Measurement and interpretation of chlorophyll fluorescence: a most dangerous game

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with contributions from
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2008 HAWAI‘I SUMMER COURSE ON MICROBIAL OCEANOGRAPHY

Microbial Oceanography: Genomes to Biomes
A laboratory field training course at the University of Hawaii at Mānoa
Chlorophyll Fluorescence Simplified

<table>
<thead>
<tr>
<th>Equation</th>
<th>Equation number</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\frac{dA}{dt} = -\dot{E}\sigma_{PSII}^0 A + \frac{BNADP}{\tau}$</td>
<td>3.41</td>
</tr>
<tr>
<td>$\frac{dB}{dt} = \dot{E}\sigma_{PSII}^0 A - \frac{BNADP}{\tau} + K_{\text{rep}} C - \Psi_d \sigma_{PSII}^0 \dot{E}B$</td>
<td>3.42</td>
</tr>
<tr>
<td>$\frac{dC}{dt} = -K_{\text{rep}} C + \Psi_d \sigma_{PSII}^0 \dot{E}B$</td>
<td>3.43</td>
</tr>
<tr>
<td>$\frac{dk_{qE}}{dt} = \begin{cases} k_{\text{ind}} (k_{qE}^\text{st} - k_{qE}) &amp; k_{qE}^\text{st} &gt; k_{qE} \ k_{\text{rel}} (k_{qE}^\text{st} - k_{qE}) &amp; k_{qE}^\text{st} &lt; k_{qE} \end{cases}$</td>
<td>3.28 and 3.29</td>
</tr>
<tr>
<td>$\frac{dNADP}{dt} = -\frac{B}{\tau} \text{NADP} + K_{\text{Calvin}} + k_{\text{sinks}}$</td>
<td>3.8</td>
</tr>
<tr>
<td>$k_{qE}^\text{st} = \gamma_d \dot{E} \left( 1 - \left[ A + C \left( \frac{n_{\text{Calvin}} k_{\text{Calvin}}^{\text{max}} - P_e^{\text{RC}}}{n_{\text{Calvin}} k_{\text{Calvin}}^{\text{max}}} \right) \right] \right)$ and $\gamma_d = \gamma_o + \gamma_{\text{NPQ}}$</td>
<td>3.27</td>
</tr>
<tr>
<td>$\sigma_{PSII}^1 = \sigma_{PSII}^0 \varphi_{pA} = \sigma_{PSII}^0 \frac{k_p}{k_d + k_f + k_p + k_{qE}}$</td>
<td>3.32</td>
</tr>
<tr>
<td>$K_{\text{Calvin}} = n_{\text{Calvin}} \frac{k_{\text{Calvin}}^{\text{max}} \text{NADPH}}{\text{NADPH}_{1/2} + \text{NADPH}}$</td>
<td>3.9</td>
</tr>
<tr>
<td>$K_{\text{rep}}^N = \frac{k_{\text{rep}} C}{C_{1/2} + C} N_{\text{status}}$</td>
<td>3.33 and 3.34</td>
</tr>
<tr>
<td>$P_e^{\text{RC}} = A \varphi_{pA} \sigma_{PSII}^0 \dot{E} = A \sigma_{PSII}^1 \dot{E}$</td>
<td>3.36</td>
</tr>
<tr>
<td>$\varphi_f = A \frac{k_f}{k_d + k_f + k_p + k_{qE}} + B \frac{k_f}{k_d + k_f + k_{qE}} + C \frac{k_f}{k_d + k_f + k_f + k_{qE}}$</td>
<td>3.12</td>
</tr>
<tr>
<td><strong>Acclimation</strong></td>
<td></td>
</tr>
<tr>
<td>$\frac{d\sigma_{PSII}^0}{dt} = \begin{cases} \kappa_{\sigma_{PSII}} \sigma_{PSII}^0 \left( \frac{A}{A + B} \left( \sigma_{PSII}^0 / \sigma_{PSII}^{\text{opt}} \right)^x - 0.3 \right) &amp; \dot{E} &gt; 0 \ 0 &amp; \dot{E} = 0 \end{cases}$</td>
<td>3.44</td>
</tr>
</tbody>
</table>
only kidding...
A Biological Property

Sensitive to

• Physiology
• Acclimation
• Adaptation
Figure 4.13 Chlorophyll a measured using stimulated fluorometers at 10, 35, 65, and 80m at the central mooring.
Fluorescence is measured to detect and quantify phytoplankton.

Bozone 1993: Wedell-Scotia Sea

Vertical Profiles
Specifically, fluorescence is measured to estimate chlorophyll.

All phytoplankton have chlorophyll $a^*$.

*Prochlorococcus* has divinyl Chl.
...and chlorophyll is used as a measure of biomass

However, the relationship between fluorescence and chlorophyll is variable

Fig. 1. Frequency polygon for all 250 measurements of fluorescence number made in coastal waters, Lake Tahoe, and Central North Pacific Gyre

Kiefer, Marine Biology, 1973a
As is the relationship between chlorophyll and biomass.

Variability of fluorescence can be related to environmental conditions, species and physiological condition.

Information on physiological status is perhaps the ultimate reward

Fluorescence can yield information on species composition.
Flow Cytometry

- Identification
- Physiological properties

www.bigelow.org/cytometry/Examples.html#GB
Active Fluorescence

Behrenfeld and Kolber 1999, Science

- FRRF
- PAM
- FlRe
- Benchtop
- Submersible
- Physiology
- Controversies
- Technical issues
...and the rate of photosynthesis

\[ f(t) = F_o + (F_m - F_o) \left( C(t) \frac{1-p}{1-C(t)p} \right) \]

\[ \frac{\partial C(t)}{\partial t} = \sigma_{PS II} \frac{1-C(t)}{1-C(t)p}. \]

\[ \frac{dC(t)}{dt} = \sigma_{PS II} \frac{dI}{dt} \frac{1-C(t)}{1-C(t)p} = \sigma_{PS II} i(t) \frac{1-C(t)}{1-C(t)p} \]

\[ C(t) = \int_0^t \sigma_{PS II} i(\nu) \frac{1-C(\nu)}{1-C(\nu)p} d\nu, \]

\[ C(t) = \int_0^t \sigma_{PS II} i(\nu) \frac{1-C(\nu)}{1-C(\nu)p} g(t-\nu) d\nu \]

\[ g(t-\nu) = g(\Delta t) = \alpha_1 \exp(-\Delta t/\tau_1) \]
\[ + \alpha_2 \exp(-\Delta t/\tau_2) + \alpha_3 \exp(-\Delta t/\tau_3). \]

\[ f_n = F_o + (F_m - F_o) C_n \frac{1-p}{1-C_n p} \]

\[ C_n - C_{n-1} \sum_{k=1}^m A_{n,k} + I_n \sigma_{PS II} \frac{1}{1-p} \left( \frac{C_{n-1} \sum_{k=1}^m A_{n,k}}{1-C_{n-1} \sum_{k=1}^m A_{n,k}} \right) \]

\[ A_{n,k} = (A_{n-1,k} + C_{n-1} \alpha_k / \sigma_{PS II}) \exp(-\Delta t/\tau_k). \]

\[ C(t) = \sigma_{PS II} \int_0^t i(\nu) \frac{F_m - f(\nu)}{F_m - F_o} d\nu = \sigma_{PS II} \int_0^t [i(\nu)q_p(\nu)] d\nu. \]

\[ \sigma_{PS II} = \left[ \int_0^\infty [i(\nu)q_p(\nu)] d\nu \right]^{-1} \]

Sun Induced Chlorophyll Fluorescence:
The only signal emitted from the ocean and detectable from space that can be unambiguously ascribed to phytoplankton.
The big goal:
Interpreting natural variability of $\phi_f$ as detected from space
What does an oceanographer need to know?
What does an oceanographer need to know?

• Principles of measurement
What does an oceanographer need to know?

- Principles of measurement
- Physiological processes
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• Principles of measurement
• Physiological processes
• Environmental influences
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• Principles of measurement
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• Taxonomic variability
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…and interactions among all of these
Measurement of Fluorescence

[Image of a vintage Fluorometer with calibration labels on it]
History: 1966

In the beginning...


A method for the continuous measurement of *in vivo* chlorophyll concentration*

CARL J. LORENZEN†

(Received 7 December 1965)

Abstract—*In vivo* chlorophyll, like many other organic molecules, possesses the ability to fluoresce. This fluorescence was measured continuously with a modified model III Turner fluorometer at sea. Reliable readings were obtained over the range of 0.04–2.0 mg chlorophyll a m⁻³ while on a 21-day cruise off the coast of Baja California. Since the relationship between fluorescence and chlorophyll was linear on all scales, it should be possible to continuously monitor chlorophyll from 0.04 to between 10 and 15 mg m⁻³, a range adequate for all open ocean studies.
Benchtop Fluorometer

- Blue Excitation
- Red emission
- Discrete samples
- Flow-through
- Low excitation
- High sensitivity

Turner, Turner Designs (brown), Turner Designs (black)
(watch out for lamp changes)
Pandora’s Box
Lorenzen covered the bases

Calibration, linearity, possible interference

Lorenzen 1966
Early application: Transects of Chlorophyll

Fig. 3. A portion of the trace obtained on cruise TO-65-1 showing variations in chlorophyll $a$ concentrations and temperature as the ship proceeded from 24° 55′ N–115° 4′ W to 26° 0′ N 112° 45′ W.
Continuous vertical profiles

*Strickland: Ecology of the Plankton off La Jolla, California*

**Chl a (mg m⁻³)**

**Depth (m)**

**Fluorescence**

*Carl Lorenzen*

**Nutrients, too!**

*John Strickland and the Food Chain Research Group 1967 Red Tide Study*
Lorenzen described the measurement of blanks.

THE BLANK CAN MAKE A BIG DIFFERENCE IN OCEANOGRAPHIC MEASUREMENTS

John J. Cullen and Richard F. Davis, Department of Oceanography, Dalhousie University, Halifax, NS B3H 4J1 Canada;
john.cullen@dal.ca, richard.davis@dal.ca
**In situ Fluorometer**

- Profilers
- Moorings
- Pulsed
- High sensitivity
- Ambient irradiance influences fluorescence yield

Frequency, intensity and spectral quality of excitation varies with manufacturer.
Now an indispensable tool

Six vertical profiles of chlorophyll fluorescence (mg m$^{-3}$) and sigma-t (kg m$^{-3}$) from a 13 hr time series of 90 profiles.

Tim Cowles, Oregon State University
...but what do fluorometers measure?
chlorophyll fluorescence, not chlorophyll
Physiological effects on fluorescence yield (Fl/Chl) were recognized early


Natural variability of fluorescence yield was quantified and tentatively interpreted: effects of nutrition and irradiance.

Fig. 1. Frequency polygon for all 250 measurements of fluorescence number made in coastal waters, Lake Tahoe, and Central North Pacific Gyre.


Variability in the quantum yield of fluorescence:

\[ \varphi_f (\text{mols photons emitted per mol photons absorbed}) \]

can be expressed in terms of rate constants \( (k, \text{s}^{-1}) \) for the three possible fates of absorbed photons:

**FLUORESCENCE, PHOTOSYNTHESIS, HEAT**

\[ \varphi_f = \frac{k_f}{k_f + k_p + k_H} \]
Variability in the quantum yield of fluorescence:

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From Yannick Huot
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Fluorescence (Constant)

\[ \varphi_f = \frac{k_f}{k_f + k_p + k_H} \]

Photosynthesis (Variable)

*From Yannick Huot*
Variability in the quantum yield of fluorescence:

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can be expressed in terms of rate constants ($k$, s\(^{-1}\)) for the three possible fates of absorbed photons:

**FLUORESCENCE, PHOTOSYNTHESIS, HEAT**

Fluorescence (Constant)

$$\varphi_f = \frac{k_f}{k_f + k_p + k_H}$$

Photosynthesis (Variable)

Heat (Variable)

*From Yannick Huot*
Nutrient Stress
Leading to Higher Fluorescence Yield
(reduced photochemical quenching)

\[ \phi_f = \frac{k_f}{k_f + k_p + k_H} \]

\( k_p \) decreases and \( \phi_f \) increases

Excess Irradiance Leads to Lower Fluorescence Yield (increased nonphotochemical quenching)

...and photosynthetic yield is reduced as well

$$\Phi_f = \frac{k_f}{k_f + k_p + k_H}$$

Solar Irradiance (old-fashioned units)

$\Phi_f$ decreases and $k_H$ increases

Photosynthetic Efficiency Explored by Measuring Change in Fluorescence upon Closure of Reaction Centers (e.g., Fv/Fm with DCMU)


Parkhill et al. 2001
The first continuous measurements of $F_v / F_m$ employed the Turner Designs

See also
Results were more provocative than conclusive

We may not know exactly what we are measuring, but the patterns observed are too strong to ignore.

Conclusion (early 80’s):

PERSPECTIVES

The Deep Chlorophyll Maximum: Comparing Vertical Profiles of Chlorophyll a

JOHN J. CULLEN

Department of Fisheries and Oceans, Marine Ecology Laboratory, Bedford Institute of Oceanography, Dartmouth, N.S. B2Y 4A2

Physiological and taxonomic influences on fluorescence yield are sources of both errors and useful information.
Conclusion (early 80’s):

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The Deep Chlorophyll Maximum: Comparing Vertical Profiles of Chlorophyll α

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Physiological and taxonomic influences on fluorescence yield are sources of both errors and useful information.

We should measure and interpret the variability of fluorescence yield in nature.
1980 - 2006: Systematic comparison of yields fell by the wayside as other powerful approaches were pursued.
Meantime, sun-induced chlorophyll fluorescence was studied on and off


Gordon, H. R. (1979), Estimation of the for the remote sensing of chlorophyll a via 1883-1884.

Topliss, B. J., and T. Platt (1986), Passive Implications for remote sensing, Deep-Sea

Fisher, J., and U. Kronfeld (1990), Sun-stimulated of oceanic properties, International Journ


Possibly the only hope for detecting relatively low concentration of phytoplankton in the presence of CDOM and MODIS FLH from Chris Jones.

Solar fluorescence-based algorithm after Huot et al., JGR Oceans (in press).

Doesn’t work

\[ R^2 = 0.28 \]
\[ n = 115 \]

Works pretty well

\[ R^2 = 0.57 \]
\[ n = 134 \]
Fluorescence line height (FLH): A proxy for $F$
Fluorescence line height (FLH): A proxy for $F$

it can sometimes provide reasonable estimates of Chlorophyll (especially when the range of Chl is very large)
But fluorescence yield is highly variable in nature

Huot et al. 2005
L&O Methods
Apparently huge variability of fluorescence yield in nature (ca. 10x) is clearly tied to environmental forcing.

Estimated fluorescence quantum yield: Huot et al. 2005
L&O Methods
Satellites detect fluorescence in full sunlight. *In situ* radiometers can measure $F$ vs $E$. It also varies greatly!
This signal should not be ignored!

Estimated fluorescence quantum yield: Huot et al. 2005
L&O Methods
A big goal: Interpreting natural variability of $\phi_f$ as detected from space.
A number of processes must be considered to relate FLH quantitatively and mechanistically to the biomass and physiology of phytoplankton.

\[ FLH = L_{uf} \left( 683, 0^- \right) = \]

\[ \frac{1}{4\pi} \cdot \frac{1}{C_f} \cdot \phi_f \cdot \theta \cdot \varepsilon(\text{PAR}, 0^-) \cdot chl \cdot a_\phi^* \cdot Q_a^*(683) \cdot [K_{abs} + a_f(683)]^{-1} \]

Recent examples:
- Babin et al. (1996)
- Ostrawska et al. (1997)
- Maritorena and Morel (2000)
- Huot et al. (2005)
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A number of processes must be considered to relate FLH quantitatively and mechanistically to the biomass and physiology of phytoplankton.

Correction for backscatter and Raman scatter

\[
FLH = L_{uf}(683,0^-) = \frac{1}{4\pi} \cdot \frac{1}{C_f} \cdot \varphi_f \cdot E(PAR,0^-) \cdot chl \cdot \bar{a}_\varphi \cdot Q_a^*(683) \cdot \frac{K_{abs} + a_f(683)}{[K_{abs} + a_f(683)]^{-1}}
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A number of processes must be considered to relate FLH *quantitatively and mechanistically* to the biomass and physiology of phytoplankton.

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\frac{1}{4\pi} \cdot \frac{1}{C_f} \cdot \varphi_f \cdot \varnothing (\text{PAR}, 0^-) \cdot \text{chl} \cdot a^* \cdot Q^*(683) \cdot [K_{abs} + a_f(683)]^{-1}
\]

Volume emission to upwelling radiance

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Full spectral emission to 683 nm
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Quantum yield of fluorescence — function of $E$ & physiology

$$FLH = L_{uf}(683,0^-) = \frac{1}{4\pi} \cdot \frac{1}{C_f} \cdot \phi_f \cdot E(PAR,0^-) \cdot chl \cdot a^*_\phi \cdot Q^*_a(683) \cdot [K_{abs} + a_f(683)]^{-1}$$

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Chl * irradiance-weighted specific absorption coefficient
A number of processes must be considered to relate FLH quantitatively and mechanistically to the biomass and physiology of phytoplankton

$$FLH = L_{uf}\left(683,0^-\right)= \frac{1}{4\pi} \cdot \frac{1}{C_f} \cdot \varphi_f \cdot \overrightarrow{E}(\text{PAR,}0^-) \cdot \text{chl} \cdot \overline{a_{\varphi}} \cdot Q_a^*(683) \cdot \left[K_{abs} + a_f(683)\right]^{-1}$$

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Internal reabsorption of fluoresced photons

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A number of processes must be considered to relate FLH quantitatively and mechanistically to the biomass and physiology of phytoplankton.

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- Babin et al. (1996)
- Ostrawska et al. (1997)
- Maritorena and Morel (2000)
- Huot et al. (2005)
- Laney et al. (2005)

\[
FLH = L_{uf}(683,0^-) = \frac{1}{4\pi} \cdot \frac{1}{C_f} \cdot \varphi_f \cdot \theta \cdot E_{(PAR,0^-)} \cdot chl \cdot a^* \cdot Q^*(683) \cdot [K_{abs} + a_f(683)]^{-1}
\]
It’s not really all that bad, and it’s needed to retrieve physiological variables

$$FLH = L_{uf}(683,0^-) =$$

$$\frac{1}{4\pi} \cdot \frac{1}{C_f} \cdot \phi_f \cdot \hat{E}(PAR,0^-) \cdot \text{chl} \cdot a^*_\phi \cdot Q^*_a(683) \cdot [K_{abs} + a_f(683)]^{-1}$$

Recent examples:
- Babin et al. (1996)
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These all contribute to the observed nonlinear relationships between FLH and Chlorophyll

Huot et al. 2005 model predicted relationship with \textit{constant quantum yield and surface irradiance}

\[ \text{FLH (W m}^{-2} \text{ sr}^{-1} \text{ nm}^{-1}) \]

\[ \text{Chlorophyll (mg m}^{-3}) \]

See Babin et al. 1996; Gower et al. 2004

Roots in papers by Morel and Prieur 1977, Neville and Gower (1977), Gordon (1979)
But $\varphi_f$ varies — a lot
...we observed the same kind of variability in the Bering Sea

Data from optical drifters
Schallenberg et al., submitted (JGR Oceans)
Goal: Explain this kind of variability in fluorescence yield in terms of $\Phi_f$ and the optical properties of phytoplankton and the water.
Approach
Approach

• Retrieve fluorescence normalized to absorbed radiation \( F_{\text{abs}} \) and surface irradiance, \( E \).
Approach

• Retrieve fluorescence normalized to absorbed radiation ($F_{abs}$) and surface irradiance, $E$
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• Ascribe variation of \(F_{abs}\) vs \(E\) to natural variability of \(\phi_f\) vs \(E\)
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Approach

- Retrieve fluorescence normalized to absorbed radiation ($F_{abs}$) and surface irradiance, $E$

- Ascribe variation of $F_{abs}$ vs $E$ to natural variability of $\phi_f$ vs $E$

- Relate inferred variability of $\phi_f$ vs $E$ to phytoplankton physiology
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Approach

• Retrieve fluorescence normalized to absorbed radiation ($F_{abs}$) and surface irradiance, $E$

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• Relate inferred variability of $\varphi_f$ vs $E$ to phytoplankton physiology

• Relate physiological status to environmental factors
The working hypothesis from the drifter studies was that high fluorescence yield corresponds to nutrient stressed assemblages.

Chlorophyll natural fluorescence response to upwelling events in the Southern Ocean

Letelier et al. 1997, GRL

FLH/Chl vs $E$ slope varied, reflecting variation in $\phi_f$

$\phi_f$ covaried with inferred upwelling: high nutrient input - low fluorescence yield
The underlying model

INCREASING NUTRIENT STRESS

DECREASING PHOTOCHEMICAL QUENCHING

$\frac{\mu}{\mu_{\text{MAX}}}$

FLUORESCENCE / CHLOROPHYLL
But nonphotochemical quenching was recognized as a factor:

Each data set is normalized to a different irradiance of maximal fluorescence yield.

But nonphotochemical quenching was recognized as a factor:

Photosynthesis decreases $\varphi_f$ (subsaturating irradiance)

But non-photochemical quenching was recognized as a factor:

Photosynthesis decreases $\varphi_f$ (subsaturating irradiance)

Heat dissipation decreases $\varphi_f$ (supersaturating irradiance)

Moving beyond FLH:
Direct estimation of quantum yield vs irradiance relationship

\[ F_{rs}^{abs} = \frac{(L_u(683) - L_{ub}(683)) \cdot 4\pi \cdot C_f}{chl_{rs} \cdot \bar{\alpha}_\phi(chl_{rs}) \cdot Q_a^*(chl_{rs})} \cdot \left[ K_{abs}(chl_{rs}) + \kappa_f(chl_{rs}) \right] \]

Optical drifter in the Bering Sea: quantum yield is

\[ F_{rs}^{abs} / E(PAR,0^-) \]

Schallenberg et al. 2008, JGR Oceans
Results from the Bering Sea

Conclusion is the same: large variation, with high fluorescence yield associated with nutrient stress.

Schallenberg et al. 2008, JGR Oceans
Conclusion is the same: large variation, with high fluorescence yield associated with nutrient stress.

Backed up with direct measures of photosynthetic efficiency, $F_v/F_m$.

*Schallenberg et al. 2008, JGR Oceans*
What is the effect of nutrient stress on $F$ vs $E$ in this system?

Fluorescence

$F_{\text{abs}}$ ($\mu$mol quanta m$^{-2}$ s$^{-1}$)

Irradiance ($\mu$mol quanta m$^{-2}$ s$^{-1}$)

Nutrient replete: $F_{v/Fm} = 0.67$

Fluorescence Yield

Quantum Yield of Fluorescence

$F_{v/Fm} = 0.67$

Irradiance ($\mu$mol quanta m$^{-2}$ s$^{-1}$)

Schallenberg et al. 2008, JGR Oceans

Analysis after Morrison (2003) L&O
Not much!
(considering only photochemical quenching)

Fluorescence

Fluorescence Yield

Nutrient replete: Fv/Fm = 0.67
Nutrient stressed: Fv/Fm = 0.22

Schallenberg et al. 2008, JGR Oceans

Analysis after Morrison (2003) L&O
Big differences in near-surface sun-induced fluorescence yield are not due to effects of nutrition on photochemical quenching.
Morrison (2003, L&O):

**A third process must be considered:** $q_i$
Morrison (2003, L&O):

**A third process must be considered: \( q_i \)**

Degrees of ‘slow’ quenching (\( q_i \)) associated with inhibition of photosynthesis.
Morrison (2003, L&O):

**A third process must be considered: \( q_i \)**

This quenching leads to reduced photosynthetic efficiency in low light.
Inference: Variability in surface $F$ vs $E$ is dominated by nonphotochemical quenching (NPQ), not effects of nutrition on photochemical quenching.

Schallenberg et al. 2008, JGR Oceans
An influence of nutrition on NPQ?

\[ F \text{ vs } E \]

Schallenberg et al. 2008, JGR Oceans
Working hypothesis: variability in the quantum yield of near-surface SICF in the ocean is driven by the slow component of nonphotochemical quenching, $q_I$.

...the phenomenology of which is nearly unknown
Interpreting this…

…thus requires an understanding of NPQ vs $E = f$ (physiological state, species)
Careful, quantitative analysis of variable fluorescence vs $E$ vs time

Parallel incubations: rapid light curves cause artifacts

A. Barnett (M.Sc. thesis)
see also Laney et al 2005
The FIRe Brigade is pursuing robust, quantitative procedures

*Comprehensive characterization of a variable fluorescence assessment system: the Fluorescence Induction Relaxation fluorometer*

Audrey B. Barnett, Flavienne Bruyant, Caitlin B. Newport, Richard F. Davis, John J. Cullen

Analysis of raw data!
Reference standards!
Blanks!
Statistical estimates of errors!

$F$ vs $E$ vs time

[Image of a graph showing fluorescence intensity ($F_{RFU}$) vs sample number (Sample #), with a note that units are expressed relative to rhodamine dye.]
Relating $F$ vs $E$ to $P$ vs $E$ during parallel incubations

$E_{\text{opt}} = 283 \, \mu\text{mol m}^{-2}\text{ s}^{-1}$

Not rapid light curves

A. Barnett (M.Sc. thesis)
And retrieving similar information from vertical profiles using “any old fluorometer”

irradiance at optimal fluorescence ($E_{opt}$)
Systematic analysis of natural variability of $\phi_f$

$R^2 = 0.58$
$n = 22$

Large, densely pigmented cells
High $\phi_f$

Small, weakly pigmented cells
Low $\phi_f$

high packaging
low packaging
Summary

Thank you!
Summary

Fluorescence yield is variable for many reasons

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We don’t know enough about the physiological influences on sun-induced chlorophyll fluorescence to interpret the variability effectively. We can propose explanations, but these would be hypotheses only.

Careful, quantitative analysis — both in the lab and in the field — will provide new and powerful interpretations of SICF.

Thank you!