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Practical Chlorophyll Fluorometry:

Vicarious Calibration of the WETLabs WETStar Chlorophyll Fluorometer

- Fluorescence as a proxy for chlorophyll concentration and absorption
- Chlorophyll as a proxy for primary productivity
- Primary productivity as a tool for examining major ecological issues: Eutrophication, Harmful Algal Blooms, Carbon Cycling, etc.

O.T.S. Fluorometers

- Affordable, portable, off-the-shelf technology, but…
- Proper usage requires proper calibration and interpretation…**It's a fluorometer, not a thermometer!**

A (very brief) Background

- Fluorometer emits excitation beam at 470nm (in the blue) absorbed by organisms – much of it goes to photosynthetic processes
- Some photons are reemitted with lower energy at 685nm (in the red) ($F = a(\lambda) * E(\lambda) * \Phi_F$)
- Fluorometer detects at 685nm, and reports in volts

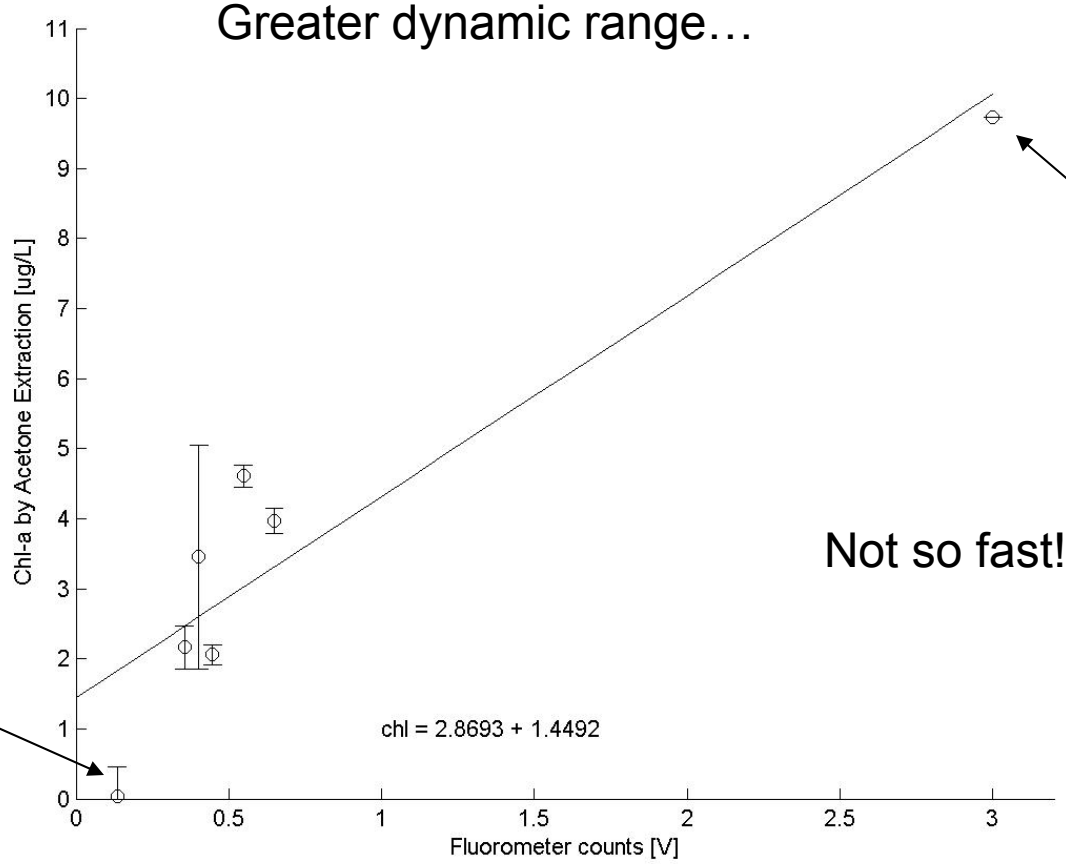
(Vicarious) Calibration Methods

- Empirical linear fit to field samples (standards analyzed by filter pad/acetone extraction method
- Extraction technique (Yentsch and Menzel, 1963; Holm-Hansen et. al., 1965)
  - chl-a = $K(Fm/Fm-1)*(Fo-Fa)*(V_{acetone}/V_{sample})$
  - phe-a = $K(Fm/Fm-1)*[(Fm*Fa-Fo)]*(V_{acetone}/V_{sample})$
- Maximize the dynamic range of sampling (filtered seawater, dilution series)
- Find your instrument dark current signal (DI water)
- Consider environmental parameters: photoquenching, sedimentary scatter, community distribution, cell size…

Improved Empirical Fit?

Greater dynamic range…

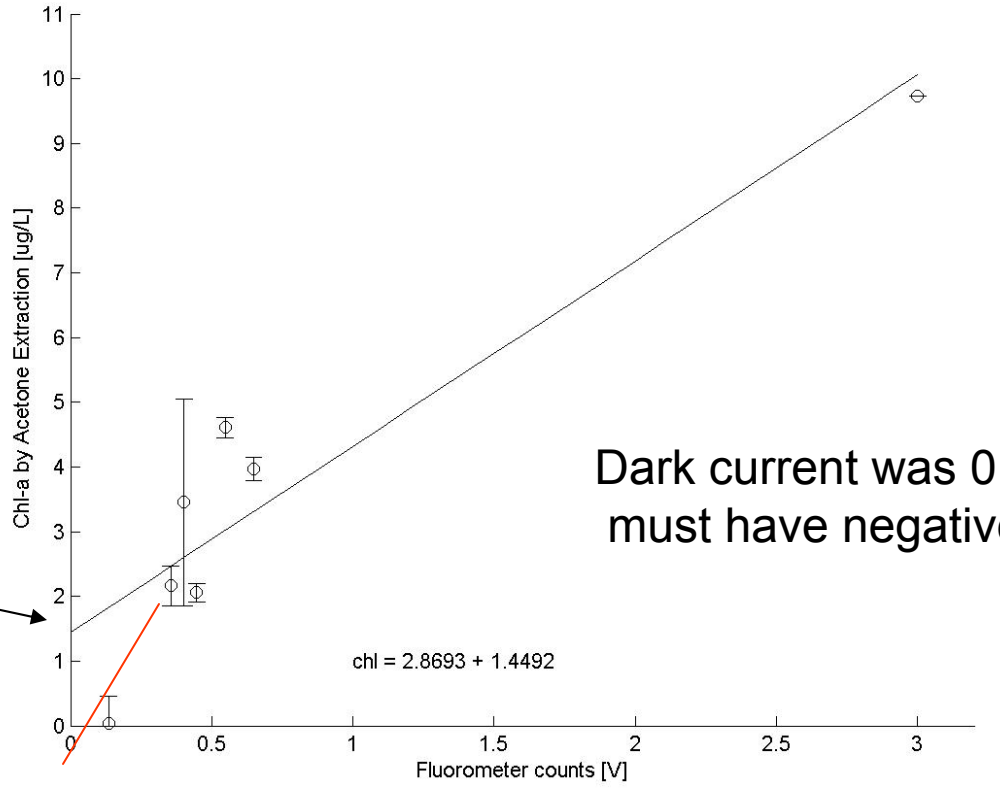
High chl culture (*Thallasiosira pseudonanna*)



Fsw sample

Not so fast!

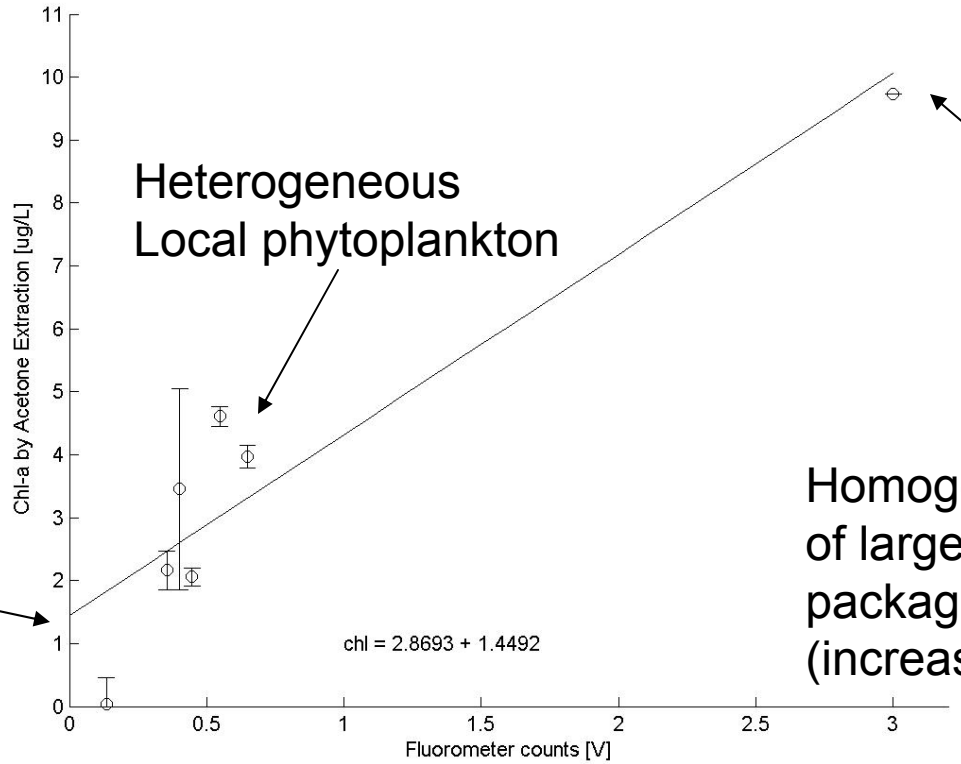
Improved Empirical Fit?



Red flag!

Dark current was 0.1, so fit must have negative intercept

Improved Empirical Fit?



Heterogeneous
Local phytoplankton

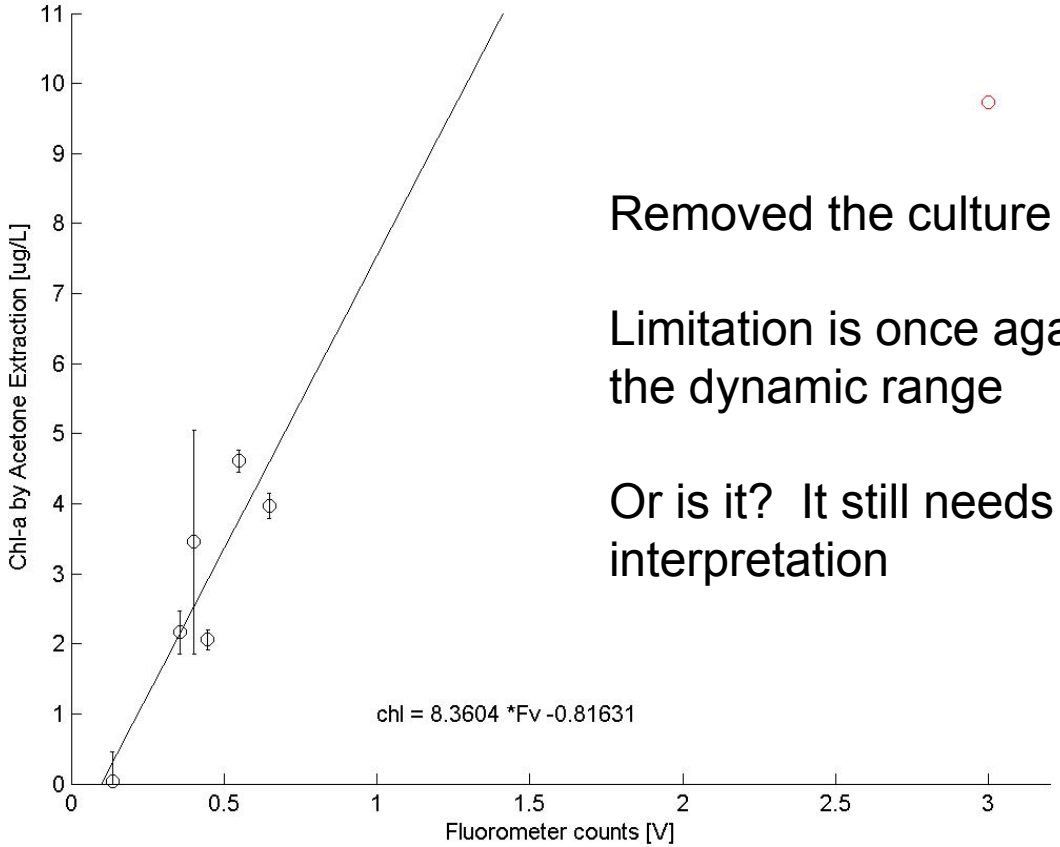
Thallasiosira
pseudonanna

Red flag!

Homogeneous population of large cells, high packaging effect (increased Fv)

Near saturation Concentration?

Call it a day?



Removed the culture from fit

Limitation is once again primarily the dynamic range

Or is it? It still needs proper interpretation

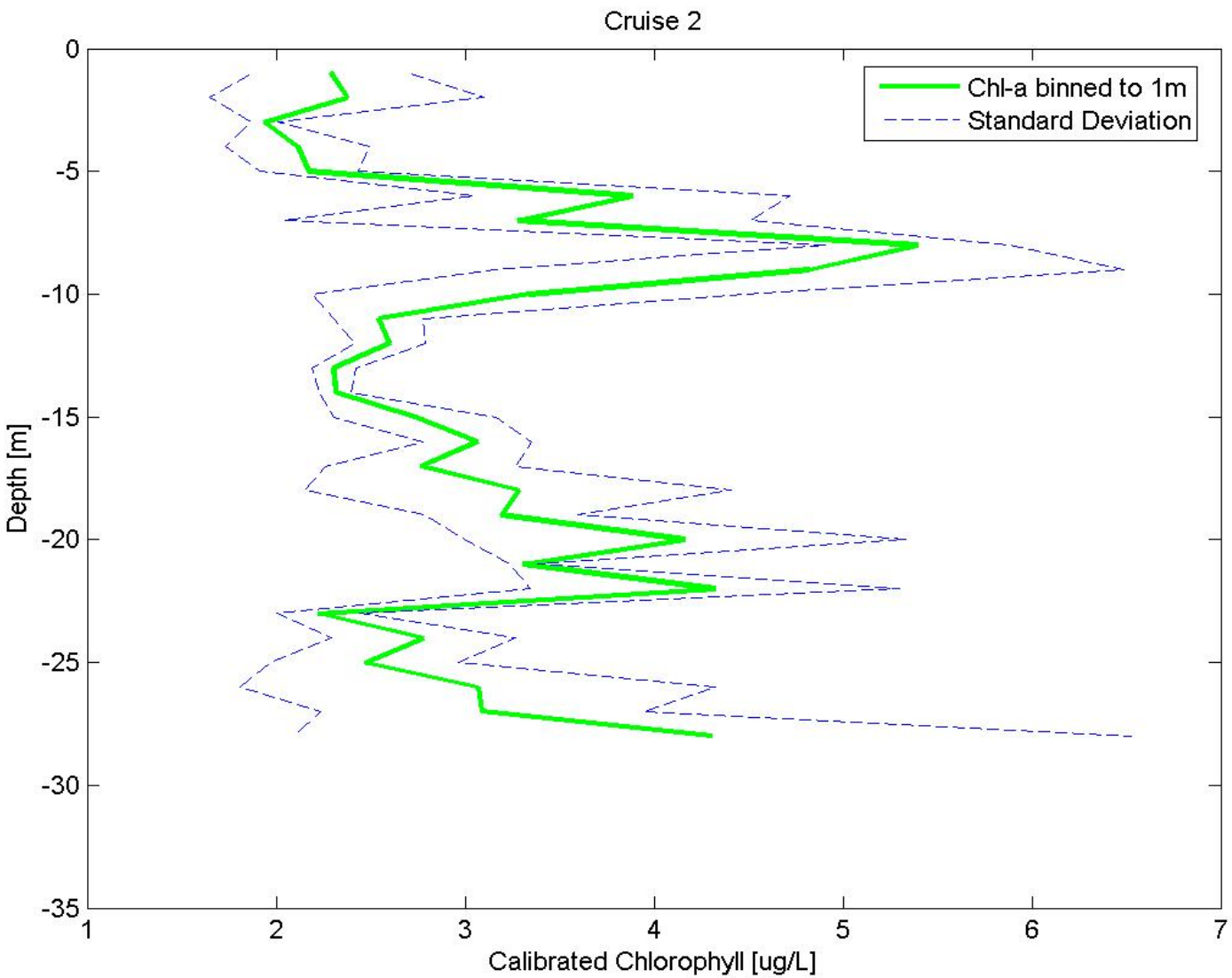
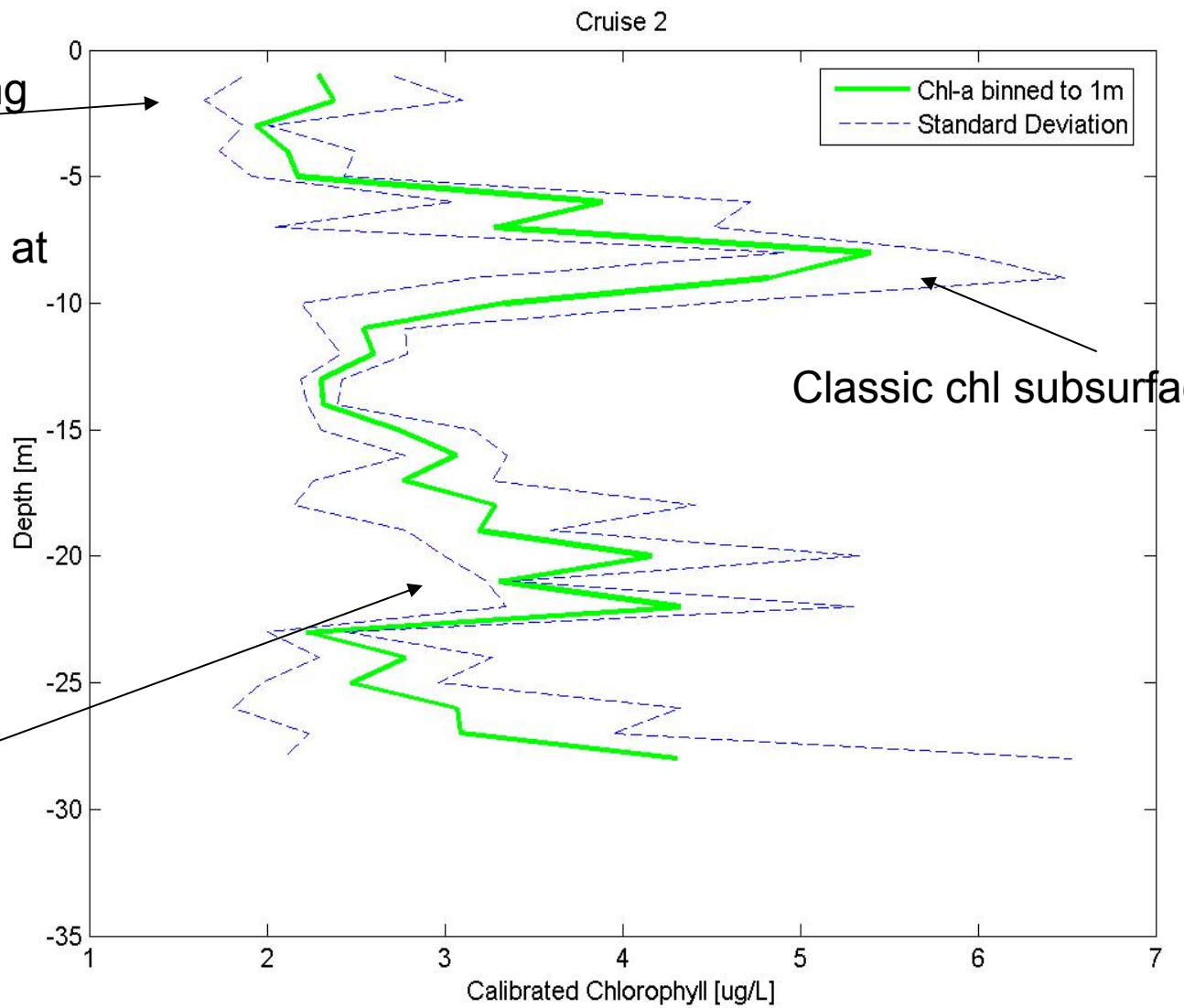

Cruise 2
Chl-a binned to 1m
Standard Deviation
Depth [m]
0
-5
-10
-15
-20
-25
-30
-35
1
2
3
4
5
6
7
Calibrated Chlorophyll [ug/L]

Photo-quenching and nutrient limitation at the surface

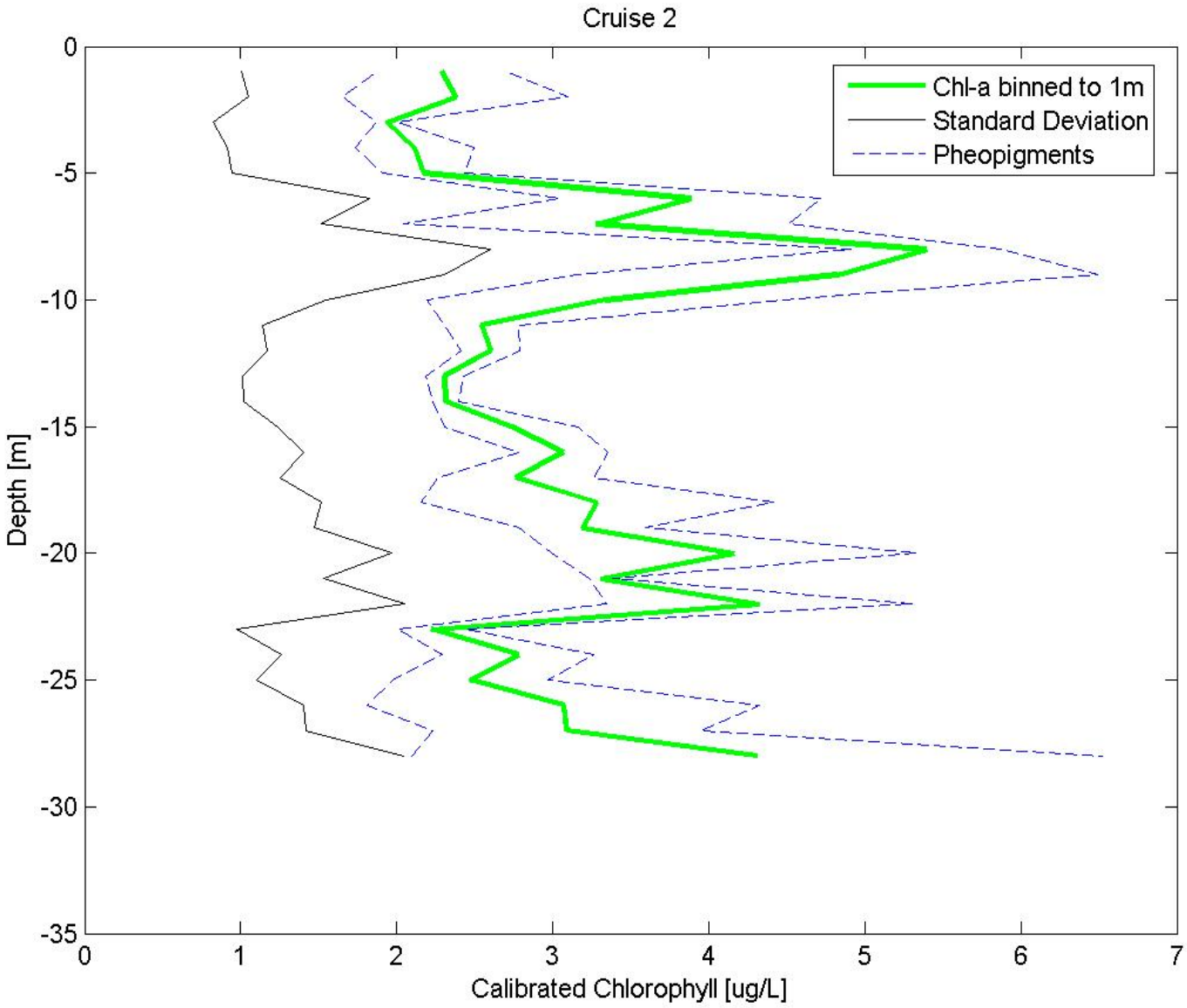


Classic chl subsurface max

Chl or sediment mixing up from the bottom in current?

Time to break out the EcoVSF backscatter ratio and take a closer look!

…if only I had more time…


Cruise 2
Chl-a binned to 1m
Standard Deviation
Pheopigments
Depth [m]
0
-5
-10
-15
-20
-25
-30
-35
0
1
2
3
4
5
6
7
Calibrated Chlorophyll [ug/L]

Take-Home Message

- Just because it doesn't take an solid-angle integral to correct doesn't mean it should be taken lightly.
- A carefully calibrated fluorometer can be the standard by which many other optical proxies/instruments are validated…
- Coherent matlab code α hrs. sleep