

Part I: Field Measurements

Flow-through measurements: For all field studies, phytoplankton fluorescence properties were monitored with a bench-top Fast Repetition Rate fluorometer (FRRf) (Kolber & Falkowski 1993; Falkowski & Kolber 1995). Seawater (~3 m depth sampling) from the ship's flow-through system was plumbed in darkness to a 1 cm³ custom quartz flow cell and then exposed to a rapid sequence (1 ms spacing) of blue-light flashes. Fluorescence induction was recorded from an initial dark-adapted level (F_0) to the maximum level (F_m). Normalized variable fluorescence was calculated as: $F_v/F_m = (F_m - F_0)/F_m$. Functional absorption cross sections for PSII (σ_{PSII}) were derived from fluorescence saturation kinetics. Turnover times for photosynthetic electron transport were determined from fluorescence decay kinetics following the saturating flash sequences. During the 1994 OliPac study (gray line in Fig. 1A of manuscript), flow-through FRRf measurements were only made during the nighttime ship transects, while discrete samples were collected during the day when the ship remained on station (1.5° latitude spacing between stations). On all other cruises, flow-through FRRf measurements were conducted continuously. All field studies were conducted under non-El Niño conditions, except during 2002 (white line in Fig. 1A) when we repeated a section of the 2000 study (purple line in Fig. 1A) to investigate the influence of El Niño conditions on nutrient stresses. Transect variable fluorescence and supporting data are available at: <http://science.oregonstate.edu/ocean.productivity> or by contacting M.J. Behrenfeld (mjb@science.oregonstate.edu).

Interpolation of FRRf data to 2-dimensional fields of dawn F_v/F_m maxima and percent nocturnal decrease in F_v/F_m (Figures 2A,B of manuscript) was accomplished using the 'zgrid' gridding routine in the PlotPlus software package. This gridding routine applies a thin plate spline interpolation that converts irregularly spaced observations into a regular grid for contouring and display. The routine can yield a pure Laplacian solution (minimum curvature) or a pure spline solution, depending on the tension applied. For our calculations, a near-minimum curvature solution was chosen.

Downwelling photosynthetically active solar radiation (PAR) was continuously monitored at 15 s intervals with a Licor cosine-collecting light sensor (Model LI-1400). Discrete 500 ml seawater samples were also collected for determination of chlorophyll concentrations. Chlorophyll samples were gently filtered through Whatman GF/F® filters, which were then placed in glass scintillation vials with 10 ml of 90% acetone and stored in a freezer for 24 to 36 h. Chlorophyll concentration was determined from the acetone extracts using a calibrated Turner Designs® fluorometer. Chlorophyll concentrations in the tropical Pacific ranged from 0.06 $\mu\text{g L}^{-1}$ in nitrate-depleted waters to 0.36 $\mu\text{g L}^{-1}$ in nitrate-enriched (<0.1 $\mu\text{g L}^{-1}$) upwelling waters.

Trace-metal sampling: Samples for dissolved Fe determination were collected similarly to methods described in Field and Sherrell (2003). Briefly, seawater was pumped through a Teflon-lined polyethylene tube (lowered to ~10m at each station) using an air-powered polypropylene body, Teflon diaphragm deck pump, directly into a HEPA filtered laminar flow bench. Polypropylene switching valves in the flow bench allowed the flow to be directed either out to waste, for flushing the line, or through a 0.45 μm pore size all polypropylene cartridge filter (Calyx, GE Osmonics) to collect clean filtered seawater samples. All surfaces contacting seawater were rigorously cleaned using trace-metal grade 1% HCl, followed by rinsing with deionized water (Milli-Q) and with ~60L of seawater before sampling began.

Filtered seawater samples were frozen on board ship and shipped to Rutgers University,

where they were thawed and acidified to pH ~1.8 using ultra-clean grade HCl (Fisher Optima). After sitting at this pH for at least two weeks, samples were analyzed using a modified version of the Mg(OH)₂ co-precipitation isotope dilution ICP-MS method (Wu and Boyle, 1998). Aliquots of 5mL were spiked with enriched ⁵⁷Fe (95%), allowed to equilibrate, then precipitated by raising the pH to ~10 using ultra-clean NH₄OH. The resulting pellet was dissolved in 4% ultra-clean HNO₃ and analyzed for Fe 56/57 ratio on a ThermoFinnigan Element-1 high resolution ICP-MS set to medium resolution (M/DM~4000) to resolve oxide and hydroxide interferences. Each sample was determined with 2-8 replicate analyses. Blanks were determined by using 50uL samples of Pacific surface seawater (Fe ~0.1nM) in place of the normal 5mL sample. Blank values were typically 0.05-0.07 ±0.01-0.02 nM, depending on the run day, for a mean detection limit (3 times SD of blank) of about 0.04 nM. The same methods were used to determine iron in samples from the first iron international intercomparison exercise (Bowie et al., 2005; lab #24), during which precision was better than ±0.016nM (1 SD; n=8). Ambient iron concentrations at all experiment locations were at trace levels and ranged from 0.04 to 0.20 nM.

Incubation Experiments: Nutrient enrichment experiments were conducted at 25 locations during 3 cruises between 2000 and 2002 (purple, yellow, and white lines in Figure 1A of manuscript). Unfiltered, trace-metal clean seawater (see above) was dispensed into 10 L acid-washed carboys and either unaltered (i.e., control) or inoculated with 5 μM NO₃, 5 μM NH₄, 1 μM PO₄, or 4 nM Fe. Samples were then incubated at ambient surface temperature and exposed to ~20% incident light to avoid excessive photoinhibitory light-stress. Subsamples were collected for the subsequent 21 to 36 hours and immediately analyzed with the FRRf, with triplicate measurements made for each treatment. Incubation carboys were maintained in the light or dark (depending on time of day) prior to sample collection for FRRf analysis to avoid any short-term changes in photosynthetic parameters. At the end of each incubation experiment, 500 ml samples were collected from each treatment carboy and analyzed for chlorophyll concentration following the methods described above.

Regional Productivity Calculations: Iron-stressed phytoplankton growing under ample reduced nitrogen concentrations (i.e., HNLC regions) synthesize special pigment-protein complexes that are functionally ‘decoupled’ from PSII, causing a rise in background fluorescence and a decrease in F_v/F_m . When abundant, these structures increase the apparent ‘greenness’ of cells and give a false impression of enhanced photosynthetic activity compared to non-HNLC conditions. If not accounted for, this phenomenon can lead to overestimates of satellite-based regional production. To quantify this effect, we implemented an iron-stress correction in two satellite productivity models: the Carbon-based Production Model (CbPM), which uses satellite-derived phytoplankton chlorophyll-to-carbon ratios to estimate growth rates (Behrenfeld et al. 2005) and the Vertically Generalized Production Model (VGPM) (Behrenfeld and Falkowski 1997), which uses sea surface temperature to estimate chlorophyll-specific photosynthetic efficiencies. For the VGPM, an exponential temperature function was employed, as this model was found to perform better in the tropical Pacific than the original VGPM polynomial function (Campbell et al. 2002).

For the 1998 to 2004 period, the standard parameterization of the CbPM gives annual tropical Pacific production values of 13.2 to 14.2 Pg C y⁻¹ (Pg = 10¹⁵ g), while the VGPM variant gives smaller values ranging from 9.3 to 10.0 Pg C y⁻¹. Our *in situ* and incubation results indicate that an $F_v/F_m \geq 0.5$ is indicative of efficient photosystems for the tropical Pacific region. A correction for the special iron-induced pigment-protein complexes can thus be made for waters

where F_v/F_m is <0.5 by adjusting the physiological variables in the two models by the factor: $0.5/(1-F_v/F_m)$, where F_v/F_m is from Figure 2A of the manuscript. With this correction, CbPM annual productivity decreases by 1.8 to 2.5 Pg C y^{-1} for the 1998-2004 period, which is more than twice the 0.8 Pg C y^{-1} global productivity change the CbPM assigns to the 1997-1999 El Niño to La Niña transition. Applying the same correction to the VGPM variant decreases regional annual production by 1.2 to 1.3 Pg C y^{-1} , which again significantly exceeds this model's 0.4 Pg C y^{-1} estimate for the global El Niño to La Niña productivity change. Thus, while the two models provide different absolute original and corrected values for tropical ocean productivity and global El Niño effects, our conclusions are robust across different productivity modeling approaches.

References:

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Part II: Photoinhibition and Nocturnal Changes in the Water Column

Two striking diel features in Photosynthetic quantum efficiencies (F_v/F_m) are the nocturnal decrease that we associate with iron limitation and the typically symmetric mid-day suppression associated with photoinhibition (Behrenfeld et al. 1998). During the 1994 OliPac study (gray line in Fig. 1A of manuscript), time-series stations were occupied at 5°S, 150°W and 16°S, 150°W (Dandonneau 1999) that permitted an investigation into how these surface features extend through the water column. What we find is that the daytime depression in F_v/F_m from photoinhibition decreases through the water column in proportion to the submarine light level, while the nocturnal decrease proceeds in parallel in all surface samples and is negligible in deeper, more light-limited phytoplankton (Fig. S1). These results are consistent with photoinhibition reflecting a light-dependent, reversible down-regulation of photosystem II (PSII) and the nocturnal decrease reflecting a nutrient-sensitive back-transfer of electrons to PSII that requires significant night photosynthate metabolism to effectively reduce the plastoquinone pool.

References:

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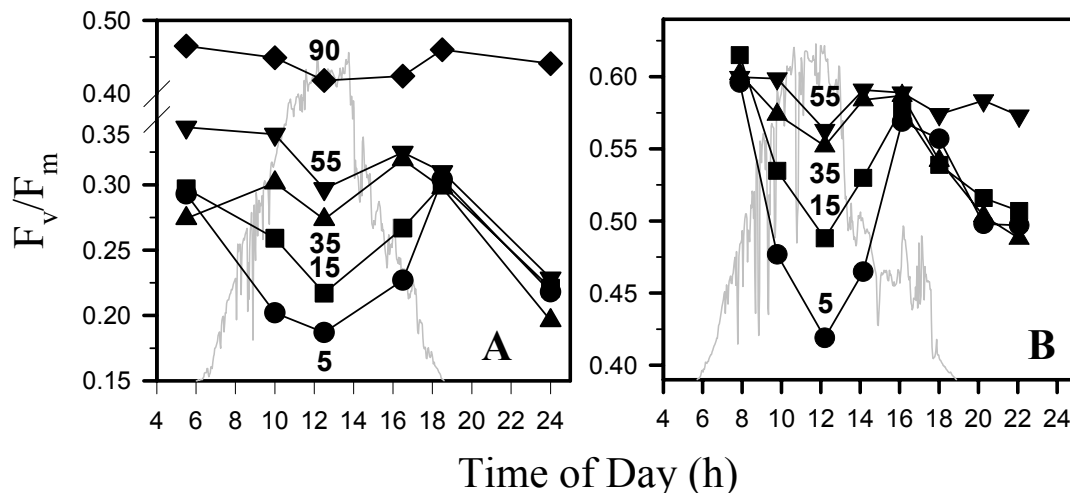


Figure S1: Depth-dependent diel changes in F_v/F_m observed at the two OliPac time-series stations [depths indicated next to each trace (m)]. Data in panel A are from the high-nitrate equatorial upwelling region, while data in panel B are from an extremely low nutrient, low phytoplankton biomass region. Light gray line indicates surface sunlight.

Part III: Potential Artifacts from Blanks

Cullen and Davis (2003) describe how problems in blank determinations can have a significant influence on interpretations of oceanographic data where comparisons between absolute values are made between ocean regions. They suggest that issues with blanks can be particularly problematic in fluorescence data. Variations in blank values can result from a variety of factors; such as differences in instruments between studies, cuvette biofouling over time, or unintentional contamination of cuvettes (e.g., oils from your hands). Changes in background fluorescence from all these sources can have a non-negligible influence on derived variable fluorescence parameters if their contribution is significant relative to fluorescence yields of the sampled phytoplankton populations.

To test for significant contributions of background fluorescence to observed patterns in photosynthetic parameters in the tropical Pacific, we routinely measured blank fluorescence values using distilled water and 0.45 μm syringe-filtered seawater each midnight before beginning the following day's continuous flow-through fluorescence record (Fig. S2). We find that our background values are more stable for freshly filtered seawater than for DI water stored in a polycarbonate vessel. In both cases, though, blank values were negligible relative to total sample fluorescence, even in highly oligotrophic waters. DI and filtered seawater blanks averaged 0.04 and 0.02 relative fluorescence units, respectively. When compared to the minimal F_0 values measured during the subsequent diurnal period (i.e., yielding the maximum potential artifact), these blank values generally represented only a few percent of total fluorescence. Clearly, blanks were not a significant problem during our studies. We note, however, that great care was taken during all our field studies to minimize fouling and we concur with Cullen and Davis (2003) that attention to blanks is an important consideration for all optical measurements.

Reference:

Cullen, J.J., R.F. Davis. The blank can make a big difference in oceanographic measurements. *Limnol. Oceanogr. Bull.* **12**, 30 (2003)

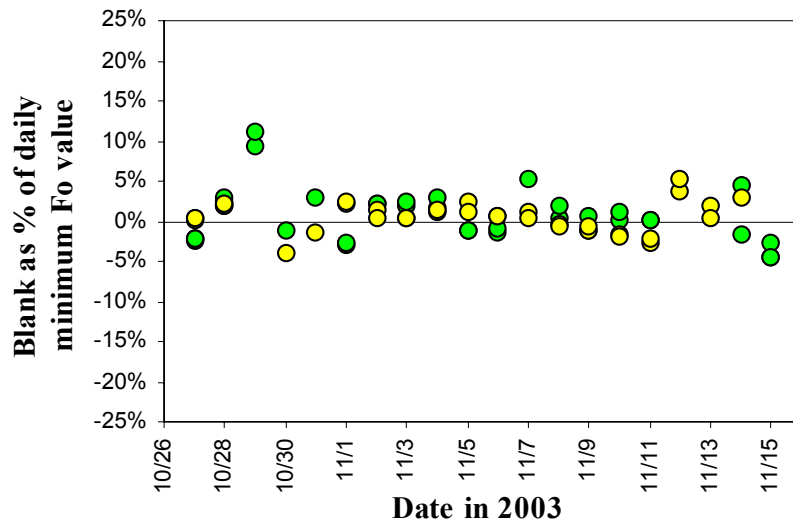


Figure S2: Blank values measure for freshly filtered seawater (green circles) and stored DI water (yellow circles) during the 2003 field study. Values are expressed relative to the minimum fluorescence value measured the following day with raw seawater.