Development and Application of Picosecond Lifetime Analyses in the Upper Ocean for the Interpretation of Solar-Induced Chlorophyll Fluorescence Signals

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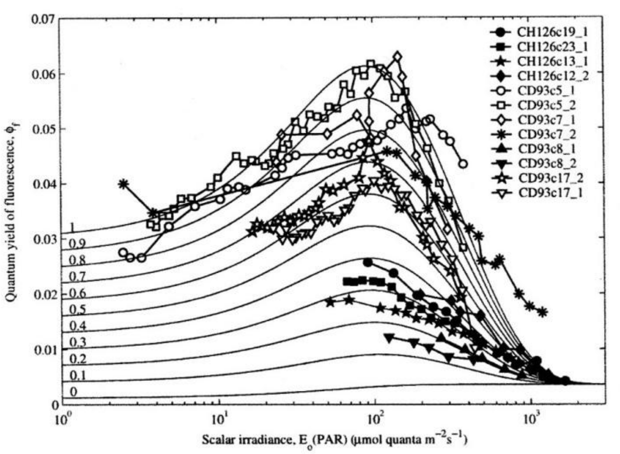
Outline

- Why to measure fluorescence lifetimes?
- Development of Picosecond Lifetime Fluorometer for oceanographic research
- Laboratory program to understand physiological mechanisms behind the variability in solar-induced fluorescence (SIF) yields
- Field studies in biogeochemically diverse regions of the global ocean
- Future directions

Publications:
Gorbunov et al., *Biochim. Biophys. Acta*, 1807: 1591-1599 (2011);
Kuzminov et al., *Biochim. Biophys. Acta*, in press (2012);
Fadeev VV, Gorbunov MY, Gostev T, *J. of Biophotonics*, in press (2012)

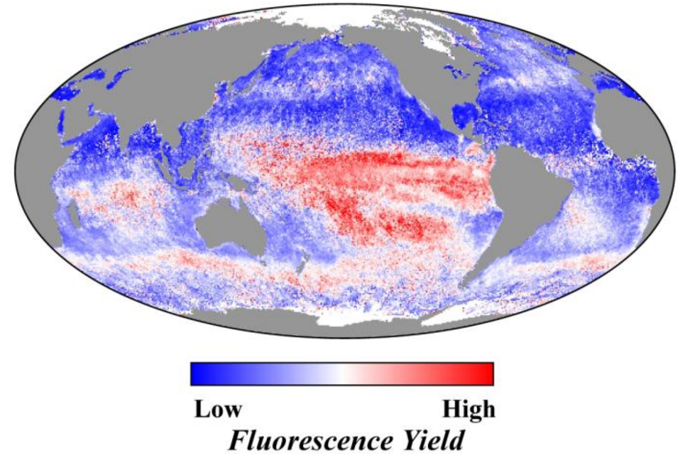
Background

- MODIS maps of solar-induced fluorescence (SIF) and *in-situ* measurements of SIF revealed a huge variability (ca. 10x) in SIF yields in the ocean.
- Mechanisms and interpretation of this variability remain poorly understood.
- Very limited field studies of related processes.



Morrison, L&O, 2003

NASA MODIS



Behrenfeld et al., Biogeosciences, 2009

Objectives

- To understand physiological mechanisms and factors that control the variability in SIF yields
- To develop a set of sea-going instrumentation that helps to interpret satellite-based SIF signals

Problem

- Measurements of the **quantum yields** of fluorescence are the key to our understanding of the variability in SIF.
- Quantum yields are very difficult to measure even in the lab and virtually impossible to measure directly in the open ocean.

Solution

- Picosecond fluorescence **lifetimes** are directly related to the quantum yields.

Theory of Fluorescence Lifetime
(Why measure lifetimes?)

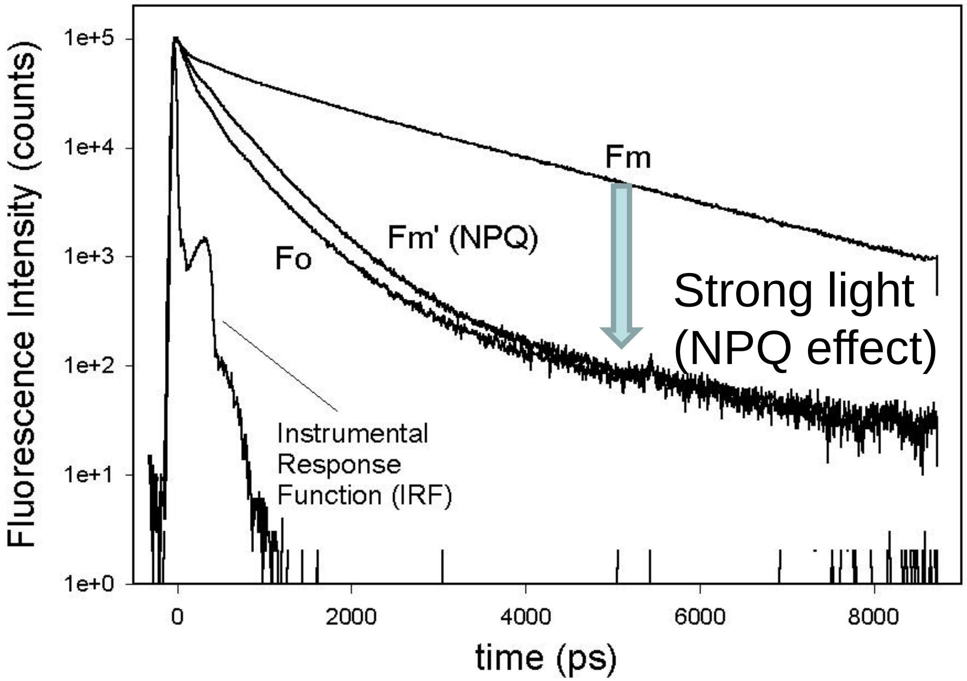
- Fluorescence is a delayed emission which is characterized by the lifetime of fluorescence.
- The lifetime is measured in absolute units.
- The lifetime is **directly proportional to the quantum yield of fluorescence** (ϕ_f):

$$\tau = \phi_f \times \tau_0$$

where τ is the observed lifetime of the excited singlet state of the molecule; τ_o is its natural lifetime.

- The lifetimes of Chl-a fluorescence in living cells are strongly affected by physiology.
- Chl-a fluorescence lifetimes vary between **0.3 and 2.5 ns**.

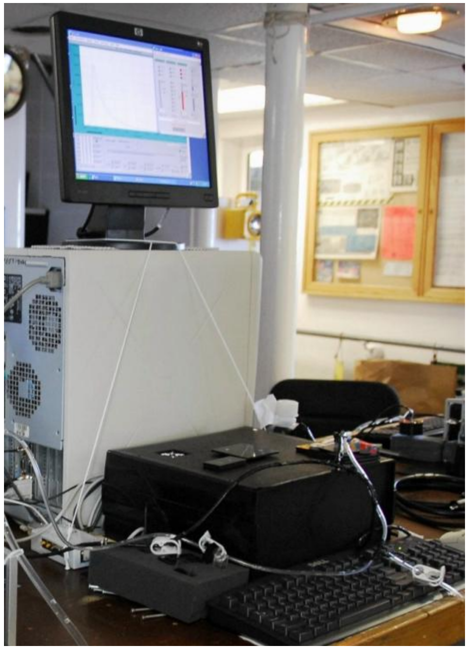
Picosecond Fluorescence Kinetics

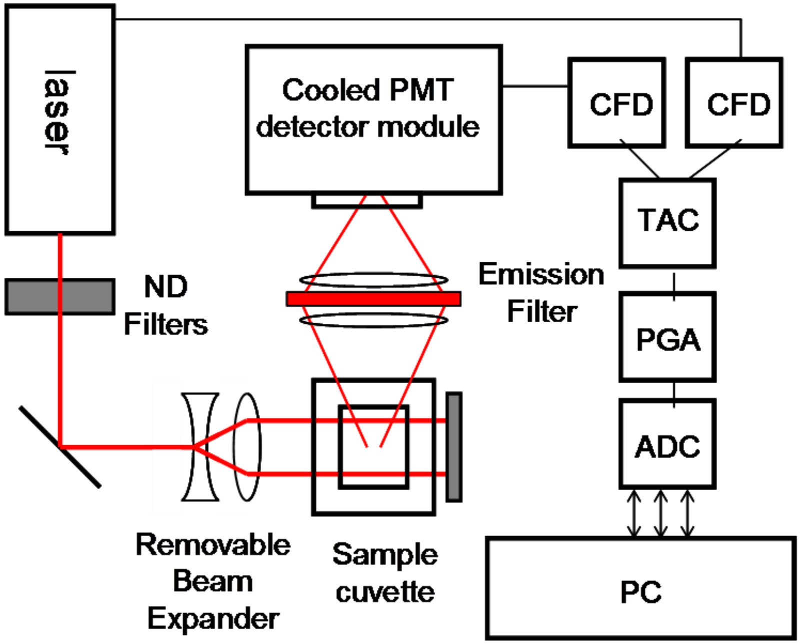


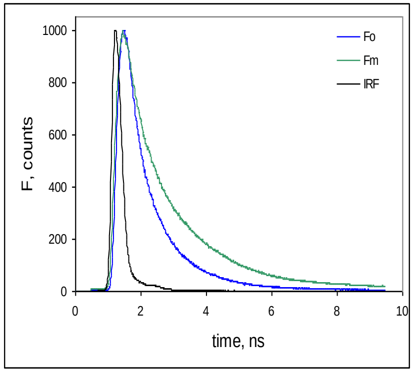
- Multi-component analysis if critical for *in vivo* Chl-a fluorescence kinetics

F_o		F_m		F_m’	
t_i *(ps)*	a_i	t_i *(ps)*	a_i	t_i *(ps)*	a_i
69	0.275	67	0.316	69	0.247
194	0.336	200	0.201	205	0.381
530	0.385	994	0.136	640	0.365
2690	0.004	**2270**	0.347	2550	0.007
Average lifetime					
298 ps		984 ps		346 ps	

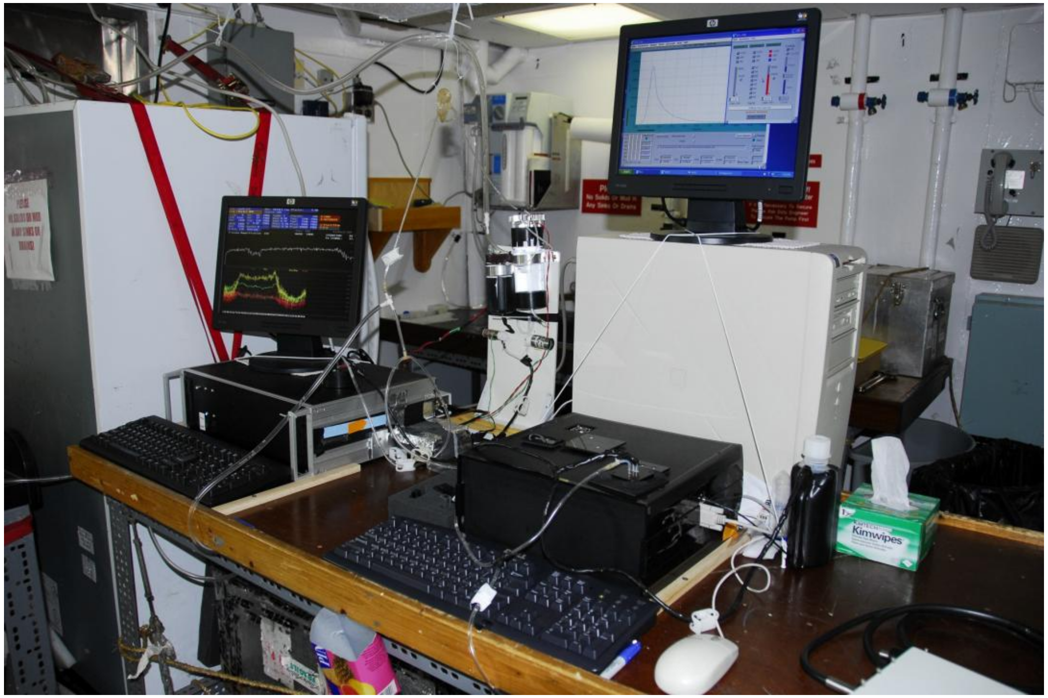
Instrumental Objective:
to develop a sea-going Picosecond Lifetime Fluorometer





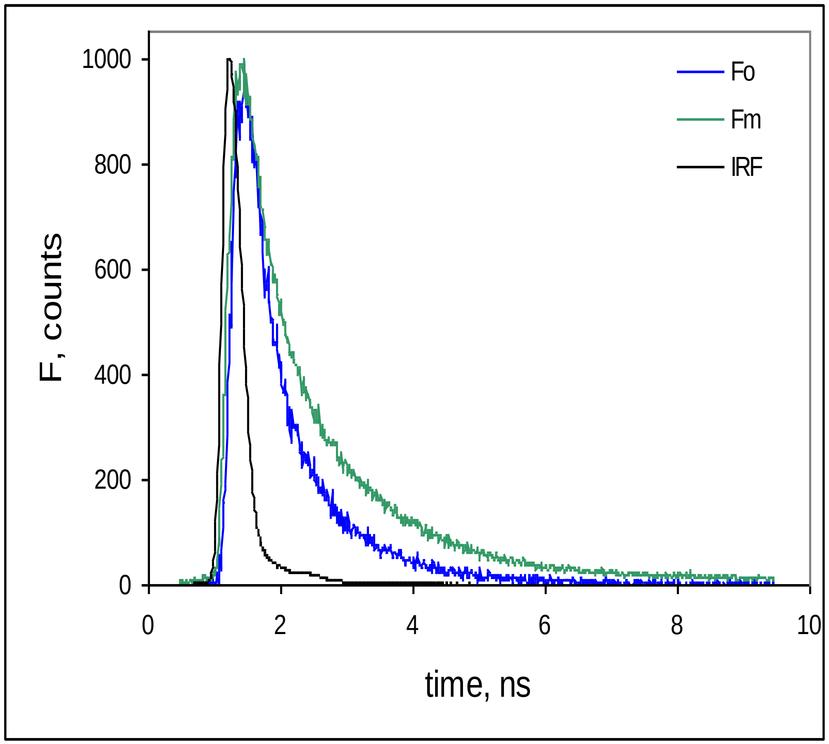


Picosecond Laser LifeTime Fluorometer & FIRe Fluorometer onboard R/V Oceanus



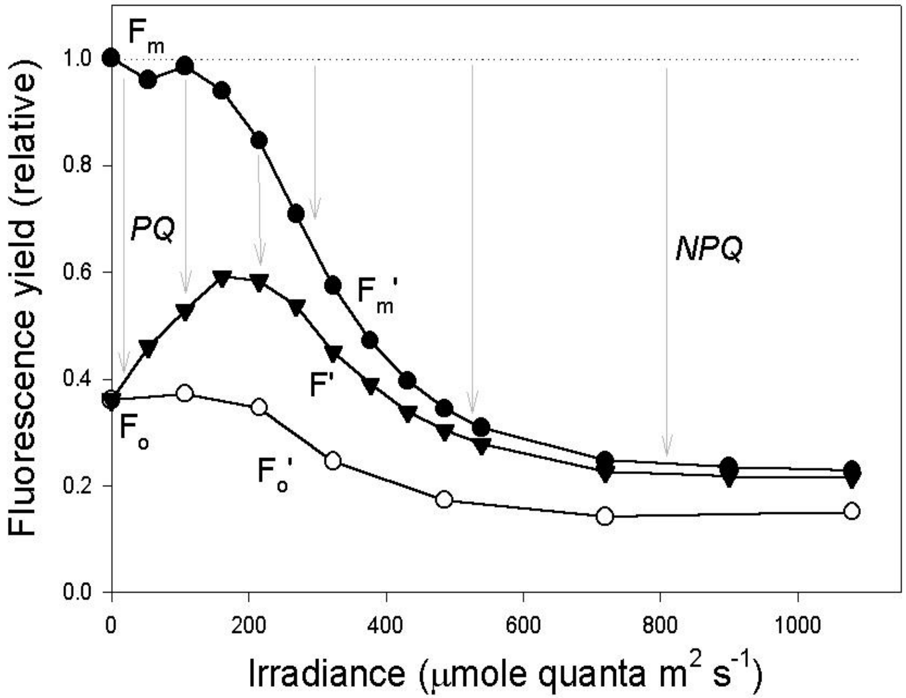
- Picosecond Fluorometry => quantum yields and lifetimes
- FIRe (Fluorescence Induction and Relaxation), or FRR => phytoplankton physiology (photosynthetic light-harvesting processes, photochemistry, electron transport rates)

Chl-a Fluorescence Decay Kinetics at Chl-a = 0.04 mg/m3 (Sargasso Sea)



- Extreme sensitivity (down to 0.01 ug/L of Chl-a)
- ~2 orders of magnitude more sensitive than Ciencia Phase-Shift Fluorometer.
- 2-, 3-, or 4-component analysis

The Irradiance Dependence of Chlorophyll Fluorescence Yields



NPQ = (Fm – Fm')/Fm'

NPQ varies from 0 to ~3

- Quantum yields of fluorescence under high light (e.g., SIF) are controlled by the process of **non-photochemical quenching (NPQ).**
- NPQ is a photoprotective mechanism that is activated under supra-optimal irradiance and thermally dissipates excess absorbed energy.

Laboratory Program

❖ Designed to understand the mechanisms of non-photochemical quenching (NPQ) in diverse phytoplankton taxa.

❖ How is NPQ affected by

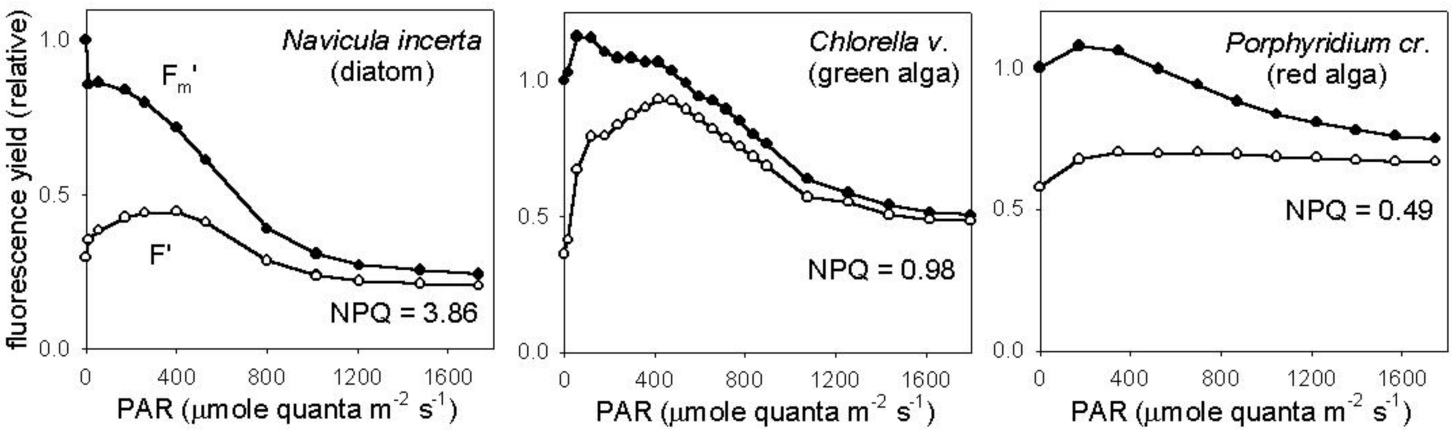
- Taxonomy;
- Nutrient status;
- Photoacclimation?

Practical implications for SIF analysis:

How variable are NPQ properties?

Can we apply a uniform NPQ correction procedure for the global ocean?

Taxonomic Variability in NPQ capacity



- ❖ All oxygenic photosynthetic organisms evolved the NPQ mechanism, but the mechanisms differ between taxa.
- ❖ Brown (diatoms and dinoflagellates) and green algae exhibit the maximum NPQ capacity.
- ❖ NPQ capacity in cyanobacteria and red algae is much lower.

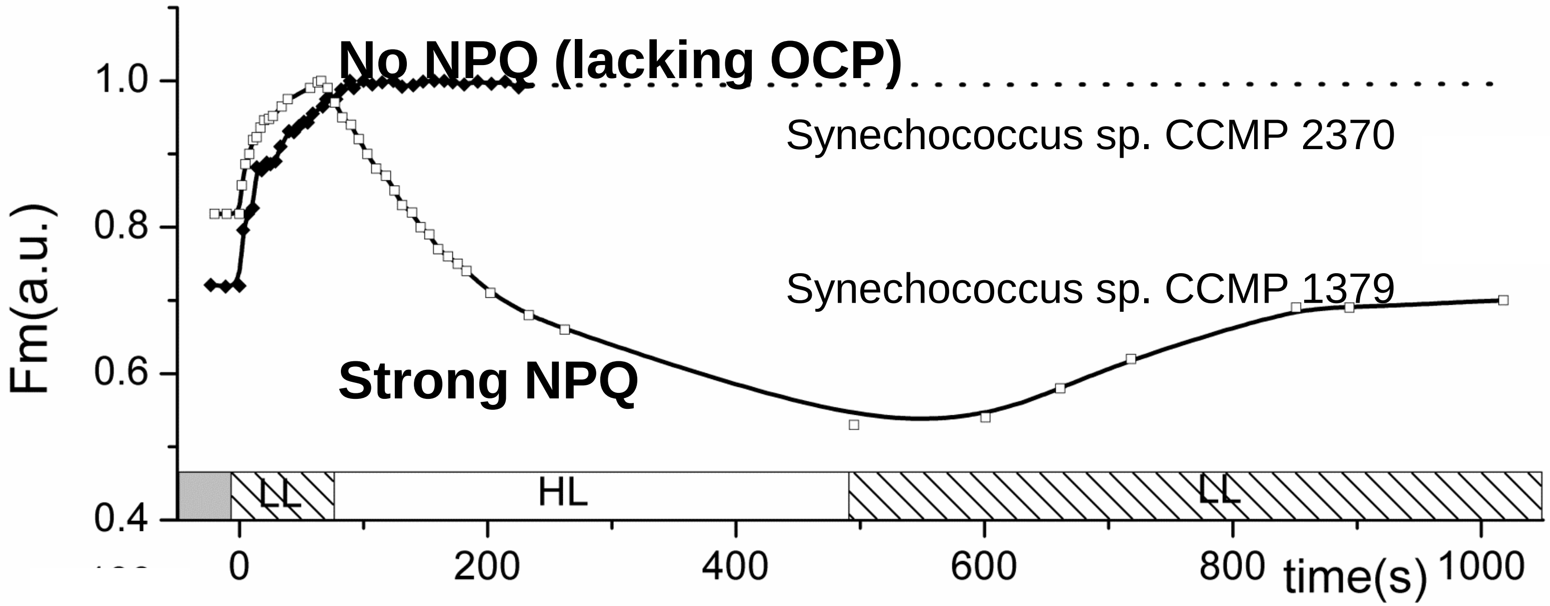
Biophysics and Biochemistry of NPQ

- In eukaryotic algae and higher plants, NPQ is induced by ΔpH across the thylakoid membrane (**energy-dependent quenching**) and involves the **xanthophyll cycle** and **conformational changes in LHCII**.
- Red algae exhibit **ΔpH-dependent quenching**, but **no xanthophyll cycle**
- Cyanobacteria lack both pH-dependent quenching and xanthophyll cycle

NPQ in cyanobacteria: Key role of Orange Carotenoid Protein (OCP)

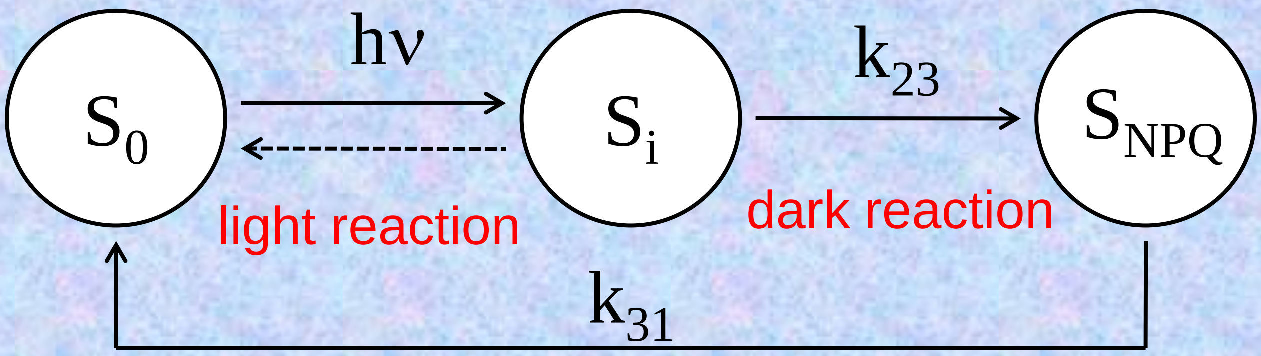
❖ NPQ is not observed in cyanobacteria lacking OCP gene or OCP-deficient mutants

Wilson et al., Plant Cell 18 (2006) 992-1007.
Boulay et al., Biochim. Biophys. Acta 1777 (2008) 1344–1354.

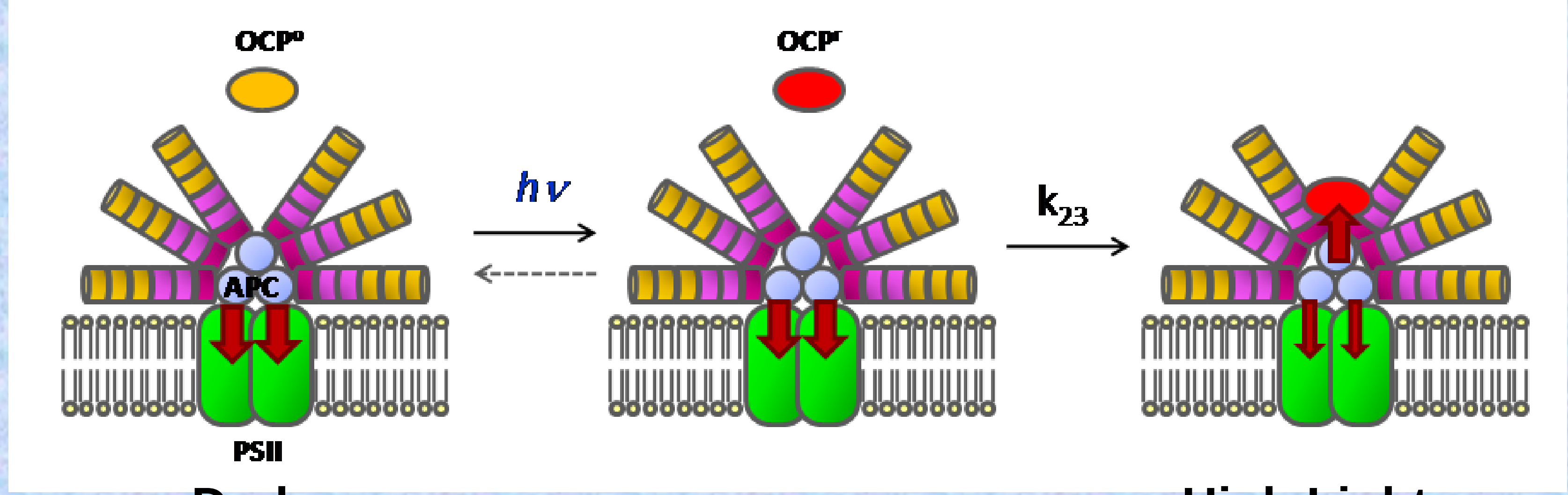


Gorbunov et al., Biochim. Biophys. Acta, 1807: 1591-1599 (2011).

Kinetic Model for NPQ in Cyanobacteria



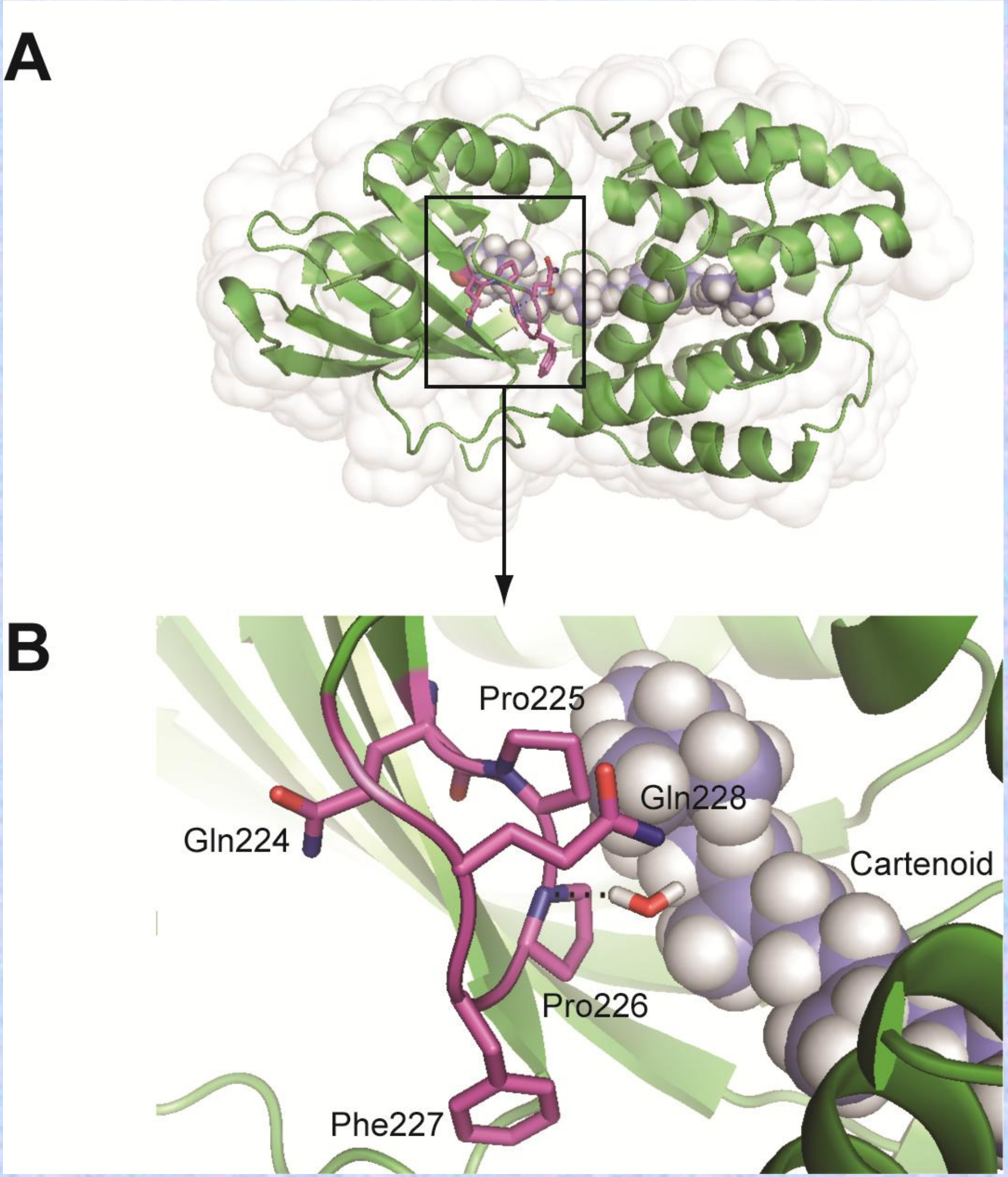
OCP^o → OCP^r → OCP^r+PBS



Dark **High Light**

Gorbunov et al., Biochim. Biophys. Acta, 1807: 1591-1599 (2011).

OCP Protein Structure Analysis:



…QPPFQ… - prolyl rich motif

highly conserved in all known OCP genes

Pro225 and Pro226 located near the photoactive site of the carotenoid

Prolyl *cis-trans* isomerisation of Pro225 and/or Pro226 is presumably the rate limiting step in NPQ activation

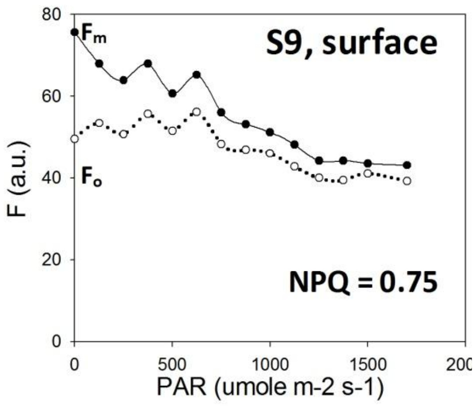
Gorbunov et al., Biochim. Biophys. Acta, 1807: 1591-1599 (2011).

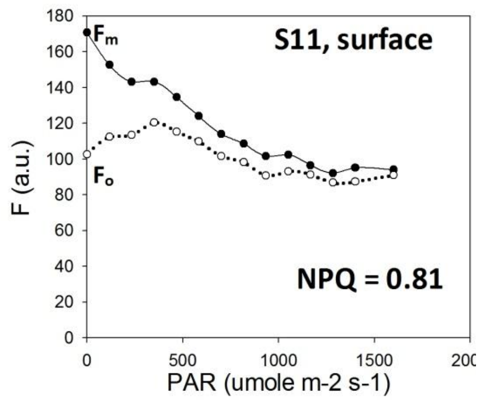
Variability in NPQ in the ocean

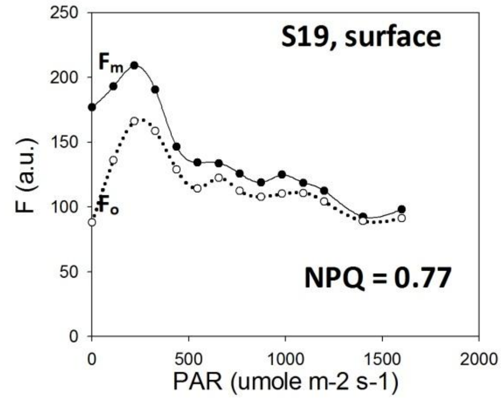
- ❑ **Lab research reveals that NPQ may vary from 0 to ~ 3.0 (=> 5x variability in quantum yields) depending on species and physiological state**
- ❑ **Taxonomy has a significant effect on satellite-based SIF yields (may account more than 50% of variability)**
- ❑ **What is the range of variability in NPQ in the real world?**

Variability in NPQ in the ocean

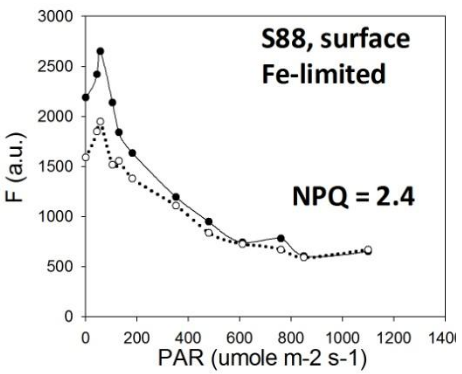
Sub-tropical Atlantic

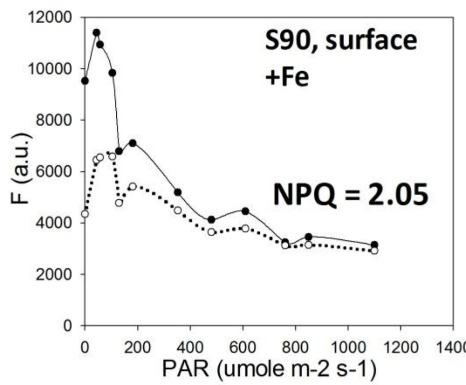




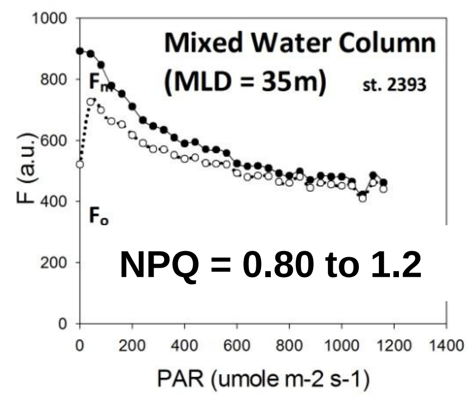


Southern Ocean





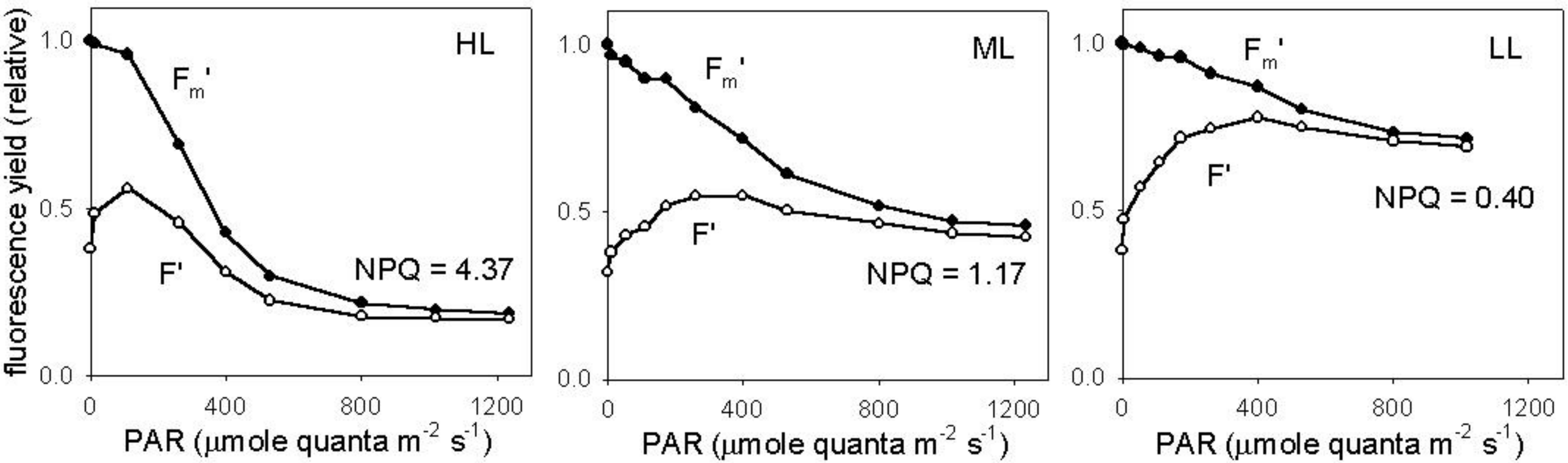
Sub-Arctic Atlantic



4x variability in NPQ capacity in the global ocean

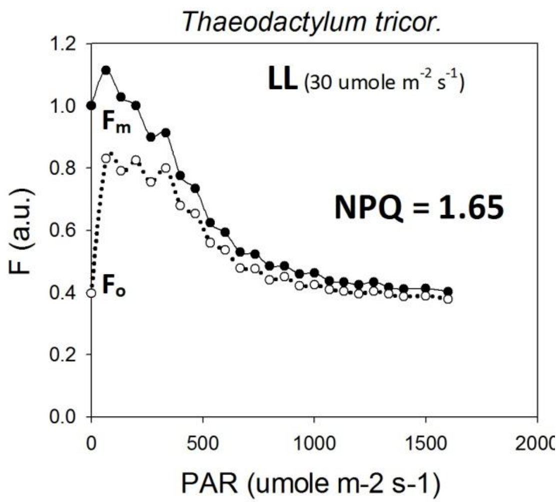
Effect of Photoacclimation on NPQ

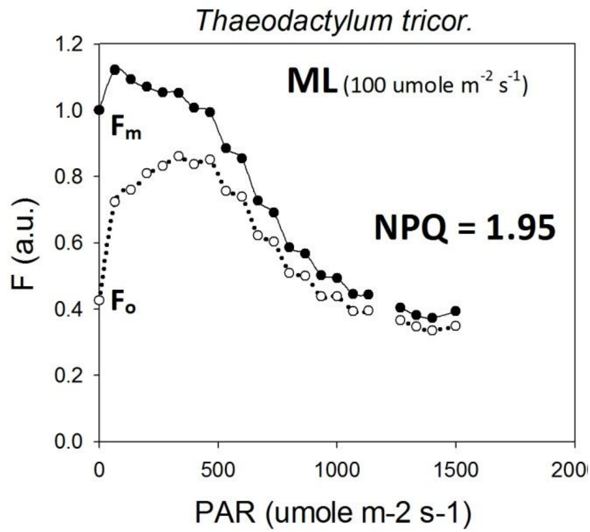
diatom *Dilytum brightwellii*

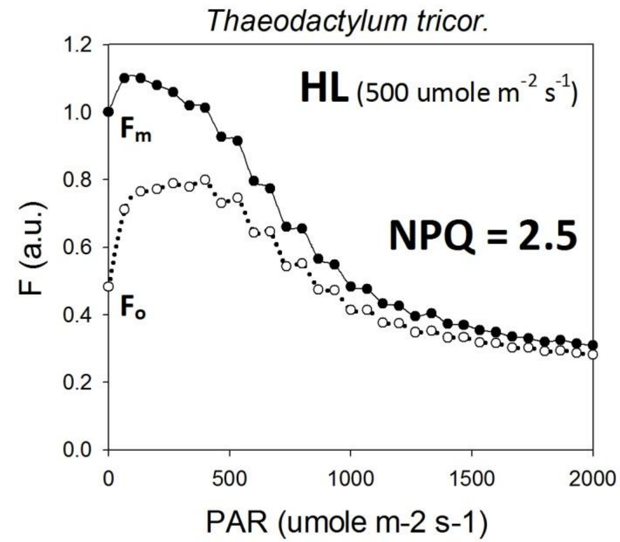


- ❑ **NPQ increases with growth light intensity**
- ❑ **Cells synthesize more xanthophyll pigments under HL**

Effect of Photoacclimation on NPQ

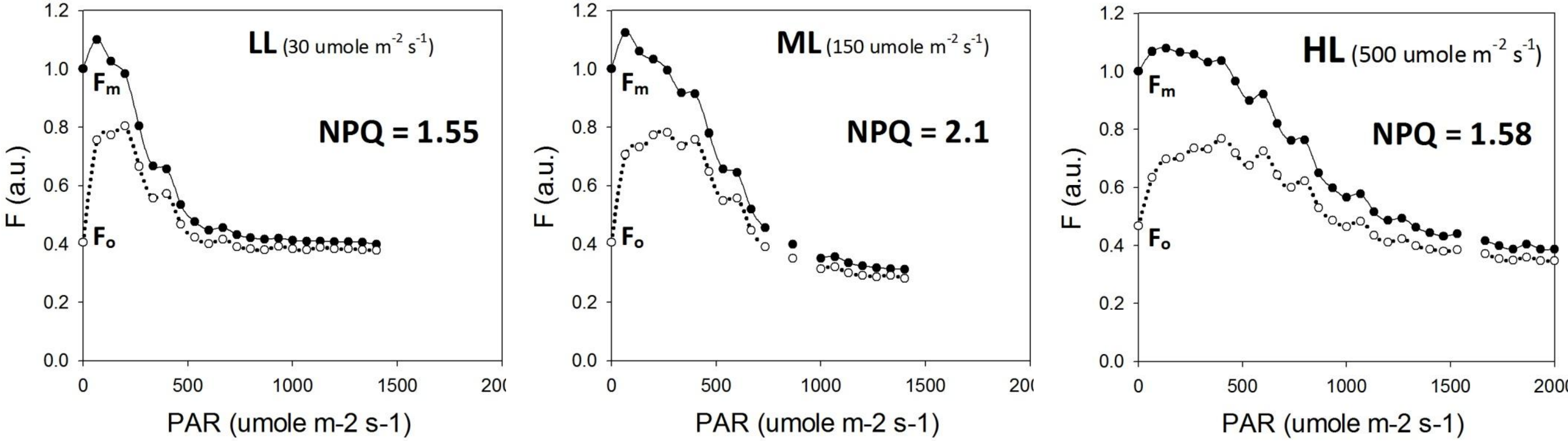






Effect of Photoacclimation on NPQ

diatom *Thalassiosira weissflogii*



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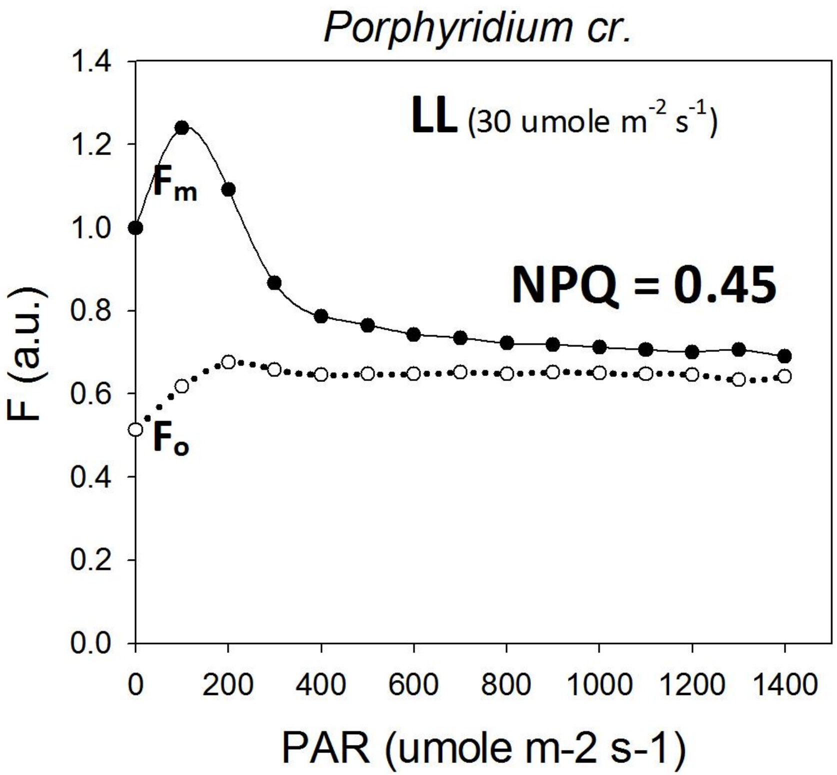
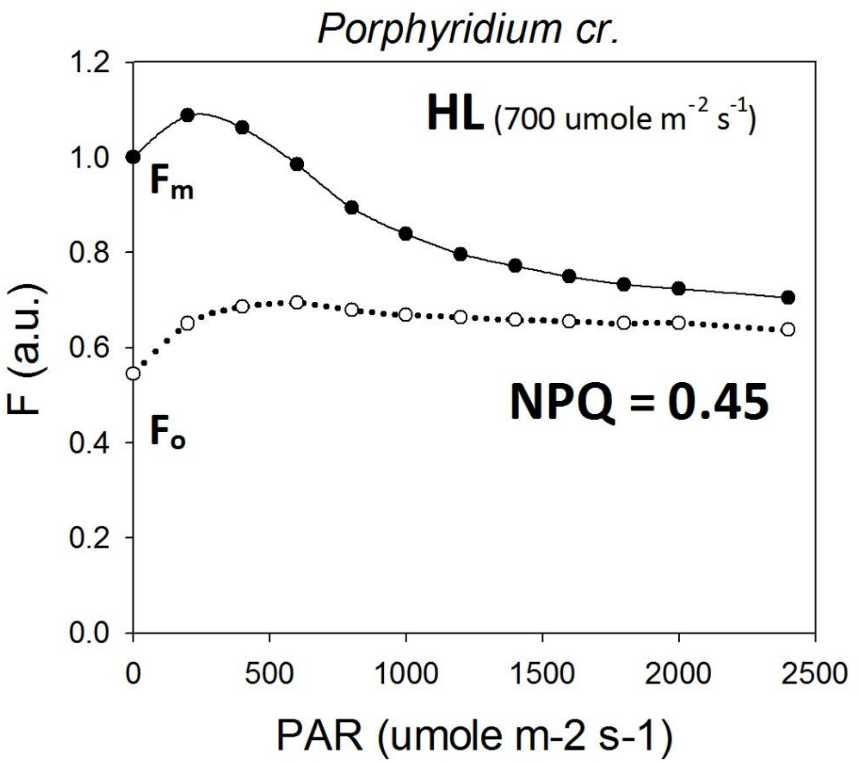
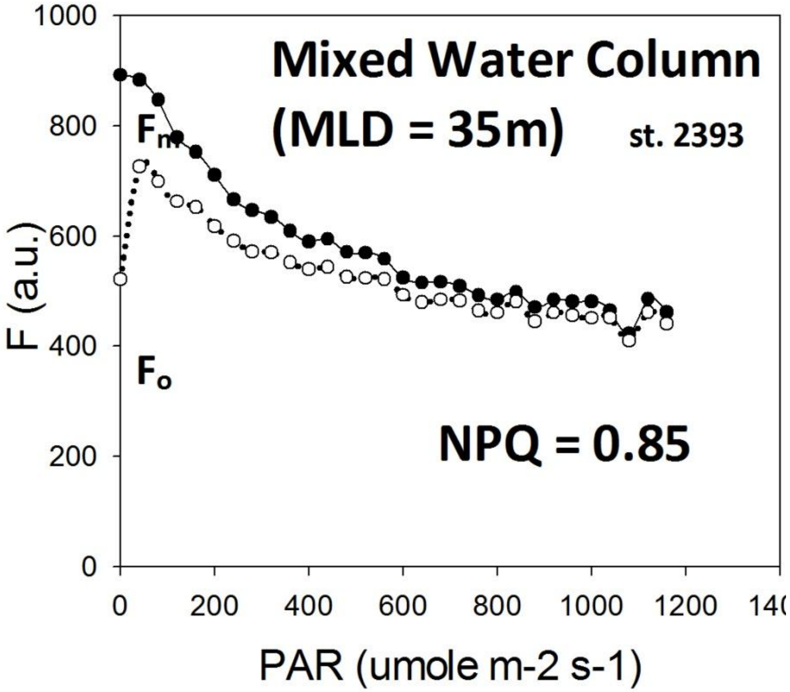
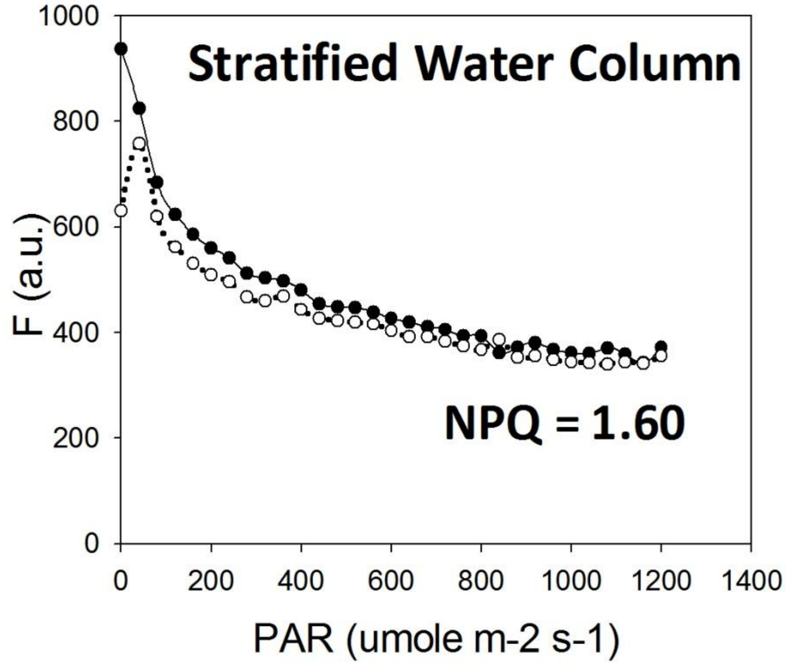




- **Photoacclimation has no effect on NPQ capacity in red algae**
- **NPQ is pH-dependent, but no xanthophylls cycle**
- **Low NPQ capacity (=> higher quantum yields of satellite-based SIF)**

Effect of Vertical Mixing on Yields of SIF (North Atlantic, Sept. 2010)





- Acclimation to High Light in a stratified water column increases NPQ capacity and, hence, decreases the quantum yield of SIF
- Acclimation of NPQ to HL is fast (~4-6 hours), even in cold sub-Arctic waters

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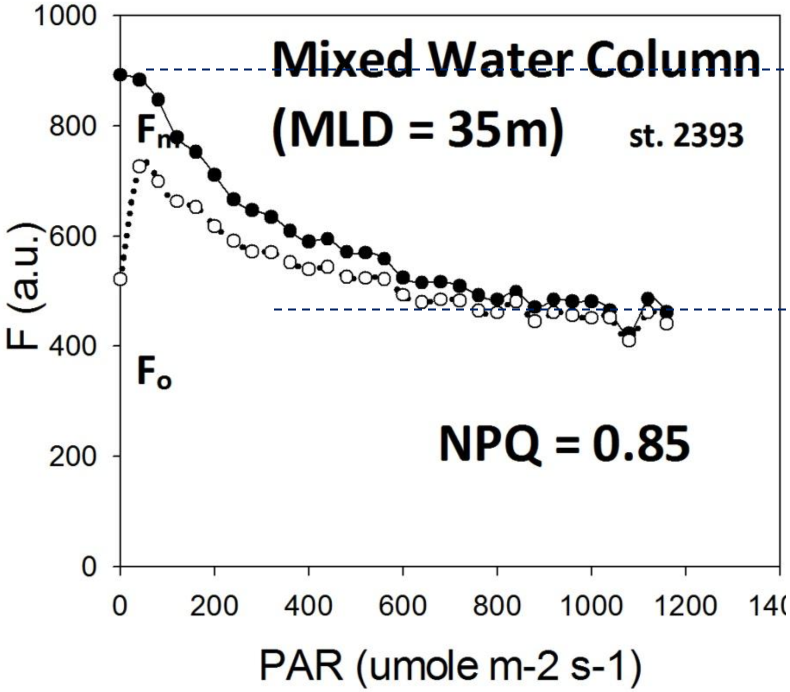

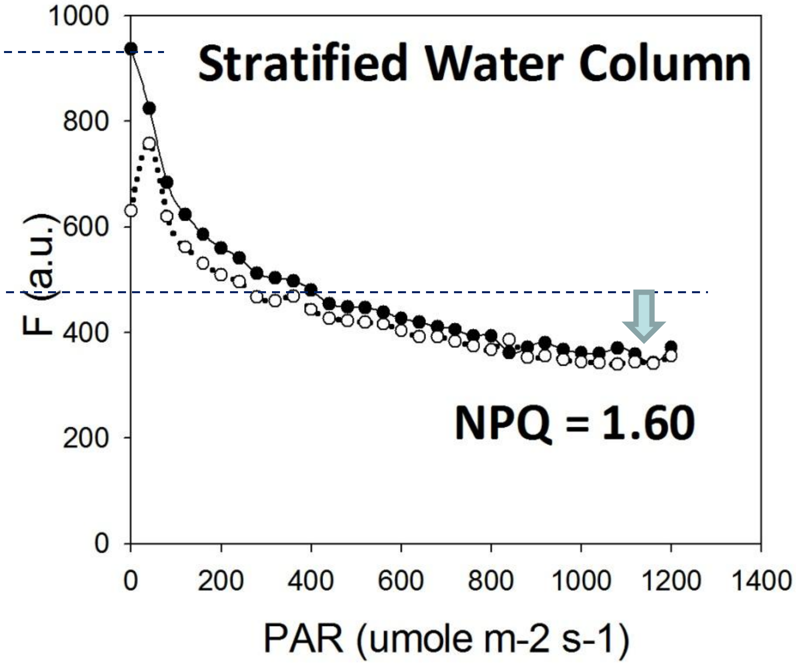

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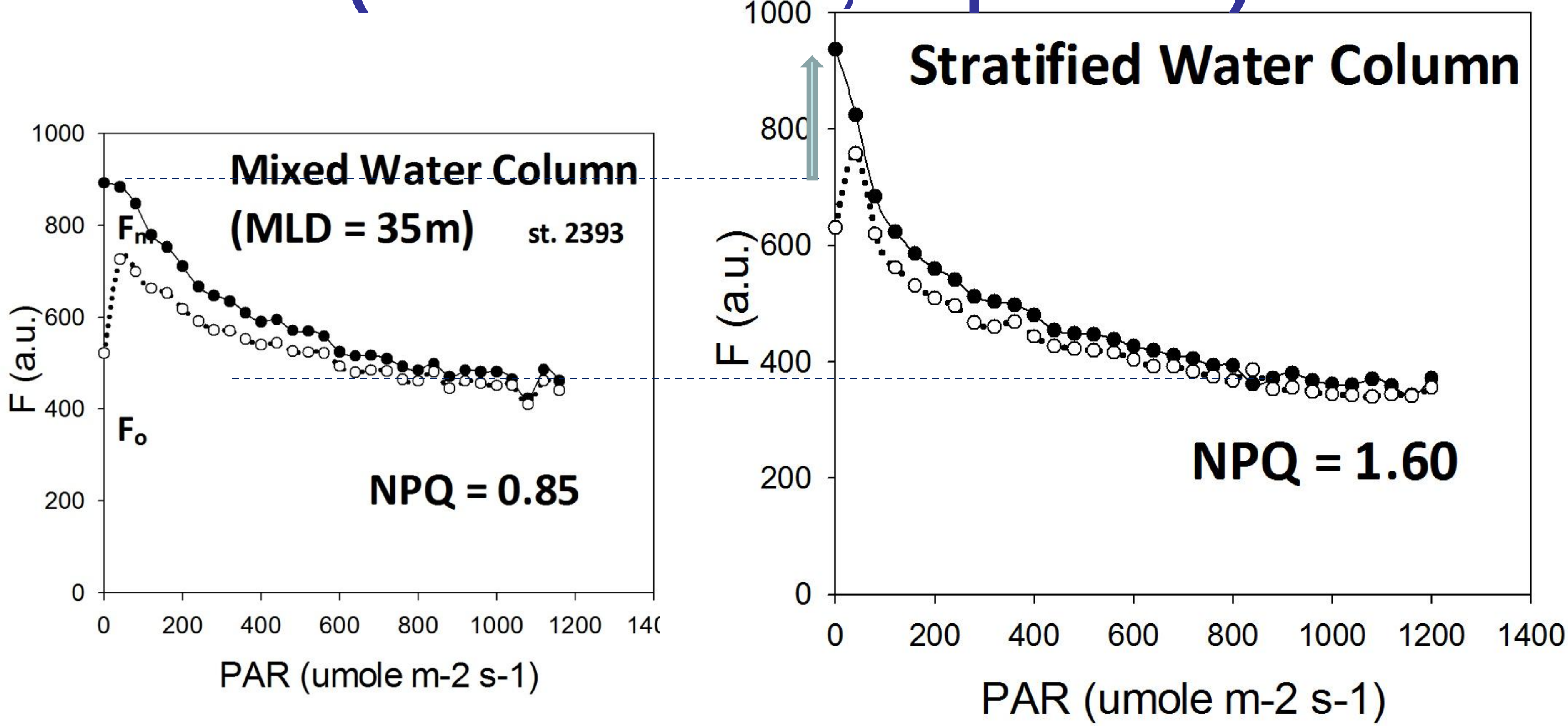

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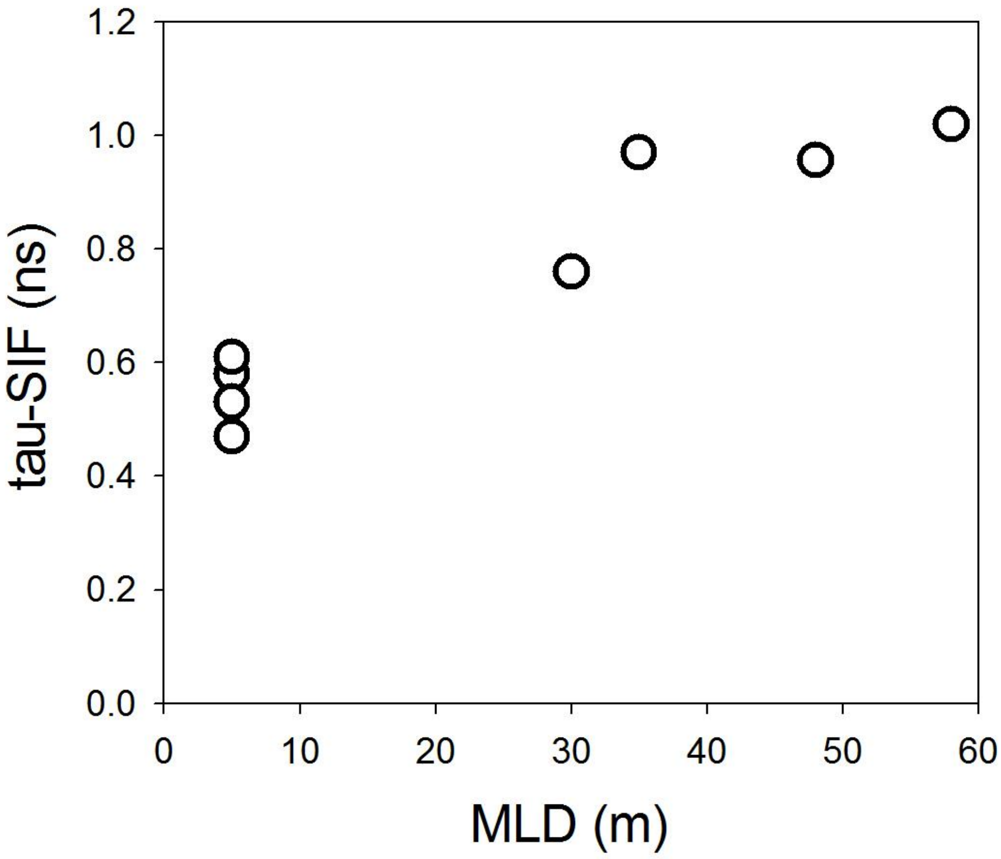
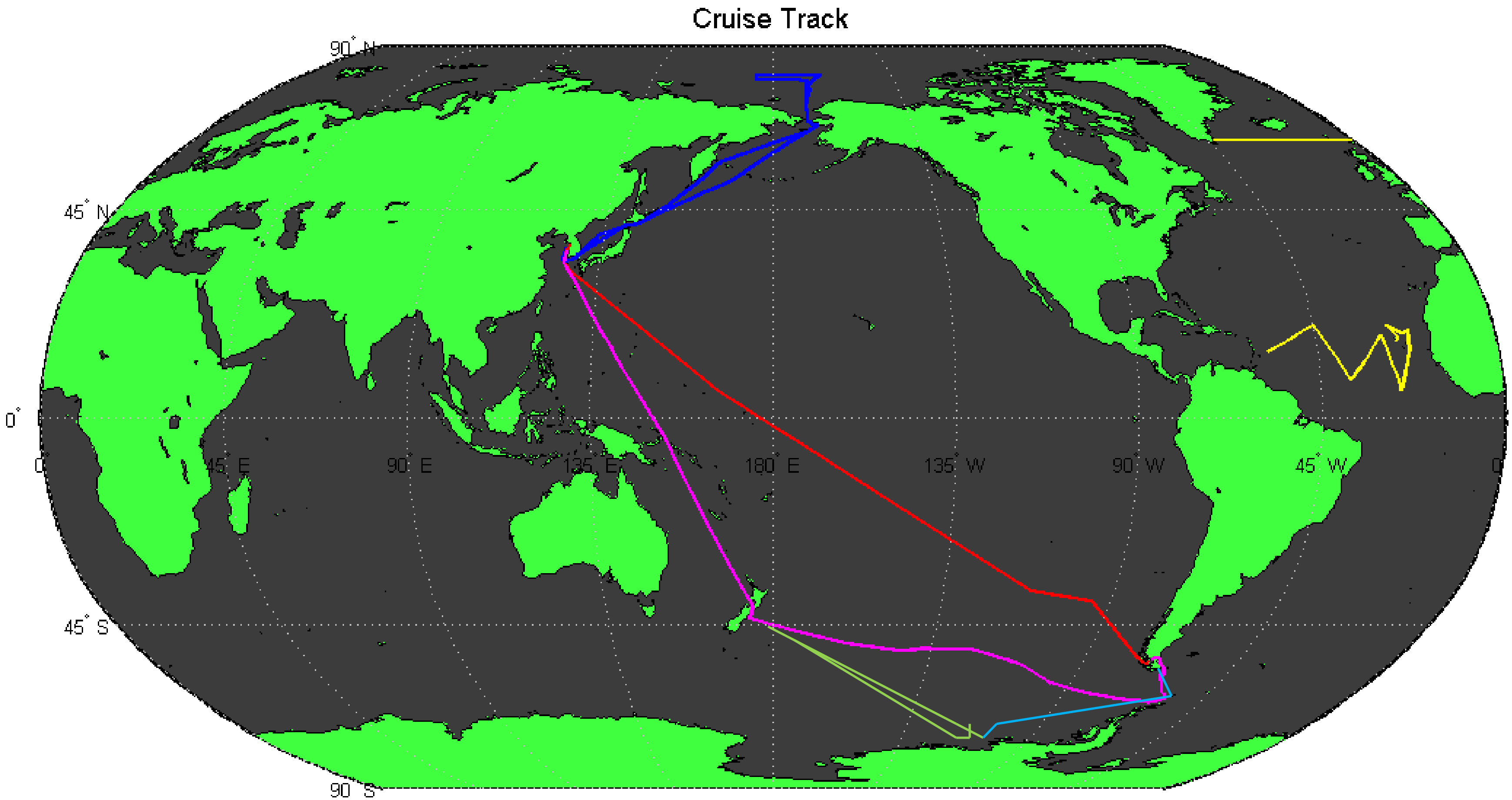


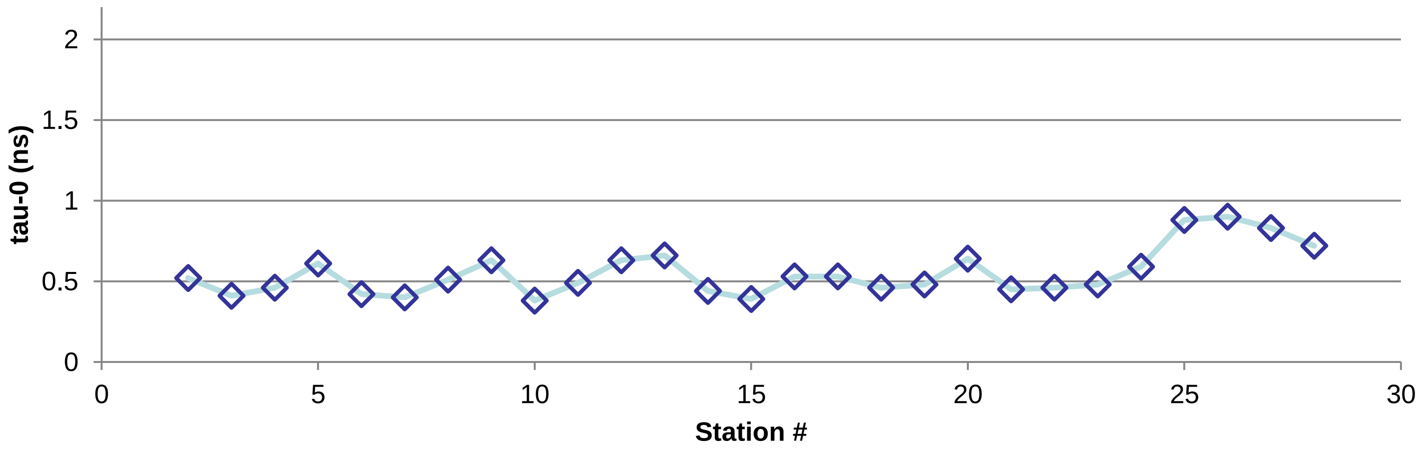
- **Acclimation to High Light in a stratified water column decreases fluorescence lifetimes and, hence, decreases the quantum yields of SIF**

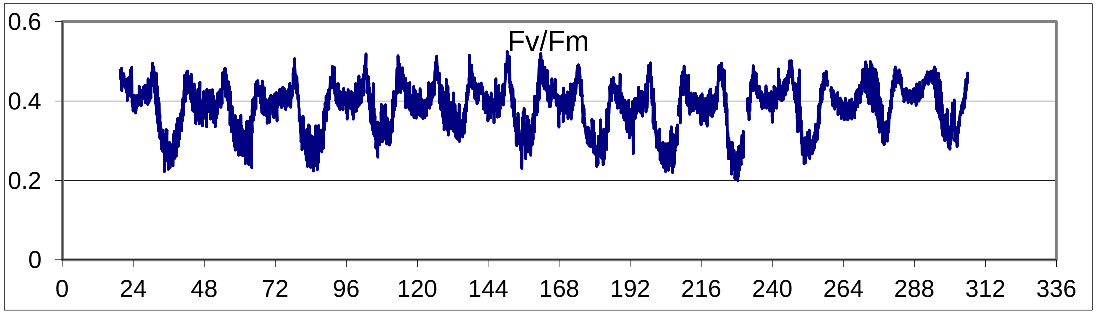
Field Program Completed

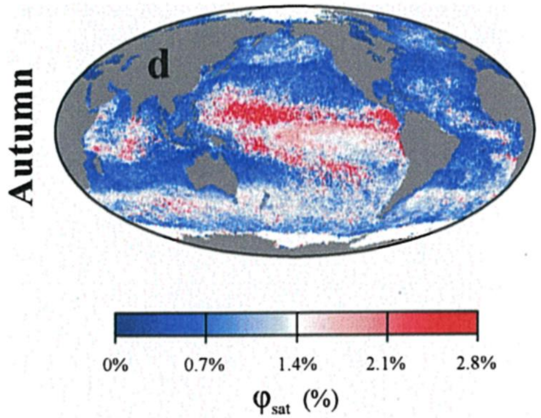


- Over 300,000 measurements of fluorescence yields collected;
- ~ 40,000 miles of transects.

Variability of Fluorescence Lifetimes in Sub-tropical Atlantic (Aug.-Sept. 2008)



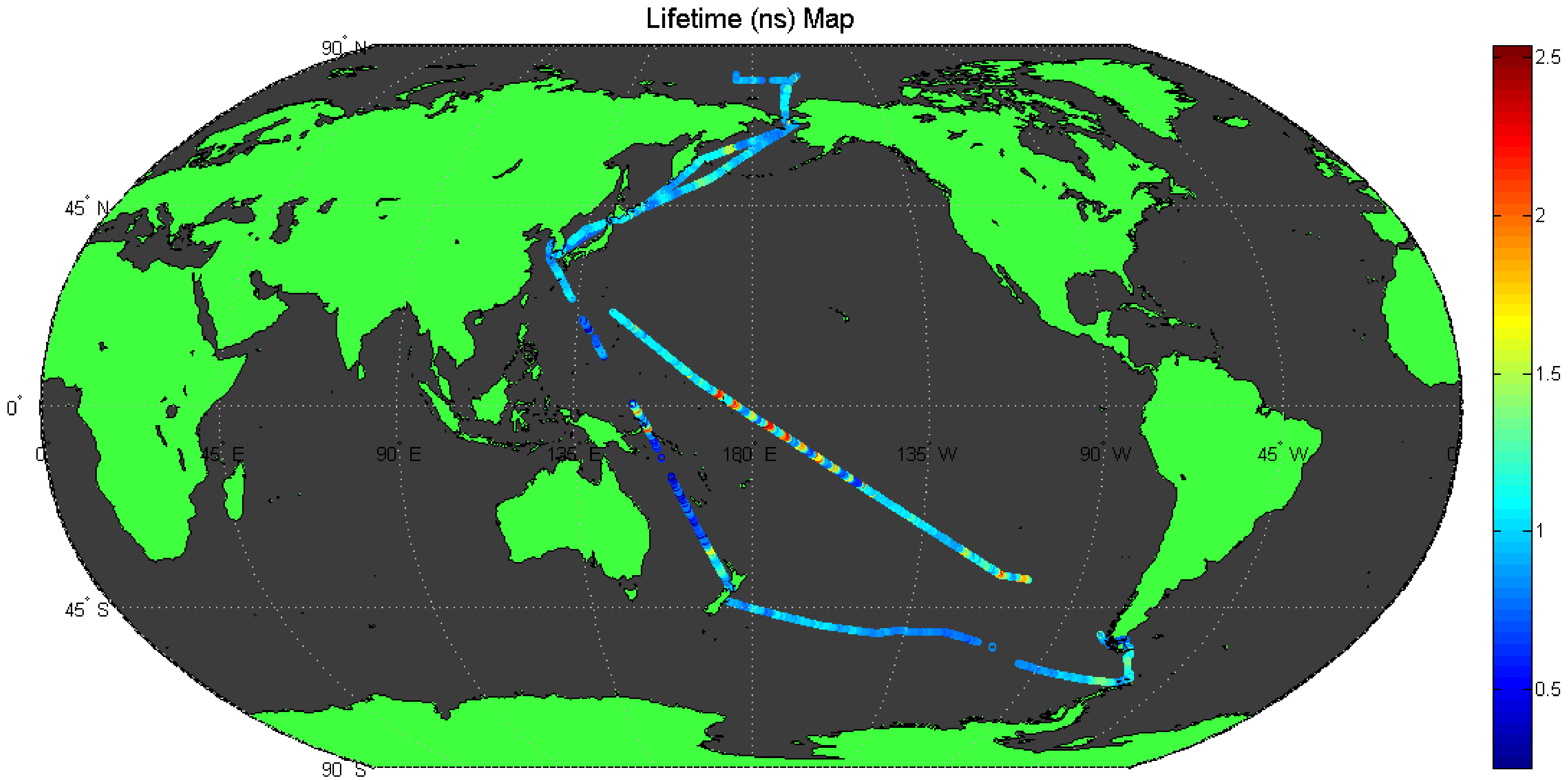




Behrenfeld et al., Biogeosciences, 2009

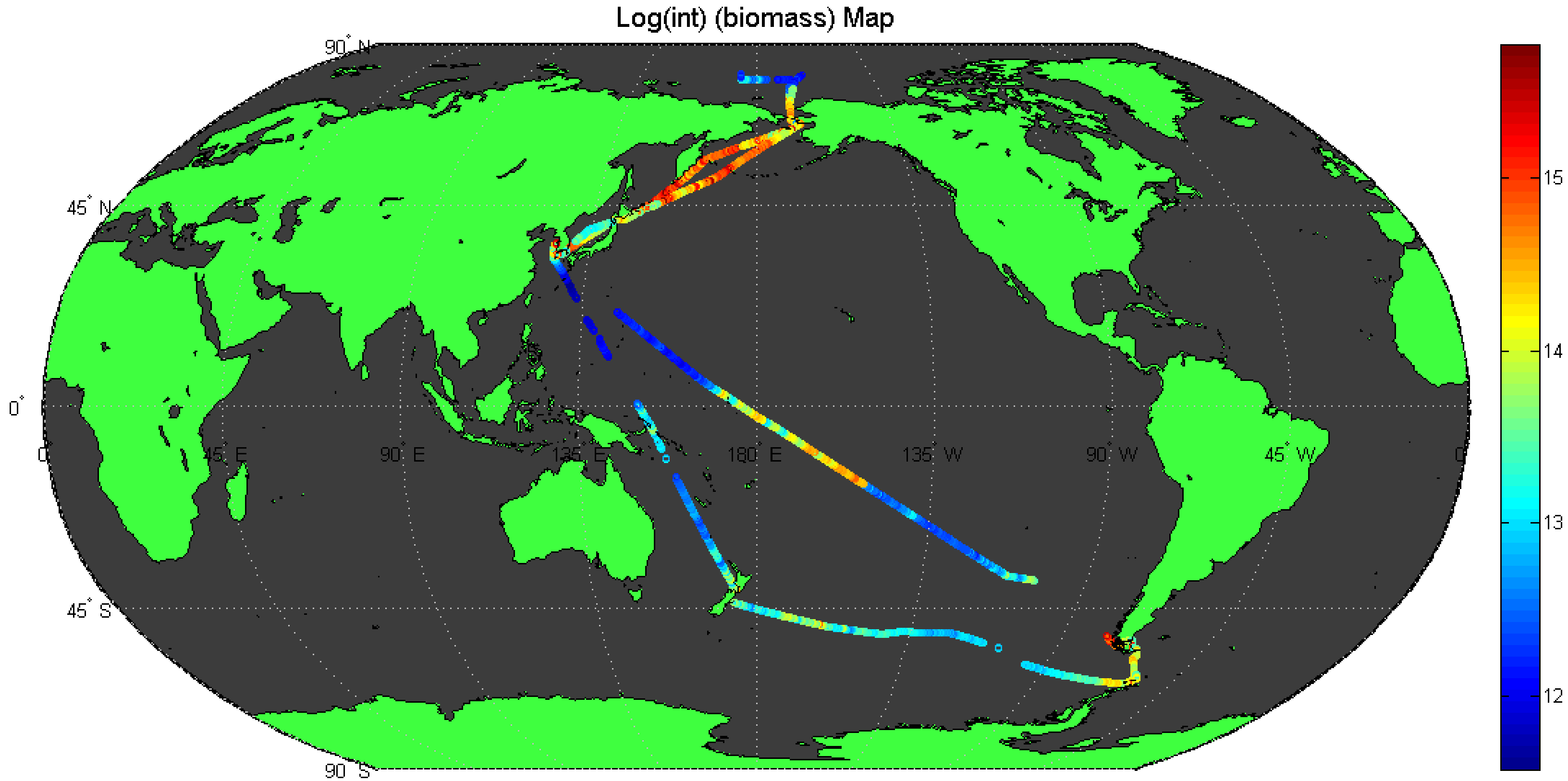
- ❑ **Fluorescence lifetimes and quantum yields are highly constrained across the Atlantic**

Variability of Fluorescence Lifetimes in the Pacific

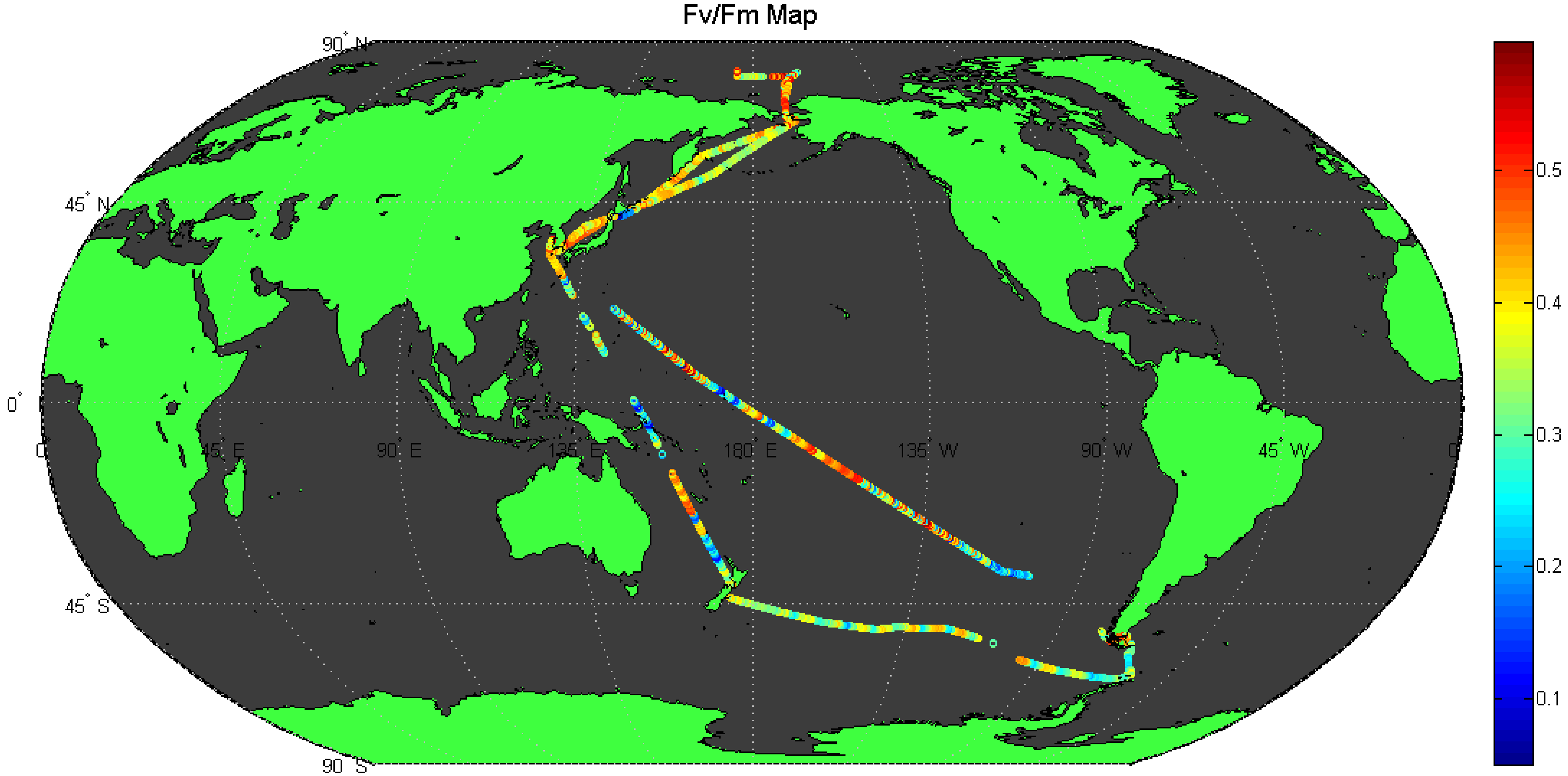


- ~5x variability in fluorescence lifetimes in the Pacific
- Strong physiological effects (due to nutrient status and taxonomy)

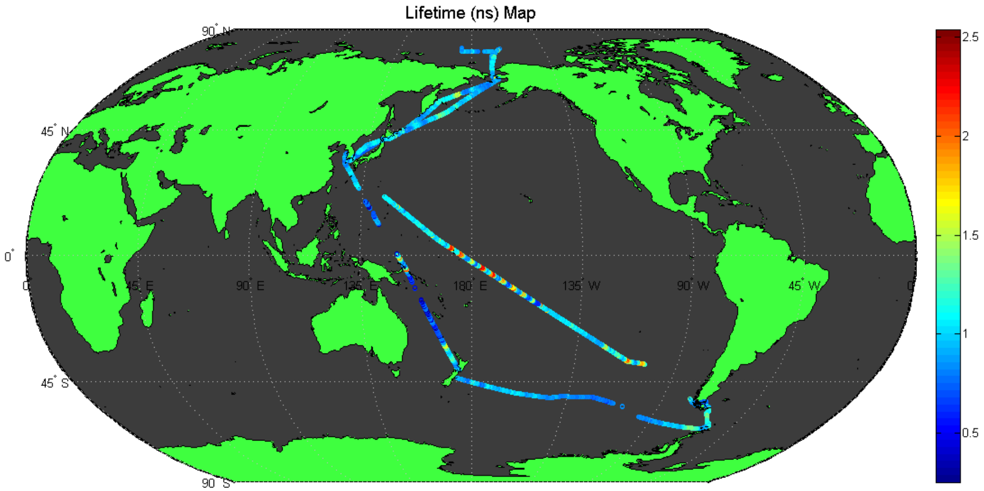
Variability in Chlorophyll Biomass

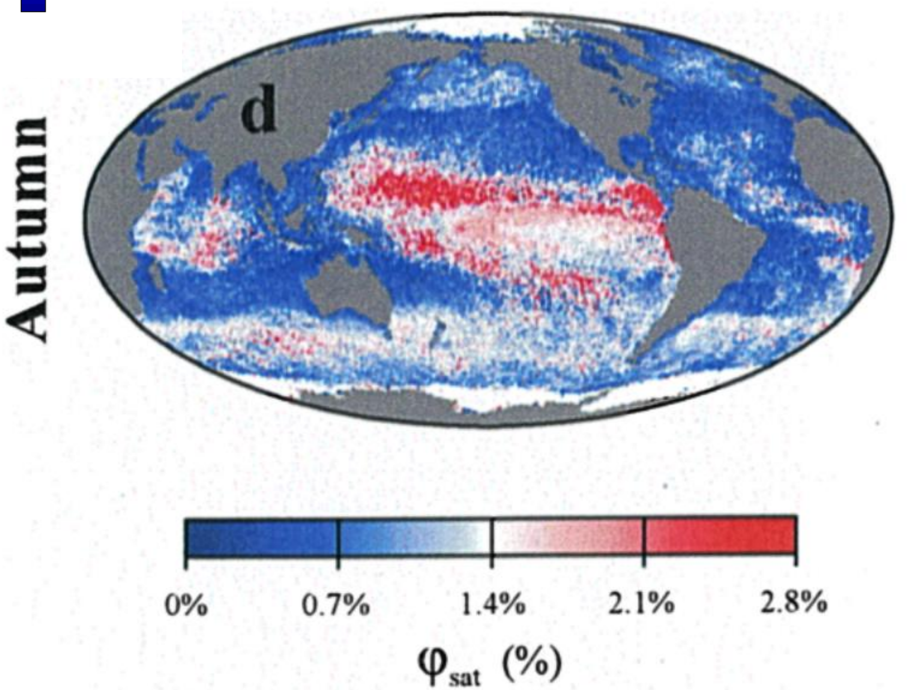


Variability in Photosynthetic Efficiency of PSII

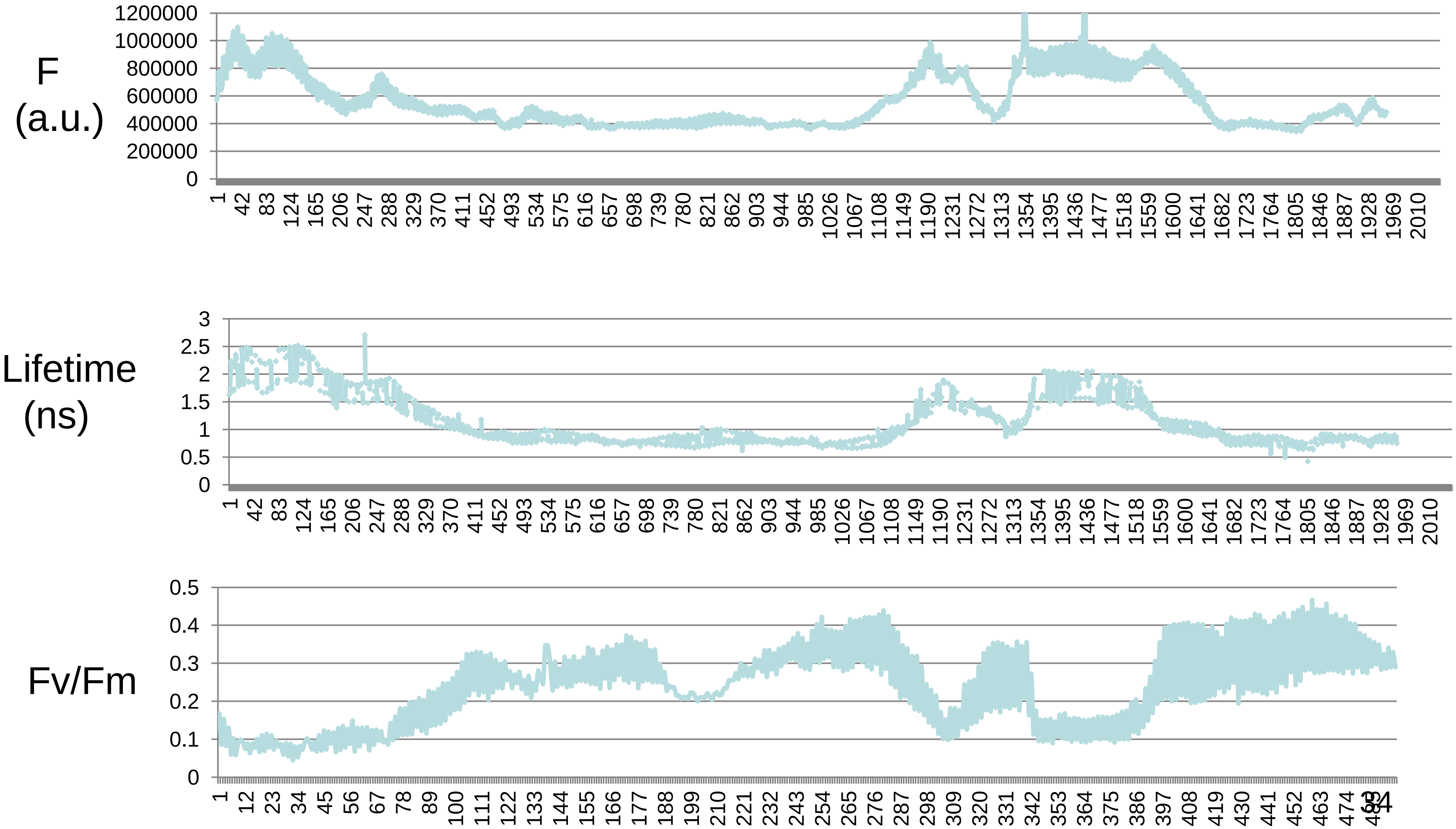


Interpreting the Variability in MODIS SIF Yields





Chl-a Fluorescence Lifetime Distribution in Near-Surface Layer (Araon, Oct 17, 2010)



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Conclusions

- SIF yields reflects phytoplankton physiology and taxonomy in the ocean.
- Phytoplankton taxonomy is as important in regulating satellite-based SIF yields as nutrient status (may account >50% of variability in SIF yields).
- Photoacclimation (vertical mixing) may also contribute to the variability in SIF yields (may account ~20% of variability in SIF yields).

Future Directions

- to complete an extensive field program in the Arctic, Pacific, and Southern oceans to complement the database of fluorescence lifetimes acquired previously.
- to reconstruct the global distribution of fluorescence lifetimes and fluorescence yields in the ocean.
- to correlate the distributions of fluorescence lifetimes and quantum yields with the variability in SIF yields retrieved from MODIS products.
- to correlate the variability in fluorescence yields with chemical, hydrological, and biological data (nutrient availability, taxonomy, and physical forcing).

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