

Photosynthesis and photoprotection in symbiotic corals

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Abstract

In zooxanthellate corals, excess excitation energy can be dissipated as heat (nonphotochemical quenching), thereby providing protection against oxidative damage by supraoptimal light in shallow reefs. To identify and quantify the photoprotective mechanisms, we studied the diel variability of chlorophyll fluorescence yields and photosynthetic parameters in situ in corals, using moored and SCUBA-based fast-repetition-rate fluorometers. The results reveal that nonphotochemical quenching is triggered prior to saturation of photosynthetic electron transport by down-regulation of the reaction centers of Photosystem II (PSII). This process dissipates up to 80% of the excitation energy. On a sunny day in shallow waters, the daily integrated flux of photons absorbed, and subsequently dissipated as heat, is ~4 times that used for photosynthesis. Fluorescence quenching is further accompanied by a slight reduction in the functional absorption cross section for PSII that results from thermal dissipation of excitation energy in the light-harvesting antennae. These two processes are highly dynamic and adjust to irradiance changes on timescales consistent with the passage of clouds across the sky. Under supraoptimal irradiance, however, up to 30% of PSII reaction centers become photoinhibited, and these are repaired only after several hours of low irradiance. In shallow corals, between 10% and 20% of the reactions centers are chronically photoinhibited and appear to remain permanently nonfunctional throughout the year. Our results establish, for the first time, the suite of biophysical mechanisms that optimize photosynthesis while simultaneously providing photoprotection in symbiotic corals in situ.

In zooxanthellate corals, light is essential for growth of the organisms, yet the light environment of the algal symbionts is dictated primarily by where the animal host lives (Falkowski et al. 1990). Although there is considerable genetic variability within the algal symbionts (Rowan 1998), clonal cultures of the symbiotic dinoflagellates are not readily distinguishable on the basis of their photosynthetic attributes (Chang et al. 1983; Iglesias-Prieto and Trench 1994; Kinzie and Falkowski unpubl. data). Thus, to first order, photosynthetic performance does not appear to be a critical determinant of phenotypic selection. Since the irradiance regimes experienced by zooxanthellate associations are

inevitably variable (for review, *see* Falkowski et al. 1990), zooxanthellae must be “euryphotic”—that is, capable of acclimating to a wide range of irradiance levels (Falkowski and Dubinsky 1981). Although the ability to photoacclimate (on timescales of ≥ 1 month) to low light may govern the ecological distributions of symbiotic corals, rapid (approximately minutes to hours) photoacclimation is required for photoprotection against supraoptimal light in shallow waters. How does this come about in nature?

A wide variety of protective mechanisms have been identified that dissipate excess excitation energy to heat (nonphotochemical quenching) in aquatic photoautotrophs (Falkowski and Raven 1997). Although nonphotochemical quenching has been the subject of intense studies for ~2 decades, the biophysical mechanisms at a molecular level remain largely unknown (Li et al. 2000). All photoprotective processes appear to suppress oxidative damage to the photosynthetic apparatus—but at a cost—they simultaneously lower the quantum yield of photosynthesis (Long et al. 1994). One photoprotective mechanism is associated with the xanthophyll cycle in light-harvesting complexes (reviewed by Demmig-Adams and Adams 1996). The light-stimulated conversion of diadinoxanthin to diatoxanthin has been observed in many species of Bacillariophyceae, Chloromonadophyceae, Chrysophyceae, Euglenophyceae, Xanthophyceae, and Dinophyceae (Demers et al. 1991; Olaizola

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et al. 1994; Falkowski and Raven 1997), including endosymbiotic dinoflagellates (Brown et al. 1999). This conversion leads to a competing energy dissipation pathway within the antenna that simultaneously reduces the functional absorption cross section of the reaction centers (Genty et al. 1990; Falkowski et al. 1994). Another nonphotochemical quenching mechanism is attributed to enhanced thermal dissipation in the Photosystem II (PSII) reaction centers themselves (Weis and Berry 1987; Schweitzer and Brudvig 1997). This phenomenon leads to a down regulation of photochemistry; the reaction center temporarily ceases to operate but is not actually damaged (i.e., restoration of activity does not require de novo synthesis of proteins and, hence, proceeds rapidly, on a timescale of minutes). Finally, irradiance-induced phosphorylation of light-harvesting complexes can induce migration of a fraction of the complexes from PSII to PSI, thereby diverting a portion of excitation energy away from PSII (the so-called State-1 to State-2 transitions) (for review, see Falkowski and Raven 1997).

Photoinhibitory damage to the reaction centers of PSII occurs when the capacity of these protective mechanisms is exhausted. Classically, photoinhibition results from the degradation of the PSII reaction center protein, D1, which is normally repaired within a few hours (Kyle et al. 1984; Prasil et al. 1992). The damaged reaction centers continue to trap excitation energy but dissipate it as heat (Krause 1988). The extended exposure of the photosynthetic apparatus to excessive light can lead to even more severe degradation of PSII, referred to as "chronic photoinhibition," because of its very slow recovery (approximately days or longer) (Krause 1988; Osmond 1994). Chronic photoinhibition has been observed in low-light acclimated plants exposed to irradiance levels far in excess of those experienced during normal growth or during normal light levels but under unfavorable environmental conditions, such as low or high temperature, limited CO₂ supply, or nutrient limitation (see Osmond 1994 for review).

The present study identifies and quantifies the mechanisms responsible for light regulation and photoprotection in endosymbiotic corals. We recorded the in situ diel variability of a comprehensive suite of fluorescence and photosynthetic parameters, using a moored fast repetition rate (FRR) fluorometer. In a series of field experiments, we examined the irradiance dependence of these parameters and their relaxation kinetics after removal from bright sunlight. Our goals are (1) to identify the major regulatory and protective mechanisms, (2) to distinguish between thermal dissipation of excessive excitation energy in the light-harvesting antennae and reaction centers, (3) to identify dynamic and/or chronic photoinhibition, and (4) to explain the interplay of these processes and the realized quantum efficiencies of photosynthesis in zooxanthellate corals.

Materials and methods

Field site and collection of corals—Field studies were performed at two sites near Lee Stocking Island (23°46' N, 76°05' W), Bahamas, during the Coastal Benthic Optical Properties field experiment. The first data were acquired in

shallow waters (2 m depth), where in situ irradiance reached 1,600–1,800 $\mu\text{mole quanta m}^{-2} \text{s}^{-1}$ at 12:00 h. At the second site, Horseshoe Reef (10 m depth), the maximum recorded in situ irradiance (400–700 nm) during the measurements was $\sim 650 \mu\text{mole quanta m}^{-2} \text{s}^{-1}$. Water temperature ranged from 24 to 26°C in January and 28 to 31°C in May.

Laboratory measurements of fluorescent and photosynthetic parameters were performed on samples of *Montastraea faveolata*, *M. cavernosa*, and *Porites astreoides* collected at both sites in May 1998 and May 1999. Corals were placed in aquaria with running seawater, where the measurements were made with an FRR fluorometer, as described by Gorbunov et al. (2000). The aquaria were exposed to natural sunlight. To simulate different light regimes, one aquarium was screened to limit the maximum irradiance to a level of $\sim 400 \mu\text{mole quanta m}^{-2} \text{s}^{-1}$, and the second one was illuminated by full sunlight with maximum irradiance of $\sim 2,000 \mu\text{mole quanta m}^{-2} \text{s}^{-1}$. In situ measurements were also made on coral heads of *M. faveolata*, *M. cavernosa*, *P. astreoides*, *P. porites*, *Diploria strigosa*, and *Manicina areolata* in January and May 1999. All FRR measurements were made on top south-facing coral surfaces.

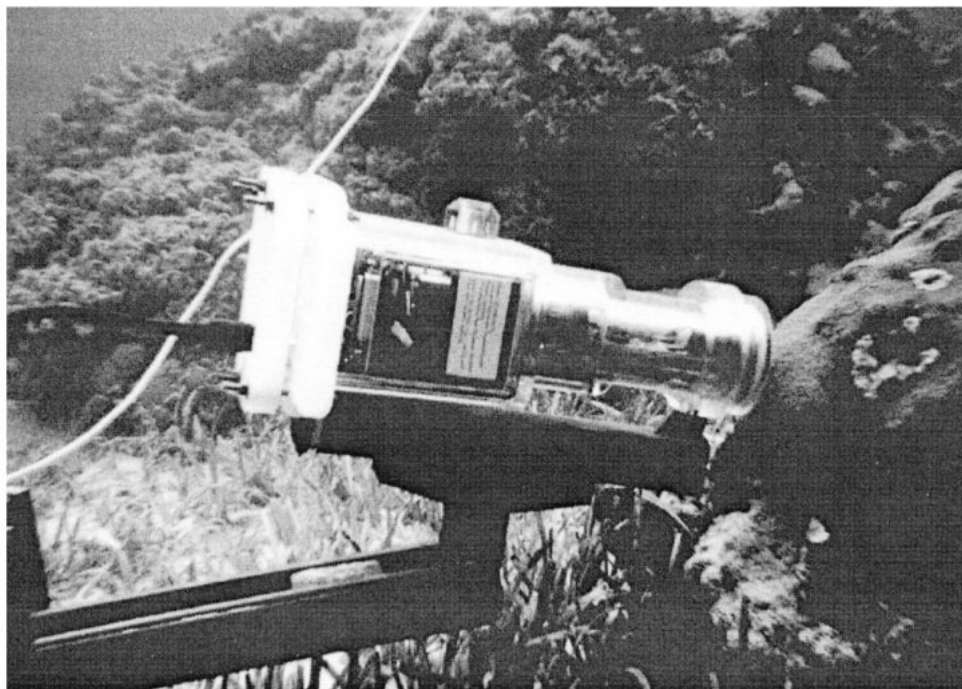
Irradiance measurements—During in situ diel series, irradiance was recorded continuously by use of a top-facing photosynthetically active radiation (PAR) sensor incorporated into the FRR instrument (Gorbunov et al. 2000). Simultaneously, the normal to coral surface irradiance was measured several times a day by a separate PAR 2π quantum sensor. These measurements allowed a cosine correction factor and sun altitude/azimuth function to be calculated over the course of a day. When this function was used, the continuously recorded PAR was converted to the PAR component normal to the coral surface. This normal PAR component is given below.

FRR fluorescence measurements—Chlorophyll fluorescence and photosynthetic parameters were measured by use of two modified SCUBA-based FRR fluorometers: a diver-operated instrument (Gorbunov et al. 2000) for measurements in laboratory aquaria, and a moored instrument (Fig. 1) for prolonged (daily to monthly) operation in situ.

FRR fluorometry records chlorophyll fluorescence transients induced by a series of subsaturating flashes that cumulatively saturate PSII within $\sim 100 \mu\text{s}$ —i.e., a single photochemical turnover (Kolber et al. 1998). The FRR technique permits rapid measurements of a suite of fluorescent and photosynthetic parameters (Table 1).

In photosynthesis, the energy of absorbed light may be used for photochemical conversion, dissipated to heat, or reemitted as fluorescence. The quantum efficiencies of the three pathways sum to unity, but each is strongly controlled by ambient irradiance.

The maximum (i.e., potential) quantum yield of photochemistry in PSII is determined in a dark-adapted state as the ratio $F_v/F_m = (F_m - F_o)/F_m$ (e.g., see Butler 1972). As irradiance increases, the quantum yield of ongoing photochemistry in PSII decreases because of a gradual increase in thermal energy dissipation and can be assessed from $\Delta F'/F_m'$ (Genty et al. 1989).



10 cm

Fig. 1. Moored Fast Repetition Rate Fluorometer, developed for recording in situ temporal (from diel to seasonal) variability of fluorescence yields and photosynthetic activity in benthic photosynthetic organisms. The instrument is installed on a benthic platform near the coral head of *M. faveolata*, for monitoring diel cycles.

Nonphotochemical quenching is classically defined as the increase in thermal dissipation induced by excess light, which, by inference, results in quenching of F_m fluorescence. Several parameters have been used elsewhere to quantify nonphotochemical quenching, including the coefficient of nonphotochemical quenching, q_N (e.g., see van Kooten and Snel 1990) and the nonphotochemical quenching parameter, $NPQ = (F_m - F_m')/F_m'$ (Bilger and Bjorkman 1990). Here, we use the ratio $(F_m - F_m')/F_m$, which gives a straightforward estimate of the portion of thermally dissipated photon flux (i.e., the quantum yield of nonphotochemical quenching). This calculation is analogous to that used to derive the quantum yield of photochemistry from variable fluorescence.

The rate of photosynthetic electron transport per PSII reaction center is given by

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

where both σ_{PSII}' and $\Delta F'/F_v'$ are functions of irradiance (E). When nonphotochemical quenching is caused by thermal dissipation in the light-harvesting antennae, the light-induced decline in σ_{PSII}' is directly proportional to that in F_v'/F_m' (see "Irradiance dependence of fluorescent and photosynthetic parameters" in the Results section):

$$\sigma_{\text{PSII}}' = \sigma_{\text{PSII}} \times [(F_v'/F_m')/(F_v'/F_m)], \quad (2)$$

In this case, Eq. 1 can be modified to

$$P_f = E \times \sigma_{\text{PSII}} \times [(\Delta F'/F_m')/(F_v'/F_m)], \quad (3)$$

where $\Delta F'/F_m'$ is the only irradiance-dependent variable.

On an organismal level, the rate of photosynthetic electron transport per unit area of coral surface can be estimated by

$$P = C_a \times E \times (\Delta F'/F_m'), \quad (4)$$

where the factor C_a represents the average portion of incident photon flux absorbed by the coral surface (i.e., absorptivity) and averages ~ 0.9 (i.e., 90%) in the studied corals (C. Mazel pers. comm.).

The flux of photons absorbed and subsequently dissipated thermally by nonphotochemical quenching is given by

$$D_{\text{npq}} = C_a \times E \times [(F_m - F_m')/F_m]. \quad (5)$$

Results

In situ measurements of diel cycles—At the shallow sampling site (2 m depth), the diel variations of fluorescence yields were driven by oscillations in ambient light (Fig. 2). Although nocturnal variability was absent, the diurnal patterns of F' and F_m' were generally characterized by a decrease in the morning, a minimum at noon, and a reciprocal increase in the afternoon (Fig. 2A). F' declined by a factor of 2–3 during the day, and F_m' declined by a factor of 4–5. Under clear skies, the diurnal patterns were roughly sinu-

Table 1. Notation.

PSII	Photosystem II
σ_{PSII}	Functional absorption cross section of PSII (\AA^2) in a dark-adapted state
σ_{PSII}'	Functional absorption cross section of PSII in a light-adapted state (the prime indicates that the measurements are made under ambient light)
F_o, F_m	Minimum and maximum yields of Chl <i>a</i> fluorescence measured in a dark-adapted state (relative units)
F_v	Variable fluorescence ($=F_m - F_o$)
F_v/F_m	Maximum quantum yield of photochemistry in PSII, measured in a dark-adapted state (dimensionless)
p	“Connectivity factor,” defining the exciton energy transfer between individual photosynthetic units (dimensionless)
F_o', F', F_m'	Minimum, steady-state, and maximum yields of Chl <i>a</i> fluorescence measured under ambient light, relative units
$\Delta F'$	Change in the fluorescence yield measured under ambient light ($=F_m' - F'$)
F_v'	Variable fluorescence measured under ambient light ($=F_m' - F_o'$)
$\Delta F'/F_v'$	Coefficient of photochemical quenching characterizing the fraction of open reaction centers in a light-adapted state
$\Delta F'/F_m'$	Quantum yield of photochemistry in PSII, measured under ambient light [$= (F_m' - F')/F_m'$] (dimensionless)
F_o'/F_m'	Quantum efficiency of photochemistry in open reaction centers of PSII, measured in a light-adapted state [$= (F_m' - F_o')/F_m'$] (dimensionless)
P^{max}	Maximum rate of photosynthetic electron transport, normalized to unit area of coral surface ($\mu\text{mol } e \text{ m}^{-2} \text{ s}^{-1}$)
D_{npq}	Flux of photons absorbed and subsequently dissipated thermally ($\mu\text{mol } e \text{ m}^{-2} \text{ s}^{-1}$)
E_k	Light-saturation parameter ($\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$)

soidal and symmetrical relative to local noon; with clouds present, however, fluorescence intensities were characterized by deep oscillations that were in synchrony with fluctuations in irradiance (Fig. 2A).

The in situ measurements, conducted with high temporal resolution, indicated a slight ($\sim 10\%$) rise in F' early in the morning and late in the afternoon (at moderate irradiances of 150–200 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$) (arrows in Fig. 2A). This response is strongly indicative of the accumulation of dynamically closed reaction centers. However, with an increase in irradiance above $\sim 200 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$, both F' and F_m' declined because of progressive nonphotochemical quenching (Fig. 3A). The decline in these two parameters paralleled a reduction in the quantum yield of photochemistry ($\Delta F'/F_m'$) (Fig. 3B). Consequently, the quantum yield of nonphotochemical quenching, $(F_m - F_m')/F_m$, increased, reaching maximum values of ~ 0.7 – 0.8 at $\sim 1,000 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ (Fig. 3B).

With an increasing irradiance, the rate of photosynthetic electron transport in PSII, P_f , became saturated at $\sim 500 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ (Fig. 3C). In *M. faveolata*, the light-saturation parameter, E_k , was $\sim 300 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ (Fig. 3C). The maximum values of P_f were slightly higher

in the afternoon than in the morning (Fig. 2E). Nonphotochemical quenching showed an opposite trend (not shown), revealing that a lower level of thermal dissipation is sufficient for photoprotection when photosynthetic efficiency increases. Under clear skies, the maximum value of P_f at 1200 h was slightly lower than that in the morning or afternoon (e.g., 1st day in Fig. 2E), indicating partial photoinhibition of photosynthesis by full sunlight. The daily integrated photosynthetic electron transport during a sunny day (Fig. 2E) was 3.5 mole $e \text{ m}^{-2} \text{ d}^{-1}$, which is not significantly different than that during a cloudy day (3.8 mole $e \text{ m}^{-2} \text{ d}^{-1}$).

Although the flux of photons used for photosynthesis, P_f , was saturated under supraoptimal irradiance, the flux of photons dissipated thermally, D_{npq} , increased markedly with increasing irradiance (Fig. 3C). The maximum level of D_{npq} was ~ 8 times higher than the maximum rate of photosynthetic electron transport, P^{max} . Consequently, the daily integrated flux of photons dissipated thermally was ~ 3 times higher during a sunny day than during a partly cloudy day (15.3 mole $\text{m}^{-2} \text{ d}^{-1}$ vs. 5.7 mole $\text{m}^{-2} \text{ d}^{-1}$, respectively).

At sunrise (but not sunset), at ~ 1 – $10 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$, $\Delta F'/F_m'$ exhibited a slight ($\sim 20\%$) reversible decrease (arrows in Fig. 2B), which was accompanied by $\sim 10\%$ quenching of F_m' without a detectable change in F' (Fig. 2A). Such a pattern was also observed in other corals, *M. cavernosa* and *P. astreoides*. This “morning reduction” in $\Delta F'/F_m'$ is likely due to a time lag in the activation of the Calvin cycle.

During field measurements, we observed an unexpected diel pattern in the functional absorption cross section of PSII (Fig. 2C). Although σ_{PSII} exhibited no nocturnal variability, the diurnal patterns had two distinctive features. First, σ_{PSII}' showed a rapid increase of 30% at sunrise and a reciprocal decrease at sunset. The increase in σ_{PSII}' occurred at very low light (~ 1 – $5 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$) and was accompanied by a slight reduction in F_m' without a change in F' (Fig. 2A). Second, when irradiance rose above $\sim 500 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$, σ_{PSII}' declined in parallel with a decrease in $\Delta F'/F_m'$ (not shown).

Rapid changes in σ_{PSII} at sunrise and sunset (Fig. 2C) were correlated with variations in the efficiency of energy transfer between PSII units (Fig. 2D). The “connectivity factor” was very low at night, drastically increased at sunrise, and returned to low values after sunset (Fig. 2D). Thereby, the increase in energy transfer between PSII units was directly proportional to change in σ_{PSII}' (Fig. 4).

At the second sampling site (Horseshoe Reef, 10 m depth), fluorescence yields exhibited much lower variability than in shallow water. F_m' did not change (within 5%–10%) during the day (not shown), implying the absence of nonphotochemical quenching even at the highest observed irradiance (400 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$). F' , however, increased $\sim 20\%$, with an increase in irradiance due to a reduction in the photochemical quenching efficiency, suggesting that $\sim 25\%$ of the active PSII reaction centers became dynamically closed at an irradiance of 400 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$. Because of a slight reduction in photochemical quenching, $\Delta F'/F_m'$ declined only to 0.32 on the first day (irradiance at 1200 h 400 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$) and to 0.38 on the second day (150–200 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$), indicating that pho-

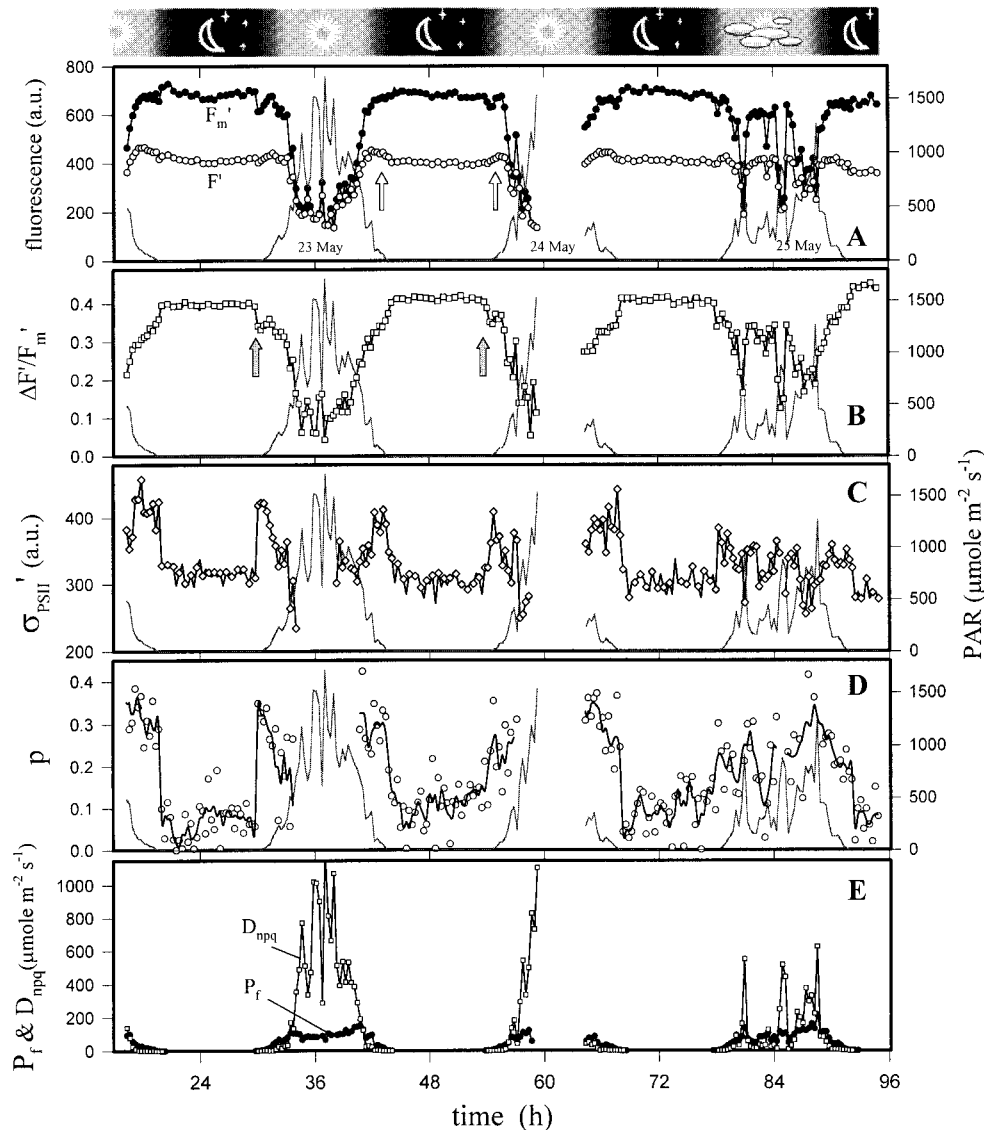


Fig. 2. Diel cycles of (A) fluorescence at steady-state (F') and maximum (F_m') levels, (B) the quantum yield of photochemistry in PSII ($\Delta F'/F_m'$), (C) functional absorption cross section for PSII, (D) energy transfer between PSII units ("connectivity factor"), and (E) the flux of photons absorbed and subsequently utilized for photosynthesis (P_f) and dissipated as heat (D_{npq}). The patterns were measured in situ on the reef-building coral, *M. faveolata* (see Fig. 1), in shallow water (2 m depth). Gray line = variations in in situ PAR.

tosynthetic electron transport was not saturated, even at local 1200 h (not shown). In contrast to shallow water corals, the fraction of excitation energy dissipated thermally was negligible, compared with the flux used for photosynthesis.

Relaxation of nonphotochemical quenching—To quantify the contributions of thermal energy dissipation in the pigment bed and in the reaction centers to protection from excess sunlight in symbiotic zooxanthellae, we measured the kinetics of fluorescence recovery in samples of *M. faveolata* and *M. cavernosa*. The samples were preadapted to a midday irradiance of $\sim 1,500 \mu\text{mole quanta m}^{-2} \text{s}^{-1}$ in the course of natural diurnal cycles, then the recovery in fluorescence was

monitored under low light ($\sim 1\text{--}2 \mu\text{mole quanta m}^{-2} \text{s}^{-1}$) for a period of 1.5 h.

An example of the recovery kinetics of F_v/F_m , σ_{PSII} , and fluorescence yields in *M. faveolata* is shown (Fig. 5). Two components in the relaxation of F_v/F_m appear (Fig. 5A). The fast component ($t_1 = 2.5 \text{ min}$) correlated with a recovery in σ_{PSII} (Fig. 5B), whereas the slow phase ($t_2 = 50 \text{ min}$) proceeded without a change in σ_{PSII} (Fig. 5B). The long lifetime of this latter component is consistent with photoinhibitory damage to the reaction centers.

The fluorescence yields at both F_o and F_m levels also recovered rapidly (Fig. 5C), reaching maximum values typical of nighttime measurements in 15–20 min. The slow com-

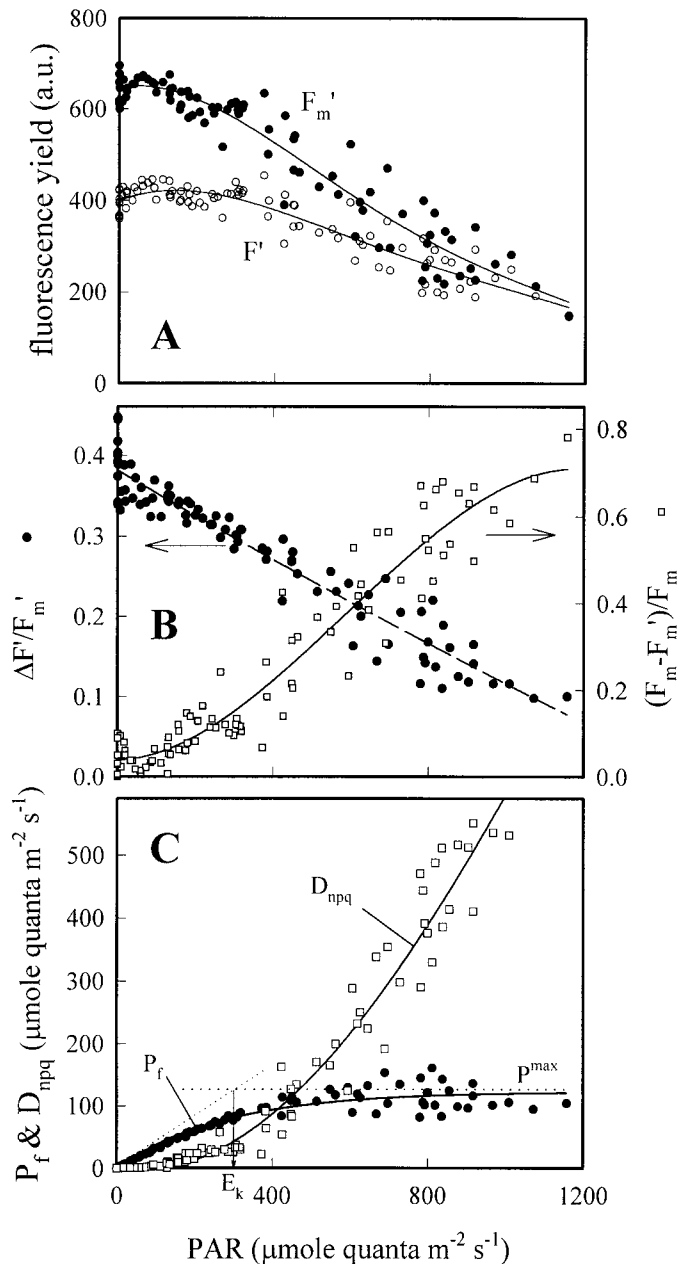


Fig. 3. Effect of irradiance on (A) the quantum yields of chlorophyll fluorescence at F' and F_m' levels, (B) the quantum yield of photochemistry in PSII $\Delta F'/F_m'$ and nonphotochemical quenching $[(F_m - F_m')/F_m]$, and (C) the flux of photons absorbed and subsequently utilized for photosynthesis (P_f) and dissipated thermally (D_{npq}). The irradiance dependencies have been retrieved from the diel cycles of fluorescent parameters presented in Fig. 2.

ponent, observed in F_v/F_m relaxation (Fig. 5A) and associated with photoinhibition, was not detectable. The decrease of F_m' induced by full sunlight was significantly larger (3–4-fold) (Fig. 5C) than that of σ_{PSII}' (only ~30% in *M. faveolata* (Fig. 5B) and ~10% in *P. astreoides*; not shown).

Irradiance dependence of fluorescent and photosynthetic parameters—Fluorescence and photosynthetic parameters,

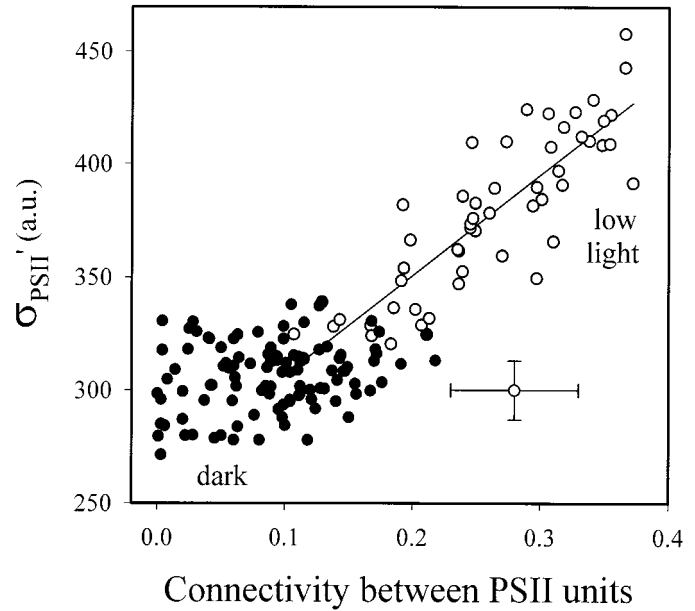


Fig. 4. Relationship between the functional absorption cross section for PSII (σ_{PSII}') and the energy transfer between PSII units ("connectivity factor") observed during the shift from dark (●) to low light (1–50 $\mu\text{mole quanta m}^{-2} \text{s}^{-1}$; ○). The data are adapted from the diel cycles for *M. faveolata* presented in Fig. 2. Standard deviations for both variables are plotted as error bars in the lower right corner.

measured as a function of irradiance (Fig. 6), provide further insight into the nature of nonphotochemical quenching. Irradiance, provided by a 100 W halogen lamp, was increased gradually from 0 to ~1,500 $\mu\text{mole quanta m}^{-2} \text{s}^{-1}$, and, at each light level, the parameters were recorded at steady state after adaptation to this level for 5 min. To measure minimum fluorescence F_o' , the ambient light was turned off for 2–5 s, allowing the reaction centers to reopen. No detectable photoinhibition was observed, even at the highest irradiance; the measured parameters recovered completely within ~10 min after dark adaptation. Therefore, the observed patterns represent the effect of rapidly reversible nonphotochemical quenching.

Nonphotochemical quenching of F_m developed at irradiance levels $>200 \mu\text{mole quanta m}^{-2} \text{s}^{-1}$ and was paralleled by a decline in $\Delta F'/F_m'$. The decline in photosynthetic quantum yield was due to a decrease in the fraction of open reaction centers and an increase in the quantum yield of nonphotochemical quenching $(F_m - F_m')/F_m$ (Fig. 6). As irradiance increased to ~600 $\mu\text{mole quanta m}^{-2} \text{s}^{-1}$, σ_{PSII}' did not change, suggesting that nonphotochemical quenching originated in the reaction centers of PSII. At higher irradiance, antennae quenching complemented thermal dissipation in the reaction centers, as evidenced from a reduction in σ_{PSII}' .

Chronic photoinhibition—In symbiotic corals, the quantum yield of PSII is almost always less than optimal, as a consequence of nutrient limitation of zooxanthellae in hospite, regardless of the irradiance regime in which the coral lives. During the day, F_v/F_m may be further reduced because

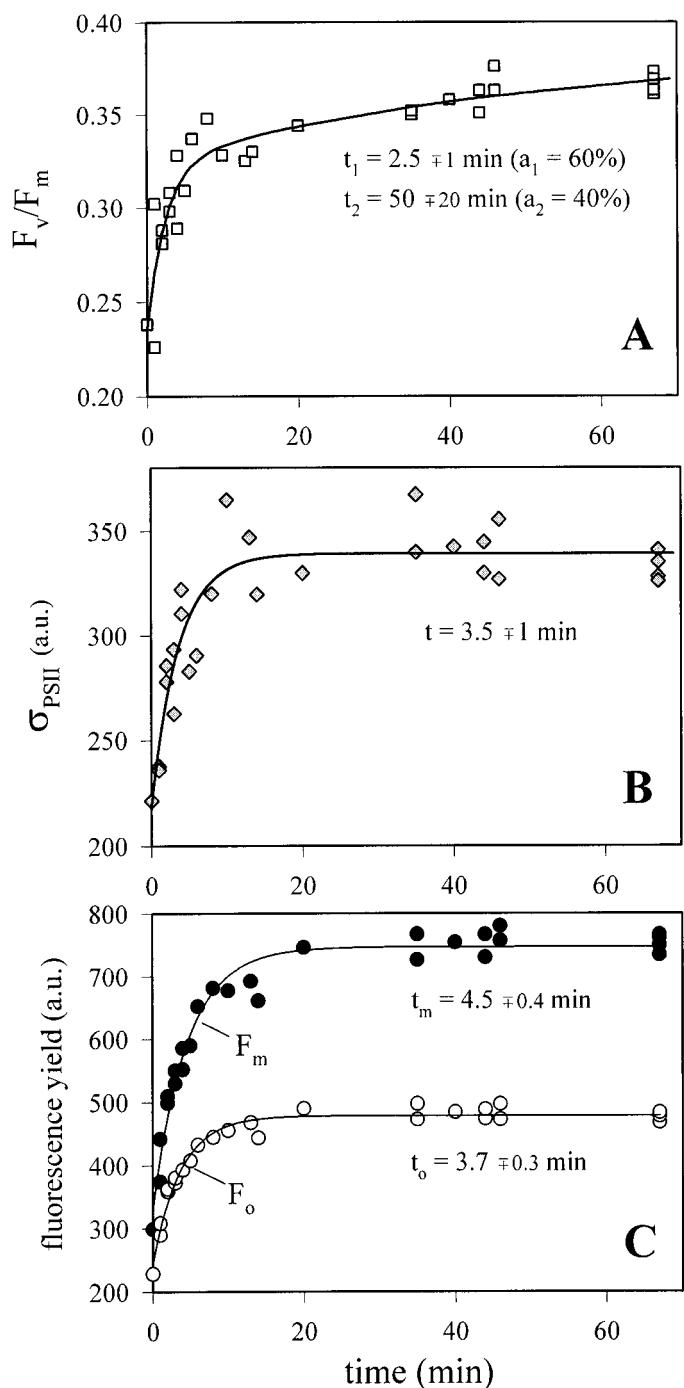


Fig. 5. Recovery kinetics of photosynthetic parameters: (A) quantum yield of photochemistry in PSII (F_v/F_m), (B) the functional absorption cross section for PSII (σ_{PSII}), and (C) fluorescence yields at minimum (F_o) and maximum (F_m) levels. The recovery was measured at low light ($1\text{--}2 \mu\text{mole quanta m}^{-2} \text{s}^{-1}$) at 1200 h in a sample of *M. faveolata* acclimated to $1500 \mu\text{mole quanta m}^{-2} \text{s}^{-1}$ before the measurements. Results of a two exponential fit for F_v/F_m and a single exponential fit for σ_{PSII} , F_o , and F_m are plotted with line.

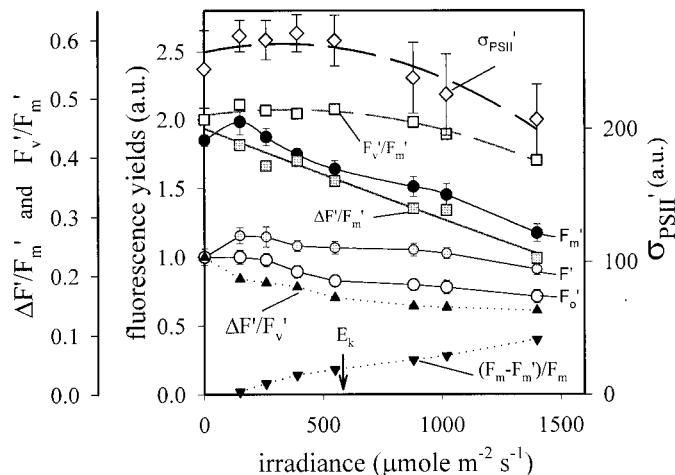


Fig. 6. Effect of irradiance on steady-state chlorophyll fluorescence yields (F_o' , F' , and F_m'), variable fluorescence (parameters $\Delta F'/F_m'$ and F_v'/F_m'), functional absorption cross section for PSII (σ_{PSII}), the coefficient of photochemical quenching ($\Delta F'/F_v'$) and the quantum yield of non-photochemical quenching ($(F_m - F_m')/F_m$). The patterns were measured in the coral *M. cavernosa* collected at 5 m depth. The scale for both $\Delta F'/F_v'$ and $(F_m - F_m')/F_m$ is the same as for fluorescence yields.

of dynamic photoinhibition. Here we distinguish between these two factors and true, chronic photodamage. Because of its extremely slow relaxation, chronic photoinhibition can be inferred in situ from a comparison of F_v/F_m measured at night, preceded by a sunny or a cloudy day. In *M. faveolata*, the night values of F_v/F_m averaged at 0.46 ± 0.02 ($n = 26$) when the preceding days were cloudy and decreased to 0.41 ± 0.03 ($n = 50$) after sunny days (Fig. 7A). This decrease was significant (t -test; $P < 0.001$) and was accompanied by a 6%–8% increase in F_o fluorescence (not shown), an indicator of chronic photoinhibition (Krause 1988; Osmond 1994). Such variations in night values of F_v/F_m and F_o have been observed on a coral head of *M. faveolata* in the summer of 1999, during three months of continuous measurements with a moored FRR fluorometer (not shown). The decrease in F_v/F_m by full sunlight persisted ($\pm 1\%$ – 2%) throughout the night (e.g., Fig. 2B) and recovered by the next night only when the following day was cloudy. Thus, the recovery from chronic photoinhibition proceeded very slowly (>20 h) and only in the presence of moderate light.

Under the assumption of full recovery from chronic photoinhibition after a cloudy day, the fraction of chronically damaged reaction centers can be estimated as a difference between $(F_v/F_m)_{\text{cloudy}}$ and $(F_v/F_m)_{\text{sunny}}$, normalized to $(F_v/F_m)_{\text{cloudy}}$ (superscripts indicate the night measurements following a cloudy or sunny day, respectively). In *M. faveolata*, this difference shows that 10% of the reaction centers were chronically damaged by daily exposure to full sunlight (Fig. 7B).

Further evidence for chronic photoinhibition in shallow-coral zooxanthellae comes from a comparison of night values of F_v/F_m measured in corals growing on the open (i.e., sun-exposed) and shaded sides (3–4-fold lower irradiance) of the reef. Our in situ measurements on the corals *P. as-*

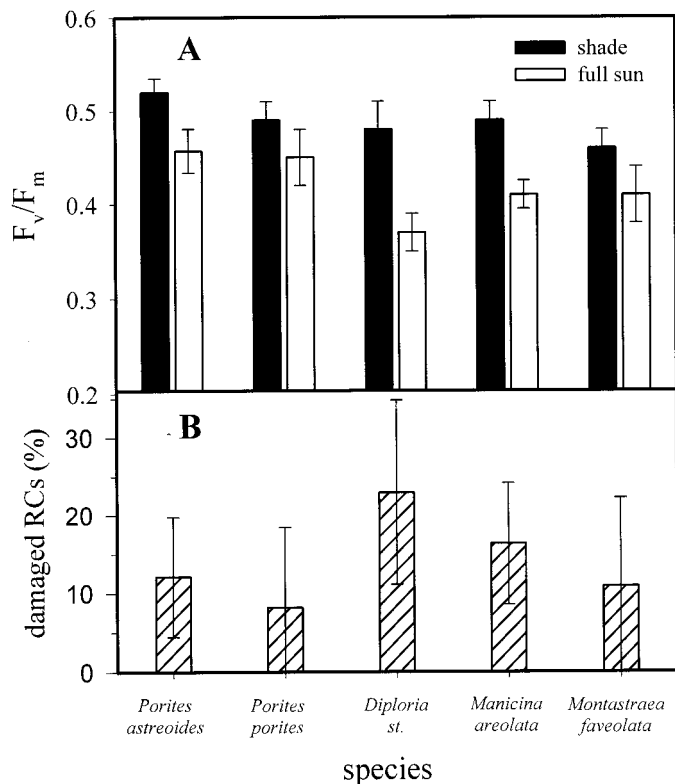


Fig. 7. Effect of chronic photoinhibition on the maximum quantum efficiency of PSII (F_v/F_m) in the corals living in shallow waters. (A) The night values of F_v/F_m in the corals exposed to full sunlight throughout the previous day and in the corals growing in shade with a 3–4 times lower irradiance. (B) The fraction of PSII reaction centers (RCs) being damaged by chronic photoinhibition was calculated as a difference between $(F_v/F_m)^{\text{shade}}$ and $(F_v/F_m)^{\text{sun}}$, normalized to $(F_v/F_m)^{\text{shade}}$ (superscripts indicate the night measurements on shaded and top-facing (sunny) sides of a reef).

treoides, *P. porites*, *Diploria strigosa*, and *M. areolata* (Fig. 7A) showed that daily exposure to full sunlight led to a 10%–15% reduction (*t*-test; $P < 0.005$) in the night values of F_v/F_m .

Discussion

This study provides direct evidence that a range of biophysical mechanisms are involved in light regulation of the photosynthetic activity of endosymbiotic dinoflagellates and the photoprotection of their photosynthetic apparatus against excess light (summarized in Table 2). Our in situ measurements support observations elsewhere that thermal dissipation in the pigment bed, associated with the xanthophyll cycle (light-induced conversion of diadinoxanthin to diatoxanthin; Brown et al. 1999) is responsible for photoprotection. However, down-regulation of the reaction centers of PSII appears to be more significant in thermal dissipation (Table 2). Under supraoptimal irradiance, dynamic photoinhibition also contributes to photoprotection.

Rapidly reversible nonphotochemical quenching—Under supraoptimal irradiance ($\geq 1,000 \mu\text{mol quanta m}^{-2}\text{s}^{-1}$), the

Table 2. Summary of photosynthetic events and photoprotective mechanisms in symbiotic dinoflagellates.

Event	Molecular site	Threshold irradiance	Relaxation time	Estimated energetic contribution to photoprotection
Saturation of photosynthetic electron transport in PSII	Plastoquinone pool	full saturation at $E \approx 2^* E_k$, where E_k is a light-saturation parameter	milliseconds	
Nonphotochemical quenching through down-regulation of PSII reaction centers	Presumably accumulation of Chl_a^{+*}	starts at $E \leq E_k$	minutes	50%–80%
Nonphotochemical quenching in the light-harvesting antennae (accompanied by a decrease in σ_{PSII})	Xanthophyll cycle (light-stimulated conversion of diadinoxanthin to diatoxanthin)	when $E \geq 2^* E_k$	minutes	10%–30%
Dynamic photoinhibition	Damage to D1 protein in PSII	$E \geq 1000 \mu\text{mol m}^{-2} \text{s}^{-1}$	~30–150 min	$\leq 10\%$
Chronic photoinhibition	Degradation of D1 and (probably) other PSII proteins	$E \geq 1500 \mu\text{mol m}^{-2} \text{s}^{-1}$	~30–50 h	0

* Schweitzer and Brudivig (1997).

quantum yield of nonphotochemical quenching ($(F_m - F_m')/F_m$) increases to 0.7–0.8 (Fig. 3B), indicating that up to 80% of absorbed photon flux dissipates as heat. As a consequence, in shallow waters under clear skies, the daily integrated flux of photons dissipated thermally is 4–5 times higher than that used for photosynthesis. The rapid variation of fluorescence yields observed under partly cloudy skies (Fig. 2) clearly suggest that this mechanism is highly dynamic.

Such behavior in chlorophyll fluorescence has been reported in terrestrial plants under rapid sunfleck-stimulated changes in nonphotochemical quenching (Adams et al. 1999), implying a common photoacclimation strategy. Rapid induction of thermal dissipation provides the photoprotection necessary to prevent oxidative damage to the photosynthetic apparatus, whereas its relaxation permits a rapid recovery of the efficiency of photosynthetic energy conversion.

Thermal dissipation in the pigment bed, coupled with a xanthophyll cycle, is thought to be the dominant mechanism of nonphotochemical quenching in higher plants (Demmig-Adams and Adams 1996; Li et al. 2000) and most non-phycobilin-containing algae (Falkowski and Raven 1997). Biophysical models predict that fluorescence quenching should be accompanied by a proportional decrease in σ_{PSII} (Genty et al. 1990; Falkowski et al. 1994). In contrast, thermal dissipation in the reaction center should decrease fluorescence without a change in σ_{PSII} (Falkowski et al. 1994; Vassiliev et al. 1995). With the recent observation of a xanthophyll conversion in corals (Brown et al. 1999), the mid-day decline in σ_{PSII} (Fig. 2C) suggests that the antenna quenching contributes to photoprotection.

However, the irradiance-induced decrease of σ_{PSII} in zooxanthellae is much lower than that in plants (Genty et al. 1990) and free living phytoplankton (Vassiliev et al. 1994). Moreover, under moderate irradiance ($\leq 500 \mu\text{mole quanta m}^{-2} \text{ s}^{-1}$), quenching of F_m proceeds without reduction in σ_{PSII} (Fig. 6), implying that nonphotochemical quenching starts with down-regulation of the reaction centers of PSII.

Quenching of maximum fluorescence (F_m') is directly proportional to a reduction in the quantum yield of photochemistry in PSII ($\Delta F'/F_m'$) (Fig. 6). Hence, the quantum yield of nonphotochemical quenching, $(F_m - F_m')/F_m$, is roughly equal to the parameter 1, $[(\Delta F'/F_m')/(F_v/F_m)]$, which characterizes the saturation of photosynthetic electron transport in PSII. Therefore, the level of thermal dissipation is directly driven by the extent to which the photosynthetic electron transport is saturated at any given irradiance.

In phytoplankton growing in nutrient-replete media, non-photochemical quenching is triggered when electron transport is almost fully ($\sim 90\%$) saturated (Falkowski et al. 1986). As irradiance rises, fluorescence at the steady-state level (F') increases significantly (approximately twofold) before it starts to decline because of progressive nonphotochemical quenching (Falkowski et al. 1986; Gorbunov and Chekalyuk 1992; Kolber and Falkowski 1993). In contrast, symbiotic dinoflagellates in hospite show only a slight increase in F' at intermediate irradiance (Fig. 2A), followed by a drastic decrease of F' at higher irradiances. Such fluorescence patterns suggest that nonphotochemical quenching

in zooxanthellae is triggered well before the photosynthetic electron transport is saturated (Fig. 3).

Dynamic and chronic photoinhibition in situ—Two types of photoinhibition can be distinguished on the basis of their recovery times. The first, dynamic photoinhibition is attributed to transient damage to the reaction centers of PSII, recovers relatively rapidly (on a timescale from tens of minutes to several hours), and can serve as a *photon protection* mechanism. The second, chronic photoinhibition is associated with much more severe damage to PSII, recovers extremely slowly, and is interpreted as *photon damage*. Although dynamic photoinhibition occurs widely in phytoplankton (Falkowski et al. 1994), macroalgae (Hanelt et al. 1993; Franklin et al. 1996), and corals (Brown et al. 1999; Hoegh-Guldberg and Jones 1999; Ralph et al. 1999; this study), signs of chronic photodamage have previously been observed only in corals exposed to elevated temperatures (Jones et al. 1998; Warner et al. 1999) or intertidal corals under solar bleaching (Brown et al. 2000). Our data on a number of shallow corals clearly suggest that the daily exposure to full sunlight leads to chronic photoinhibition, even at moderate temperatures (24–29°C).

Dynamic photoinhibition can be quantified from the relaxation kinetics of variable fluorescence quenching (Fig. 5A). We interpret the slow phase in F_v/F_m relaxation (Fig. 5A) as a recovery from dynamic photoinhibition—thus, the fraction of damaged reaction centers is estimated from the amplitude of the slow component relative to the maximum F_v/F_m . In *M. faveolata* (Fig. 5), only 15% of PSII reaction centers were transiently damaged at an irradiance of $1,500 \mu\text{mole quanta m}^{-2} \text{ s}^{-1}$. In general, in samples of corals, *M. faveolata*, *M. cavernosa*, and *Porites astreoides*, the fraction of damaged centers ranges from 10% to 30% under irradiances of $\sim 1,500$ – $1,800 \mu\text{mole quanta m}^{-2} \text{ s}^{-1}$, implying that dynamic photoinhibition plays a minor role in the photoprotection in these corals.

A portion of the reaction centers may become chronically damaged and not be repaired by night (Fig. 7). The reduction in F_v/F_m and a proportional increase in F_o fluorescence suggest that chronically damaged centers are not capable of dissipating excitation energy into heat and, thus, do not provide photo protection.

Excitation energy transfer between reaction centers—When a fraction of reaction centers are transiently closed by ambient light, energy transfer between neighboring photosynthetic units provides efficient redistribution of excitons, thus protecting these centers from excess energy flux. The energy transfer between PSII units has traditionally been assessed from either the shape of a fluorescence induction curve (Joliot and Joliot 1964) or the efficiency of singlet-singlet exciton annihilation (Pailotin et al. 1983).

Our FRR fluorescence measurements, which are measurements of fluorescence induction on the microsecond timescale, suggest very low energy transfer between PSII units in dark-adapted zooxanthellae (Fig. 2D). The “connectivity factor” (parameter p in “Notations”) ranges from 0 to 0.15 in the intact corals, *M. cavernosa*, and *M. faveolata* (Gorbunov et al. 2000; present study) and in a variety of zoo-

xanthellae cultures isolated from corals (*P. porites* and *Montipora* sp.), clams (*Tridacna* sp.), sea anemone (*Aiptasia* sp.), and jellyfish (*Cassiopea*) (M.Y. Gorbunov unpubl. data). Although these values are well below 0.4–0.7 reported in phytoplankton species and plants, the connectivity factor in zooxanthellate corals increases to ~0.35 during the day (Fig. 2D). The increase in energy transfer between PSII units proceeds with a proportional increase in the functional absorption cross section of PSII (Fig. 4), implying a common biophysical mechanism.

We propose the following working model of the underlying mechanism for this transition. At night, the connectivity between PSII units is low, implying energetic segregation of the units in the thylakoid membrane. A transition to low light enhances the energy transfer between PSII units, stimulating energy exchange between active and inactive centers and, thus, increasing the apparent absorption cross section of the active centers. The extent of this increase depends on the fraction of inactive centers in the thylakoid membrane; for instance, a significant change in σ_{PSII} may occur only if this fraction is large. The fraction of inactive centers can be assessed from variable fluorescence measurements as $1 - [(F_v/F_m)/0.65]$, where 0.65 is the maximum F_v/F_m ratio observed in isolated zooxanthellae nutrient-replete cultures, measured when all reaction centers are active (Kolber and Falkowski 1993; Gorbunov et al. 2000). Under the assumption of $F_v/F_m = 0.42$ for *M. faveolata*, the fraction of inactive centers is 35%, which is large enough to explain the observed (~30%) change in σ_{PSII} .

Our results clearly establish multiple photoprotection strategies in zooxanthellate corals in situ. Such strategies mitigate against the loss of photosynthetic capacity in shallow-water coral reef ecosystems. Although photoinhibitory damage to the reaction centers of PSII occurs in shallow reef environments, the fraction of damaged centers appears to be low enough to prevent a significant reduction in maximum photosynthetic electron-transport rates. This reduction may be prevented by a compensatory increase in electron turnover rates in the remaining functional centers (Behrenfeld et al. 1998). Simply put, zooxanthellate corals exposed to supraoptimal irradiance levels on shallow reefs are not limited by the rate of production of organic matter, and, hence, a loss of photosynthetic electron transport capacity does not necessarily lead to a reduction in growth or net community production. Thus, after 200 million yr of evolution (Wells 1957), coral symbioses have adapted to persistent (i.e., chronic), low-level photodamage, without seriously compromising photosynthetic efficiency or, apparently, fitness.

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