# Calibration and Correction of the Chlorophyll Fluorometer on GoMOOS Buoy A01: Nov 2005 – Apr 2011

**Massachusetts Water Resources Authority**
**Environmental Quality Department**
**Report 2011-15**

Roesler, CS. 2011. **Calibration and Correction of the Chlorophyll Fluorometer on GoMOOS Buoy A01: Nov 2005 – Apr 2011**. Boston: Massachusetts Water Resources Authority. Report 2011-15. 8 p.

# Calibration and Correction of the Chlorophyll Fluorometer on GoMOOS Buoy A01:Nov 2005 – Apr 2011


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| Principal Investigator: | Dr. Collin Roesler |
| Institution: | Bowdoin College<br>Department of Earth and Oceanographic Science |
| Address: | 6800 College Station<br>Brunswick, ME 04011 |
| Phone/Fax: | 207-725-3842 / 207-798-7037 |


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| Period of Performance: | 20 January 2011 - 30 June 2011 |

## Introduction

The Gulf of Maine Ocean Buoy Array (formerly GoMOOS) has nearly completed its tenth year of real-time hourly observations in the Gulf of Maine. Initially, four buoys were equipped with optical sensors (B, E, I, and M; figure 1); Buoy A was instrumented in 2005 through support from MWRA; Buoy F (Penobscot Bay) through support from NASA, and more recently Buoy D02 (Harpswell Sound) through support from NSF and NASA (Pettigrew & Roesler, 2005). Buoy A optical sensors consist of a WETLabs combination chlorophyll fluorometer/turbidity sensor (ECO-series FLNTU) and a DH4 data handler that provides the mean values of 30-second burst sampling each hour to the buoy data logger for real time transmission. The time course observations of calibration chlorophyll fluorescence at buoy A is presented in this report from the perspective of the sources of variability in determining phytoplankton biomass from *in situ* fluorescence and from the perspective of observing phytoplankton dynamics.

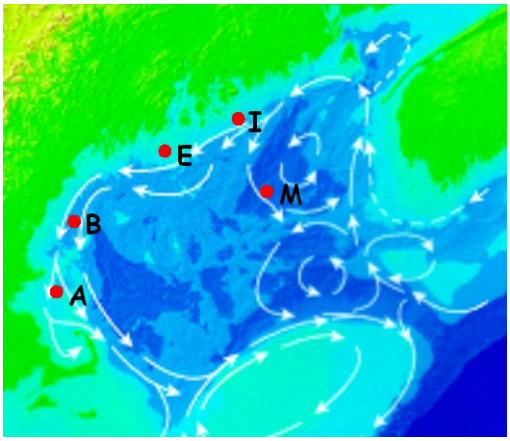


*Figure 1. Map of the Gulf of Maine bathymetry (courtesy R. Signell) with summertime surface circulation indicated by white arrows (Pettigrew et al. 2005). The locations of optically-instrumented buoys are indicated by red symbols.*

## Methods

The two FLNTU sensors are serviced and calibrated by the factory in between each deployment. Additionally, the fluorometers are calibrated in Roesler's lab using a monospecific culture of the diatom *Thalassiosira pseudonana* grown on a 24h light cycle (to minimize diel variations in pigment concentration and photosynthetic parameters), in replete nutrients and light levels that maximize growth rates (i.e. ~300 μmol photons $m^{-2}$ $s^{-1}$) and minimize pigment packaging due to low light acclimation. The fluorescence efficiencies of the culture have been shown to be repeatable over years (Proctor and Roesler 2010). This approach to calibration thus provides not only a consistent fluorescence response between deployments, it also provides a more realistic estimate of *in vivo* chlorophyll concentrations from *in situ* fluorescence.

Drift and biofouling are assessed in two ways. Instrumental drift is quantified as the different in dark reading before and after each deployment. Over the lifetime of the sensors, this drift has been negligible. Biofouling is quantified by the difference in calibrated observations collected on the last day of a deployment and the first day of the subsequent deployment. The offset is then projected backward in the prior deployment to the time point when the offset trend disappears (Figure 2).

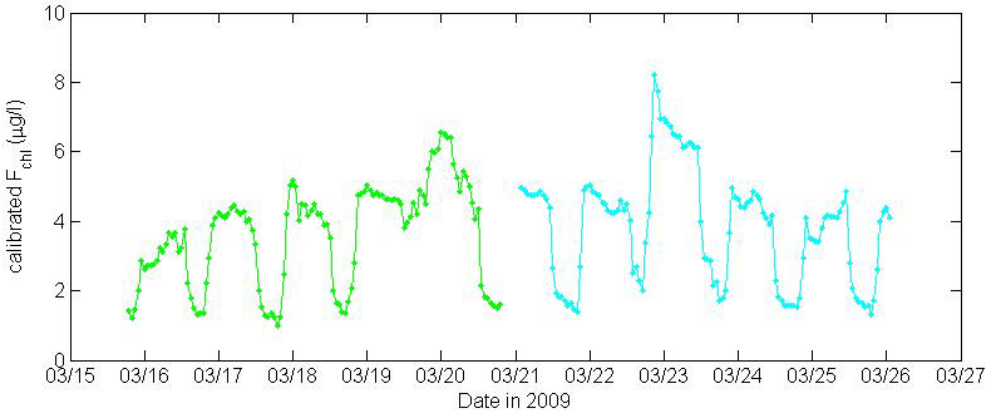


*Figure 2. Example of the transition between deployments in which the offsets between observations over the preceding week have been matched to the following week.*

**Results**

The complete hourly time series of calibrated chlorophyll fluorescence is shown in Figure 3.

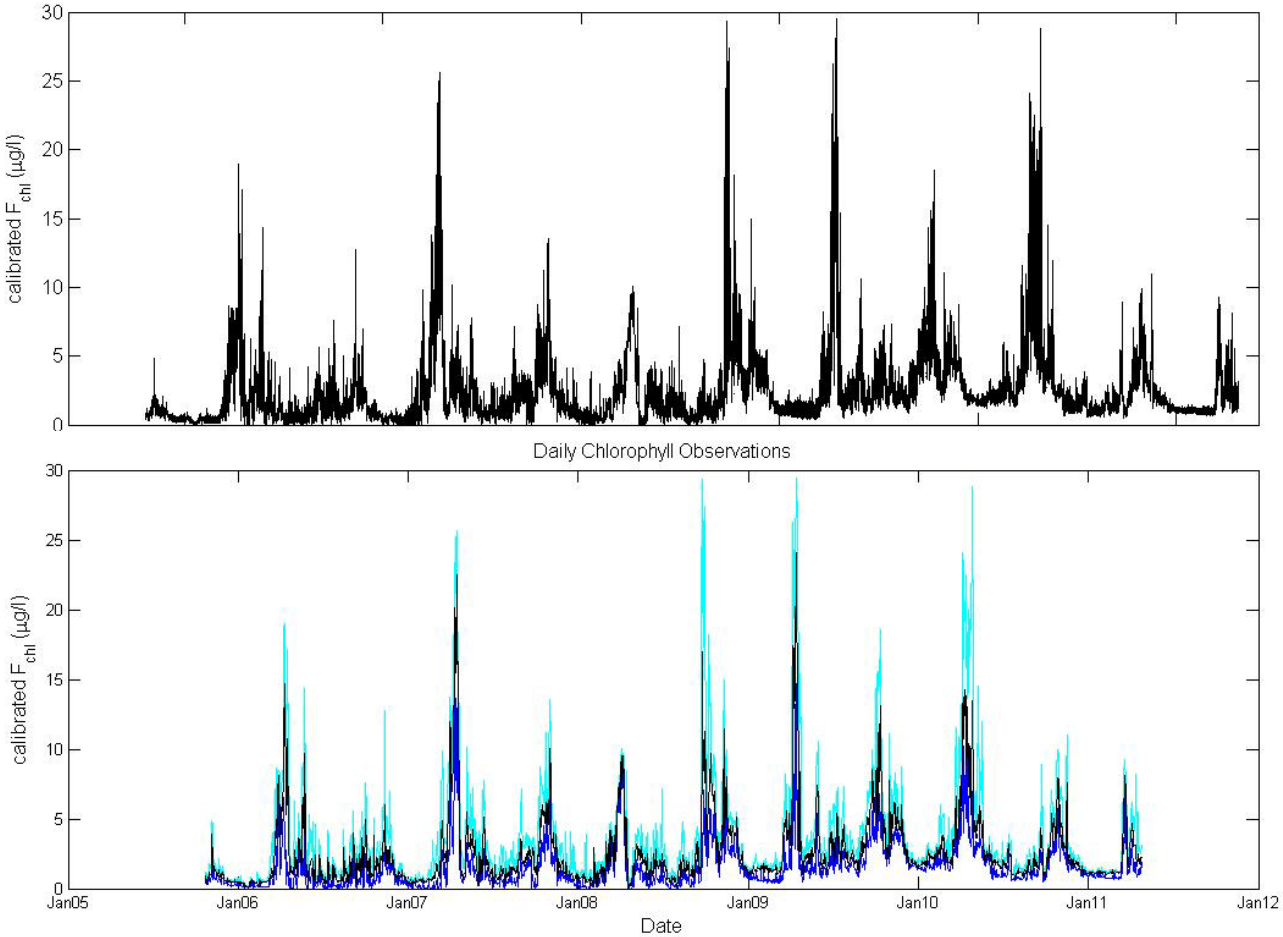


*Figure 3.* ***A.*** *Hourly observations of calibrated chlorophyll fluorescence at buoy A.*
***B.*** *Daily median values of calibrated chlorophyll fluorescence (black) bracketed by daily minima (blue) and maxima (cyan) induced by diel non-photochemical quenching.*

Spring and fall blooms are apparent each year with lower chlorophyll concentrations evident in winter due to light limitation and in summer due to nutrient limitation. Significant diel variations are due to non-photochemical quenching of fluorescence (shown in detail in Fig. 2).

**Sources of variations in the observed chlorophyll fluorescence.**

*Diel variations in apparent chlorophyll concentration*

*In vivo* fluorescence is often observed to undergo a midday non-photochemical quenching due to photoinhibition (Figure 2). This leads to apparent noisiness in the hourly observations of the time series (Figure 3A) and an apparent significant variation in the estimated chlorophyll concentration over a 24 hour period. The max/min ratio is typically about 3 but may be as high as 10. This leads to uncertainties in the estimation of actual *in situ* chlorophyll concentration and to large variations between *in situ* fluorescence-based estimates and those obtained by extraction of discrete water samples. Figure 3B shows the daily median estimated chlorophyll concentration and the daily minima and maxima which will bracket the actual values. So which value provides the best estimate of the actual chlorophyll concentration? When sensors are calibrated against a culture, the culture is sensitive to the light environment during calibration. To minimize induced non-photochemical quenching, which would lead to low fluorescence to chlorophyll ratios and ultimately overestimation of *in situ* chlorophyll, ambient lights are kept dim in the calibration room. Simulated recovery of non-photochemical quenching in response to dark adaptation indicates that an approximately 10- 15% increase in fluorescence can occur over timescales of 60 minutes, comparable to that observed *in situ*. Thus the calibration conditions are more similar to the mid morning or late afternoon conditions *in situ* and thus daily median values are likely the most robust estimate of *in situ* chlorophyll based upon non-photochemical quenching behavior.

*Seasonal variations in chlorophyll at buoy A.*

The phytoplankton community at buoy A exhibit strong spring and fall blooms (Figure 4).

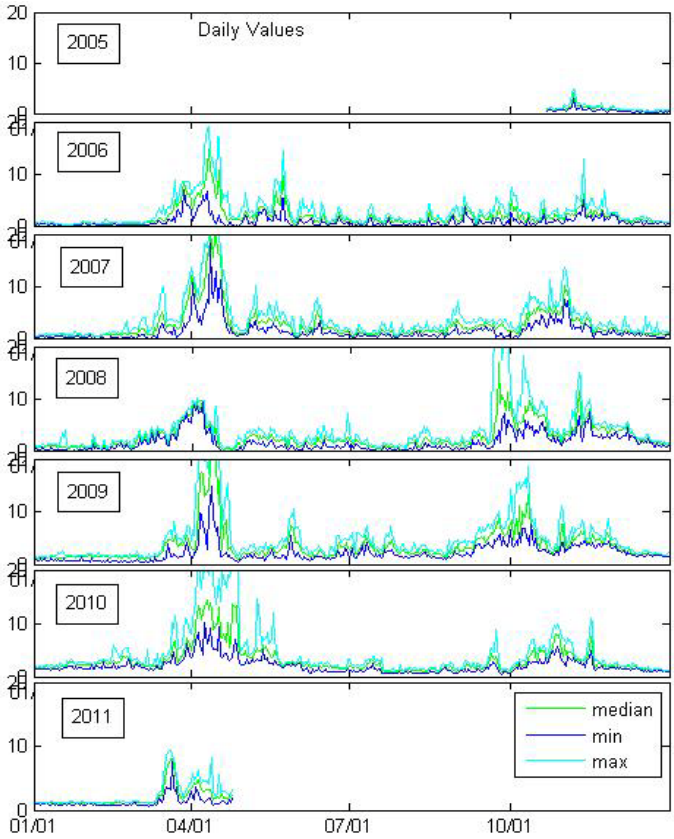


*Figure 4. Annual patterns of calibrated chlorophyll fluorescence at buoy A. Daily median, minimum and maximum values shown for each year.*

Spring blooms are initiated at the end of March and can last until May. Fall blooms generally initiate at the end of September although some years they can occur into November. 2005 and 2006, years of record autumn river discharge are notable for their near absence of fall blooms. Summer and winter time values are low, generally below 2 µg/l, while concentrations of approximately 10µg/l are observed during blooms. The daily climatological pattern is shown in Figure 5. The error bars represent the year to year standard deviation in daily concentrations. Spring blooms are more intense and shorter in duration than are the fall blooms.

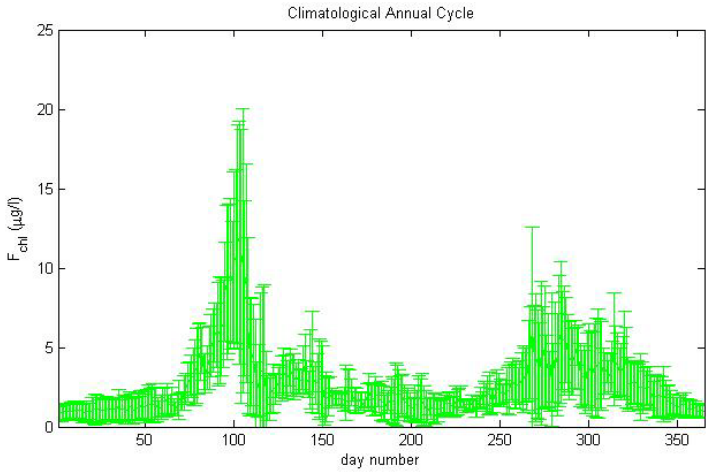


*Figure 5. Climatological daily values of calibrated chlorophyll fluorescence determined from the median daily observations from 2005 to 2011. Error bars indicate the standard deviation in daily observations over the 6 years.*

The time course of daily calibrate chlorophyll fluorescence can be used to estimate the specific growth rates of the phytoplankton population. Daily specific growth rates were computed from the daily derivative of the natural log transformed observations. As an example, the spring bloom for 2011 exhibited a classical Gaussian shape (Figure 6).

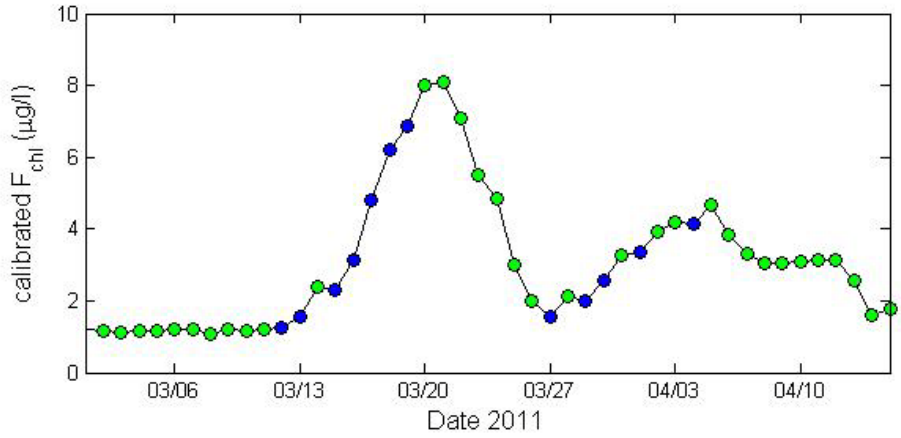


*Figure 6. Daily calibrated chlorophyll fluorescence for the spring bloom at buoy A in spring 2011. Times when daily specific growth rates exceeded 0.3 $d^{-1}$ are shown by blue symbols.*

Specific growth rates during the initial bloom and even in the secondary bloom were in excess of 0.3 $d^{-1}$. Maximum growth rates were 0.5 $d^{-1}$.

**Sources of Variability in Calibrated *in vivo* Chlorophyll Fluorescence**

In addition to variations induced by non-photochemical quenching, which are significant, there are also natural variations in the fluorescence to chlorophyll ratio induced by species

composition, light history and growth phase. In addition, *in vivo* fluorescence provides an estimate of the effective chlorophyll (i.e. the packaged chlorophyll) while the extracted chlorophyll method provides quantification of total chlorophyll. The details of these sources of variability are the subject of the paper by Proctor and Roesler (2010). Briefly, however, the fluorescence response does vary significantly between species (Figure 7).

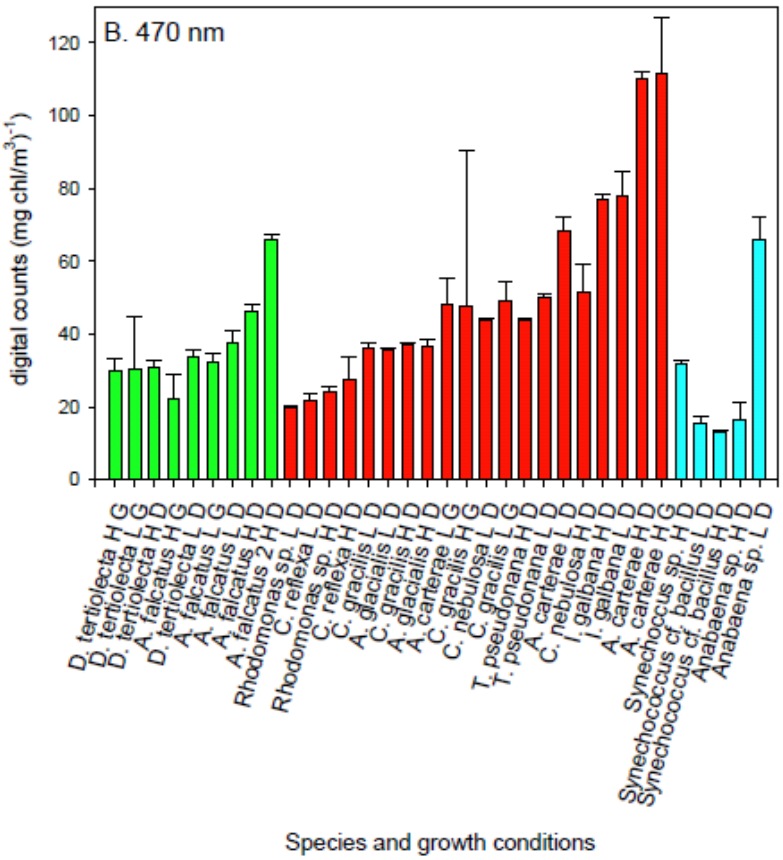


*Figure 7. Chlorophyll-specific fluorescence response (digital counts per mg chl/m$^3$) for a typical WETLabs ECO-type chlorophyll fluorometer with 470 nm LED excitation and emission centered on 695 nm for thirteen species of phytoplankton grown in high and low irradiance (H, L, respectively) and measured either at exponential growth phase or as a function of time through a growth curve(D, G, respectively). Color of bars indicate pigment-based lineage, error bars indicate standard deviation of response of each experiment (triplicate observations). From Proctor and Roesler (2010), data were rank ordered by response at 440 nm excitation.*

The thirteen species of phytoplankton used in this investigation were separated by pigment lineage into Green, Red and Cyan lineages based upon taxonomically-specific accessory pigmentation (i.e. chlorophyll b, chlorophyll c and phycobilipigments). The fluorescence response is the calibration coefficient that would be applied to the fluorometer to retrieve the chlorophyll concentration from *in situ* fluorescence observations. The variations in the calibration slope range by nearly an order of magnitude indicating that the derived chlorophyll concentration obtained from *in situ* fluorescence can be a factor of 10 different from the actual chlorophyll concentration depending upon the species used for calibration and the *in situ* phytoplankton composition. These variations are independent of the actual fluorometer used and are solely due to natural variations in the fluorescence per chlorophyll response. *Thalassiosira pseudonana* has a fluorescence response that is very close to the median response for this array of species and growth conditions. Thus, this species represents a robust estimator for natural populations. Variations in fluorescence per chlorophyll response due to photoacclimation (light history) were of order 12%, while variations due to growth phase were of order 17%. Thus even for the same phytoplankton community, variations of order 10-20% in estimated chlorophyll

concentration can be observed due to the phase of the bloom and/or stratification/mixing which will induce photoacclimation to varying light levels.

**Conclusions**

The estimation of chlorophyll concentration from *in situ* fluorometry is a complicated endeavor due to both the factors related to both the sensor and its deployment but also due to natural variations in the chlorophyll-specific fluorescence response. Issues related to the deployment of the sensor are concerned with calibrations and corrections due to biofouling and drift. Issues related to algal physiology are concerned with variations in the chlorophyll-specific fluorescence response which can be caused by species-specific variations, light acclimation variations, growth-phase variations, and non-photochemical quenching. Given these large natural variations, it is natural to ask the question, what does the *in vivo* chlorophyll fluorescence signal mean? It may be less useful to try to interpret the *in vivo* chlorophyll fluorescence in terms of chemically-extracted chlorophyll molecules. Rather, *in vivo* chlorophyll fluorescence provides a more accurate assessment of the effective chlorophyll concentration. When quenching is observed at noon time in response to high light, the reduced fluorescence provides an indication of reduced photosynthetic potential rather than actual reduced biomass. Once these diel variations are removed from the fluorescence signal, it becomes more of a biomass indicator. While the specific relationship between the fluorescence signal and the mass of chlorophyll is variable, the patterns in fluorescence provide unparalleled resolution of the dynamics of phytoplankton growth and decline.

## References

Pettigrew NR, Roesler CS (2005). Implementing the Gulf of Maine Ocean Observing System (GoMOOS), IEEE.

Proctor CW, Roesler CS (2010). New insights on obtaining phytoplankton concentration and composition from *in situ* multispectral chlorophyll fluorescence. Limnol. Oceanogr. Methods 8, 695–708.


Massachusetts Water Resources Authority
Charlestown Navy Yard
100 First Avenue
Boston, MA 02129
(617) 242-6000
http://www.mwra.state.ma.us