

# Temporal Variations of Photoacclimation

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## INTRODUCTION

Interpreting variability in fluorescence yield, defined as fluorescence per unit biomass, under different environmental conditions is problematic if fluorescence data are used to estimate chlorophyll *a* (but see Holm-Hansen *et al.*, 2000 for a solution). This variability is in part, due to light exposure, species composition, and nutrient status (Cullen, 1982) and has been well documented for a long time (eg. Keifer, 1973; Cullen, 1982). Variability in fluorescence yield with incident light can be observed by a reciprocal relationship over the course of 24 hours or as a near surface decrease in vertical profiles of fluorescence yield (Keifer, 1973; Cullen, 1982).

Near surface decrease of fluorescence yield can be measured with commercial fluorometers, such as those from WetLabs, under high irradiance conditions. One cause for this near surface decrease, is the triggering of photoprotective mechanisms when phytoplankton are exposed to high light. One photoprotective mechanism is called energy dependent non-photochemical quenching ( $q_E$  [unitless]). It competes with photosynthesis and fluorescence for light by converting pigments that would normally be used for harvesting light energy to pigments that dissipate light energy as heat. This photoprotective mechanism can operate on time scales as short as seconds to minutes, and can decrease fluorescence yield up to 75% (Sakshaug *et al.* 1987). The threshold irradiance at which  $q_E$  begins to decrease fluorescence yield ( $E_{f_{opt}}$  [ $\mu\text{mol m}^{-2}\text{s}^{-1}$ ]) is thought to be linked with the photoacclimation state of phytoplankton (Falkowski and Raven, 1997), and has been correlated with the photosynthesis saturation parameter ( $E_k$  [ $\mu\text{mol m}^{-2}\text{s}^{-1}$ ]) in lab studies (Barnett, 2005).

Being able to estimate a photoacclimation parameter with instruments common to most oceanographic cruises, gliders, AUV's, and environmental monitoring buoys will bring new high resolution (temporally and spatially) datasets that can be further used to understand its variability. Understanding the variability of  $E_{f_{opt}}$  will provide insights to the variability of  $E_k$  which could improve models of primary production. This study will provide two methods to estimate  $E_{f_{opt}}$  *in situ* with common fluorometers and examine its daily and seasonal variability.

## METHODS

The threshold irradiance at which  $q_E$  begins to observably quench fluorescence yield can be measured different ways. This study will provide two methods to estimate  $E_{f_{opt}}$ , one from vertical profiles of fluorescence and irradiance, and the other from the diel variation of near surface fluorescence yield at a fixed mooring (fig. 1). The assumptions and drawbacks of each method will be discussed.

*Profiler method*

In this study,  $E_{fopt}$  was estimated from profiles by fitting the two best fit straight lines to a vertical fluorescence yield profile (fig. 1a). The shallowest line captures the decrease of fluorescence due to  $q_E$ , and the deepest line represents fluorescence unaffected by  $q_E$ . The irradiance at the intercept of both lines is the estimate of  $E_{fopt}$ . The two best fit line model will be referred hereafter as the bilinear model, and is based on minimizing the residuals of these two equations and the fluorescence yield profile:

$$F_1(d) = b_1 + m_1 \cdot (d \cdot (d \leq d_{qE})) \quad (1)$$

$$F_2(d) = b_1 + m_1 \cdot d_{qE} + (m_2 \cdot d \cdot (d > d_{qE}) - d_{qE}) \quad (2)$$

where,  $F_1(d)$  [rfu] is a straight line relating fluorescence yield as a function of depth, with an intercept,  $b_1$  [rfu] and slope  $m_1$  [rfu ( $\mu\text{mol m}^{-2}\text{s}^{-1})^{-1}$ ], until the threshold depth  $d_{qE}$  [m].  $F_2(d)$  is the second straight line fit to the profile, with a slope of  $m_2$  [rfu ( $\mu\text{mol m}^{-2}\text{s}^{-1})^{-1}$ ] (see fig. 1a for an example but note the axis are reversed, ie: x - fluorescence, and y - depth). A MATLAB routine was used to retrieve the parameters  $b_1$ ,  $m_1$ ,  $m_2$ , and  $d_{qE}$ , with confidence limits using a least-squares fit.  $E_{fopt}$  was then estimated by finding the irradiance at  $d_{qE}$ .

This method has been applied to a data set obtained from the SeaHorse™ (Hamilton et al., 1999), which is a stationed autonomous profiling CTD with an irradiance sensor and *in vivo* fluorometer. It is moored 30 kilometers off the coast of Nova Scotia, Canada, and measures a vertical profile from 80 meters to the surface approximately once every hour. Unfortunately, the SeaHorse™ profiler does not provide an estimate of chl *a* concentration independent of *in vivo* fluorescence; a key parameter for retrieving fluorescence yield. If photoacclimation, species composition, and chl concentration are assumed constant throughout the surface mixed layer, *in vivo* fluorescence can be used in place of a calculated fluorescence yield. For this study, the mixed layer was defined from the surface to the depth at which density was 0.02 [ $\text{kg m}^{-3}$ ] higher than surface values.

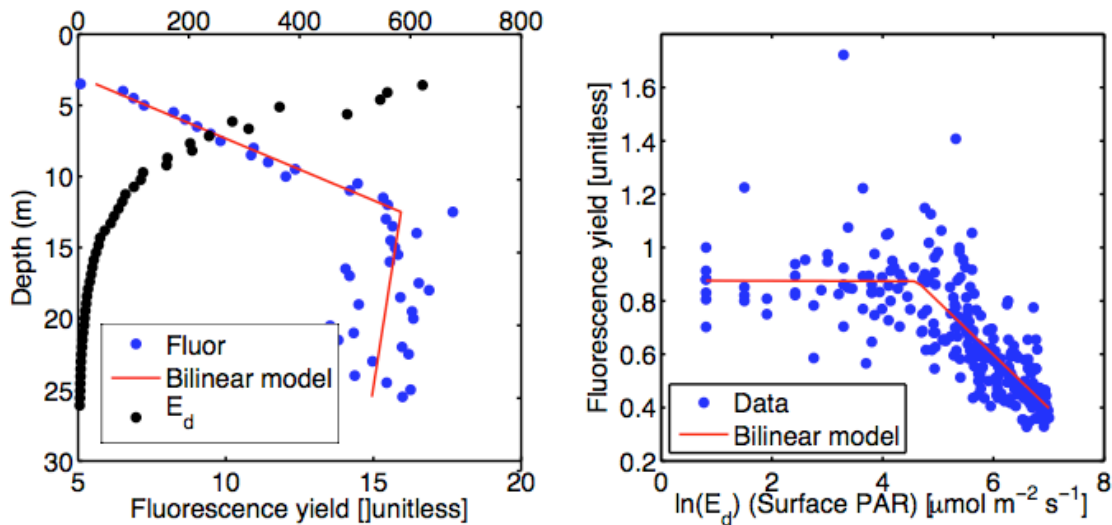
#### *Surface mooring method*

In order to obtain estimates of  $E_{fopt}$  from surface moored fluorometers the bilinear model was fit to a plot of the natural logarithm of irradiance versus fluorescence yield by replacing the *depth* ( $d$ ) variable with the natural logarithm of irradiance according to equations 3 and 4:

$$F_1(\ln(E_d)) = b_1 + m_1 \cdot (\ln(E_d) \cdot (\ln(E_d) \leq E_{fopt})) \quad (3)$$

$$F_2(\ln(E_d)) = b_1 + m_1 \cdot E_{fopt} + (m_2 \cdot \ln(E_d) \cdot (\ln(E_d) > E_{fopt}) - E_{fopt}) \quad (4)$$

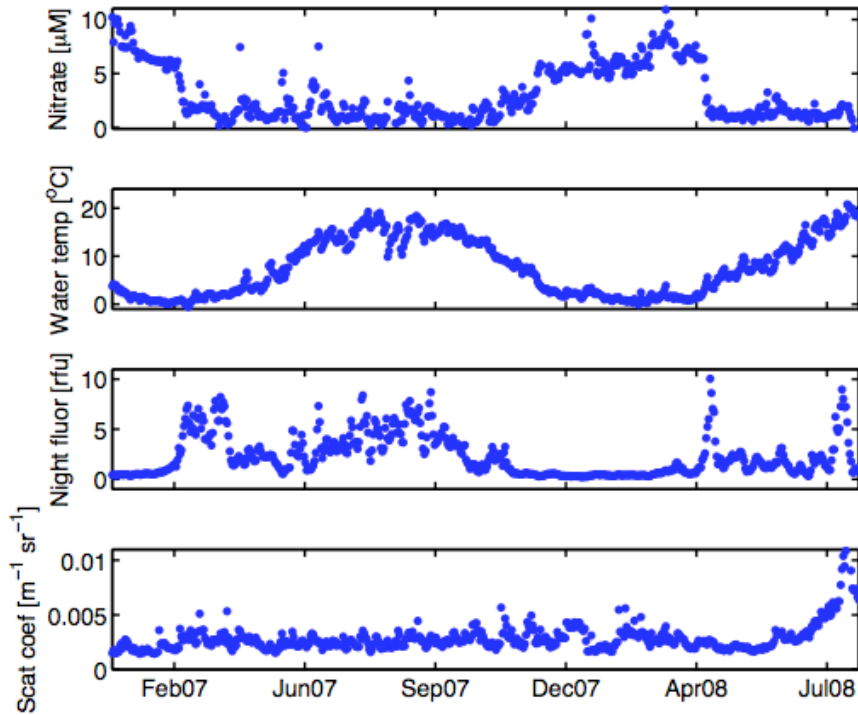
where,  $F_1(\ln(E_d))$  [rfu] is a straight line relating fluorescence yield as a function of the natural logarithm of irradiance, with an intercept,  $b_1$  [rfu] and slope  $m_1$  [rfu ( $\mu\text{mol m}^{-2}\text{s}^{-1})^{-1}$ ], until the threshold irradiance  $E_{fopt}$  [ $\mu\text{mol m}^{-2}\text{s}^{-1}$ ].  $F_2(\ln(E_d))$  [rfu] is the second straight line fit to the profile, with a slope of  $m_2$  [rfu ( $\mu\text{mol m}^{-2}\text{s}^{-1})^{-1}$ ] (see fig. 1b for an example).



**Figure 1. Two methods to estimate  $E_{f_{opt}}$ .** a) A depth profile of fluorescence yield illustrating near surface fluorescence quenching. The inflection point in the bilinear model is the depth at which  $E_{f_{opt}}$  occurs.  $E_{f_{opt}}$  is then estimated by obtaining the irradiance at that depth. b) Using fluorescence yield and irradiance data from a surface mooring the decrease of fluorescence yield can be seen as irradiance increases. An estimate of  $E_{f_{opt}}$  can be obtained from the irradiance where fluorescence yield begins to decrease.

This method has been applied to a dataset obtained from the Land-Ocean Biogeochemical Observatory (LOBO) located in the Northwest Arm, an estuary in Nova Scotia, Canada, produced by Satlantic, see Comeau *et al.* (2007) for a description. This observatory has a sampling rate of once per hour. Data from the beginning of May 2007 to the beginning of August 2008 were analyzed in this study. With a sampling frequency of once per hour, only ~12 data points can be collected during the day, therefore, 14 days of data were pooled to produce one fluorescence yield versus irradiance curve to which the bilinear model was fit to estimate  $E_{f_{opt}}$ . The same process was also repeated with one month of data pooled together to produce one fluorescence versus irradiance curve.

Since fluorescence yield cannot be measured directly, it was estimated by normalizing fluorescence data to a median of the nighttime (between 10 pm and 2 am local time) values before and after each day. This method doesn't account for any advective gains or losses to phytoplankton during the day, and ideally some other independent estimate of phytoplankton biomass would be used to normalize the data such as the scattering coefficient. Although the scattering coefficient was measured simultaneously, it did not correlate with the fluorescence signal (fig. 2), likely because the measurements take place in a relatively shallow tidally influenced estuary. This method can operate in stratified environments, however any advective processes or daily changes in photoacclimation may result in changes fluorescence yield not due to  $q_E$  and potentially lead to sources of error.



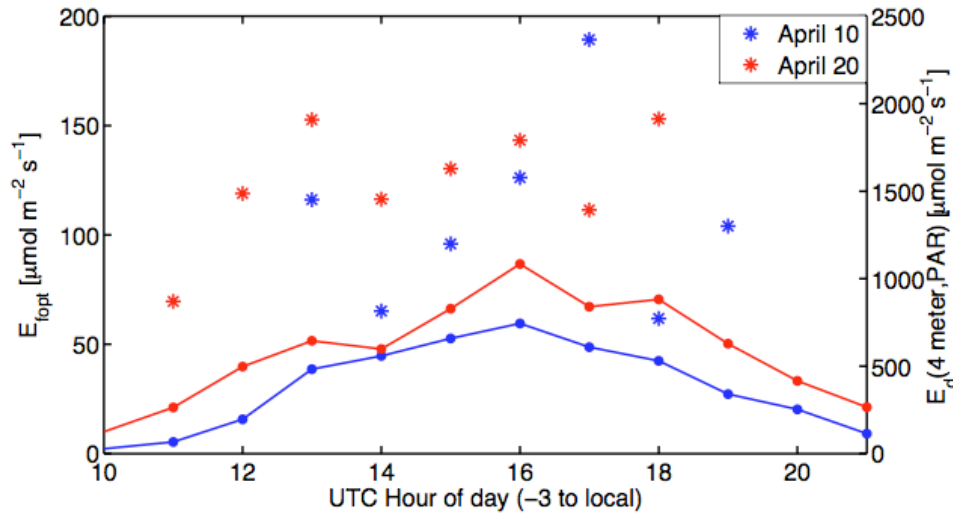
**Figure 2.** *A time series demonstrating seasonal patterns of environmental parameters measured with the LOBO. High nitrate was observed in both winters and low nitrates occurred throughout the spring fall and summer. Water temperature was low in both winters and high in the summer. Night time fluorescence which is a direct approximation of chlorophyll concentration showed two spring blooms, as well as high values in the summer of 2007. The scattering coefficient was relatively constant throughout the year, and did not covary with phytoplankton night time fluorescence.*

A difference between the surface mooring method and the profiler method is one is plotted with fluorescence yield as a function of depth, and the other a function of the natural logarithm of irradiance. The reason the profiler method was plotted as a function of depth is to eliminate the variability of the irradiance profile caused by wave focusing and defocusing of light.

## RESULTS

### *Daily variability*

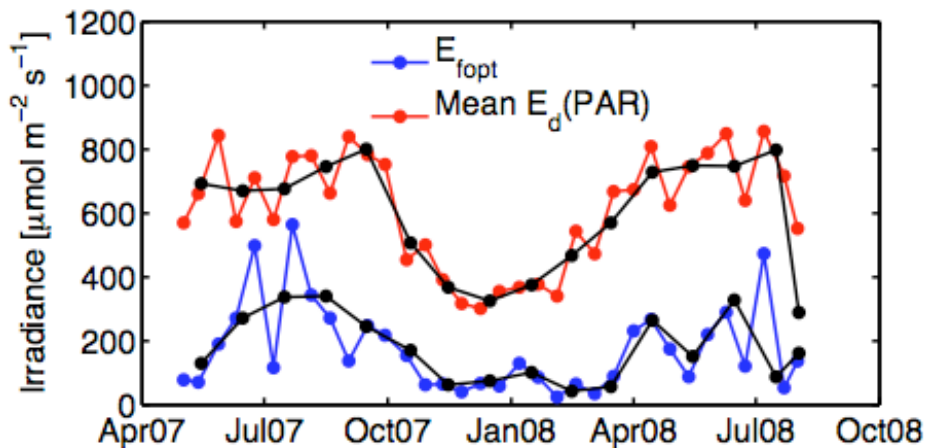
Since the SeaHorse™ profiles once an hour, hourly estimates of  $E_{f_{opt}}$  can be obtained from the profiler method, provided sufficient range of irradiance within the mixed layer. To demonstrate the daily variability of  $E_{f_{opt}}$ , data from the 10th and 20th of April were chosen since they had high surface irradiance and deep mixed layers. There is no clear daily pattern observed in  $E_{f_{opt}}$  on either of these two days (fig. 3). There was some scatter around the mean for both days. The standard deviation was 40% of the daily mean for April 10<sup>th</sup>, and 22% of the mean for April 20<sup>th</sup>.



**Figure 3.** This figure demonstrates the diel variability of  $E_{f_{opt}}$  estimated from profiles of the SeaHorse™, from two days. The stars represent estimates of  $E_{f_{opt}}$  and the lines with dots represent measured downwelling irradiance at 4 meters depth ( $E_d(4m,PAR)$ ). There was no surface irradiance sensor, so  $E_d(4m,PAR)$  is meant as an approximation of surface irradiance. The data show no clear daily trend over a wide range of near surface irradiances.

#### Seasonal variability

Using the surface mooring method the lobo was used to estimate  $E_{f_{opt}}$  once every 14 days, and once a month (fig. 4). There is more variation in the 14 day compiled estimate of  $E_{f_{opt}}$  than the one month compiled estimate, however both measures show similar seasonal patterns. By comparing fig. 2 and fig. 4 estimates of  $E_{f_{opt}}$  follow the same seasonal patterns as surface water temperature, and to a lesser extent nitrate. As expected, our estimates of  $E_{f_{opt}}$  are higher in the summer than in the winter.



**Figure 4.** This figure demonstrates the seasonal variability of  $E_{f_{opt}}$  estimated from the LOBO. The blue line represents an estimate of  $E_{f_{opt}}$  by the surface mooring method, compiling 14 days worth of data and the red line is the average irradiance during that time period. The black line is similar in both cases however one month of data was compiled instead of 14 days. As expected, winter estimates of  $E_{f_{opt}}$  are lower than summer estimates.

## DISCUSSIONS

### *Limitations and assumptions of the profiler method to estimate $E_{f_{opt}}$*

Both methods of estimating  $E_{f_{opt}}$  require knowledge of fluorescence yield, and assumptions have to be made. In this study, the profiler method assumed that the mixed layer was mixing fast enough to provide vertically uniform photoacclimation, species composition and chlorophyll *a* concentration, so fluorescence yield is assumed from the fluorescence profile. If one would want to use this method in stratified environments and obtain estimates of fluorescence yield by normalizing fluorescence to some independent measure of phytoplankton biomass, it is important to note that since fluorometers measure fluorescence from chlorophyll *a* an independent measure of chlorophyll *a* that is not influenced by  $q_E$  must be used as the denominator. If backscattering, which has been correlated to chlorophyll *a* concentration (Huot *et al.*, 2008), is used to normalize fluorescence, depth changes in the carbon to chlorophyll *a* ratio (Fennel and Boss, 2003) could mask changes in fluorescence yield due to  $q_E$ . The profiler method for estimating  $E_{f_{opt}}$  is recommended only for sufficiently mixed water columns.

### *Limitations and assumptions of the surface mooring method to estimate $E_{f_{opt}}$*

Fluorescence yield was obtained by normalizing daytime fluorescence by the median nighttime fluorescence of the night before and after each day. This method implicitly assumes that fluorescence would be constant throughout the day in the absence of  $q_E$ . Of course there are many reasons for fluorescence not to be constant, such as growth, death, advection, and photoacclimation and these add scatter to the fluorescence yield versus irradiance plots, however, when many days of data are pooled together, an average  $E_{f_{opt}}$  is apparent from the plot.

## **CONCLUSIONS**

Two methods to estimate  $E_{f_{opt}}$ , a measure of photoacclimation, that rely on commonly used oceanographic instruments, a fluorometer and a radiometer, have been described. Using these two methods, the daily and seasonal variability of  $E_{f_{opt}}$  was examined. This method is promising because its simple requirements allow it to be obtained from surface moorings, vertical profiles, and even from the new generation of optically equipped gliders. Further studies that correlate field estimates of  $E_{k_s}$ , the light saturation parameter, with  $E_{f_{opt}}$  will be important for possible use in models of primary productivity.

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