SOCCOM A12 HPLC Data
Emmanuel Boss, University of Maine, 13 October 2016

(6) HPLC and POC

36 near-surface samples from SOCCOM CTD stations were taken for HPLC analysis. 1-2L of sample was filtered in the dark through glass fiber filters. Filters were immediately stored in aluminium foil packages in a dewar of liquid nitrogen. 36 near-surface samples from SOCCOM CTD stations were also taken for POC analysis. 1-2L of sample was filtered in the dark through pre-combusted glass fiber filters. Filters were immediately stored in pre-combusted aluminium foil packages in a dewar of liquid nitrogen. At each station one set each of HPLC and POC samples was a duplicate. Samples were packed for shipping (dry shipper) via air freight back to University of Maine (Emmanuel Boss).

(From project document: http://soccom.princeton.edu/sites/default/files/files/SOCCOM_2015-1_PS89_floats.pdf

From Analysis Report:

	SeaBASS and current report abbreviation	**SeaHARRE and/or previous report abbreviation**	**full name**	**notes**
Primary Pigments	Allo	Allo	alloxanthin	
	alpha-beta-Car	Caro	carotenes	alpha (beta, epsilon) + beta (beta, beta) carotene. Unresolved and therefore undifferentiated
	But-fuco	But fuco	19'-butanoyloxyfucoxanthin	
	Diadino	Diad	diadinoxanthin	
	Diato	Diato	diatoxanthin	
	Fuco	Fuco	fucoxanthin	
	Hex-fuco	Hex fuco	19'-hexanoyloxyfucoxanthin	
	Perid	Perid	Peridinin	
	Tot_Chl_a	TChl a	total chlorophyll a	DV_Chl_a + MV_Chl_a + Chlide_a + Chl_a allomers + Chl_a epimers

	Tot_Chl_b	TChl b	total chlorophyll b	DV_Chl_b + MV_Chl_b + Chl_b epimers
	Tot_Chl_c	TChl c	total chlorophyll c	Chl_c3 + Chl_c12
	Zea	Zea	Zeaxanthin	
Secondary Pigments	Chl_c3	Chl c3	Chlorophyll c3	
	Chlide_a	Chlide a	chlorophyllide a	
	DV_Chl_a	DVChl a	divinyl chlorophyll a	
	DV_Chl_b	DVChl b	divinyl chlorophyll b	
	MV_Chl_a	Chl a	monovinyl chlorophyll a	
	MV_Chl_b	Chl b	monovinyl chlorophyll b	
		Chl c12	Chlorophyll c2 + chlorophyll c1 + MGDVP	
		MGDVP	Mg-2,4-divnyl pheoporphyrin a5 monomethyl ester	
Tertiary Pigments	Lut	Lut	Lutein	
	Neo	Neo	Neoxanthin	
	Phide_a	Phide a	total pheophorbide a	multiple peaks
	Phytin_a	Phytin a	total pheophytin a	pheophytin a + pheophytin a'
	Pras	Pras	Prasinoxanthin	
	Viola	Viola	Violaxanthin	
Ancillary Pigment	Gyro	Gyr diester	Gyroxanthin diester	

Replicate filters

The replicate filter precision page summarizes our results for any replicate filters you submitted.

Replicate injections

The analysis precision page summarizes our results for the same sample extract injected twice. Typically, we reinject the first sample analyzed on a given at the end of the day (the ".5" injection). For example, sample 03-0001 and 03-0001.5 are replicate injections of the same extract, injected approximately 24 hours apart (all samples extracted on a particular day require about 24 hours to complete the HPLC analyses). We do this to measure our analysis precision and any effects caused by a sample's residence time in the refrigerated autosampler compartment. Please note that individual results with very large CV% are usually caused by pigments present in very low concentrations.

Effective Limit of Quantitation

On the effective LOQ page, we calculate an effective limit of quantitation based on our calculated LOQs (calculated in used with your samples. We make these calculations because our LOQ information is most useful to the data user if it is different filtration volumes. For example, the LOQ of 0.25 ng will result in very different effective LOQs when carried filtration volume of 2800 ml, the calculated effective LOQ would be 0.002 ug/L. However, if the filtration volume were has no way of knowing that both of these concentrations were acquired at detection-limited concentrations.

Zeros

Instead of including zeros, pigments that were "not found" (not detected) are noted with a replacement value of -111. Pigments that were "not found" are considered to below detection limits. For pigments that have a replacement value in the respective cell, the pigment was investigated and determined to be "not found" (this is different than a "missing" value, which would imply that the measurement was not performed).

Analysis method description

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 Crystal 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 at wavelengths used to quantify pigments and can be used in very high concentrations with S:N ratios much higher than 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 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).

SeaBASS submission

Please refer to the "Data Contributors" menu on the SeaBASS website (http://seabass.gsfc.nasa.gov) for information on how to prepare your data files for submission. If your data file contains measurements that were below detection limits ("not found", see Zeros section), those values were set to -111, and the following information should be

/below_detection_limit=-111
! Comments
!
! Measurements below detection limits are assigned the value -111

SeaBASS abbreviation	description	notes
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