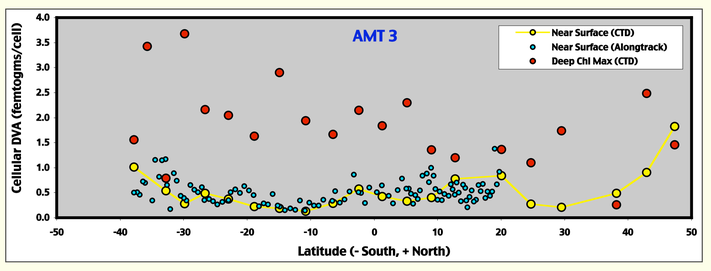

Fig. 11. Latitudinal distributions of divinyl chl *a* cell^{-1} for *Prochlorococcus* for samples take from the CTD and from the flow through system (6 m).

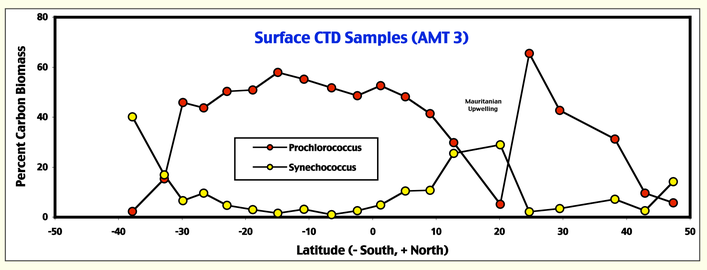

Fig. 12. Latitudinal distributions of the contribution of carbon biomass by *Prochlorococcus* and *Synechococcus* to total picoplankton biomass for AMT 3. Samples were collected from surface CTD bottles (2 to 13 m).

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