Fluorescence class at DMC-- 22 October 2012

HANDS-ON:

Meet in meet in Perry lab and head to the dock:
1) water sampling from the dock
    2 students sample three replicates (~ 130 mL)
    one student – sample three volumes (~ 60, 130, 280 mL)
    remaining students – one sample (~ 130 mL)
2) BB2F on the dock, during water sampling
3) net tow to collect phytoplankton

Perry lab:
1) Filter water samples and put in acetone (Ivona Cetinic will sonicate).
2) Tour of Perry lab – Turner Designs fluorometer; lab safety
3) spinach extract – visual of chlorophyll fluorescence

Mayer Lab:
At end of class – if time – back to the Perry lab to read the chlorophyll extracts.

LECTURE/DISCUSSION:

1) Who in the class has used chlorophyll fluorescence? When? Why? How?

2) Big picture
    - what do we really want to know about phytoplankton?
        - (biomass, number of phytoplankton, size distribution, species, ?)?
    - why the interest in chlorophyll fluorescence?
        - you might want it to five chlorophyll concentration
        - is chlorophyll concentration a good proxy for phytoplankton?
        - proxy for phytoplankton what?
            - 'biomass', but requires chlorophyll to element ratio (Chl/C)
            - does not give cell number
            - phytoplankton size or size distribution?
                - maybe, with size fractionations of chlorophyll (Nitex)
                - maybe mean size with variance/mean ratio (Nathan)
    - what does fluorescence actually provide?
    - do coupled measurements get us closer? (other optical measurements – bbp)

3) Overview – different ways to think about phytoplankton (molecule, membrane, cell)

4) Fluorescence in general and chlorophyll fluorescence in particular; relationship between absorption and fluorescence

5) BRIEF overview and history of measurement of chlorophyll – or different aspects of chlorophyll (place fluorescence in context and reference for 'calibration')
   -- *in vivo*: by eye (color of the water) and by smell
   -- *in vivo*: by eye (Munsell color chart)
   -- *in vitro*: spectrophometry of extracts
   -- *in vitro*: fluorescence of extracts
   -- *in vitro*: HPLC of extracts
   -- *in vivo*: chlorophyll fluorescence profiles by pump, then *in situ* fluorometry
   -- *in vivo*: epifluorescence
   -- *in 'vitro'*: spectrophometry of filter pads (a_676)
   -- *in vivo*: spectrophometry with ac9/acs (a_676), profiles and underway
   -- *in vivo*: flow cytometry on bench and *in situ*
   -- remote sensing: ocean color remote sensing reflectance
   -- remote sensing: ocean color remote sensing fluorescence line height
   -- *in vivo*: pump and probe fluorescence for physiology (profiles and bench top)

6) general principles of fluorometric measurement - excitation/emission, light source, filters, detectors, geometry, temporal resolution.
   -- bench top and *in situ* manufacturers
   -- choice of wavelength (absorption spectra, excitation/emission spectra)
   -- calibration for fluorometers – *in vitro* (extracts) and *in vivo* (of living cells); what is calibrated?
   -- drift – electronics, filter degradation
   -- temperature effects – electronics, fluorescence quenching

7) interpretation of data, and challenges therein, and potential workarounds
   – fluorescence quenching due to sun and innate diel rhythms
   – pigment packaging – cell size and photoadaptation
   – variable ratio of photosynthetic pigments
   – nutrient limitation

8) synthesis

Assigned readings

Basic readings on fluorescence and fluorometers in general, and chlorophyll fluorescence in particular:

Turner Designs– the Basics.pdf

Turner Designs website - practical info.docx

Primer on fluorescence.pdf (some redundancy with Turner Designs – the Basics)

Suggett et al. 2010 – table of contents.pdf (Chapters 3 & 7 are from Suggett et al. – available as an e-book from UMaine library)

Historical use of chlorophyll fluorescence – the classic paper:

Lorenzen 1966.pdf

The method you will use to measure chlorophyll concentration:

JGOFS_chl_method.pdf

Additional reading, if you are interested, mostly on 'variable fluorescence':

Chapter 3 Huot Babin overview.pdf

Chapter 7 Richardson taxonomic discrimination.pdf