SYKE

Jerico Best Practices Workhop

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CALIBRATION BEST PRACTICES CHLOROPHYLL AND TURBIDITY

JUKKA SEPPÄLÄ

FINNISH ENVIRONMENT INSTITUTE, SYKE

Calibration Best Practices: Chlorophyll and turbidity

TASK 4.1: CALIBRATION

SubTask 4.1.2: Optical sensors Chl-a, Turbity, PAR

3) Designation of best practices for the use of optical sensors. This includes recommendations on time of day and frequency for sampling, calibration procedures, anti fouling measures and procedures to combine different data to produce high quality products.

1. Primary instrument calibration
2. Conversion from optical signal to concentration

Calibration Best Practices: Chlorophyll and turbidity

1. Primary instrument calibration

- Fluorescence intensity is given in arbitrary units (bits, V), calibration with other physical units is not practical (spectral issues, geometry of optics)
- Aim of calibration is to provide a solid reference point
- Typically primary calibration is carried out using material with constant quantum yield

2. Conversion from optical signal to concentration

- Provide relationship between fluorescence intensity and Chl*a* concentration (which is NOT constant)
- Without primary calibration, the variability in the above mentioned relationship cannot be understood or modeled

Calibration Best Practices: Chlorophyll and turbidity

1. Primary instrument calibration

Why:

To get stable response from the instrument, allowing comparison

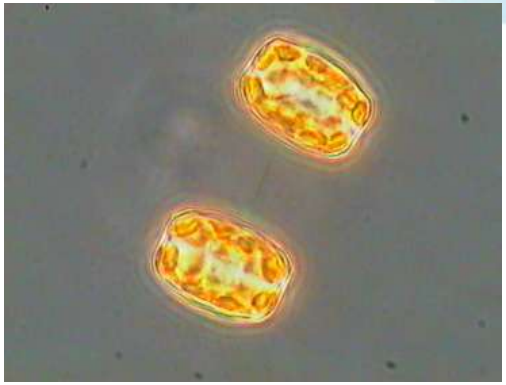
- between cruises/deployments
- between years
- between instruments (with the same optical setup) in different platforms

How:

- Factory calibration
- Algae cultures
- Chemical standards in water/solvents
- Solid standards

Calibration Best Practices: Chlorophyll and turbidity

1. Primary instrument calibration



Factory calibration

+ professional check
+ certificate
+ technical inspection & repair

- expensive
- time consuming
- inflexible

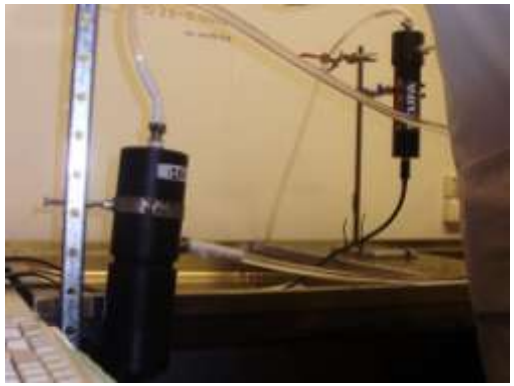
? Calibration material
? Traceability

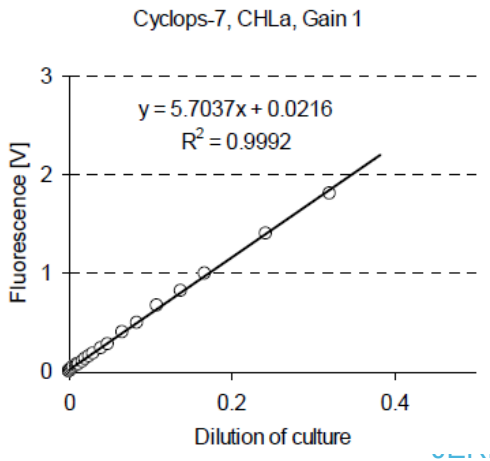
Calibration Best Practices: Chlorophyll and turbidity

1. Primary instrument calibration

Algae cultures

+ may be used directly in Chla concentration estimation
 - requires specific infrastructure
 - variable fluorescence to [Chla] ratio (taxonomy, physiology)
 - no traceability
 - not applicable for calibration check in platforms





In vivo Chl*a* fluorescence ≠ Chl*a* concentration

$$F = [Chla] \cdot R$$

R varies 2-4 fold for single species,
and up to 50-fold between different species.

$$F(\lambda_{ex/em}) = [Chla] \cdot E_{ex} \cdot \bar{a}_{PSII} * \cdot Q_a^*(\lambda_{em}) \cdot \phi_F$$

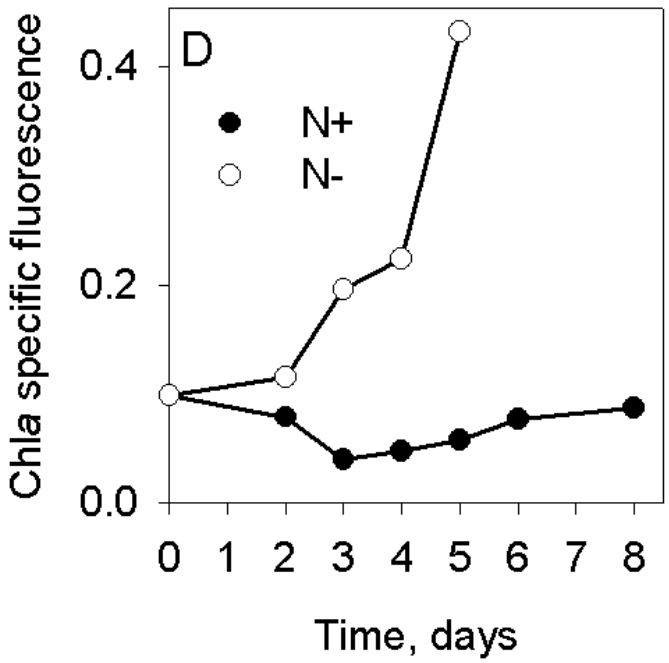
Biomass | Instrument | Species | Pigmentation | Physiology

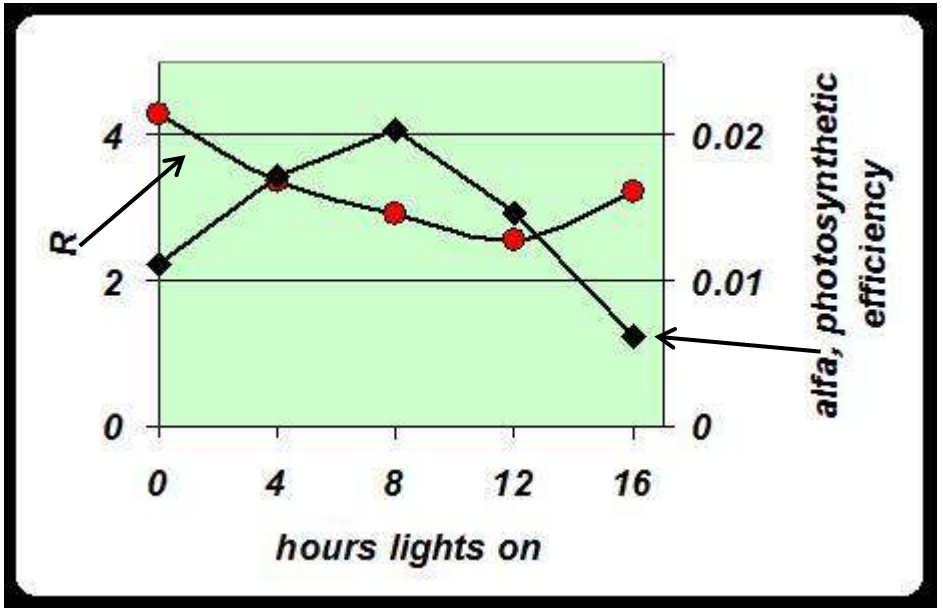
Calibration Best Practices: Chlorophyll and turbidity

1. Primary instrument calibration

Algae cultures

- variable fluorescence to [Chla] ratio (taxonomy, physiology)





Calibration Best Practices: Chlorophyll and turbidity

1. Primary instrument calibration

Chemical standards in water/solvents

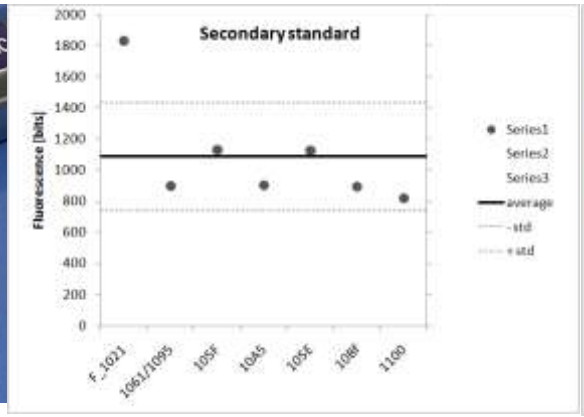
+ principally a good solution, but no agreement on substance/solvent
+/- Chl*a* in acetone (or other solvent) may be solution for some instruments but may not be compatible with other
- Other chemicals (like fluorescein) are not stable or do not match the wavelengths of Chl*a* to yield a good calibration

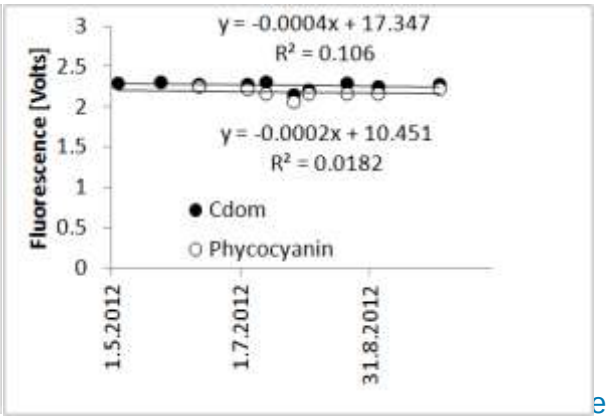
Calibration Best Practices: Chlorophyll and turbidity

1. Primary instrument calibration

Solid secondary standards

+(/-) stable and traceable signal, thus instrument performance can be tracked
- secondary standard does not allow direct instrument-instrument comparisons





Calibration Best Practices: Chlorophyll and turbidity

1. Primary instrument calibrationc

The perfect chromophore-based fluorescence standard should

- be simple to use,
- be sufficiently stable in solution or as a solid
- absorb and emit in the same general regions as the compounds under study,
- have a constant fluorescence quantum yield
- reveal a negligible small temperature dependence of its fluorometric properties,
- be easy to purify/manufacture
- dissolve in solvent compatible with field fluorometers
- inexpensive
- flexible
- traceable

Modified from
Resch-Genger & DeRose 2010 Pure Appl. Chem.

Calibration Best Practices: Chlorophyll and turbidity

1. Primary instrument calibration

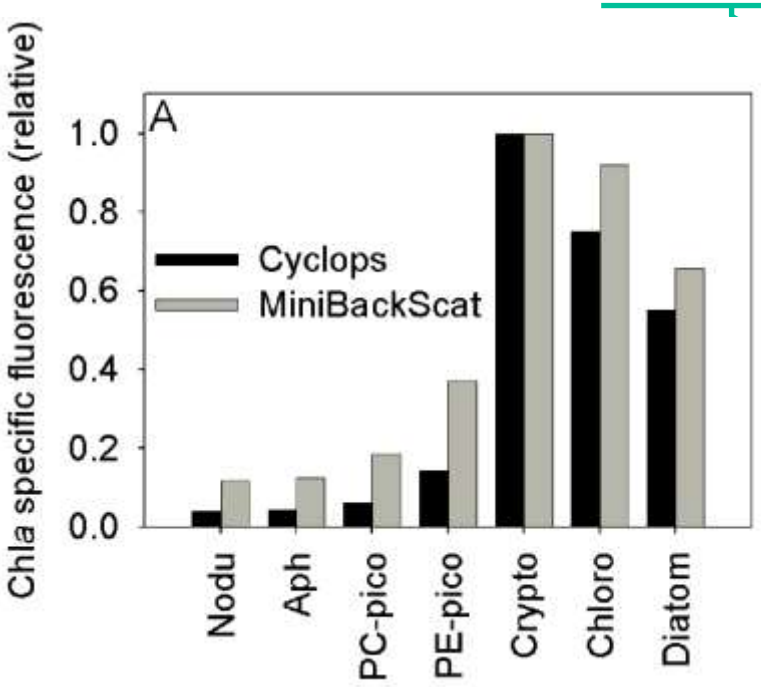
	simple	Stable fluorescence	spectral match	compatible	transferable	cost	traceability	flexible
Factory	-	+	+	+	+	-	?	-
Culture	-	-	+	+	+	+	-	-
Chl*a* in solvent	+(-)	+	+	-/+	+	+	+	+/-
Fluorescein	+(-)	?/-	-	+	+	+	+	+/-
Chla in water	?	?	+	+	+	?	?	?
Solid	+	+(?)	+	+	-	+	?	+

Calibration Best Practices: Chlorophyll and turbidity

1. Primary instrument calibration

Comparison of instruments with different optics may be a mess...

$$F(\lambda_{ex/em}) = [Chla] \cdot E_{ex} \cdot \bar{a}_{PSII}{}^{*} \cdot Q_a{}^{*}(\lambda_{em}) \cdot \phi_F$$



Spectral variability between calibration and field samples

Calibration Best Practices: Chlorophyll and turbidity

1. Primary instrument calibration

to support best practises:

1. Review of calibration questionnaire, individual methodological descriptions → possible further questions

2. Questionnaire to manufacturers
   - Method of calibration
   - Traceability
   - Availability of secondary standard, material, durability
   - Recommendations

3. Testing artificial Chl*a* dissolved in water, as proposed by Rajesh Nair
   - Stability, traceability, spectral match etc. to be studied