SOCCOM IN2016\_V01 HEOBI HPLC

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**Sample collection**

Near-surface samples from SOCCOM CTD stations were taken for HPLC analysis. Clean transfer from the niskins (<210 μm to remove zooplankton) to a closed dedicated filtration rig. The samples were size fractionated in: total and <20 μm. 20% of the filter area was removed for biogenic silica digest. Volume filtered varied from 1 to 2 L to achieve maximum loading without blockage. Filters used were GF/F 25 mm diameter.

More information on the cruise are available at:

<https://soccom.princeton.edu/content/shipboard-data-reports>

**Analysis method**

Analysis was performed by Lesley Clementson (lesley.clementson@csiro.au) at CSIRO on

February 27, 2016 following the protocol of Hooker et al. 2012.

**Reference**

Hooker, Stanford B., Lesley Clementson, Crystal S. Thomas, Louise Schlüter, Merete Allerup, JosephineRas, Herve Claustre, Claire Normandeau, John Cullen, Markus Kienast, Wendy Kozlowski, Maria Vernet,Sumit Chakraborty, Steven Lohrenz, Merritt Tuel, Donald Redalje, Paulo Cartaxana, Carlos R. Mendes,Vanda Brotas, S.G. Prabhu Matondkar, Sushma G. Parab, Aimee Neeley, and Einar Skarstad Egeland. The Fifth SeaWiFS HPLC Analysis Round-Robin Experiment (SeaHARRE-5), 2012, NASA/TM-2012-217503.

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METHODS # DO NOT INCLUDE THIS SECTION IN PDF

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Near-surface samples from SOCCOM CTD stations were taken for HPLC analysis. 1-2 L of sample was filtered in the dark through glass fiber filter (GF/F) having a diameter of 25 mm. Filters were immediately stored in aluminium foil packages in a dewar of liquid nitrogen (-80 ºC). Samples were shipped and analyzed at NASA GSFC.

The HPLC analysis method can be cited as Van Heukelem and Thomas (2001), further described in Hooker et al. (2005). For a more detailed description, please see below; contact Crytal Thomas ([crystal.s.thomas@nasa.gov)](mailto:crystal.s.thomas@nasa.gov)) for a tailored description.

The HPLC used for pigment analysis is an Agilent RR1200 with a programmable autoinjector (900 ul syringe head), refrigerated autosampler compartment, thermostatted column compartment, quaternary pump with in-line vacuum. degasser, and photo-diode array detector with deuterium and tungsten lamps. The HPLC is controlled by Agilent Chemstation software.

The 4.6 x 150 mm HPLC Eclipse XDB column (Agilent Technologies, Palo Alto, CA) is filled with a C8 stationary phase (3.5 um stationary phase); the mobile phase consists of a linear gradient from 5-95% solvent B over 27 minutes, for which solvent A is 70 parts methanol, 30 parts 28 mM tetrabutylammononium acetate (pH 6.5) and solvent B is methanol. The column temperature is 60 C and the photo diode array detector is set to plot chromatograms at 450, 665, and 222 nm to acquire visible absorbance spectra between 350 and 750 nm.

Vitamin E acetate is used as the internal standard (ISTD) for determining extraction volumes. Its absorbance is monitored at 222 nm; it has negligible absorbance at 450 nm and none at 665 nm. Therefore, it does not interfere at wavelengths used to quantify pigments and can be used in very high concentrations with S:N ratios much higher than are possible with pigments. The high signal:noise ratio contributes to excellent analysis precision, for which injection repeatability averages 0.6%. It is stable under conditions of extraction and analysis.

Calibration is performed with individual pigment standards, whose concentrations have been determined spectrophotometrically using absorption coefficients in common with those used by most other laboratories (Hooker et al. 2005) and the commercial vendor, DHI Water and Environment (Horsholm, Denmark). Standards are either purchased from DHI (in solution with concentrations provided) or purchased in solid form and suspended in solvent at GSFC.

Thirty-six peaks are individually quantified by HPLC, from which 26 pigments are reported (some pigments contain individual components that are summed and reported as one pigment).

**Abbreviations**

|  |  |  |
| --- | --- | --- |
| **Primary Pigments** | Allo | alloxanthin |
|  | alpha-beta-Car | carotenes |
|  | But-fuco | 19'-butanoyloxyfucoxanthin |
|  | Diadino | diadinoxanthin |
|  | Diato | diatoxanthin |
|  | Fuco | fucoxanthin |
|  | Hex-fuco | 19'-hexanoyloxyfucoxanthin |
|  | Perid | Peridinin |
|  | Tot\_Chl\_a | total chlorophyll a |
|  | Tot\_Chl\_b | total chlorophyll b |
|  | Tot\_Chl\_c | total chlorophyll c |
|  | Zea | Zeaxanthin |
|  |  |  |
| **Secondary Pigments** | Chl\_c3 | Chlorophyll c3 |
|  | Chlide\_a | chlorophyllide a |
|  | DV\_Chl\_a | divinyl chlorophyll a |
|  | DV\_Chl\_b | divinyl chlorophyll b |
|  | MV\_Chl\_a | monovinyl chlorophyll a |
|  | MV\_Chl\_b | monovinyl chlorophyll b |
|  |  | Chlorophyll c2 + chlorophyll c1 + MGDVP |
|  |  | Mg-2,4-divnyl pheoporphyrin a5 monomethyl ester |
|  |  |  |
| **Tertiary Pigments** | Lut | Lutein |
|  | Neo | Neoxanthin |
|  | Phide\_a | total pheophorbide a |
|  | Phytin\_a | total pheophytin a |
|  | Pras | Prasinoxanthin |
|  | Viola | Violaxanthin |
|  |  |  |
| **Ancillary Pigment** | Gyro | Gyroxanthin diester |

**Other abbreviations**

|  |  |  |
| --- | --- | --- |
| DP | total diagnostic pigments | PSC + allo + zea + Tot\_Chl\_b |
| PPC | photoprotective carotenoids | allo + diadino + diato + zea + alpha-beta-car |
| PPC\_TCar | ratio of photprotective carotenoids to total carotenoids | [PPC]/[Tcar] |
| PPC\_TPg | ratio of photoprotective carotenoids to total pigments | [PPC]/[Tpg] |
| PSC | photosynthetic carotenoids | but-fuco + fuco + hex-fuco + perid |
| PSC\_TCar | ratio of photsynthetic carotenoids to total carotenoids | [PSC]/[TCar] |
| PSP | phosynthetic pigments | PSC + TChl |
| PSP\_TPg | ratio of photsynthetic pigments to to total pigments | [PSP]/[TPg] |
| TAcc | total accessory pigments | PPC + PSC + Tot\_Chl\_b + Tot\_Chl\_c |
| TAcc\_TChla | ratio of total accessory pigments to total chlorophll a | [Tacc]/[Tchla] |
| TCar | total carotenoids | PPC + PSC |
| TChl | total chlorophylls | Tot\_Chl\_a +Tot\_Chl\_b +Tot\_Chl\_c |
| TChl\_TCar | ratio of total chlorophyll to total carotenoids | [TChl]/[TCaro] |
| TChla\_Tpg | raito of total chlorophyll a to total pigments | [TChla]/[TPg] |
| TPg | total pigments | TAcc + Tot\_Chl\_a |

SOCCOM AU1603 K-Axis HPLC

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More information on the cruise are available at:

<https://soccom.princeton.edu/content/shipboard-data-reports>

**Analysis method**

Analysis was performed by Karen Westwood (karen.westwood@aad.gov.au) at the Australian

Antarctic Division following the methods of Wright et al. 2010.

**Reference**

Simon W. Wright, Rick L. van den Enden, Imojen Pearce, Andrew T. Davidson, Fiona J. Scott, Karen J. Westwood. Phytoplankton community structure and stocks in the Southern Ocean (30–80°E) determined by CHEMTAX analysis of HPLC pigment signatures, Deep Sea Research Part II: Topical Studies in Oceanography, Volume 57, Issues 9–10, 2010, Pages 758-778, ISSN 0967-0645, https://doi.org/10.1016/j.dsr2.2009.06.015.