Calibration report of the 3X1M-001 fluorometer used on THEORETICAL-001 cruise

Name: WET Labs 3X1M ("Triplet" custom optical sensor)
Model and S/N: 3X1M-001
Purchase date: 07/2005

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I) Introduction

The 3X1M is a custom in situ fluorometer in the ECO Triplet class (WET Labs, Inc). It consists of three light emitting diodes (LED) that provide excitation energy in a narrow waveband at 435 nm, 470nm, and 532 nm. A photodiode detector to measures chlorophyll a fluorescence emission at 695 nm (3X1M: 3 eXcitation 1 eMission). Excitation wavelengths were selected to provide isolation of the in vivo Chl a fluorescence intensity resulting from direct Chl a excitation (435 nm) and from energy transfer from the accessory chlorophylls, carotenoids, and phycobilipigments. Excitation beams enter the water at approximately 55-60 degrees with respect to the end face of the unit and fluoresced light is received at an acceptance angle of approximately 140 degrees. An interference filter is used to reject scattered excitation light. Scaling factors are used to convert the voltage readings to values representing chlorophyll concentration.

II) Calibration / Maintenance

A. Manufacturer calibration and coefficients

Manufacturer calibrations are provided here for reference, although self-calibrations were used to generate reported data.

Calibration Date: ~03/01/2005

Instrument	Wavelength	Scale Factor	Dark Counts	Maximum Output
3X1M-001	435 nm	X (chl (mg/m^3)/DC)	102 ± 15	4120
3X1M-001	470 nm	Y	49 ± 15	4100
3X1M-001	535 nm	Z	111 ± 15	4115

*Chl [mg m^3] = scale factor * (DC_measured – dark counts)*

DC: Digital Counts (units of raw sensor output)
Dark Counts: Signal measured in clean water with black tape over the detector face
Scale factor determined from phytoplankton monoculture (*Thalassiosira weisflogii*) and concentration measured using an absorption method.

B. Self calibration methods and results

Standard curves (i.e. the response of the instrument to dilution series of the calibrating constituents) were performed to calibrate the 3X1M to specific species of phytoplankton with different pigment

compositions. *Thalassiosira pseudonana, Duneliella tertiolecta* and *Synechoccus sp cf bacillus* cultures were obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP, Boothbay Harbor, USA.)

The 3X1M-001 was calibrated by quantifying the fluorescence response in the set of monospecific cultures grown in batch under controlled light conditions. Cultures were grown in f/2 media from the CCMP (Anderson 2005) maintained at 20 degrees C and grown under a 12:12 days-night cycle illuminated by cool white fluorescent bulbs. Cultures were grown at irradiances of 270 and 60 mol photon $m^{-2}s^{-1}$ (for eukaryotes and cyanobacteria respectively.) Irradiance was measured using a LICOR quantum sensor. Fluorescence response was measured over a two order of magnitude dilution series (approximately 0.5 to 50 mg chl/m3). Triplet readings were performed in a 1-L glass beaker set on a black cloth in a dim room. Cultures were given half an hour to adapt to the room's light after being removed from the growth chamber, to avoid the effects of rapid photosystem changes such as the xanthophyll cycle (Lutz et al., 2001). Dilutions were performed with 0.2 mm-filtered seawater. Concentration was determined from triplicate samples of each dilution collected for fluorometric chl analysis (Yentsch and Menzel 1963; Holm-Hansen et al. 1965).

Calibration slopes were determined by performing linear regressions between Chl concentration and the fluorescence response (DC minus dark counts) for each of the 3X1M's excitation channels. Fluorescence response slopes were generated for each of the 3X1M excitation channels plus or minus the absolute value of the difference between the slope and 95% confidence intervals. Slope units are digital counts of 3X1M digital counts divided by Chl concentration.

Chl [mg m^3] = $slope^{-1}$ * (DC_measured – dark counts)

Calibration Date: 08/12/2008

Species	Ex. Wavelength	Slope (DC/chl(mg/m3))
Thalassiosira pseudonana	435	66.60 ± 1.71
(Bacillariophceae)	470	43.66 ± 0.66
	532	10.78 ± 0.22
Duneliella tertiolecta	435	42.24 ± 1.92
(Chlorophyceae)	470	30.84 ± 1.75
	532	3.09 ± 0.26
Synechoccus sp cf bacillus	435	21.34 ± 1.35
(Cyanophyceae)	470	13.07 ± 0.67
	532	36.73 ± 2.93

Dark Counts

Measurements of dark counts were taken to derive the instrument noise and so that the instrumental offset could be subtracted from sample observations. Dark counts were collected after placing black tape over the face of the instrument and submerging it in tap water. Median values and the standard deviations of 30 s of ~1 Hz samples were collected before each calibration and before and after deployments since 2005. No significant drift has occurred.

Wavelength	Dark counts
435	210 ± 8
470	65 ± 1
532	69 ± 1

Temperature characterization

Temperature calibrations were performed on the sensor to enable correction for affects of media temperature on sensor dark counts. The topical faces were coated with a layer of black electrical tape and measurements were taken in a non-reflective black bucket filled with filtered Milli-Q water. A Neslab/Endocal RTE-100 unit controlled temperature over a range of 1-30ºC with measurements recorded at 5ºC increments. A Hanna digital thermometer was used to verify the water temperature with 0.1ºC increments. The instruments were submerged and allowed to equilibrate at each temperature for 3 minutes to replicate thermal equilibrium that would occur during deployment. To simulate how field readings were collected, the sensors were powered on, a burst sample of 30 seconds was collected, and the sensor powered down.

Calibration Date: 11/01/2008

Ex. Wavelength	ΔDC / ΔT
435	0.45 (per degree C)
470	0.31 (per degree C)
532	0.36 (per degree C)

III) Deployment / Sample Collection

A) Measurement Methods

Data were collected every 15 minutes for 30 s at a 1 Hz sampling rate.

B) Package Design

The sensors were deployed 1 m below the surface, attached to an anchored stainless steel frame suspended by a buoy. Surfaces were coated with black tape to minimize any reflection interference from the frame or anchor. The sensors were controlled and powered by a WET Labs DH4 data handler and 2 ECO battery packs.

C) Blanks

While moored, the 3X1M was maintained bi-weekly which included retrieving it, collecting pure-water blank measurements and then cleaning it. Trends were not observed in the blank measurements so no corrections for biofouling were necessary.

IV) Data Processing

A) Data Analysis

Post processing included binning the 30 s sampling intervals then applying temperature corrections, subtracting dark counts and applying chlorophyll calibrations. Matlab code was used to discard the first

5 s of each sampling to avoid any effects of instrument warm up. The remainder of the 30 s of data collected every 15 minutes were binned by taking the median value along with the standard deviation.

Thalassiosira slopes from the calibration table were selected to derive chlorophyll concentration (selection based on a combination of channel ratios and microscopy identifying dominant diatom populations in the study region). The values from the 3X1M's 435 nm excitation channel were used to calculate the reported Chl concentration values on the THEORETICAL-001 deployment cruise.

Below are the sequence of equations to compute Chlorophyll a concentration from in situ Chl a fluorescence measurements for any of the three excitation wavelengths. Dark counts are corrected based on field temperature readings from a thermistor, then subtracted from field measurements then multiplied by the appropriate slope to convert DC to Chl a.

(eq 1) $Chl = CorrectedDCsample \times (Slope\_phyto\_cal)^{-1}$
(eq 2) $CorrectedDCsample = DC\_measured - DC\_dark\_counts$
(eq 3) $DC\_dark\_counts = DC\_dark\_counts(based\ on\ T\_cal) + (T\_cal - T\_insitu) \times (\Delta DC / \Delta T)$

V) Additional Information

Cautionary notes

- Many factors affect fluorescence response per chlorophyll (i.e. calibration slope). Different species often have very different pigment types and packaging. Culture health, light acclimation and pigment composition are just some of the other factors that affect estimates of chlorophyll concentration based on fluorescence.

- The presence of CDOM may impact fluorescence measurements (particularly when present in high concentrations, e.g. some coastal waters.) Correction calibrations can be calculated and used if this instrument is paired with CDOM measurements.

References

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