Chapter 30

Development and Application of Variable Chlorophyll Fluorescence Techniques in Marine Ecosystems

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Summary

Since its introduction in the early 1960s, in vivo chlorophyll fluorescence has been used as an index of photosynthetic biomass in marine ecosystems. In the late 1980s, however, active fluorometric techniques, originally based on the pump and probe method, were used to derive estimates of photosynthetic electron transport, the overall quantum efficiency of photosynthetic energy conversion, and the effective cross section of Photosystem II. It was quickly realized that nutrient limitation, but not acclimation to light or temperature, had a profound influence on photosynthetic energy conversion efficiency (reported as, e.g., $F_v/F_m$). Subsequently, variable

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fluorescence techniques were employed to assess physiological control of oceanic photosynthesis by nutrients that potentially limit photosynthetic electron transport. The pump and probe technique was subsequently supplanted by a fast repetition rate (FRR) fluorescence method, which greatly improved the precision and efficiency of variable fluorescence measurements at sea. The FRR method has revealed how the availability of iron, fixed inorganic nitrogen, and phosphate control photosynthetic electron transport rates throughout the world oceans. The technique has been further applied to corals, seagrasses, single cells of free-living marine phytoplankton, and anoxygenic aerobic photosynthetic bacteria. Variable fluorescence data reveal extraordinary physiological plasticity of the photosynthetic apparatus in the genetically diverse group of organisms that comprise the primary producers in contemporary oceanic ecosystems.

I. Introduction

In contrast to terrestrial ecosystems, primary production in the oceans is the result of the photosynthetic activity of a taxonomically heterogenous group of single celled organisms (called phytoplankton), macroalgae, submerged angiosperms (seagrasses) and symbiotic invertebrates such as zooxanthellate corals, Table 1 (Falkowski et al., 2004). In aggregate, these organisms comprise less than 1% of the total photosynthetic carbon-based biomass on Earth (Falkowski and Raven, 1997). Despite their extremely low biomass, marine net primary production accounts for nearly half of the global total (Field et al., 1998; Behrenfeld et al., 2001). A corollary of the extraordinarily high productivity/biomass ratio is that, on average, the turnover time of oceanic photosynthetic biomass is approximately one week. Over hundreds of millions of years, natural selection, driven by the high turnover rates, has resulted in highly dynamic responses of the photosynthetic apparatus to the highly variable conditions that organisms inevitably encounter in the oceans. The application of variable chlorophyll fluorescence techniques has provided an unparalleled window into many of these responses. In this chapter, we discuss the application of variable fluorescence techniques to understanding the photophysiological responses of marine photosynthetic organisms.

A. Brief History of the Application of Chlorophyll Fluorescence in Marine Ecosystems

1. Estimating Photosynthetic Biomass

Chlorophyll (Chl) a is the most convenient and specific measure of photosynthetic biomass in marine ecosystems. However, the concentration of Chl a in the water column of the upper ocean is extremely small, ranging from 0.02 to up to ca. 20 µg/L (under rare bloom conditions); the global mean Chl a concentration in the upper ocean is 0.31 µg/L. Below the upper 200 m in the open ocean, Chl a concentrations decrease markedly, approaching no more than a few ng/L. It is possible to filter water samples to concentrate the organisms containing photosynthetic pigments, and to measure the concentration of individual pigments in solvent extracts by spec-

Abbreviations: \( a_{psii} \) – the optical absorption cross section of Photosystem II; \( a^* \) – the chlorophyll normalized optical cross section in vivo (m²/mg Chl a); AAP – aerobic anoxygenic photrophs; \( a_{psii} \) – the optical absorption cross section (m²/mg Chl a); BChl – bacteriochlorophyll; Chl a – chlorophyll a; DCMU – 3-(3,4-dichlorophenyl)-1,1-dimethyl urea; \( E_a \) – absorbed irradiance; \( E_0 \) – incident spectral irradiance (quanta m⁻² s⁻¹); ETR – (photosynthetic) electron transport rate; \( \text{ETR}^{b} \) – (photosynthetic) electron transport rate normalized to chlorophyll a; \( F \) – fluorescence (arbitrary units); \( f \) – fraction of functional Photosystem II reaction centers; \( F_m \) – maximal value of fluorescence, measured in darkness; \( F_o \) – minimal value of fluorescence, measured in darkness; FRR – fast repetition rate (fluorescence method); \( F_v \) – variable fluorescence (\( F_m \) – \( F_o \)); HNLC – high nutrient-low chlorophyll region; IRFRR – infrared fast repetition rate fluorescence method; \( J_{up} \) – rate of light absorbed by Photosystem II; \( k_1 \) – first order rate constant for fluorescence (s⁻¹); \( k_2 \) – first order rate constant for non-radiative energy dissipation (heat) (s⁻¹); \( k_3 \) – first order rate constant for photochemistry (s⁻¹); LHII (or 2) – Light Harvesting Complex II (or 2); MODIS – moderate resolution imaging spectroradiometer; \( n_h \) – density of Photosystem II reaction centers (Chl a/Reaction center II); \( p_h \) – net primary production normalized to chlorophyll; PAM – pulse amplitude modulated fluorescence method; PS II – Photosystem II; \( q_p \) – photochemical quenching (dimensionless); \( Q_{s} \) – self-shading attenuation coefficient; RCII – reaction center II; SCUBA – self contained underwater breathing apparatus; \( \alpha \) – distribution of excitation energy between photosystems I and II; \( \Delta F_{psii} \) – the change in the quantum yield of fluorescence following a single turnover saturating actinic flash; \( \sigma_{psii} \) – the effective cross section of Photosystem II; \( \phi_{psii} \) – fluorescence yield of Photosystem II; \( \phi_{psii} \) – maximum quantum yield of fluorescence; \( \phi_{psii} \) – minimum quantum yield of fluorescence; \( \phi_{psii} \) – quantum yield of fluorescence in PS II; \( \phi_{psii} \) – quantum yield of photochemistry in PS II (electrons/quanta)
Table 1. The higher taxa of oxygenic photoautotrophs, with estimates of the approximate number of total known species, and their distributions between marine and freshwater habitats.* (Original sources cited in Falkowski and Raven, 1997.)

<table>
<thead>
<tr>
<th>Taxonomic Group</th>
<th>Known Species</th>
<th>Marine</th>
<th>Freshwater</th>
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<tr>
<td>Empire: Bacteria (= Prokaryota)</td>
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<td>2</td>
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<td>5,000</td>
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<tr>
<td>Fucoxophyceae (brown algae)</td>
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<td>250</td>
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<tr>
<td>Tribophyceae (Xanthophyceae)</td>
<td>600</td>
<td>50</td>
<td>500</td>
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</tbody>
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*The difference between the number of marine and freshwater species, and that of known species, is accounted for by terrestrial organisms. Dashes indicate that no species are known (by us) for their particular group in this environment.
trophotometric (Jeffrey and Huphrey, 1975; Bidigare et al., 1988), (spectro)fluorometric (Yentsch and Menzel, 1963; Holm-Hanson et al., 1965), or chromatographic techniques (Jeffrey, 1974; Falkowski and Schuder, 1981; Mantoura and Llewellyn, 1983; Jeffrey et al., 1997). While all these approaches have the advantage of high precision and accuracy, they are invariably time consuming and labor intensive. Sample throughput, a factor that is frequently critical in ecological or ecophysiological studies, becomes a limiting factor in determining patterns of distributions of photosynthetic organisms and rates.

In an attempt to semi-quantify measurements of Chl \( a \) in situ, Carl Lorenzen (1966) introduced an in vivo fluorescence technique, where water samples were simply pumped from the ocean through a simple fluorometer on the deck of a ship. The excitation source and fluorescence emission were isolated by broadband and band-pass filters, respectively. By occasional calibration using solvent extraction on parallel samples, the absolute concentration of Chl could be estimated. This technique, which is widely used to this day in various instrumental incarnations, is extremely convenient and versatile. It can provide reasonable estimates of phytoplankton Chl biomass as a ship transverses large areas (Platt, 1972), or, when instruments are moored at a fixed position, it can provide a time series of phytoplankton Chl biomass (Wirick, 1993) (Fig. 1). Indeed, the basic concept has even been applied to space-based sensors, such that solar induced fluorescence of Chl \( a \) on the surface of the world ocean is detected on a daily basis (Fig. 2 and Color Plate 3). This approach is especially useful for estimating photosynthetic biomass in coastal waters, where dissolved organic matter and suspended sediments frequently interfere with the more accurate estimations of Chl based on backscattered radiance estimates of spectral absorption (Abbott and Barksdale, 1991).

2. The Rationale of in Vivo Chlorophyll Fluorescence as an Estimate of Photosynthetic Biomass

Formally, the rationale behind the application of in situ fluorescence for estimating Chl \( a \) is based on the

![Image](https://example.com/image.png)

Fig. 1. An example of a time-series analysis of in vivo chlorophyll fluorescence and sea surface temperature, recorded from an instrument package located 40 m below the surface in the Red Sea. The sharp drops in temperature in April and May are the consequence of storm-induced mixing of cold, deep waters with the more buoyant warm water in the upper mixed layer. These storms profoundly influence the chlorophyll distribution. As the season progresses, solar heating slowly results in an increased thermal gradient, leaving a quasi-stable warmer upper mixed layer. The phytoplankton in the mixed layer bloom in June and July subsequently become nutrient limited in August when the heating rate of the upper ocean is so great as to virtually isolate this layer from the nutrient-rich deeper waters (see Falkowski, 1994 for a brief review of this phenomenon). The 'fuzzy' nature of the fluorescence and temperature records is not 'noise,' but a manifestation of high-frequency fluctuations in these variables. (Figure courtesy of Creighton Wirick, Brookhaven National Laboratory.)
assumption that accessory pigments, including Chl $b$ and Chl $c$ do not fluoresce in vivo, and that at physiological temperatures all Chl emission arises from pigment-protein complexes associated with Photosystem II (PS II), with a peak emission wavelength of 685 nm. Although most phycobilin containing organisms (cyanobacteria and cryptomonads), often fluoresce at shorter wavelengths, the fluorescence does not interfere with the retrieval of Chl. The rate of light absorbed ($I_{\text{abs}}$) by PS II in an optically thin suspension at a given excitation wavelength is related to the product of the excitation spectral irradiance,
$E_{\alpha,\lambda}$ the concentration of PS II reaction centers within the suspension, $n_{\lambda}$, their optical absorption cross section, $a_{PSII}$, and the mean fraction of the reduction in incident irradiance (Morel and Bricaud, 1981) due to self-shading within the plastids, $*Q_a$:

$$\ J_{\alpha,\lambda} = n_{\lambda} \times E_{\alpha,\lambda} \times a_{PSII} \times *Q_a \quad (1) $$

Assuming that $*Q_a$ is constant at a given excitation irradiance, then the fluorescence emission is given by:

$$\ F = \Phi_{II} \times n_{\lambda} \times E_{\alpha,\lambda} \times a_{PSII} \times *Q_a \quad (2) $$

where $F$ is the PS II-normalized fluorescence and $\Phi_{II}$ is the quantum yield of fluorescence for PS II reaction centers. While in reality, all four of the biological variables in Eq. 2, namely, $\Phi_{II}$, $n_{\lambda}$, $a_{PSII}$, and $*Q_a$ vary with species and physiological state, nonetheless, this relationship can be empirically calibrated and is highly useful in deriving estimates of Chl in situ.

B. The Discovery of Non-photochemical Quenching in the Oceans

Early on in the approach of in vivo Chl fluorescence in aquatic ecosystems, it was realized that $\Phi_{II}$ varied systematically with background irradiance (Abbott et al., 1982) (Fig. 3). This phenomenon was most obvious when comparing yields obtained during the day or at night: Night-time yields were almost always higher (Owens et al., 1980) (Fig. 4). Several approaches were used to reduce the variability, including adding 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) to the measured sample (Slovacek and Hannan, 1977), dark-adapting the sample prior to measuring the fluorescence yields, and controlling the intensity of the excitation light source. Although somewhat successful, none of these measures removed the effect. Only after the 'discovery' of non-photochemical fluorescence quenching, the xanthophyll cycle, and the photodegradation of PS II reaction centers (Fig. 5), did it become clear that the time scale for the quenching process, on order of minutes to hours, will inevitably confound the measurement of Chl biomass in situ. (For a discussion of non-photochemical quenching and xanthophylls cycle, see Chapters 19, Bruce and Vasil'ev; 20, Golan et al.; 21, Gilmore; and 22, Adams and Demming-Adams.) Nevertheless, the basic approach toward assessing phytoplankton biomass from their fluorescence signal remains extremely useful in aquatic ecosystems.

II. Fluorescence-based Estimation of Photosynthetic Electron Transport

A method for real-time, non-invasive, non-destructive, estimation of photosynthetic electron transport has been a goal of marine ecologists for a long time.

Fig. 3. Variations in chlorophyll a fluorescence (F) and surface irradiance (L) measured in Lake Tahoe with a towed fluorometers at 35 m. The correlation between the changes in irradiance, which is caused by clouds passing across the sky, and fluorescence is due to nonphotochemical quenching. T = surface temperature (flat line). (Modified from Abbott et al., 1982.)
This goal is potentially attainable from measurements of variable fluorescence using modulated light techniques, however, the application of the method requires some assumptions.

**A. Problems with Incubations**

The motivation for estimating photosynthetic rates in marine ecosystems stems from the desire to quantify the fluxes of organic matter through food webs and in biogeochemical cycles (Falkowski et al., 1998). In marine ecosystems, photosynthetic rates have traditionally been measured by following the uptake of radioactive inorganic carbon into particulate organic matter. This method, introduced by Steemann Nielsen in 1952 is extremely sensitive, but requires an incubation of samples in a confined space. By the late 1970s, it became increasingly clear that incubation of samples in bottles, especially in the oligotrophic open ocean, could lead to large artifacts due to trace metal contamination (Carpenter and Lively, 1980; Fitzwater et al., 1982), and from alterations in the community structure during the incubation (Eppley, 1980). Because of these complications, alternative, instantaneous estimates of photosynthesis were sought.

**B. Theoretical Basis of Active Fluorescence Techniques**

Based on theoretical treatment of fluorescence in relation to the competing energy dissipation pathways, Warren Butler (1972) showed that, in absence of energy transfer between PS II reaction centers, the photosynthetic yield, $\Phi_p$, can be expressed as:

$$\phi_p = q_p \times \frac{k_p}{k_p + k_f + k_i} = q_p(E_o) \times \phi_{p,max}$$

where $k_p$, $k_f$, and $k_i$ are the rate constants for photosynthetic, fluorescence, and thermal deactivation pathways for the excitation energy, $q_p$ is photochemical quenching (which, in the absence of energy transfer, defines the oxidation level of PS II reaction centers), and $\Phi_{p,max}$ is the maximum quantum yield of photosynthesis, observed when all reaction center (RC)IIIs are open.

The quantum yield of fluorescence, $\Phi_{FII}$, is expressed as:

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Fig. 4. Vertical profiles of chlorophyll $a$, extracted in 90% acetone, taken from discrete depths, and simultaneous measurements of in vivo chlorophyll fluorescence taken at 08:00 and noon local time. Note the non-photochemical quenching of fluorescence in the upper 20 m. (Owens et al., 1980.)

Fig. 5. The Stern Volmer relationship for nonphotochemical quenching as a function of diatozanthin/chlorophyll ratio in a marine diatom. $SV_m =$ Stern Volmer coefficient at $F_m$; $SV_o =$ Stern Volmer coefficient at $F_o$. (Modified from Olaizola et al., 1994.)
\[ \phi_{III}(E_o) = q_p(E_o)\phi_{F,o} + (1 - q_p(E_o))\phi_{F,m} \]

\[ \phi_{F,o} = \frac{k_f}{k_p + k_f + k_i} \]

\[ \phi_{F,m} = \frac{k_f}{k_f + k_i} \]

(4)

where \( \phi_{F,o} \) is the minimum fluorescence yield, observed when all reaction center (RC) IIs are open, and \( \Phi_{F,m} \) is the maximum fluorescence yield, observed when all RCIIIs are closed. From these, \( \phi_{p,\text{max}} \) can be calculated as:

\[ \phi_{p,\text{max}} = \frac{\phi_{F,m} - \phi_{F,o}}{\phi_{F,m} - \phi_{F,o}} = \frac{F_m - F_o}{F_m} \]

(5)

where \( F_o \) and \( F_m \) are the fluorescence signals measured in situation when all RCIIIs are open, and closed, respectively. Assuming that energy is not transferred between reaction centers, \( q_p \), which is a function of ambient irradiances, can be calculated from Eq. 4 (also see Eq. 5) as:

\[ q_p = \frac{\phi_{F,m} - \phi_{F,o}}{\phi_{F,m} - \phi_{F,o}} = \frac{F_m - F}{F_m - F_o} \]

(6)

where \( F \) is the fluorescence signal measured under ambient irradiances. Combining Eqs. 5 and 6 allows us to express the photosynthetic yields as:

\[ \phi_p = \frac{F_m - F_o}{F_m} \times \frac{F_m - F}{F_m - F_o} = \frac{F_m - F}{F_m} \]

(7)

The electron transport rate can therefore be calculated as:

\[ ETR = E_o \times \alpha \times \frac{F_m - F}{F_m} \]

(8)

where \( E_o \) is the light absorbed by the photosynthetic pigments, and \( \alpha \) is a coefficient describing distribution of the absorbed light between PS II and PS I (usually assumed to be 0.5). Non-photochemical quenching, \( q_{q0} \), resulting from either an increase of the rate constant, \( k_q \), or a decrease in \( k_o \), affects both the photochemical and the fluorescence yield, preserving the integrity of Eq. 8 under a wide range of ambient irradiances.

III. The Functional Absorption Cross Section of Photosystem (PS) II

As elegant and simple Eq. 8 is, it requires an accurate estimate of the flux of excitation energy to PS II antenna, \( E_a = E_o \times a_{PSII} \). While in terrestrial plants, \( a_{PSII} \) can be assumed to be \( \sim 0.85 \) (i.e., most of the light incident on the leaf is absorbed), in marine ecosystems, the determination of \( E_a \) from the measurement of absorbance is virtually impossible. First, the low signal-to-noise ratio, and complicated relationship between beam attenuation and scattering due to the presence of particulate materials (such as bacteria and sediments) complicates assessment of the light absorbed by the photosynthetic pigments (Morel, 1978). Second, there is a very large variety of pigments in the marine environment, many of which are non-photosynthetic. This further confounds discrimination between the absorbed and the photosynthetically available radiation. Third, pigment packaging strongly influences the distribution of the absorbed light among layers of pigment, decreasing optical cross section (Falkowski et al., 1985). Finally, because of wide variations in PS II/PS I ratios and their respective cross sections, the distribution of the excitation flux between PS II and PS I varies between phytoplankton species, and is affected by their physiological state.

This problem can be addressed by directly measuring the light absorbed by PS II reaction centers. The PS II absorption can be conveniently quantified in terms of the functional absorption cross section, \( \sigma_{PSII} \) (Ley and Mauzerall, 1982) where

\[ \sigma_{PSII} = -\frac{\partial q_p}{\partial I_o} \bigg|_{q_p=1} = -\frac{\partial q_p}{\partial I_o} \bigg|_{\text{max}} (0 < q_p < 1) \]

and \( I_o \) is the incident energy

\[ I_o = \int_o^{\Delta t} E \, dt \]

(9a)

delivered over a time \( \Delta t \), when \( \Delta t \) is much shorter than the time constant for electron transport (negligible reoxidation of PS II). Alternatively, \( \sigma_{PSII} \) can be expressed as a product of the optical absorption cross-section of PS II, \( a_{PSII} \), and the maximum quantum yield of charge separation, \( \phi_{p,\text{max}} \).
\[
\sigma_{\text{PSII}} = a_{\text{PSII}} \times \phi_{p,\text{max}} = a_{\text{PSII}} \times \phi_p \times \frac{1}{q_p} \quad (10)
\]

\(a_{\text{PSII}}\) represents the RCII-normalized efficiency of the photosynthetic pigments for light harvesting, and \(\phi_{p,\text{max}}\) represents the ability of the RCII to convert the absorbed light into electrons in the process of charge separation. The RCII-normalized rates of photosynthetic electron transport, \(\text{ETR}_{\text{RCII}}\), can therefore be calculated as:

\[
\text{ETR}_{\text{RCII}} = E_o \times a_{\text{PSII}} \times \phi_p = E_o \times \sigma_{\text{PSII}} \times q_p \quad (11)
\]

\(\sigma_{\text{PSII}}\) can be assessed experimentally by applying a series of short flashes of varying intensity, and fitting the measured \(q_p\) to a negative exponential equation (Mauzerall, 1986)

\[
q_p = e^{-\sigma_{\text{PSII}} \times E} \quad (12)
\]

Alternatively, \(\sigma_{\text{PSII}}\) can be measured very precisely and rapidly using fast repetition rate (FRR) fluorometry (Kolber et al., 1998).

In order to calculate the bulk photosynthetic electron transport rate per unit Chl \(a\), \(\text{ETR}^a\), Eq. 9 has to be multiplied by the concentration of the functional reaction centers. This parameter is proportional to the bulk concentration of PS II reaction centers, \(n_{\text{RCII}}\), scaled by the fraction, \(f\), of the functional reaction centers, \(f_{\text{RCII}}\). We calculate \(f_{\text{RCII}}\) from the \(F_o/F_m\) ratio measured in dark adapted samples. Assuming that, in darkness, the functional reaction centers display fluorescence yield \(F_o\) while the nonfunctional reaction centers display a yield approaching \(F_m\) (i.e., by definition, a non-functional reaction center does not trap excitation energy and has no variable fluorescence), \(F_o/F_m\) in the dark can be expressed as:

\[
\left(\frac{F_o}{F_m}\right)_{f=1} = \frac{F_m - (F_o)_{f<1}}{F_m} = \frac{F_m - (f \times F_o + (1-f) F_m)}{F_m} = f \times \left(\frac{F_o}{F_m}\right)_{f=1} \quad (13)
\]

Empirically, this ratio varies between 0.20 to 0.65 in the ocean, depending primarily on the local nutrient conditions (Kolber et al., 1990, 1994; Behrenfeld et al., 1996). Equation 13 leads to the postulate that, under optimal environmental conditions, when all reaction centers are functional (i.e., \(f_{\text{RCII}} = 1\), \(F_o/F_m\) approaches 0.65. We therefore can simplify Eq. 13 and empirically express \(f_{\text{RCII}}\) (Kolber and Falkowski, 1993) as

\[
f = \frac{F_o}{F_m} \times \frac{1}{0.65} \quad (14)
\]

and calculate the photosynthetic electron transport rates normalized to Chl \(a\) as:

\[
\text{ETR}^a = E_o \times \sigma_{\text{PSII}} \times q_p \times n_{\text{RCII}} \times \frac{F_o}{F_m} \times \frac{1}{0.65} \quad (15)
\]

or

\[
\text{ETR}^a = E_o \times \sigma_{\text{PSII}} \times n_{\text{RCII}} \times \frac{F_o}{F_m} \times \frac{1}{0.65} \times \frac{F_m - F}{F_m} \quad (16)
\]

Assuming eight photons are required for each molecule of oxygen evolved, and the photosynthetic quotient is 1.5, net primary production, in terms of carbon fixed per unit Chl \(a\), can be calculated as:

\[
p^a = \frac{1}{12 \times 0.65} \times E_o \times \sigma_{\text{PSII}} \times n_{\text{RCII}} \times \frac{F_m - F}{F_m} = 0.128 \times E_o \times \sigma_{\text{PSII}} \times n_{\text{RCII}} \times \frac{F_m - F}{F_m} \quad (17)
\]

Equation 17 operates on the incident irradiance, \(E_o\), while Eq. 8 requires knowledge of the light absorbed by the photosynthetic pigments, \(E_a\). Comparing these two equations allows one to express the relationship between \(E_o\) and \(E_a\) as:

\[
E_a = E_o \times \sigma_{\text{PSII}} \times n_{\text{RCII}} \times \frac{1}{0.65} \times \frac{1}{\alpha} \approx E_o \times \sigma_{\text{PSII}} \times n_{\text{RCII}} \quad (18)
\]

indicating that the light absorbed by the PS II pigments is proportional to the incident irradiance, the functional absorption cross-section of PS II, and the concentration of PS II reaction centers.
IV. Measuring Variable Chlorophyll Fluorescence in Marine Environment

The fluorescence yield of PS II, \( \Phi_{\text{II}} \), varies in response to the level of photochemical activity in PS II. This activity can be manipulated using an actinic light, and assessed from the measured changes in \( \Phi_{\text{II}} \). Using this approach, a variety of active fluorescence methods have been developed, where the actinic light of controlled intensity, duration, and spectral quality is used to selectively excite various components of the photosynthetic apparatus, and fluorescence yield is measured using either the actinic excitation, or is derived from the fluorescence signal induced by a separate, non-actinic (‘probe’) source. The simplest of such protocols is based on the ‘fluorescence induction technique’ (Kautsky and Hirsh, 1931; Govindjee, 1995) in which a brief exposure to a strong actinic source induces a fluorescence transient. The kinetics of such a transient are complex. On the time scale of milliseconds to seconds, the transient is primarily related to the redox state of the electron carriers on the acceptor side of PS II (\( Q_A, Q_B \)) plastoquinone pool). On time scales of minutes the transient is influenced by the activation of ribulose 1,5-bisphosphate carboxylase/oxygenase, and the reduction of inorganic carbon.

By the late 1970s, there was interest in applying a fluorescence induction technique to estimate photosynthetic electron transport in aquatic ecosystems. In part, the inspiration for the initial work was based on the difference in fluorescence induction curve obtained in the presence and absence of DCMU (Malkin and Kok, 1966). The basic assumption is that, in the absence of DCMU, the integrated area over the induction curve represented the total number of electrons that can be injected into the electron transport chain between PS II and PS I, while that obtained in the presence of DCMU gave the single turnover of PS II alone. Normalization of the former to the latter provided a ‘working potential’, that is the maximum rate of electron transport per unit absorbed photons (Ishimaru et al., 1985). The technique was never widely adopted, primarily because it was more time consuming than informative for ecological studies, and because the equipment required for the measurements needed to be custom made (for further use of fluorescence induction technique, see Chapter 12, Strasser et al.).

An additional issue that is unique to measurements of variable fluorescence in aquatic ecosystems is the problem of the ‘blank’. Because the concentration of chlorophyll is so low, and water contains many dissolved fluorescent as compounds and non-fluorescent particles that scatter light, photons from these sources can leak into the chlorophyll emission wavelengths, giving an erroneously large \( F_v \) value. Hence, determining the background fluorescence becomes crucial to an accurate measurement of \( F_v \). Empirically, determination of \( F_v \) in aquatic ecosystems requires a measurement of the background ‘fluorescence’. One way this is achieved is by subtracting the fluorescence of water filtered through a 0.22 \( \mu \)m pore size filter.

A. Pump and Probe Fluorescence

The introduction of flash photolysis by George Porter in the mid 1950s paved the way for the application of a ‘pump and probe’ method (Delosme, 1971; Mauzerall, 1972) to investigate photochemical reactions with much greater control over the experimental protocol than afforded by an induction technique. The pump and probe approach uses a microsecond, saturating ‘pump’ flash to instantly reduce \( Q_A \) (but not the plastoquinone pool), and a weak, subsaturating probe flash to measure the fluorescence changes induced by the saturating flash. Mauzerall (1972) used this technique to investigate the kinetics of changes in fluorescence yields, from picoseconds to milliseconds. In the mid 1980s, Falkowski et al., (1984, 1986) had examined the application of the pump and probe technique for deriving \( \sigma_{\text{PSII}} \) and the quantum yield of photochemistry (Fig. 6). The change in the quantum yield of the probe flash following a saturating pump flash, \( \Delta \Phi_{\text{sat}} \) (the equivalent of \( F_v/F_m \)) appeared to be extremely constant when measured in the dark for all \textit{nutrient replete} eukaryotic algae, averaging ca. 0.65. By varying the intensity of the pump flash, a flash intensity saturation profile could be derived, from which \( \sigma_{\text{PSII}} \) could be calculated by applying a cumulative one-hit Poisson function (Ley and Mauzerall, 1982; Falkowski et al., 1986b).

By following the change in \( \Delta \Phi_{\text{sat}} \) with background irradiance, it was possible to identify specific irradiance-dependent ‘domains’. At extremely low irradiance, \( \Delta \Phi_{\text{sat}} \) decreased by ca. 15% from the dark-adapted value (Fig. 7). The reduction was attributed to the generation of a \( \Delta pH \) gradient. At low irradiance levels, \( \Delta \Phi_{\text{sat}} \) was relatively constant; this region corresponds to the initial, linear portion of the photosynthesis-irradiance curve, where the probability of encountering a closed reaction center at the time of measurement is
components of $\Delta \phi_{sat}$, namely the $F_p$ (the fluorescence yield of the probe flash prior to pump flash) and $F_s$ (the fluorescence yield of the probe flash following a saturating pump flash), decreased markedly, but $\Delta \phi_{sat}$ itself remained remarkably constant. In this region of the photosynthesis-irradiance curve it was clear that a ‘non-photochemical’ quencher was generated, and that it was virtually impossible to close all the reaction centers by light on the acceptor side of PS II. Falkowski et al. (1986) proposed that the non-photochemical quencher was a carotenoid. Further, based on simultaneous measurements of oxygen produced by the saturating flash, it appears that 10–15% of electrons from the donor to acceptor side of PS II at high light did not originate from water, but rather cycled around the reaction center (Falkowski et al., 1986a; Prasil et al., 1996).

**B. Pulse Amplitude Modulated Fluorescence**

Simultaneously and independently, a second fluorescence technique was developed based on ‘pulse amplitude modulated’ (or PAM) method (Bradbury and Baker, 1984; Schreiber et al., 1986). Like the pump and probe method, PAM uses weak modulated measuring light to obtain a fluorescence signal. This approach makes it possible to accurately measure fluorescence yields even in the presence of a back-

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**Fig. 6.** The relationship between pump energy and the change in the fluorescence yield of a high light (●) and low light (○) adapted diatom. Both curves follow a cumulative one hit Poisson distribution where the slope is proportional to the effective cross section of Photosystem II.

Vanishingly small. As irradiance increased however, $\Delta \phi_{sat}$ decreased, reflecting an increasing fraction of closed reaction centers due to competition with the background light. At very high irradiances, the two

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**Fig. 7.** Change in the quantum yields of variable chlorophyll fluorescence and its component yields in relation to continuous background irradiance. Also plotted is the relative photosynthetic rate measured simultaneously on a bare platinum electrode. $F_s$ is the fluorescence induced by the continuous background light. (After Falkowski et al., 1988.)
ground light. Unlike the pump and probe technique, however, PAM based fluorescence yields are typically assessed with an actinic ‘flash’ lasting about 0.5 s, long enough so that it reduces all the quinones on the acceptor side of PS II. Because the PAM method uses relatively long actinic flashes (relative to the photochemical turnover of QA), the resulting reduction of the plastoquinone pool can lead to an elevated value of FM. The exact biophysical cause of the rise in fluorescence yield (in eukaryotic algae and higher plant chloroplasts) upon reduction of the pool is not clear (Samson et al., 1999). One possibility is that the change in the membrane potential resulting from the reduction of the PQ pool (and the accompanying proton gradient) induces a Stark effect that modifies the fluorescence yield of chlorophyll molecules in core antenna complexes close to the PS II reaction center (Gottfried et al., 1991). PAM-based measurements of variable fluorescence are as much as 50% higher than those obtained using a single turnover flash approach. The resulting retrieval of the maximum quantum yield of photochemistry (e.g., Fv/Fm) is correspondingly 10–15% higher. Despite this problem, the PAM method became widely applied in assessing photochemical and non-photochemical quenching in terrestrial environments and more recently in aquatic ecosystems. The technique and its application are described in Chapter 11, Schreiber.

**C. Fast Repetition Rate Fluorescence**

The most common fluorescence parameter, Fv/Fm, is frequently interpreted as a measure of the quantum yield of charge separation. This parameter is extremely sensitive to the experimental protocol (Samson et al., 1999), complicating interpretation, and regrettably, the validity of the experimental protocols used to measure Fv/Fm (Schreiber et al., 1986; Samson and Bruce, 1996; Kolber et al., 1998). The problem is confounded by the fact that both photochemical quenching, qph (which, strictly speaking should reflect the redox state of the first stable electron acceptor, QA, and not the plastoquinone pool) and non-photophysical quenching, qNP, conspire to modulate the measured fluorescence signal over the length of the experimental protocols.

In an effort to accommodate both single and multiple turnover flash techniques while simultaneously making more precise and rapid measurements of variable fluorescence a ‘fast’ repetition rate (FRR) fluorescence technique was developed (Kolber et al., 1998). The FRR fluorescence method uses a series of submicrosecond ‘flashlets’ to both selectively manipulate the photosynthetic status of PS II electron carriers (QA and/or PQ pool), and to measure the corresponding fluorescence transients. The excitation energy in the FRR fluorescence method is controlled by changing the length and the interval between flashlets, while maintaining the intensity of the flashlets constant. As a result, the measured fluorescence transients directly reflect the changes in the fluorescence yield. Moreover, in contrast to both the pump and probe and the PAM methods, the fluorescence yield is derived using a single excitation source, and is measured using the full power of the excitation signal, simplifying the design of the instrumentation and greatly improving the signal-to-noise ratio. In addition, the FRR fluorescence technique allows one to derive the functional absorption cross section, the extent of excitation transfer between PS II reaction centers, the yield of charge separation, and the kinetics of the photosynthetic electron transport on the acceptor side of PS II with a single excitation sequence. By controlling the excitation energy and the length of the excitation protocol, both single turnover, and multiple-turnover flash can be generated (Fig. 8).

**V. Variations in the Maximum Quantum Yield of Fluorescence in Marine Environments**

By the mid-1980s we began a series of experiments to assess how the quantum yield was influenced by simple, ecologically relevant variables, including acclimation to light, temperature, and nutrients. Photoacclimation in eukaryotic algae (see Falkowski and Chen, 2002) appeared not to significantly influence ΔΦsat, that is, under nutrient replete conditions cells that were allowed to fully acclimate to changes in irradiance had a maximum change in the quantum yield of variable fluorescence (Fv/Fm) of ca. 0.65 independent of species. Temperature also had little effect. However, in a study of the effect of nitrogen limitation using chemostats, there was a hyperbolic relationship between ΔΦsat and nutrient supply (Kolber et al., 1988; Fig. 9). Specifically, as the rate of growth (as determined by dilution rate) decreased, Fv increased and the maximum quantum yield declined. It was hypothesized that the decline was due to an ‘uncoupling’ of light harvesting pigment
A. Testing a Limiting Nutrient Hypothesis

The question of whether rates of photosynthesis (and ultimately, growth) were limited by nutrients developed in the middle of the 20th century, and by the late 1960s a view, championed primarily by Richard Dugdale and John Goering (see Dugdale, 1967) emerged, suggests that in many regions of the world oceans the flux of nutrients, especially fixed, inorganic nitrogen, from deep waters into the euphotic zone was insufficient to maintain maximum photosynthetic rates. This view was challenged by Joel Goldman (1980), who suggested that although the concentration of dissolved inorganic nutrients such as ammonium or nitrate were indeed vanishingly low in the tropical and subtropical ocean gyres, the recycling rate of these nutrients was exceedingly high, such that cells were always growing near to the maximum rates. Variable fluorescence provided a way to test these two hypotheses. If Dugdale and Goering’s hypothesis was correct, there should be a significant spatial variation in \( \Delta \Phi_{sat} \) in the ocean; however, if Goldman was correct, \( \Delta \Phi_{sat} \) should be relatively constant.

In 1989, the first seagoing pump and probe fluorometer was used to derive real-time measurements of the changes in the maximum quantum yields in the ocean (Kolber et al., 1990). The initial studies, conducted off the northeast coast of the United States, revealed that \( \Delta \Phi_{sat} \) varied significantly, and non-randomly. Changes in \( \Delta \Phi_{sat} \) appeared to be related to physical mixing along the coastal region, such that higher values of
\(\Delta \phi_{\text{sat}}\) were related to the gradient in fixed inorganic nitrogen. This fundamental observation was repeated in a transect from the oligotrophic central Atlantic gyre across the Gulf Stream onto the nutrient-rich continental shelf (Kolber and Falkowski, 1992; Fig. 10). These studies presaged an effort to elucidate other elements that potentially limited the quantum yield of photochemical energy conversion in the ocean, and clearly supported Dugdale and Goring's view. Simply put, photosynthetic energy conversion efficiency in the ocean is physiologically limited by the availability of nutrients, especially fixed inorganic nitrogen. Could it be limited by other elements?

**B. Iron**

There are three major areas of the world ocean where dissolved inorganic nitrogen and phosphate (two ‘macronutrients’) are never depleted in the euphotic zone, despite high irradiance. These areas are further characterized by lower than expected Chl concentrations, and hence are called ‘high nutrient-low Chl’ or HNLC regions. In the late 1980s, a chemical oceanographer, John Martin, suggested that the HNLC regions were the consequence of limitation of primary production by a trace element, iron. Despite the extraordinary abundance of iron in Earth's crust, the concentration of the transition metal in the upper ocean seldom exceeds 500 pM, and in some regions is less than 100 pM. Iron is delivered to most of the remote regions of the ocean via wind-blown (aeolian) dust from continental sources. The three HNLC regions, namely the sub-arctic Pacific, the eastern equatorial Pacific, and the Southern Ocean, are far removed from aeolian iron supplies, and Martin's very careful measurements of iron in the upper portion of the water column clearly suggested that this element was exceedingly scarce.

The 'iron limitation' hypothesis was initially met with skepticism; the marine sciences community did not believe that a trace element could actually limit phytoplankton production. In an effort to prove this hypothesis, Martin organized a cruise to the equatorial Pacific in which iron, in the form of ferrous sulfate, would be added directly to a 8 x 8 km section of the upper ocean and the effect would be compared with stations outside of the fertilized area. On that cruise we deployed a pump and probe fluorometer in an attempt to document the changes in \(\Delta \phi_{\text{sat}}\) and other biophysical parameters. The results were dramatic; within 24 h following the addition of 2 nM iron, \(\Delta \phi_{\text{sat}}\) increased by over 50%, and the change in the quantum yield clearly was in response to the iron alone (Kolber et al., 1994). The experiment was repeated in 1996, using the fast repetition rate fluorescence system, and it became immediately clear that the changes in quantum yields were a 'leading' indicator of the physiological response (Behrenfeld et al., 1996) (Fig. 11). Simultaneously, laboratory studies on the mechanism for iron limitation of photochemical
energy conversion in PS II suggested that a restriction in the synthesis of hemes prevented cytochrome \textit{b}559 from forming functional reaction centers (Greene et al., 1992; Vassiliev et al., 1995; Fig. 12). Studies with \textsuperscript{59}Fe suggested that the rapid effect of iron on changes in the quantum yield of photochemistry could be attributed to the recruitment of previously synthesized protein subunits of the cytochrome, which could become inserted into the PS II reaction center to form functional units once iron was made available. Subsequent application of variable fluorescence methods have been used to follow iron fertilization experiments in the Southern Ocean, and to provide ‘diagnostic’ evidence of iron limitation of photosynthesis in the South Atlantic and Pacific Oceans (Gervais et al., 2001; Coale et al., 2004).

\textbf{VI. Fluorescence-based Estimates of Primary Production}

Calculations of primary production using Eq.17 are in good agreement with the uncertainties in estimates of net photosynthesis obtained using radiocarbon tracer techniques (Kolber and Falkowski, 1993) (Fig. 13). Uncertainties arise in the fluorescence based estimates primarily from variability in the \(n_{\text{RCI}}\) and the variability in distribution of Chl \(a\) between PS II and PS I. Assuming a constant Chl \(a/n_{\text{RCI}}\) ratio of 300 (mole/mole), we calculated primary production (mgC/day) over a 120 km long section across the Middle Atlantic Bight (Fig. 14). These results reveal a high level of spatial correlation between the Chl distribution and variable fluorescence measured by using FRR fluorescence, as well as between the photosynthetic yields and functional absorption cross section.

Measurements of photosynthetic properties, such as \(F_/F_o\) and the functional absorption cross-section, over spatial scales of hundreds of kilometers, further reveal strong correlation with temperature (Fig. 14A). This is not a direct effect, rather the change in tempera-

\textbf{Fig. 11.} The change in the photochemical quantum efficiency (\(F_/F_o\)) within the first 24 hours following the addition of iron in the eastern equatorial pacific. The inset shows the simultaneous change in the effective absorption cross section in PS II. (Behrenfeld et al., 1996.)

\textbf{Fig. 12.} (A) Whole cell and (B) Membrane associated protein profiles of iron replete and iron deficient cultures of the marine chlorophyte \textit{Dunaliella tertiolecta}. (C) Western blots showing the relative abundance of selected photosynthetic proteins. Note the over expression of the apoproteins of Cytochrome \textit{b}559 in the iron deficient cells. Upon addition of iron there is a rapid induction of cytochrome synthesis that leads to the formation of functional Photosystem II reaction centers.
Fig. 13. Correlation between fluorescence based estimates of photosynthetic electron transport inferred from equation 16 and that derived from radiocarbon based estimates of primary production for 55 discrete samples taken from the North Atlantic over a period of two years (after Falkowski et al., 1991).

Fig. 14. (A) Sea Surface Temperature (Temp), Photochemical yield (Fv/Fm) and functional absorption cross section (σ_{psii}) measured along 140 km long cruise track in Middle Atlantic Bight; (B) Net Primary Productivity (NPP) and Chl concentration along the same cruise track.
ture reflects mixing of cold, nutrient-rich waters into the upper ocean. The nutrients enhance photoconversion efficiency (Fig. 14A). The relationship between the temperature and the functional absorption cross section is much less clear, yet strong discontinuities are usually observed at temperature gradients. As a result, measured net primary production (Fig. 14B) is negatively correlated with temperature, and relatively independent of the Chl concentration.

VII. Applications of Variable Fluorescence in Benthic Ecosystems

Many oxygenic photoautotrophs live on or in sediments in coastal areas. These photosynthetic activities are poorly understood and usually ignored. The development of SCUBA (self-contained underwater breathing apparatus)-based FRR fluorometers allowed for fluorescence yields and photosynthetic parameters to be measured in situ in a variety of benthic organisms, including seagrass, macrophytes, algal turfs, and corals (Gorbunov et al., 2000). The instruments acquire fluorescent parameters and an image of the target simultaneously. The former is extremely valuable in assessing the impact of environmental factors, such as irradiance, nutrients, temperature etc., on the photosynthetic performance, while the latter is for post-dive morphological examination of sampled targets and species identification. Physical parameters, such as in situ irradiance, temperature and depth, are simultaneously recorded by the sensors incorporated within the instrument. A diver-operated instrument allows for more than a hundred targets to be sampled within a single dive (Gorbunov et al., 2000). A moored instrument is installed on a benthic platform to record a high-resolution temporal (from diel to monthly and seasonal) variability of target parameters (Gorbunov et al., 2001).

SCUBA-FRR fluorometry allows for specific biooptical signatures of benthic organisms to be identified and quantified. The variability in fluorescence yields and photosynthetic characteristics in four major functional groups of benthic organisms (corals, macroalgae, seagrass, and algal turfs on sediments) is illustrated in Fig. 15. To avoid modulation of fluorescence data by non-photochemical quenching, we analyzed measurements in a dark or low light adapted state. The analysis revealed that fluorescence yields are highly variable within each functional group and overlap between the groups (Fig. 15A). In contrast to fluorescence yields, the photosynthetic parameters \(F_e/F_m\) and \(\sigma_{PSII}\) are much more conserved and specific for each group of organisms. The combination of fluorescence yields and photosynthetic parameters provides signatures that are unique for each functional

![Fig. 15. Fluorescence and photosynthetic signatures of major functional groups of benthic photosynthetic organisms (Jacques et al. 1979). (A) The effective absorption cross sections of PSII \(\sigma_{PSII}\) plotted as a function of Chl fluorescence yield; (B) \(\sigma_{PSII}\) as a function of the quantum efficiency of light utilization in PSII \(F_e/F_m\). Note four clusters that represent different types of benthic organisms.](image-url)
group (note four clusters in Fig. 15 representing different groups of benthic organisms). Below we analyze the range and sources of variability in fluorescence and photosynthetic characteristics within individual groups of benthic organisms.

A. Corals

In corals, the quantum yields of Chl fluorescence vary by a factor of ~3 between species (Fig. 16), with lower values in shallow corals (*M. areolata, Favia f., P. astreoides*) and generally higher values in deep reef corals (*Agaricia sp., Colpophyllia sp., Diploria sp., Montastrea spp., and Scolamia*). At the same time, ~ two-fold variability in fluorescence yields occurs even within common species.

Like leaves of terrestrial plants, the majority of benthic organisms are optically thick and absorb most, or at least a significant fraction, of the incident visible irradiance. The fluorescence emitted from deep layers of the tissue may be re-absorbed, that decreases the measured quantum yield of Chl a fluorescence. Therefore, the observed quantum yields of fluorescence are primarily determined by the molecular quantum yield and the extent of fluorescence re-absorption. Our measurements of fluorescence lifetimes in a variety of corals and isolated zooxanthellae showed that the molecular quantum yields of Chl fluorescence at the F_o level inversely correlate with F_o/F_m ratio, thus reflecting the general relationship between photosynthetic efficiency and Chl a fluorescence. (For further discussion of lifetime of fluorescence measurements, see Chapter 19, Bruce and Vasil'ev and Chapter 21, Gilmore.)

The narrow range of variability of F_o/F_m in corals (Fig. 16) suggests that the molecular quantum yields in dark-adapted corals are rather invariant. In contrast, the re-absorption of emitted fluorescence is highly variable, thus being the main factor controlling the observed quantum yields in dark-adapted organisms.

In contrast to fluorescence yields, both F_o/F_m ratio and \( \sigma_{PSII} \) vary within a very narrow range in ‘healthy’ corals (Fig. 16), both being statistically independent of fluorescence yields and species. Two natural processes were found to affect F_o/F_m ratios, deviating them from the ‘normal’ values. First, chronic photoinhibition, that develops in shallow (< 2m depth) waters, decreases F_o/F_m by 10% to 15% (Gorbunov et al., 2001). Second, elevated temperatures and subsequent coral bleaching reduce F_o/F_m by 30% to 50% (Warner et al., 1999; Lombardi et al., 2000; Lesser and Gorbunov, 2001).

B. Algal Turfs and Macroalgae

Algal turfs exhibit the highest \( \sigma_{PSII} \) and high photosynthetic efficiency (F_o/F_m averages 0.50). Fluorescence intensities vary by an order of magnitude, depending on algal density. Seagrasses exhibit the lowest \( \sigma_{PSII} \) and very high photosynthetic efficiency (F_o/F_m = 0.65 to 0.70), except in senescent and dead leaves (F_o/F_m = 0.25 to 0.4). Macroalgae are characterized by the highest level of variability in both fluorescence yields and photosynthetic efficiency, that appears to be due to variations in the nutrient status of these benthic organisms.

VIII. Aerobic Anoxogenic Phototrophs

Aerobic anoxogenic phototrophs (AAPs) were discovered in the late 1970s in the coastal waters of Tokyo Bay, Japan (Harashima et al., 1978; Shiba et al., 1979). These organisms contain type-2 BChl a containing reaction centers similar to those of purple non-sulfur bacteria; however, they are strict aerobes, requiring oxygen for both growth and photosynthesis (Yurkov and Beatty, 1998). Moreover, contrary to anaerobic photosynthetic bacteria they contain only small amounts of the reaction centers, and appear to be unable of photoautotrophic growth as they require a source of organic carbon (Kolber et al., 2001). Until recently, AAPs were thought to be confined to small, organic-rich ecological niches such as beach sands and stones, tidal zones, stromatolites and seaweed surfaces. Using an infrared FRR (IRFRR) instrument, equipped with 880 nm detector, and excitation sources at 470 nm and 790 nm, we detected fluorescence transients, characteristic of bacterial photosynthesis, throughout the eastern equatorial Pacific (Kolber et al., 2000). Our laboratory measurements with marine AAP isolates revealed high values (0.8) of F_o/F_m ratios and rather small effective absorption cross-sections \( \sigma_{BC} = 40-43 \text{Å}^2 \) at 470 nm. Such a small cross-section, and the lack of an absorption band at 790–800 nm suggest the absence of the outer photosynthetic antennae (LH2) in these organisms.

Oceanic AAPs are capable of light-dependent CO_2 fixation (Kolber et al., 2001). Using IRFRR fluorometry, we found that the rates of light-dependent CO_2 fixation in laboratory-grown AAP isolates are relatively low, averaging at about 0.43 mol C/mol RC/s, with a maximum quantum yield of about 1% (mol carbon/mol quanta absorbed). These yields are about an order of magnitude lower than in phyto-
Fig. 16. Depth dependence of chlorophyll fluorescence yields at minimum ($F_o$) and maximum ($F_m$) levels, the functional absorption cross section of PS II ($\sigma_{PSII}$), the maximum quantum yield of photochemistry in PS II ($F_o/F_m$), and time constant for photosynthetic electron transport in PS II ($\tau_{Qe}$) in the coral *M. cavernosa*. Right axis - the maximum in situ irradiance at noon, estimated from SCUBA-based measurements with a hand-held photon detector.
plankton. From these measurements, we estimated the daily cellular rates of CO₂ fixation at about 0.08 fmol of carbon, or 3% of the cellular content. As the cells were grown in 2–20 mM organic medium, maintaining specific growth rates of four per day, CO₂ fixation contributed to about 1% of the total carbon anabolism. In the open ocean, however, where organic carbon is three orders of magnitude less abundant, the relative contribution of the CO₂ fixation may be significantly higher. Nevertheless, the potential contribution of AAPs to the oceanic carbon cycle is determined by their ability to supplement, or substitute respiration with the light-driven generation of ATP and reductants for carbon anabolism, preserving the existing organic carbon. Isolates of oceanic AAPs grown in carbon limited chemostat under a light/dark cycle displayed about two times higher biomass accumulation compared to heterotrophs. Such a mechanism of light-enhanced preservation of the organic carbon will affect the extent of the new production in the upper ocean, given that AAPs are abundant within the microbial community.

IX. Concluding Remarks

The human experience is closely tied to terrestrial ecosystems, but the oceans contain the evolutionary history of a continuous ecological parade of photosynthetic organisms that have acclimated and adapted to, and ultimately transformed, the geochemistry of Earth. Photosynthesis is a constant participant in the ongoing stream of key biogeochemical and ecological processes. Biological oceanographers have spent decades trying to elucidate the major ecological factors that control photosynthetic processes in the contemporary ocean. The tools they have traditionally appropriated, including radiocarbon based measurements of primary production, and solvent-extracted measurements of pigments, have, over the past decade, been supplemented by satellite observations of ocean color and solar stimulated fluorescence, and extremely sensitive active fluorometers that permit exploration of biophysical and physiological responses to changes in oceanic conditions in real-time. This revolution in ocean sciences will inevitably continue; however, the power of fluorescence techniques in revealing mechanisms and processes in the photosynthetic apparatus in marine phototrophs is just beginning to be appreciated. The future of these techniques will be most profound in connecting subcellular, indeed, molecular processes to global scale biogeochemical cycles.

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