

# Lecture 16

# Fluorescence

by phytoplankton pigments and CDOM

Collin Roesler

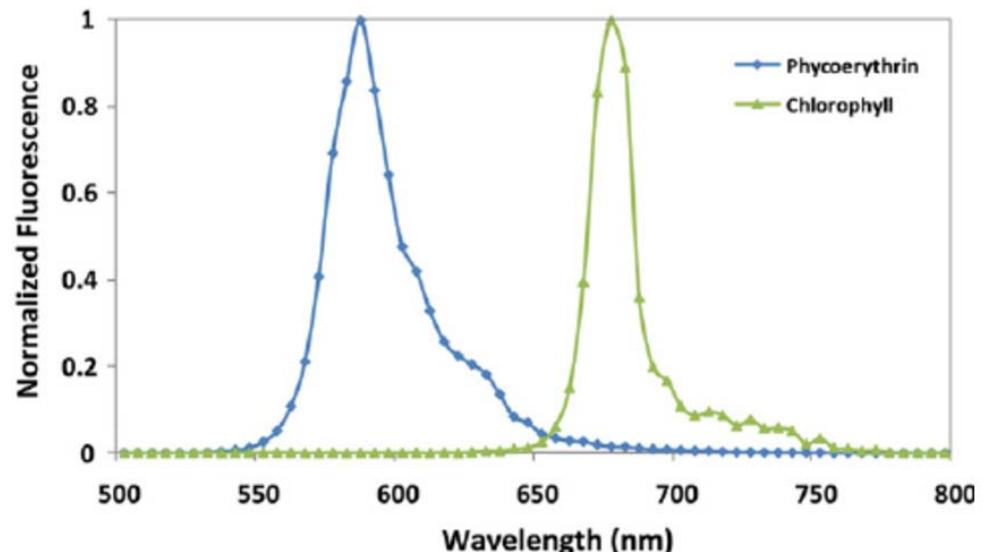
18 July 2017

# Fluorescence

- What is it
- Who does it
- Physics of fluorescence
- Physiology of fluorescence
- Given all of the sources of variability, what can we learn

# Fluorescence

- The property of a molecule to re-emit absorbed light energy as a photon of longer wavelength (lower energy)
- In the ocean we have fluorescence by:
- Phytoplankton fluorescence is due to
  - Chlorophyll a (red)
  - Phycoerythrin (orange)



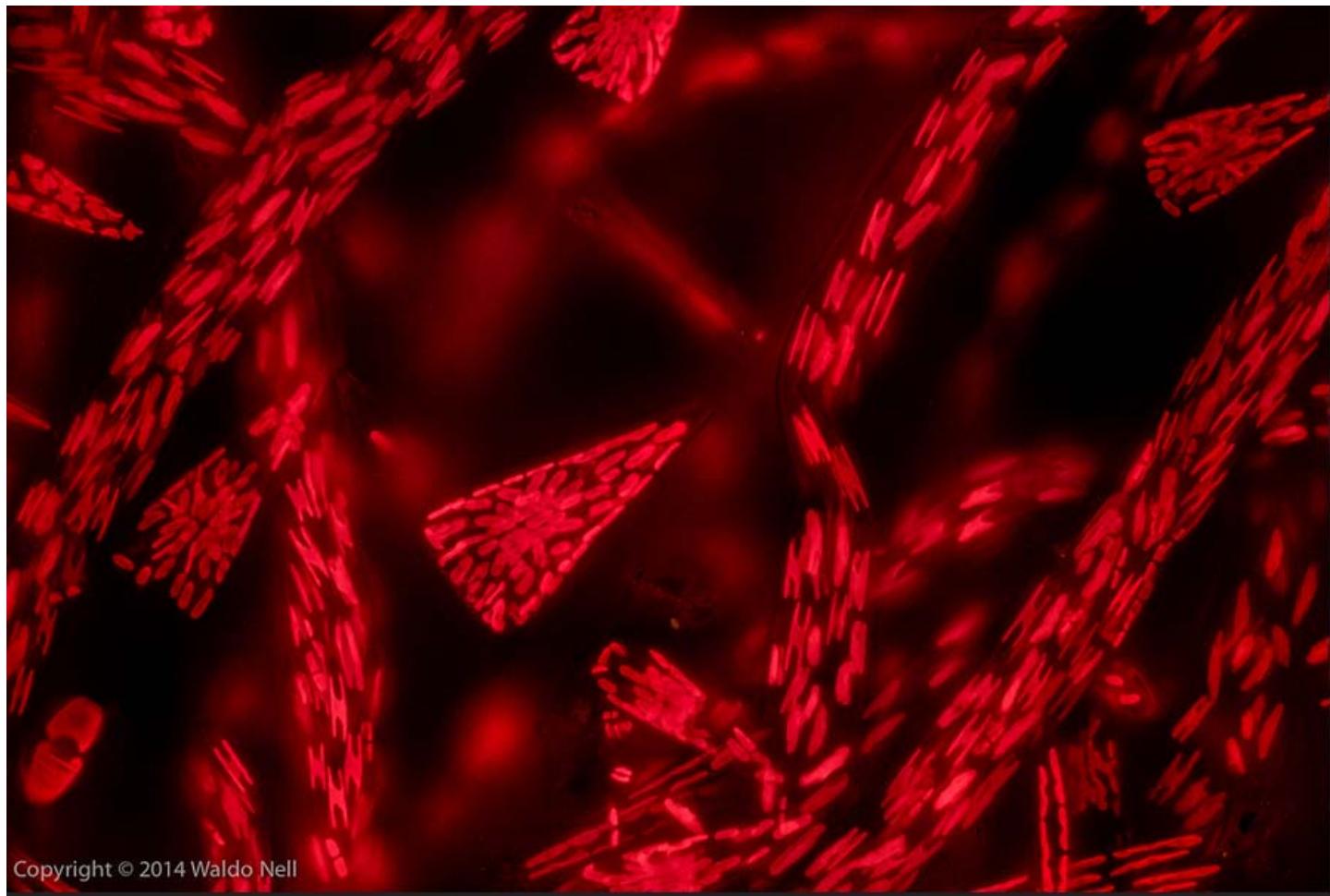
# Light micrograph of diatoms

- 



# Epifluorescent microscopy

- 



# Fluorescence of chl extracts

cellular versus  
solution level

- Extract appears green under ambient light
- When exposed to 440 nm light, appears red

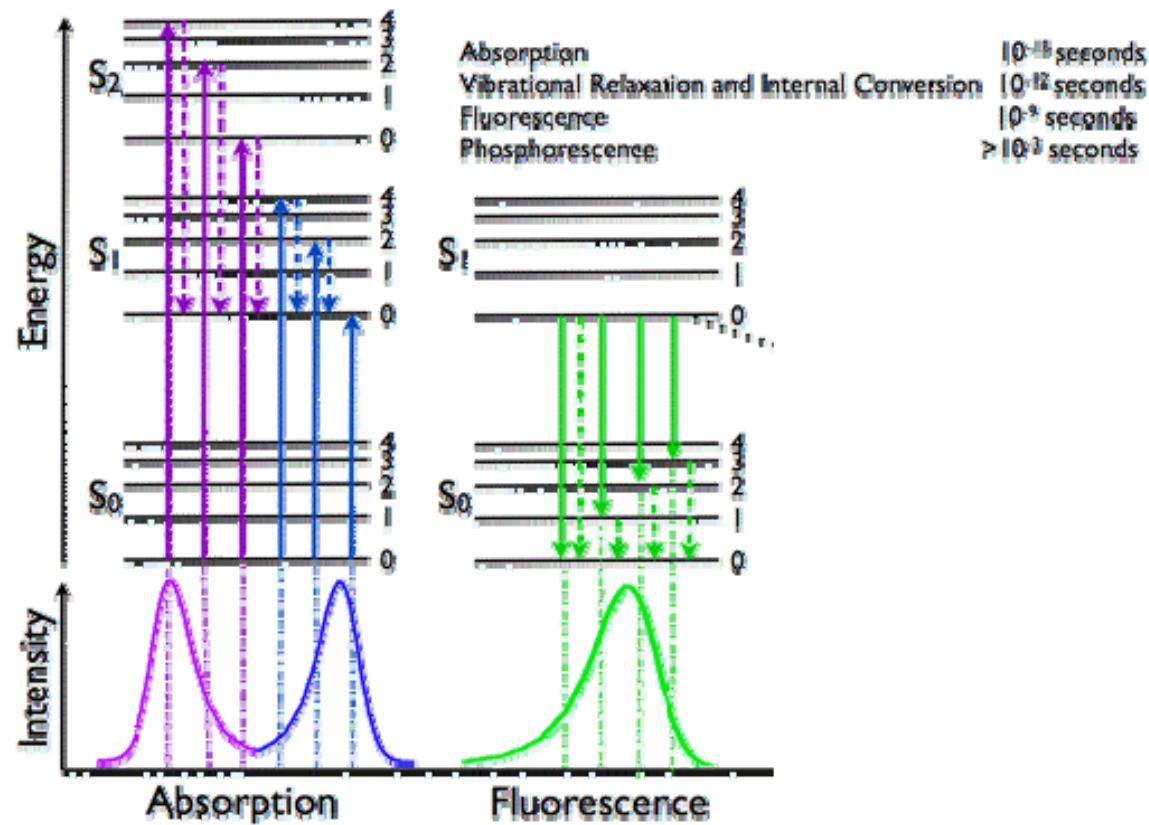


Credit Laura Cinti, <http://c-lab.co.uk>

# Fluorescence

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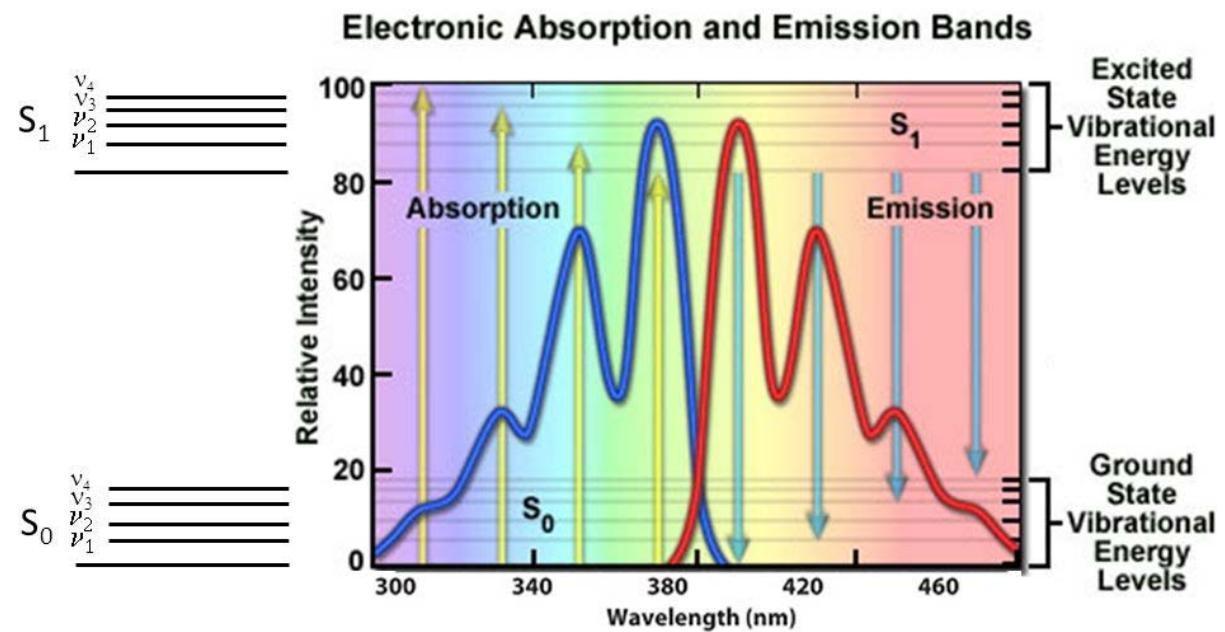
# Fluorescence physics



# Fluorescence physics

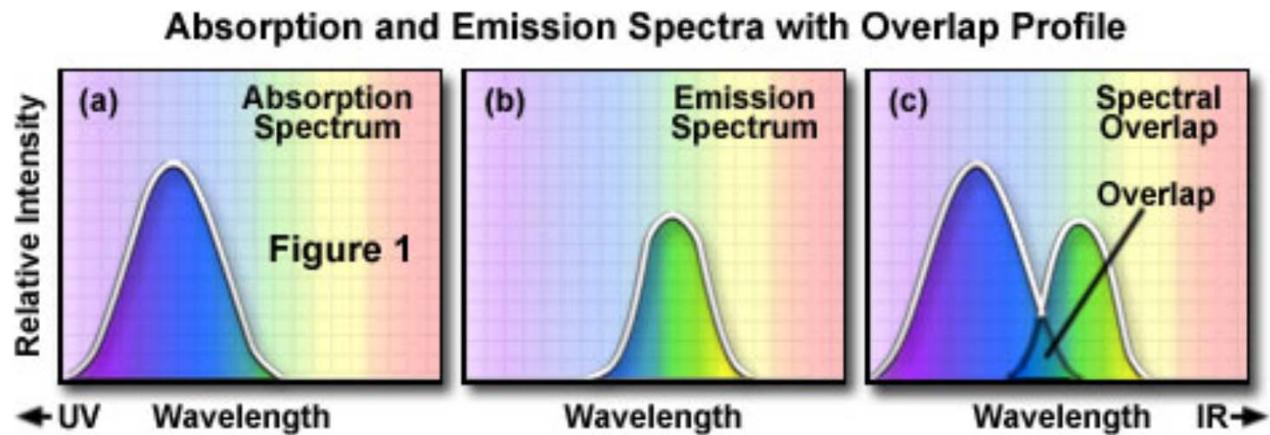
FLuorescence is a mirror image of absorption

## Mirror Image Rule



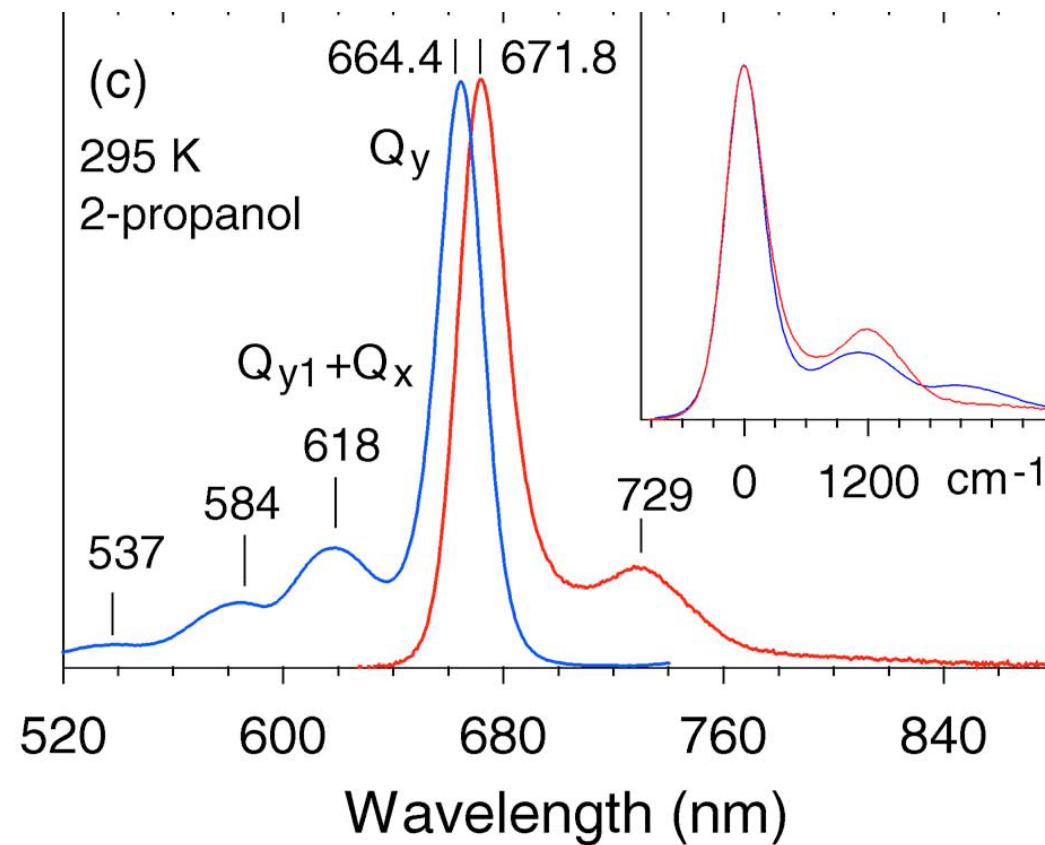
# Fluorescence Physics

- Stokes shift



# Fluorescence physics

- Mirror image absorbance/fluorescence Chl

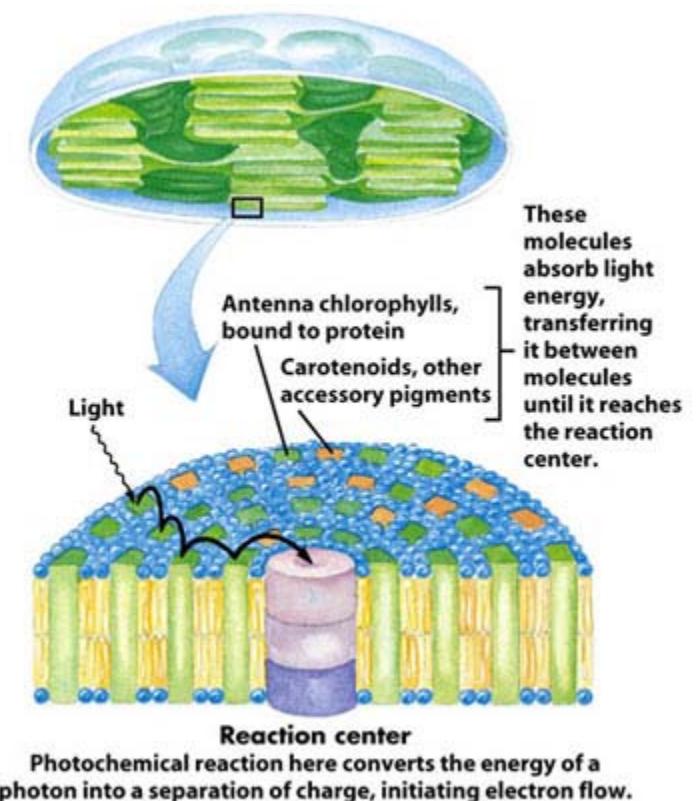


# Fluorescence

- What is it
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# Fluorescence Physiology

- Phytoplankton absorb light in their light harvesting complexes which are imbedded in the thylakoid membranes of the chloroplasts
- Antenna Chl *a* absorb on the blue
- Carotenoids and other chls absorb in the green range
- They transfer energy toward reaction center chls for PS
- This is where fluorescence occurs



# Fluorescence Physiology

- Three fates for absorbed light energy,  $E(\lambda) * a(\lambda)$ 
  - Charge separation (photosynthesis)
  - Heat dissipation
  - Fluorescence
- $F = E(\lambda) * a(\lambda) * \Phi_f$ 
  - Available light (spectral)
  - Absorption coefficients (spectral)
  - Fluorescence efficiency (quantum efficiency) 
- The “loss” of absorbed energy to photosynthesis is called “photochemical quenching”
- The loss of absorbed energy to heat can occur on many time scales (<seconds to > hours), can be reversible or results in structural damage to the photosystem, “non-photochemical quenching”

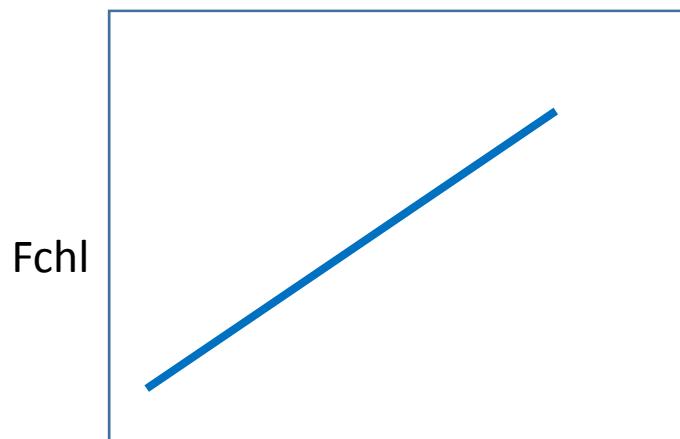
scalar irradiance \* absorption coefficient  
-they don't care what direction it comes from

don't use PAR - use spectra of light \*  
spectra of absorption

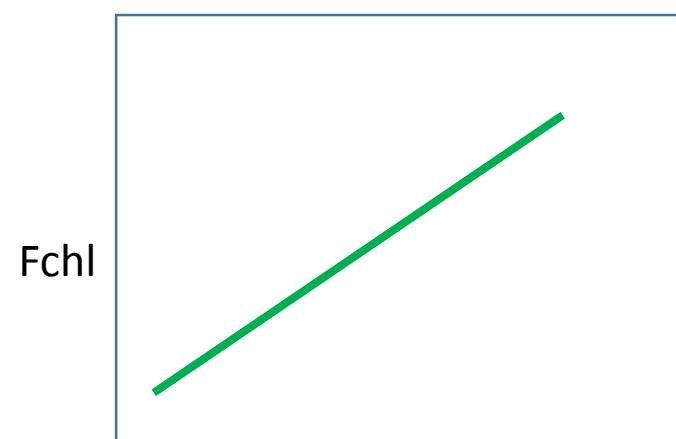
two kinds of  
quenching on  
fluorescence -

# Fluorescence Physiology

- $F = E(\lambda) * a(\lambda) * \Phi_f$ 
  - Available light (spectral)
  - Absorption coefficients (spectral)
  - Fluorescence efficiency (quantum efficiency, 1-3%)



$E$  (hold  $a, \Phi$  constant)



$a$  (hold  $E, \phi$  constant)

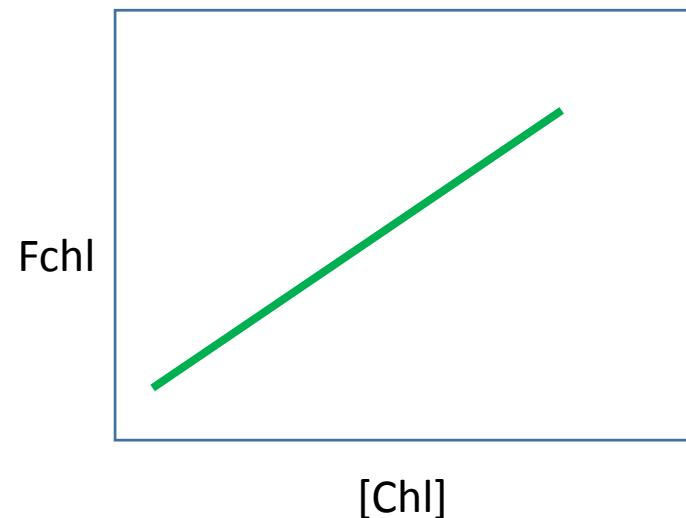
hold irradiance  
constant (left)  
hold fluorescence  
stable too

$a \sim [chl]$

Fluorescence is  
proportional to  
chlorophyll-a  
concentrations

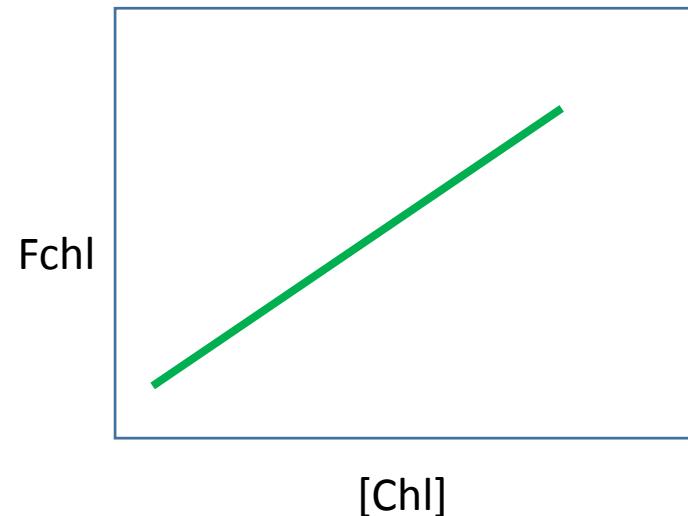
# Fluorescence in extract

- Chl molecules in solution
- No variations in quantum yield
- No physiological pathways
- Maintain constant E
- Fluorometers relative units
- Calibrate with chl standard
- Extractive method



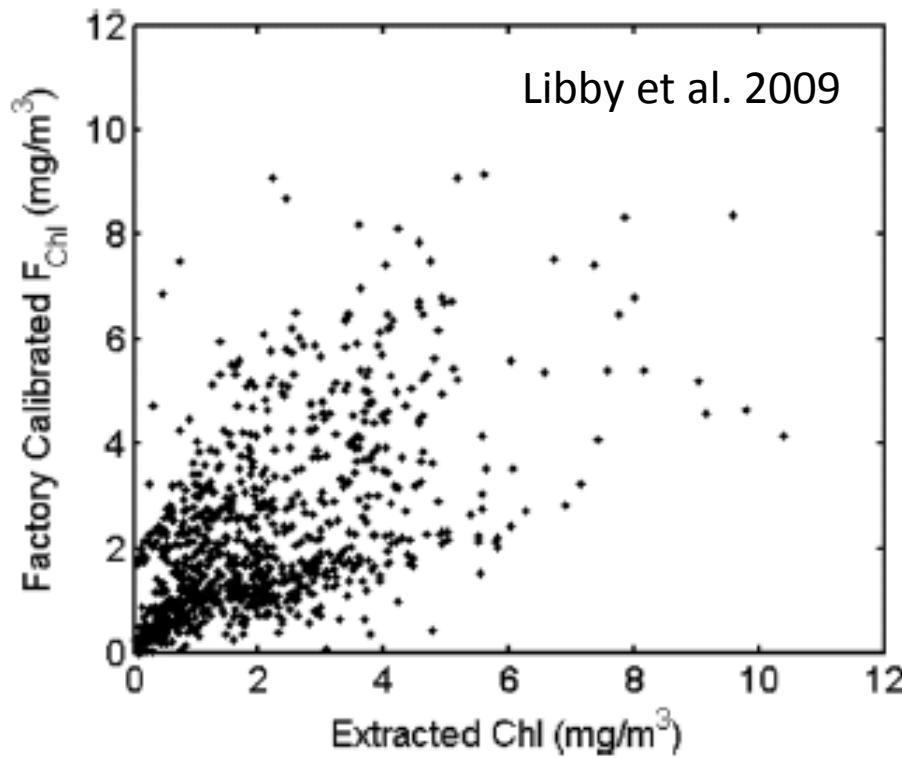
# Fluorescence in cells

- Chl molecules bound to proteins, membranes
- other physiological pathways
- variations in quantum yield
- maintain constant E
- fluorometers relative units
- Calibrate with chl standard
- How bad can it be



# How many have experienced this?

- Freshly calibrated fluorometer
- Paired *in situ* calibrated fluorescence and extracted chlorophyll concentration for validation
- ugh



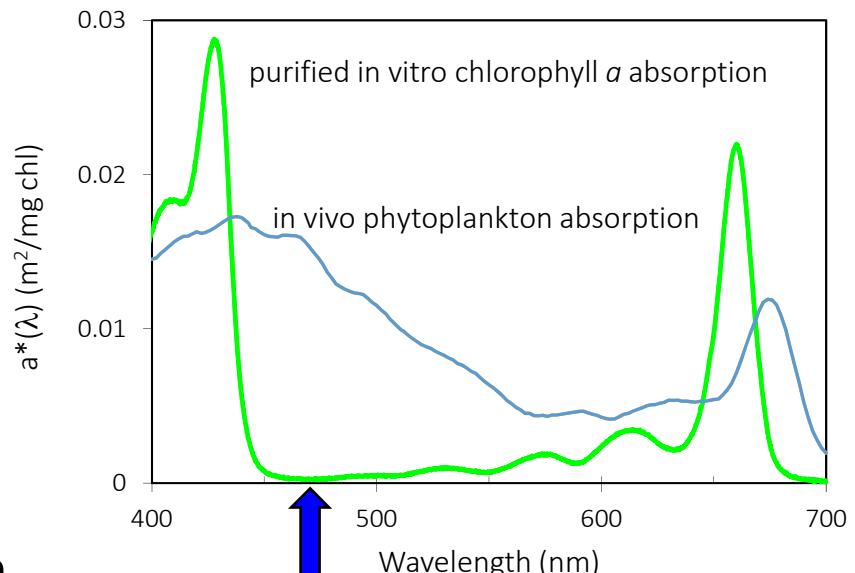
- Mass Bay, 1069 paired samples

# Calibration – from volts to mg/m<sup>3</sup>

- Standards
- *In vitro* chlorophyll
  - Purified chlorophyll *a*
  - Solvent effects (wavelength shift and packaging)
  - LED excitation mismatch
    - Many sensors 470 nm excitation
    - Chlorophyll in extract doesn't absorb at 470 nm
    - 470 nm absorbed by accessory pigments, transferred to chlorophyll *a*, then fluoresced
- “solid standards”

can't use extracted chlorophyll as a standard because it doesn't absorb at 470

they all peak at the same place, but they have difference accessory

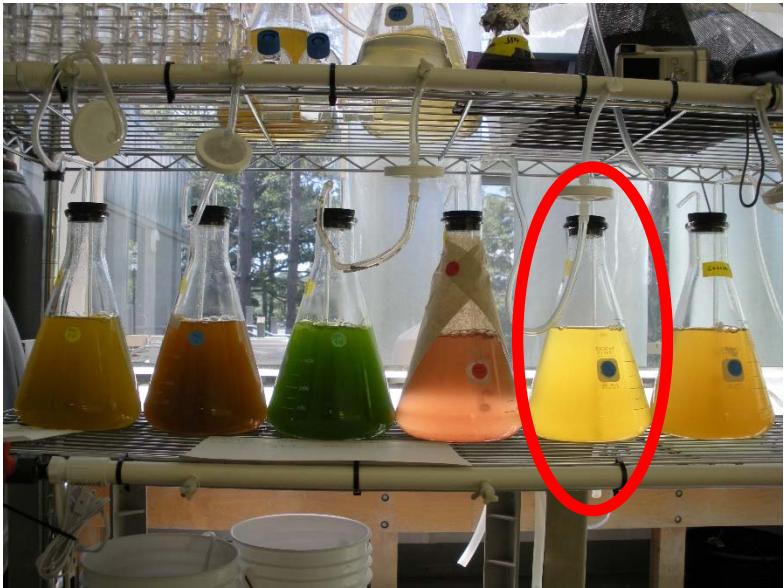


# Calibration – from volts to mg/m<sup>3</sup>

- Standards
- *In vivo* chlorophyll *a* (living culture)
  - *Traceable?*
    - Easy to culture
    - Ubiquitous
    - Robust optical properties
    - *Thallasiosira pseudonana*
    - Growth conditions
      - Replete but not inhibiting light 250 µE/m<sup>2</sup>/s
      - 24h to discourage diel cycles/phases
      - Replete nutrients
      - Exponential growth
    - Database of chlorophyll, HPLC pigments, absorption, size, POC

# Calibrating in situ fluorometers

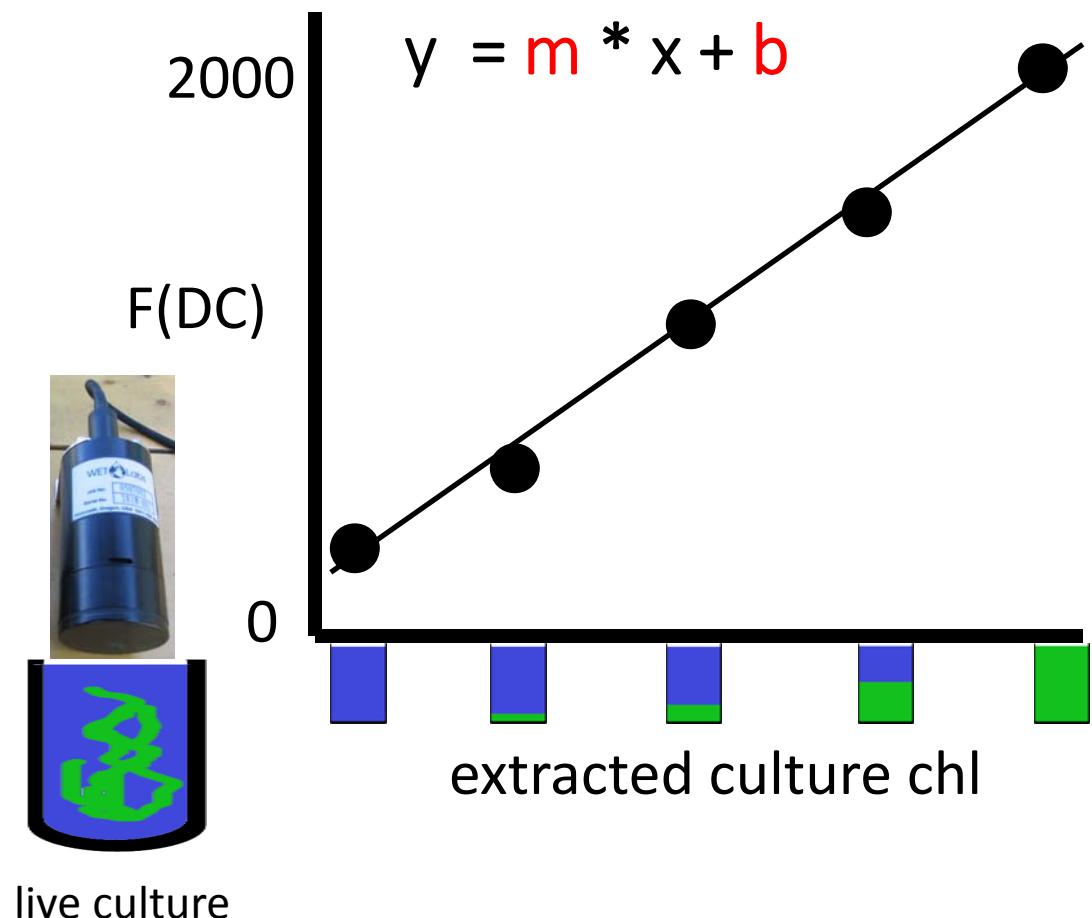
- Grow a culture
- Make a standard curve



Thibodeau et al. 2014

# Calibration Standard Curve

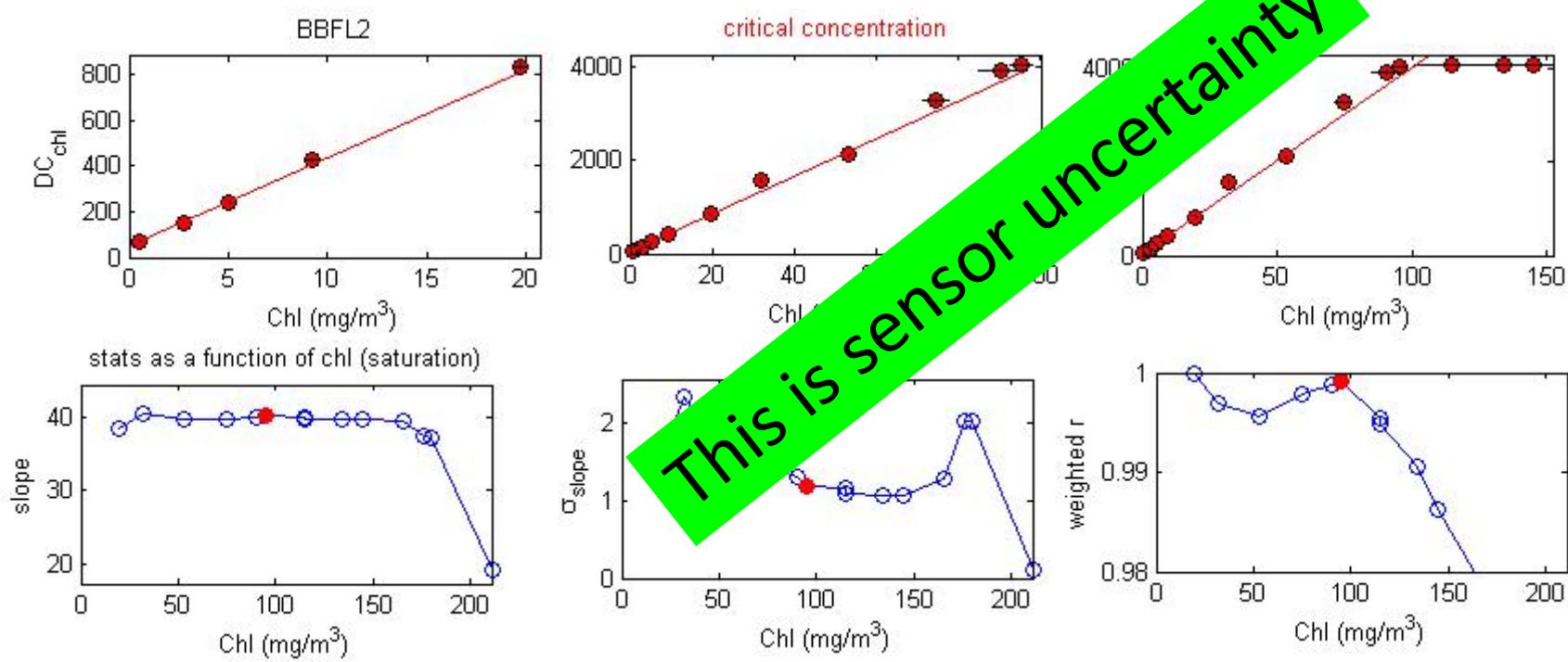
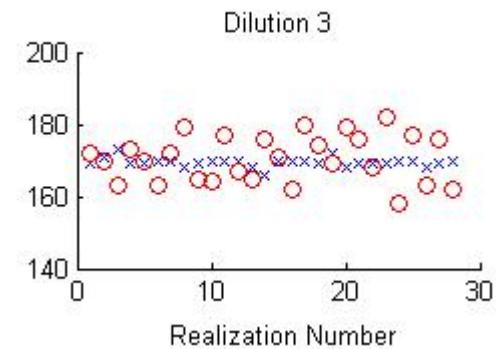
- Dilution series
- Fluorescence response
- Extracted chlorophyll each dilution
- Determine ( $F/\text{chl}$ )  
Fluorescence yield = calibration slope



$$\text{Chl (mg/m}^3\text{)} = (\text{F}_{\text{meas}} - \text{F}_{\text{dark}})/\text{slope}$$

# Steps to calibration - Slope

- 20 dilutions (typically 9)
- 30 second burst sample
- Saturation (30-150 mg/m<sup>3</sup>)
- Slope and intercept statistics (type 2 regression)



# Steps to calibration – Dark reading

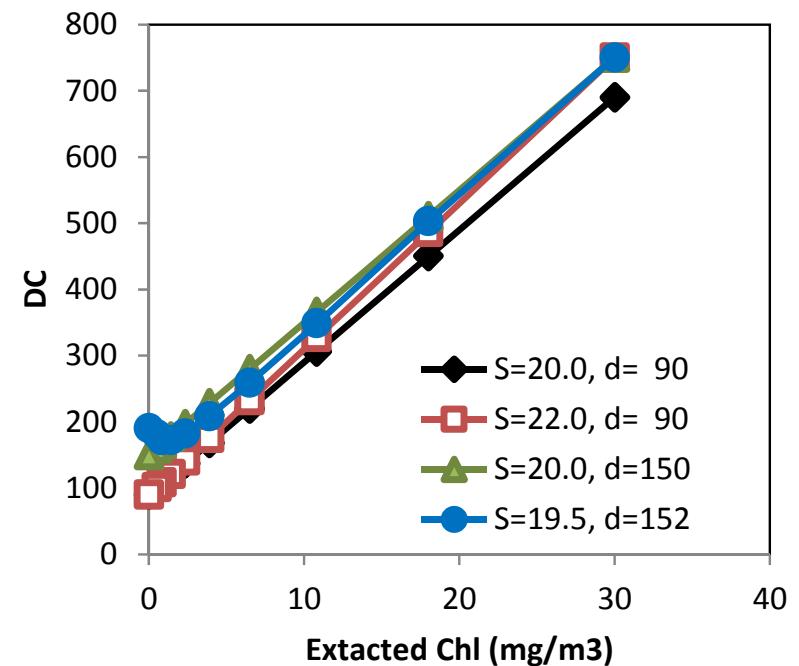
- **Dark reading is instrument signal**
  - Dark air reading
  - Taped in water
  - Determine in deployment configuration
- Dark is not the calibration intercept

$$\text{Chl (mg/m}^3\text{)} = \frac{(F_{\text{meas}} - F_{\text{dark}})}{\text{Slope}_{\text{cal}}}$$
$$F_{\text{meas}} \text{ (DC)}$$
$$F_{\text{dark}} \text{ (DC)}$$
$$\text{Slope}_{\text{cal}} \text{ (DC/(mg/m}^3\text{))}$$

The intercept is actually the blank, NOT the dark  
It is filtered seawater and tells you how much fluorescence signal there is in your lbank, but it doesn't tell you what the dark reading is

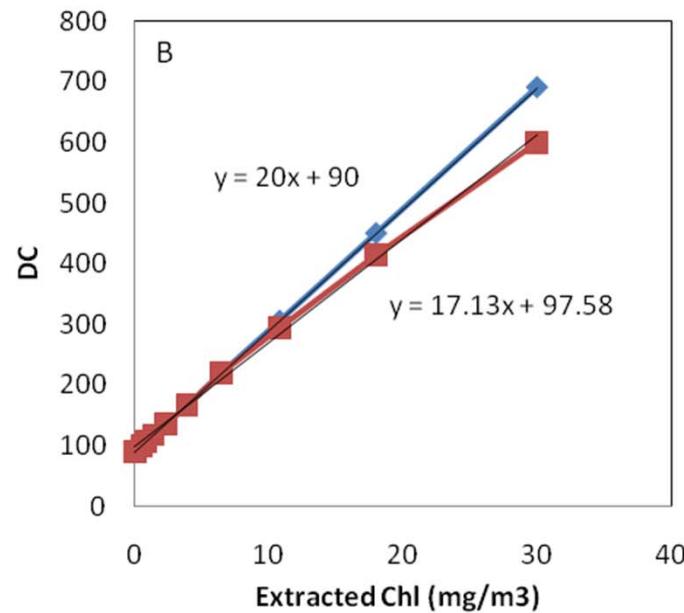
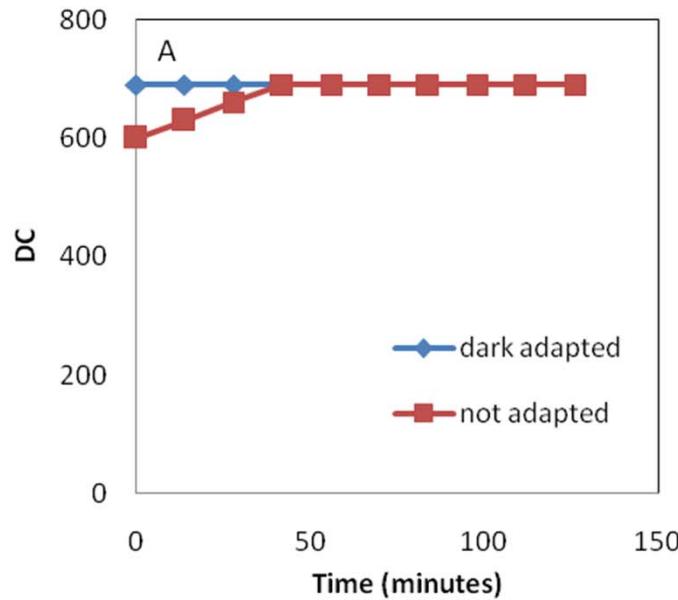
# Considerations for *in vivo* Calibration

- Diluent for the dilution series makes a difference
  - Medium may or may not have signal
  - Diluent may or may not have signal
  - Impacts derived slope (10%)
- **Dilute using culture filtrate**
  - Not culture media
  - Not filtered seawater
  - Not pure water



# Considerations for *in vivo* Calibration

- The culture is alive and therefore subject to change in environmental conditions during calibration
- Example of impact of photo-recovery on calibration
- **Allow culture to acclimate to calibration conditions**

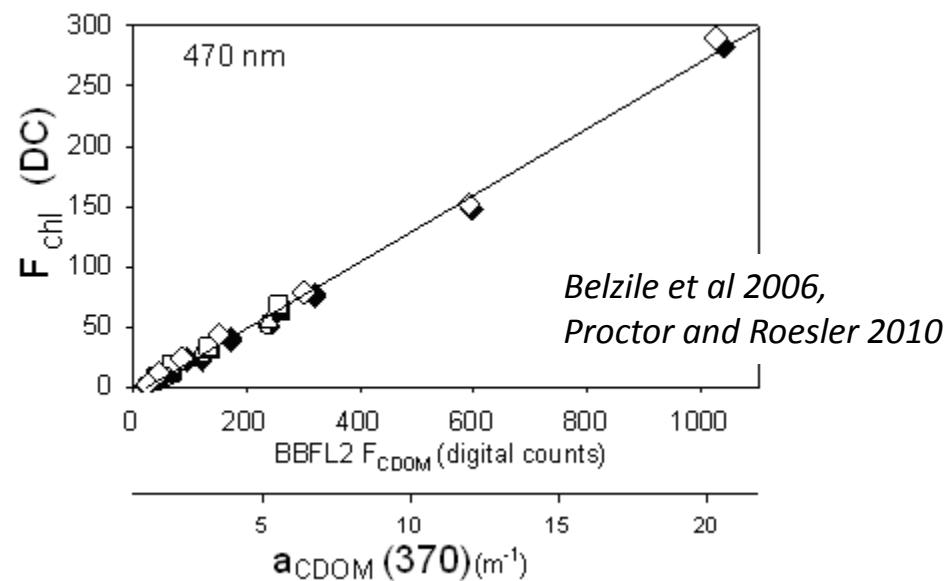
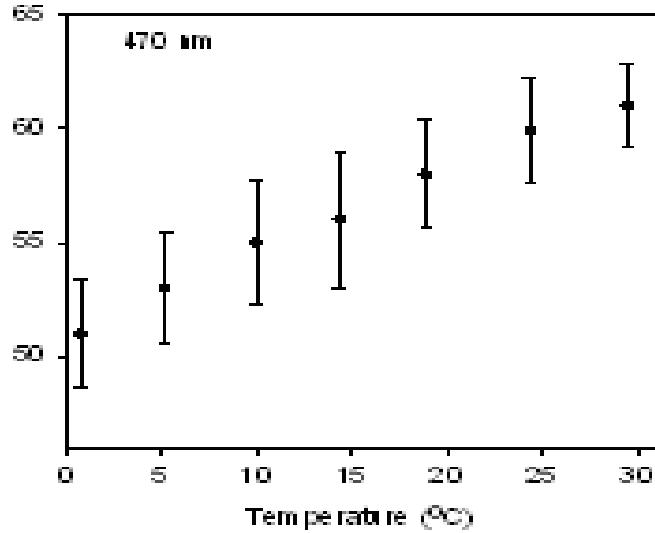


# So what is the *Blank*?

Cullen and Davis 2003

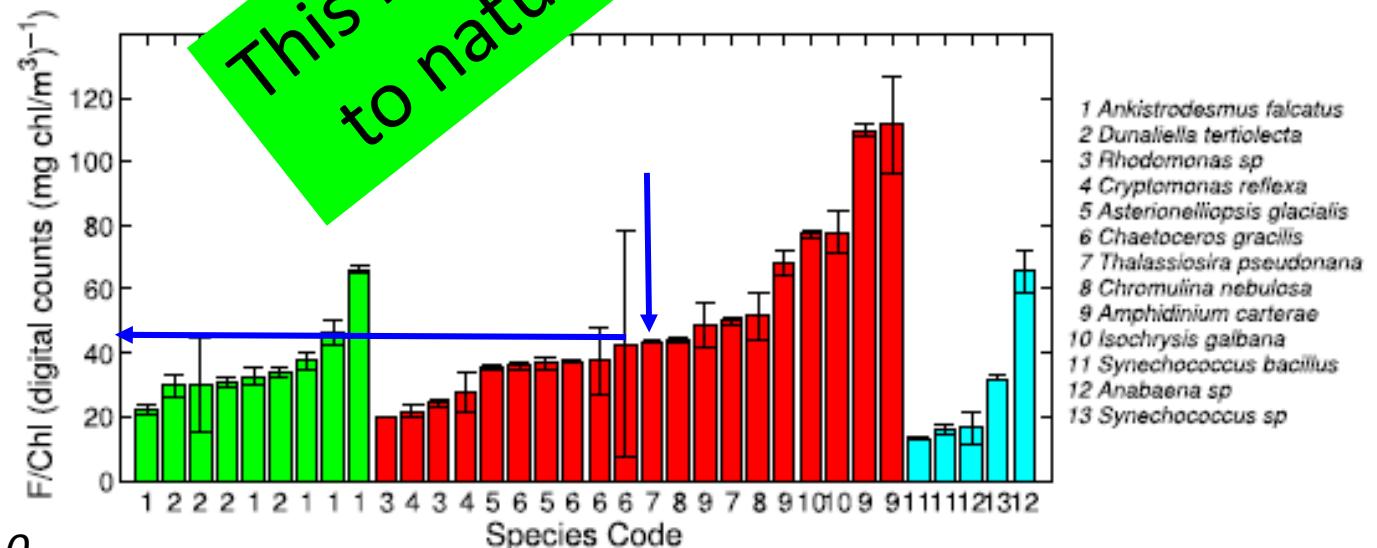
- The blank is **not** the dark reading
- Corrects for environmental signal contamination
  - e.g. Temperature
  - CDOM
    - CDOM fluorescence contributes to apparent chlorophyll
    - Collect measurement of in situ filtrate

y = conc chla



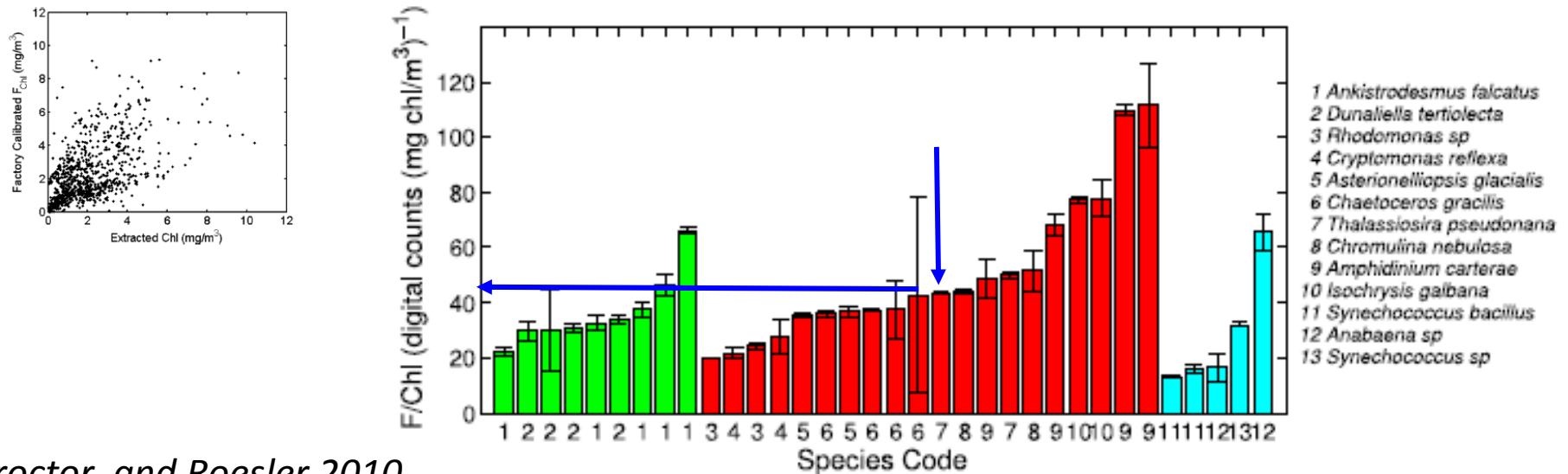
# Considerations for *in vivo* Calibration

- What about other species?
  - 6 fold variation in calibration slope (fluorescence yield)
  - Light history
  - Growth phase
- Why *Thalassiosira pseudonana*?
  - Ubiquitous
  - Easy to grow – provides a reference
  - Median slope



# Considerations for *in vivo* Calibration

- What do the units really mean on the calibration?
- *Thallasiosira*-equivalent chlorophyll
- Good precision (repeatable calibration)
- Can improve accuracy (with taxonomy knowledge)
- Not the biggest uncertainty



Proctor and Roesler 2010

# Best Practice Deployment Protocols

- Obtained from Factory
- In laboratory
  - Dark reading (air)
  - Pure water reading
  - Characterization (T, CDOM)
  - Culture calibration
- Deployment Configuration
  - Dark reading (air)
  - Deploy
- Recovery
  - Dirty pure water reading
  - Cleaned pure water reading
  - Post-recovery culture calibration
  - Dark reading (air)
- Dark Readings (air)
  - Assess configuration
  - Quantify drift
- Pure water readings
  - Assess biofouling
- Culture calibration
  - Assess response variations

# Instrument characterization/calibration

- Calibrations

- Pre-deployment calibration (1)
- Post-recovery pre-clean calibration (2)

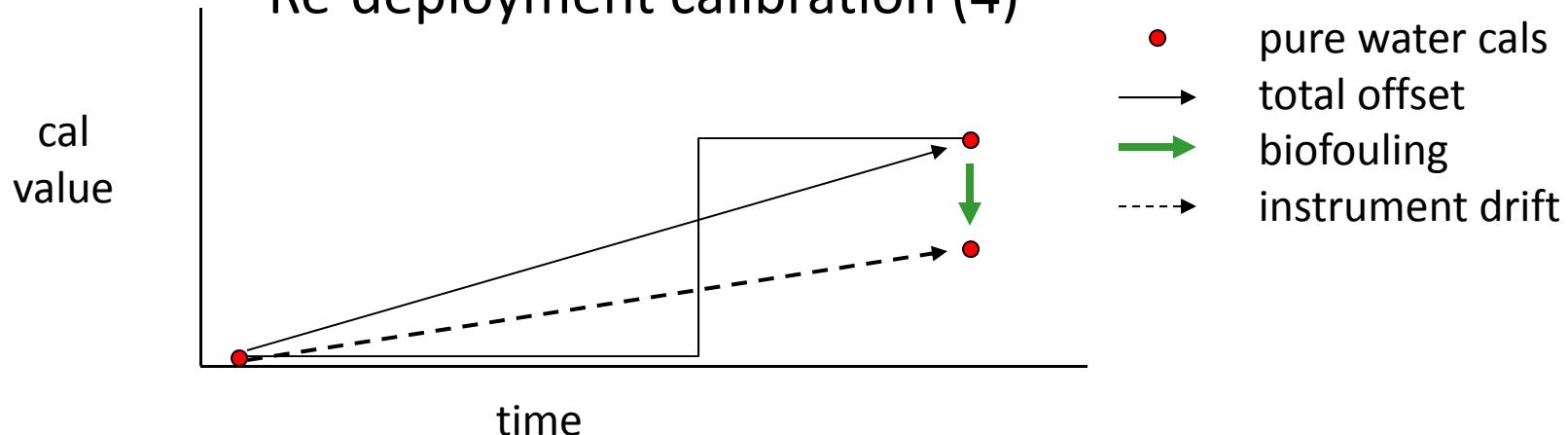
$$\text{Total offset} = (2) - (1)$$

- Post-recovery post-clean calibration (3)

$$\text{biofouling} = (3) - (2)$$

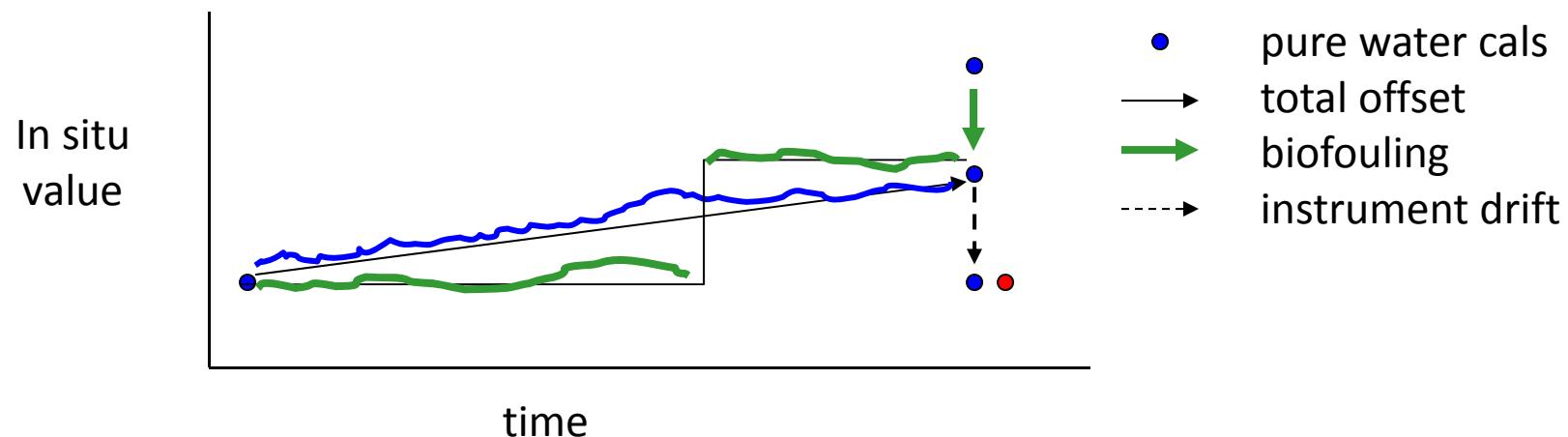
$$\text{drift} = (3) - (1)$$

- Re-deployment calibration (4)

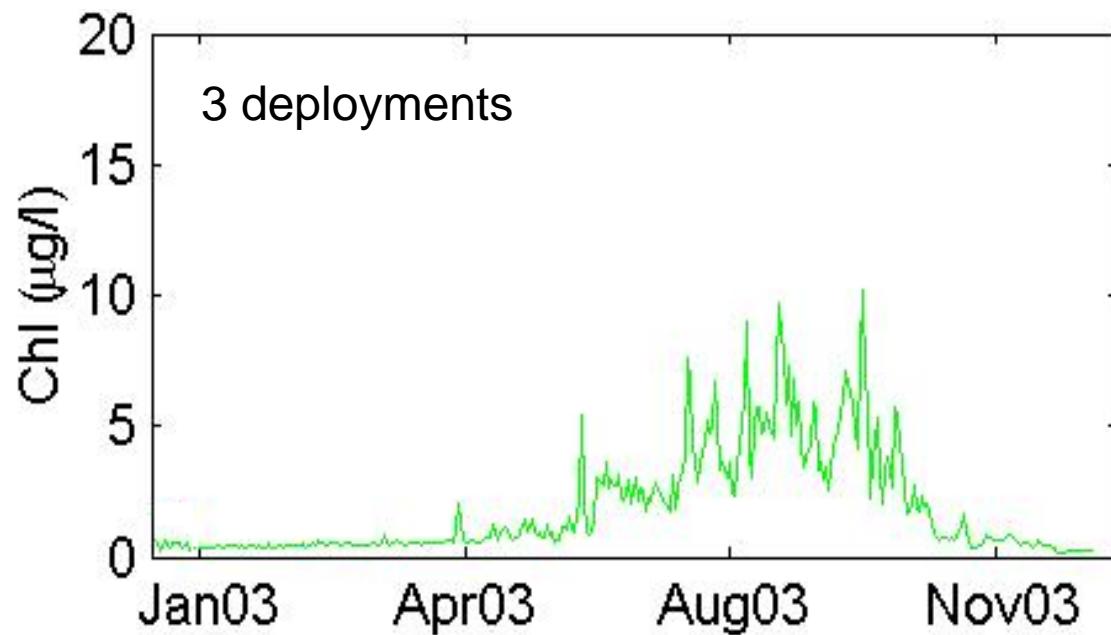
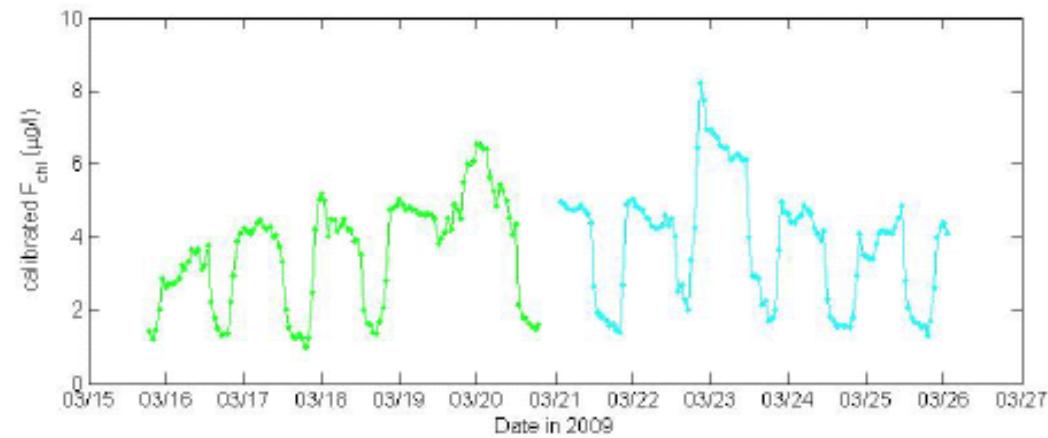


# Instrument characterization/calibration

- Calibrations
  - Linear trend
  - Step function trend
  - Validation (new deploy – corrected)



# After Drift and Biofouling Corrections



Roesler and Boss 2009

# Drift and Biofouling Corrections

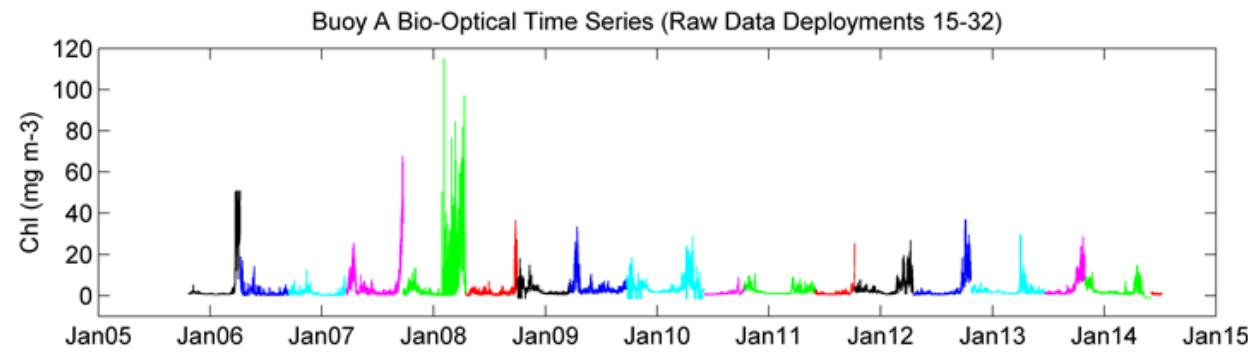
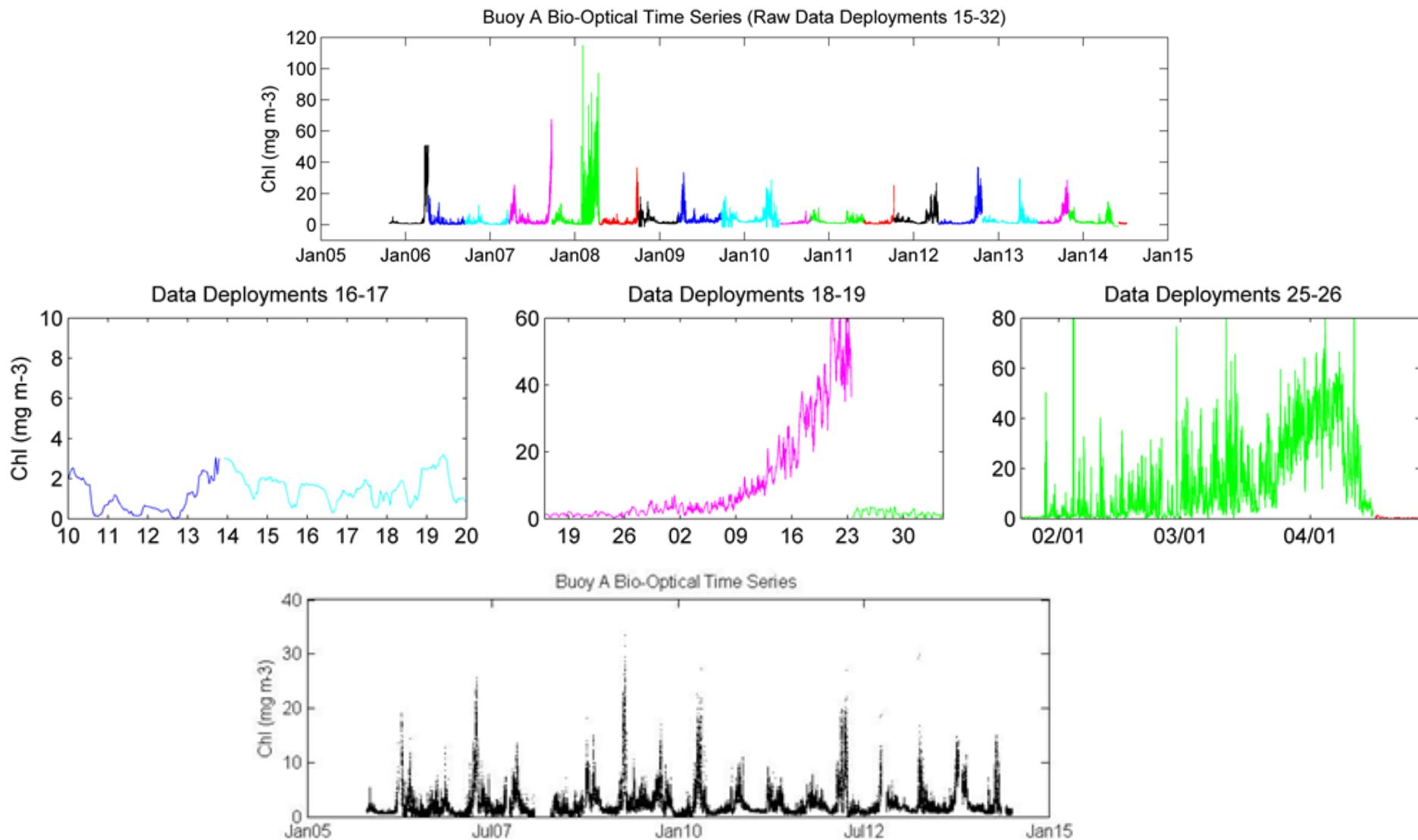


photo credit M. Mickelson



# Drift and Biofouling Corrections

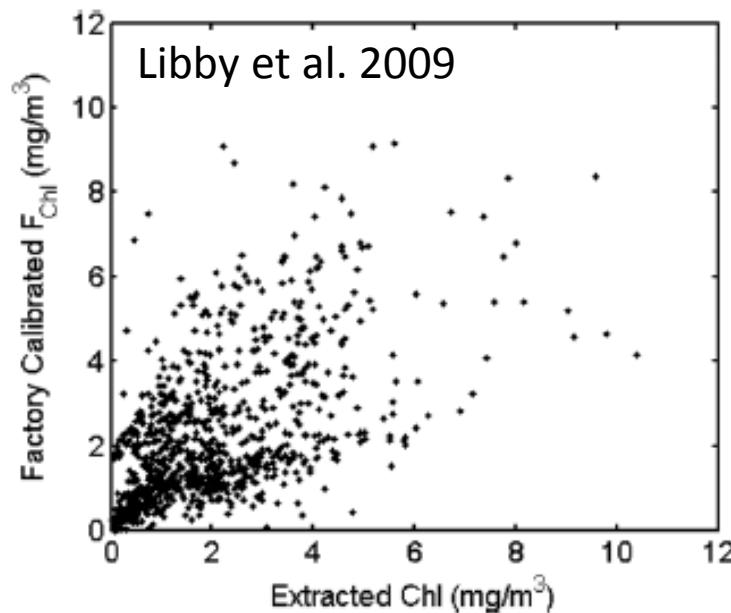


Roesler 2014

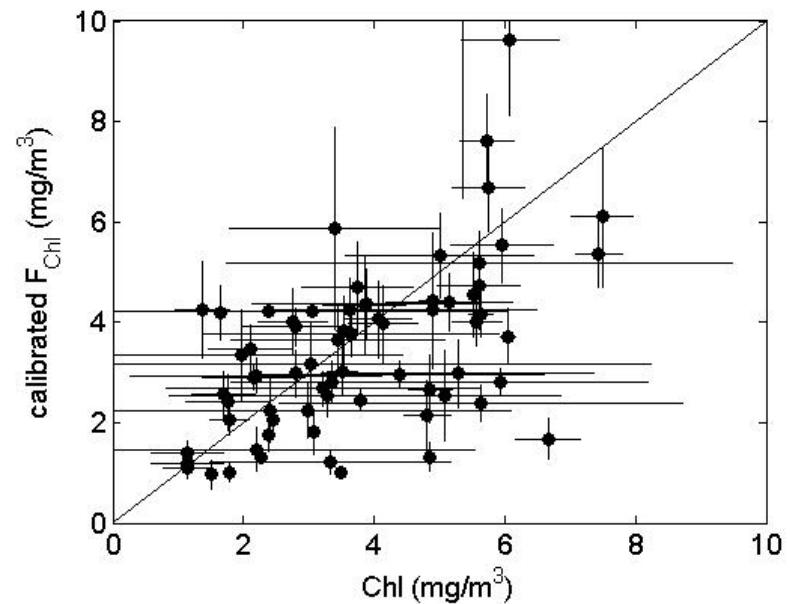
# Validation

- Paired *in situ* calibrated fluorescence and extracted chlorophyll concentration
- *Still* ugh

- Mass Bay, 1069 paired samples

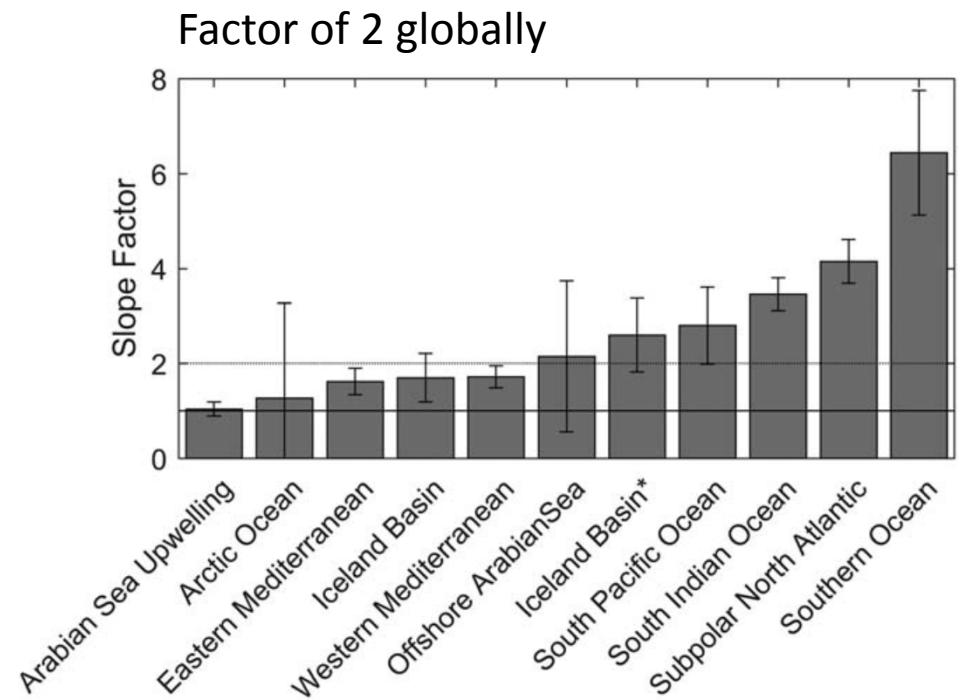
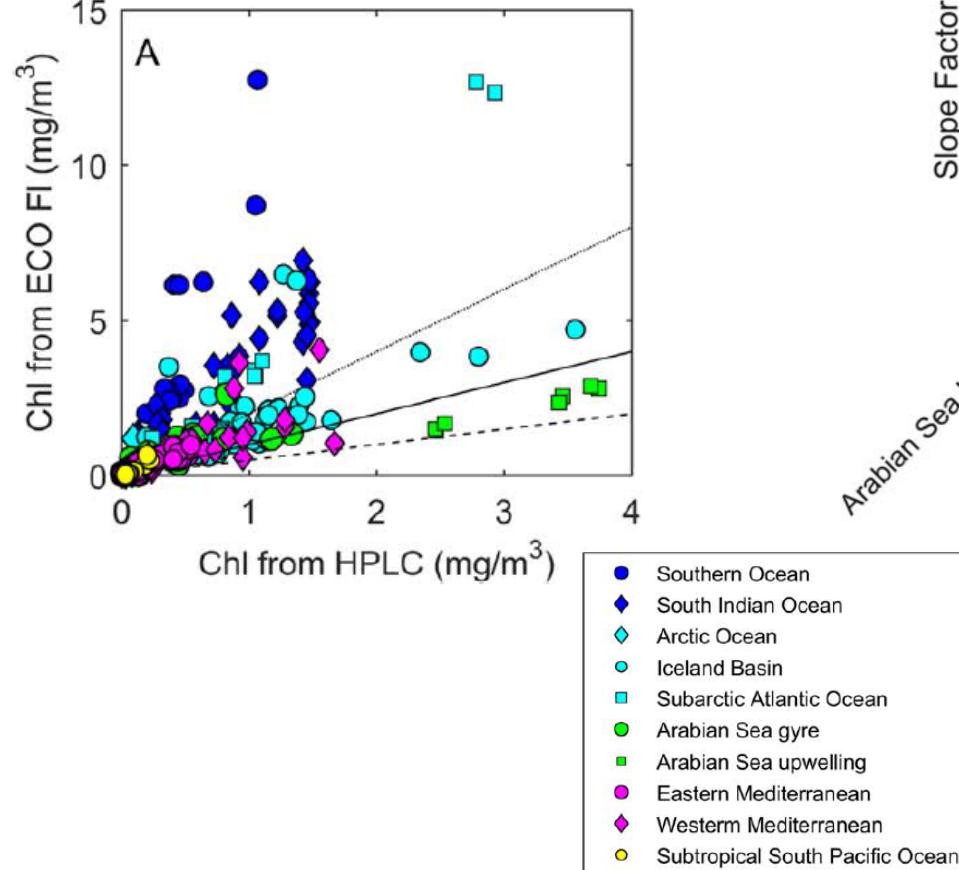


- *in situ* samples and buoy obs
- March-August, 2008 - 2011



includes spatial-temporal sampling variations, species natural variability

# The single calibration factor provided by the company may be wrong

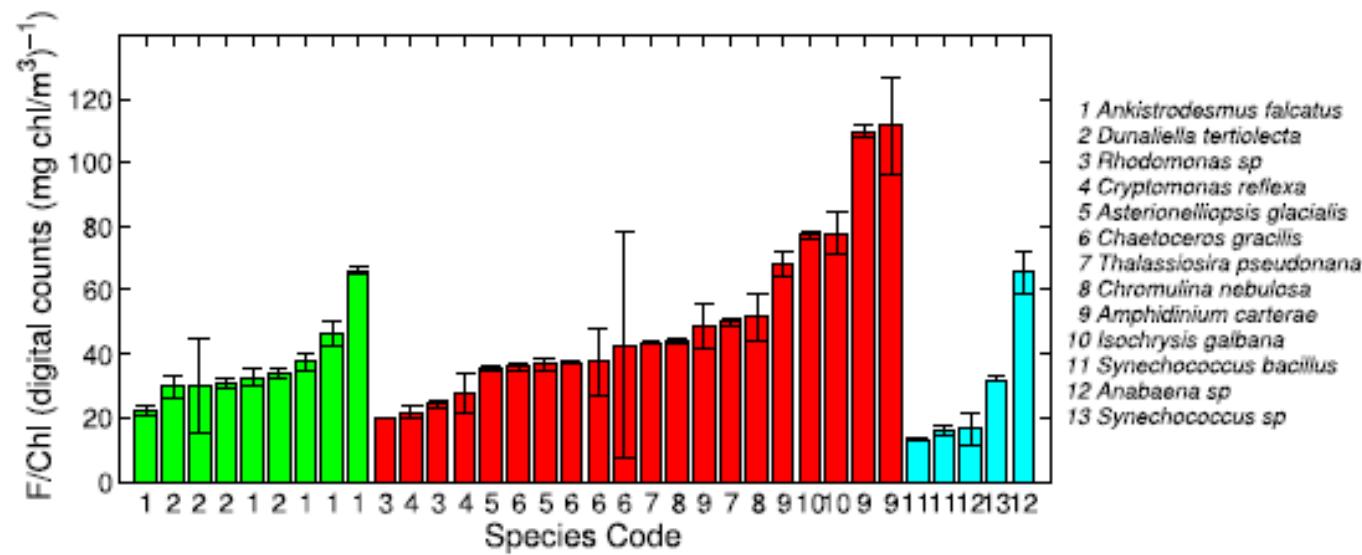


But there is still tremendous natural variability

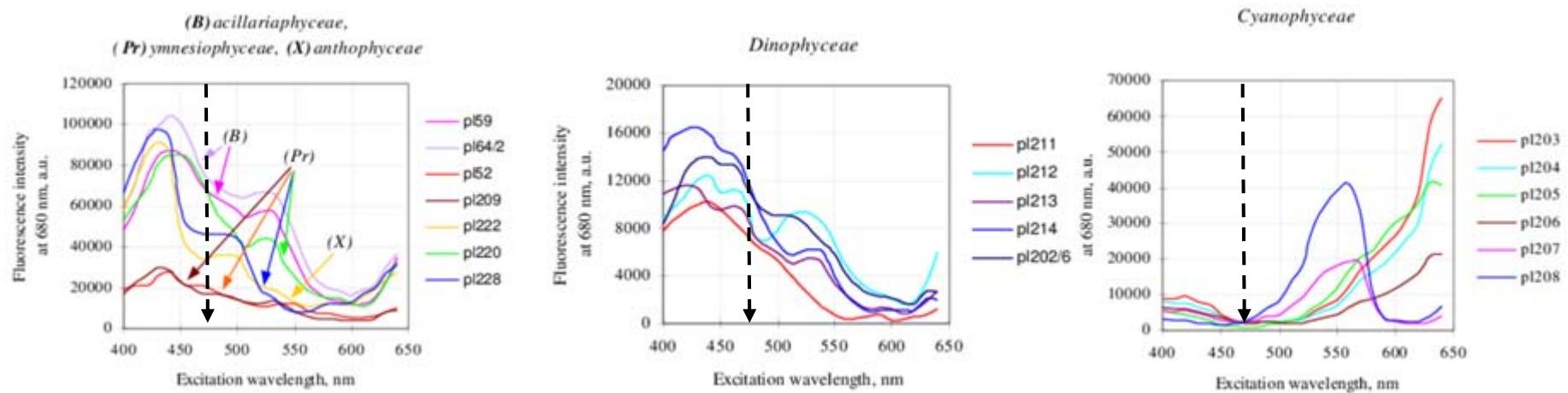
Roesler et al. 2017

# Natural variations in $F_{chlorophyll}$

- Part 1: Species composition
- Solution
  - If you have some taxonomic information
  - Use appropriate calibration slope



# The calibration slope varies significantly between species due to variations in pigment composition (fluorescence excitation)



Depends on the  
fluorescence  
excitation  
wavelength

Larisa Poryvkina, Sergey Babichenko and Aina Leeben

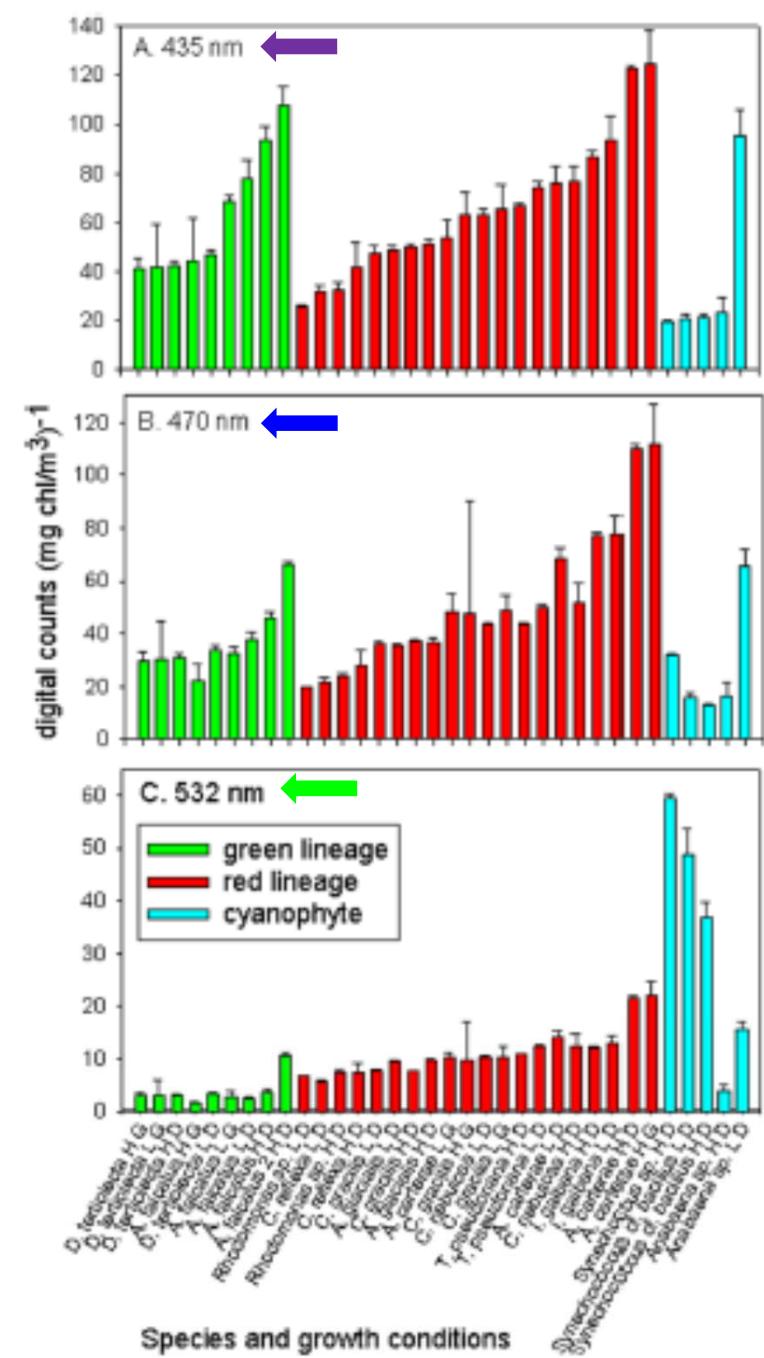
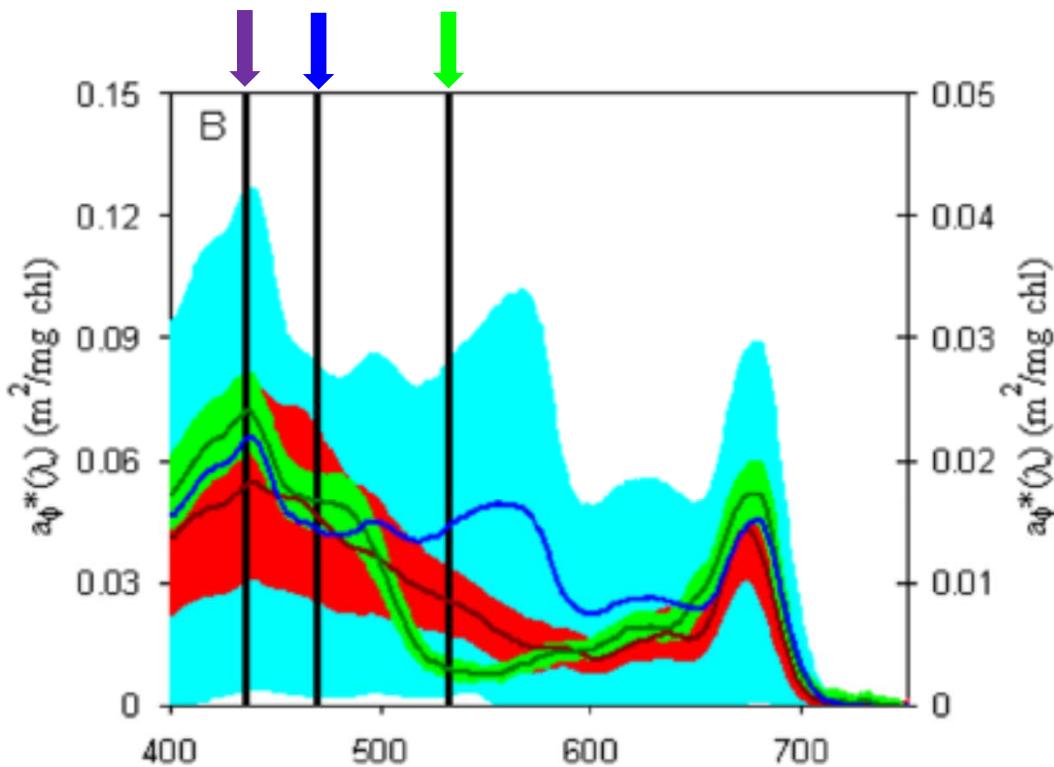
ANALYSIS OF PHYTOPLANKTON PIGMENTS BY EXCITATION SPECTRA OF FLUORESCENCE

Proceedings of EARSeL-SIG-Workshop LIDAR, Dresden/FRG, June 16 – 17, 2000

EARSeL eProceedings No. 1 224, Institute of Ecology / LDI, Tallinn, Estonia

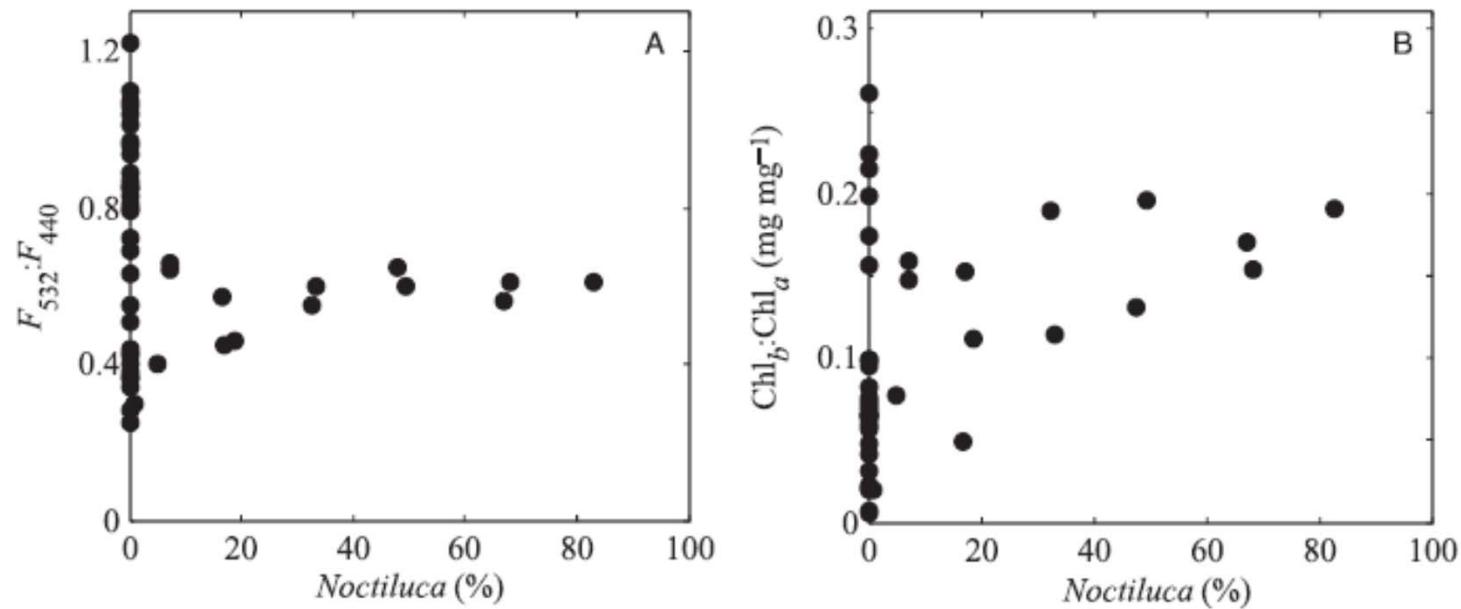
# Calibrate 3 channels of $F_{chl}$ with cultures

- Species response is a fcn of:
    - Absorption
    - Pigment composition
- F ratios are distinct



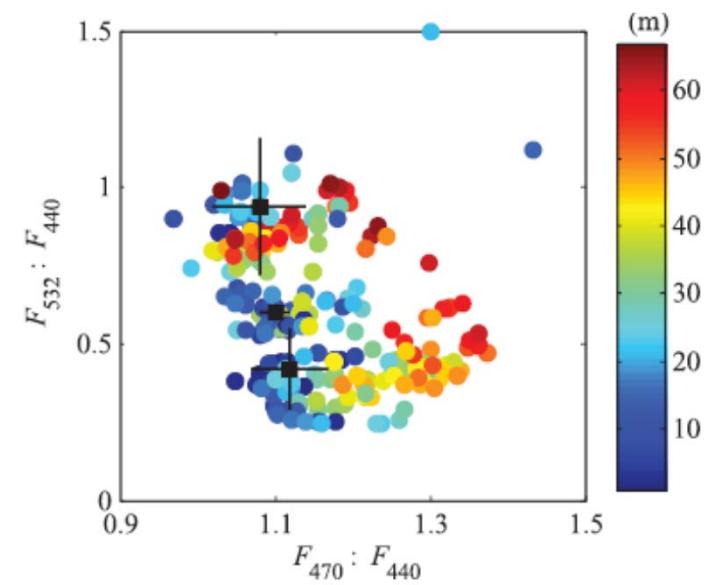
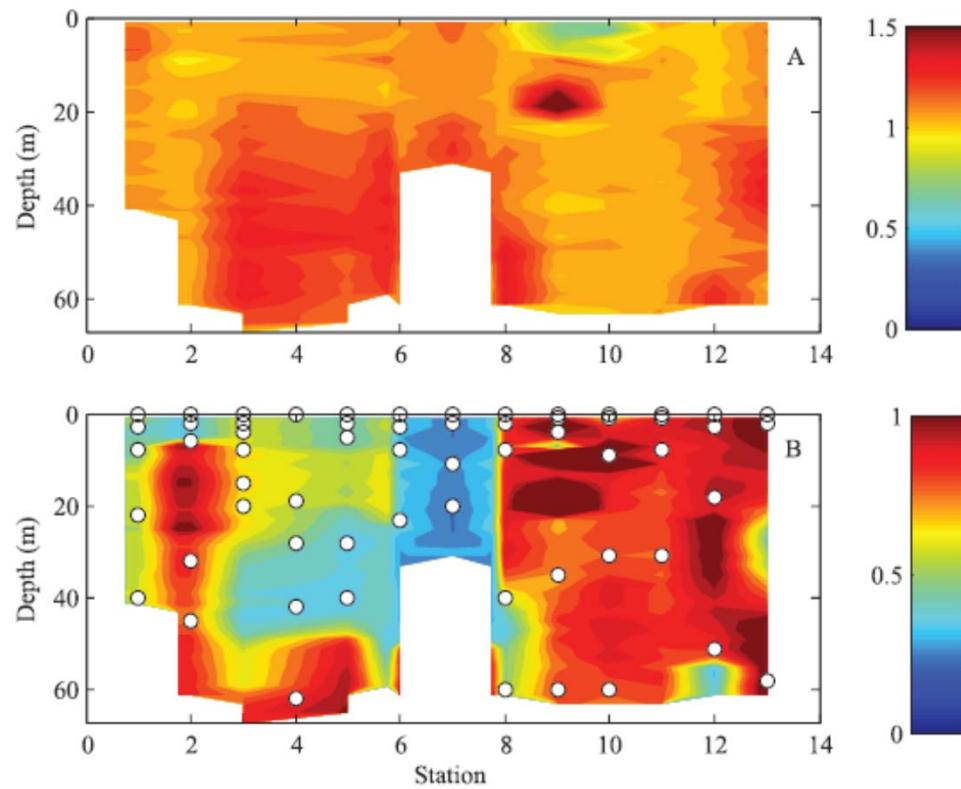
# $F_{470}:F_{440}$ and $F_{532}:F_{440}$ statistically related to pigment ratios

- *Noctiluca* determined by microscopy
- *Pigment ratios determined by HPLC*



# $F_{470}:F_{440}$ and $F_{532}:F_{440}$ statistically distinct between pigment groups

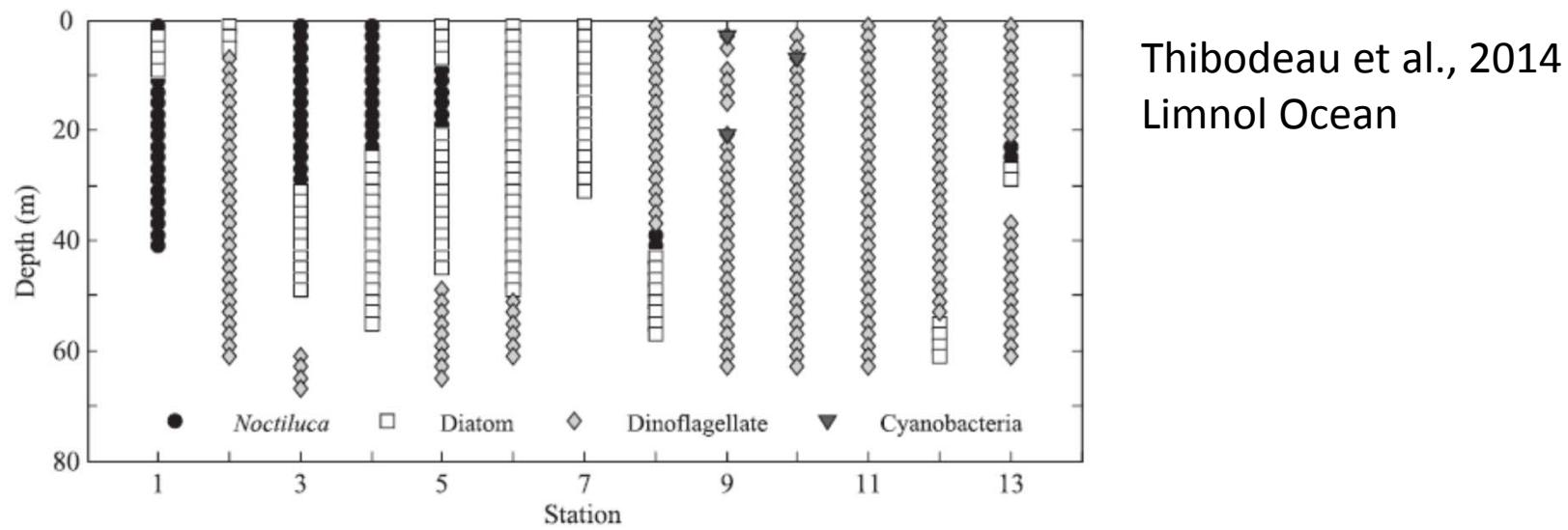
- Along a transect in the Arabian Sea



- Data points cluster
  - Diatoms
  - Dinoflagellates
  - *Noctiluca*

# Assign pigment-based taxonomic dominance to each depth interval

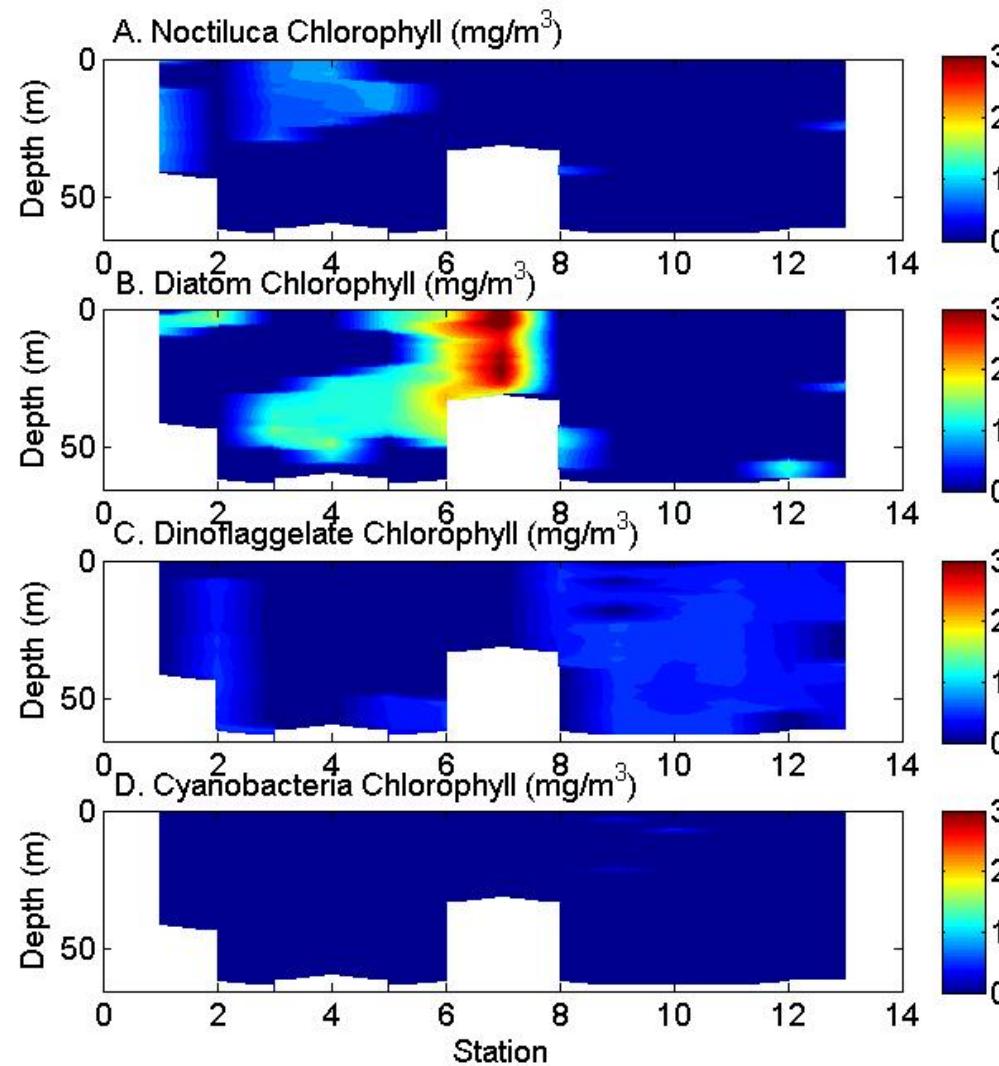
- Along a transect in the Arabian Sea



Thibodeau et al., 2014  
Limnol Ocean

- Then  $F_{chl}$  can be partitioned into PFTs
- Apply PFT-specific calibration slope to  $F_{chl}$  to retrieve more accurate chlorophyll estimates

# Apply taxon-specific calibrations to each bin to partition chl



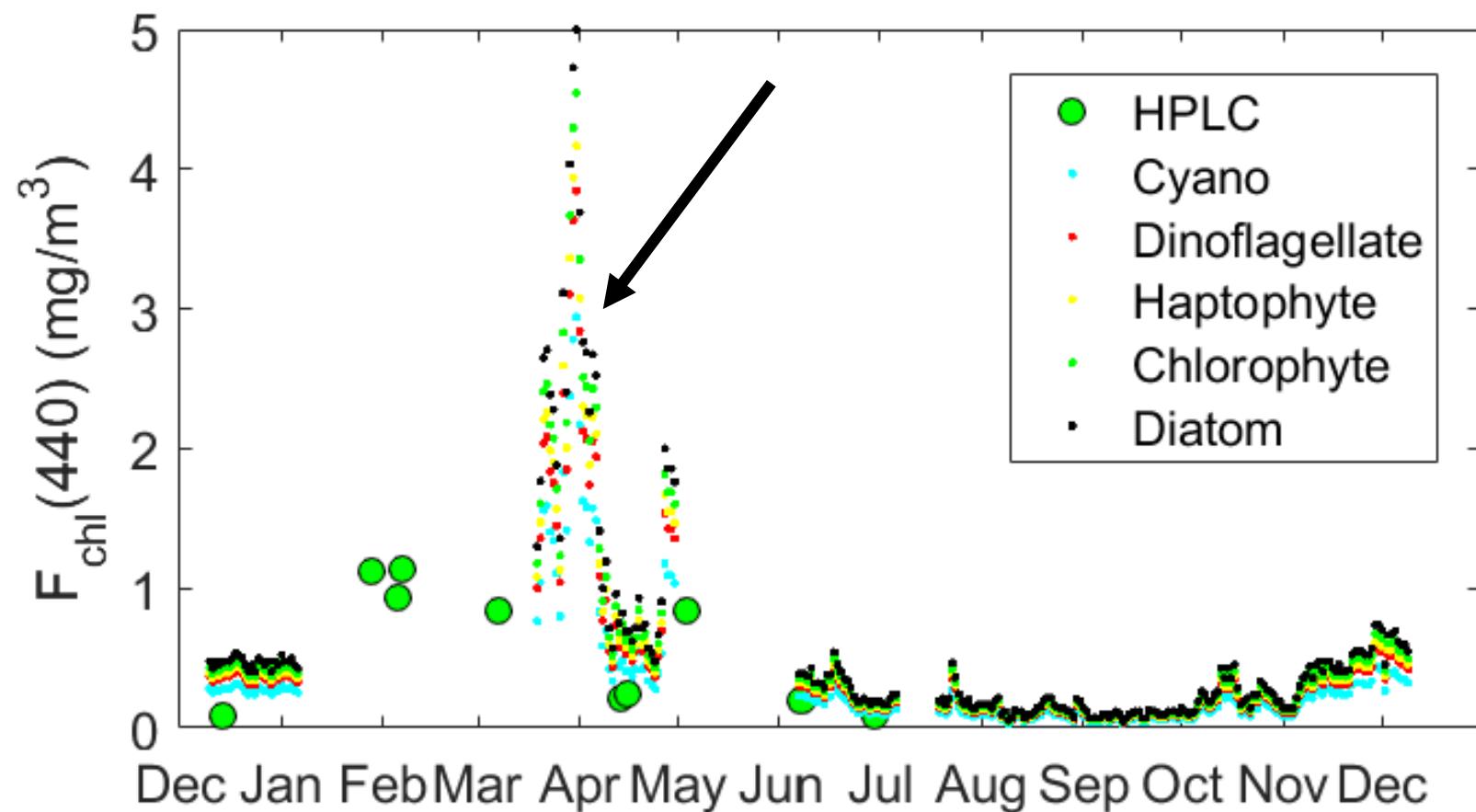
# Boussole Mooring in the Mediterranean Sea

- Sensor deployed at 9 m for one year
- Monthly HPLC samples collected

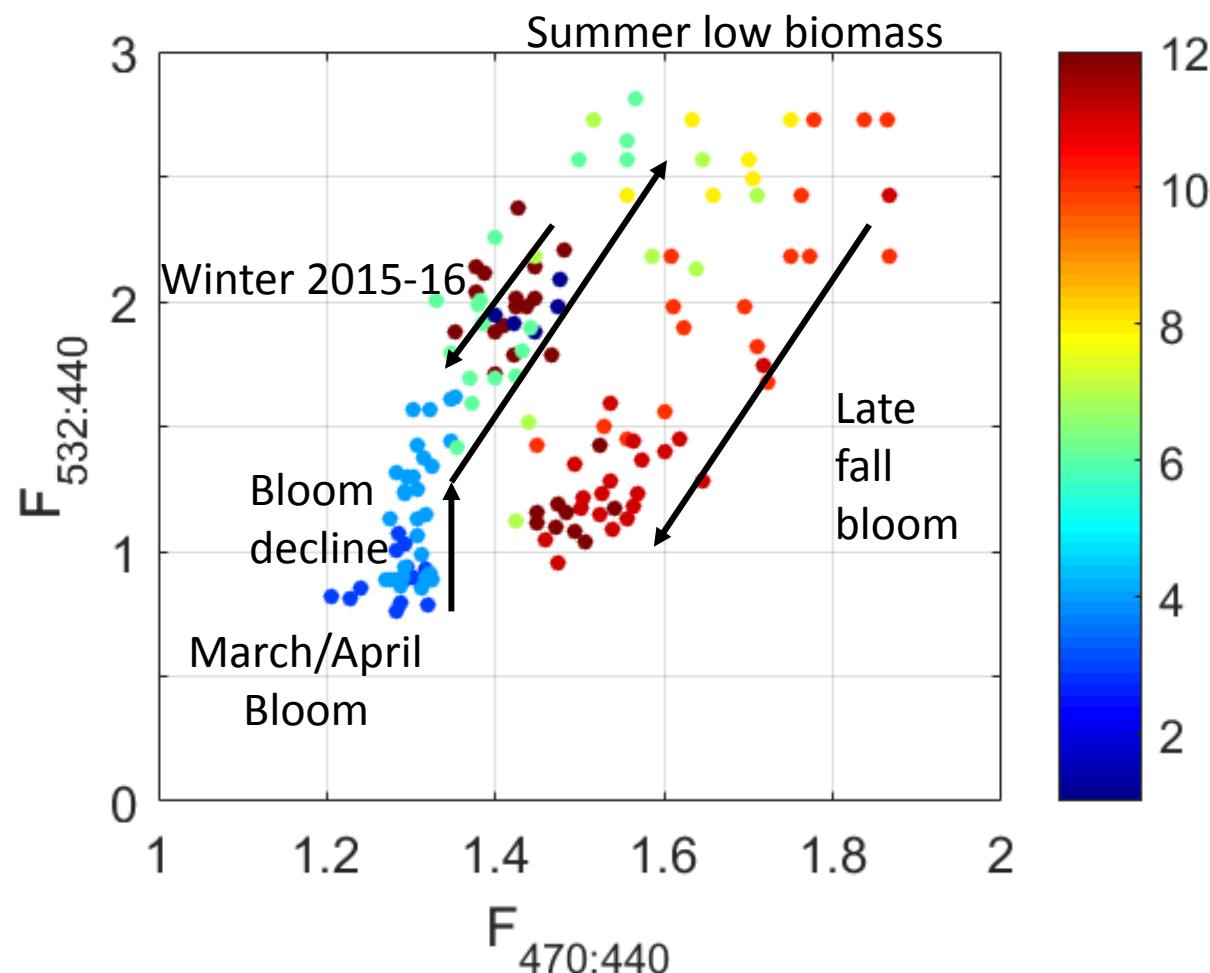
<http://www.obs-vlfr.fr/Boussole>

# Time Series Chl *a*

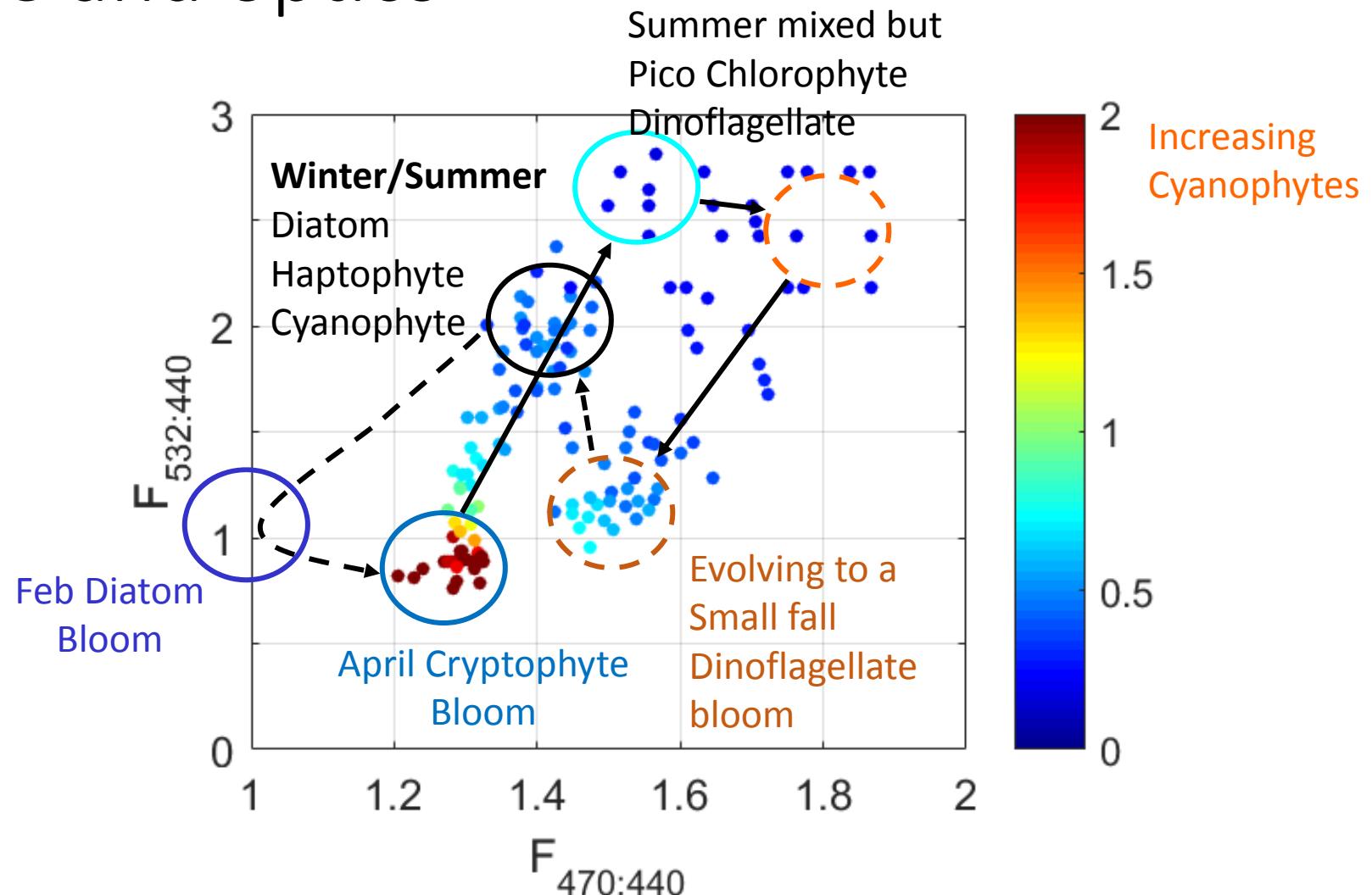
FLourescence  
goes down at noon



# Evolution of phytoplankton succession in Fluorescence ratio space

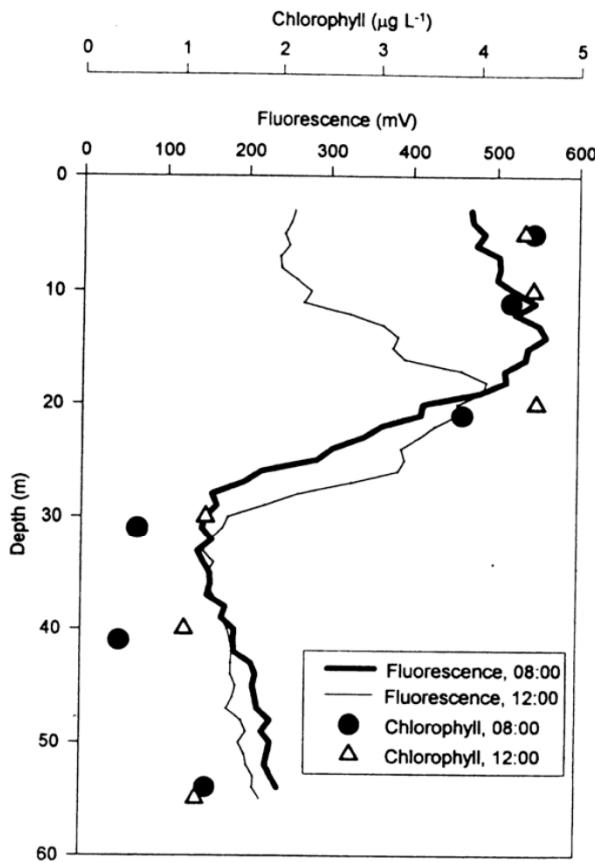


# Complex biomass and taxonomic composition evolution documented with HPLC and optics



# Other sources of variability - NPQ

- profiling

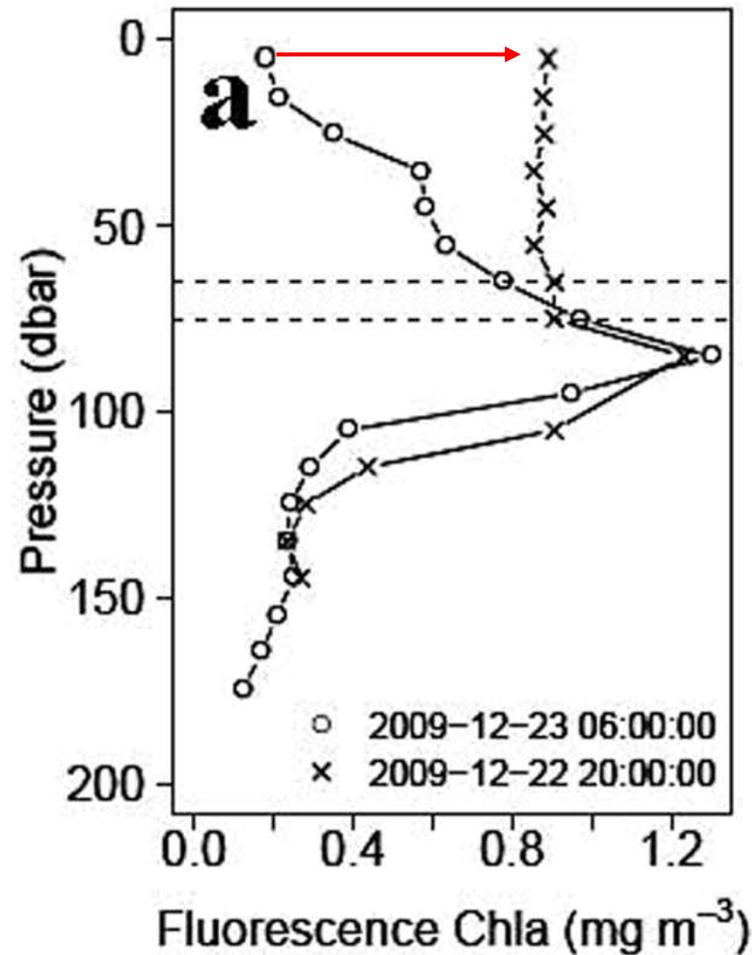


**Figure 9.6** An example of nonphotochemical quenching of in vivo fluorescence in the ocean. An in situ fluorometer, with a xenon flash excitation source, was lowered from the surface to 55 m at 0800 and 1200 hours local time in the northwestern Atlantic in April. The fluorescence intensity was shown in real time on the deck of the ship and recorded in engineering units as a voltage. During the vertical profile, water samples from discrete depths were obtained and the chlorophyll *a* was extracted in 90% acetone and analyzed independently. A comparison of the in vivo fluorescence profiles between early morning and midday reveals a sharp decrease in the fluorescence intensity in the upper 20 m that is not reflected in the extracted chlorophyll analyses.

# Other sources of variability - NPQ

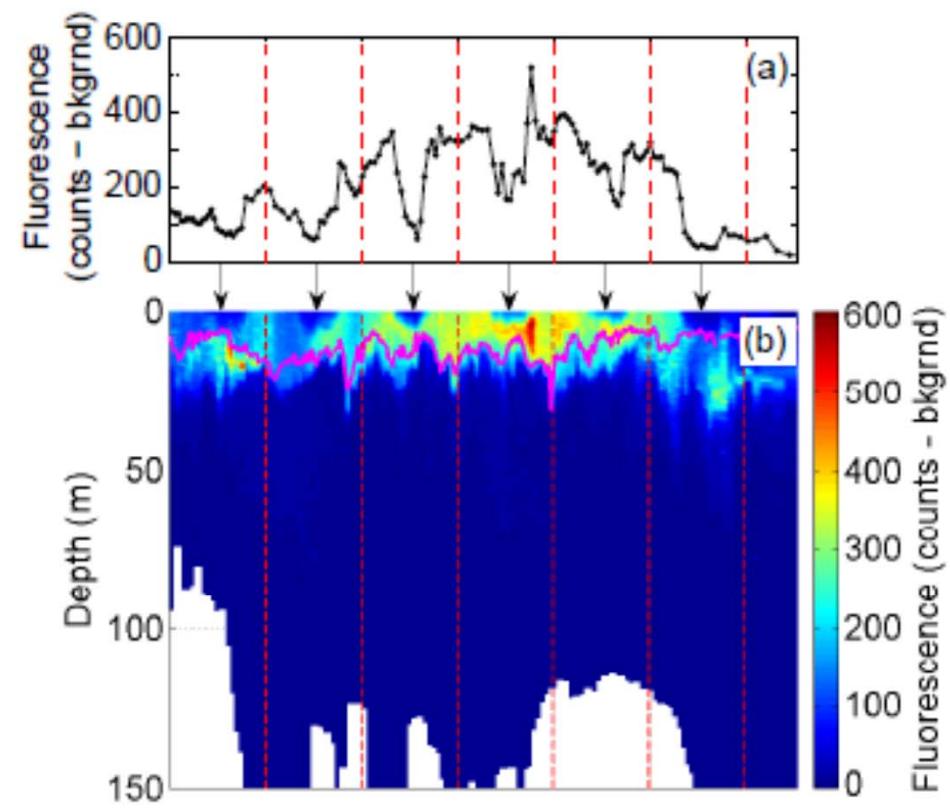
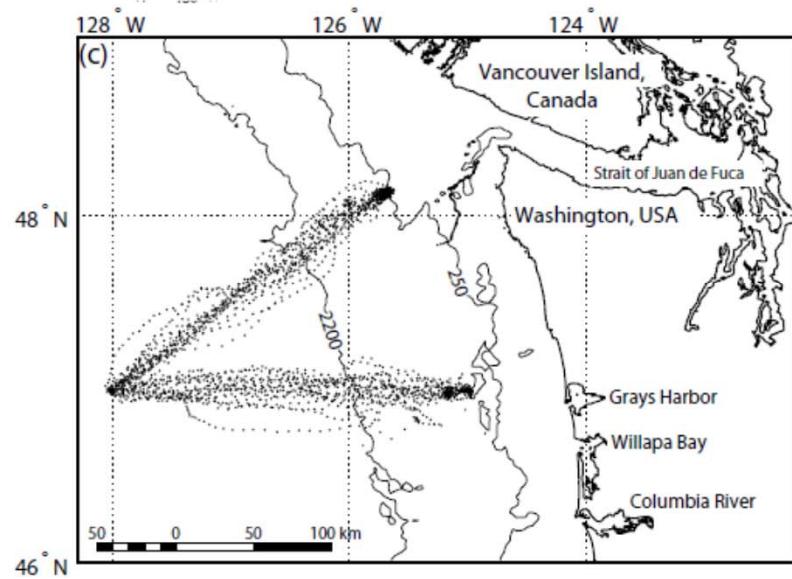
- On a profiling float

F goes way down  
because energy is  
going to heat



# Other sources of variability - NPQ

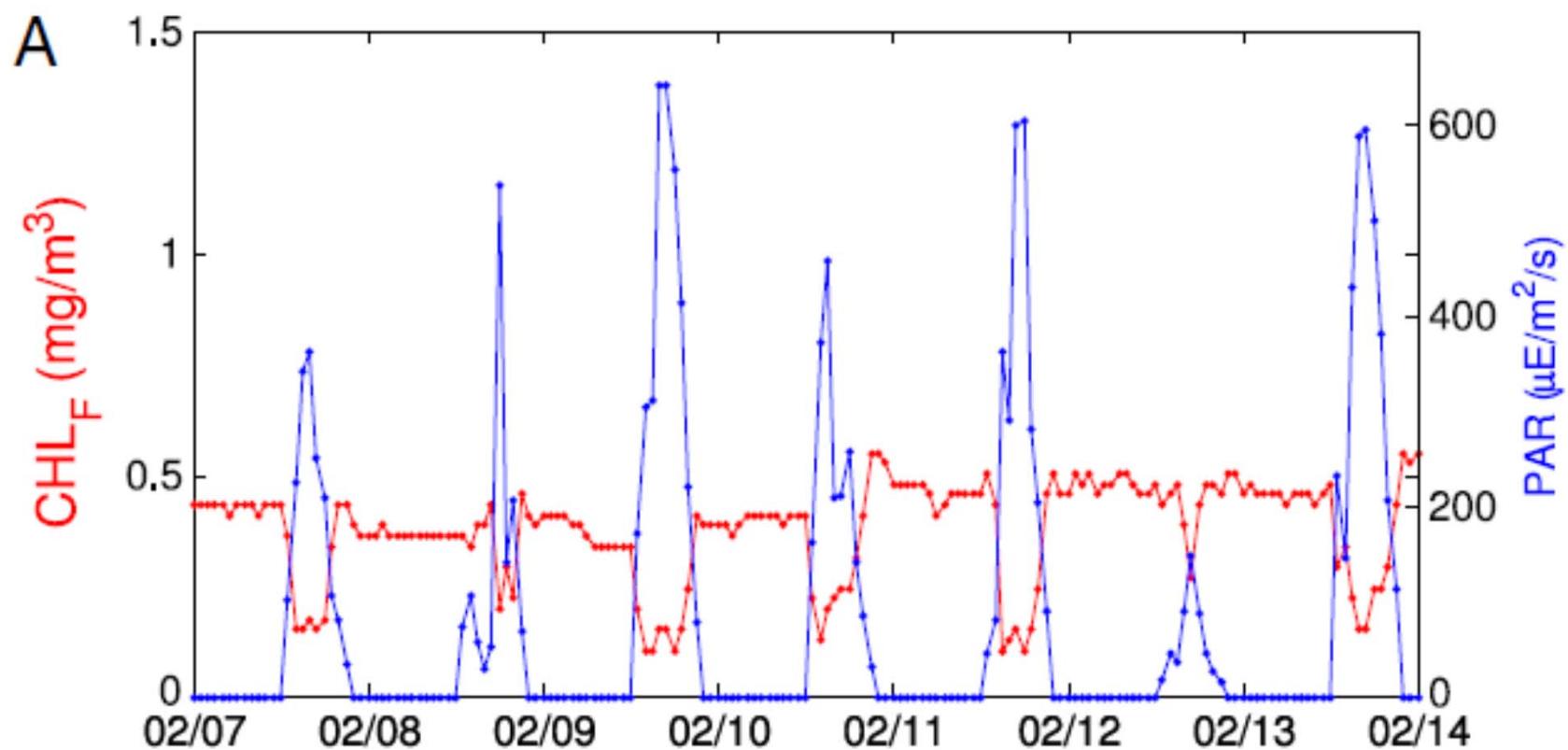
- Glider
- Correct to  $b_b$



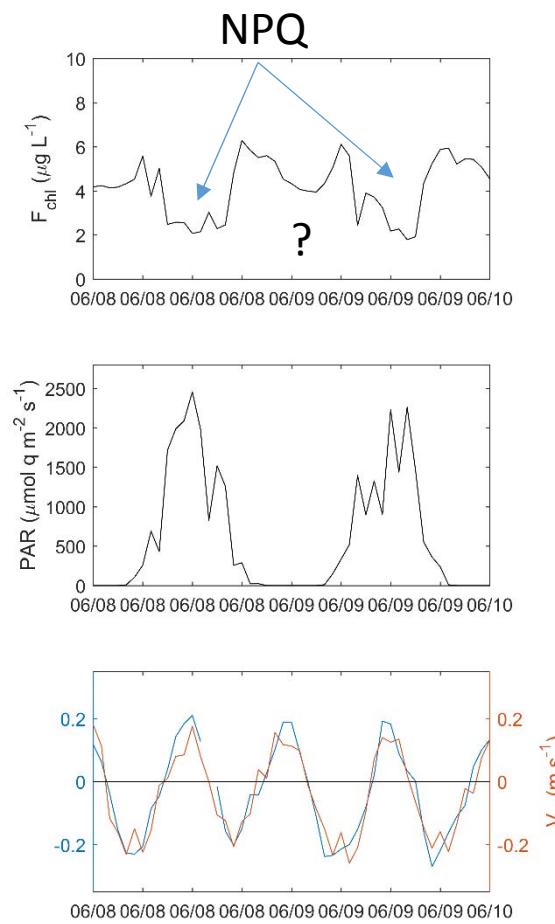
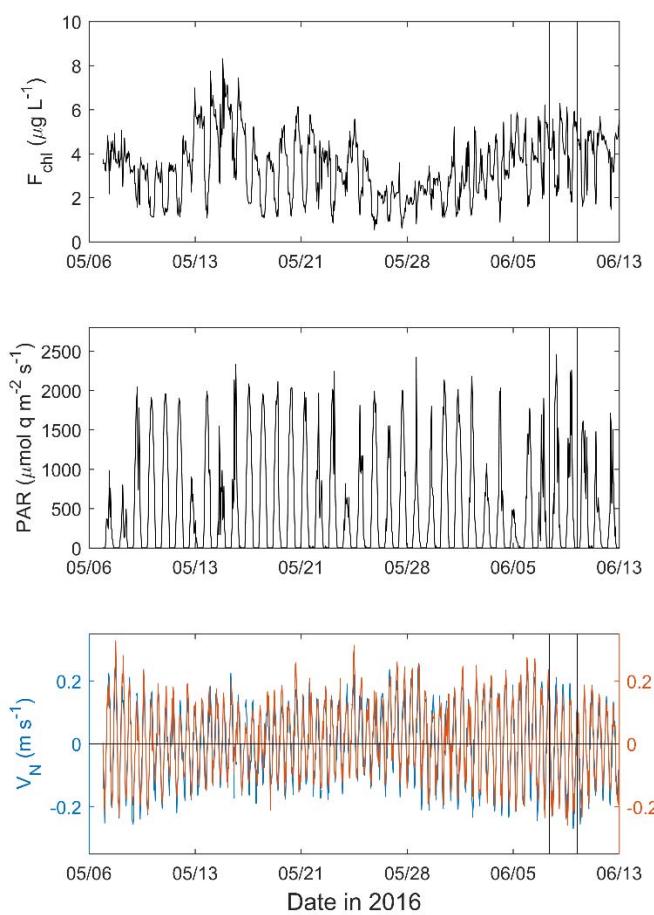
Sackman et al. 2008

# Other sources of variability - NPQ

- On a mooring

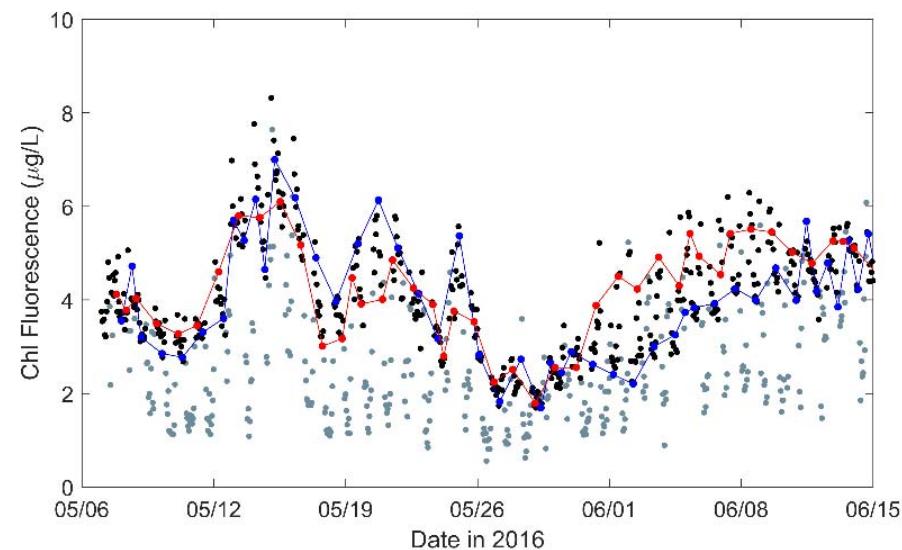


# Diel and Tidal variability



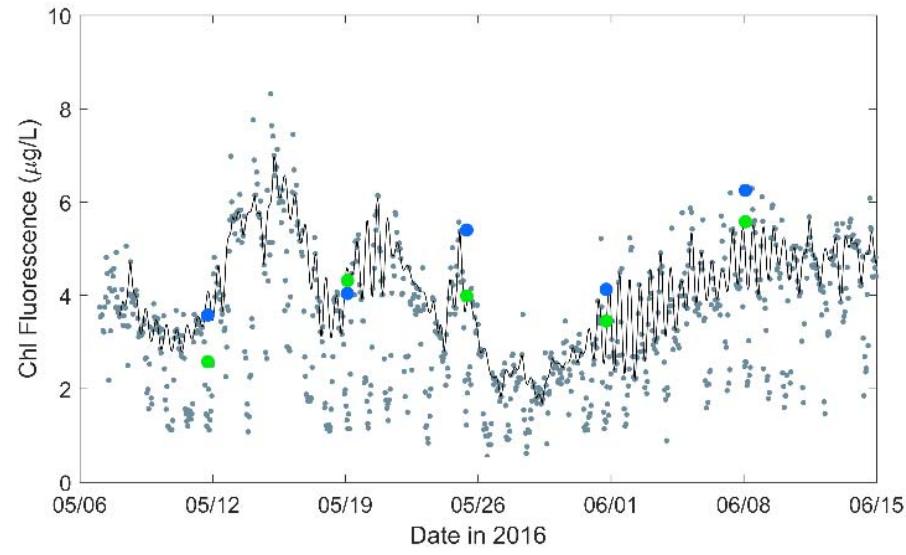
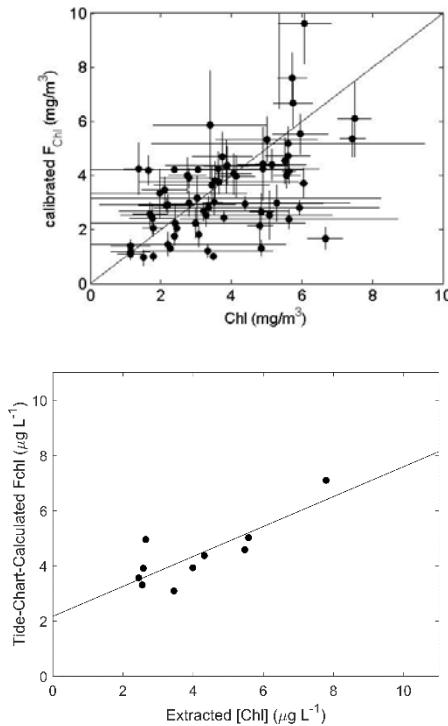
# Diel and Tidal variability

- Identified NPQed observations
- Identified high and low tide conditions
- Identified unquenched high and low tide observations



# Diel and Tidal variability

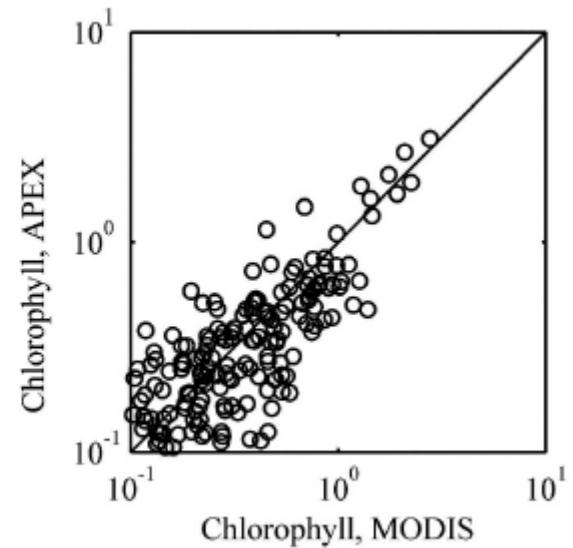
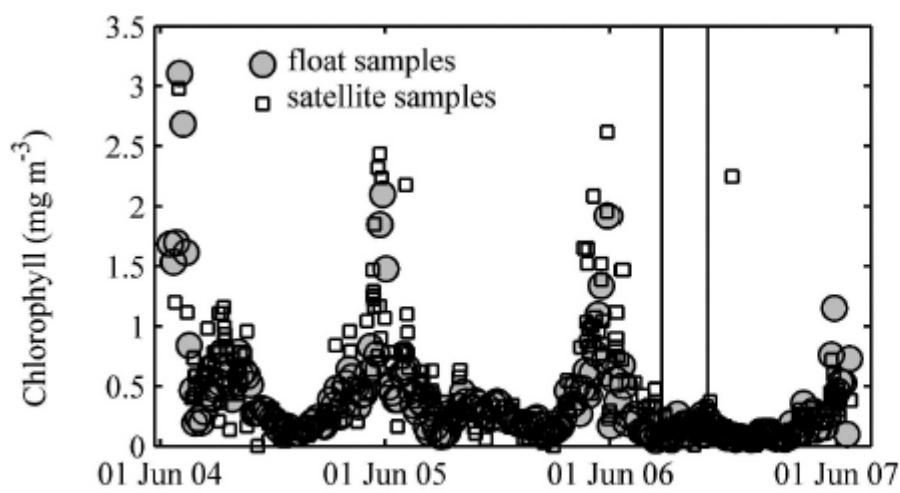
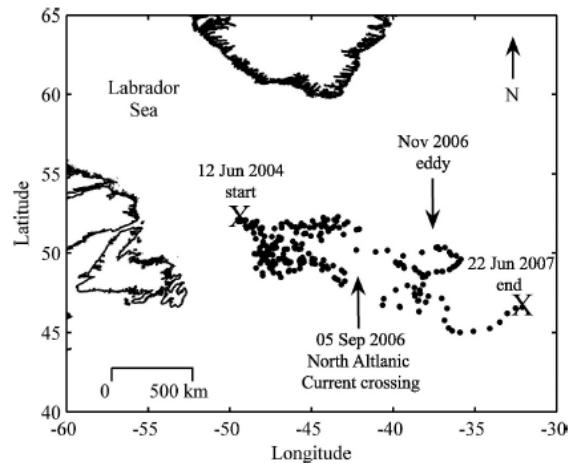
- Fit hourly tidal sinusoid to unquenched high and low tide observations
- Validated with weekly discrete chl observations



# Fluorescence

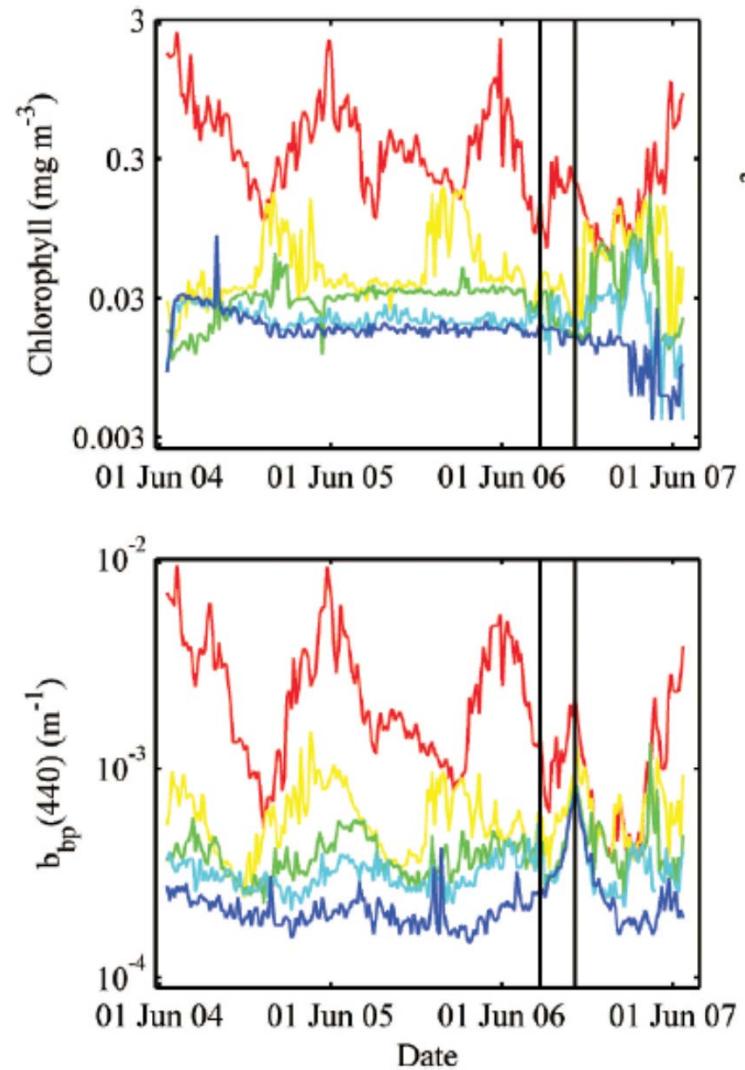
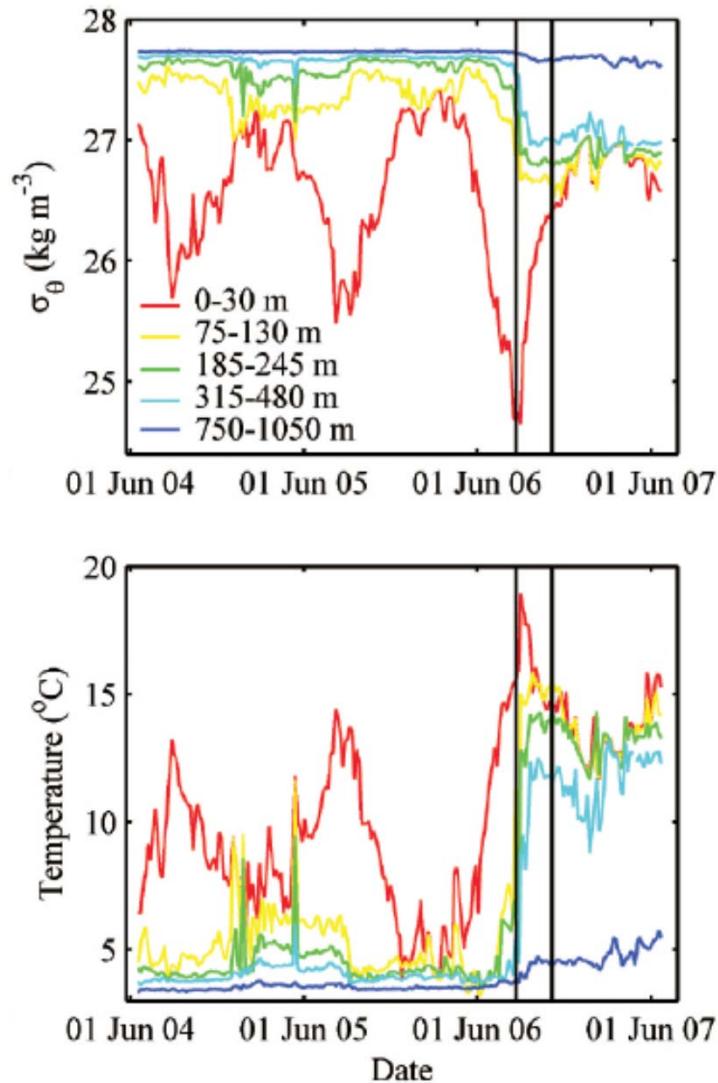
- What is it
- Who does it
- Physics of fluorescence
- Physiology of fluorescence
- Given all of the sources of variability, what can we learn

# Interannual variability



Boss et al. 2008

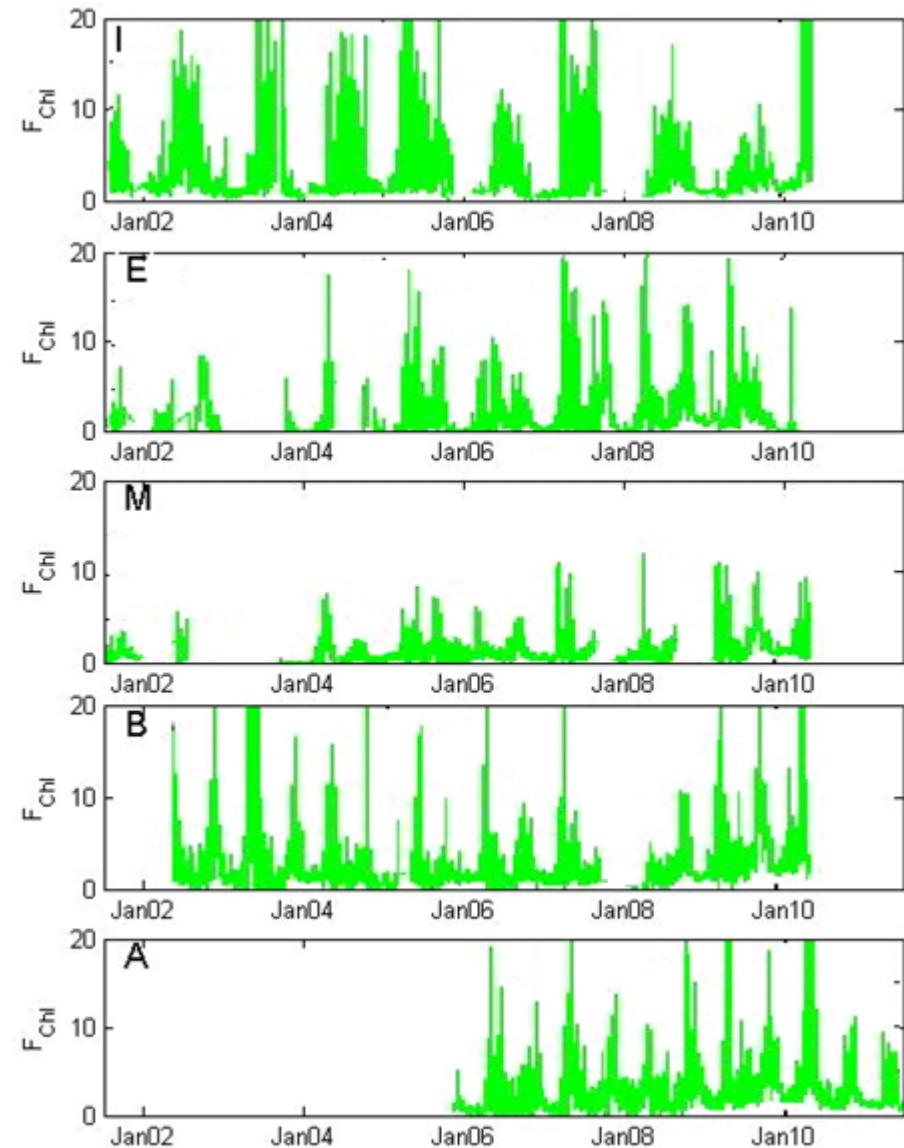
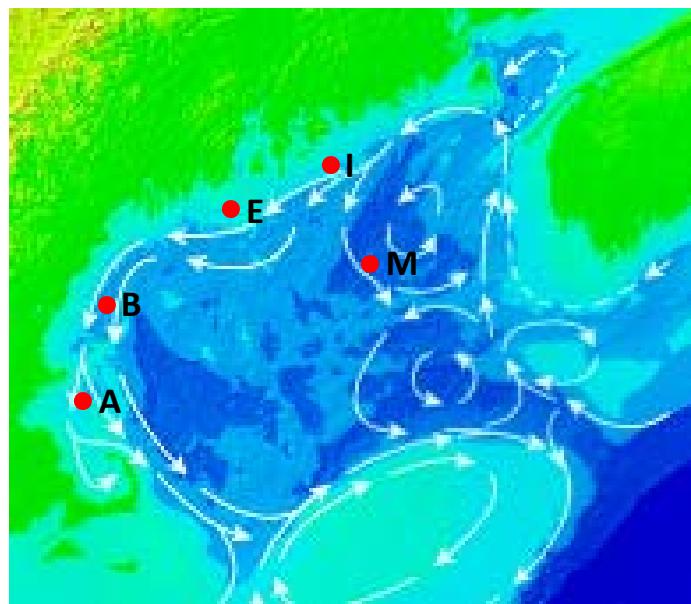
# Interannual variability



Boss et al. 2008

# Gulf of Maine Moored Array\*, July 2001

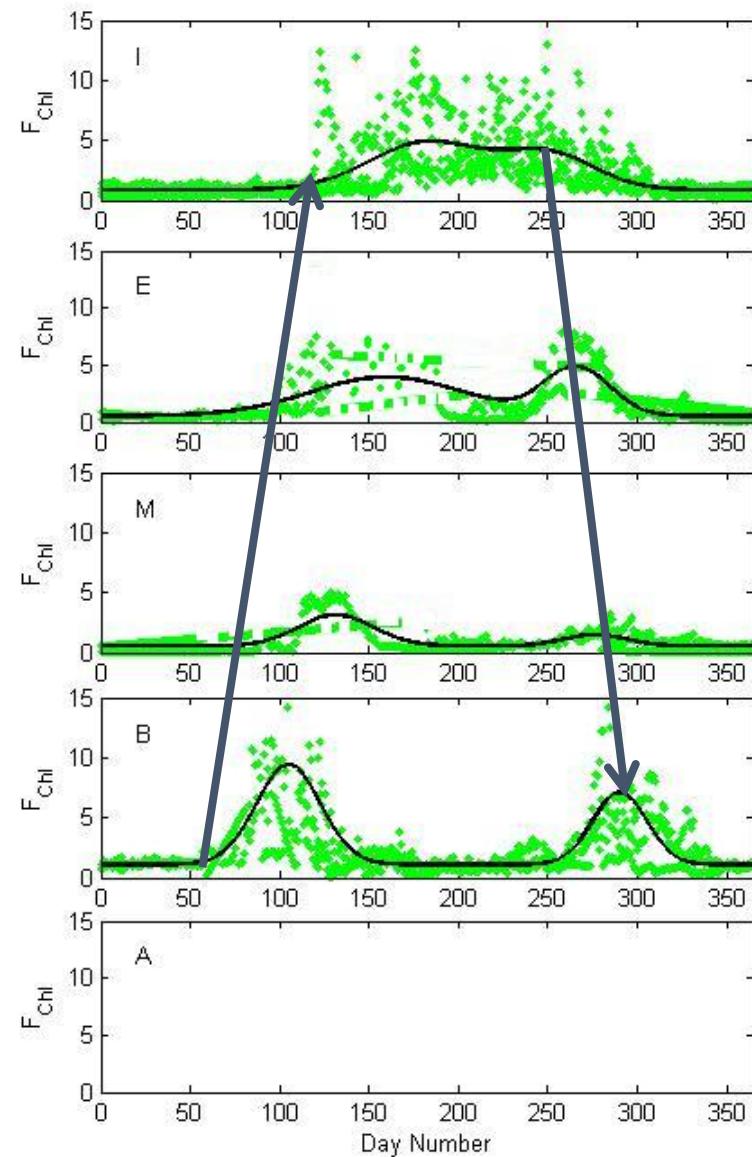
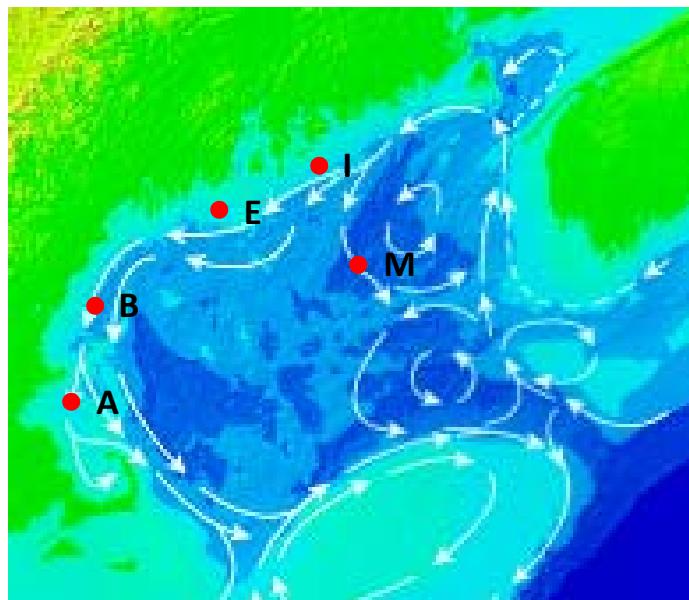
Over a decade of hourly  
calibrated, traceable chlorophyll  
estimates at 8 buoys in the  
Gulf of Maine



\* GoMOOS, NERACOOS...

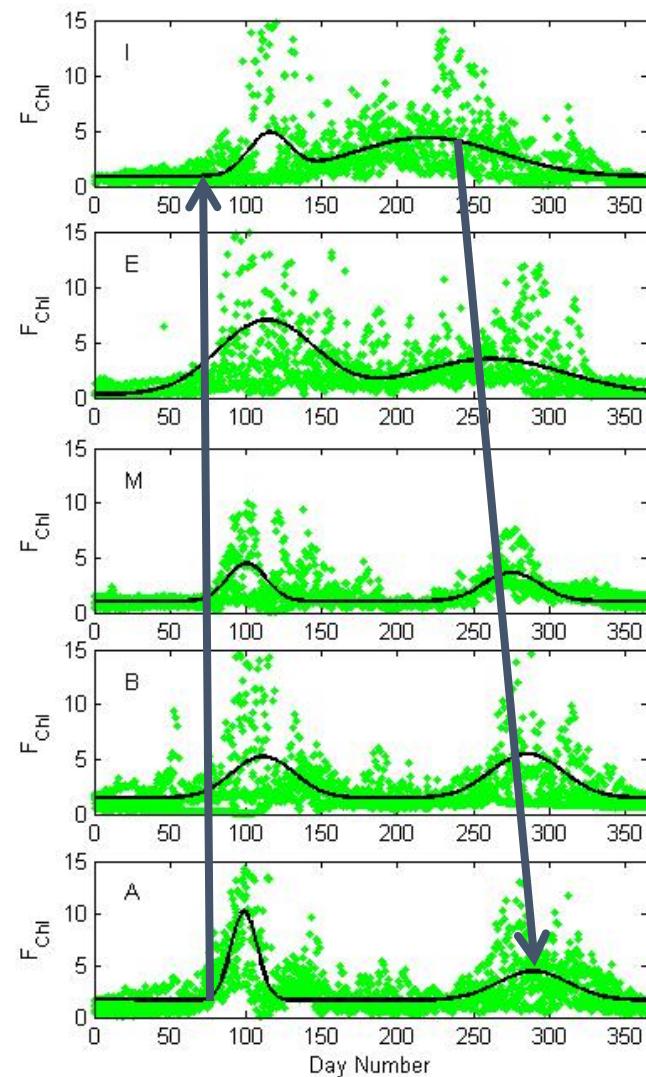
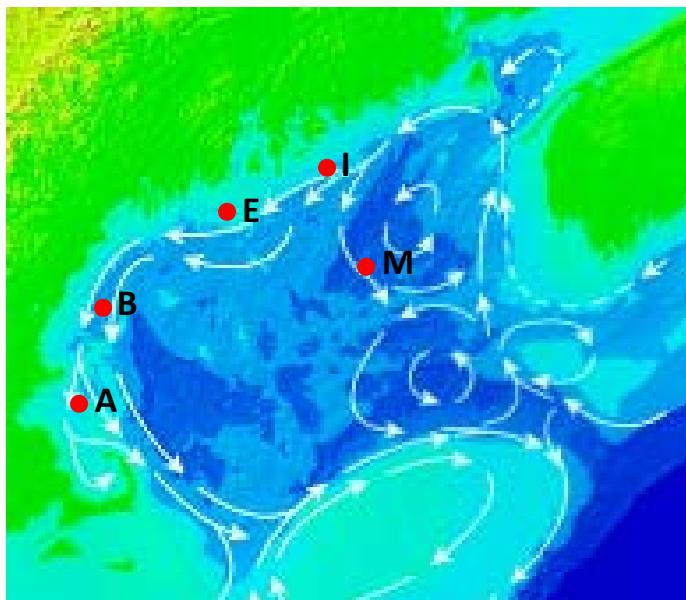
# 2001-2004 seasonal cycle

- Spring blooms initiate in the south coincident with thermal stratification
- fall blooms initiate north following destratification
- Disappearance after both



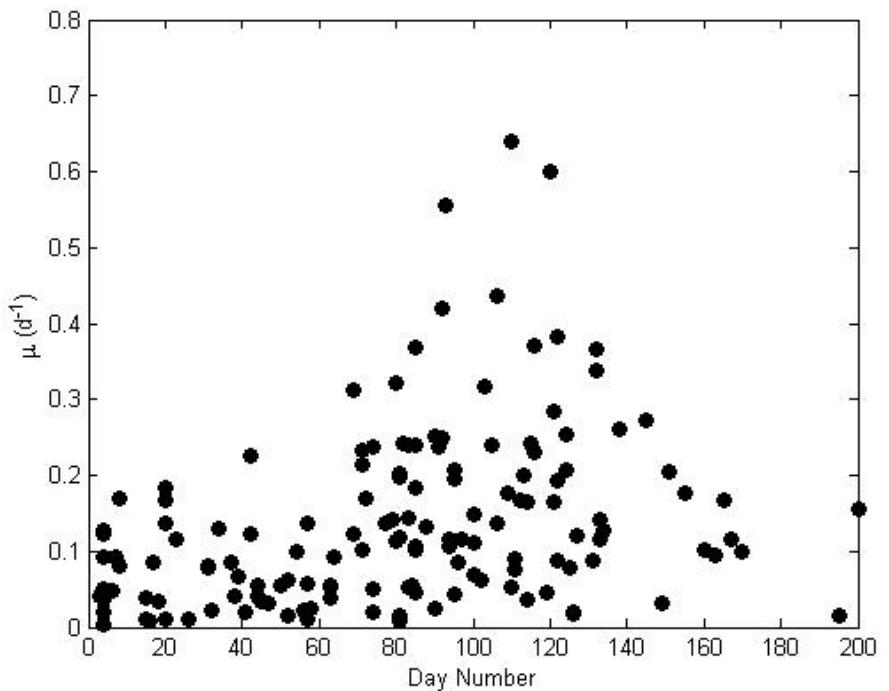
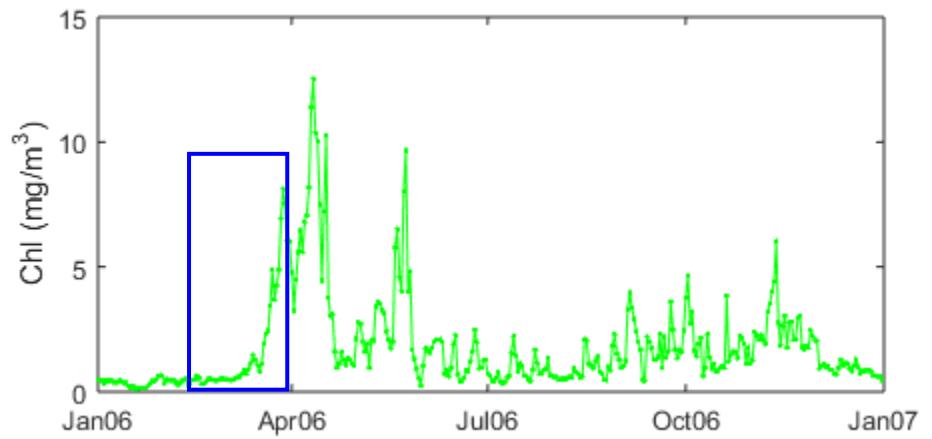
# 2005-2012 seasonal cycles

- Changing hydrologic patterns in 2005-2012 fundamentally impacted timing and intensity of spring bloom patterns
- Early intense river discharge lead to near-simultaneous salinity-driven stratification
- Some regions experienced blooms 2-3 months earlier



# Growth rates

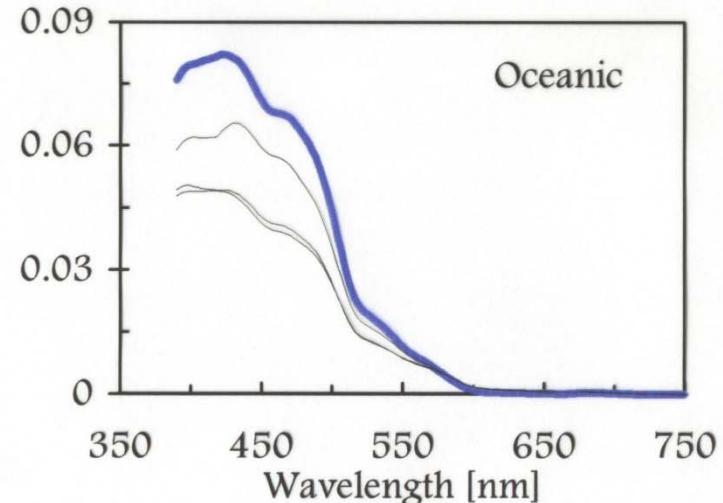
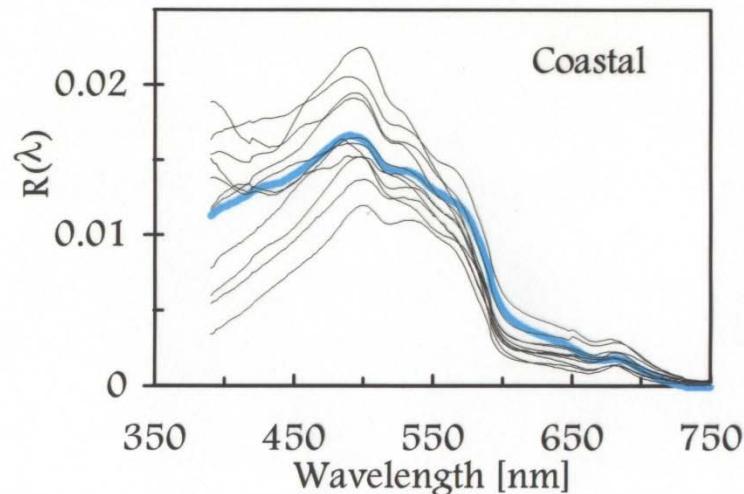
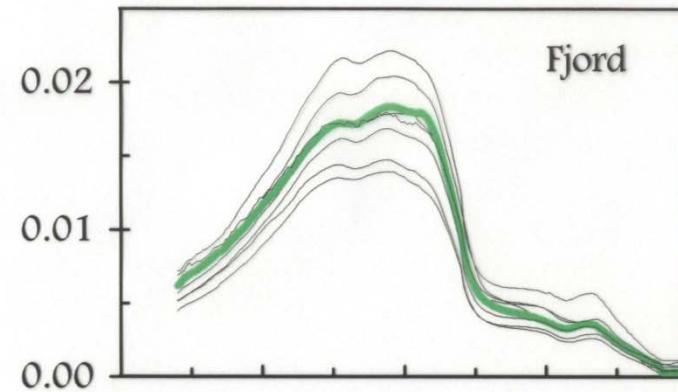
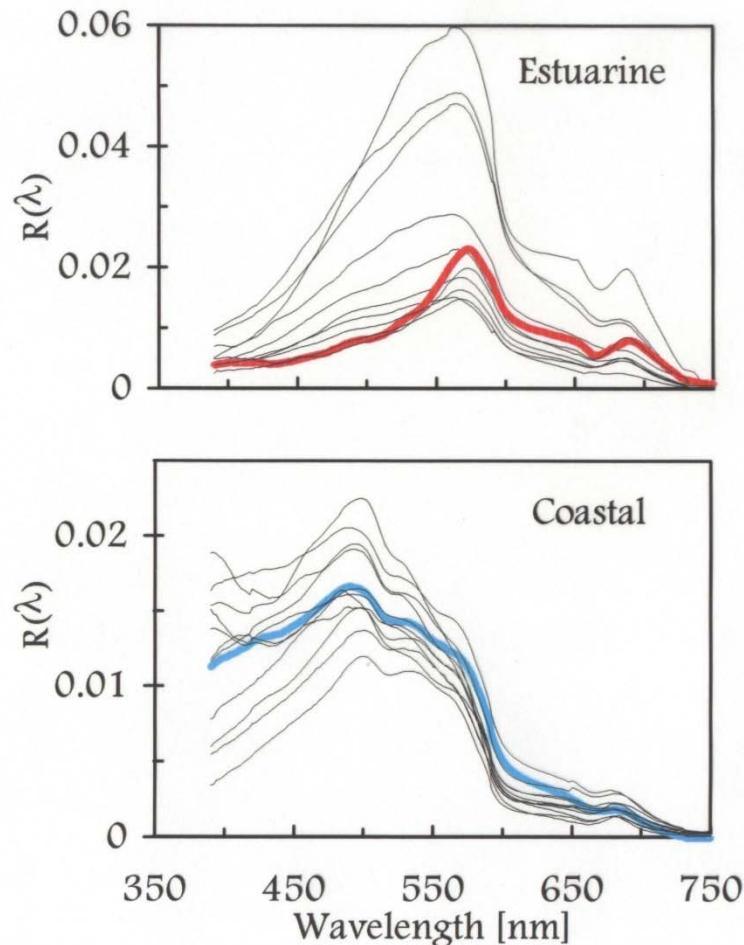
- specific growth rate computed for each bloom event
- growth rate statistics compiled
- Earlier blooms are slower growing blooms
- → important changes in Gulf of Maine phenology
- These interpretations require high quality observations and data products



# Natural or solar stimulated fluorescence

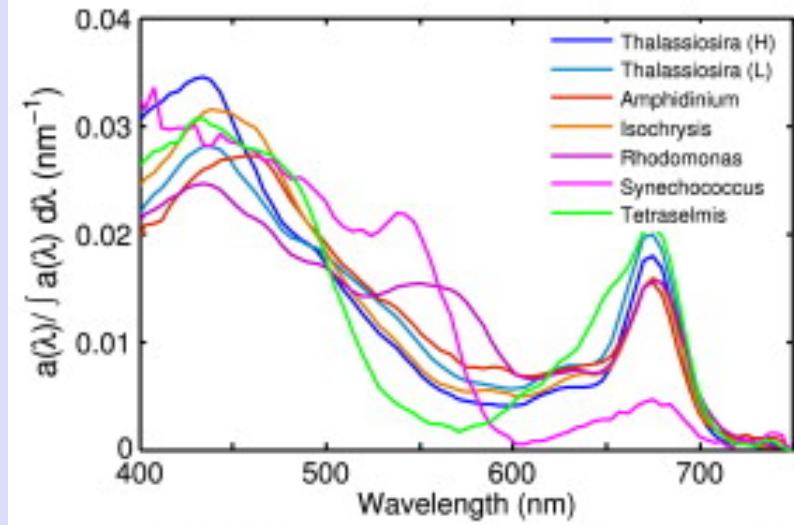
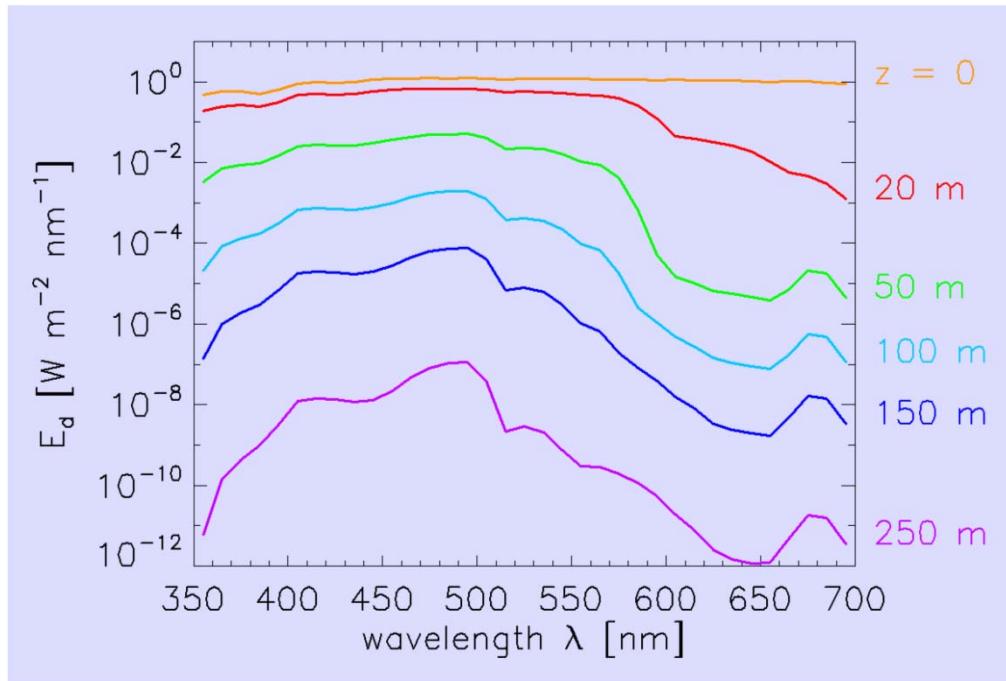
- We have been discussing fluorescence stimulated by light sources in a variety of instruments
- What about fluorescence stimulated by the sun?
- What might it look like?

Identify the fluorescence signature in the irradiance reflectance spectra below



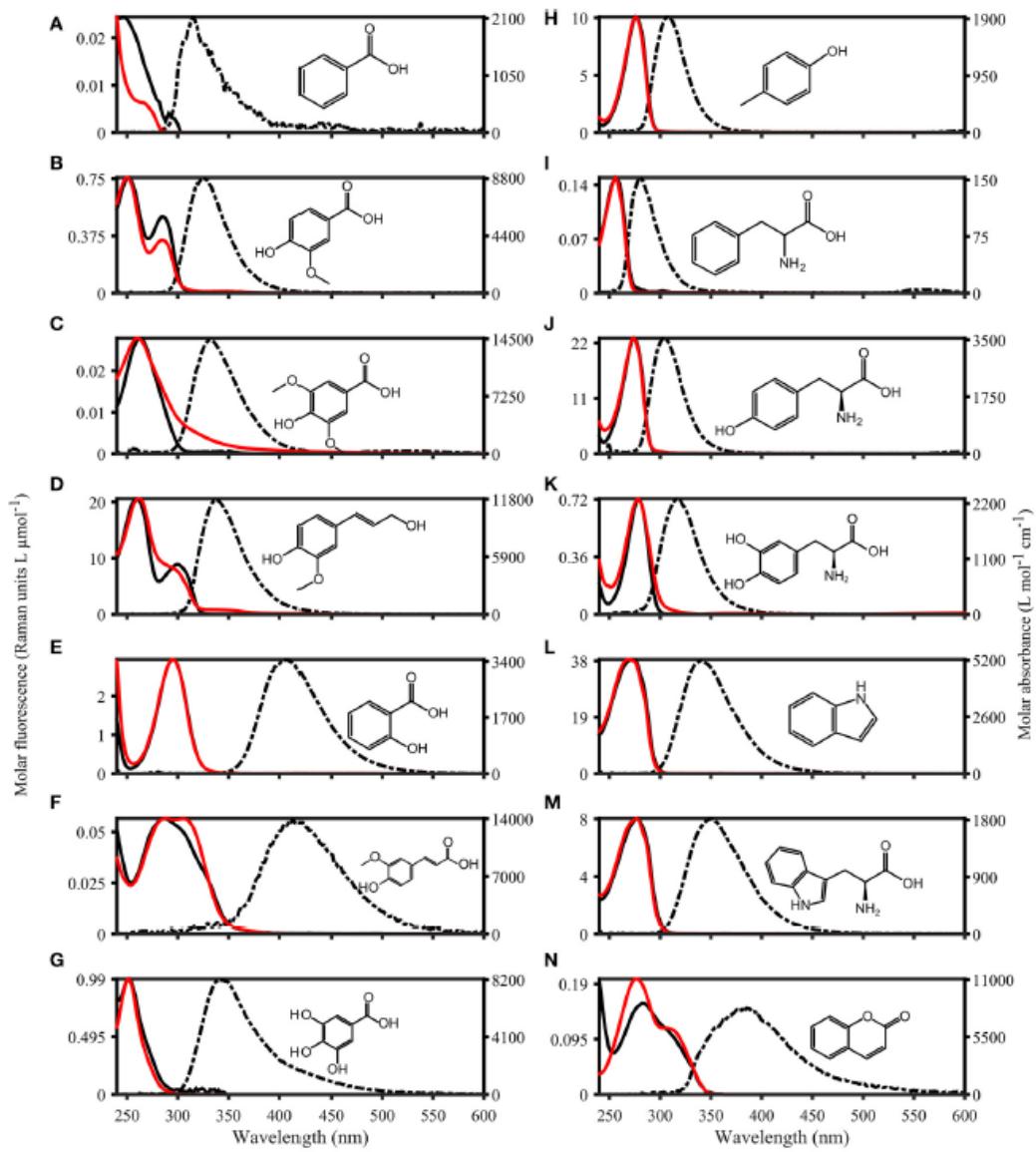
# Fluorescence Physiology

- $F = E(\lambda) * a(\lambda) * \Phi_f$ 
  - Available light (spectral)
  - Absorption coefficients (spectral)
  - Fluorescence efficiency (quantum efficiency)



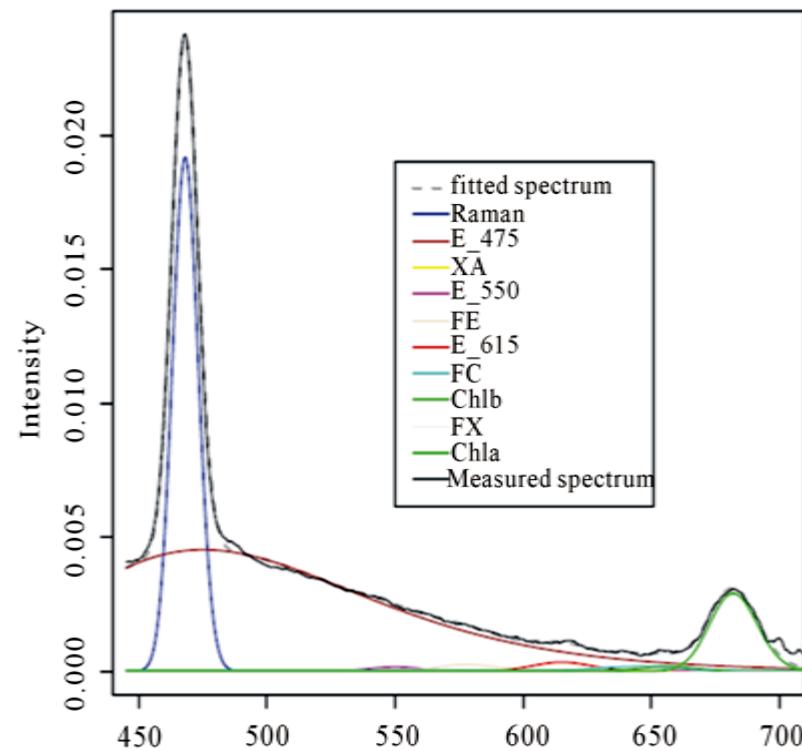
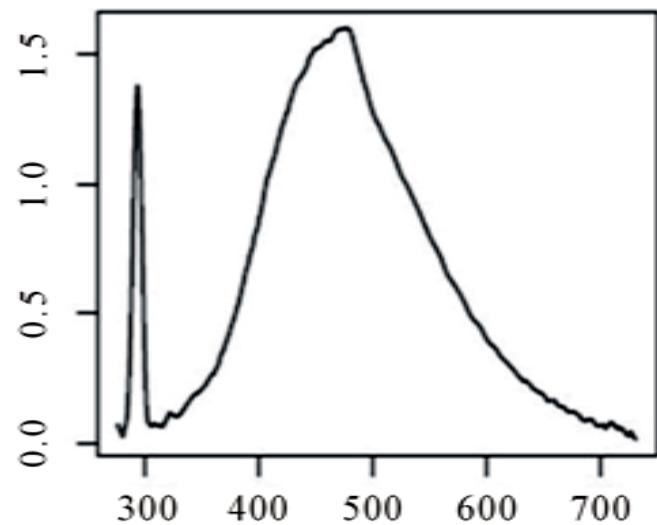
# CDOM fluorescence

- Many fluorescing compounds
- Range of abs, fluor spectra
- very complicated
- Molar absorption (red); Fluorescence (black dash)



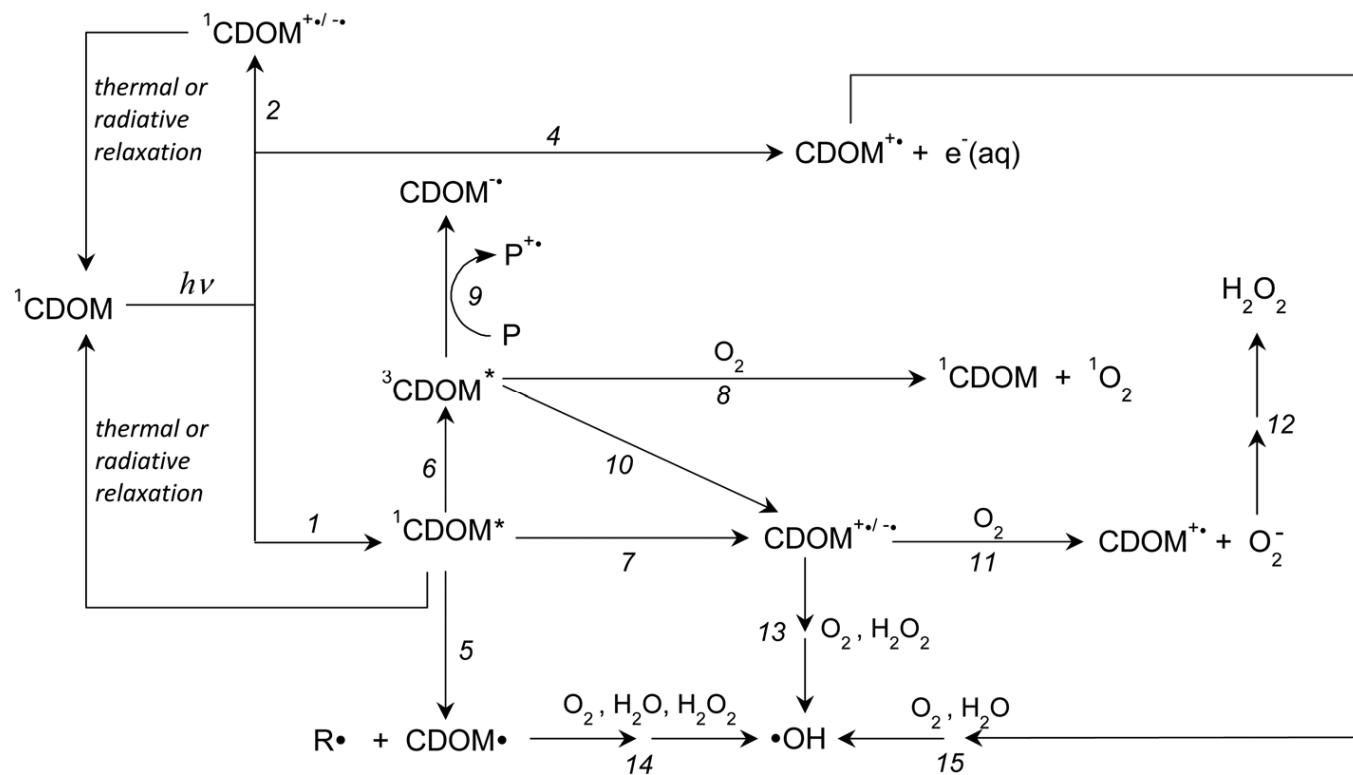
# CDOM fluorescence

- Ex 266
- Ex 405



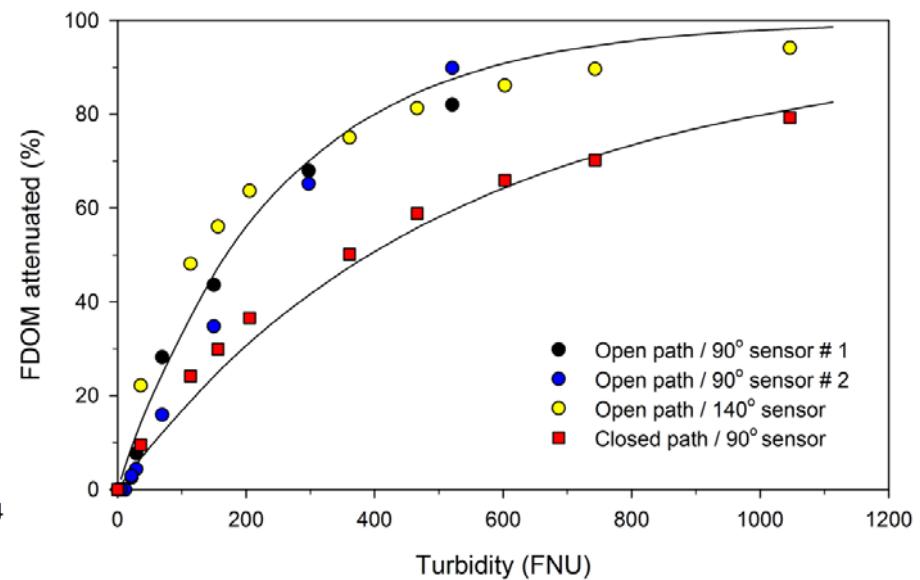
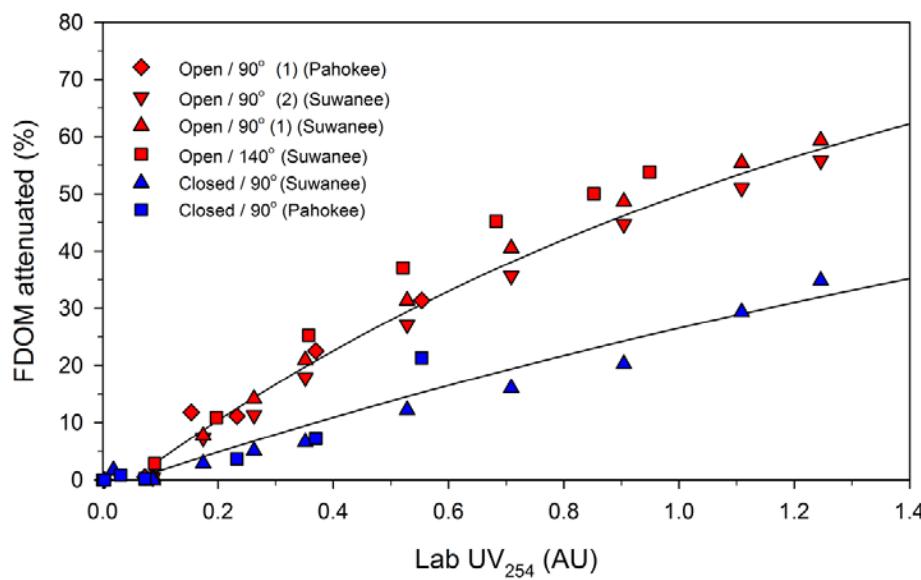
# Model of CDOM photophysics and photochemistry

- 



# Sensitivity of FDOM to environmental parameters

- Absorption and scattering

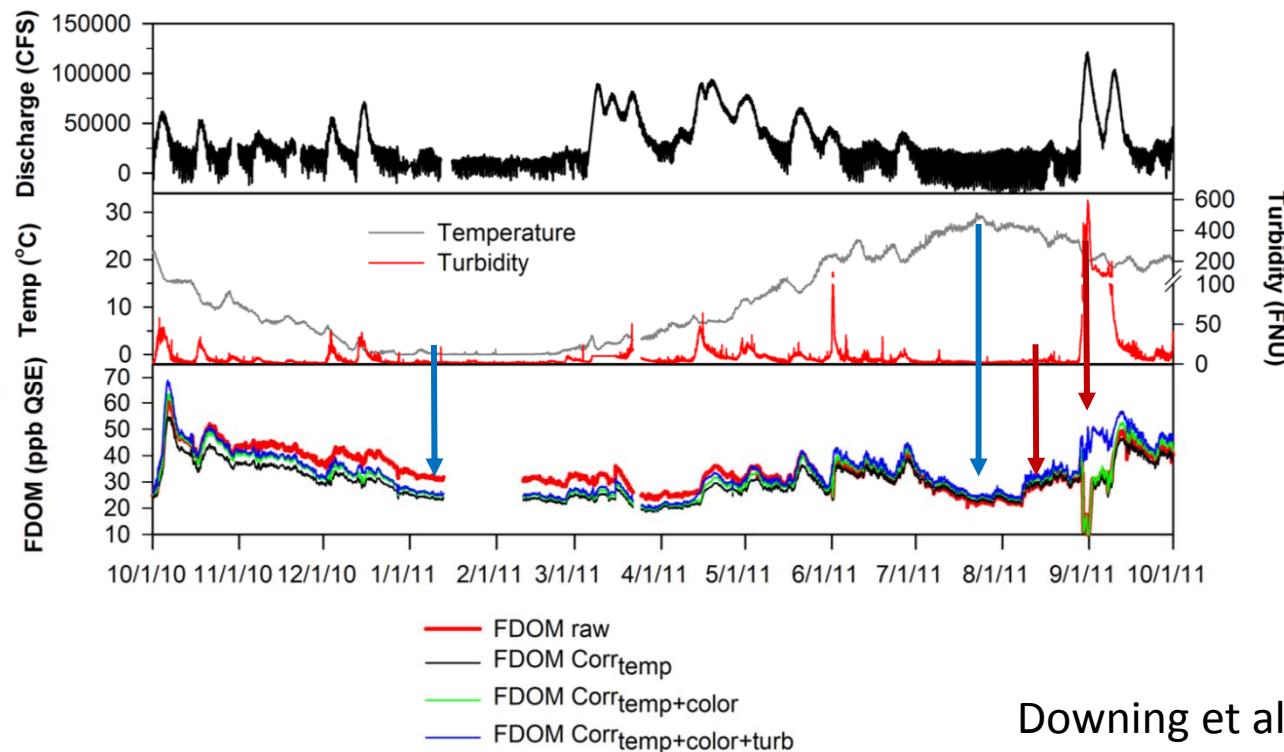


Downing et al. 2012

# Sensitivity of FDOM to environmental parameters

- Absorption and scattering
- Temperature

$$FDOM_{corr} = FDOM_{raw} + \rho(T_{meas} - 25) / r_p(FNU) \propto r_d(A_{254})$$



Downing et al. 2012

# Take home

- Know your instruments
- Characterize your instruments
- Calibrate your instruments
- Take advantage of sources of variability in your signal to exploit measurements for more information