Widespread Iron Limitation of Phytoplankton in the South Pacific Ocean

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Diel fluorescence patterns were discovered in phytoplankton sampled over 7000 kilometers of the South Pacific Ocean that appear indicative of iron-limiting growth conditions. These patterns were rapidly lost after in situ iron enrichment and were not observed during a 15,000-kilometer transect in the Atlantic Ocean where iron concentrations are relatively high. Laboratory studies of marine *Synechococcus* sp. indicated that the patterns in the South Pacific are a unique manifestation of iron limitation on the fluorescence signature of state transitions. Results suggest that primary productivity is iron limited not only throughout the equatorial Pacific but also over much of the vast South Pacific gyre.

Evaluating the response of biospheric photosynthesis to alterations in global climate requires an understanding of the geographic distribution of rate-limiting factors for primary production (*1*). Resolving such distributions in the open ocean has remained at the forefront of oceanographic research for nearly a century (*2*), but progress has suffered from the lack of physiological diagnostics for particular limiting factors that can be surveyed over vast regions. In situ enrichment experiments (*3–5*) and molecular probes (*6*) have now provided critical evidence of iron limitation in two high-nutrient, low-chlorophyll (HNLC) regions, but such techniques are expensive, labor intensive, and limited in their spatial and temporal coverage. Here we describe readily observable diel patterns in photosynthetic parameters that appear to be unique to iron-limited phytoplankton. Our results indicate that iron limitation is not only commonplace in the HNLC equatorial Pacific but also widespread throughout the low-nutrient South Pacific gyre, a vast region classically assumed to be nitrogen limited.

We evaluated photosynthetic parameters from variable fluorescence measurements (*7, 8*) conducted on phytoplankton collected during two studies in the South Pacific Ocean [OliPac transect, 16°S to 1°N 150°W (1900 km); IronExII transect, 11°S 136°W to 2°S 103°W (5000 km)] and one study in the Atlantic Ocean [AMT-1 transect, 48°N 10°W to 49°S 56°W (15,000 km)]. From these measurements, we derived initial (F_0) and maximal (F_m) fluorescence rates, photochemical quantum efficiencies [$F_v/F_m = (F_m - F_o)/F_m$], and functional absorption cross sections of photosystem II (σ_{PSII}) (*8*). During both the OliPac and the IronExII transects, we collected samples from the low-nutrient, low-chlorophyll South Pacific gyre (SPG) and the HNLC equatorial upwelling (EU) region. Flow cytometer (*9*) and size-fractionated fluorescence measurements identified *Prochlorococcus* sp. and *Synechococcus* sp. as prominent components of the prokaryote-dominated phytoplankton assemblages in the SPG and EU (*10*). Similar assemblages likewise populate central Atlantic gyres (*10*).

During OliPac, we observed an unexpected diel pattern in F_v/F_m throughout the upper 50 m of the water column, which was characterized by a rapid decrease at sunset followed by a reciprocal increase at sunrise. This pattern was further resolved during the IronExII transect and found to occur throughout the SPG and EU (Fig. 1). During each diel cycle, F_o and F_m exhibited large fluctuations (Fig. 1A) that, in part, reflected protective quenching processes commonly observed under saturating irradiances (*11*). F_v/F_m consistently decreased by 35% to 60% at sunset and subsequently recovered at sunrise, giving a pillared appearance to the transect profile (Fig. 1B). Nocturnal decreases in F_v/F_m were accompanied by similar decreases in σ_{PSII} in the SPG and portions of the EU (Fig. 1C) but were not correlated with changes in chlorophyll concentration.

Evidence that the nocturnal fluorescence changes were a consequence of iron limitation was first provided during the in situ iron-enrichment experiment conducted immediately after the IronExII transect (*4, 5*). The initial enrichment (2 nM iron) resulted in a rapid decrease in F_0 (Fig. 2A), a small decrease in F_m (Fig. 2B), and loss of the extensive nocturnal decreases in F_v/F_m (Fig. 2C) and σ_{PSII} (Fig. 2D). These responses occurred before any change in species composition. Outside the enrichment region, the same diel cycles observed throughout the South Pacific persisted (Fig. 2). These results indicate that the nocturnal decreases in F_v/F_m and σ_{PSII} were a consequence of iron limitation within the enrichment area and suggest that similar iron-limiting conditions prevail throughout the SPG and EU.

Across the Atlantic Ocean, midday decreases in F_v/F_m from photoinhibition (*12*) were often pronounced, but the large nocturnal decreases in F_v/F_m and σ_{PSII} observed in the South Pacific were absent (Fig. 1, E and F). On average, F_v/F_m in the Atlantic gyres—that is, those regions where phytoplankton assemblages are most comparable to those in the South Pacific (*10*)—decreased by ≈15% shortly after sunset and then gradually recovered during the night (Fig. 3A). As the aeolian flux of iron to the central Atlantic is one to two orders of magnitude greater than in the South Pacific (*13*), these results are consistent with an iron-dependent mechanism for the diel patterns in the South Pacific and a general lack of iron limitation in dominant phytoplankton of the Atlantic gyres.

Despite the difference between nocturnal F_v/F_m patterns in the Atlantic and South Pacific (Fig. 3A), changes in F_v (that is, $F_m - F_o$) were remarkably similar (Fig. 3B). However, in the Atlantic gyres, the ≈25% decrease in F_v at sunset (Fig. 3B) primarily reflected a decrease in F_m, whereas in the South Pacific the same decrease in F_v was associated with an increase in background fluorescence, leading to the much larger decrease in F_v/F_m (Fig. 3A). Nevertheless, similarity between diel F_v patterns implied a common physiological mechanism. Coincident changes in σ_{PSII} in the South Pacific (Fig. 1C) suggested that this underlying mechanism was an effect of iron limitation on a phenomenon referred to as a state transition (*14*).

In both eukaryotic and prokaryotic photoautotrophs, the light reactions of photosynthesis require two photosystems (PSII and PSI) for light harvesting and charge separation, along with plastoquinone (PQ) molecules and cytochrome b_6–f complexes for proton and electron transport. When photosynthetic cells are exposed to light that preferentially excites PSII, the pool of PQ molecules becomes reduced, which triggers conformational changes in light-harvesting complexes that increase PSI excitation and decrease σ_{PSII} by lowering energy transfer to PSII (termed a state 2 transition) (*14*). These changes may entail a migration of light-harvesting complexes from PSII to PSI [as in eukaryotic algae (*14*), cyanobacteria (*15*), and Prochlorophytes (*16*)] or changes in spillover of excitation energy from PSII to PSI (*17*) or both. Likewise, when exposed to PSI-specific far red light, the PQ pool becomes oxidized and energy transfer to PSII increases (a state 1 transition).

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In prokaryotic photoautotrophs, state 2 transitions also occur upon exposure to darkness (*18*). This feature of prokaryotes results from common PQ and cytochrome b_6–f pools being used for both photosynthesis and metabolism (*19*). Consequently, photosynthate metabolism at night leads to a reduction of the PQ pool and induction of a state 2 transition.

To investigate the influence of iron limitation on fluorescence parameters during a dark-induced state transition, we grew cultures of marine *Synechococcus* sp. under nutrient-replete, nitrate-limiting, and iron-limiting conditions (*20*). As observed in the Atlantic Ocean (Fig. 3), F_v decreased by about 25% and F_v/F_m decreased by about 15% (k = 0.102 s^{-1}) upon exposure to darkness in both nitrate-limited (Fig. 4A) and nutrient-replete *Synechococcus* (*21*). These changes reflected a decrease in F_m and were rapidly reversed (k = 0.026 s^{-1}) upon exposure to PSI-specific far-red light, which induced a state 1 transition (Fig. 4A). Thus, the state 2 transition decreased F_v/F_m by increasing energy transfer to PSI, which has a very low fluorescence yield (*22*).

In iron-limited *Synechococcus*, the dark-induced state 2 transition likewise resulted in an ≈25% decrease in F_v, but it was associated with an increase in background fluorescence (Fig. 4B). Consequently, the decrease in F_v/F_m was more extensive, following a kinetic rate (k = 0.024 s^{-1}) that was nearly identical to that in the South Pacific (*23*). Iron-limited *Synechococcus* also exhibited a PSI-to-PSII ratio that was lower than in nutrient-replete or nitrate-limited cells by a fac-

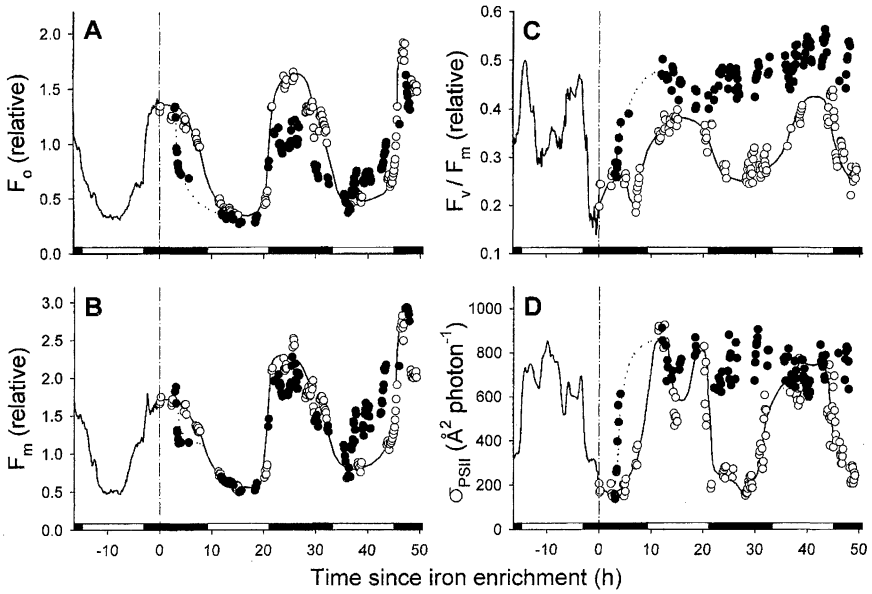

Fig. 2. Effects of in situ iron enrichment (2 nM iron) on diel fluorescence patterns in the South Pacific. (**A**) Initial fluorescence (F_0), (**B**) maximal fluorescence (F_m), (**C**) photochemical quantum efficiencies (F_v/F_m), (**D**) functional absorption cross sections of PSII (σ_{PSII}). Vertical dash-dot line indicates end of iron enrichment. Solid circles, fluorescence data collected inside the iron enrichment area; open circles, fluorescence data collected outside the enrichment area. Fluorescence patterns from the final day of the transect study (Fig. 1) are shown to the left of the vertical dash-dot line (negative time on *x* axis). Solid and open bars indicate night and day. Methods are described in (*5, 7*).

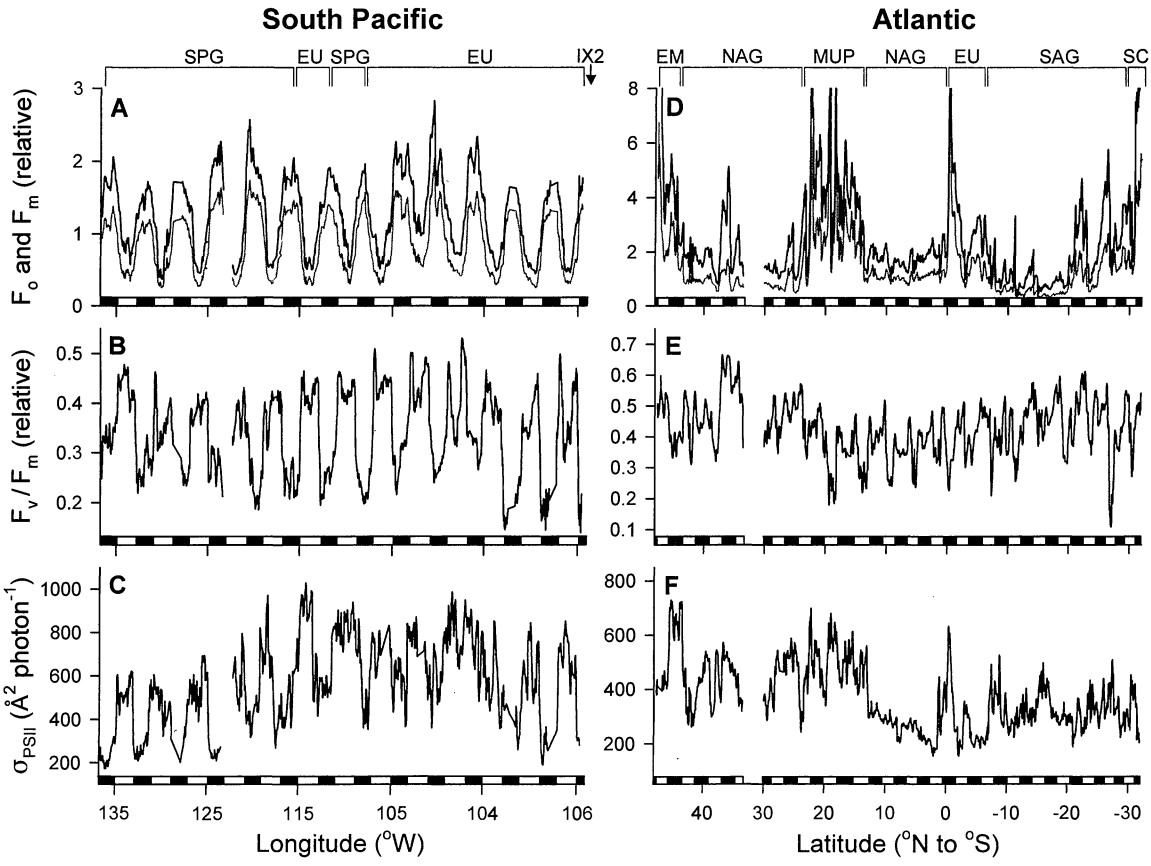

Fig. 1. Diel fluorescence patterns in the South Pacific (**A** to **C**) and the Atlantic (**D** to **F**). (A and D) Initial (F_0 = lower gray line) and maximal (F_m = upper black line) fluorescence; (B and E) photochemical quantum efficiencies [$F_v/F_m = (F_m - F_0)/F_m$]; (C and F) functional absorption cross sections of PSII (σ_{PSII}). IX2 indicates beginning of the in situ iron enrichment experiment; EM, eastern margin, NAG, North Atlantic gyre; MUP, Mauritanian upwelling plume; SAG, South Atlantic gyre; SC, subtropical convergence. Curves are based on >13,000 and >21,000 measurements in the South Pacific and Atlantic, respectively (*7*). Solid and open bars indicate night and day.

tor of 3. Accordingly, recovery of F_v/F_m upon exposure to PSI-specific far-red light was an order of magnitude slower (k = 0.002 s^{-1}) (Fig. 4B).

We thus propose the following underlying mechanism for the divergent Pacific and Atlantic fluorescence patterns. In the South Pacific, iron limitation leads to a decrease in cellular constituents with high iron requirements, such as cytochrome b_6–f (5 Fe per complex) and PSI (12 Fe per complex) (*24*). At sunset, the PQ pool becomes reduced through photosynthate metabolism and a state 2 transition is induced, just as in the Atlantic. However, low PSI-to-PSII ratios in the South Pacific prevent complete association of antennae complexes with PSI. Consequently, during variable fluorescence measurements, antennae complexes decoupled from PSII but not associated with PSI emit absorbed excitation energy, which is observed as an apparent increase in background fluorescence and a decrease in F_v/F_m. At sunrise, PSI turnover assists in reoxidation of the PQ pool, and the associated state 1 transition leads to a full recovery of F_v/F_m.

We propose that iron fluxes in the Atlantic Ocean are sufficient to support relatively high PSI-to-PSII ratios. Thus, nocturnal changes in F_v/F_m are modest because antennae complexes decoupled from PSII during the state 2 transition at sunset are completely associated with PSI, as in nitrate-limited and nutrient-replete *Synechococcus*. Likewise, iron enrichment in the South Pacific induces synthesis of iron-dependent cellular constituents (*5, 24*), which results in rapid decreases in background fluorescence and subsequent increases in F_v/F_m.

The redox-controlled mechanism thus described is based on iron-dependent alterations in photosystem composition. Similar processes may also occur in eukaryotic photoautotrophs when cytochrome b_6–f is sufficiently diminished by iron limitation to cause PQ pool reduction during nocturnal chlororespiration (*19*), thereby enhancing the general utility of the fluorescence diagnostic. Our results indicate state transitions as the underlying mechanism for the diel fluorescence patterns, although processes such as a back-transfer of electrons from a highly reduced PQ pool to PSII may also be involved (*19*). Irrespective of the underlying mechanism, a physiological diagnostic for a specific growth-limiting factor proffers a powerful tool for ecological studies and may now be available for iron limitation. Our results evidence widespread iron limitation in both the South Pacific gyre and HNLC equatorial Pacific, thereby greatly expanding the spatial distribution of iron-limited ecosystems in the global ocean.

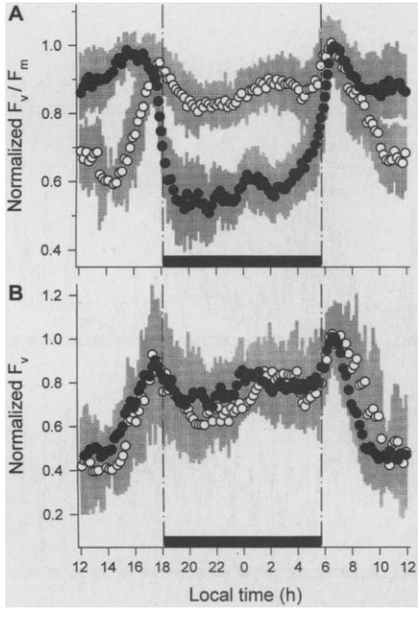

Fig. 3. Average diel cycles in photochemical quantum efficiencies (F_v/F_m) (**A**) and variable fluorescence ($F_v = F_m - F_o$) (**B**) for the South Pacific (solid circles) and central Atlantic gyres (open circles). Solid bars indicate night, with vertical dash-dot lines indicating sunset (left) and sunrise (right). Average F_v/F_m and F_v values were calculated by normalizing each diel cycle to one near sunrise. This normalization removes the influence of changes in phytoplankton biomass on absolute fluorescence values. Shaded area indicates ±1 standard deviation.

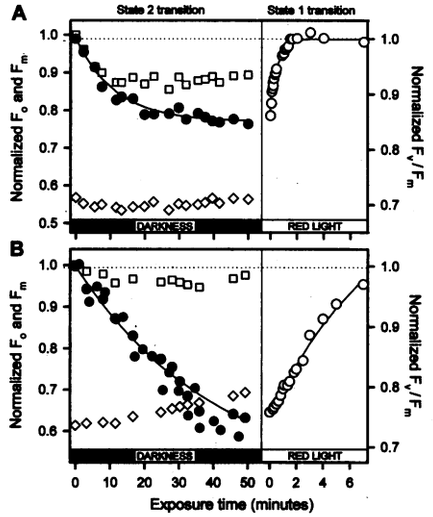

Fig. 4. Changes in variable fluorescence during state transitions in nitrate-limited (**A**) and iron-limited (**B**) cultures of marine *Synechococcus* sp. (*20*). Data to the left and right of the solid vertical line correspond to dark-induced state 2 transitions and far-red light–induced state 1 transitions, respectively. Open squares, F_m; diamonds, F_0 (left axis); solid circles, F_v/F_m during state 2 transition; open circles, F_v/F_m during state 1 transition (right axis). For comparison, changes in F_v/F_m observed in the South Pacific during the first 50 min after sunset (Fig. 3A) are indicated as shaded circles in (B). Results for nutrient-replete cells were similar to those for nitrate-limited cells. Data are normalized to account for differences in absolute values between replicate experiments. F_v/F_m (circles) was normalized to the value at the beginning of the dark period. F_0 and F_m were normalized to the value of F_m at the beginning of the dark period.

References and Notes

1. C. B. Field, M. J. Behrenfeld, J. T. Randerson, P. G. Falkowski, *Science* **281**, 237 (1998).
2. H. J. W. deBaar, *Prog. Oceanogr.* **33**, 347 (1994).
3. J. H. Martin *et al.*, *Nature* **371**, 123 (1994).
4. K. H. Coale *et al.*, *ibid.* **383**, 495 (1996).
5. M. J. Behrenfeld, A. J. Bale, Z. S. Kolber, J. Aiken, P.G. Falkowski, *ibid.*, p. 509.
6. J. LaRoche, P. W. Boyd, R. M. McKay, R. G. Geider, *ibid.* **382**, 802 (1996).
7. We conducted variable fluorescence measurements with a fast repetition rate fluorometer (FRRf) (*8*) on discrete samples collected at depths between 0 and 50 m during OliPac and on a continuous flow-through of seawater sampled from a depth of about 3 m during IronExII and AMT-1. The FRRf exposes phytoplankton to subsaturating light flashes and measures changes in red-light in vivo fluorescence from the initial, dark-adapted state (F_o), when all functional PSII reaction centers are oxidized, to the light-saturated state (F_m), when all PSII reaction centers have been photochemically reduced. σ_{PSII} is calculated from the saturation rate from F_o to F_m. Field studies used a blue-green excitation flash spectrum (Schott BG-39 bandpass filter). Fluorescence values were corrected for background by using measurements conducted on Milli-Q water. An ~3-min dark adaptation period was used to ensure complete oxidation (that is, opening) of PSII reaction centers before FRRf measurements. To avoid fouling, we frequently replaced the cuvette during flowthrough measurements. Phytoplankton chlorophyll concentrations ranged from 0.05 to 0.35 mg m^{-3} during OliPac, from 0.08 to 0.24 mg m^{-3} during IronExII, and from 0.07 to 6.47 mg m^{-3} during AMT-1. We sampled the South Pacific gyre over about 53% and 67% of the OliPac and IronExII transects, respectively.
8. Z. Kolber and P. G. Falkowski, *Limnol. Oceanogr.* **38**, 1646 (1993); P. G. Falkowski and Z. Kolber, *Aust. J. Plant Physiol.* **22**, 341 (1995); Z. Kolber, O. Prasil, P. G. Falkowski, *Biochim. Biophys. Acta* **1367**, 88 (1999).
9. D. Vaulot and D. Marie, *J. Geophys. Res.*, in press; K. K. Cavender-Bares, E. Mann, S. W. Chishlom, M. E. Ondrusek, R. R. Bidigare, *Limnol. Oceanogr.*, in press.
10. *Synechococcus* ranged from about 3000 to 25,000 and from about 5000 to 10,000 cells per milliliter and *Prochlorococcus* ranged from about 100,000 to 200,000 and from about 70,000 to 160,000 cells per milliliter during the OliPac and IronExII transects, respectively (*9*). *Synechococcus* and *Prochlorococcus* are likewise prominent in the central Atlantic gyres [L. S. Murphy and E. M. Haugen, *Limnol. Oceanogr.* **30**, 47 (1985); W. W. Gieskes and G. W. Kraay, *Mar. Biol.* **91**, 567 (1986); R. J. Olsen, S. W. Chisholm, E. R. Zettler, M. A. Altabet, J. A. Dusenberry, *Deep Sea Res.* **37**, 1033 (1990); M. J. W. Velduis and G. W. Kraay, *Mar. Ecol. Prog. Ser.* **68**, 121 (1990); F. Partensky, J. Blachot, F. Lantoine, J. Neveux, D. Marie, *Deep Sea Res.* **43**, 1191 (1996)].
11. G. H. Krause and E. Weis, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **42**, 313 (1991). It is noteworthy that Y. Dandonneau and J. Neveux [*Deep Sea Res.* **44**, 1869 (1997)] observed F_m patterns in the equatorial Pacific similar to those shown in Fig. 1A. They attributed the diurnal changes to nonphotochemical quenching and the nocturnal changes to an unresolved circadian rhythm; our results suggest that the latter result from iron limitation effects on fluorescence yields.
12. M. J. Behrenfeld, O. Prasil, Z. S. Kolber, M. Babin, P. G. Falkowski, *Photosynth. Res.*, in press; P. J. Neale, in *Photoinhibition*, D. J. Kyle, C. B. Osmond, C. J. Arntzen, Eds. (Elsevier, Amsterdam, 1987), pp. 123–144; S. P.

Long, S. Humphries, P. G. Falkowski *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **45**, 633 (1994).
13. A primary source of new iron in the South Pacific is atmospheric deposition, with one of the lowest annual budgets of Fe on the globe at ≤1 mg m^{-2} $year^{-1}$ (particulate plus dissolved). Aeolian deposition of Fe in the Atlantic ranges from 10 to 100 mg m^{-2} $year^{-1}$ [R. A. Duce and N. W. Tindale, *Limnol. Oceanogr.* **36**, 1715 (1991)].
14. J. F. Allen, *Biochim. Biophys. Acta* **1098**, 275 (1992); J. Allen, J. Bennett, K. E. Steinback, C. J. Arntzen, *Nature* **291**, 25 (1981).
15. C. W. Mullineaux, M. J. Tobin, G. Jones, *Nature* **390**, 421 (1997).
16. T. Burger-Wiersma and A. F. Post, *Plant Physiol.* **91**, 770 (1989).
17. O. Salehian and D. Bruce, *J. Luminesc.* **51**, 91 (1992).
18. M. Aoki and S. Katoh, *Biochim. Biophys. Acta* **682**, 307 (1982); C. W. Mullineaux and J. F. Allen, *FEBS Lett.* **205**, 155 (1986); S. Scherer, A. Helmar, P. Böger, *Photosynth. Res.* **15**, 95 (1988).
19. In eukaryotic photoautotrophs, photosynthesis and general cell metabolism occur separately in the chloroplast and mitochondria. Thus, under normal conditions, eukaryotic plants remain in state 1 at night. However, R. Belkhodja *et al.* [*Photosynth. Res.* **56**, 265 (1998)] reported a nocturnal reduction of the PQ pool under iron-limiting conditions in terrestrial plants and eukaryotic algae (presumably by chlororespiration), which caused increased background fluorescence.
20. *Synechococcus* was grown at 25°C under a 12-hour light–12-hour dark cycle at 250 μmol of quanta per square meter per second. State 2 transitions were studied during the first ≈55 min of darkness after the 12-hour photoperiod using the FRRf (*7, 8*). State 1 transitions were induced immediately thereafter by exposing cells to PSI-specific far-red light [light-emitting diode (LED) source with Schott RG-695 long-pass filter]. State transition experiments were conducted during the first few days after the onset of nitrate or iron starvation. PSI and PSII concentrations were determined according to methods described by Dubinsky *et al.* [Z. Dubinsky, P. G. Falkowski, K. Wyman, *Plant Cell Physiol.* **27**, 1335 (1986)].
21. In cyanobacteria, decreases in σ_{PSII} are observed after state 2 transitions when fluorescence excitation spectra include green or orange wavelengths, as in our field studies (*7*). When measured with a blue excitation spectrum, no change in σ_{PSII} is observed (D. Bruce, personal communication). The mechanism responsible for this discrepancy is unknown. During our laboratory studies, the primary fluorescence excitation source was blue-light LEDs. Consequently, no changes in σ_{PSII} (mean 702 ± 29 A^2 per photon) were observed despite the clear induction of state 2 transitions and their reversal upon exposure to far-red light (Fig. 4).
22. H. Dau, *Photochem. Photobiol.* **60**, 1 (1994)
23. Greene *et al.* [R. M. Greene, Z. S. Kolber, D. G. Swift, N. W. Tindale, P. G. Falkowski *Limnol. Oceanogr.* **39**, 1061 (1994)] did not observe the large nocturnal decreases in F_v/F_m in the equatorial Pacific illustrated in Fig. 1B. Based on the kinetic rates we observed, this discrepancy resulted from inadvertent induction of a state 2 transition during the prolonged dark adaptations (>20 min) during the previous study.
24. J. A. Guikema and L. A. Sherman, *Plant Physiol.* **73**, 250 (1983); G. Sandmann, *Photosynth. Res.* **6**, 261 (1985); N. A. Straus, in *The Molecular Biology of Cyanobacteria*, D. A. Bryant, Ed. (Kluwer Academic, Boston, 1994), pp. 731–750; I. R. Vassiliev *et al.*, *Plant Physiol.* **109**, 963 (1995); R. M. Greene, R. J. Geider, Z. Kolber, P. G. Falkowski, *ibid.* **100**, 565 (1992).
25. Supported by NASA grants NAG5-7437 and UPN161-35-05-08. We thank A. Bale, K. Coale, and K. Johnson for assistance during IronExII; S. Laney and J. Aiken for AMT-1 data; D. Vaulot, B. Coste, and the crew of *L'Atalante* for assistance during OliPac; K. Wyman for laboratory assistance; and especially P. Falkowski, L. Sherman, D. Bruce, C. Mullineaux, R. Geider, R. Barber, D. Mauzerall, S. Seitzinger, and O. Prasil for helpful suggestions and encouragement.

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The Dynamics of Zooxanthellae Populations: A Long-Term Study in the Field

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Coral bleaching characterized by the expulsion of symbiotic algae (zooxanthellae) is an increasing problem worldwide. Global warming has been implicated as one cause, but the phenomenon cannot be fully comprehended without an understanding of the variability of zooxanthellae populations in field conditions. Results from a 6-year field study are presented, providing evidence of density regulation but also of large variability in the zooxanthellae population with regular episodes of very low densities. These bleaching events are likely to be part of a constant variability in zooxanthellae density caused by environmental fluctuations superimposed on a strong seasonal cycle in abundance.

Coral bleaching is normally characterized by expulsion of the endosymbiotic zooxanthellae (the unicellular dinoflagellate *Symbodinium* spp.), loss of algal pigmentation, or both. Coral bleaching events, defined here as concomitant with very low zooxanthellae density, have had serious effects on corals and reefs worldwide (*1*). Given the dependence of the coral on its symbiotic algae (*2*), it is important to determine the cause of these bleaching events. A number of explanations for coral bleaching have been proposed, including unusually high seawater temperatures (*3, 4*), high doses of ultraviolet light (*5*), bacterial infection (*6*), and changes in salinity (*7*). What is crucial to our understanding of zooxanthellae expulsion and bleaching is how the density of zooxanthellae within the coral is changing, if at all, under the prevailing range of environmental conditions. Here we present the results of a long-term field study (August 1991 to March 1997), with data collected on a weekly basis, during which the population density of zooxanthellae within the coral *Acropora formosa* (Dana 1846) in a shallow lagoon in Mauritius was monitored (*8*) and environmental variables were measured (*9*).

The time series of the zooxanthellae density over the study period is shown in Fig. 1. The mean density was 1.7 × 10^6 cm^{-2} (SD = 2.4 × 10^6 cm^{-2}), comparable to densities of about 1 × 10^6 to 2 × 10^6 cm^{-2} previously reported (*10, 11*). There is evidence of some regulatory mechanism, as the change in zooxanthellae density from one week to the next is dependent on the density during the preceding week (*12*). The time series reflects sampling over the whole coral colony and does not reflect changes in individual tips from one week to the next; the density dependence detected in the time series thus indicates trends through time within the coral colony as a whole.

In addition to the density dependence, there is considerable variation in density, with fluctuations over three orders of magnitude. Although densities from 0.5 × 10^6 to 5.0 × 10^6 cm^{-2} have been reported in different studies (*13–15*), here such variability is reported from a single coral colony over time. In particular, there was a bleaching event in the spring and summer of 1993 (density < 0.1 × 10^6 cm^{-2} from 28 October to 17 December 1993; weeks 109 to 117 in

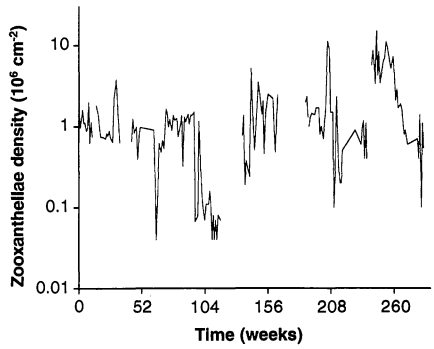

Fig. 1. Time series of zooxanthellae density from August 1991 to March 1997. The coral samples were collected on approximately a weekly basis, and density was determined by a standard methodology (*13*). At three points in the time series, there are gaps because no data were collected during these periods (for logistical reasons). The total number of data points is 159.

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