Sampling and Analytical Methodology

Water samples were collected in opaque bottles and filtered onto 25-mm Whatman GF/F glass fiber filters (nominal porosity of 0.7 µm) using vacuum filtration (7-10 in Hg). Sample filters were folded in half and individually stored in folded packets of heavy-duty aluminum foil, then placed immediately in liquid nitrogen. (Please modify or replace sampling methodology based on actual sample collection procedures during GEOTRACES cruise).

Filters for pigment analyses were extracted in 3 mL of HPLC-grade acetone in culture tubes along with 50 µL of an internal standard (canthaxanthin) at 4°C for 24 hours. The extracts were then brought to room temperature, vortexed, and centrifuged for 5 minutes to remove cellular and filter debris. Mixtures of 1-mL extract plus 0.3-mL HPLC grade water were prepared in opaque autosampler vials and 200 µL injected onto a Varian 9012 HPLC system equipped with a Varian 9300 autosampler, a Timberline column heater (26° C), and a Waters Spherisorb® 5-µm ODS-2 analytical (4.6 x 250 mm) column and corresponding guard cartridge (7.5 x 4.6 mm). Pigments were detected with a ThermoSeparation Products UV2000 detector ($\lambda_1 = 436$, $\lambda_2 = 450$). A ternary solvent system was used for pigment analysis: Eluent A (methanol:0.5 M ammonium acetate, 80:20, v/v), Eluent B (acetonitrile:water, 87.5:12.5, v/v), and Eluent C (100 % ethyl acetate). Solvents A and B contained an additional 0.01 % 2,6-di-ter-butyl-*p*-cresol (0.01 % BHT, w/v; Sigma-Aldrich) to prevent the conversion of chlorophyll a into chlorophyll a allomers. The linear gradient used for pigment separation was a modified version of the Wright et al. (1991) method: 0.0' (90 % A, 10 % B), 1.00' (100 % B), 11.00' (78 % B, 22 % C), 27.50' (10 % B, 90 % C), 29.00' (100 % B), 30.00' (100 % B), 31.00' (95 % A, 5 % B), 37.00' (95 % A, 5 % B), and 38.00' (90 % A, 10 % B) (Bidigare et al., 2005). Eluent flow rate was held constant at 1.0 mL min^{-1}.

Pigment peaks were identified by comparison of retention times with those of pure pigment standards (DHI Lab Products, Hørsholm, Denmark) and extracts prepared from phytoplankton reference cultures. Pigment concentrations were calculated using internal and external standards, and expressed as concentrations (ng pigment per L of filtered seawater). A dichromatic equation was used to resolve mixtures of monovinyl and divinyl chlorophyll *a* spectrally (Bidigare and Trees, 2000).

Stable detector response was confirmed on a weekly basis using freshly prepared chlorophyll *a* standard. Chlorophyll *a* standard injections were also run concurrently with samples as a daily QC check. An internal standard was used to account for extraction volume variability.

Lower limit of detection for the HPLC analytical method is 0.39 ng inj^{-1} for carotenoids and 0.59 ng inj^{-1} for chlorophylls. Precision for the analytical method is 0.81 %RSD for carotenoids and 1.40 %RSD for chlorophylls as determined from the mean ($\bar{x}$) and standard deviation (s) of replicate injections (n = 30) and calculated as 100 times $|s/\bar{x}|$. Accuracy for this analytical method is estimated as ~0.5% based on the uncertainty in the absorbance measurement of chlorophyll a standard.

Bidigare, R.R., Van Heukelem, L., Trees, C.C., 2005. Analysis of algal pigments by high-performance liquid chromatography. In: Andersen, R. (Ed.), Algal Culturing Techniques. Academic Press, pp. 327-345.

Wright, S.W., Jeffrey, S.W., Mantoura, R.F.C., Llewellyn, C.A., Bjornland, T., Repeta, D., Welschmeyer, N., 1991. Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton. Marine Ecology Progress Series 77, 183-196.

Bidigare, R.R., Trees, C.C., 2000. HPLC phytoplankton pigments: sampling, laboratory methods, and quality assurance procedures. In: Mueller, J.L., Gargion, G. (Eds.), Ocean Optics Protocols for Satellite Ocean Color Sensor Validation, Revision 2, NASA Technical Memo, 2000209966, pp. 154-161.

Data Processing

Individual pigment concentrations were calculated as follows:

$$C^{i}_{\text{Sample}} = \frac{A^{i}_{\text{Sample}} V_{\text{IS}} A^{\text{Cantha}}_{\text{IS}}}{F^{i} V_{\text{Injected}} V_{\text{Sample}} A^{\text{Cantha}}_{\text{Sample}}},$$

where C^{i}_{Sample} is the individual pigment concentration (µg L^{-1}), A^{i}_{Sample} is the area of individual pigment peak for a sample injection, V_{IS} is the volume of the IS spike added to the sample (mL, measured to the nearest 0.001 mL), V_{Injected} is the volume injected (mL, measured to the nearest 0.001 mL), V_{Sample} is the sample volume filtered (L, measured to the nearest 0.001 L), $A^{\text{Cantha}}_{\text{IS}}$ is the area of the canthaxanthin peak in the IS spike solution, and $A^{\text{Cantha}}_{\text{Sample}}$ is the area of the canthaxanthin peak in the sample, and F^{i} is the HPLC system response factor (area μg^{-1}).

Detection limits for these samples (in situ ng L^{-1} concentrations) were calculated using the analytical method LODs (chlorophylls 0.59 ng inj^{-1}; carotenoids 0.39 ng inj^{-1}), an injection volume of 0.2 mL, an extract volume of 3 mL, and a filtered volume corresponding to the amount filtered (L) for a particular sample, resulting in the following:

LOD Estimates

Volume Filtered (L)	**1.84**	**2.00**	**3.80**	**4.00**
LOD for chlorophylls (ng L^{-1})	**4.8**	**4.4**	**2.3**	**2.2**
LOD for carotenoids (ng L^{-1})	**3.2**	**2.9**	**1.5**	**1.5**

Pigment values <LOD were removed from the data set and reported as zeroes. Zeroes for this data set should be considered to mean <LOD.