

## **Method: In-line Multi-excitation Chlorophyll Fluorescence, WET Labs ECO 3X1M**

**Document author and contact info:** Collin Roesler, croesler@bowdoin.edu

**Date:** 1 August 2019

**Brief description of protocol:** Multi-excitation chlorophyll fluorescence was obtained from continuous inline observations collected with a WETLabs ECO 3X1M fluorometer using the differencing method between unfiltered and filtered observations following Slade et al. (2010). The fluorometer excites with 3 LEDs (nominally 440 nm, 470 nm, and 532 nm) and measures chlorophyll fluorescence (695 nm). The instrument is calibrated against a dilution series of the monospecific culture of the diatom *Thalassiosira pseudonana* to quantify the relative fluorescence response to each of the 3 excitations. Earlier studies have demonstrated that the fluorescence ratios vary as pigmentation varies (Yentsch and Phinney 1985), primarily due to taxonomically-driven variations in pigment composition with secondary dependence on photoacclimation and growth phase (Proctor and Roesler 2010; Thibodeau et al. 2014). The standard method for estimating chlorophyll *a* concentration is to calibrate in situ observations of chlorophyll fluorescence derived from a 470 nm LED to paired in situ HPLC TChl measurements. The fluorescence at the other channels is scaled according to the *T. pseudonana* response. Thus if the in situ phytoplankton was *T. pseudonana* then all three channels would yield the same calibrated chlorophyll values. Variations in the derived calibrated chlorophyll between channels is then interpreted as pigment variations *relative* to those observed in *T. pseudonana*, as published in Proctor and Roesler (2010).

**Deployment methodologies:** A diaphragm pump installed in the sea chest of the R/V Armstrong delivered continuous seawater to the wet lab, with minimal particle disruption. The inline optical system consists of serial flow in the following order: MSRC VDB-1 vortex debubbler, Seabird thermosalinograph, Sequoia Flow Meter, WET Lab acs, WET Labs ECO 3X1M, WET Labs ECO BBFL2 and WET Labs ECO BB3. The ECO 3X1M was configured in a WET Labs cylindrical ECO flow cell. The flow meter automatically switched flow from unfiltered to filtered through a large volume 0.2  $\mu\text{m}$  cartridge filter. The automated switch operated continuously through the day such that during each hour 50 minutes is unfiltered and 10 minutes is filtered. Approximately twice per day, an additional filtered configuration was manually triggered such that the hour was parsed into 20 minutes of unfiltered, 15 minutes of 20- $\mu\text{m}$  filtration, 15 minutes of 5- $\mu\text{m}$  filtration and 10 minutes of 0.2- $\mu\text{m}$  filtration. Discrete water samples were collected from each of the size fractions and processed for spectrophotometric particulate absorption, HPLC and POC.

**Data processing:** All flow through data were processed into 1 minute bin median and standard deviation values. Observations compromised by bubbles were removed. Data were time-merged with the ship navigation GPS data streams, also processed into 1-minute bin median values. Data stream was parsed into unfiltered and filtered intervals, with transitional data removed. Filtered observations were interpolated to 1-minute intervals and subtracted from unfiltered data, thus yielding blank-subtracted fluorescence values (Slade et al. 2010). Non-negligible non-photochemical quenching was observed within 2 hours of local midnight. Match-ups between

discrete HPLC TChl values and unquenched inline chlorophyll fluorescence values were used to generate an in situ calibration for the 470 nm excitation channel, with  $M \left( \frac{mg}{m^3} dc^{-1} \right)$ , representing the slope of the matchup relationship

$$Chl \left( \frac{mg}{m^3} \right) = M \left( \frac{mg}{m^3} / dc \right) \times (F_{unfilt} - F_{filt}).$$

The calibration was scaled to the 440 nm and 532 nm channels using the 440:470 and 532:470 blank-corrected digital count ratios established with the *T. pseudonana* culture. All values are reported in units ( $mg m^{-3}$ ); non-photochemically quenched values are provided and should not be interpreted as chlorophyll concentration.

**Uncertainties and quality control concerns:** Uncertainties associated with natural variations in chlorophyll fluorescence were determined from the standard deviations of the one-minute bin median data for filtered,  $\sigma_{filt}$ , and unfiltered,  $\sigma_{unfilt}$ , observations. Uncertainty in the chlorophyll calculation,  $\sigma_{chl}$ , was propagated (Jcgm 2008) as:

$$\sigma_{chl} = Chl \times \sqrt{\sigma_{unfilt}^2 + \sigma_{filt}^2 + \left( \frac{\sigma_M}{M} \right)^2}$$

where  $\sigma_M$  is the uncertainty in the regression slope of the HPLC and fluorescence match-up.

**Data products originating with this method:**

Parameter	Symbol	Units
Calibrated Fchl (ex470 nm)	Fchl (470)	mg m <sup>-3</sup>
<i>Thalassiosira pseudonana</i> -equivalent Fchl (excitation 440 nm)	Fchl(440)	mg m <sup>-3</sup>
<i>Thalassiosira pseudonana</i> -equivalent Fchl (excitation 532 nm)	Fchl(532)	mg m <sup>-3</sup>

**Key method references:**

- Jcgm, W. G. 2008. Evaluation of measurement data - Guide to the expression of uncertainty in measurement, p. 134. *In* B. I. d. P. e. Mesures [ed.], GUM: Guide to the Expression of Uncertainty in Measurement.
- Proctor, C. W., and C. S. Roesler. 2010. New insights on obtaining phytoplankton concentration and composition from in situ multispectral Chlorophyll fluorescence. *Limnology and Oceanography: Methods* **8**: 695-708.
- Slade, W. H. and others 2010. Underway and Moored Methods for Improving Accuracy in Measurement of Spectral Particulate Absorption and Attenuation. *Journal of Atmospheric & Oceanic Technology* **27**.

Thibodeau, P. S., C. S. Roesler, S. L. Drapeau, S. Prabhu Matondkar, J. I. Goes, and P. J. Werdell. 2014. Locating *Noctiluca miliaris* in the Arabian Sea: An optical proxy approach. *Limnology and Oceanography* **59**: 2042-2056.

Yentsch, C. S., and D. A. Phinney. 1985. Spectral fluorescence: an ataxonomic tool for studying the structure of phytoplankton populations. *Journal of Plankton Research* **7**: 15.