DISCUSSION ON FLUORESCENCE CHLOROPHYLL-A DATA DMQC

DECEMBER 2018

ADMT-19
Chlorophyll-a fluorescence

\[ F(\lambda_{em}) = E(\lambda_{ex}) \times [\text{Chla}] \times a^{*}_{ph}(\lambda_{ex}) \times \phi \times Q^{*}_{a}(\lambda_{em}) \]

Emission Light intensity \( E(\lambda_{ex}) \) is assumed constant (If no sensor decay);

Reabsorption coefficient \( Q^{*}_{a}(\lambda) \) is close to 1 at 700nm (Receiver band of Wetlabs ECO Chla fluorometer);

Fluorescence quantum yield \( \phi \) is related to physiology;

Specific-absorption coefficient \( a^{*}_{ph}(\lambda_{ex}) \) is related to physiology, algal species, and package effect.

IF \( (a^{*}_{ph}(\lambda_{ex}) \times \phi) \) is assumed as constant, a linear relationship between Fluorescence signal \( (F(\lambda_{em})) \) and [Chla] may be expected.
ECO Chlorophyll-a Fluorometer

1. Linear assumption and calibration
   \[ F_{\text{Chla}} = \text{Slope} \times (\text{Counts} - \text{Dark}) \]

2. Calibration

   “The relationship between fluorescence and chlorophyll-a concentrations in-situ is highly variable. The scale factor listed on this document was determined using a mono-culture of phytoplankton (Thalassiosira weissflogii). The population was assumed to be reasonably healthy and the concentration was determined by using the absorption method. To accurately determine chlorophyll concentration using a fluorometer, you must perform secondary measurements on the populations of interest.” – ECO calibration sheet
FChla Data correction needs to consider of

1. Dark correction
   (Due to the change of dark currents of sensor on float)

Xing et al. (2014) JGR
BACKGROUND 3

- FChla Data correction needs to consider of

  2. NPQ correction

  (If profiling at daytime, Due to the fluorescence dynamics of in vivo chlorophyll-a)

Sackmann et al. (2008) BGD
FChla Data correction needs to consider of

3. Slope correction

(Due to the factory-calibration issue and fluorescence variability)

Proctor and Roesler (2010) LOmet

Roesler et al. (2017) LOmet
1. Dark correction

Based on the median of deep FChla profile
2. NPQ correction

Xing12 extrapolation method

(Based on MLD)

Figure 4: We illustrate the following part of the flowchart of the RTQC for the Chlorophyll-A. (Following equation 6, on the Figure 2)
3. Slope correction

Roesler factor = 0.5

Fig. 2. Mean slope factors derived from observations of paired HPLC
and in situ Chl fluorescence from major oceanographic regions (Table
1). Error bars indicate 95% confidence limits on slope from linear regres-
sion of all observations within each region. Lines indicate slope factors of
1 (solid) and 2 (dotted).

Roesler et al. (2017) LOmet
NOW, IN DMQC

- All these processing above are SIMPLE & ROBUST and potentially adapted for RTQC;
- But in DMQC, we re-consider if they are sufficient enough or not.
1. DARK CORRECTION IN DMQC

- Option 1: On-Float-Measurement (OFM)?
  - FC-Dark = Factory-Calibrated Dark
  - OFM-Dark = On-Float-Measured Dark

<table>
<thead>
<tr>
<th>WMO</th>
<th>ECO_SN</th>
<th>FL_FC-Dark</th>
<th>FL_OFM-Dark</th>
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</table>

Difference of 3-5 counts, which means, for FChla, 0.02 to 0.035 mg m$^{-3}$ (without considering of the slope correction)
1. DARK CORRECTION IN DMQC

- Option 1: On-Float-Measurement (OFM)?

Using the OFM dark, we get a closer-zero deep values (900-950m).

It means, then, we could better evaluate the “deep fluorescence” signal (which appears in the OMZs and subtropical gyres).

If possible, could we ask the float manufacturer to do OFM?
1. DARK CORRECTION IN DMQC

- Option 2: Median of all minima (MAM) for each float?

_Xing et al. (2014) JGR_
1. DARK CORRECTION IN DMQC

Option 3: Minimum-offset correction (MOC) for each profile?

- Wojtasiewicz et al. (2018) JMS
- Xing et al. (2011) JGR
- Xing et al. (2017) Lomet
- Wojtasiewicz et al. (2018) JMS

Diagram images showing chl-α concentration profiles with and without correction.
1. DARK CORRECTION IN DMQC

- Option 0: Keep RTQC dark correction
- Option 1: On-Float-Measurement (OFM)
- Option 2: Median of all minima (MAM) for each float
- Option 3: Minimum-offset correction (MOC) for each profile
- Other Options?
2. NPQ CORRECTION IN DMQC

- Backgrounds:
  1. Xing12 has the “over-correction” issue in very deep waters
  2. Xing12 (and other extrapolation methods) cannot solve the NPQ correction in the shallow mixing waters (NPQ effect in the stratified layer)

- Option 1: Xing18?

  Improved correction for non-photochemical quenching of *in situ* chlorophyll fluorescence based on a synchronous irradiance profile

  *Xiaogang Xing, ¹,* Nathan Briggs, ² Emmanuel Boss, ³ and Hervé Claustre ⁴
2. NPQ CORRECTION IN DMQC

- **Xing18:**
  1. Using PAR profile to determine a NPQ threshold depth ($z_{i\text{PAR15}}$), the extrapolation of Xing12 is applied from surface to $\min(z_{i\text{PAR15}}, \text{MLD})$ for well-mixing waters

\[
X_{12+}(z) = \begin{cases} 
F\text{Chla}(z_{X_{12}}) & (z \leq z_{X_{12}}) \\
F\text{Chla}(z) & (z > z_{X_{12}})
\end{cases}
\]

2. Using PAR profile and empirical relationship (XB18) to correct NPQ for shallow-mixing waters (DCM$>\text{MLD}$)

\[
XB_{18}(z) = \begin{cases} 
\frac{F\text{Chla}(z)/\left(0.092+0.908/\left(1+(i\text{PAR}(z)/261)^{22}\right)\right)}{(z \geq 10m)} & (z \geq 10m) \\
XB_{18}(z=10m) & (z < 10m)
\end{cases}
\]
2. NPQ CORRECTION IN DMQC

Xing18:

If PAR observation is unavailable, Plant18 is suggested, i.e. Estimate first the Kd(PAR) profile, and then euphotic layer depth ($z_{eu}$), finally apply Xing12 in the layer from surface to $\min(z_{iPAR15}, MLD)$.

\[
\begin{align*}
[Chla] &= FChla \times 0.5 \\
K_d(PAR) &= 0.0232 + 0.074 \times [Chla]^{0.674} \\
&= z \left( \exp \left( -\int_0^z K_d(PAR) \, dz \right) = 0.01 \right) \\
&= z \left( FChla = \max \left( FChla \left( z \leq \min(MLD, z_{eu}) \right) \right) \right) \\
P18(z) &= \begin{cases} 
FChla(z_{P18}) & (z \leq z_{P18}) \\
FChla(z) & (z > z_{P18}) 
\end{cases}
\end{align*}
\]
2. NPQ CORRECTION IN DMQC

- Option 0: Keep Xing12
- Option 1: Xing18 based on irradiance
- Option 2: Josh’s method based on estimated euphotic layer depth (zeu) for other percentage light level (like 2% or 5%)
- Other Option?
3. SLOPE CORRECTION IN DMQC

- **Option 1: Onboard-based correction**
  - 1. Water-sampling at deployment place
  - 2. [Chla] is determined by HPLC or fluorometery
  - 3. A linear regression on [Chla] vs. FChla (after Dark and NPQ correction) without intercept to acquire SLOPE

\[
[\text{Chla}] = \text{SLOPE} \times \text{FChla}
\]

HPLC measurement is not valid always, and in fact, very few floats have HPLC matchup data.
3. SLOPE CORRECTION IN DMQC

- **Option 2: Satellite-based correction**
  1. Ocean Color Satellite remotely-sensed [Chla] product (like AMODIS OCI) at same position and same day of each profile float
  2. Get the ratio between OCI [Chla] and Surface FChla (after Dark and NPQ correction)

\[
Slope_i = \frac{[\text{Chla}]}{\text{FChla}}
\]

  3. SLOPE is determined with the median of \(Slope_i\) for each float

\[
\text{SLOPE} = \text{median}(Slope_i)
\]
3. SLOPE CORRECTION IN DMQC

- Option 3: Irradiance-based correction?

\[ F \text{ factor (F490)} \text{ retrieved from Ed490 profile based on Xing et al. (2011) JGR} \]
3. SLOPE CORRECTION IN DMQC

- Option 0: Keep to use the Roesler factor
- Option 1: Onboard-based correction
- Option 2: Satellite-based correction
- Option 3: Irradiance-based correction
- Other Options? or Any Priority?
MBARI floats are dominated by SOCCOM floats in the Southern ocean.
How well do we know Chlorophyll from fluorescence? Fluorescence : Chlorophyll ratio variability

Josh Plant, Tanya Maurer & Ken Johnson
Monterey Bay Aquarium Research Institute

ADMT19
December 5, 2018 San Diego, CA
Current MBARI protocol
(decision from ADMT18, Hamburg Germany)

RAW DATA (CHLA)
• CHLA = (Fluorescent Counts – Dark Counts) * Scale Factor
• CHLA_QC = 3

ADJUSTED DATA (CHLA_ADJUSTED)
• 1st 5 profiles > 900m?
• if YES determine In situ Dark Counts:
  = median of profile minimums (any depth)
  use instead of factory Dark Counts for all profiles
• Instrument bias correction: Float CHLA * 0.5 (Roesler et al. 2017)
• Correct for NPQ on median filtered profile (Xing et al. 2012)
  pad edges with raw, dp >3m bin = 3, otherwise bin = 5
• Add “spike data” back to profile (not NPQ corrected!)
• Set adjusted QC flags to 5 for NPQ corrected data
• QC flag for the rest of CHLA data 2 (?)
Seabird FLBB & MCOMS:

Benefits:
precise, robust, fast, easy to use & relatively affordable phytoplankton biomass proxy (Fluor => Chl => Phyto C)

Data interpretation issues:

• **Calibration bias** – chlorophyll over estimated
  
  *Roseler et al. 2017*

• **Non photochemical quenching** – CHLA underestimated
  
  *Sackman et al. 2008, Xing et al. 2012, Bierman et al. 2015, Thomalla et al. 2018, Xing et al. 2018*

• **Non chlorophyll fluorescence** – CHLA overestimated
  
  *Xing et al. 2017*

• **Variable fluorescence : chlorophyll A ratio** – poor accuracy
Chlorophyll A from fluorescence

\[ CHLA = \frac{\text{returned Fluorescence}}{\text{sensor excitation light}} \times \frac{1}{a^* \cdot \Phi_f} \]

\( a^* \) = chlorophyll a specific absorption coefficient
\( \Phi_f \) = quantum yield of fluorescence
Chlorophyll A from fluorescence

\[ CHLA = \frac{\text{returned Fluorescence}}{\text{sensor excitation light}} \times \frac{1}{a^* \phi_f} \]

\( a^* \) = chlorophyll a specific absorption coefficient
\( \phi_f \) = quantum yield of fluorescence

Major assumption: \( (a^* \phi_f) \) is constant!
What causes the absorption coefficient & quantum yield to change?

- Species composition (type, cell size, pigment distribution)
- Phytoplankton health (Fe, nutrient status)
- Light level
- Depth in water column
- Time of year

13 monospecific lab cultures
Different light levels & growth phases
6x range in Fluor / Chl ratio!

from: Roesler and Barnard, 2013

from: (Roesler et al., 2017)
What can we learn from the SOCCOM project?

~170 paired HPLC T_CHLA & profiling float samples from Southern Ocean

- **HPLC analyses** - considered the “gold standard” to reference

- **Float data processing:**
  - in situ dark count corrected
  - NPQ corrected (Xing et al. 2012)
  - interpolated to the bottle sample depth

- **Gain = Float CHLA / HPLC CHLA** (point measurements!)

- **SOCCOM floats** – a subset of BGC Argo floats from the Southern Ocean
- **HPLC Pigment samples** - from bottle casts during float deployments
- **HPLC pigment data** from NASA SeaBass, search “SOCCOM”
Gain variability in the Southern Ocean

- Point measurements of gain vary by 10x!
- No apparent correlation with latitude
- Gain variability highest between 70S & 50S
Gain variability within profiles

- Within profiles & within the surface mixed layer gains are constant
- Between profiles they are not
Gain variability within profiles

- Gain often decreases dramatically below the mixed layer (in these cases all close to 2)
- Point ratios get noisy at low concentrations

![Graphs showing variability in ratios across different WMO numbers](image-url)
Is there a relationship between HPLC & float CHL?

<table>
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<th></th>
<th>$m$</th>
<th>$b$</th>
<th>$R$</th>
<th>$my$</th>
<th>$by$</th>
<th>RMSE</th>
<th>MAPE</th>
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<td>-0.19</td>
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<td>0.43</td>
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<td>0.04</td>
<td>1.75</td>
<td>0.73</td>
<td>195.76</td>
</tr>
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</table>
Why so much scatter in regression data? How good is the gold standard?

If POC & Chl are correlated.....
bottle vs bottle should have a better correlation than float vs float –
This is not the case!

If gain variability is due to phytoplankton physiology fluor chl vs BBP
should have similar variability as fluor vs HPLC Tot Chl a.
This is also not the case!
How good is the gold standard?

- Float BBP is also well correlated to bottle POC
- The worst correlate to any parameter is HPLC Tot Chl a!
- If HPLC analysis in the lab is sound, sample handling & transport may be adding to the variability in HPLC measurements & to the variability in gain?
Float CHL vs modeled gain?

- Linear & log fits show a rapid gain increase at low CHLA
- Does this make sense?
- If real should show up in Float chl vs BBP. It does not
- Suggest poor correlation at low concentration
Gain Variability Summary

• Southern ocean bias in gain appears greater than Roesler et al’s 2X
• Gain varies considerably over space & time & depth
• Poorly constrained due to high variability in reference data (4-8) and environmental variability. Can this be improved?
• If HPLC samples are degraded this would not be random error (can only decrease concentration)
• Error estimate should be based on this variability
• Should a different gain be used for Southern Ocean floats 5.5?
• If so how would that be done?