Photophysiology, FRR/FIRe fluorometry: How it works and how we can do it better

Combining amplitude-based variable fluorescence with kinetic approach to improve estimates of growth and PP rates and nutrient stress

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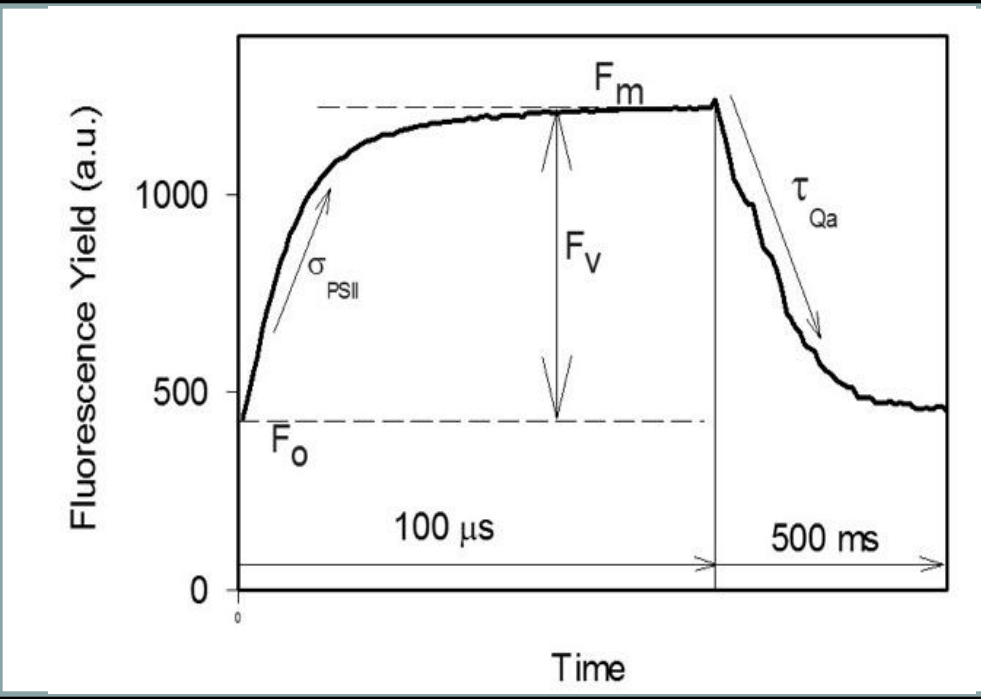
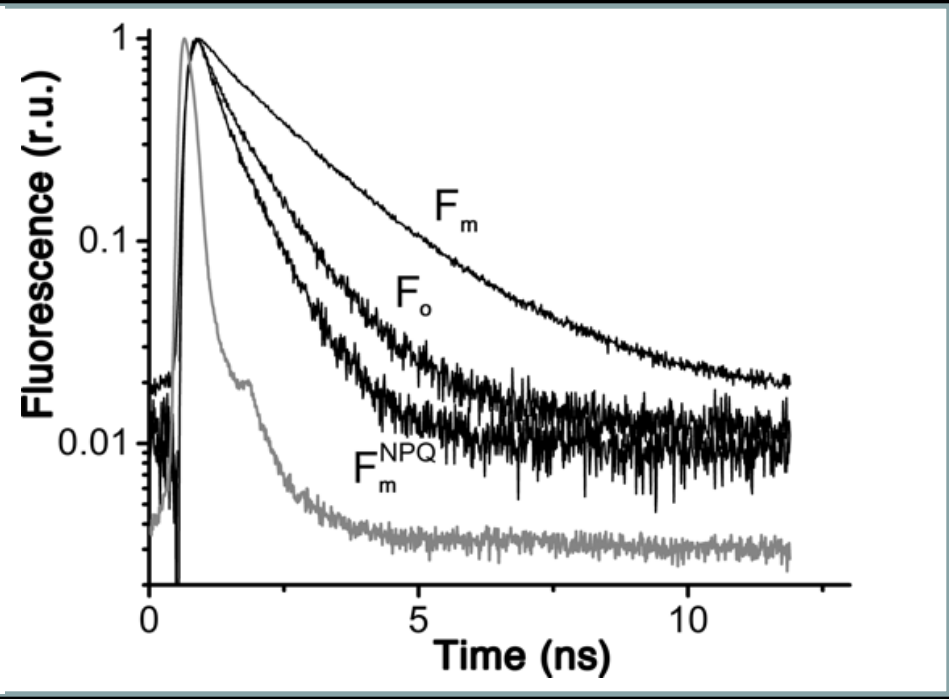
Concepts of amplitude-based variable fluorescence versus picosecond lifetime kinetics



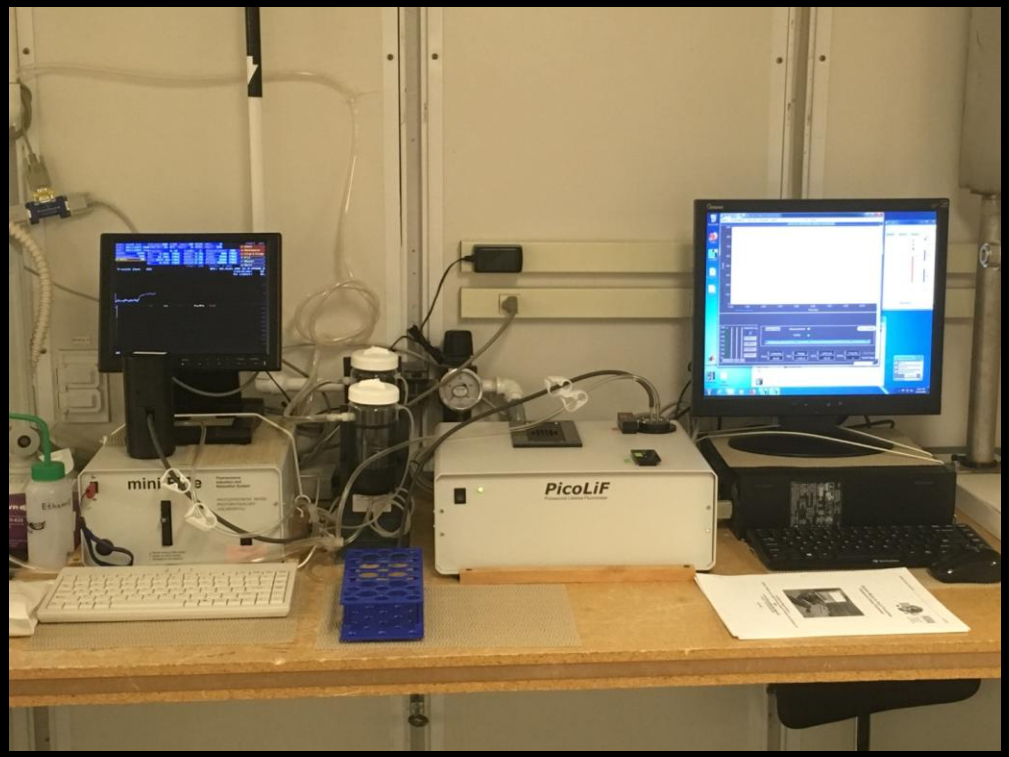


Note difference between :

Relaxation in fluorescence yield on µs time scale

Fluorescence decay kinetics on ps time scale

Mini-FIRe and PicoLIF fluorometers installed aboard R/V Neil Armstrong for robotic underway sampling of photophysiology and quantum yields



Mini-FIRe is conceptually similar to FRR/FIRe, but exhibits

ca. 20x higher sensitivity and

incorporates new, more robust kinetic analyses (below)

Combination of amplitude-based Fv (mini-FIRe) and picosecond lifetime measurements

FIRe – photophysiology, estimates of ETRs, PP rates and growth rates, in relation to N and Fe stress

PicoLIF – absolute quantum yields of Chl-a fluorescence *in situ*; the only practical tool for *in situ* cal/val of MODIS-based retrievals of SIF quantum yields.

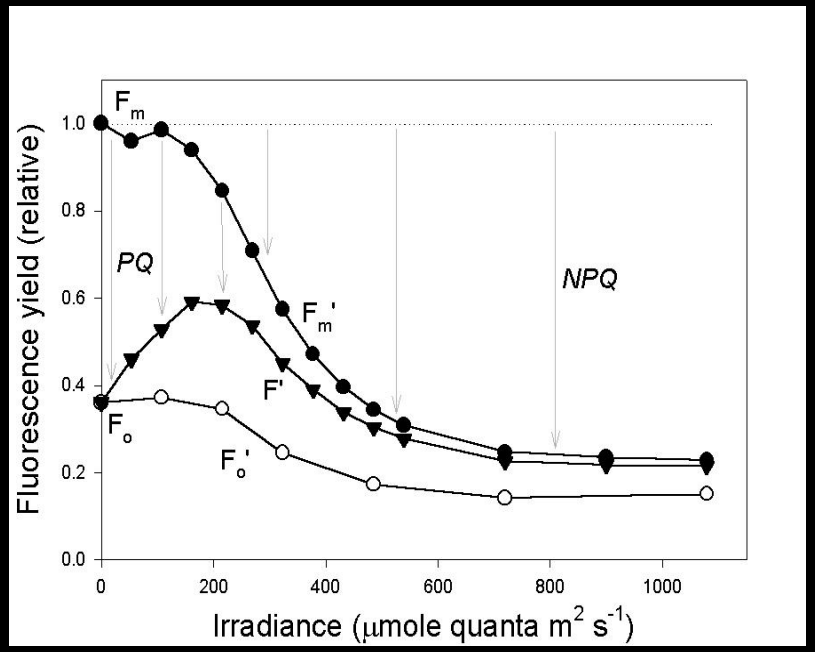
Rationale for FRR/FIRe variable fluorescence

1. Variable fluorescence from Chl-a is coupled to photochemistry in PSII and can be used to derive/model photosynthetic electron transport rates (ETRs) in PSII reaction centers (i.e., ETR per reaction center)

Rationale for FRR/FIRe variable fluorescence

2. Assuming the size of PSII unit (Chl-a/RC), ETR per RC can be converted to ETR per unit Chl-a (ETR_{Chl}).
3. To convert ETR_{Chl} to CO_2 (or O_2) rates, one need to know "electron requirement for carbon fixation" – how many e- are required to fix one CO_2

Mathematical Formalism for amplitude-based fluorescence modeling of photosynthetic rates



1. ETRs per reaction center:

ETR(E) = $E \times \sigma_{PSII}' \times q_P$

or

ETR(E) = $E \times \sigma_{PSII} \times (\Delta F'/F_m')/(F_v/F_m)$

here $\Delta F'/F_m'$ *is the only E-dependent variable*

2. **ETRChl =** $E \times \sigma_{PSII} \times (\Delta F'/F_m')/(F_v/F_m)\ n_{PSII}$

where n_{PSII} *is the size of PSU (RC/Chl-a ~ 0.002)*

Mathematical Formalism for amplitude-based fluorescence modeling of photosynthetic rates (cont.)

3. Chl-specific rates of PP:

$$\mathbf{P_{Chl} = ETR_{Chl}\ 0.25\ /\ PQ}$$

Here 0.25 is the quantum yield of O_2 evolution (4 e- needed per one O_2);

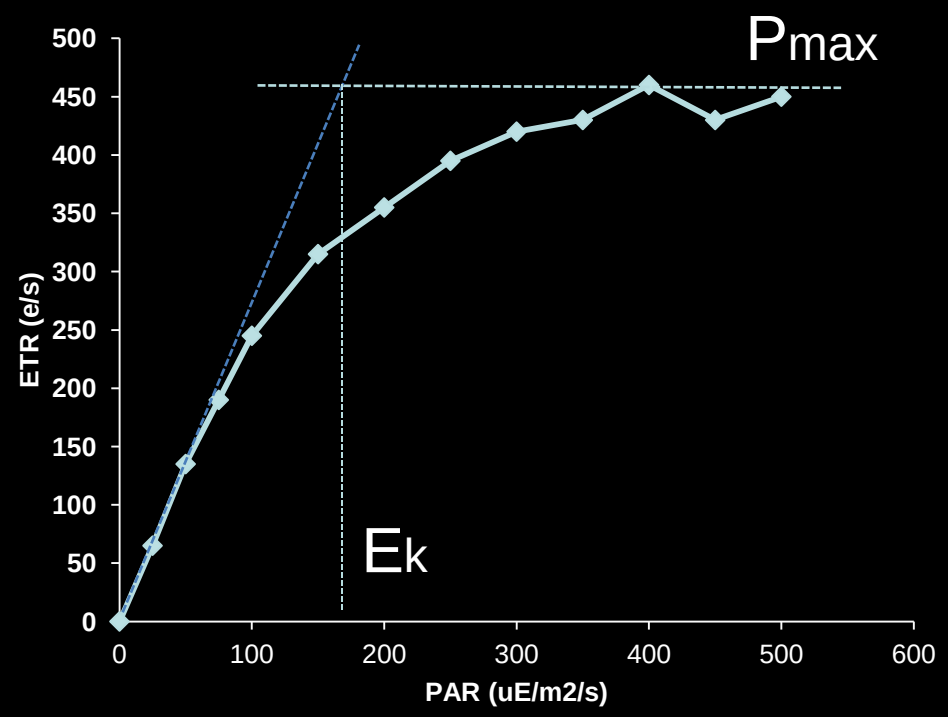
PQ is called the photosynthetic quotient (the ratio of O_2/CO_2);

PQ = 1.4 under N-replete conditions and increases under severe N-limitation. => <u>Quantitative (and better) proxies of N stress are important</u>.

$$\mathbf{P_{Chl}\ (CO_2) =} E\ \sigma_{PSII}\ (\Delta F'/F_m')/(F_v/F_m)\ n_{PSII}\ 0.25\ /\ PQ$$

$$\mathbf{PP =} E\ \sigma_{PSII}\ (\Delta F'/F_m')/(F_v/F_m)\ n_{PSII}\ 0.25\ /\ PQ\ \text{[Chl-a]}$$

What are the key photophysiological parameters that determine PP rates?

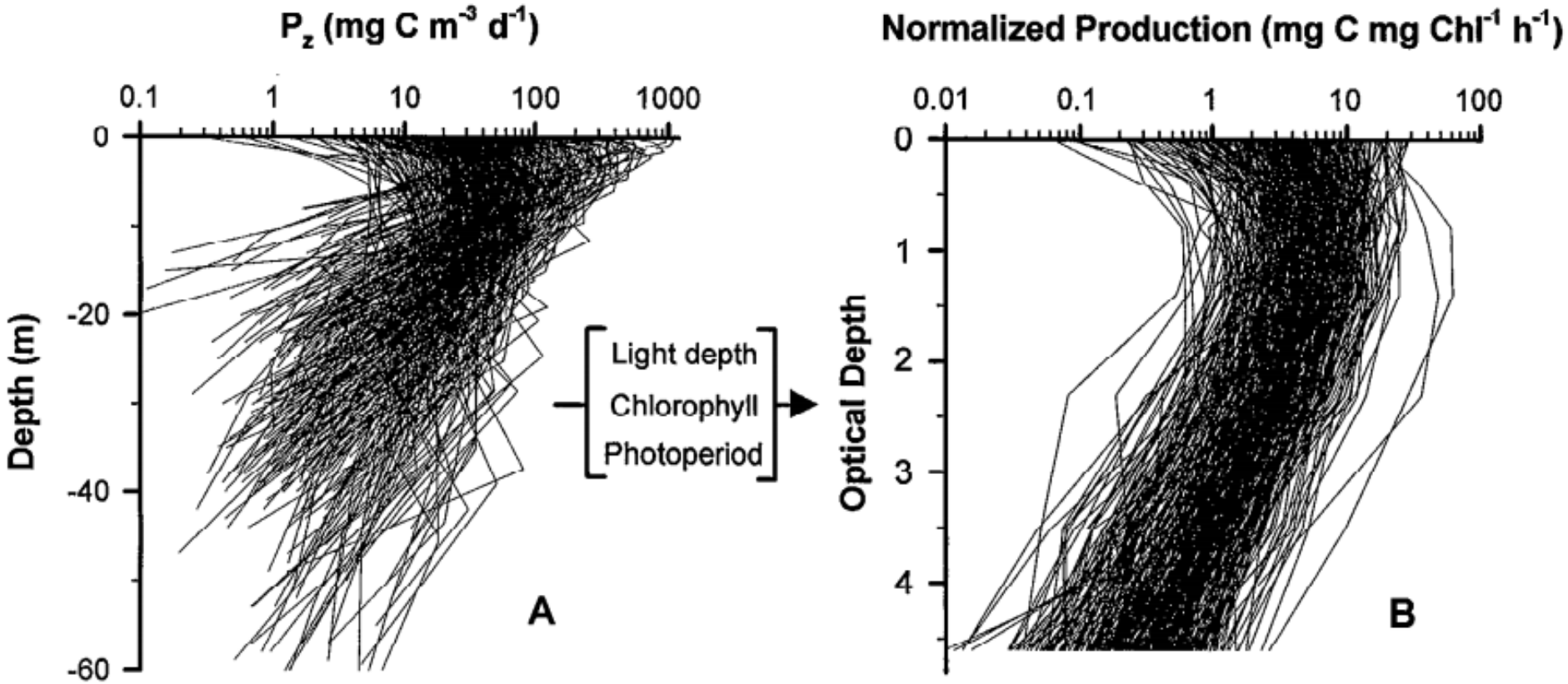


Low PAR: *Fv/Fm &* σ_{PSII} (i.e. light absorption and the quantum yield of photochemistry)

High PAR (> Ek): Maximum photosynthetic turnover rate

($1/\tau = E_k \ \sigma_{PSII}$)

Maximum turnover rate ($1/\tau$) also determines P_b^{opt}, the key parameter in remote sensing models for the water-column integrated NPP



Behrenfeld and Falkowski 1997

Maximum turnover rate ($1/\tau$) is fundamentally important to measure/model PP rates, especially the water-column integrated rates

- $1/\tau$ **cannot be measured directly using FRR technique,**
- **but it can be retrieved (instantaneously) using a new kinetic-based protocol in mini-FIRe instruments, which offers a significant improvement in Fv-based estimates of PP and growth rates.**

Limitations of Amplitude-Based Fv Technique(s)

- **Photosynthetic rates are not measured directly from Fv signals; the rates are modeled.**
- **Many parameters in the model => many sources of errors …**

$$P_{Chl}(CO_2) = E\ \sigma_{PSII}\ (\Delta F'/F_m')/(F_v/F_m)\ n_{PSII}\ 0.25 / PQ$$

$$PP = E\ \sigma_{PSII}\ (\Delta F'/F_m')/(F_v/F_m)\ n_{PSII}\ 0.25 / PQ\ [\text{Chl-a}]$$

Limitations of Amplitude-Based Fv Technique(s)

- There are different models to convert Fv signals to ETR.
- Different models produce (slightly) different numbers.

Problems and Challenges in Measuring Photosynthetic Rates

Questions:

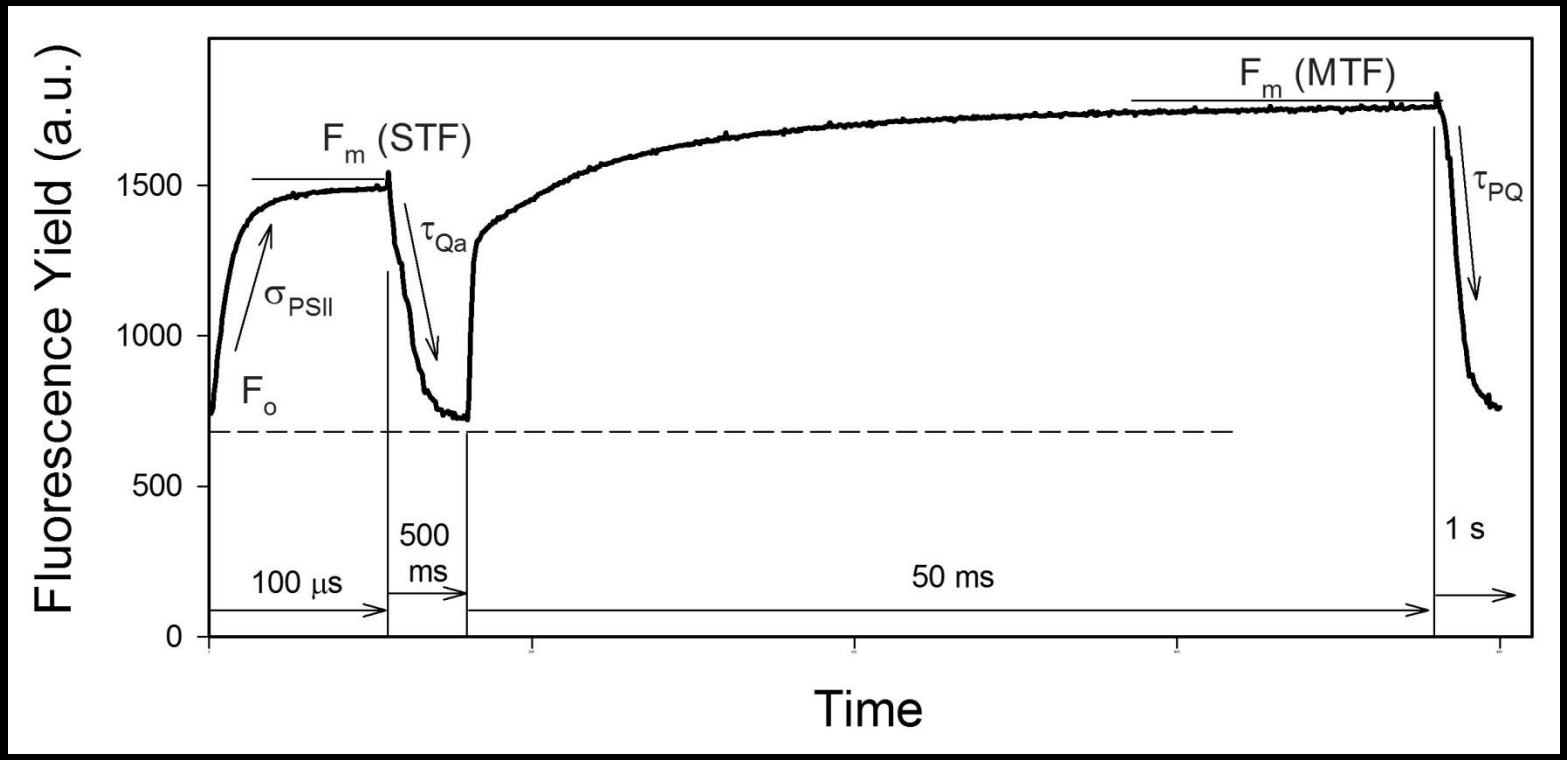
Which model is better?

Is there a better (e.g., direct) approach to measure photosynthetic rates and $1/\tau$?

Potential Solution (and Hypotheses):

- The use of **kinetic analysis** which is the most accurate way to measure the **rates** of chemical reactions.
- Develop a new approach to measure absolute photosynthetic rates directly from **time-resolved kinetic analysis of photosynthetic electron flow**
- Kinetic analysis of fluorescence relaxation on microsecond scale provides ETRs, including photosynthetic turnover rates more accurately and in absolute units

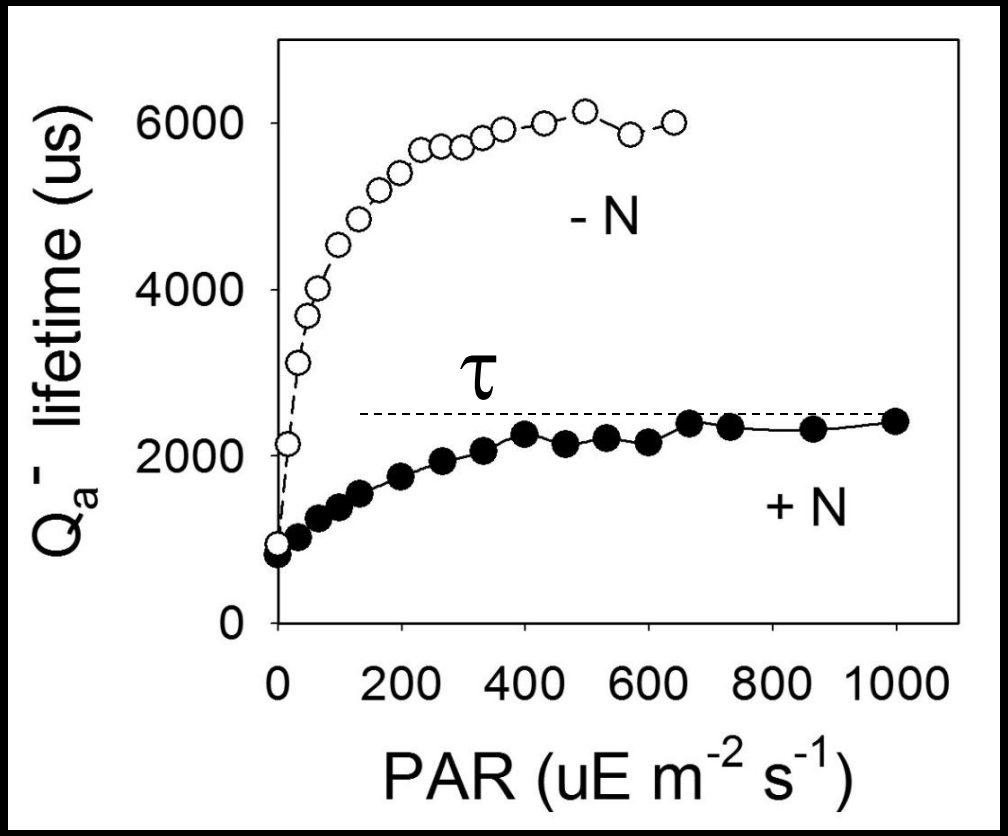
Relaxation Analysis in FRR/FIRe protocol

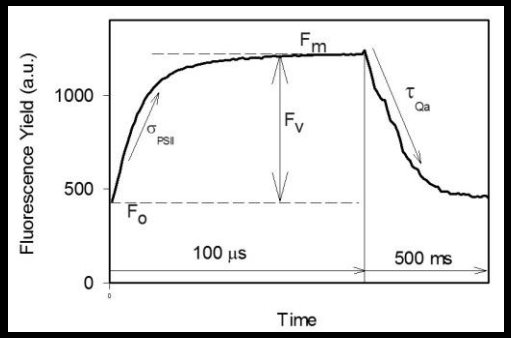


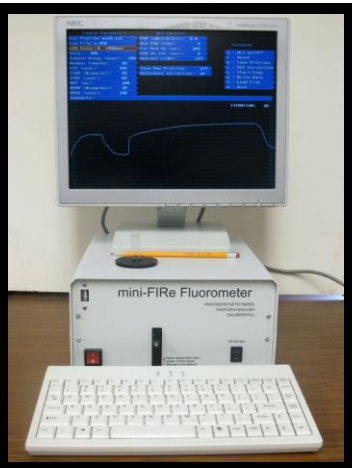
The FRR Puzzle:

- **Photosynthetic Turnover time is ~ 2 ms (from classical P-E curves)**.
- The time of Qa re-oxidation after STF is ca. 500 µs (two short).
- The time of PQ re-oxidation after MTF is ca. 5 – 10 ms (two long)?
- Can the relaxation kinetics provide actual photosynthetic rates (e.g., turnover rates)?

How do Qa re-oxidation rates vary with PAR?





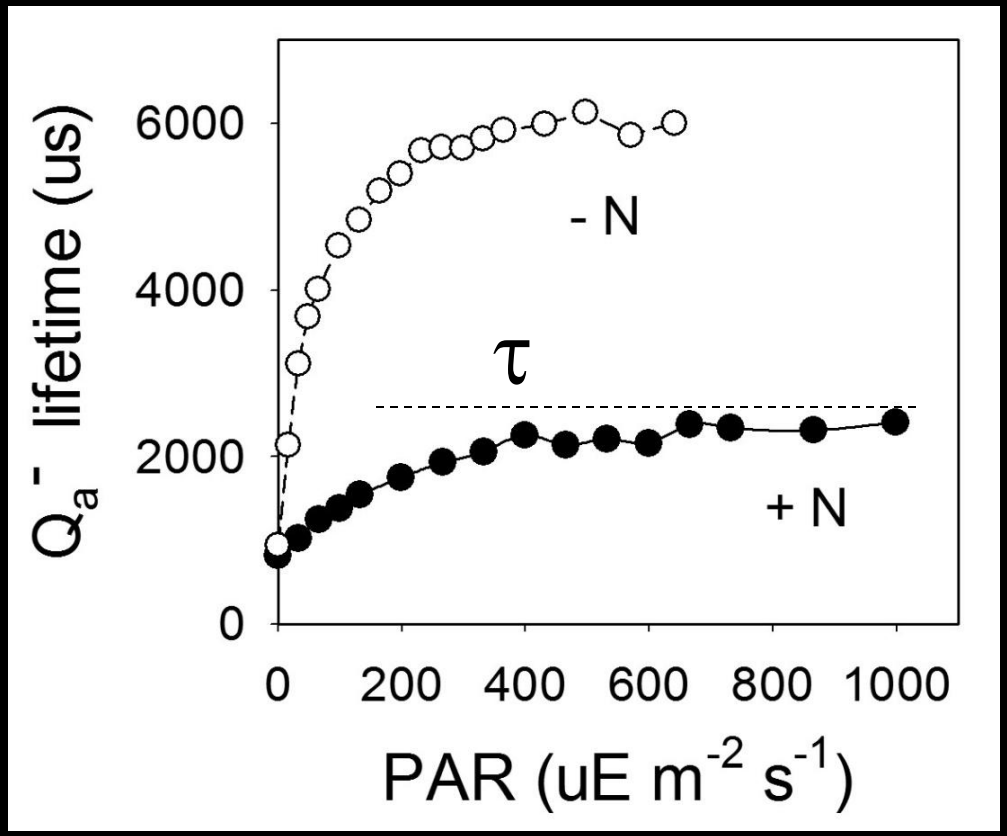


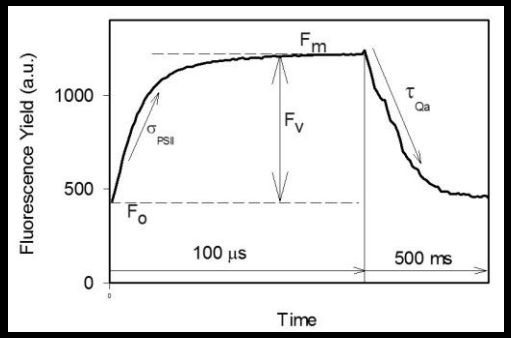
Kinetic mini-FIRe Fluorometer

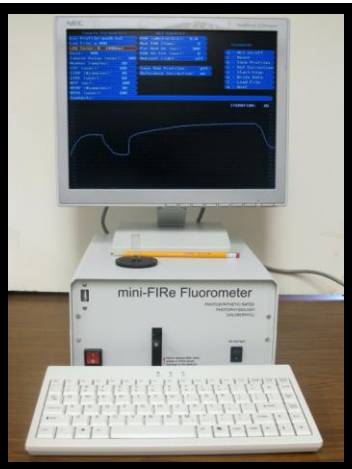
Result:

- **Under saturating PAR, the rates of Qa re-oxidation (retrieved with a new mini-FIRe protocol) are equal to photosynthetic turnover rates.**
- **These rates are sensitive to N stress (but not Fe), which provides a quantitative proxy of the N stress .**

Analysis of fluorescence relaxation kinetics measured under high ambient light provides photosynthetic turnover rates

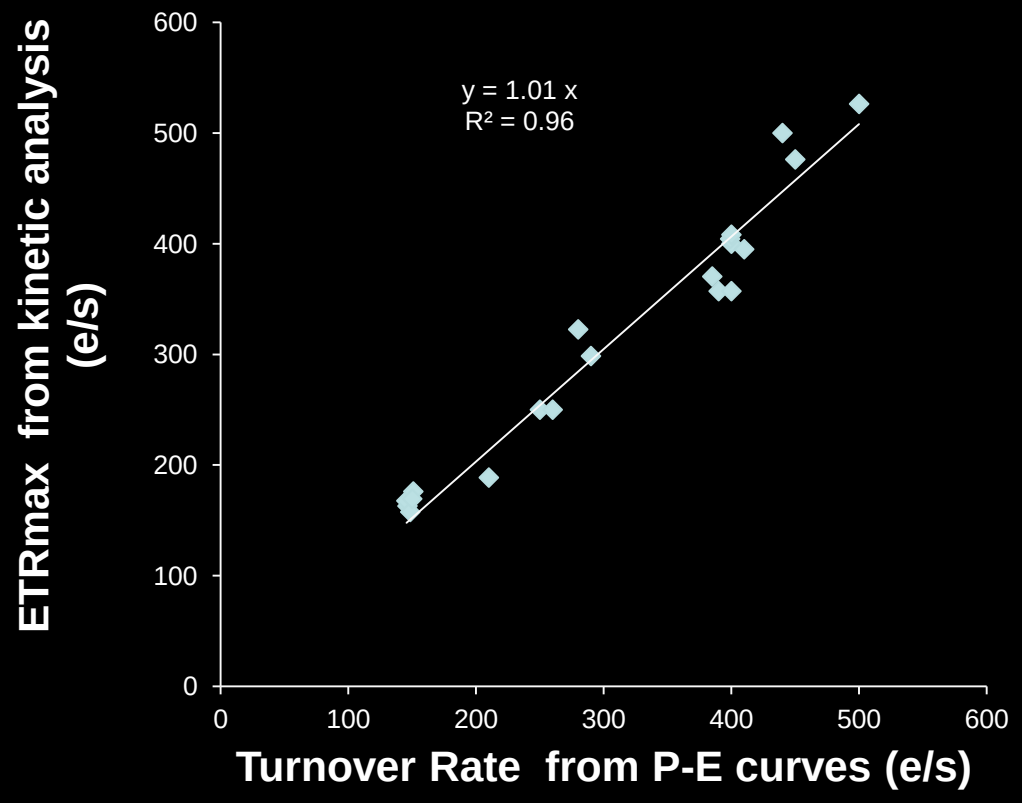






Kinetic mini-FIRe Fluorometer

Amplitude-based versus Kinetic Analyses

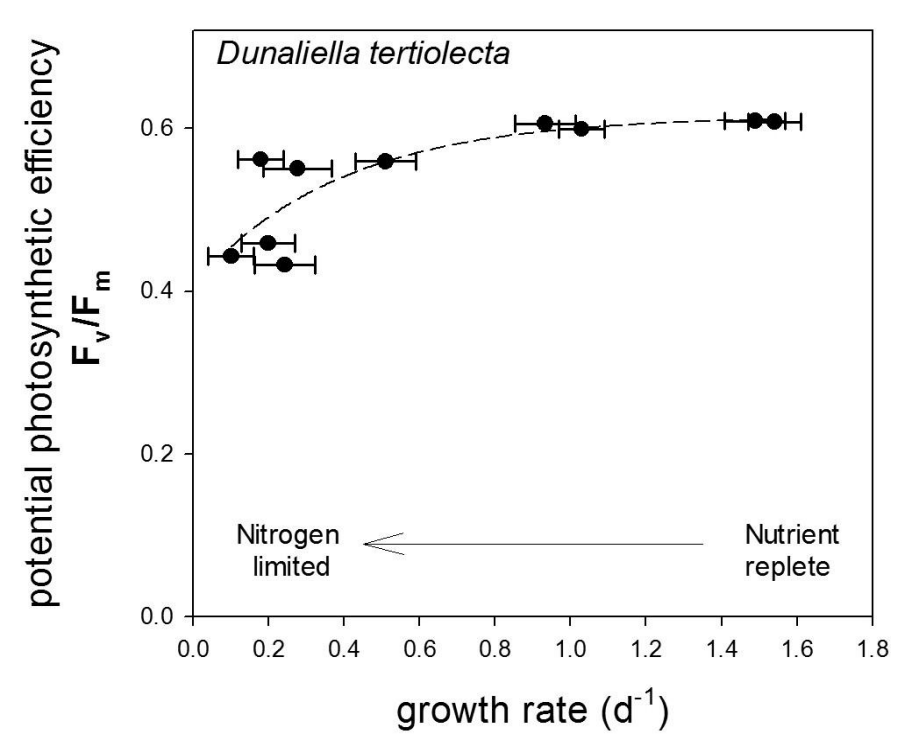


Diatoms and green algae;
Nutrient-replete cultures;
HL versus LL acclimated

- **Under N-replete conditions, ETRmax from kinetic analysis are exactly the same as photosynthetic turnover rates**
- **=> Electron transport is the rate limiting reaction is algae**

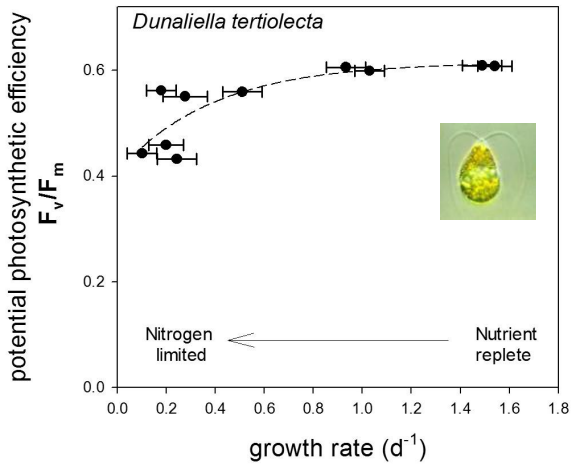
Limitations of Fv/Fm as an indicator of N stress in the ocean

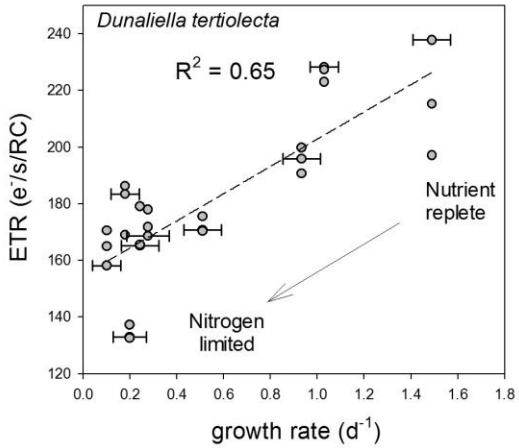
- Nitrogen is the main limiting nutrient in the ocean on the global scale
- Fv/Fm is a classical indicator of nutrient stress (and other stressors)
- But the relationship between Fv/Fm and growth rates is highly non-linear.
- Is there a better diagnostics of N stress? Does the kinetic analysis help?



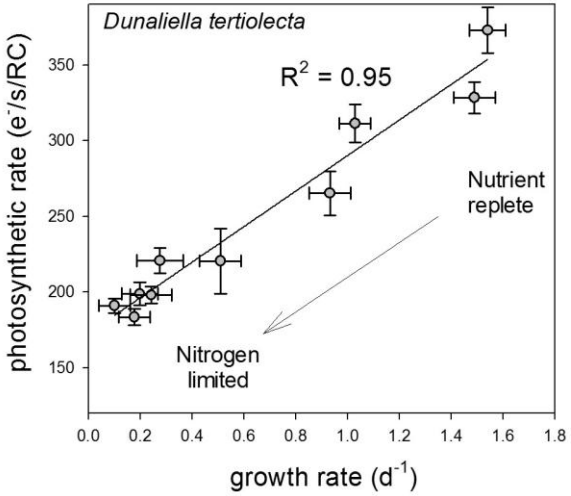
Revaluating the Relationship between Phytoplankton Photophysiology and Growth Rates: Searching for a better proxy of N stress

Amplitude-based Approach (FRR or FIRe)





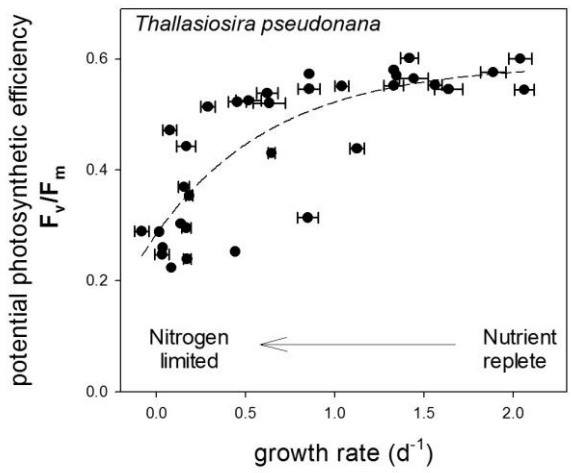
New Kinetic-based Approach

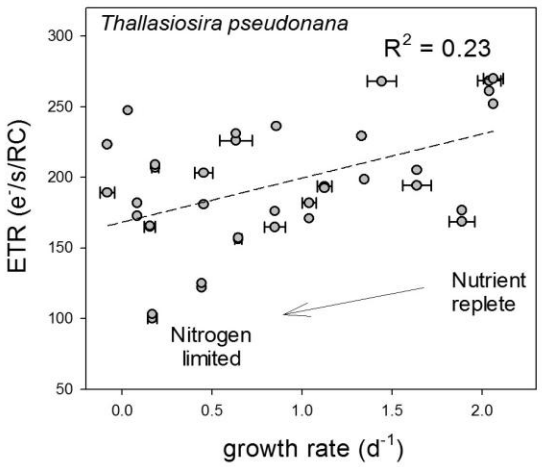


- **Kinetic analysis offers a good quantitative proxy for <u>growth rates</u>.**
- **Reduction in growth rates under N stress is primarily driven by the reduction in photosynthetic turnover rates**

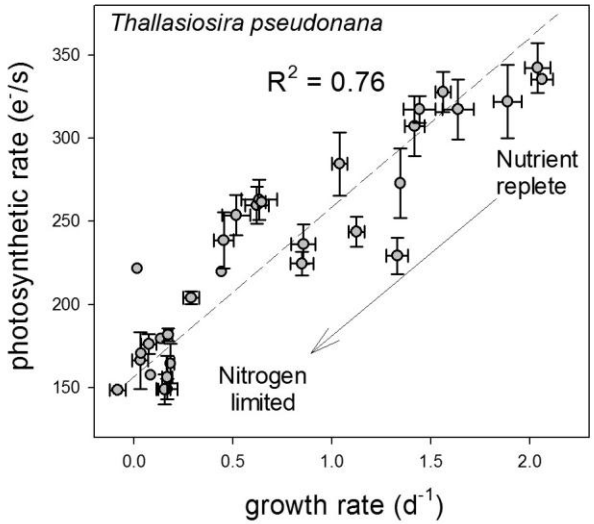
Revaluating the Relationship between Phytoplankton Photophysiology and Growth Rates: Searching for a better proxy of N stress

Amplitude-based Approach (FRR or FIRe)





New Kinetic-based Approach



Can we (better) quantify and distinguish between Fe and N limitation in the ocean using kinetic analysis?

	Fe stress	N stress
Fv/Fm	Strong decline	Little decline
Qa reoxidation in dark (low light)	Strong decline	No effect
Maximum turnover rates	No (or little) effect	Strong decline

- Based on lab and field data on <u>moderate</u> extent of N and Fe stress.

Conclusions

- The combination of amplitude-based and kinetic analyses in the new mini-FIRe instruments offers a significant improvement in the capabilities of Fv for assessment of absolute photosynthetic rates, growth rates, NPP, in relation to N and Fe stress in the ocean.
- New fluorescence kinetic protocols, implemented in mini-FIRe instruments, allow us to measure absolute ETRs and turnover rates more accurate.
- These kinetic measurements offer a better and more specific and diagnostics of N and Fe limitation in the ocean.
- Measurements of instantaneous growth rates help overcome the uncertainties of 14C technique for NPP.
- Incorporation of (more robust) proxies of N stress is important.

- Think beyond Fv/Fm

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SCIENCES
Environmental
Biophysics
& Molecular
Ecology Program

NASA

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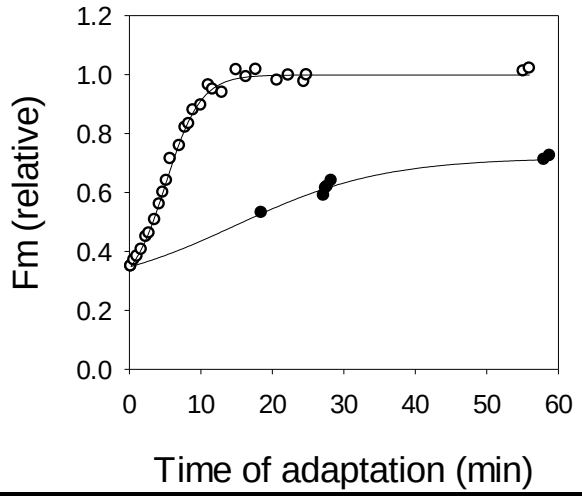
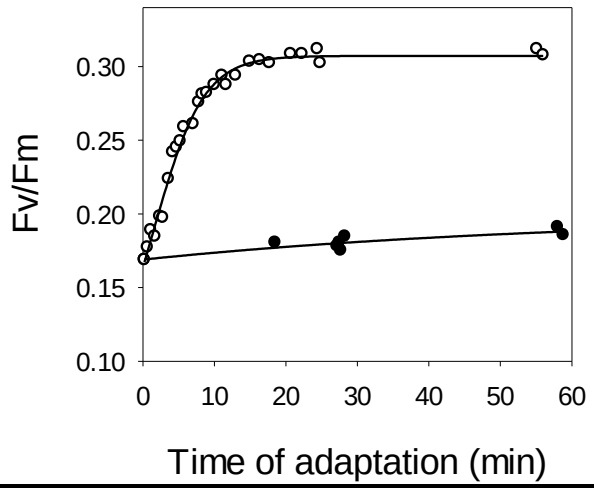
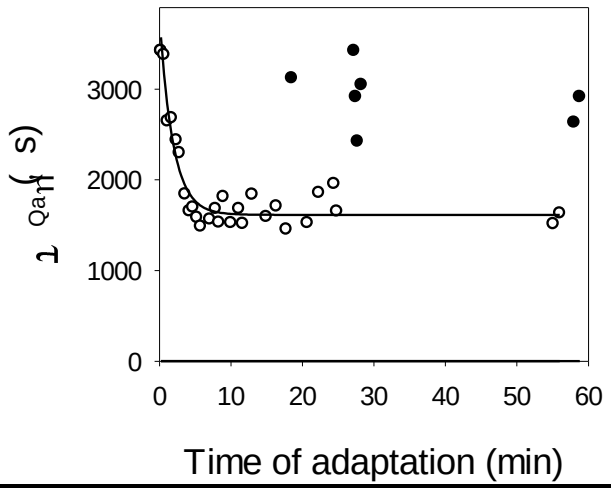
Backup slides

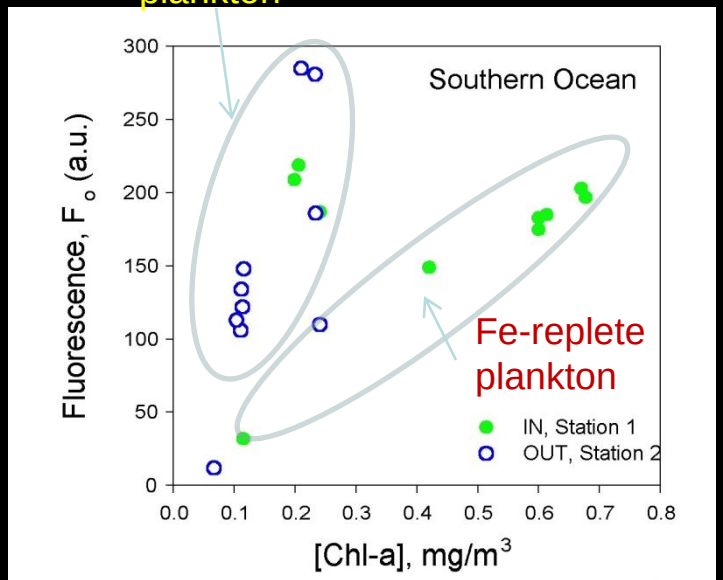
Importance of low-light (not dark !) acclimation

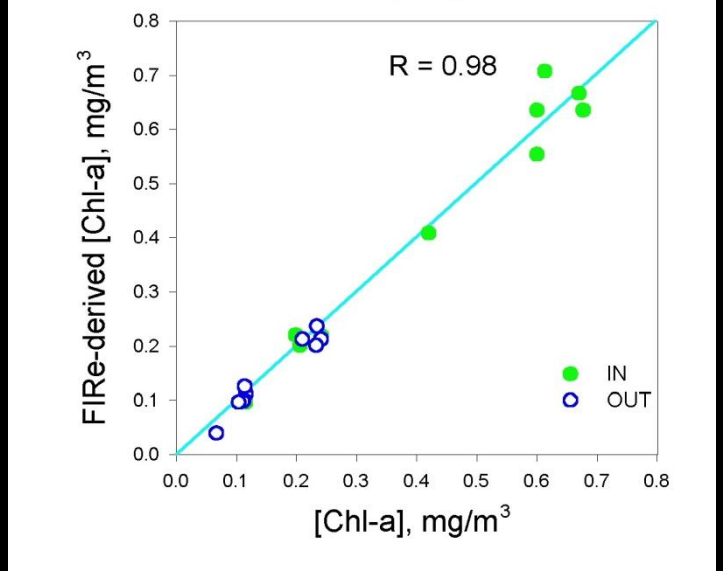






FIRe photosynthetic parameters improves conversion of Chl-a fluorescence to Chl-a concentration





- Chlorophyll fluorescence is a common tool for assessment of phytoplankton biomass, but <u>fluorescence yield is a function of physiology</u>.

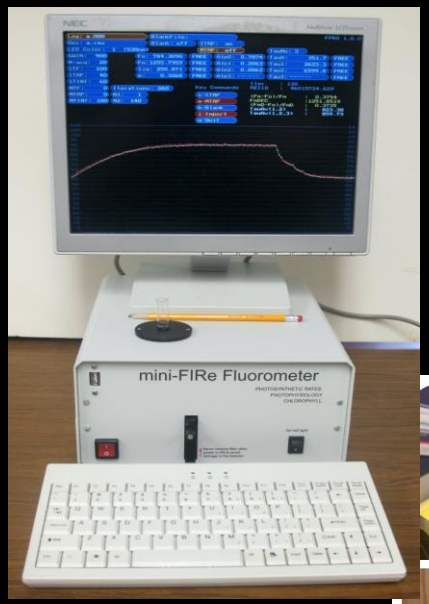
- F_o is the fluorescence yield measured by most of conventional fluorometers. F_o per unit Chl-a varies by a factor of ~3-4, depending on the functional state of the photosynthetic apparatus.

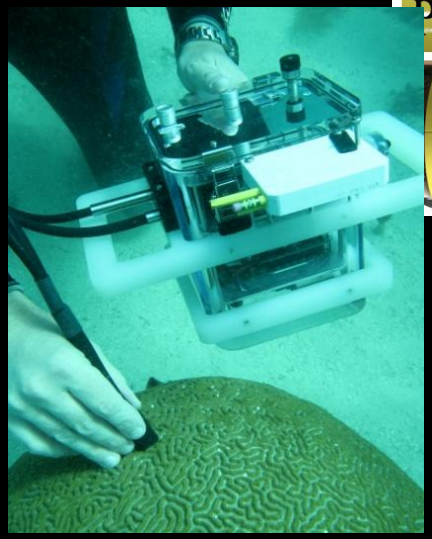
- Incorporation of photosynthetic parameters into the model increases dramatically the precision of fluorescence-based estimates of Chl-a concentration.

$$[\text{Chl-a}]_{FIRe} = C(F_v/F_m, \sigma_{PSII}) * F_o$$

C - fluorescence per unit Chl-a calculated from photosynthetic characteristics.

New Series of mini-FIRe Instruments





Lab Mini-FIRe

Lab mini-FIRe is a basic, low-cost and sensitive system.

Mini-FIRe UHS new*

Mini-FIRe UHS is an ultra-high sensitivity version (20X improvement over its predecessors).

Multi-Color mini-FIRe new*

Ultra-high-sensitive system with six excitation wavelengths is designed for dedicated oceanographic research and allows the user to monitor in real-time and automatically both photophysiological characteristics and changes in the taxonomic composition of phytoplankton communities.

Diving mini-FIRe

Diver-operated or moored instrument for underwater research on coral and other benthic organisms.

What is the photosynthetic efficiency of phytoplankton in the ocean?

Three pathways for energy use and dissipation:

$$\Sigma\Phi = k_p + k_f + k_t = 1$$

k_p is the quantum yield for photochemistry,
k_f – fluorescence,
k_t – thermal dissipation (heat).

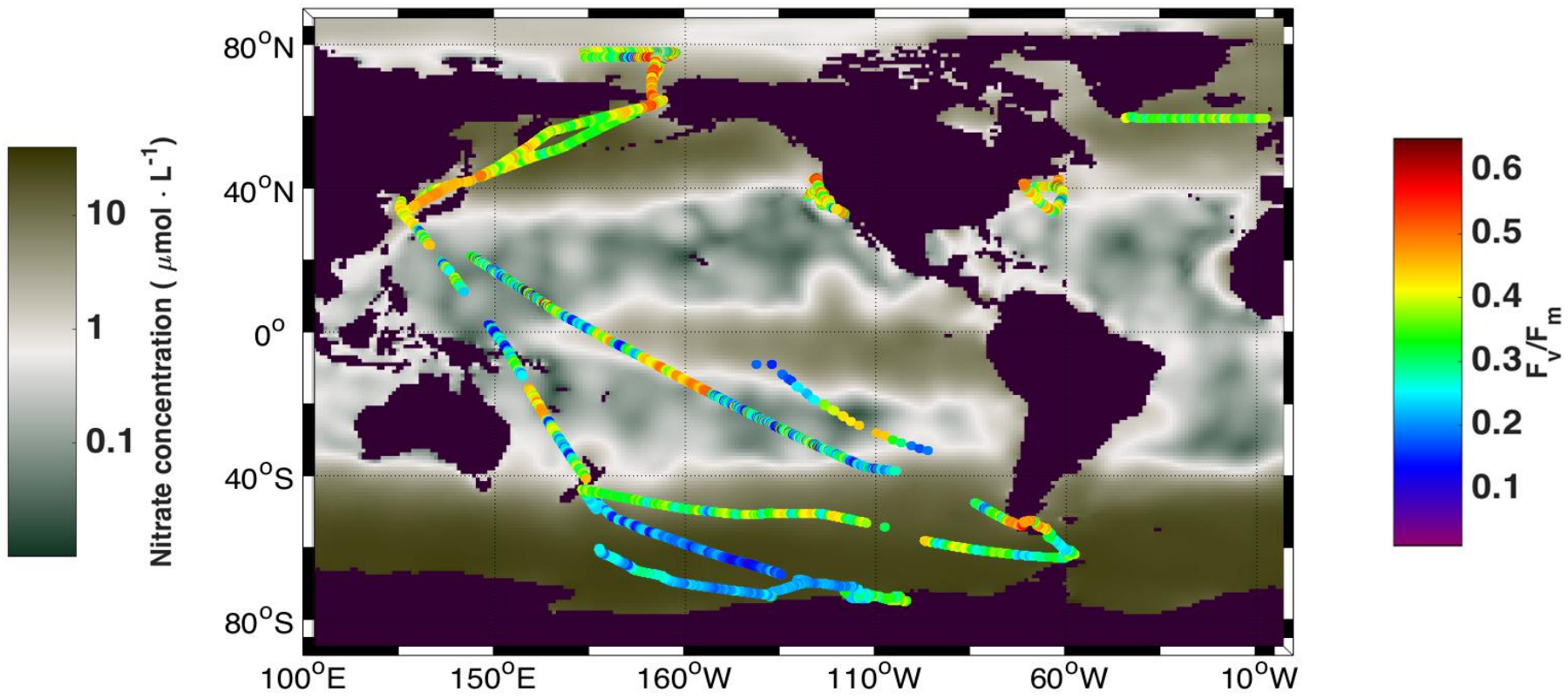
Energy Budget for Absorbed Solar Light:

$$\Sigma\Phi = k_p + k_f + k_t = 1$$

K_p and K_f can be measured *in situ* by chlorophyll fluorescence – but using two different techniques:

- K_p can be measured from changes in fluorescence yields (= F_v/F_m);
- K_f can be deduced from picosecond lifetime measurements.

Distribution of Photosynthetic Efficiency in the Global Ocean



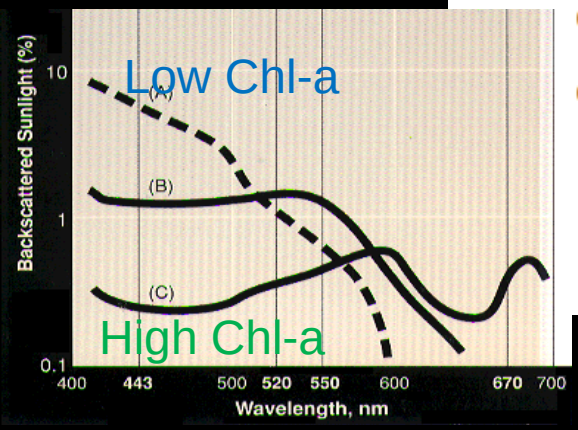
- ~4x range of variability in photosynthetic efficiency in the ocean (20% to 100% of its potential maximum);
- On average, Fv/Fm is only ~ 30% of its potential maximum;
- Nutrient (N and Fe) limitation is the major control.

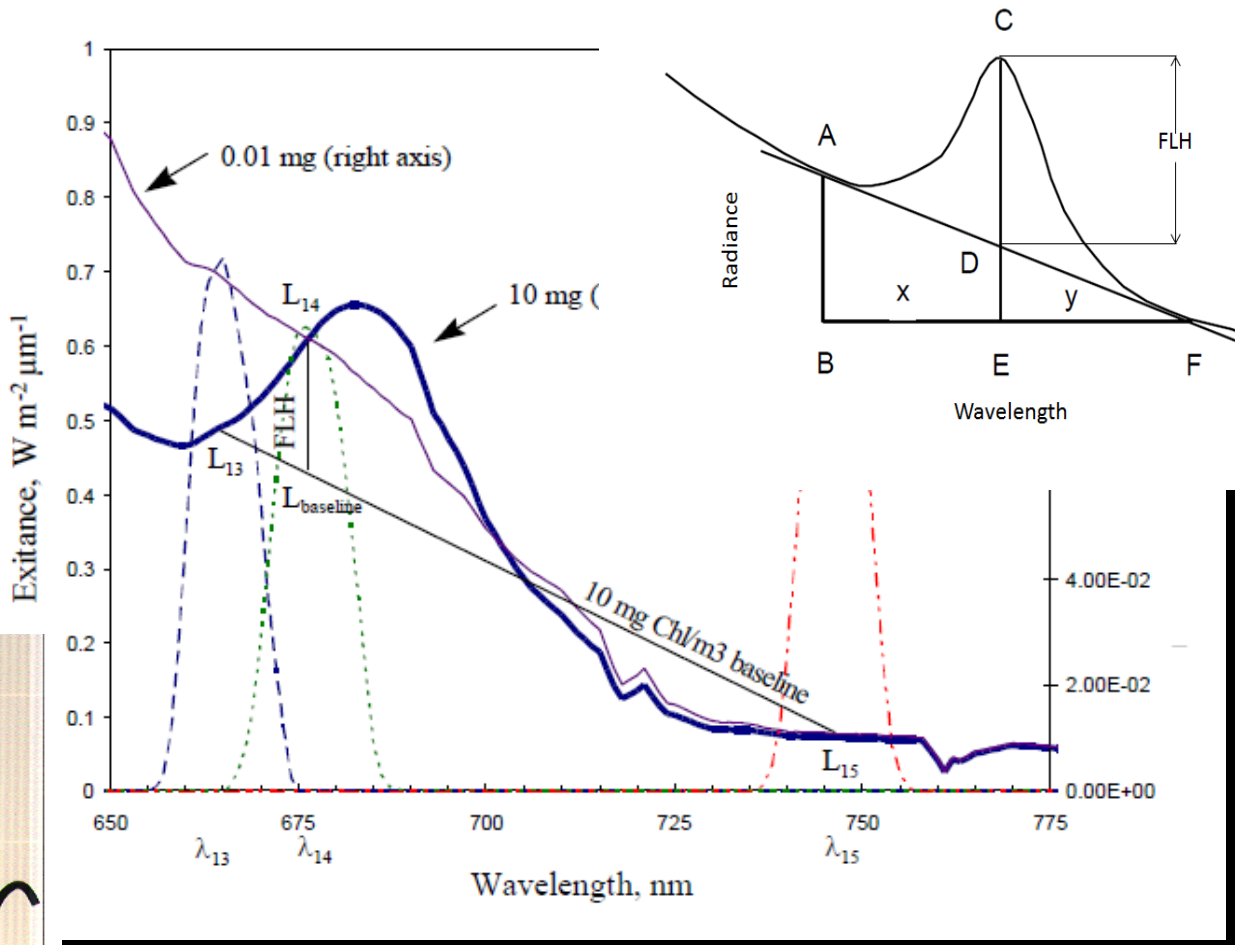
Solar Induced Fluorescence

- F_v cannot be recorded from space.
- An alternative approach to assess phytoplankton physiology is to measure quantum yields of fluorescence.
- The quantum yields of Chl-a fluorescence can be estimated from space-borne sensors (such as MODIS and MERIS)

Solar Induced Fluorescence from Space

- Solar induced fluorescence can be detected from space as a weak peak in water-leaving spectral reflectance from the surface ocean.
- The detector flies on the two MODIS satellites – and has equator crossing times of ~10:00 (MODIS AM) and 14:00 (MODIS PM.





Schematic of the MODIS FLH Algorithm

With dash/dot lines representing the normalized transmittance of MODIS bands 13, 14, and 15, the solid lines show the spectral distribution of upwelling radiance above the surface of the ocean for chlorophyll concentrations of 0.01 and 10 mg/m^3. The fluorescence per unit chlorophyll is assumed to be 0.05 W/m^2/mm/sr per mg chlorophyll. Fig was applied from Fig. 1 of MODIS ATBD20 document.

The quantum yield of Solar Induced Fluorescence

- Defined as a ratio of fluorescence emission (from FLH) to the amount of sun light absorbed by phytoplankton (from the blue-green ratio);
- Fluorescence emission and absorption are the two independent variables retrieved from satellite sensors.

Problem

- Measurements of the **quantum yields** of fluorescence are the key to our understanding of the variability in satellite-based 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,** which are directly related to the quantum yields of fluorescence.

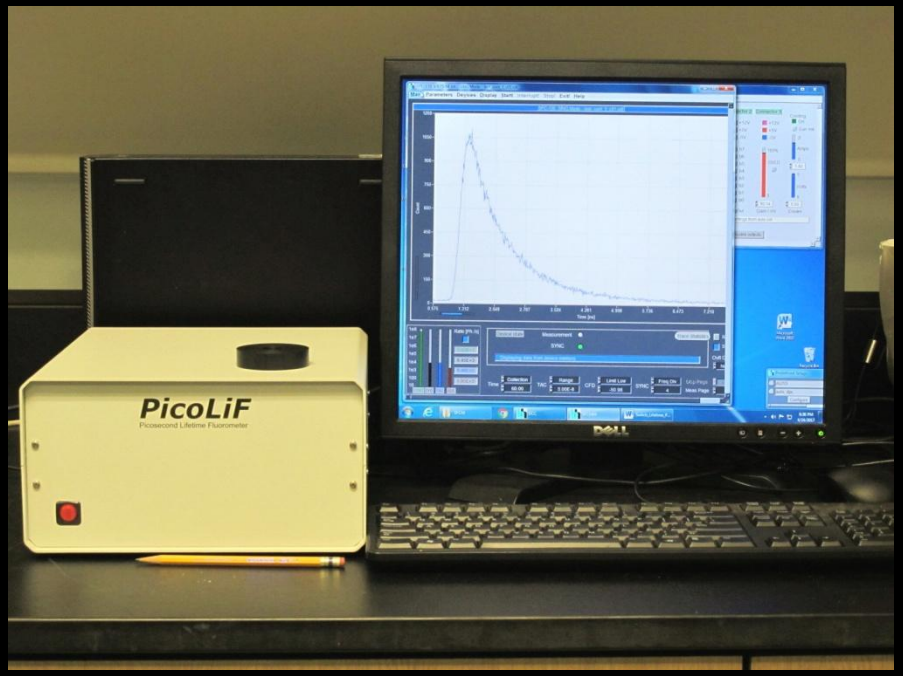
Theory of Fluorescence Lifetime (Why measure lifetimes in the ocean?)

- 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 ($\phi_f = k_f$):**

$$\phi_f = \tau / \tau_0$$

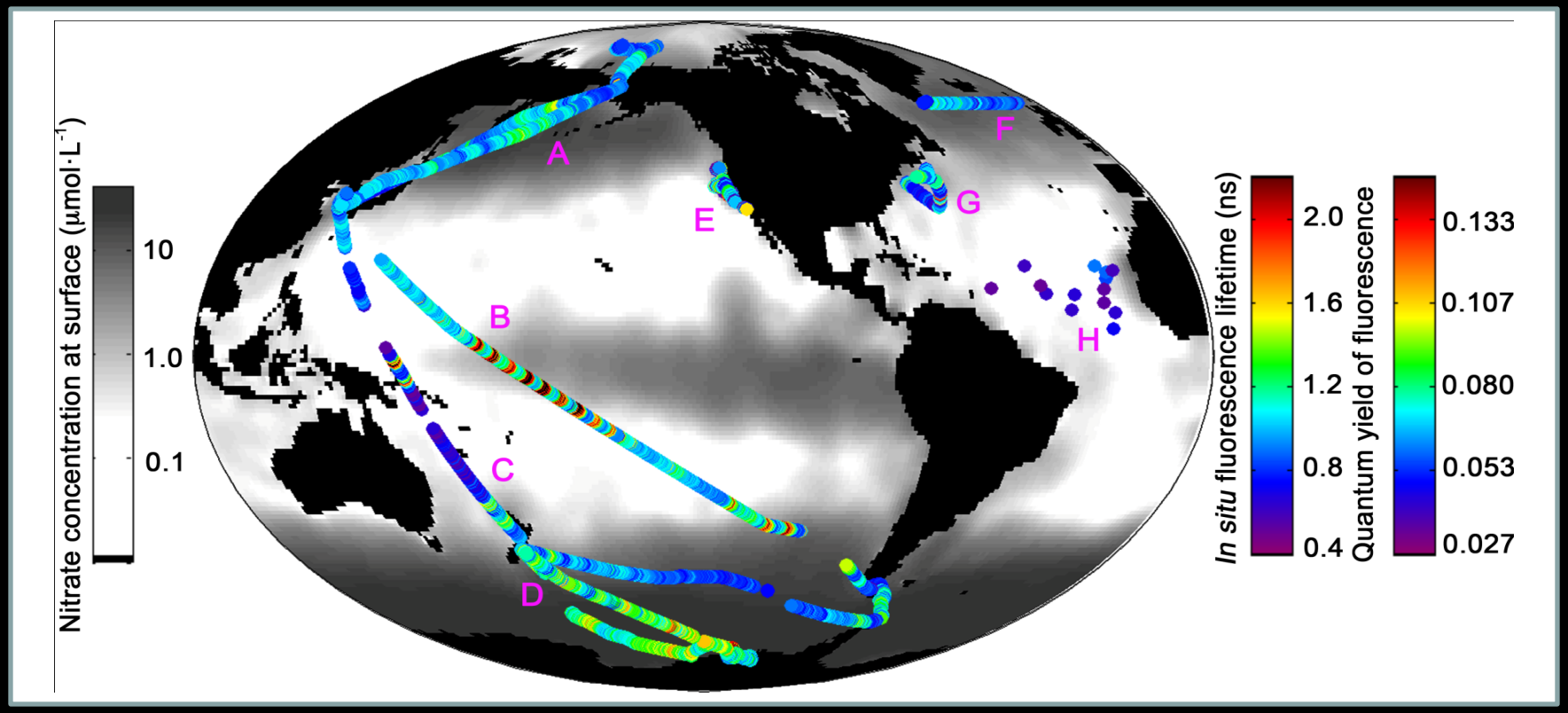
where τ is the observed lifetime of the excited singlet state of the molecule; τ_o is its natural lifetime (15 ns).

Theory of Fluorescence Lifetime (Why measure lifetimes in the ocean?)



- The lifetime of Chl-a fluorescence *in vivo* is a function of phytoplankton physiology
- *In vivo* Chl-a fluorescence lifetimes vary between **0.3 and 2.5 ns**.

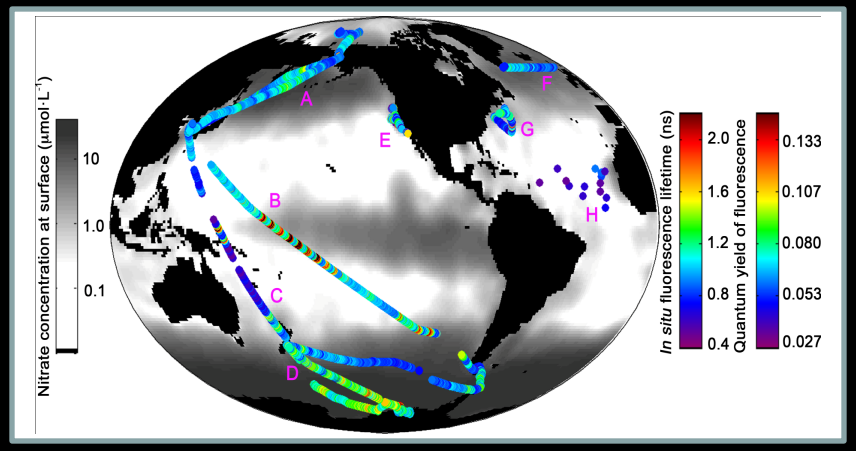
Global distribution of Chl-a fluorescence lifetimes in the ocean

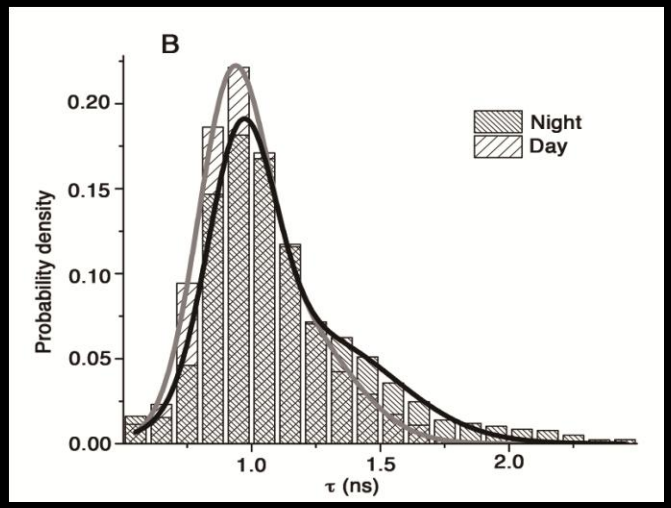


- Over 300,000 measurements of fluorescence yields collected;
- ~ 40,000 miles of transects.

Lin, Kuzminov, Park, Lee, Falkowski and Gorbunov et al. (2016), Science, 351: 264-267.

Controls of fluorescence lifetimes and quantum yields in the ocean





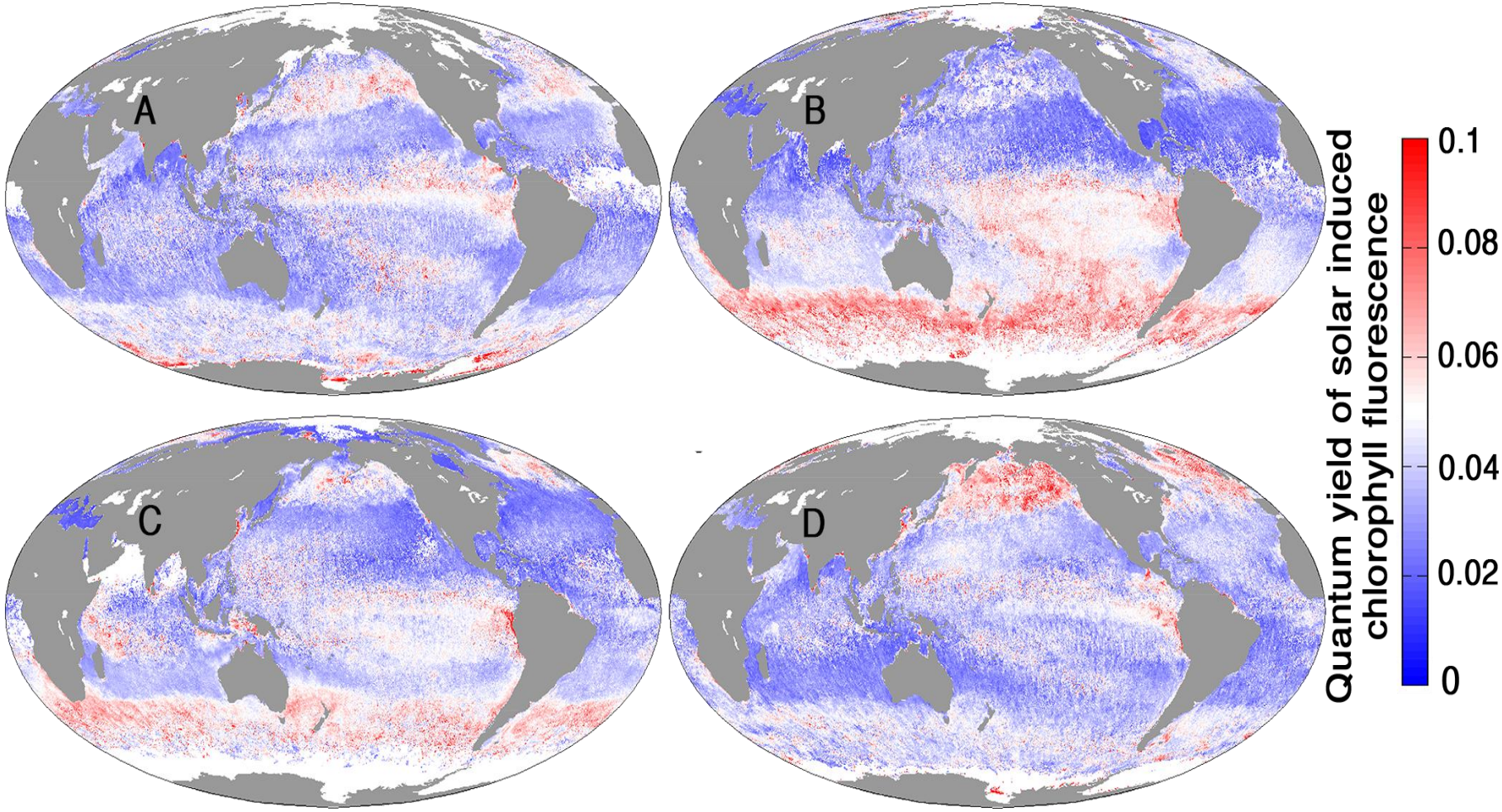
- ~ 5 times variability in fluorescence lifetimes (0.5 to 2.6 ns).
- Longest lifetimes are in Fe-limited regions.
- But NPQ under high light greatly reduces the range of variability in lifetimes.
- Average lifetimes:

  Nighttime values: 1.13 ± 0.33 ns

  Daytime: 1.02 ± 0.22 ns

Lin et al., Science, 2016

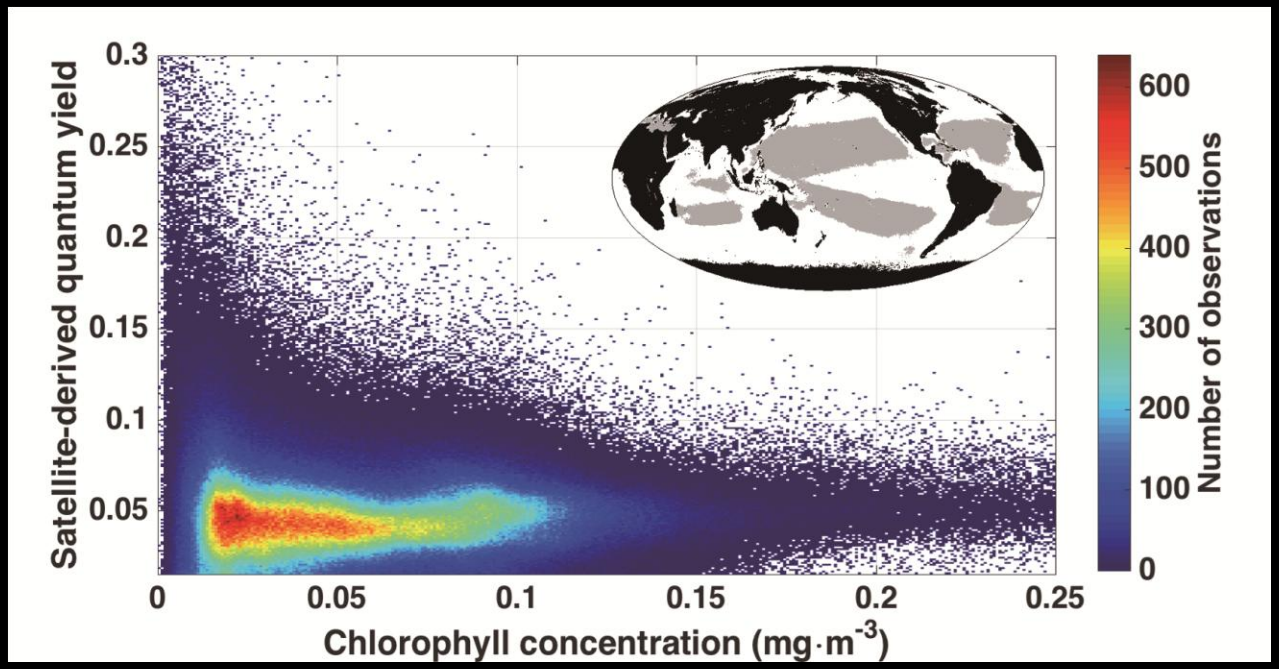
Seasonal variations in Chl-a SIF quantum yields



(A) boreal winter; (B) spring; (C) boreal summer; (D) boreal fall.

Lin et al., Science, 2016

Challenges in MODIS retrievals of Chl-a SIF quantum yields



- SIF signals are extremely weak and noisy in oligotrophic waters.
- SIF yields are largely controlled by NPQ processes rather than photochemistry in PSII
- The capacity of NPQ varies with taxa and nutrient status.

Lin et al., Science, 2016

The Result

- The average quantum efficiency for photochemistry at night (when NPQ is nil) is ~0.35, which is only ~ 50% of its maximum
- The average quantum yield of chlorophyll fluorescence at night is ~0.07
- Thus, solving for the master equation, ~ 60% of the photosynthetically active solar radiation absorbed by phytoplankton in the oceans is converted to heat.

Conclusions

1. Most of the time, phytoplankton in the real world ocean are extremely inefficient in converting absorbed solar radiation to photochemical energy.
2. The limitation is imposed by nutrients (such as N and Fe).
3. The model we have is that phytoplankton “body surf” - they wait for a pulse of nutrients to temporarily approach their maximum photosynthetic energy conversion efficiency.