Lecture 6

In situ fluorometry

Fluorescence is very easy to measure, very difficult to interpret

Why do it?

Deployment

- profilers

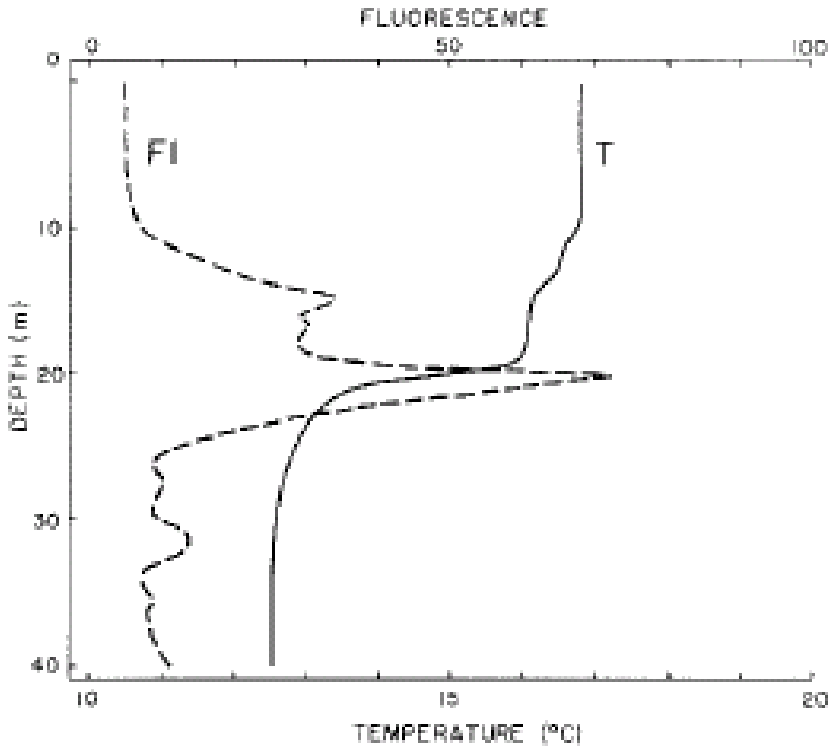
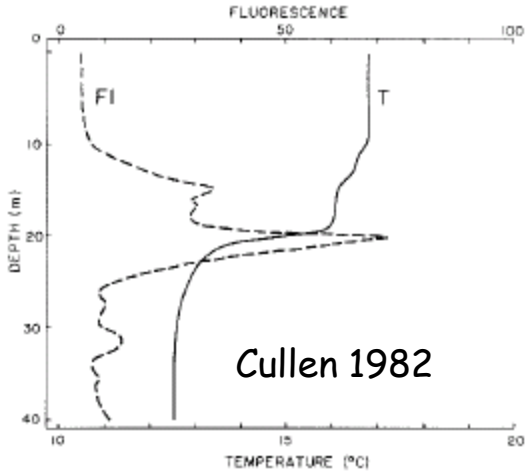


FIG. 8. Vertical profile of fluorescence (arbitrary units) and temperature from the western English Channel. 5°23'W, 49°25'N, July 1975. From Pingree et al. (1975).

Cullen 1982

Deployment

- profilers
- buoys



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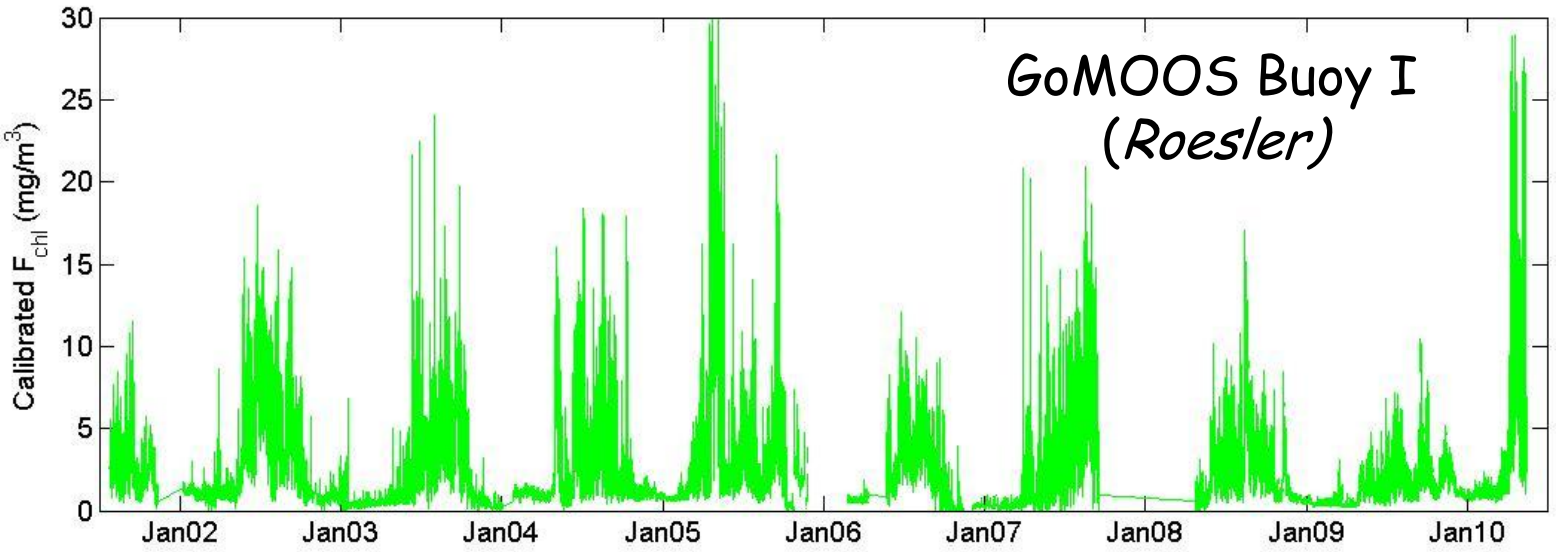
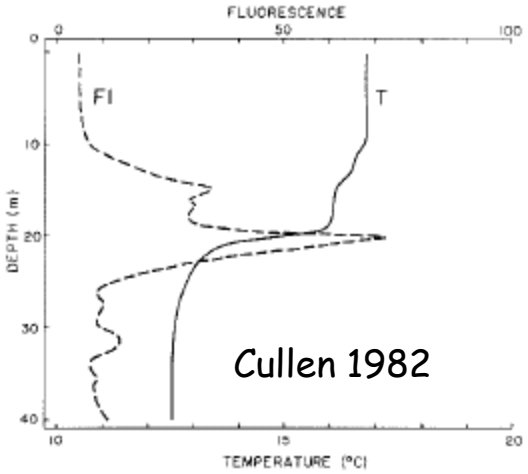


Deployment

- profilers
- buoys
- floats



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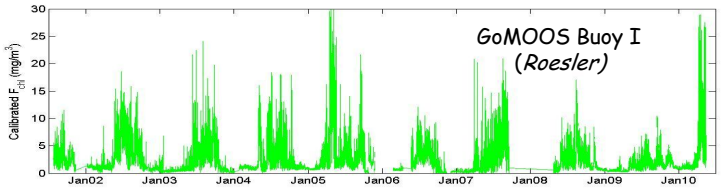
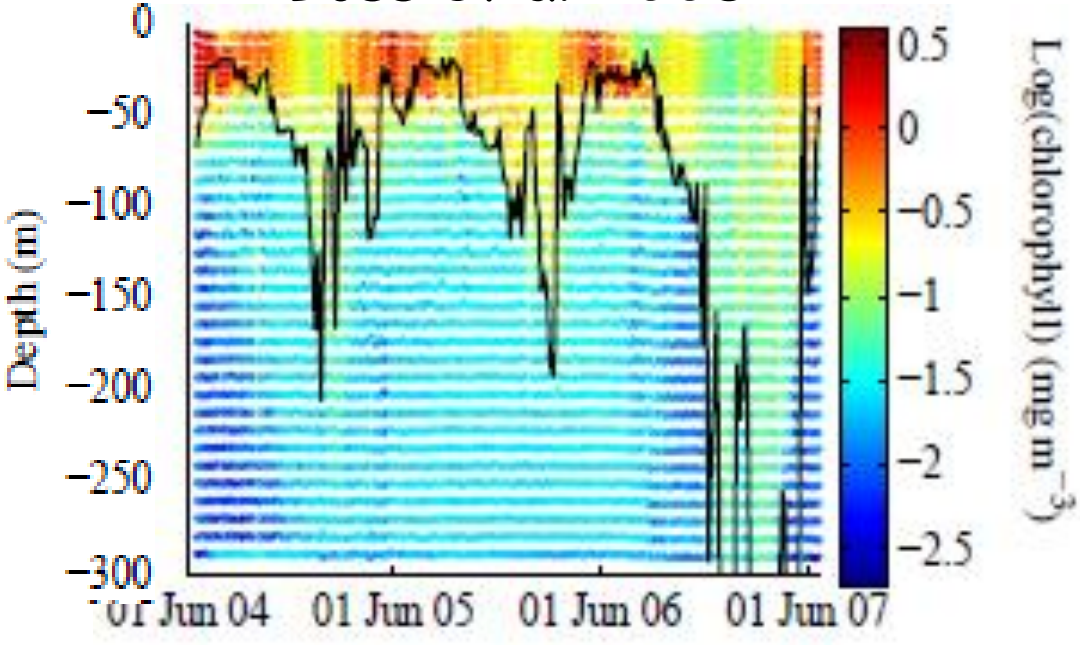
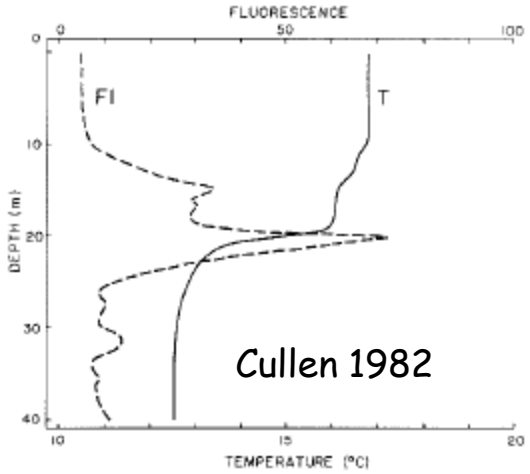


Boss et al 2008



Deployment

- profilers
- buoys
- floats
- autonomous gliders



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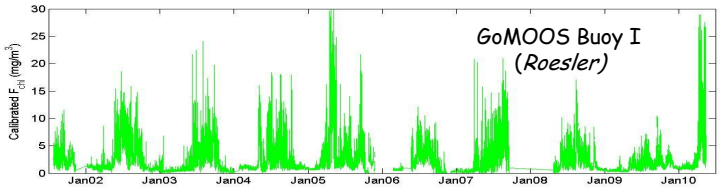
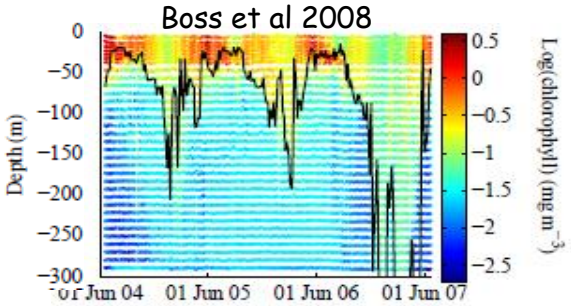
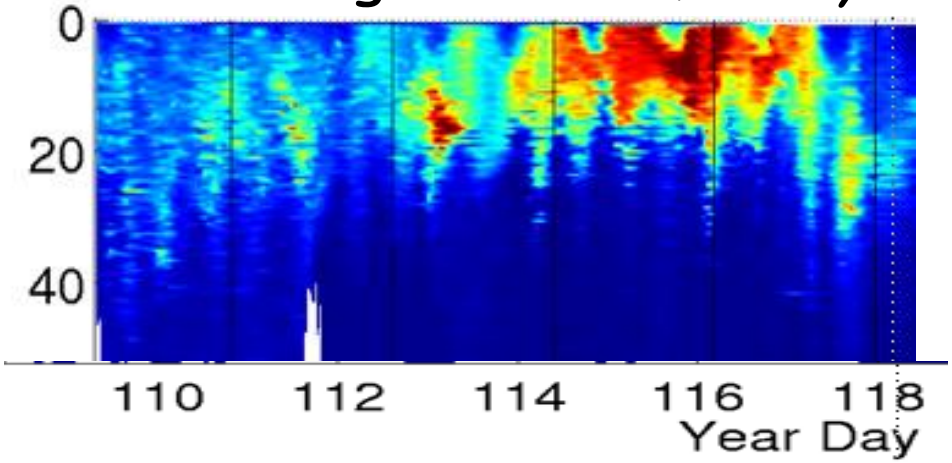




Washington Coast, *Perry*



Because of the sampling potential, it is worth dealing with the issues of interpretation

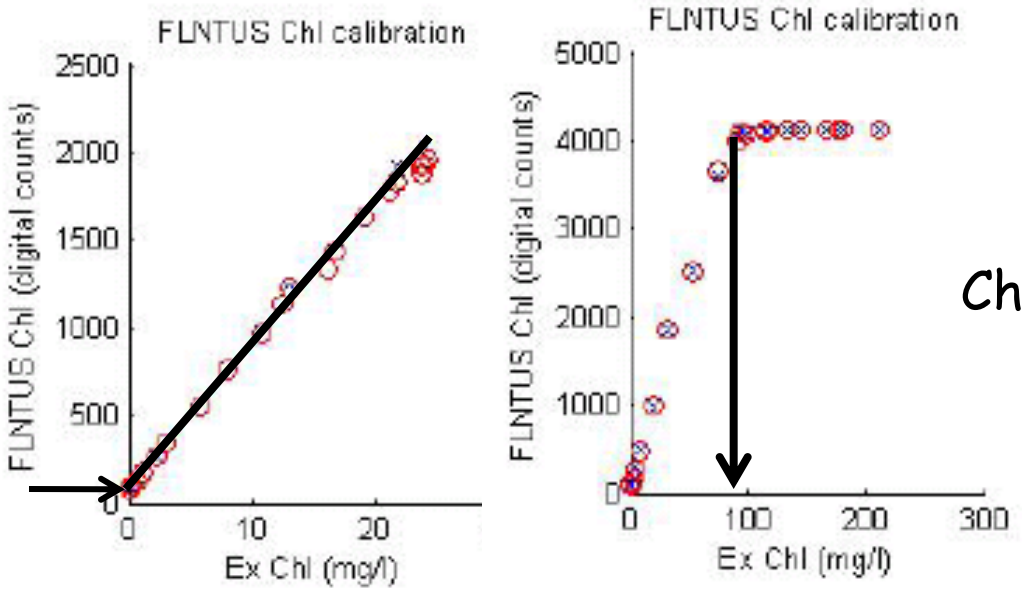
strategies

Outline

- Calibration
- Characterization
- Biofouling
- Validation
- Capabilities

Calibration

- sensor output = voltage (digital counts))
- what you want = mg chl/m^3
- Standard curve
  - slope = digital count/(mg chl/m^3) (type II regression)
  - intercept = media blank ≠ V_{dark}
  - saturation limit



$Chl(mg/m^3) = (V_{sample}-V_{dark})/Slope$

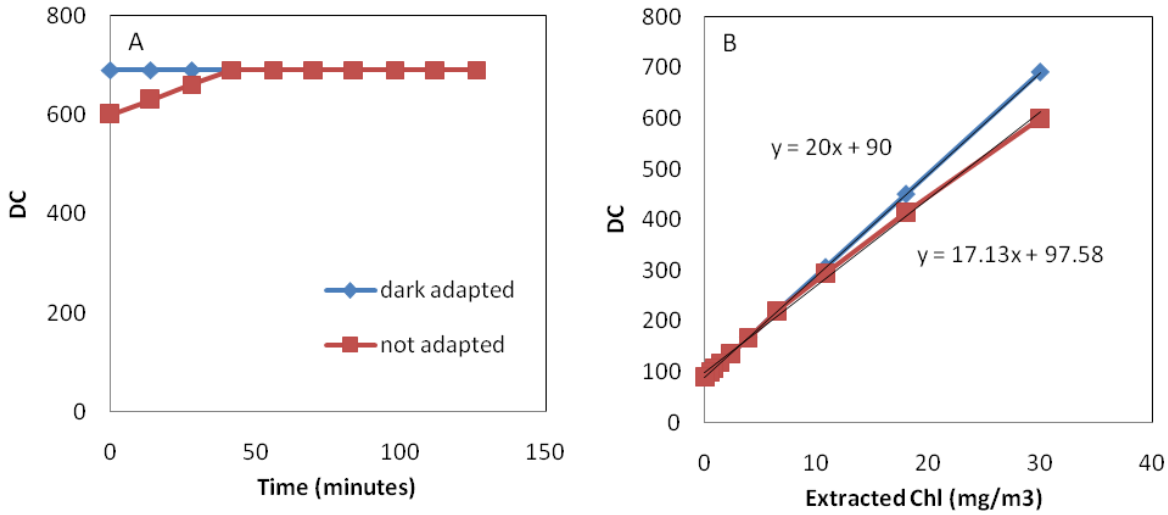
Calibration standard curve

- what do you calibrate with?
  - Chl a standard
    - not excited by 470 nm LED
    - not packaged
  - vicarious calibration in situ samples
    - changes, not really calibration
    - many sources of variability
  - culture
    - which one
    - growth conditions

Calibration standard curve

- cultures respond to calibration conditions

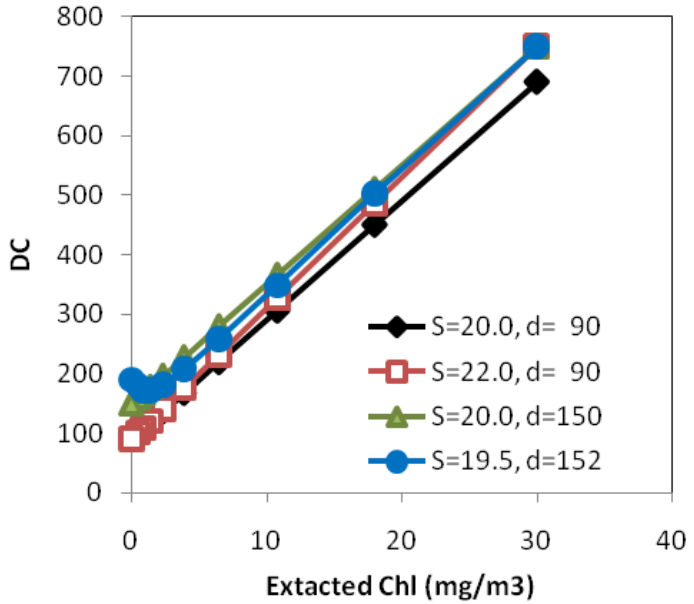
$$Chl(mg/m^3) = (V_{sample} - V_{dark})/Slope$$



Calibration standard curve

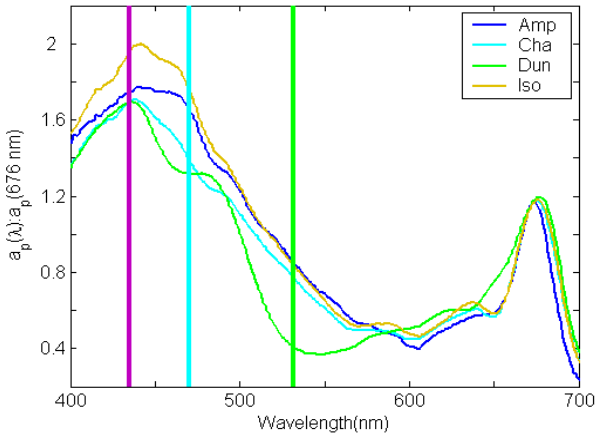
- how do you make up your standard curve samples→dilute with culture filtrate
- regression intercept vs dark reading

$Chl(mg/m^3) = (V_{sample}-V_{dark})/Slope$



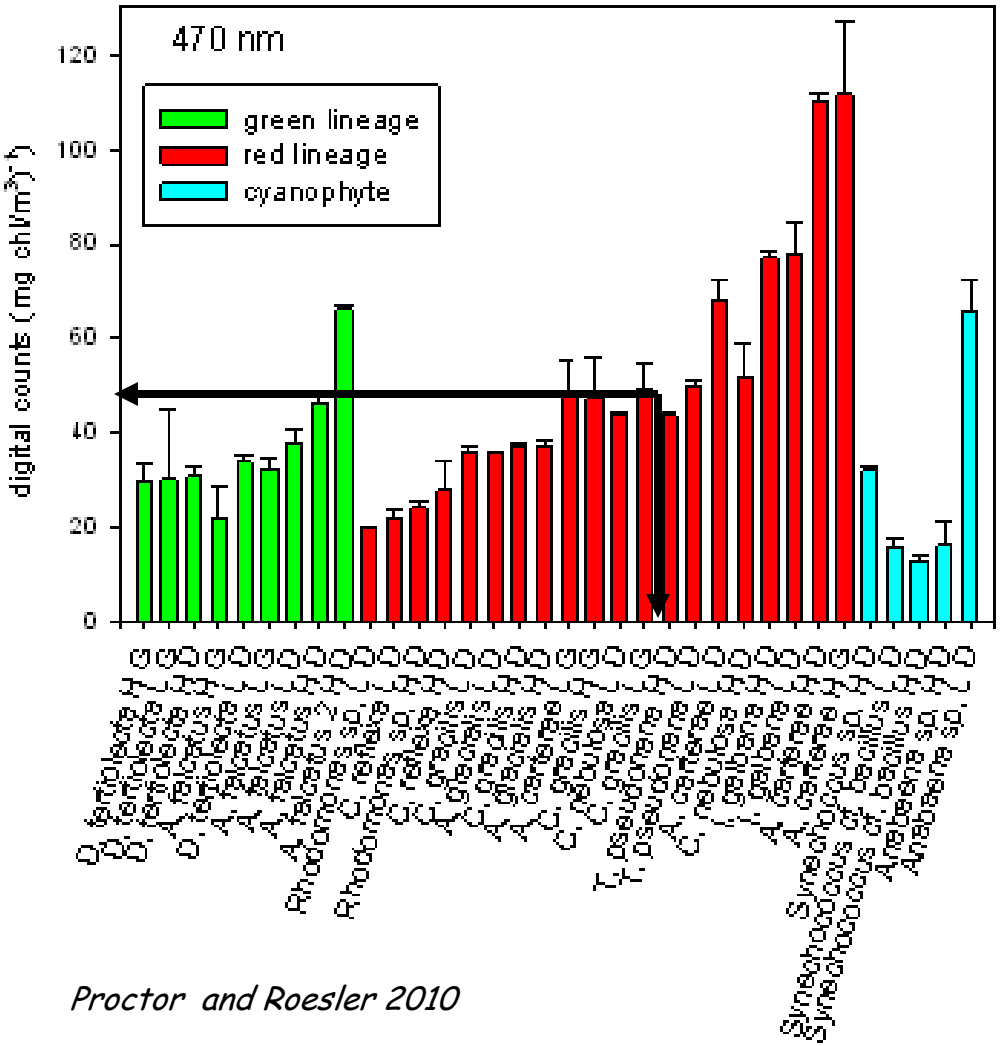
Calibration standard curve

- what culture to use?
  - 13 species
  - 2 light levels
  - growth phase



$Chl(mg/m^3) = (V_{sample}-V_{dark})/Slope$

Slope

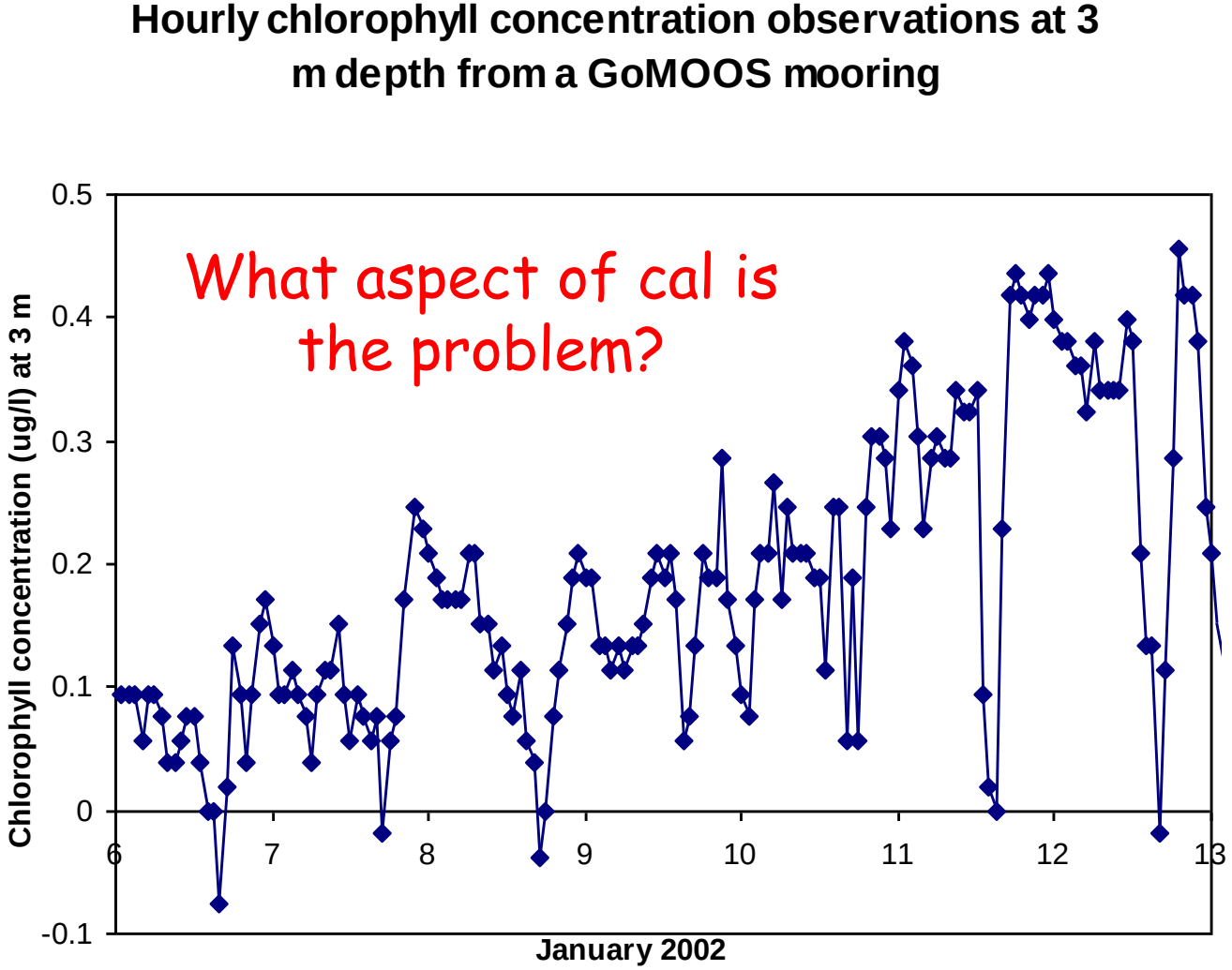


Proctor and Roesler 2010

Calibration

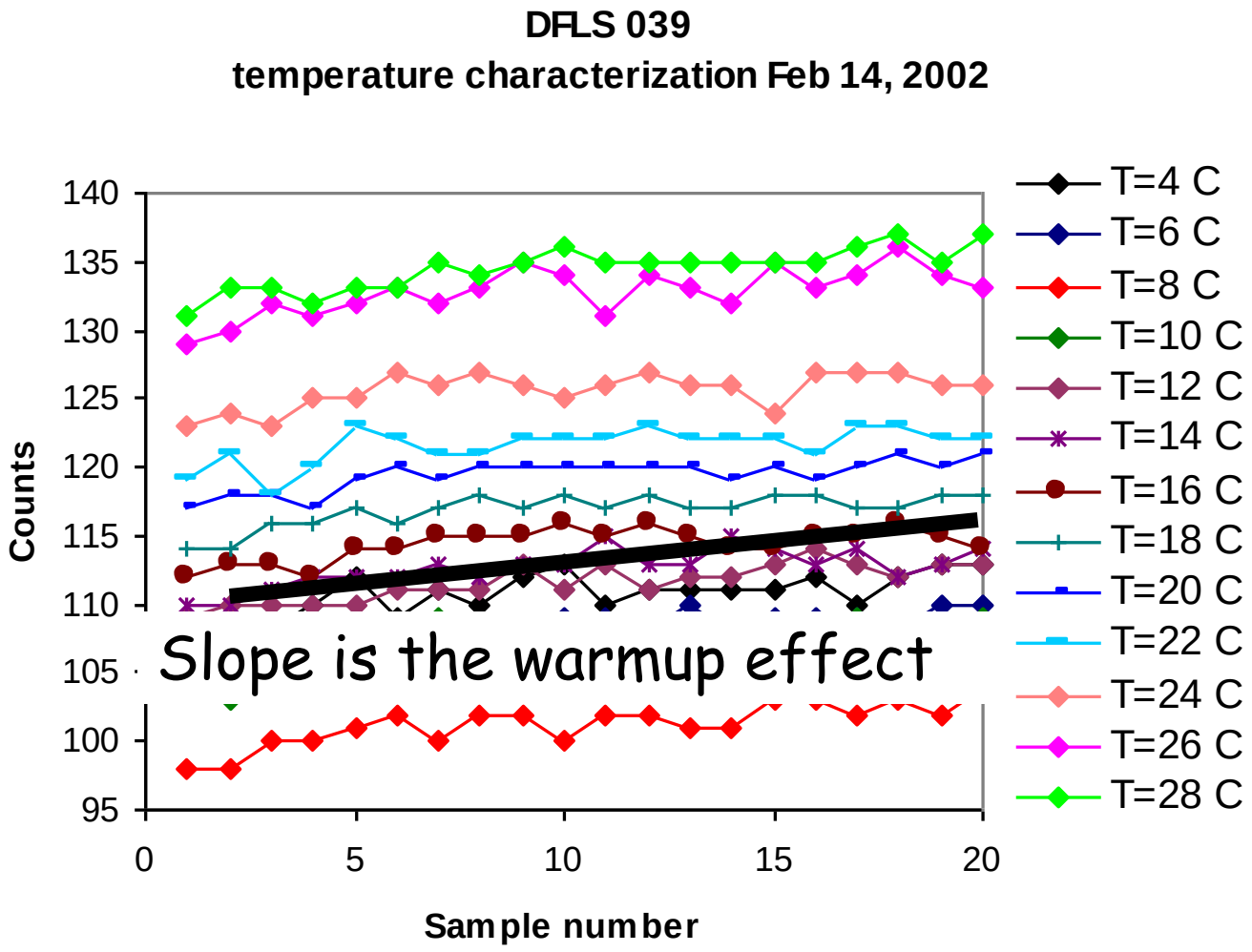
- Result is
- "nutrient-replete, moderate 24hr irradiance, exponential phase-*Thalassiosira psuedonana* – equivalent chlorophyll concentration"
- or "calibrated chlorophyll fluorescence"
- Are we done?
- Environmental characterization

Chlorophyll Fluorometer Characterization



Environmental Characterization
Temperature Dependence

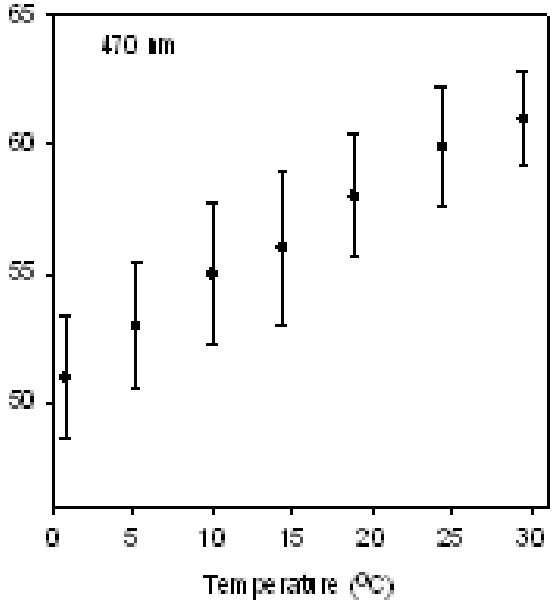
Temperature Response

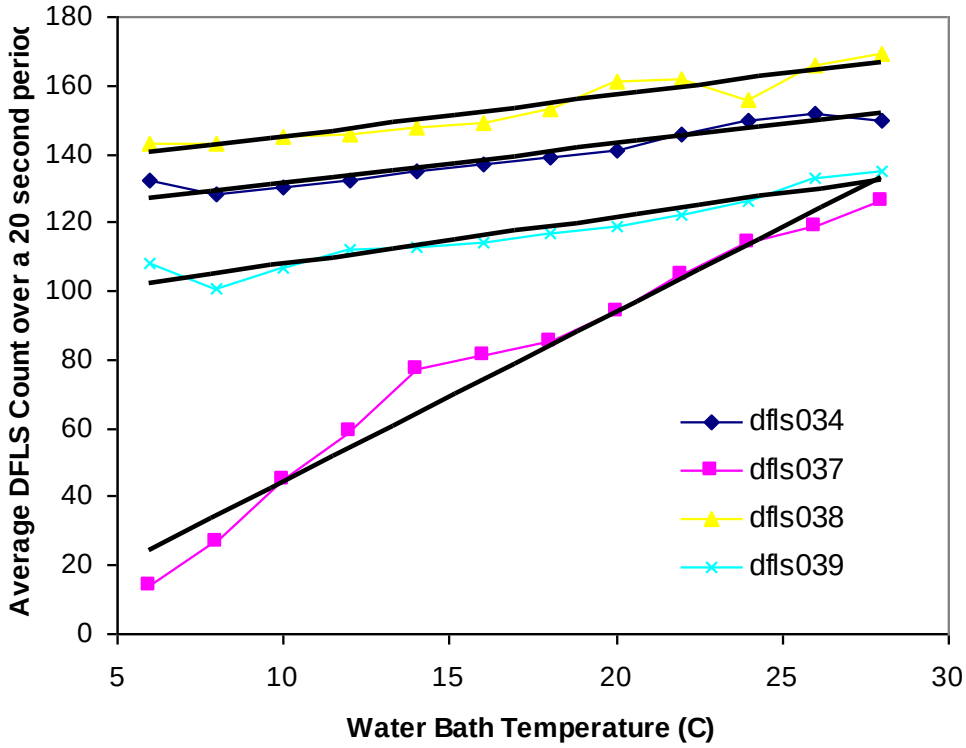


Environmental Characterization Temperature Dependence

February 14, 2002

The temperature dependence, of course, varies between sensors and between sensor type



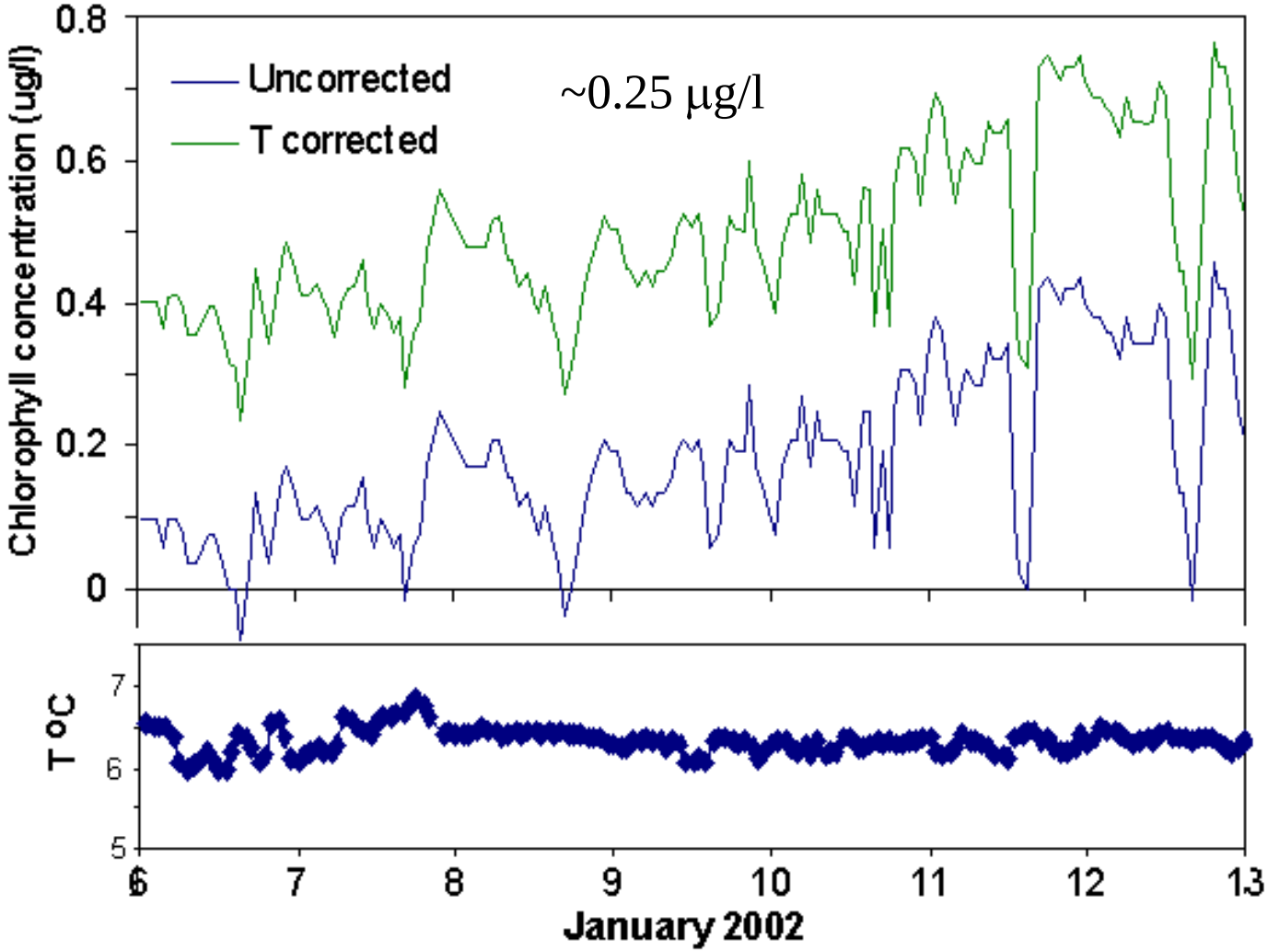


Older sensors ~ 1 to 5 counts/°C

newer sensors ~ 0.3 count/°C

Slope = $\Delta V_{dark}/\Delta T_{eq}$

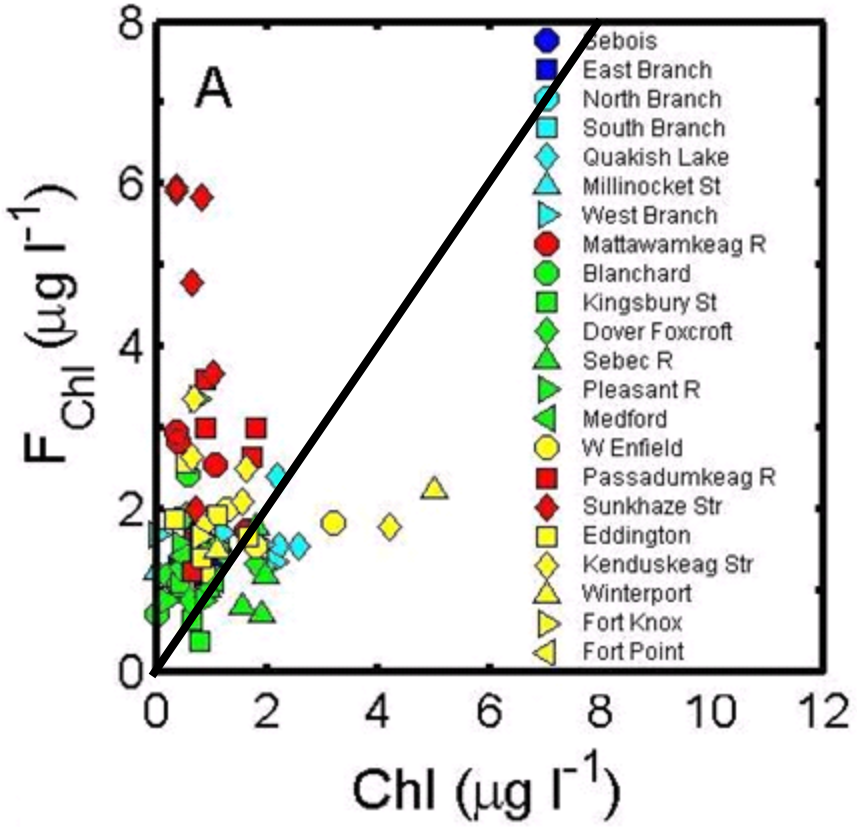
Environmental Characterization
Correction for Temperature Dependence

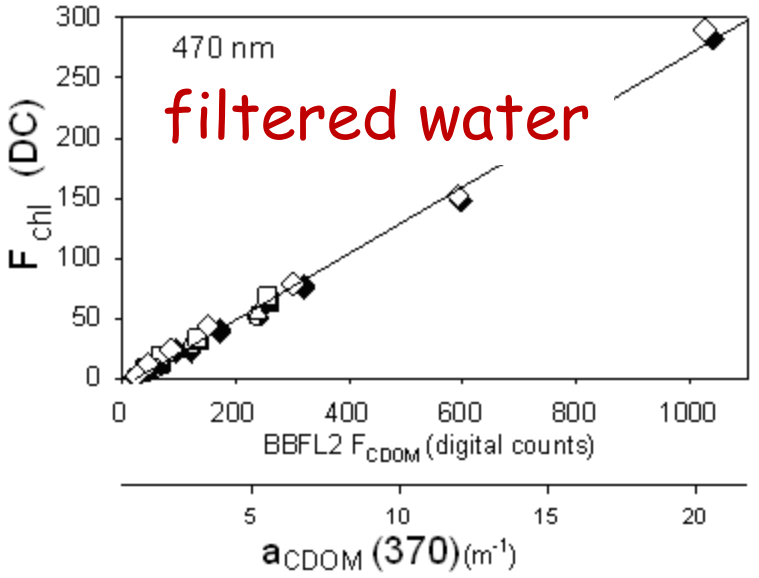


Compounding Issue:
The biggest temperature effect occurs in the winter (ΔT), and that is when chlorophyll is lowest.

Environmental Characterization
Correction for CDOM fluorescence

- high CDOM waters exhibit high F_{chl}





Belzile et al 2006,
Proctor and Roesler 2010

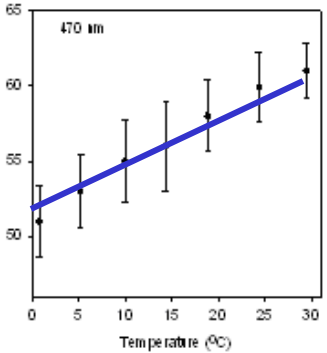
Slope = apparent F_{chl}/F_{CDOM}
(dark offset and temperature corrected)

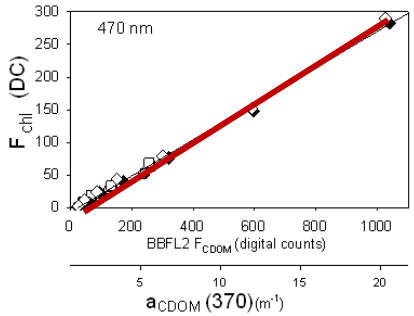
Calibration Equation

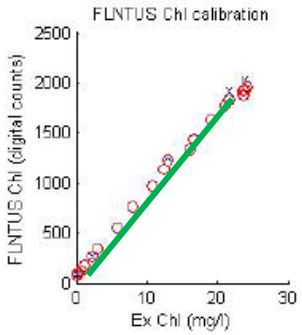
- Chl (mg/m^3) = $(V_{sample} - V_{offset})$/**Slope**
- where $V_{offset} = V_{dark} + B_{CDOM}$
- V_{dark}= temperature corrected dark reading (dc)

  $V_{dark}(T_{in\ situ}) = V_{dark}(T_{cal}) + (T_{cal} - T_{insitu}) * \Delta V_{dark}/\Delta T_{eq}$
- B_{CDOM} = CDOM blank correction for F_{chl} sensor using co-located F_{CDOM} sensor which is itself temperature corrected (dc)

  $B_{CDOM} = S_{CDOM-chl} * (V_{CDOM} - V_{dark}(T_{cal}) + (T_{cal} - T_{insitu}) * \Delta V_{dark}/\Delta T_{eq})$
- Slope is the calibration slope (dc/(mg chl/m^3))

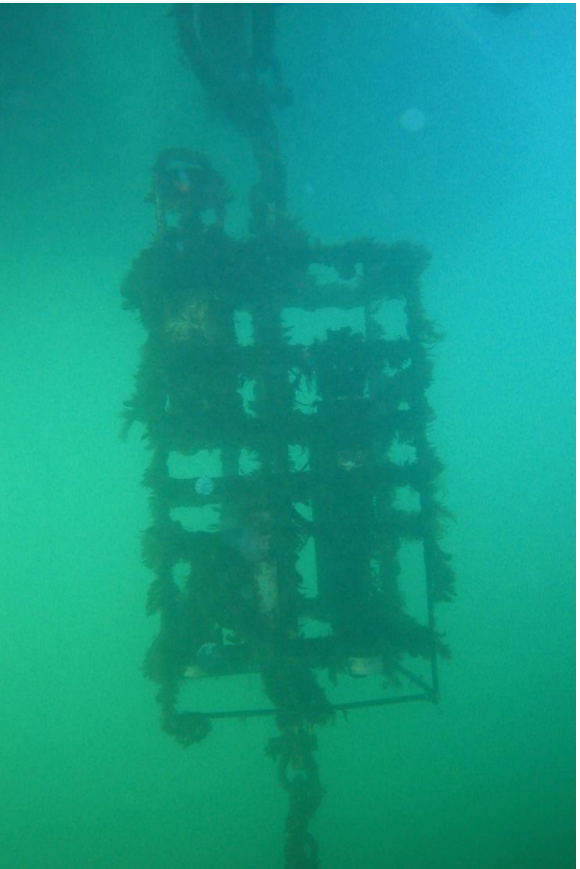






So now you can put it in the water and what happens?

stuff grows on it



sensors drift

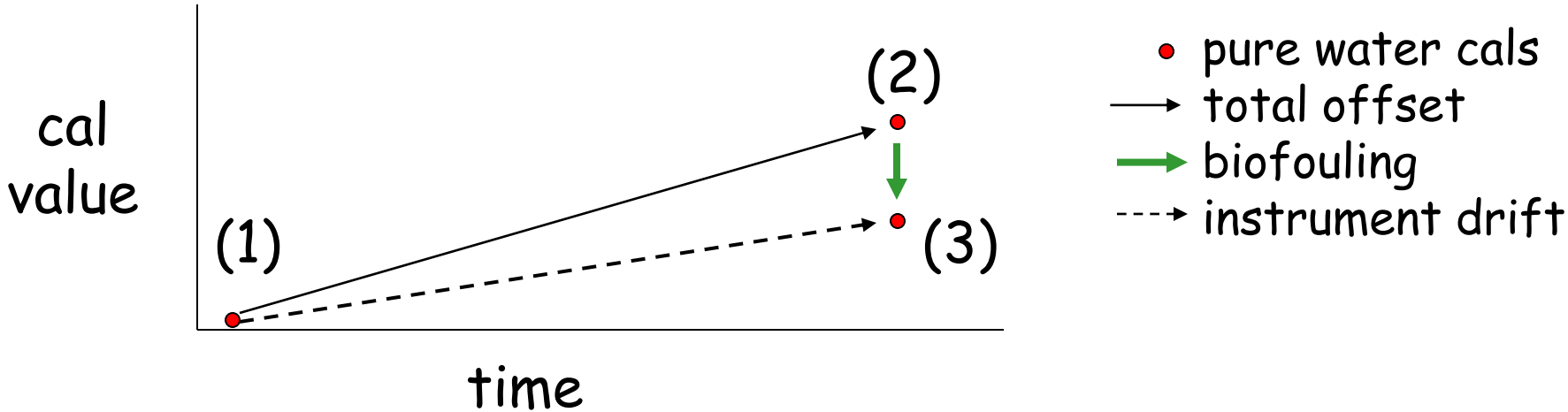
Instrument Drift and Biofouling

Roesler and Boss 2010

- Calibrations
  - Pre-deployment calibration (1)
  - Post-recovery pre-clean calibration (2)
  - Total offset = (2) – (1)
  - Post-recovery post-clean calibration (3)

    biofouling = (3) – (2)

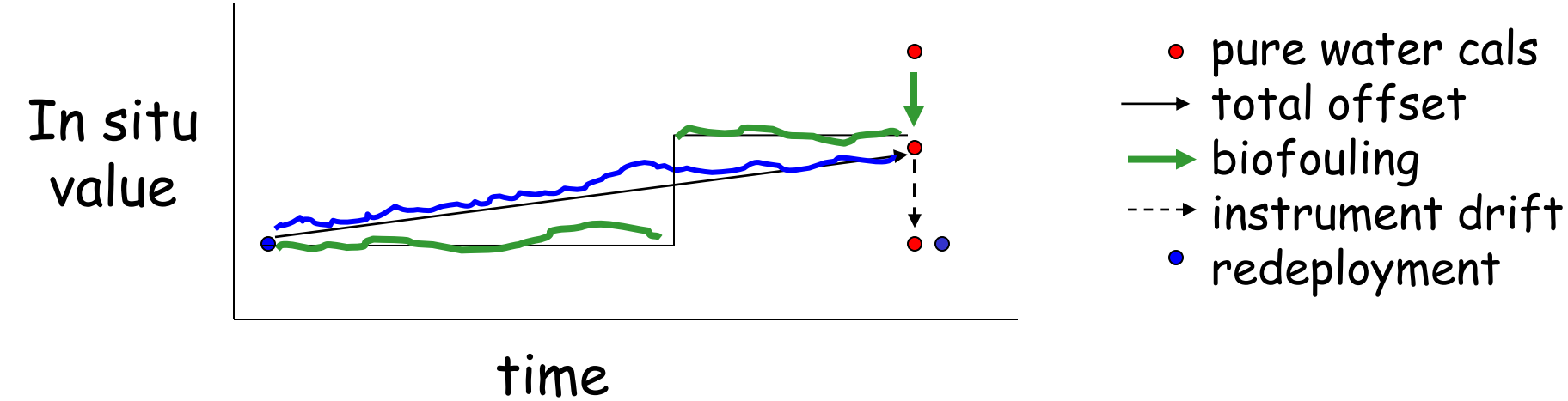
    drift = (3) – (1)



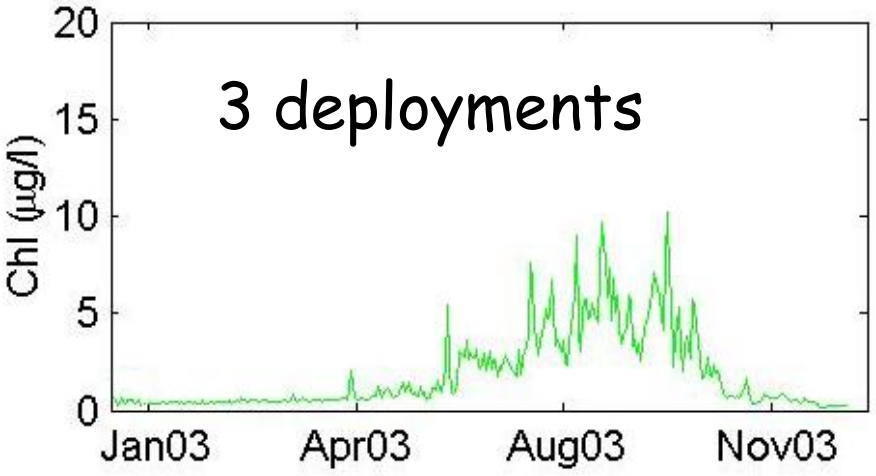
Instrument Drift and Biofouling

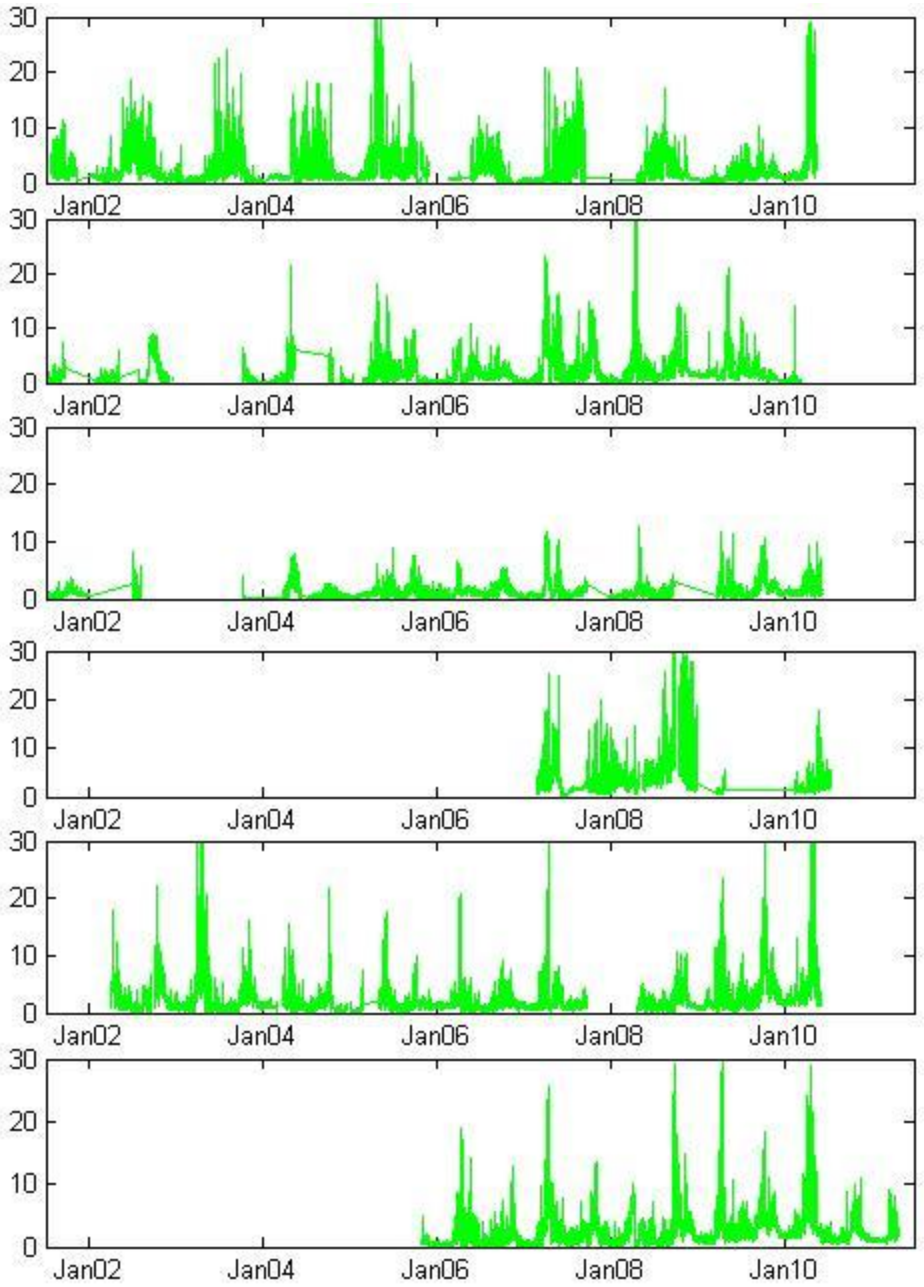
Roesler and Boss 2010

- Evaluating instrument drift
  - Linear trend
  - Step function trend
  - Validation (new deploy – corrected)



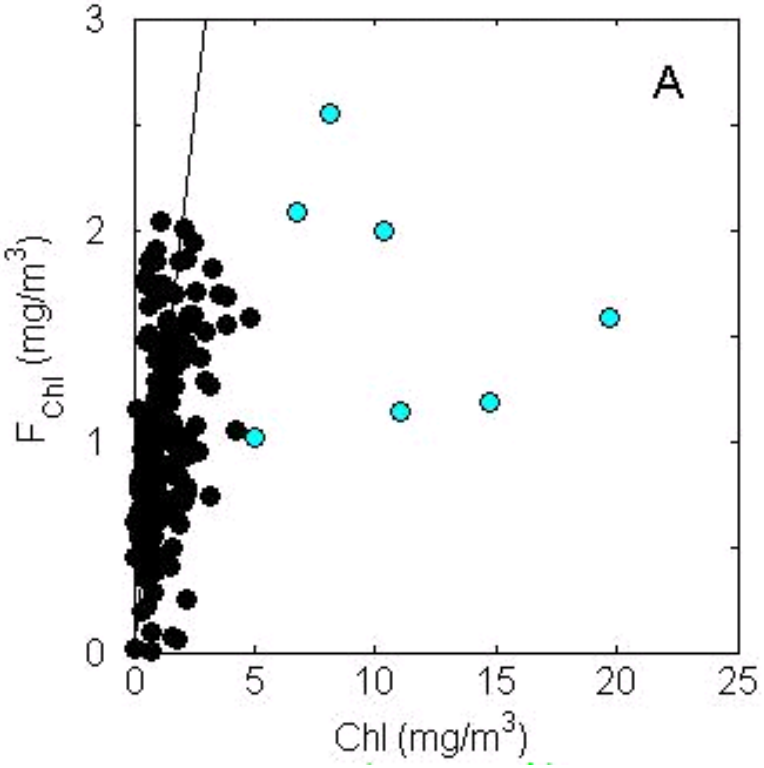
Final product





Validation (ground truth)

- why does is usually look like a scatter plot?
  - species variations

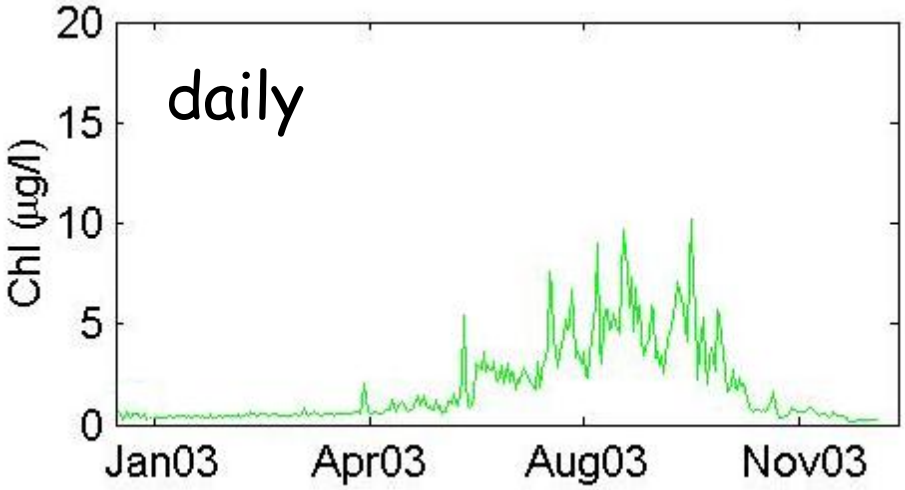


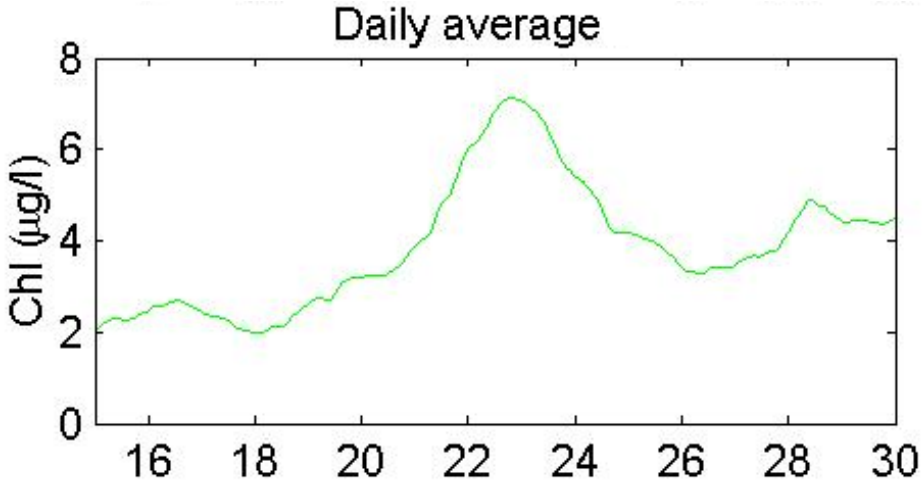
http://www.bowdoin.edu/earth-oceanographic-science/workshops/index.shtml

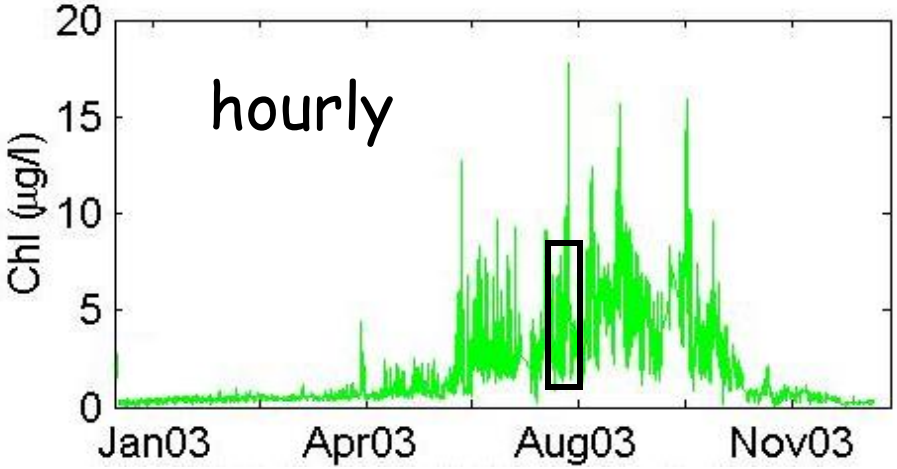
Validation (ground truth)

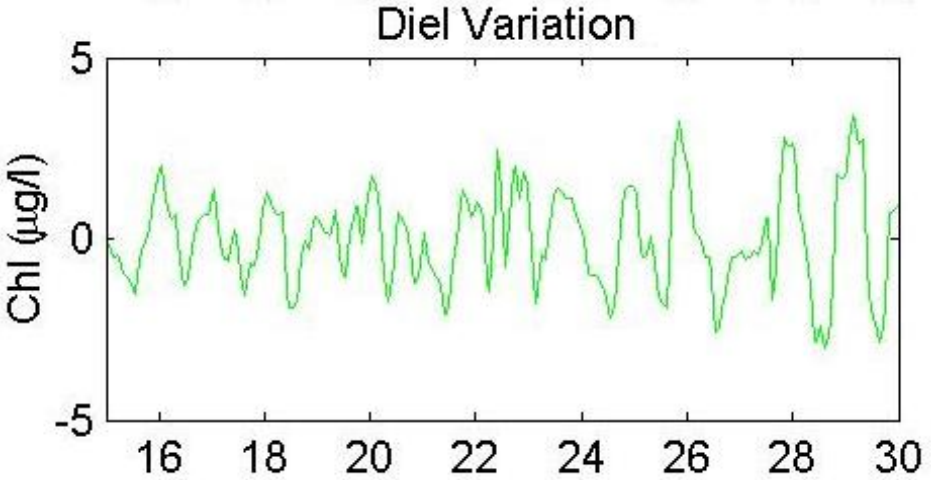
- why does is usually look like a scatter plot?
  - species variations
  - quenched fluorescence

Chlorophyll Fluorescence Seasonal Cycles



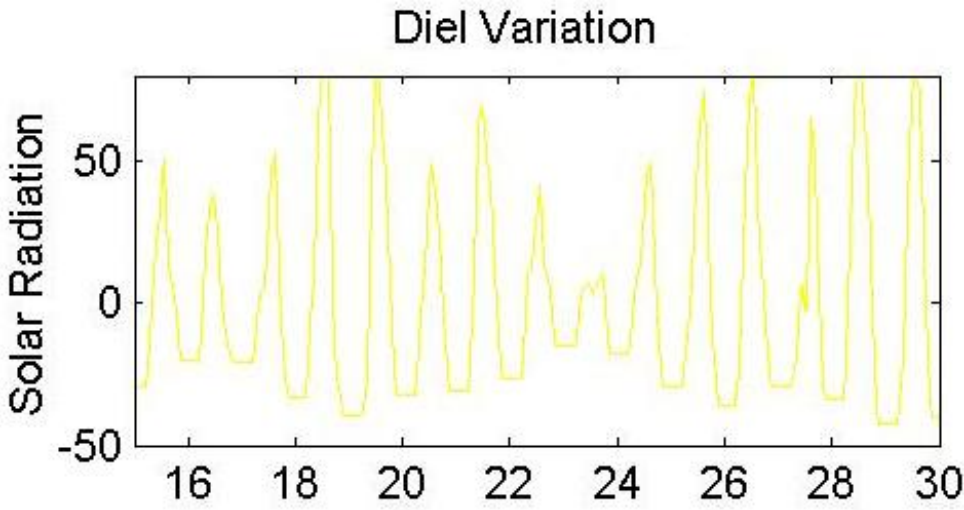


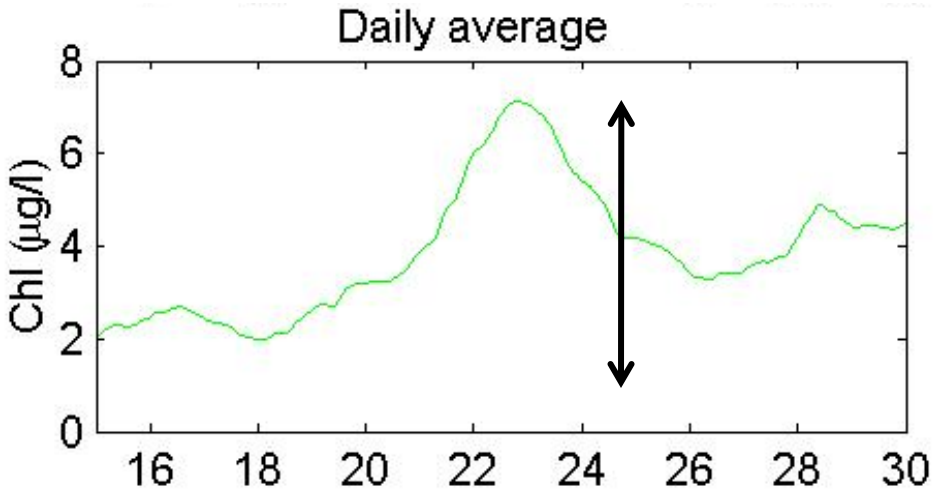


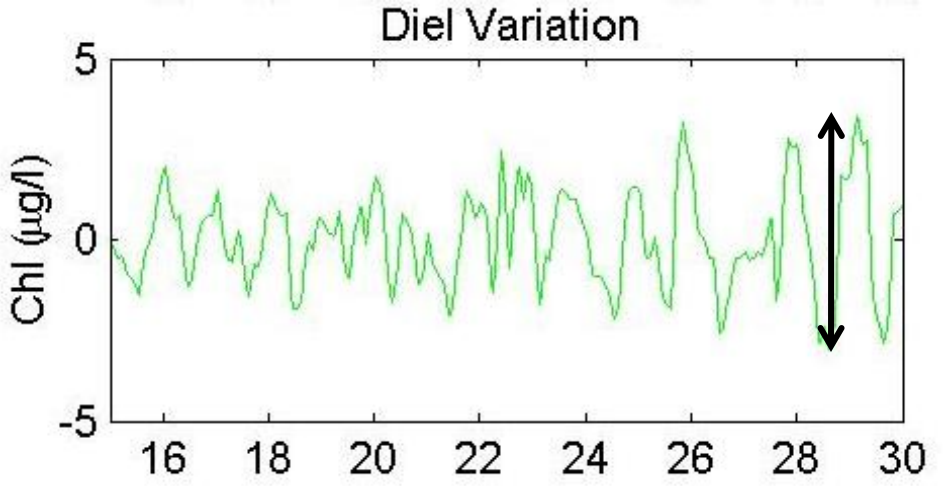


Chlorophyll Fluorescence Diel Cycles

<u>lagged correlation</u>
ρ = -0.62
lag = 0 hours



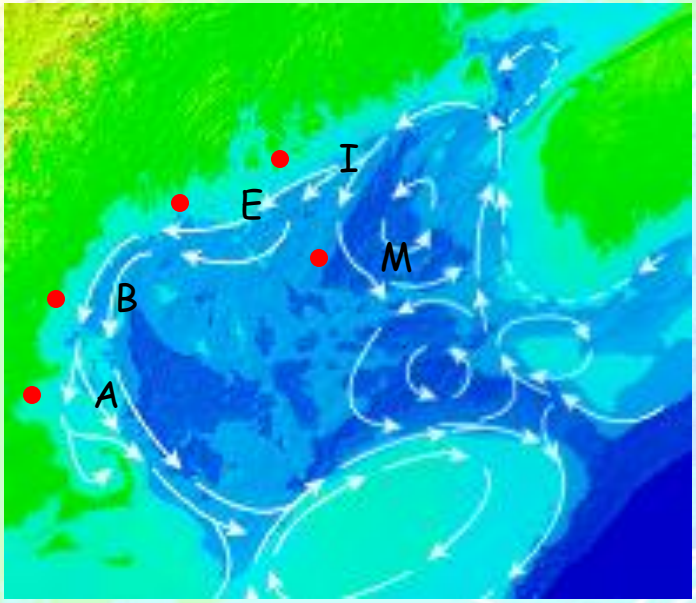


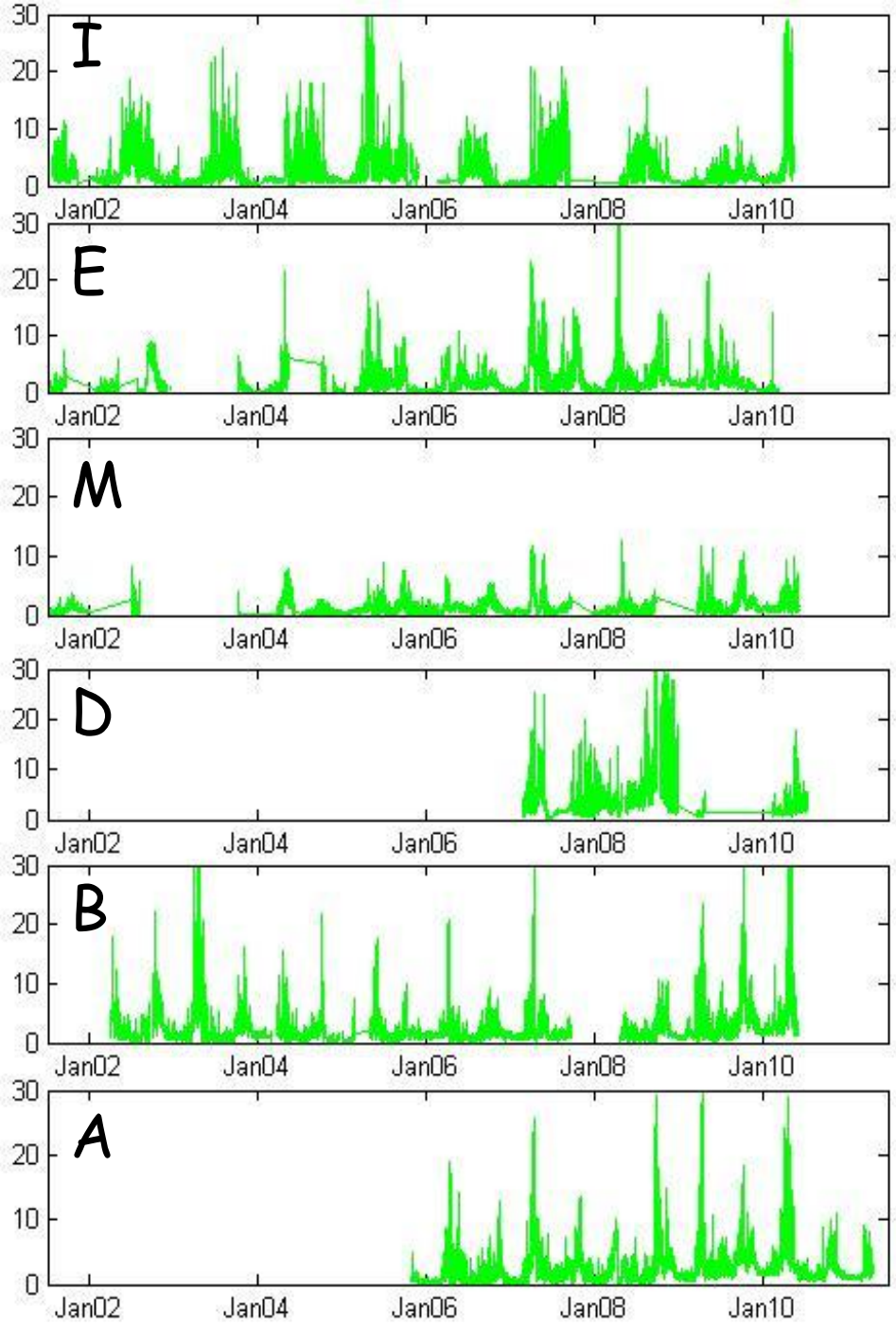


Validation (ground truth)

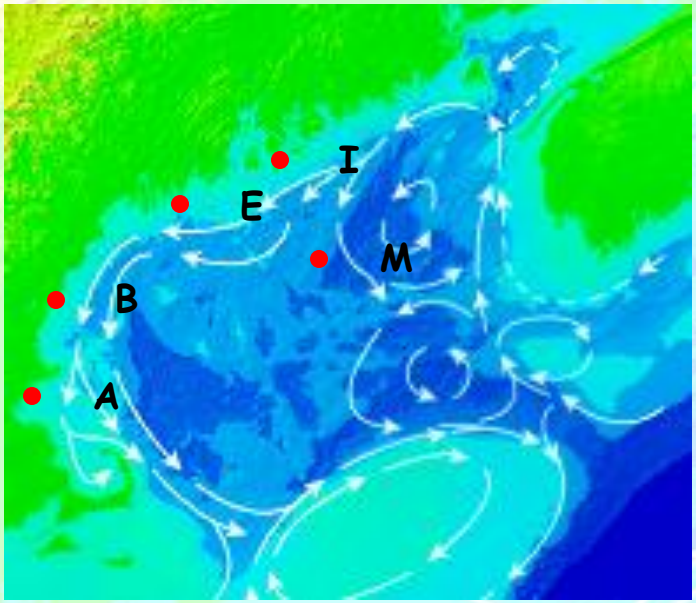
- why does is usually look like a scatter plot?
  - species variations
  - quenched fluorescence
  - …
- and what should you report to SeaBASS?

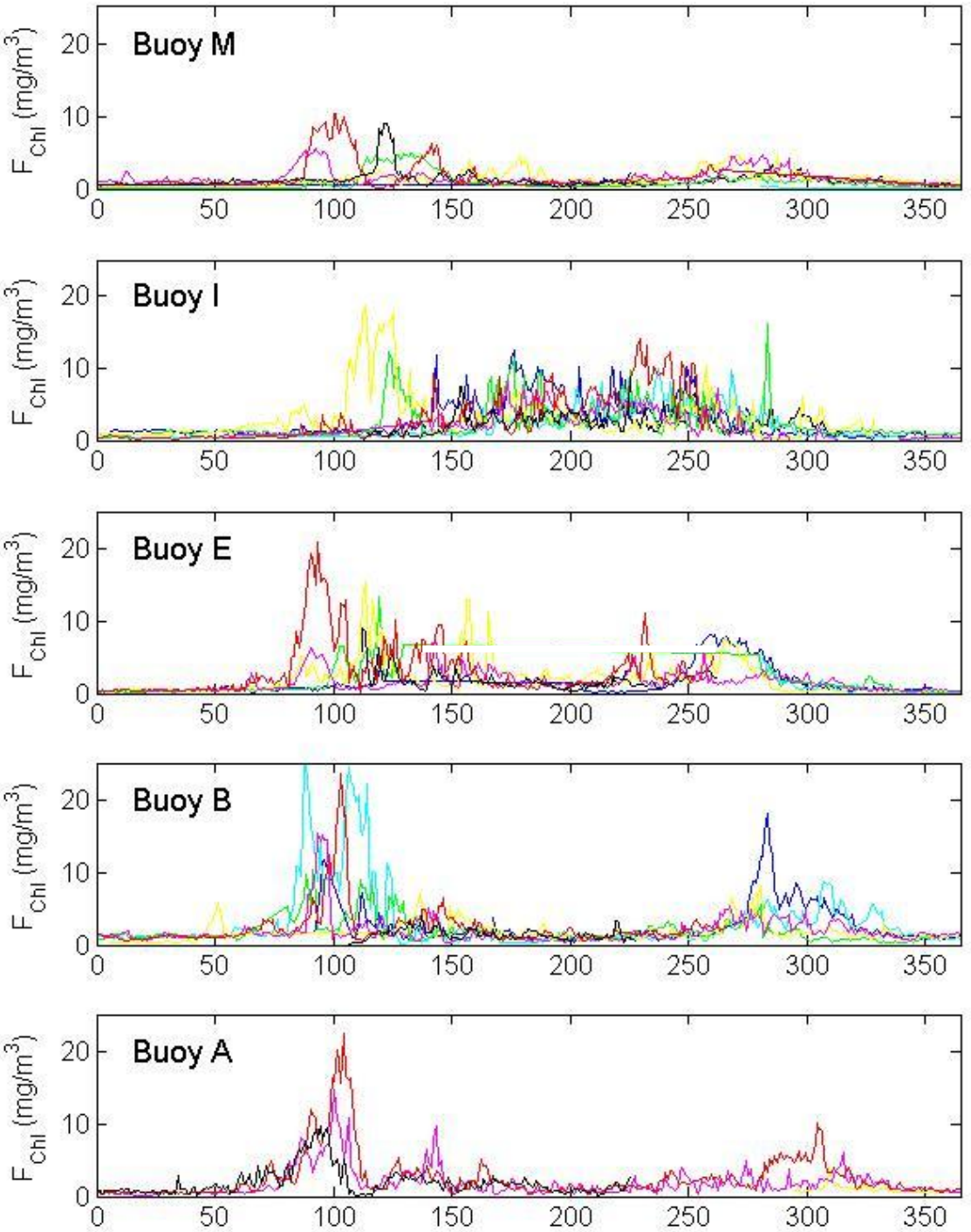
Hourly Observations





Yearly Observations





shift in bloom timing 2001-2004 and 2005-2010