

Measurement of photosynthetic parameters in benthic organisms in situ using a SCUBA-based fast repetition rate fluorometer

Abstract—Benthic photoautotrophic organisms significantly contribute to the productivity of shallow tropical coastal ecosystems. However, measurements of photosynthetic light use and dissipation in benthic organisms are complicated by taxonomic diversity, spatial heterogeneity, natural variability in local nutrient, irradiance, and temperature regimes, as well as destructive sampling protocols. To help overcome these problems, we developed a SCUBA-based fast repetition rate (FRR) fluorometer for measurements of variable chlorophyll fluorescence in corals, sea grasses, macroalgae, and algal turfs. Photosynthetic light use and electron transport can be readily calculated from variable fluorescence kinetics. Using the SCUBA-based FRR fluorometer, changes in photosynthetic processes can be measured nondestructively in situ with high spatial and temporal resolution. Here we describe the instrument design and characteristics and present representative field results.

Benthic photoautotrophic marine organisms, including corals, sea grasses, macroalgae, and algal turfs, are among the most productive photosynthetic organisms in aquatic ecosystems (Larkum 1983; Falkowski and Raven 1997). However, measurements of photophysiological responses generally require destructive sample manipulations and are limited in spatial and temporal coverage. Moreover, systematic sampling of the myriad species comprising these local communities is impractical, or impossible in some protected areas. To overcome these obstacles, we developed a SCUBA-based fast repetition rate (FRR) fluorometer with an autofocus imaging system. The instrument permits measurements of an extensive suite of photosynthetic parameters (see *Notation Table*) based on fluorescence transients induced by a sequence of brief subsaturating flashes (Kolber et al. 1998). Here we describe the concept of the SCUBA-based FRR fluorometer and present field data that characterize photosynthetic performance of representative taxa of benthic organisms.

Instrument description—The SCUBA-based FRR instrument (Fig. 1) uses a bank of 80 high-luminosity blue light-emitting diodes (LED NLPB300, Nichia Chemical Industries) to excite chlorophyll fluorescence at 460 nm with a 30-nm bandwidth. Excitation light is focused on the target using a Fresnel lens (focal length 5 cm), with a spot size of 15 mm. A computer-controlled light-emitting diode (LED) driver circuit generates a sequence of flashlets with a pulse duration from 0.5 to 2 μ s and an interval of 2.5 μ s to 1 ms. Operating at a pulsed current of 300 mA per LED, the instrument generates about 0.7 W cm⁻² of optical power density at a distance of 3 cm from the output window.

The fluorescence signal is collected from a central, 6-mm diameter, portion of the illuminated target, isolated by a red long-pass filter (RG665, Schott) and an interference filter

Notation

FRR	Fast repetition rate (fluorometry)
PSII	Photosystem II
σ_{PSII}	Functional absorption cross section for PSII (\AA^2)
F_o, F_m	Minimum and maximum yields of Chl <i>a</i> fluorescence measured after dark adaptation (relative units)
F_v	Variable fluorescence ($=F_m - F_o$)
F_v/F_m	Maximum quantum yield of photochemistry in PSII, measured on dark-adapted samples, dimensionless
p	“Connectivity factor” defining the exciton energy transfer between individual photosynthetic units, dimensionless
τ_{Qa}	Time constant for photosynthetic electron transport on the acceptor side of PSII (Q_a reoxidation) (s)
F', F_m'	Steady-state and maximum yields of Chl <i>a</i> fluorescence measured at ambient light (the prime character indicates the measurements are made under ambient light), relative units
$\Delta F'/F_m'$	Quantum yield of photochemistry in PSII measured under ambient light
E	Irradiance ($\mu\text{mol quanta m}^{-2} \text{s}^{-1}$)
P_f	Rate of photosynthetic electron transport through PSII (e s^{-1})
P^{max}	Maximum rate of electron transport (e s^{-1})
α	Initial slope of the P_f - E curve ($\text{\AA}^2 \text{e quanta}^{-1}$)
E_k	Light-saturation parameter ($\mu\text{mol quanta m}^{-2} \text{s}^{-1}$)
$1/\tau$	Maximum turnover rate of photosynthesis (s^{-1})

(S10–680-R, Corion), and detected by an avalanche photodiode module (APD C5460, Hamamatsu). A small portion of the excitation light is recorded by a PIN photodiode as a reference signal. Both the fluorescence and reference signals are integrated over a single excitation pulse by dual switched integrators (ACF2101, Burr-Brown) and digitized by 12-bit analog-to-digital converters (LTC1410, Linear Technology). By using an avalanche photodiode as a detector and integrating the fluorescence signal in the analog mode, the instrument operates with a high signal-to-noise ratio, allowing the acquisition of fluorescence transients in a single excitation sequence (see Fig. 2), i.e., quasi-instantaneously, a criterion of crucial importance for SCUBA diving operations.

The excitation protocols and data acquisition are controlled by an embedded digital signal processing (DSP) circuit based on an ADSP-2181 microprocessor (Analog Devices) interfaced to a PC/104 computer board (486DX4 100 MHz, Ampro Computers). Up to 2,000 fluorescence transients can be stored in the on-board flash memory card (PCMCIA Type II 40 Mb, SanDisk). A compact black and white video camera (V-1210, Marshall Electronics) is incorporated into the instrument, allowing the diver to monitor the target in real time. A frame grabber (CX-100–30, Imagegenation Vision System Specialists) captures the image simultaneously with the fluorescence measurements. A liquid

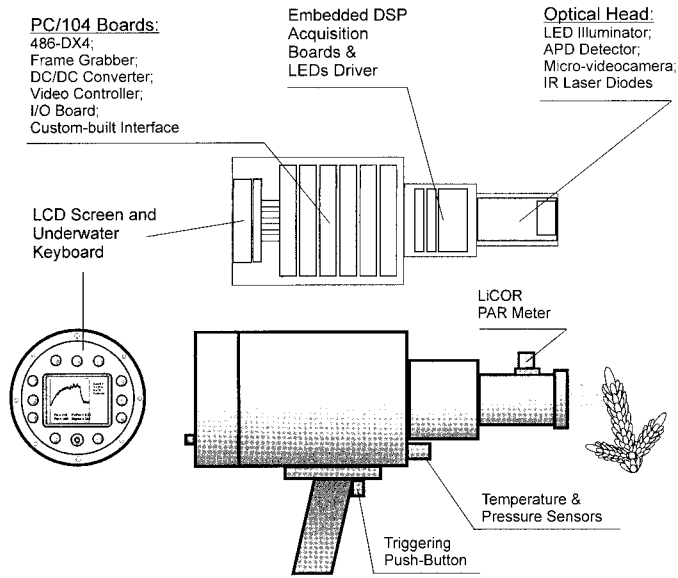


Fig. 1. Schematic block diagram of the SCUBA-based fast repetition rate fluorometer.

crystal display (LCD) screen installed on the front panel displays both the video signal from the video camera and the RGB (red-green-blue) signal from the on-board PC. A pair of orthogonal, off-axis infrared laser diodes (wavelength 780 nm, optical power 5 mW, Power Technology) are incorporated in the viewfinder to precisely control the optimal distance to the target, which is 3 cm from the output window. Using an underwater keyboard comprising 12 piezo keys (7020, Tschudin & Heid Inc.), a diver manipulates the instrument and annotates the acquired data. Photosynthetically available radiation (PAR), temperature, and depth are simultaneously measured by a LiCor 2π underwater quantum meter, a thermistor, and a pressure gauge, all incorporated within the instrument.

Results—SCUBA-based FRR fluorometers were used during the coastal benthic optical properties (CoBOP) field program at Lee Stocking Island, in the Bahamas, during 1998 and 1999 May and 1999 January to study a variety of benthic organisms, including corals, sea grasses, algal turfs, and sediments. Over 2,000 measurements were obtained both in situ and in laboratory sea tables. Typical FRR fluorescence profiles measured on a zooxanthellate coral, *Montastraea cavernosa*, the sea grass, *Thalassia testudinum*, algal turf on the sediment surface, and the brown macroalga, *Stytopo-*

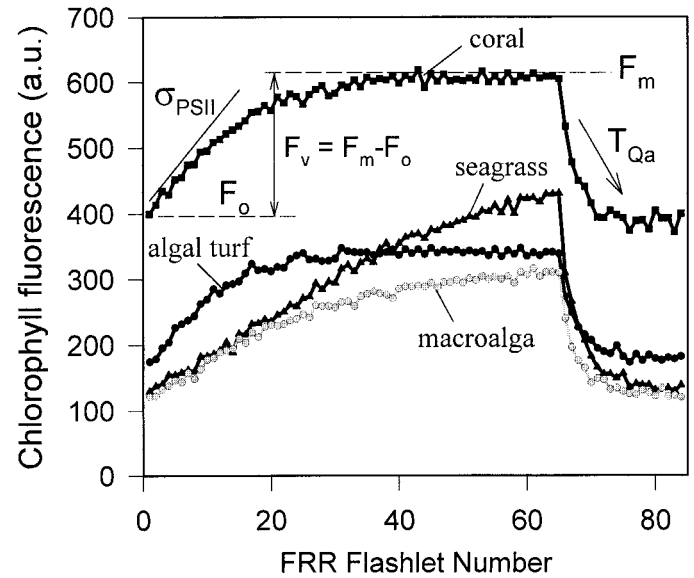


Fig. 2. Chlorophyll fluorescence transients measured with the FRR protocol on the zooxanthellate coral, *Montastraea cavernosa*, algal turf on sediment, the sea grass, *Thalassia testudinum*, and the brown macroalga, *Stytopodium zonale*. On the first phase (saturation protocol) a series of 64 subsaturating flashlets of $1.5 \mu\text{s}$ duration was used to cumulatively saturate PSII within $150 \mu\text{s}$. A magnitude of the rise in fluorescence yield is determined by the quantum yield of photochemistry in PSII (F_v/F_m), while the rate of the fluorescence rise is proportional to the functional absorption cross section of PSII (σ_{PSII}). Upon cessation of the saturation protocol, the fluorescence yield decreases, reflecting kinetics of electron transfer on the acceptor side of PSII. A series of weak flashes of $0.5 \mu\text{s}$ duration at 100 to $800 \mu\text{s}$ intervals was used to monitor the relaxation kinetics.

dium zonale, are shown in Fig. 2. The corresponding photosynthetic parameters, calculated for these FRR profiles using the procedure described in Kolber et al. (1998), are presented in Table 1. These results indicate that different benthic photoautotrophs have characteristic photosynthetic signatures. Zooxanthellae, the symbiotic dinoflagellates living in coelenterate hosts, are characterized by slightly lower (relative to algal turfs) functional absorption cross sections and low quantum yields of photochemistry ($F_v/F_m \approx 0.38$). The low photosynthetic efficiency of zooxanthellae leads to inherently high quantum yields of chlorophyll *a* fluorescence; the F_o level in corals is about threefold higher than in sea grasses and macroalgae (see Table 1). Interestingly, energy transfer between PSII units (the *p* parameter in Table

Table 1. Fluorescent and photosynthetic parameters calculated from the FRR profiles presented in Fig. 2. Standard deviations characterize precision of the fitting procedure for a single flash protocol.

	F_v/F_m	$\sigma_{\text{PSII}} (\text{\AA}^2)$	F_o (a.u.)	F_m (a.u.)	<i>p</i>	$\tau_{\text{Qa}} (\mu\text{s})$
Coral	0.38 ± 0.01	410 ± 20	380 ± 5	610 ± 5	0.15	530 ± 100
Algal turf	0.55 ± 0.01	530 ± 20	160 ± 5	350 ± 2	0.30	475 ± 80
Seagrass	0.73 ± 0.01	190 ± 10	125 ± 5	455 ± 5	0.55	560 ± 50
Macroalga	0.63 ± 0.01	230 ± 10	115 ± 4	320 ± 4	0.45	480 ± 70

1) is extremely low in zooxanthellae, suggesting that reaction center density is small relative to antenna size (Falkowski and Raven 1997). The algal turf, comprising in this study area of cyanobacteria, dinoflagellates, and diatoms, have the highest σ_{PSII} and moderately high photochemical energy conversion efficiency ($F_v/F_m = 0.55$) (Table 1). Finally, sea grasses and macroalgae have small functional cross sections but very high quantum yields of photochemistry in PSII (F_v/F_m up to 0.73). Such a high level of variability in photosynthetic parameters among benthic organisms reflects differences in adaptations to nutrient acquisition, photoinhibition, and possibly the molecular architecture of the photosynthetic apparatus.

Nutrient availability and ambient irradiance are natural factors that directly affect photosynthetic activity in the aquatic ecosystems (Kolber and Falkowski 1993; Kolber et al. 1994; Falkowski and Kolber 1995; Behrenfeld and Kolber 1999). Under nutrient-replete conditions, the F_v/F_m ratio averages at 0.65 in a wide variety of phytoplankton species, independent of growth irradiance (Falkowski and Kolber 1995). Nutrient (e.g., nitrogen or iron) limitation leads to a characteristic decline in F_v/F_m , accompanied by a rise in the F_o fluorescence level (Kolber et al. 1988). In zooxanthellae isolated from corals and cultivated in nutrient-replete media, we measured F_v/F_m values ranging from 0.62 to 0.66, i.e., similar to nutrient-replete phytoplankton (Kolber et al. 1988). The F_v/F_m ratio decreased to ca. 0.4 as the zooxanthellae were grown under nitrogen-limited conditions (Gorunov unpubl. data). Measurements in a wide variety of zooxanthellate corals revealed an average F_v/F_m of 0.39 ± 0.07 ($n = 350$), well below the level characteristic for nutrient-replete cells.

While photoinhibition of PSII reaction centers is a second natural factor that may reduce F_v/F_m in situ (Baker and Bowyer 1994), in zooxanthellate corals F_v/F_m values were always <0.5 , even when the corals were growing on shaded sides of deep reefs (maximum irradiance about $50\text{--}100 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$). These results suggest that the low efficiency of photochemical energy conversion in PSII is primarily a consequence of nutrient deficiency of zooxanthellae in *hospitace* (Falkowski et al. 1993).

In contrast to zooxanthellate corals, the high quantum yields of photochemistry in sea grasses suggest an absence of nutrient limitation or photoinhibition. Presumably, in these organisms, the presence of a true root system helps maintain a supply of nutrients, while the low functional absorption cross section reduces the probability of photoinhibition. The highest level of variability in the quantum yields of photochemistry in PSII was observed in macroalgae where the F_v/F_m ratio varied from 0.50 up to 0.75. As the functional cross sections are rather low in macroalgae, the variability in fluorescence efficiency in the organism may reflect environmental variability in nutrient status rather than photoinhibition.

Variable fluorescence is affected by ambient irradiance both directly, via photochemical processes, and indirectly, via nonphotochemical energy dissipating reactions. FRR fluorescence profiles measured on the zooxanthellate coral, *Montastraea faveolata*, at various levels of ambient irradiance are shown in Fig. 3A. Corresponding light-induced

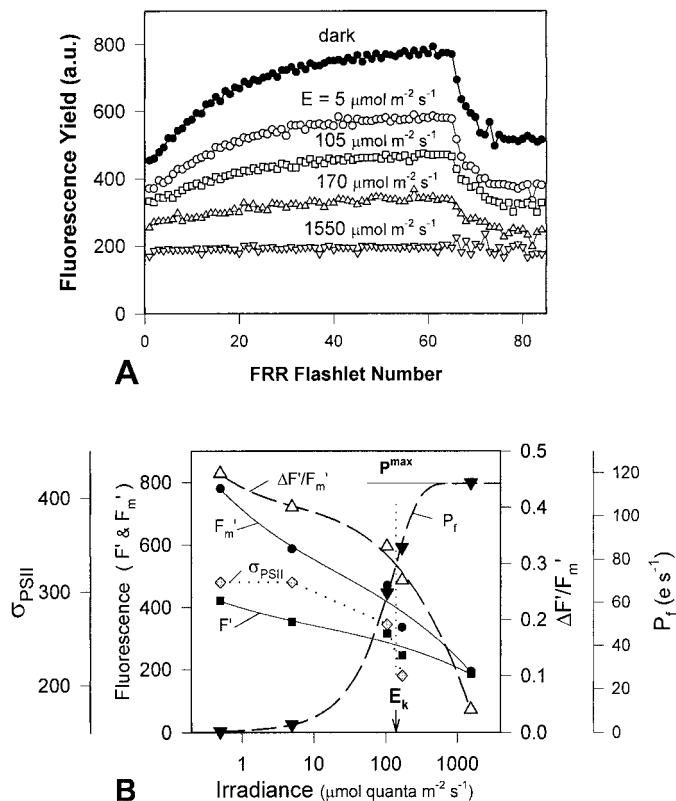


Fig. 3. (A) FRR fluorescent transients measured in the coral, *Montastraea faveolata*, at various levels of ambient light. (B) Light-induced changes in photosynthetic parameters calculated from the FRR profiles.

changes in the photosynthetic parameters are presented in Fig. 3B. As the ambient light intensity increased, both steady-state (F') and maximum (F'_m) fluorescence yields decreased due to nonphotochemical quenching. This quenching phenomenon, resulting from thermal deactivation of the absorbed excitation energy in the light-harvesting antennae, is associated with a reduction in σ_{PSII} . As ambient irradiance increased, the variable component of fluorescence ($\Delta F' = F'_m - F'$) also declined as the probability of finding a closed reaction center increased. This resulted in a reduction in the apparent quantum yield of photochemistry measured under ambient irradiance, $\Delta F'/F'_m$ (Fig. 3B). Under supra-optimal irradiance (\approx ca. $1000 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$), when photosynthetic electron transport in PSII becomes light saturated, fluorescence yields decline as a consequence of photoinhibitory damage to the reaction centers. Our in situ measurements suggest that under full noontime solar radiation in situ ($\approx 1000 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$) up to 30% of the reaction centers of PSII in symbiotic corals are transiently damaged (i.e., down regulated, Baker and Bowyer 1994). The photodamaged reaction centers are repaired in the afternoon and evening (data not shown).

The rates of photosynthetic electron transport can be calculated from fluorescence-derived parameters measured as a function of ambient irradiance (Kolber and Falkowski 1993). The rate of electron transport through PSII is given by

$$P_f = E \times \sigma_{\text{PSII}} \times \Delta F'/F'_v, \quad (1)$$

where the symbols are defined in the Notation Table. Fitting the P_f versus E curve (Fig. 3B) with a model dependence $P_f = f(E, \alpha, P_{\text{max}})$ permits calculation of the initial slope of the P_f - E curve (α) and the maximum rate of electron transport (P_{max}) (Webb et al. 1974; Jassby and Platt 1976; Platt et al. 1980; Falkowski and Raven 1997). While α can be calculated directly from fluorescence information, P_{max} cannot. The light-saturated rate of electron transport can be estimated from knowledge of the maximum rate of photosynthetic electron transport ($1/\tau$), which in turn is calculated from the light-saturation parameter, E_k . By definition,

$$E_k = P_{\text{max}}/\alpha; \quad (2)$$

$(1/\tau)$ can be derived from fluorescence parameters using the relationship (Falkowski 1992)

$$1/\tau = E_k \times \sigma_{\text{PSII}}. \quad (3)$$

As an example, using the data presented in Fig. 3, the following values of the photosynthetic parameters were calculated for PSII reaction centers for *M. faveolata*: $\alpha = 135 \text{ \AA}^2 \text{ e quanta}^{-1}$, $P_{\text{max}} = 115 \text{ e s}^{-1}$, $E_k = 140 \text{ \mu mol quanta m}^{-2} \text{ s}^{-1}$, and $1/\tau = 240 \text{ s}^{-1}$. For *T. testudinum* the following values were obtained: $\alpha = 152 \text{ \AA}^2 \text{ e quanta}^{-1}$, $P_{\text{max}} = 415 \text{ e s}^{-1}$, $E_k = 475 \text{ \mu mol quanta m}^{-2} \text{ s}^{-1}$, and $1/\tau = 530 \text{ s}^{-1}$.

While understanding the patterns of diel variability of fluorescence-derived photosynthetic parameters provides a basis for modeling daily primary production in coastal environments, our results suggest that SCUBA-based FRR fluorometry can also be used to monitor the physiological status of coral reefs in situ and for studying the dynamics of reef response to environmental changes. The precision of fluorescence measurements achieved with the instrument described allows one to follow minute variations in photosynthetic processes nondestructively in real time, making this technology an efficient and ecologically benign diagnostic tool for monitoring the physiological status of zooxanthellate corals and other benthic organisms. By analogy with active fluorescence techniques applied to phytoplankton and terrestrial vegetation, we anticipate that the application of SCUBA-based FRR technology will help identify the early indications of harmful modifications or stresses to benthic photosynthetic organisms prior to the appearance of macroscopic changes in the organisms or community structure.

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