Chapter

# Chlorophyll Estimation from Fluorescence Vertical Profiles in Ocean

*Romaissa Harid, Hervé Demarcq and Fouzia Houma-Bachari*

## Abstract

The present study deals with the correction of chlorophyll-a (Chl-*a*) estimated from fluorescence data, the proposed method test for the first time a ratio between Chl-*a* from high-performance liquid chromatography (HPLC) measurements and its corresponding fluorescence. Considering the variability of this ratio with depth, the adjustment of fluorescence data was greatly improved. This ratio increase in the oceanic surface layer probably because of the quenching effect, however, it decreases and becomes stable with depth. This approach can be used to correct fluorescence values for future large datasets of biological variables. Finally, this method is designed for a global scale and/or regional applications.

**Keywords:** chlorophyll, linear regression, euphotic layer, Algerian Basin, OLS, HPLC

## 1. Introduction

The study of chlorophyll-a (Chl-*a*) vertical structure in oceanic water bodies has been an important effort in marine biological and ecological research. Quantitative relationships between Chl-*a* concentration from high-performance liquid chromatography (HPLC) measurements and corresponding fluorescence (Fluo) profile have not thus far been considering the ratio between these two parameters. Other methods have been also proposed by [1–4] which did not consider this ratio to correct the Fluo data. Chlorophyll pigment studies using HPLC provide information about oceanic phytoplankton pigments [5–7], but is limited by the number of vertical punctual measures (generally sampled each 20 m of depth). However, the Fluo profiles are continuous measures (generally measured every 1 m of depth) and can be used to determine the vertical structure of Chl-*a* in the euphotic layer (the Fluo can be used as a proxy of Chl-*a*).

In our work, we propose for the first time a new method correct the Chl-*a* concentration estimated from Fluo measurements. The approach is based on the study of the variability of the ratio between Chl-*a* concentrations estimated from HPLC analysis and the Fluo measured at the same time as the Chl-*a* sample for HPLC.

## 2. Methods

### 2.1 Chl-a from fluorescence profiles

The Chl-*a* pigment data are obtained from the SOMBA-GE-2014 cruise [8] in the AB (**Figure 1**) (more details about the cruise are available at: https://doi.org/10.17600/14007500). The data set included 263 Chl-*a* offshore waters sampled

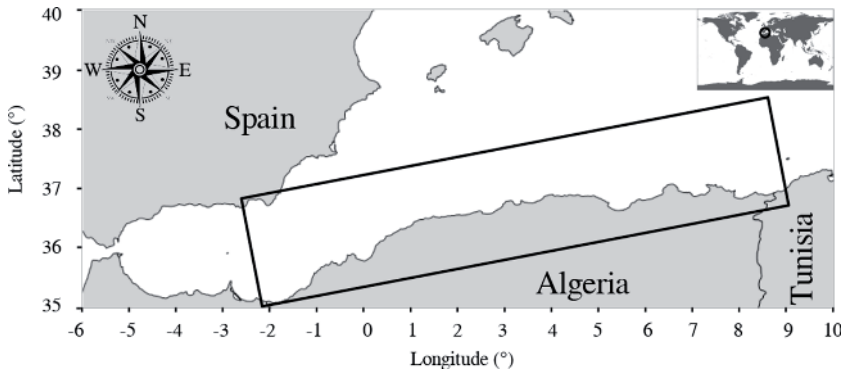


**Figure 1.**
*Geographical localization of Algerian Basin.*

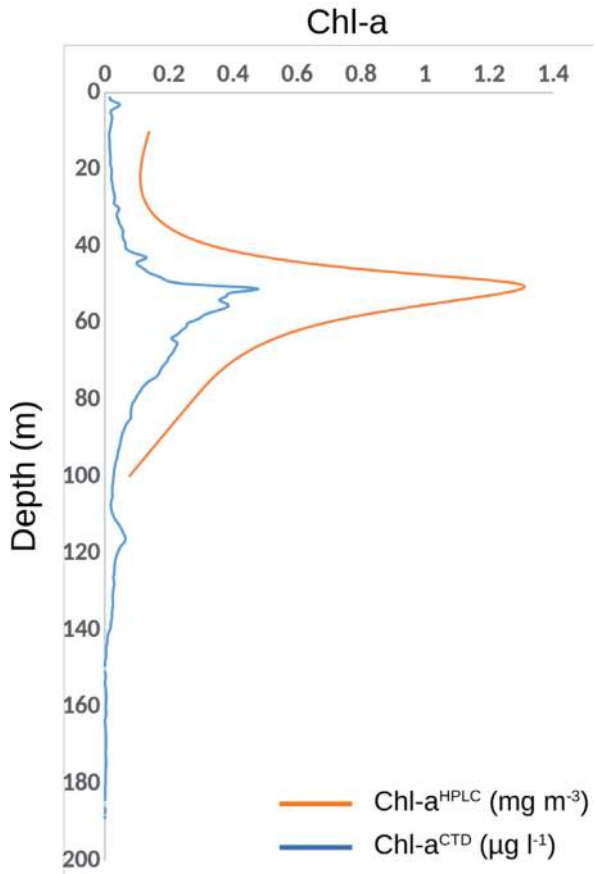


**Figure 2.**
*The vertical distribution of Chl-a concentration from Fluo (in blue) and HPLC (in orange) measurements.*

stations and ranged from 0.0007 to 2.3937 mg $m^{-3}$. The Chl-*a* pigment determinations were made using HPLC 1200 instrument. The Chelsea Acquatracka fluorometer was systematically operated in conjunction with a conductivity-temperature-depth (CTD) sensor. In our work, the vertical profiles of algal fluorescence (Fluo) were converted into equivalent Chl-*a* profiles using the HPLC measurements. The Chl-*a* values were computed by regressing the vertical continuous Fluo ($Fluo^{CTD}$) and punctual Chl-$a^{HPLC}$ profiles from the surface to 100 m.

### 2.2 Datasets normality test

The application of the normal law on a datasets is a classic step to model the relationship between two variables. In our work, we explore the normality test between Chl-$a^{HPLC}$ and $Fluo^{CTD}$ data, to determine the best linear regression model that fits our datasets. The difference between Chl-$a^{HPLC}$ and $Fluo^{CTD}$ data is illustrated in **Figure 2**, it shows the difference between these two measures. For this, we applied two normality tests: Shapiro-Wilk and Khi-2 Pearson.

### 2.3 Quenching effect

Several methods have been proposed to correct the non-photochemical quenching (NPQ) effect [1, 9, 10]. In our work, we used a new method that consist to divide the water column (from surface to 100 m) according to the variability of the ratio (Chl-$a^{HPLC}$/$Fluo^{CTD}$) calculated at each depth of the vertical profile where a HPLC measurements are available.

## 3. Results and discussion

### 3.1 Linear regression between $Fluo^{CTD}$ and Chl-$a^{HPLC}$

The results of *p*-values (**Table 1**) reflect that the Chl-$a^{HPLC}$ and $Fluo^{CTD}$ data do not follow the normal law.

In our situation, we cannot apply any simple linear regression (the data do not follow the normal law). However, simple linear regressions exist that can be applied when the data do not follow the normal law [11–13]. In contrast, the simple model-II linear regression uses the following methods: major axis (MA), standard major axis (SMA), ranged major axis (RMA), and ordinary least squares (OLS). The first three methods require the bivariate normal data [13], which is not our case. Only the OLS can be applied if the distribution of data is not normal [12]. The least absolute deviations (LAD) method also does not require the bivariate normal data. Indeed, we also tested the LAD [14] method between the Chl-$a^{HPLC}$ and $Fluo^{CTD}$ *in-situ* data.

| | **Chl-$a^{HPLC}$** | **$Fluo^{CTD}$** | **$Log_{10}$ (Chl-$a^{HPLC}$)** | **$Log_{10}$ ($Fluo^{CTD}$)** |
|---|---|---|---|---|
| Shapiro-Wilk | $2.2*10^{-16}$ | $2.2*10^{-16}$ | 0.01618 | 0.0000001068 |
| Khi-2 Pearson | $2.2*10^{-16}$ | $2.2*10^{-16}$ | 0.0009629 | 0.00117 |

**Table 1.**
*Results of normality tests.*

The LAD method minimizes the sum of the absolute deviation and not the sum of square deviation, contrary to OLS.

To better estimate the Chl-*a* continuous profiles from fluorescence profiles, we applied both previous regression models (OLS and LAD) separately on all water columns. The result shows that the OLS method was slightly better them the LAD method. This last one underestimates the Chl-*a* values in the surface layer. In our study, the OLS method is most adequate to estimate the Chl-*a* from Fluo. The OLS regression is one of the major techniques used for estimating the parameters of a mixed-effects model (to assess the agreement and the measurement errors between the two variables [15]) and is effective for estimating the slope and intercept [16, 17].

### 3.2 Chlorophyll quenching index

The average ratio ($Chl\text{-}a^{HPLC}/Fluo^{CTD}$) is regularly decreases with depth according to the light intensity as shown in **Figure 3** which represents the chlorophyll quenching index (CQI) classes from 230 concomitant measurements of chlorophyll and fluorescence. This gradient could be explained by the presence of NPQ which represents a serious problem in the Chl-*a* profiles on the surface [18].

Three depth classes were determined (**Table 2**) to quantify CQI as shown in **Figure 3**. For each CQI depth class, the $Fluo^{CTD}$ values are transformed into Chl-*a* using a different OLS linear regression as shown in **Figure 4**, which illustrate method of the correction and the adjustment of Fluo data using three different models.

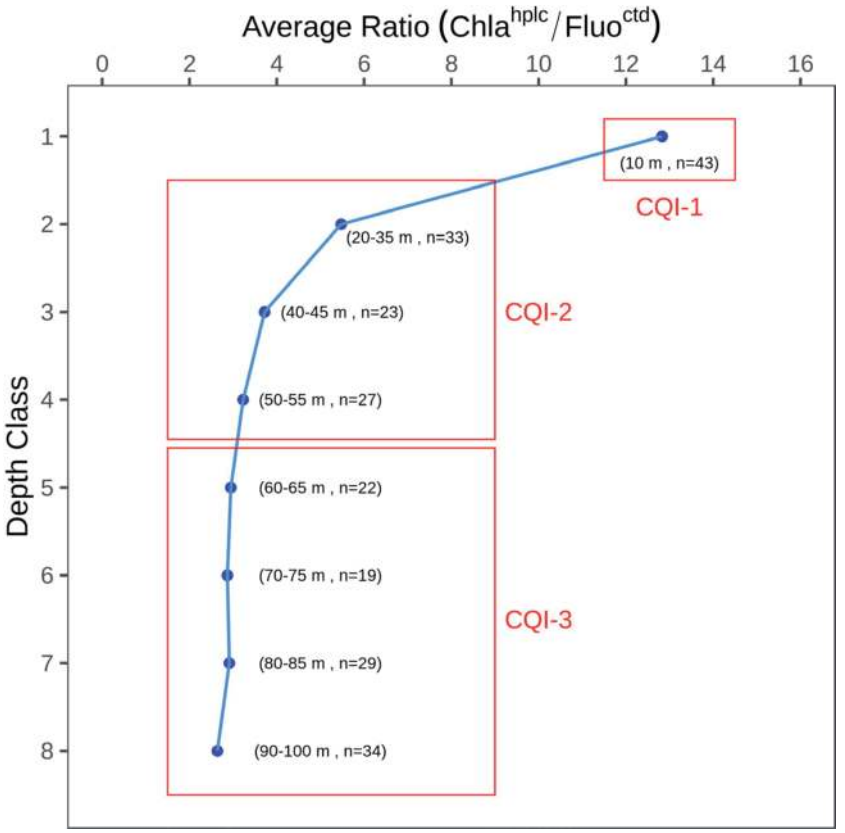


**Figure 3.**
*The chlorophyll quenching index (CQI) from the average ratio ($Chl\text{-}a^{HPLC}/Fluo^{CTD}$) into three depth classes from 230 concomitant measurements of chlorophyll and fluorescence.*

| Model | Class | Depth (m) | No. point | Chl-*a* (mg $m^{-3}$) | Slope | *r* | RMSD |
|---|---|---|---|---|---|---|---|
| OLS-1 | CQI-1 | [0–10] | 43 | [0.074, 1.000] | 3.716 | 0.95 | 0.042 |
| OLS-2 | CQI-2 | [10–60] | 83 | [0.156, 2.815] | 2.127 | 0.91 | 0.151 |
| OLS-3 | CQI-3 | [60–100] | 104 | [0.101, 1.941] | 2.490 | 0.95 | 0.100 |

**Table 2.**
*Models parameters using to estimate the Chl-a profiles from Fluo.*

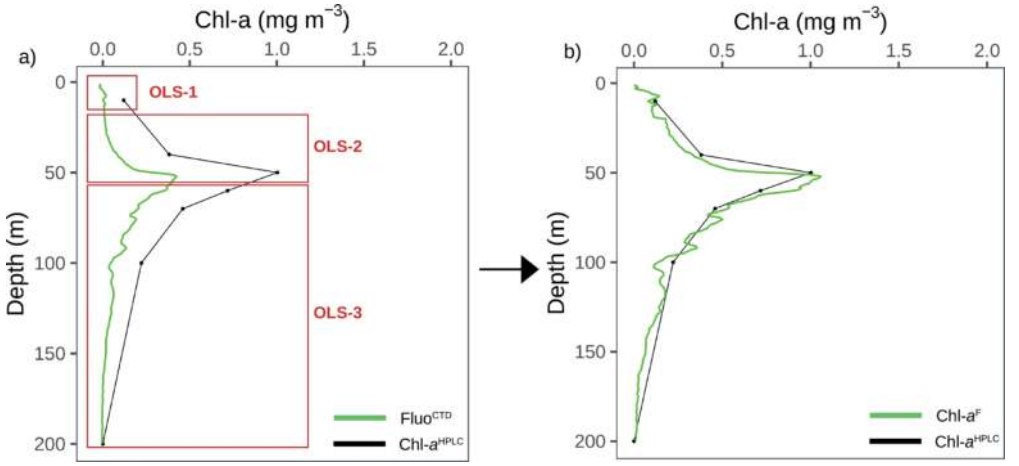


**Figure 4.**
*Illustrating method of the correction and the adjustment of Fluo data. (a) the OLSs applied for each CQI classes. (b) the Chl-a profile after correction.*

During summer, Chl-*a* concentration near the surface is very low and the Chl-*a* maximum is deeper (**Figure 4**).

## 4. Conclusion

The punctual measurements of Chl-*a* concentrations designated to HPLC analysis, requires important investigation mean and generally impractical to sampled seawater each 1 m between the surface and the maximum depth of the euphotic layer. In some regions, this depth can exceed 100 m. The fluorescence measurements are very complementary to HPLC measurements for marine biological studies related to chlorophyll pigments. **Figure 5** shows clearly the readjustment of fluorescence data which are very close to HPLC data. Considering the variability of the ratio (Chl-$a^{HPLC}$/Fluo$^{CTD}$), we were greatly improved the adjustment of the fluorescence data. In contrast, the ratio is high in the surface layer and becomes stable with depth. This increase of ratio in the surface, which is strongly related to the presence of the quenching effect, is corrected by an OLS model typical only for this part of the ocean layer.

However, the evolution of this ratio with depth is very important to determine the alternative deeper adjustment models other than that of the surface. This new method is to generate high-quality datasets used for different goals of biological marine study which are related to the first link of the marine food chain, that is, the phytoplankton production. However, our method is designed for a global scale and/or regional

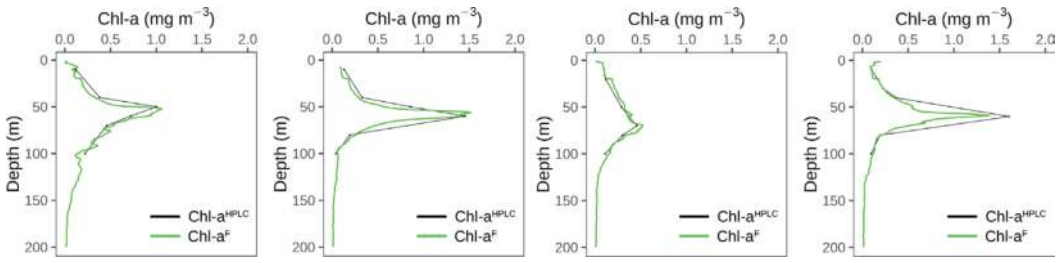


**Figure 5.**
*Comparison of Chl-a profiles measured at four stations from HPLC measurements (black line) and Chl-a adjusted from fluorescence (green line).*

applications. It is important to note that the night Chl-*a* measurements could give stable ratios from the surface to the bottom (the quenching effect is absent during the night).

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## Conflict of interest

The authors declare no conflict of interest.

## Ethical approval

All applicable international, national guidelines for the care and use of datasets were followed.

## Data availability statement

The SOMBA-GE-2014 cruise data supporting **Figures 2-4**, are available on request from Dr. Laurent Mortier (more details about the cruise are available at: https://doi.org/10.17600/14007500).

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