**High Performance Liquid Chromatography (HPLC) Method**

The current HPLC method has been used since 1999 (Van Heukelem and Thomas 2001, further described in Hooker et al. 2005).

**Replicate filters**

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

On both the replicate filter and analysis precision page, pairs with precision worse than 10% (15% for degradation products) are flagged in yellow. If a simple reason can be determined (ex. Concentration is below the effective LOQ), it is noted in a comment.

**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.

On both the replicate filter and analysis precision page, pairs with precision worse than 10% (15% for degradation products) are

flagged in yellow. If a simple reason can be determined (ex. Concentration is below the effective LOQ), it is noted in a comment.

**Effective Limit of Quantitation**

On the effective LOQ page, we calculate an effective limit of quantitation based on our calculated LOQs (calculated in ng/injection), our typical extraction volume for this sample set, and the various filtration volumes used with your samples. We make these

calculations because our LOQ information is most useful to the data user if it is available in units of concentration (ug/L seawater).

The same LOQ can end up looking very different for different filtration volumes. For example, the LOQ of 0.25 ng will result in very different effective LOQs when carried through our calculation equation to represent the ug/L seawater. For an extraction volume of 2.5 ml and a filtration volume of 2800 ml, the calculated effective LOQ would be 0.002 ug/L. However, if the filtration volume were only 100 ml, the effective LOQ would calculate to be 0.042 ug/L. Without these calculations, the end user 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 -8888 (NEW VALUE AS OF MARCH 2016, the replacement value was -111 before that date). 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 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).

**References:**

Van Heukelem, L. and C.S. Thomas, 2001: Computer-assisted high-performance liquid chromatography method development with applications to the isolation and analysis of phytoplankton pigments. J. Chromatogr. A, 910, 31-49.

Hooker, S.B., L. Van Heukelem, C.S. Thomas, H. Claustre, J. Ras, L. Schlüter, J. Perl, C. Trees, V. Stuart, E. Head, R. Barlow, H. Sessions, L. Clementson, J. Fishwick, C. Llewellyn, and J. Aiken, 2005: The second SeaWIFS HPLC Analysis Round-Robin Experiment (SeaHARRA-2). NASA Tech. Memo. 2009-215849, NASA Goddard Space Flight Center, Greenbelt, Maryland, 112 pp.

Additional information at:

<https://oceancolor.gsfc.nasa.gov/fsg/hplc/>