

Controls on tropical Pacific Ocean productivity revealed through nutrient stress diagnostics

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In situ enrichment experiments have shown that the growth of bloom-forming diatoms in the major high-nitrate low-chlorophyll (HNLC) regions of the world's oceans is limited by the availability of iron^{1–3}. Yet even the largest of these manipulative experiments represents only a small fraction of an ocean basin, and the responses observed are strongly influenced by the proliferation of rare species rather than the growth of naturally dominant populations^{4,5}. Here we link unique fluorescence attributes of phytoplankton to specific physiological responses to nutrient stress, and use these relationships to evaluate the factors that constrain phytoplankton growth in the tropical Pacific Ocean on an unprecedented spatial scale. On the basis of fluorescence measurements taken over 12 years, we delineate three major ecophysiological regimes in this region. We find that iron has a key function in regulating phytoplankton growth in both HNLC and oligotrophic waters near the Equator and further south, whereas nitrogen and zooplankton grazing are the primary factors that regulate biomass production in the north. Application of our findings to the interpretation of satellite chlorophyll fields shows that productivity in the tropical Pacific basin may be 1.2–2.5 Pg C yr⁻¹ lower than previous estimates have suggested, a difference that is comparable to the global change in ocean production that accompanied the largest El Niño to La Niña transition on record⁶.

The tropical Pacific is characterized by warm, well-stratified and nutrient-poor waters separated by a major upwelling plume of nutrient-rich water near the Equator extending from roughly the dateline (180°) to the eastern boundary (Fig. 1a). The upwelled water is rich in dissolved CO₂ that subsequently degasses to the atmosphere. Although the region is the largest natural oceanic source of CO₂ to the atmosphere^{7,8}, the extent of this CO₂ release is curtailed to about 0.7–1.5 Pg yr⁻¹ (1 Pg = 10¹⁵ g) by the photosynthetic carbon uptake of an elevated phytoplankton biomass supported by upwelled macronutrients and micronutrients^{8,9}. Throughout the tropical Pacific, variations in physical and chemical properties of the upper ocean imprint resident phytoplankton with physiological characteristics diagnostic of their specific growth constraints. These physiological expressions can be distinguished by associated diel patterns in normalized variable fluorescence (F_v/F_m)¹⁰.

We collected more than 140,000 measurements of variable fluorescence along 58,000 km of ship transects during ten field studies between 1994 and 2006 to characterize the broad-scale biological and physiological features of the tropical Pacific (Fig. 1a). Phytoplankton biomass in the study area is distributed similarly to surface nitrate (Fig. 1a), with low values north of roughly 9°N and west of the

dateline, and distinctly elevated values in the equatorial upwelling zone. Daily F_v/F_m patterns in the tropical Pacific exhibit three dominant features: first, maxima at sunrise and sunset; second, a midday suppression from photoinhibition; and third, a nocturnal decrease (Fig. 1b, c). We find dawn maxima in F_v/F_m to vary inversely with biomass, having high values in low-nitrate areas and decidedly lower values in upwelling waters (Figs 1b and 2a). Midday photoinhibition

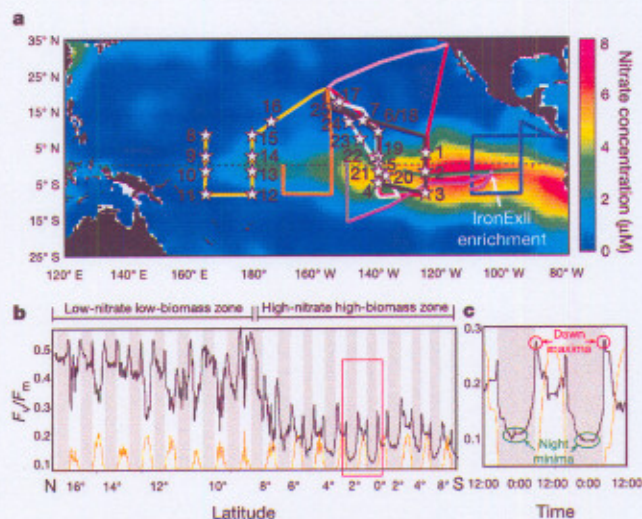


Figure 1 | The tropical Pacific study area. **a**, Ship transects (lines) and enrichment experiment locations (stars) for the ten field studies. Transect dates are as follows: grey, 1994; dark purple, 1995; black, 2000; yellow, pink, orange and green, 2001; white, 2002; blue, 2003; red, 2006. The background colour is Levitus climatological surface nitrate concentration, showing elevated values in the equatorial upwelling zone. **b**, Typical variations in normalized variable fluorescence (F_v/F_m) for the tropical Pacific, with high dawn values and large photoinhibition in low-nitrate low-biomass regions and low dawn values and large nocturnal decreases in high-nitrate high-biomass areas (data from the 2000 transect, corresponding to the black line in **a** extending from Hawaii to 8°S, 140°W). **c**, Primary diel features in F_v/F_m (from the red box in **b**) are a nocturnal decrease (circled in green), a dawn maximum (circled in red) and midday photoinhibition. To illustrate the scale of the current investigation, the 64 km² IronExII experiment of 1996, which dwarfed earlier bottle enrichments, was only 1/100 the size of the small white rectangle shown in **a** at 2°S, 104°W. The orange lines in **b** and **c** indicate incident sunlight (relative).

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is also enhanced in regions of low biomass (Fig. 1b). The remarkable nocturnal decreases in F_v/F_m are uniform in the upper water column and then disappear in the light-limited lower reaches of the photic zone (see Supplementary Information).

We delineated three physiological regimes in the tropical Pacific on the basis of dawn maxima in F_v/F_m and the extent of the nocturnal F_v/F_m decrease (Fig. 2). Regime I is largely found in the north and is characterized by high values (0.45 or more) of F_v/F_m at dawn and small (less than 25%) nocturnal decreases. Regime II is found west of the upwelling plume and has similarly elevated dawn maxima (Fig. 2a), but exhibits large nocturnal decreases in F_v/F_m (Fig. 2b). Regime III is distinguished by having chronically low dawn F_v/F_m and pronounced decreases in F_v/F_m at night (Fig. 2). The link between these three physiological regimes and specific nutritional constraints was directly tested by conducting 25 small-volume (10-litre) nutrient enrichment experiments (Fig. 1a). These 21–36-h experiments included representatives from all three regimes and involved enrichments of 5 μM NO_3 , 5 μM NH_4 , 1 μM PO_4 and 4 nM iron (see Supplementary Information). Their short duration ensured that observed responses reflected immediate physiological changes rather than growth of the dominant species or a bloom of a rare species.

The addition of NO_3 , NH_4 or PO_4 had no significant influence on photosynthetic characteristics of samples from regime III. In contrast, addition of iron to samples from regime III consistently eliminated the nocturnal decrease in F_v/F_m , markedly increased overall F_v/F_m values to regimes I and II levels (Fig. 3a), enhanced functional absorption cross-sections of oxygen-evolving photosystem II (PSII) complexes and caused a major decrease in electron

turnover times of the plastoquinone (PQ) pool (Fig. 3b, c). In regime II, iron addition similarly removed the nocturnal decrease in F_v/F_m and generally enhanced electron turnover rates, but dawn F_v/F_m values were already near maximal and did not change with iron (or PO_4) enrichment (Fig. 3a). Additions of NO_3 and NH_4 caused the most striking responses in regime II, inducing marked decreases in F_v/F_m (Fig. 3a) that were associated with increased background fluorescence levels. Finally, photosynthetic characteristics in regime I remained unaltered after the addition of iron or PO_4 , and none of the nutrient treatments significantly influenced PSII absorption cross-sections or PQ electron turnover (Fig. 3). The only clear physiological response in regime I was a decrease in F_v/F_m during four of the eight experiments after NO_3 or NH_4 amendment (Fig. 3a). Again, these decreases in F_v/F_m resulted from an increase in background fluorescence.

Clearly, the three tropical Pacific regimes correspond to different conditions of iron and nitrogen availability. Because many details on the effects of iron stress in plants are known, our population-level fluorescence diagnostics can now be associated with specific phenomena in photosynthetic membranes. Importantly, the photosynthetic apparatus is a major sink for cellular iron, with 24 iron

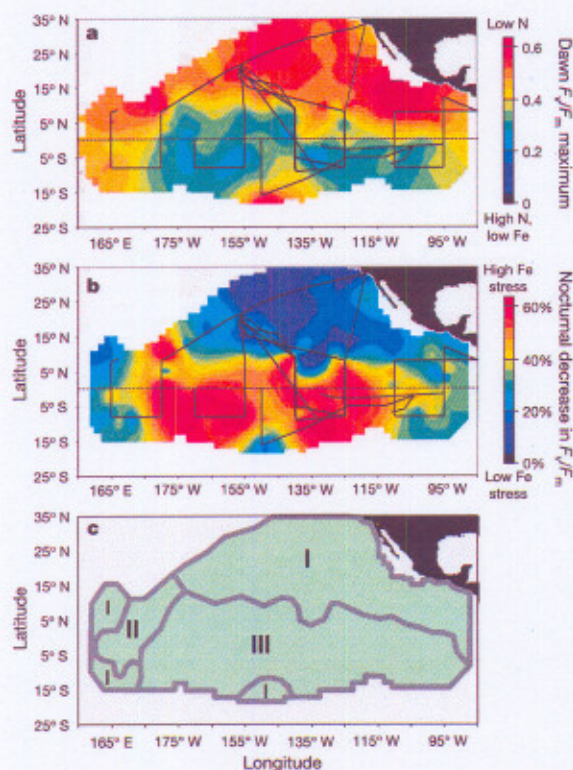


Figure 2 | Fluorescence diagnostics delineate three physiological regimes in the tropical Pacific. **a**, Dawn F_v/F_m maximum. **b**, Nocturnal decrease in F_v/F_m . **c**, The three regimes: regime I (small nocturnal decreases and high dawn values of F_v/F_m), regime II (large nocturnal decreases and high dawn values of F_v/F_m) and regime III (large nocturnal decreases and low dawn values of F_v/F_m).

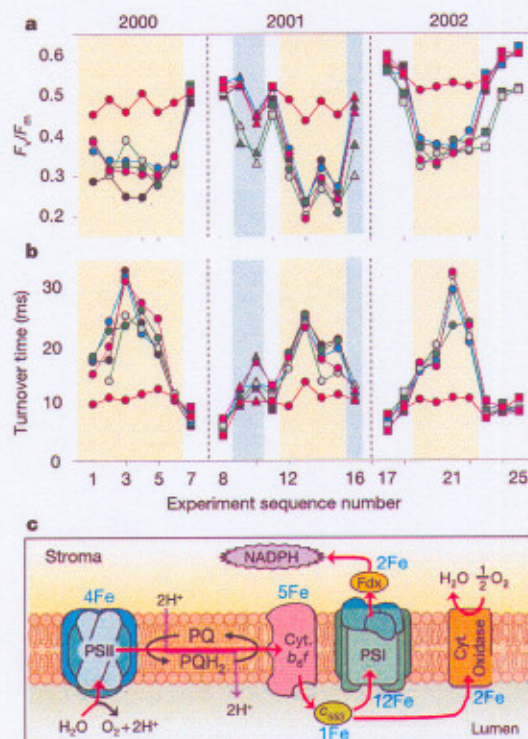


Figure 3 | Nutrient enrichment responses in the three physiological regimes. **a**, **b**, Initial and end-of-experiment treatment values of normalized variable fluorescence (F_v/F_m) (**a**) and plastoquinone pool electron turnover times during the 25 enrichment experiments (stars in Fig. 1a) (**b**). Regime I is represented by squares and no background shading; regime II by triangles and blue shading, and regime III by circles and yellow shading. Black symbols and lines, zero time control; blue, end-of-experiment control; dark green, NO_3 ; light green, NH_4 ; pink, PO_4 ; red, iron. Vertical dashed lines separate cruises (labelled at the top). Experiment sequence number (x axis) follows Fig. 1a. **c**, Primary components of a photosynthetic membrane and their iron requirements (blue text). The photosynthetic electron transport sequence is as follows: PSII to plastoquinone pool (PQ, PQH_2) to cytochrome b_6/f to a mobile cytochrome (c_{553}) and finally to either PSI and ferredoxin (Fdx) or a terminal cytochrome oxidase. In prokaryotes and eukaryotes, electron transport can also proceed directly from the PQ pool to terminal oxidases.

atoms required for a single complete copy of the electron transport chain (Fig. 3c)¹¹. Iron stress significantly depletes photosynthetic components on the acceptor side of the PQ pool (cytochrome *b₆f*, cytochrome oxidase, photosystem I (PSI) and ferredoxin) (Fig. 3c)^{12–15}. These changes are responsible for the large nocturnal F_v/F_m decreases in regimes II and III.

All phytoplankton use their photosynthetic membranes for respiratory electron transport in the dark (termed 'chlororespiration' in eukaryotes) (Fig. 3c). Consequently, the PQ pool is generally mildly reduced at night, but under iron-stressed conditions this reduction is severe because electron transport is rate-limited by diminished cytochrome concentrations (Fig. 3c)^{10,12,15–18}. Because the redox state of the primary electron acceptor on PSII (Q_A) exists in equilibrium with that of the PQ pool¹⁷, severe reduction of PQ at night results in a back-transfer of electrons to Q_A that we detected as a decrease in F_v/F_m . Being a respiration-driven phenomenon, the degree of PQ pool reduction depends on the pool size of respiratory substrates. Depletion of these substrates is responsible for the modest increase in F_v/F_m each night before sunrise (Fig. 1c) and explains the observation that the nocturnal decrease in F_v/F_m cannot be artificially induced after sunrise by re-exposure to darkness¹⁷ until an adequate photosynthate pool has been rebuilt. The immediate increase in F_v/F_m at dawn results from oxidation of the PQ pool by PSI turnover (Fig. 1c), a response that can be artificially replicated at night by exposure to PSI-specific light^{10,17}. Rapid loss of the nocturnal decrease in F_v/F_m and increased electron turnover (Fig. 3b) in regimes II and III on the addition of iron reflect an associated induction of cytochrome synthesis^{12,16}.

The distinguishing characteristic of regime III is low dawn F_v/F_m maxima (Fig. 2a). This feature is caused by unique chlorophyll-protein complexes that are synthesized when reduced nitrogen is abundant but iron is limiting^{12,18–23}. These complexes seem to function in a photoprotective manner, are to a large degree functionally 'disconnected' from PSII, and have high background fluorescence that decreases F_v/F_m (refs 11, 12, 18, 22–24). Pigment from the

complexes is immediately transferred to functional PSII antennae on iron enrichment, leading to decreased background fluorescence and increases in F_v/F_m (Fig. 3a) and PSII absorption cross-sections. This same response is regularly observed in laboratory iron-recovery experiments^{12,16,20,25,26}, proceeds in the presence of chlorophyll synthesis inhibitors²⁶ but not protein synthesis inhibitors¹⁵, and suggests that the iron-induced complexes function as pigment reservoirs to facilitate recovery from iron stress^{12,20,25}. In regime II, the process is simply reversed by the addition of NO_3 or NH_4 , which induces the synthesis of these special pigment-protein complexes that are naturally substrate limited by nitrogen availability. The occasional appearance of this 'regime II-type' response in regime I experiments (Fig. 3a) indicates that these northern waters, which are regulated by nitrogen and grazing, can easily be perturbed into iron-stressed conditions²⁷.

Our two variable fluorescence diagnostics define four possible physiological regimes, three of which are present in the tropical Pacific (Fig. 4). High dawn F_v/F_m values occur in both nitrogen-limited and iron-limited systems, as long as reduced nitrogen levels are low (regimes I and II). Low dawn F_v/F_m values result when iron is limiting and elevated nitrogen levels allow the synthesis of the special pigment-protein complexes (classic HNLC conditions). Large nocturnal decreases in F_v/F_m require both iron stress and sufficient dark respiration to drive PQ pool reduction (regimes II and III). Conversely, small nocturnal decreases in F_v/F_m result when iron stress is absent or growth is too slow to cause PQ pool reduction at night. Accordingly, the fourth physiological regime, which was not found in the tropical Pacific and is defined by low dawn F_v/F_m and small nocturnal decreases (Fig. 4), is predictably observed in polar HNLC regions where iron is limiting and nitrate is replete, but growth rates are too low for significant night-time PQ pool reduction²⁸.

The tropical Pacific basin is responsible for roughly 20% (9–14 Pg C yr⁻¹) of global ocean productivity (see Supplementary Information) and has a prominent function in air-sea CO₂ exchange. Assessing constraints on productivity in this permanently stratified region is challenging because the dominant phytoplankton are naturally growing at relatively high rates (of the order of one division per day) and their standing stock changes little with nutrient enrichment⁴. Physiological diagnostics provide a solution to this problem and here are applied on an unprecedented scale. Special pigment-protein complexes synthesized during iron stress underlie one of our key fluorescence attributes. These structures cause an enhanced greenness in HNLC regions (that is, regime III) that is not associated with elevated photosynthesis. This effect is quantitatively related to the suppression of dawn F_v/F_m values and must be accounted for when satellite surface chlorophyll fields are used to estimate ocean productivity or to evaluate ocean-circulation-ecosystem model performance. We assessed the influence of these iron-induced structures by adjusting satellite chlorophyll fields with our variable fluorescence data from the field; we found tropical Pacific production to be 1.2–2.5 Pg C yr⁻¹ lower than for uncorrected fields (see Supplementary Information). This difference is comparable to global productivity changes during major El Niño to La Niña transitions (0.4–0.8 Pg C yr⁻¹)^{7,29} and underscores the importance of characterizing nutrient constraints and their physiological consequences. Our current climatological treatment unquestionably misses seasonal and interannual variations in boundaries between physiological regimes. Future evaluations will benefit if links can be established between nutritional conditions and remote sensing properties, such as Moderate Resolution Imaging Spectrometer (MODIS) solar-stimulated fluorescence.

METHODS

The Supplementary Information contains details of all experimental methods and model calculations.

Fluorescence. A fast-repetition-rate fluorimeter (FRRf) was used to measure chlorophyll fluorescence characteristics of phytoplankton continuously sampled

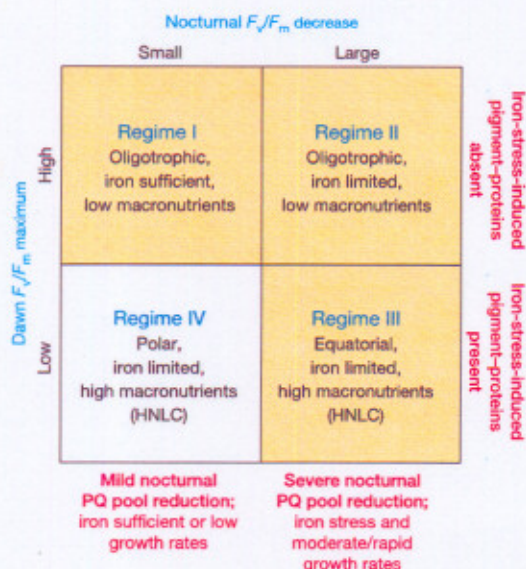


Figure 4 | Environmental conditions corresponding to the four physiological regimes. The regimes are distinguished by dawn normalized variable fluorescence values (F_v/F_m) (low, less than 0.45; high, 0.45 or more) and the percentage decrease in F_v/F_m at night (small, 25% or less; large, more than 25%). Physiological and nutritional conditions responsible for the diagnostic attributes are summarized in red text to the right and at the bottom of the matrix. The three regimes observed in the tropical Pacific are identified by a yellow background.

from the surface mixed layer. The FRRf protocol involves a rapid sequence of subsaturating light flashes that cause a rise in fluorescence *in vivo* from an initial (F_0) to a maximal (F_m) level. This change in fluorescence (F_v) is associated with absorbed light energy used for photosynthesis and is normalized to F_m (that is, F_v/F_m) to account for biomass variability. Functional absorption cross-sections of PSII can also be derived from the rate of increase between F_0 and F_m . Electron transport rates downstream of PSII are determined from fluorescence decay kinetics after a saturating sequence of light flashes.

Received 14 December 2005; accepted 19 July 2006.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank Z. Kolber, R. O'Malley, and the crew and officers of the NOAA ships *Ka'imimoana* and *Ronald Brown*. This research was funded by the National Science Foundation, the National Aeronautics and Space Administration, and the National Oceanic and Atmospheric Administration's Tropical Atmosphere Ocean array programme.

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