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The effect of iron- and light-limitation on phytoplankton communities of deep chlorophyll maxima of the western Pacific Ocean

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ABSTRACT

The deep chlorophyll maximum (DCM) is a widespread feature in most stratified, oligotrophic waters. In addition to their well-established importance for many surface phytoplankton communities, more recent evidence suggests that iron, light or co-limitation may also be important drivers for some DCM communities. To test this hypothesis, we describe the results from six grow-out experiments, four from the Equatorial Pacific Ocean (between 150°E and 140°W), one in Western Pacific Warm Pool (9°S, 170°E) and one in the middle of the Tasman Sea (36°S). Photosynthetic efficiency (Fv/Fm) and biomass response, including Chl *a* and phytoplankton community structure (pennate diatoms, photosynthetic eukaryotes, *Synechococcus, Prochlorococcus,* and major *Prochlorococcus* ecotypes), were assessed over five days in control, +Fe, +Light or +Fe +Light treatments. Photosynthetic efficiency did not change dramatically in any of the treatments at any of the locations, except at 0°N 140°W where the control and +Fe bottles had elevated efficiency relative to both +Light treatments. Except for some ecotypes of *Prochlorococcus* (eMIT9313 and eNATL2A), phytoplankton populations were most strongly limited by light in the DCM. Pennate diatoms and other photosynthetic eukaryotes showed the most enhancement with the addition of iron and light at some stations and may be co-limited, but no phytoplankton populations were enhanced by adding iron alone. Although the duration and magnitude of the responses varied depending on initial macronutrient concentrations, they were generally consistent across the locations sampled. These results suggest that light is the primary limiting resource of the DCM for this vast region, but that iron can play an important additive role in limiting phytoplankton populations in locations where flux to the DCM is reduced.

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1. Introduction

The deep-chlorophyll maximum (DCM) is a widespread feature in the stratified oligotrophic ocean and is found in all but the most well-mixed waters. The extensive occurrence of the DCM has significant ecological (Venrick, 1988), geochemical (Jochem and Zeitzschel, 1993) and remote sensing implications (Devred *et al.,* 2007). This subsurface maximum in pigment biomass is driven by at least three independent mechanisms including (1) elevated ratios of chlorophyll to phytoplankton total biomass resulting from photoacclimation or photoadaptation, (2) biomass accumulation due to cells sinking from above and (3) increased biomass due to the optimized balance of irradiance and nutrient availability (Cullen, 1982). Other mechanisms may also be operating and in particular in specialized environments like suboxic zones where secondary chlorophyll maxima are observed much deeper than the traditionally defined euphotic zone (Johnson *et al.,* 1999). Although it is clear that each of these three major mechanisms play a role in establishing and maintaining DCM, in open ocean stratified oligotrophic waters, the depth of the DCM is typically located at or near the depth of the nutracline suggesting that the balance between the supply of nutrients from below and light from above is, at least in part, regulating the depth of the DCM. While the depth of nutracline and the depth of the DCM are interdependent, it is not clear which nutrient has proximal influence because there is often a relationship among the nutrients below the euphotic zone. Either macronutrients or micronutrients could be important in regulating the deep chlorophyll maximum populations.

For surface waters there is substantial evidence that phytoplankton photosynthesis and growth may be limited by iron (Fe) over vast regions of the Pacific Ocean (Behrenfeld *et al.,* 2006; Boyd *et al.,* 2007), albeit with other processes playing an important role in community structure and dynamics. In particular, most of the surface Equatorial Pacific Ocean has fluorescence induction signals that are indicative of Fe-limitation (Behrenfeld *et al.,* 2006). Further, the photophysiology of natural populations (Lindley *et al.,* 1995) and bottle and open-ocean nutrient amendment experiments of surface phytoplankton communities (Coale *et al.,* 1996b; Martin *et al.,* 1994) from various locations in this region support Fe as the proximal limiting nutrient of the growth of surface phytoplankton populations.

Substantial variability in the flux and concentrations of Fe in the Pacific Ocean (Duce *et al.,* 1991; Gordon *et al.,* 1997) complicate these patterns. In particular, a major source of Fe to the Equatorial Pacific Ocean is the equatorial under current (Cromwell *et al.,* 1954; Mackey *et al.,* 2002), which is hypothesized to deliver Fe from coastal and shelf sources near Papua New Guinea along the equator (Coale *et al.,* 1996a; Wells *et al.,* 1999). The equatorial undercurrent depth shoals from west to east creating a gradient in Fe along the equator both in terms of concentration and depth (Slemons *et al.,* 2009; 2010). The gradient in Fe and other upwelled nutrients along the equator leads to zonal patterns in primary productivity and export production (Barber and Chavez, 1991; Dunne *et al.,* 2000). Thus variability in Fe concentrations influences surface phytoplankton populations. However, the effect of Fe on subsurface phytoplankton populations is less clear. Because the depth of

the DCM is largely coincident with the nutracline, the flux of Fe from below may regulate these deep phytoplankton populations as well. Fe quotas are elevated for the low light acclimated phytoplankton that are found deep in the water (Kettler *et al.,* 2007; Sunda and Huntsman, 1998; Timmermans *et al.,* 2001) and Fe limitation induces significant changes in the ability of phytoplankton to harvest light (Bibby *et al.,* 2001; Buitenhuis and Geider, 2010; Greene *et al.,* 1992). There is also substantial evidence that Fe and light co-limit phytoplankton in other ocean regions (Boyd *et al.,* 1999; Maldonado *et al.,* 1999; Mitchell *et al.,* 1991; Smith *et al.,* 2000) and that the interplay of light and Fe limitation is regionally and vertically complex (Galbraith *et al.,* 2010). Thus, variability in Fe concentrations in the deep euphotic zone across the Pacific Ocean may be affecting phytoplankton productivity in the DCM. Indeed, others have found deep phytoplankton populations in the eastern Pacific Ocean (Hopkinson and Barbeau, 2008; Selph *et al.,* 2010) or the NE subarctic Pacific Ocean (Maldonado *et al.,* 1999) to have complex interactions between light and Fe suggesting that in some regions Fe can regulate both surface and deep phytoplankton populations.

In addition to the effects of nutrients (in this case Fe) and light on abundance and productivity and biomass of the DCM community as a whole, these environmental variables can also affect the community composition. In particular, in this region phytoplankton populations of the DCM are typically dominated by small eukaryotes and prokaryotic phytoplankton (Landry *et al.,* 1996). However, these picophytoplankton populations are also most abundant in areas where Fe is limiting (Landry *et al.,* 1996), in part because their small size is an adaptive response to increase Fe uptake kinetics and decrease Fe requirements (Hudson and Morel, 1990). Nevertheless, other Fe addition experiments (Coale *et al.,* 2004; 1996b) and Fe and light co-limitation experiments (Boyd *et al.,* 2000; Hoffmann *et al.,* 2008; Sunda and Huntsman, 1998) have demonstrated that community structure may be strongly dependent on Fe concentrations and light levels. Thus, Fe and light fluxes to the DCM may affect both the magnitude and composition of the phytoplankton community.

Because there is evidence that phytoplankton populations from the DCM may be co-limited by Fe and light in other regions of the Pacific Ocean that have different physical and chemical characteristics (Hopkinson and Barbeau, 2008; Selph *et al.,* 2010), the goal of this study was to investigate this potential co-limitation over a broad area in the biogeochemically and climatically important western Pacific Ocean to see if this co-limitation is found more broadly. To determine the degree of Fe or light limitation (or co-limitation) of the DCM in different hydrographic environments, we performed Fe and light amendment grow-out experiments at six locations in the Western Pacific Ocean (Fig. 1). We measured the daily response of natural populations over the course of five-day grow-out experiments using chlorophyll biomass, flow cytometry (phytoplankton taxonomic groups), qPCR (*Prochlorococcus* ecotypes), and active fluorescence (Fv/Fm, σ_{PSII}) to determine the degree of Fe- and light- (or co-) limitation of bulk community and specific phytoplankton populations. We find that the bulk community and the majority of the

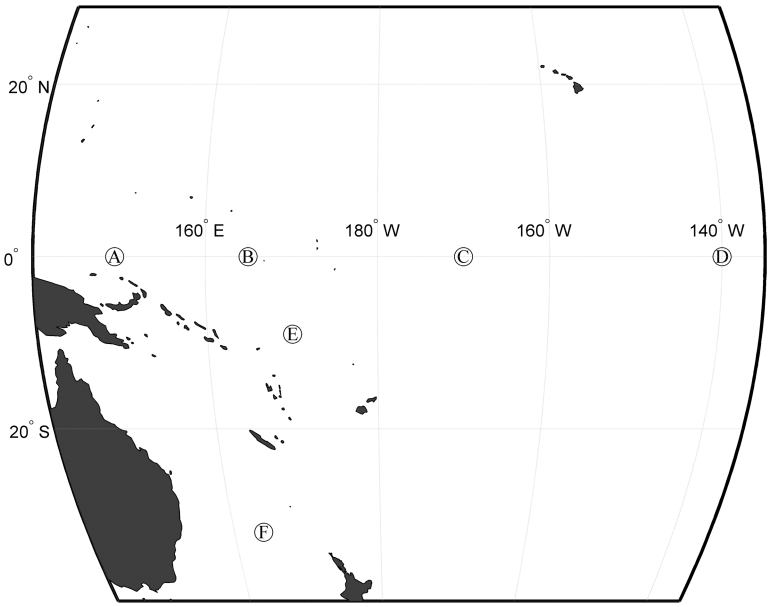

Figure 1. Locations of grow-out experiments.

specific phytoplankton populations were largely light-limited. Pennate diatoms and other photosynthetic eukaryotes were co-limited by Fe and light at some locations, but within the resolution of these experiments, no phytoplankton population appeared to be limited by Fe alone. These results suggest that light is the proximal variable regulating phytoplankton populations from the DCM over a wide region in the Pacific Ocean, but that Fe plays an important role in modulating the phytoplankton community response to light availability.

2. Methods

a. Field sampling

Samples were collected aboard the R/V *Kilo Moana* on two major ocean transects, the first from Honolulu, Hawaii to Rabaul, Papua New Guinea during August 2006 and the second from Honolulu, Hawaii to Brisbane, Australia during January–February 2007 (Fig. 1). During both cruises, *in situ* profiles of temperature, light and chlorophyll fluorescence were collected using a conductivity, temperature and depth recorder (CTD) mounted to a standard sampling rosette. The mixed layer depth was calculated from temperature profiles (Lorbacher *et al.,* 2006). The deep chlorophyll maximum sampling depth was identified from the peaks of real-time fluorescence traces (e.g. Fig. 2). The deep chlorophyll maxima of the six grow-out stations occurred at the ~2% surface irradiance isolume and were sampled at near local noon. The four samples for incubations from the equator (Sta. A–D) were collected using a trace metal clean rosette (Measures *et al.,* 2008) and sampled taking

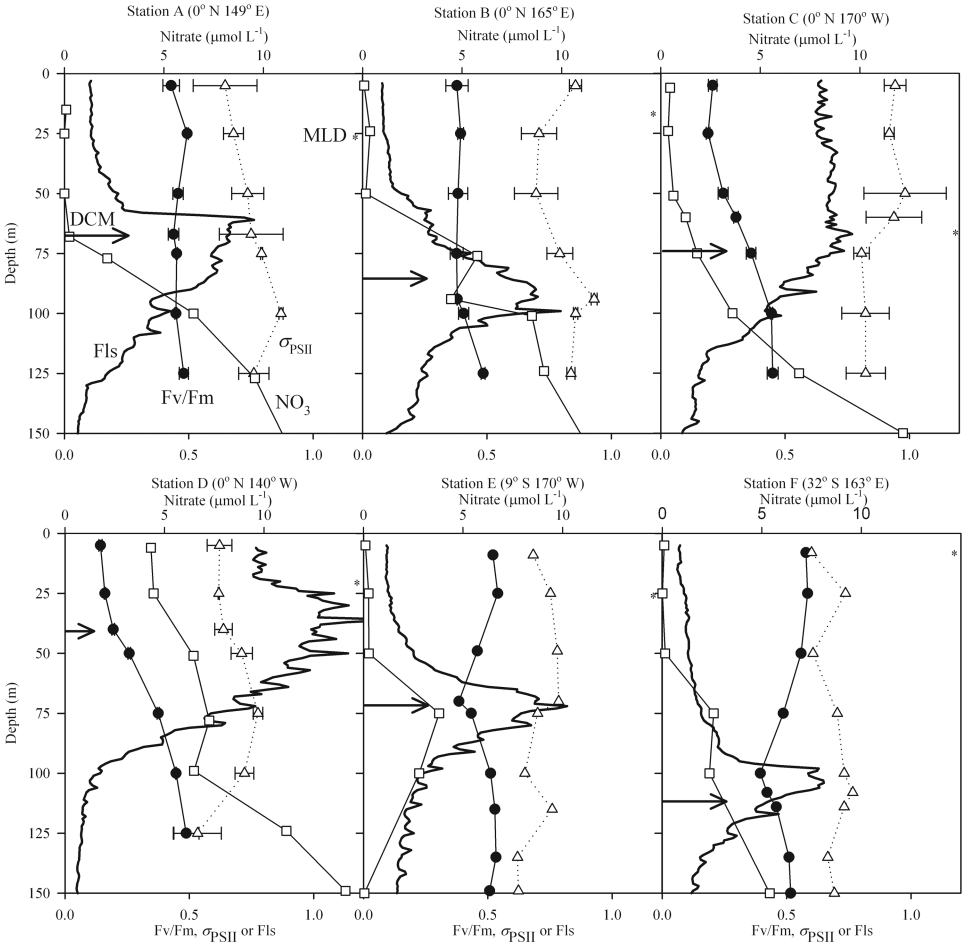

Figure 2. Characteristics of the water columns where the deep-chlorophyll maxima were sampled. Solid line with open squares is nitrate (NO_3), solid line is fluorescence (Fls), solid line with circles is Fv/Fm, and broken line with triangles is σ_{PSII}. The asterisk indicates the mixed layer depth (MLD) and the arrow represents the deep-chlorophyll maximum (DCM) sampling depth.

precautions against contamination (Fitzwater *et al.,* 1982). The two stations in the southwestern Pacific Ocean (Sta. E and F) were sampled using a standard oceanographic rosette, equipped with HCl cleaned (Fitzwater *et al.,* 1982) niskin bottles and taking similar sampling precautions to limit contamination. For all experiments, 500 mL HCl cleaned, polycarbonate bottles were rinsed three times with sample, then filled in triplicate for each time point and treatment using an acid-cleaned platinum-cured silicone filling tube, shielding the tube and sample bottle from ambient light. Each bottle was filled such that there were no visible air bubbles. Bottles were sealed tight and bagged in clear zipper bags

before being placed in the incubators. The sample collection, treatment and subsequent delivery to incubators generally took under an hour.

b. Incubations

Fe-amended bottles received an aliquot from an acidified FeCl solution (75 μmol L^{-1}) to raise the final Fe concentration by 5 nmol L^{-1}. All sample bottles were placed in custom built on-deck incubators, which were kept at surface seawater temperatures using the ship's flow through seawater system. Surface temperature was typically less than a degree warmer than at the DCM. Control (2% of surface irradiance) and amended (5% of surface irradiance) light levels were achieved using a combination of blue and neutral density stage screening (Roscolux). Incident sea surface and *in situ* incubator irradiance were monitored throughout the incubation period using a cosine (Biospherical No. QCR-2100) and 4π scalar irradiance (Biospherical No. QSP-2110) sensors, respectively. Incubator temperature, which was monitored daily throughout the incubation period using a calibrated hand probe, differed from sea surface temperature by ≤1°C. Triplicate bottles for each treatment (control, +light, +Fe, +light +Fe) were sacrificed daily at local noon and each measurement was made on each bottle.

c. Chlorophyll

Chlorophyll concentrations were measured by filtering 100 mL of sample onto a 0.22 μm polycarbonate filter using gentle vacuum (<100 mm Hg) and extracting in 100% MeOH at −20°C in the dark for >24 h following (Holm-Hansen and Riemann, 1978). Fluorescence was measured using a Turner Designs 10-AU fluorometer following (Welschmeyer, 1994) that was calibrated against a standard chlorophyll solution (Ritchie, 2006).

d. Flow cytometry

Subsamples for flow cytometry were collected and stored in the dark until live processing (within three hours) for eukaryotic phytoplankton populations or frozen with 0.125% glutaraldehyde at −80°C following (Vaulot *et al.,* 1989) for later analysis for prokaryotic phytoplankton (*Prochlorococcus* and *Synechococcus*). All flow cytometry samples were run on a Becton Dickinson FACSCalibur flow cytometer modified with a syringe pump for quantitative sample delivery. Particles were excited with 488 nm excitation (15 mW Ar laser) and forward (<15°) scatter, side (90°) scatter, green (530 ± 30 nm) fluorescence, orange fluorescence (585 ± 42 nm), and red fluorescence (>670 nm) emissions were measured. *Prochlorococcus, Synechococcus,* pennate diatoms and other eukaryotic phytoplankton (all less than ~20 μm) were classified based on their characteristic flow cytometric signatures relative to standard fluorescent microspheres (Polysciences YG) following standard population gating schemes (Olson *et al.,* 1989).

e. Prochlorococcus clades

The four most abundant clades of *Prochlorococcus* (eMIT9312, eMIT9313, eMED4, and eNATL2A) were enumerated using a quantitative polymerase chain reaction (qPCR) based approach following (Ahlgren *et al.,* 2006; Zinser *et al.,* 2006) (note: the sequence of the eMED4 forward primer lowBAIIf published in Ahlgren *et al.* (2006) was misreported and should read: 5′-TACCTCCACTGAATACCACCTCT-3′). Other clades (eSS120 and eMIT9211) were found to compose $<1\%$ of the total *Prochlorococcus* population in profiles sampled from this region and therefore were excluded from this study. Briefly, triplicate 100 mL samples were filtered on 0.22 μm polycarbonate filters using gentle vacuum (<100 mm Hg), followed by ~3 mL preservation solution (10 mmol L^{-1} Tris, 100 mmol L^{-1} EDTA, 0.5 mol L^{-1} NaCl) and stored at −80°C until later analysis. Cell lysates were made by shaking filters (≥4800 RPM) with 10 mmol L^{-1} Tris pH 8.0 in a beadbeater without beads and the lysate incubated at 95°C for 15 mins before being stored at −80°C until later analysis. qPCR protocols, based on primers and conditions specific for a given clade of *Prochlorococcus* and calibrated with cultures, utilized Sybr I Green to quantify amplicons. For each run, the purity of products was evaluated and verified using dissociation curve analyses.

f. Photosystem II photophysiology

Single turnover fluorescence induction curves were made using a FIRe fluorometer (Satlantic) to assess the photophysiology of the phytoplankton community (Gorbunov and Falkowski, 2004). Duplicate samples were taken from each incubation bottle and stored in the dark for >15 mins prior to assessment. Blanks (0.22 μm syringe filtered sample) were run identically to samples, but did not introduce a significant source of error. Raw data were collected following manufacturers protocols, and then processed using custom written software based on previous work (Johnson, 2004). Parameters of a model (Kolber *et al.,* 1998) were optimized to fluorescence induction curve data and a single exponential decay rate was optimized to fluorescence decay data. Although there are multiple components involved in fluorescence decay, a single component model was used because the signal to noise ratio of samples from this oligotrophic region precluded estimating higher orders of variability. From these models, Fo (initial fluorescence), Fm (saturated fluorescence), σ_{PSII} (photosystem II functional cross section), p (photosystem connectivity), and τ_{PSII} (turnover rate) were estimated. Fv/Fm was calculated as (Fm-Fo)/Fm.

g. Macronutrients

For Sta. A–D, major nutrients including NO_3, $SiOH_4$, and PO_4 were measured as follows: for NO_3, suspended particles were removed by filtering 30 mL of sample through a GF/F filter (0.7 μm nominal pore size) into acid-cleaned 60 mL low density polyethylene bottles. Nutrient samples were frozen at −20°C until onshore analysis. Within two months after the cruise, the nitrate (NO_3 + NO_2) was determined using an Astoria Autoanalyzer (Marchetti *et al.,* 2010). NO_2 represented $<0.05\%$ of total measured ambient N. The

detection limit for NO_3 is 0.05 μmol L^{-1}. $Si(OH)_4$ was measured on-board spectrophotometrically following (Strickland and Parsons, 1972), using a reverse-order reagent blank (Brzezinski and Nelson, 1986). For Sta. E, F, major nutrients were measured on water samples collected from a trace metal clean rosette, 0.4 μm filtered and rapidly frozen at −20°C. Dissolved inorganic phosphorus (PO_4) and nitrate + nitrite (NO_3) concentrations (detection limits of 0.006 and 0.05 μmol L^{-1}, respectively) were measured following (Hynes *et al.,* 2009).

h. Statistical treatment

For each treatment, significant differences ($p < 0.05$) between treatments or controls were determined using Student's t-tests on triplicate samples from each time point using untransformed data.

3. Results

Most of the stations sampled had hydrographic columns with a typical tropical structure; fluorescence profiles had subsurface maxima between ~40 and 100 m (Fig. 2). Of the stations reported here, the sole exception to this pattern was Sta. C, which is located at the equator on the dateline. This station had a relatively constant fluorescence trace for the upper ~80 m, with a minor peak around 75 m (which is where this nominal DCM was sampled). This station also had the deepest mixed layer depth at 62 m, whereas the other stations all were less than 25 m (Table 1).

The in situ photophysiological properties associated with the water columns could be characterized into three broad categories. Sta. A, B, located in the Western Pacific Ocean along the equator had relatively constant photosynthetic efficiency (Fv/Fm) and absorption cross section (σ_{PSII}) over the upper ~100 m of the water column, in spite of dramatic differences in the biomass profile and shallow mixed layers. In contrast, Sta. C, D, which are located in the Central Pacific Ocean and at the western edge of the cold tongue on the equator, had decreased surface Fv/Fm which gradually increased deeper into the euphotic zone. The surface values of the Fv/Fm were less than 0.3 and significantly ($p < 0.01$) below those from Sta. A, B, but the deep values of Fv/Fm were indistinguishable from Sta. A and B. The σ_{PSII} from these stations also did not follow any patterns in the upper water column. The final photophysiological category included Sta. E, F that are from the southern Western Pacific Ocean. Like Sta. A, B, these stations had relatively high surface Fv/Fm (>0.5), but these stations had sub-surface minima (<0.4) associated with the DCM. There was no associated pattern in the σ_{PSII}. Although the surface values of Fv/Fm varied between the stations, Fv/Fm was consistently less than 0.5 at the DCM suggesting stressed phytoplankton populations (Kolber *et al.,* 1994).

a. Photophysiology (Fv/Fm)

As a proximal gauge of Fe or light stress, grow-out experiments were assessed photophysiologically (Geider *et al.,* 1993). As with *in situ* profiles of photophysiology,

Table 1. Location and initial conditions of each of the six stations sampled. Depths are given next to value when depth sampled differs from the nominal deep chlorophyll maximum depth. Error bars, where present, represent the standard deviation of triplicate samples. Fe data after (Slemons *et al.,* 2010)* (total Fe) and (Chappell, 2009)$^{+}$ (<0.4 μm Fe).

Station	A	B	C	D	E	F
Location	0°N 149°E	0°N 165°E	0°N 170°W	0°N 140°W	9°S 170°E	32°S 163°E
DCM Depth (m)	67	82	75	40	70	112
°C	29	30	29	29	28	19
Mixed Layer Depth (m)	26	12	62	18	22	5
% Isolume	2	3	2	7	2	2
NO_3 (μmol L^{-1})	0.3	5.8 (76 m)	1.8	6.5 (51 m)	4.3	2.2 (100 m)
PO_4 (μmol L^{-1})	0.2	0.1 (76 m)	0.4	0.5 (51 m)	0.5	0.3 (100 m)
$Si(OH)_4$ (μmol L^{-1})	1.0	1.2 (76 m)	1.2	2.5 (51 m)	0.7	0.9 (100 m)
Fe (nmol L^{-1})	1.1 (75 m)*	0.40 (75 m)*	0.55 (75 m)*	0.52 (30 m)*	0.18 ± 0.01 (50 m)$^{+}$	0.21 ± 0.08 (0 m)$^{+}$
Fv/Fm	0.49 ± 0.00	0.50 ± 0.01	0.31 ± 0.07	0.25 ± 0.01	0.58 ± 0.00	0.56 ± 0.00
Chl (μg L^{-1})	0.402 ± 0.002	0.503 ± 0.017	0.256 ± 0.035	0.266 ± 0.012	0.162 ± 0.065	0.128 ± 0.070

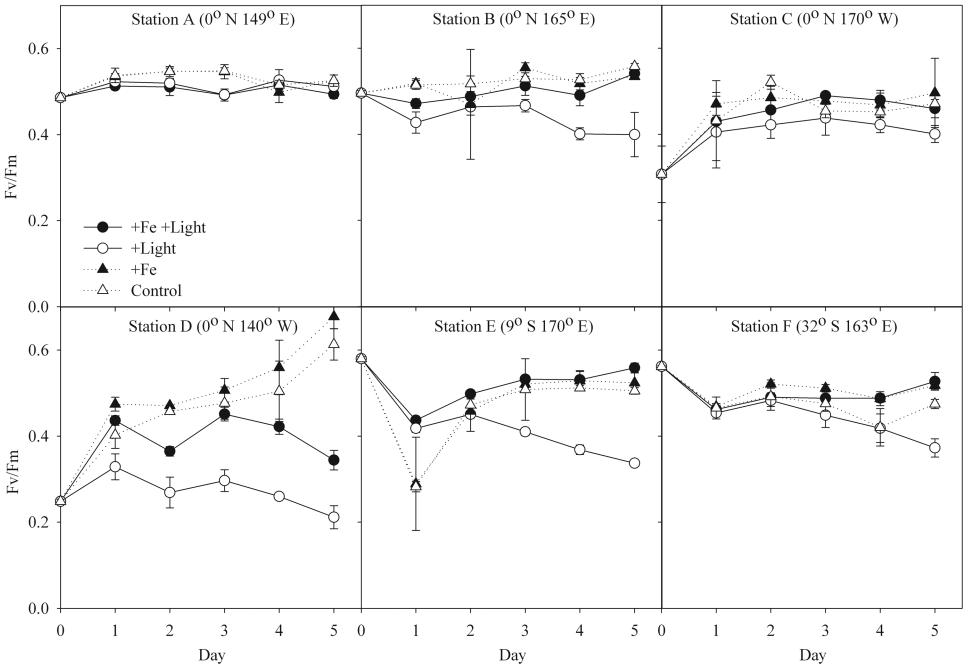

Figure 3. Fv/Fm from the six incubations. Error bars represent the standard deviation between three biological replicate bottles, and when not visible, are smaller than the symbols.

grow-out experiments could be divided into different broad categories based on their generalized response to iron (+Fe), light (+Light) or iron and light (+Fe +Light) addition (Fig. 3). All treatments from Sta. A, B had relatively invariant Fv/Fm over the duration of the experiment with the exception of +Light bottles from Sta. B, which did show a small decrease in Fv/Fm by the end of the experiment (day 5). Values of σ_{PSII} did not change significantly over the duration of the incubations (Table 2). Overall, photophysiological responses from all of these Sta. A, B bottles were minor. In contrast, values of Fv/Fm from Sta. C, D increase significantly over the duration of the experiment, regardless of treatment (or control). At Sta. C, these values start low (0.3) and all increase to ~0.5 with no major differences between treatments or control. Sta. D also started low (0.25), but +Light bottles remained relatively constant, whereas +Fe +Light bottles increased to an intermediate level (~0.4) and both the control and +Fe treatment increase steadily over the experiment peaking on day 5 at ~0.6. Sta. E, F, both of which started with Fv/Fm at ~0.55, had Fv/Fm values decrease during the grow-out experiments. This decrease in Fv/Fm was ~20%, but was most dramatic for the +Light treatment, which was significantly less for both E and F. Values of σ_{PSII} also changed during the incubations, and generally were opposite to patterns of Fv/Fm, such that when Fv/Fm increased, σ_{PSII} decreased (Table 2). The exception to this trend was for Sta. D, where both Fv/Fm and σ_{PSII} increased over the

Table 2. Mean of the ratio of treatment normalized to control from the day of maximal response (usually Day 5). Treatments that were significantly different ($p < 0.05$, $n = 3$) from control are in italics. +Fe +Light treatments that are different from +Light are in bold.

Treatment	+Fe						+Light						+Fe +Light					
Station	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F
Fv/Fm	1.0	1.0	*1.0*	*1.1*	1.0	1.1	1.0	*0.9*	*0.8*	*0.5*	*1.2*	0.9	*0.9*	***1.0***	*0.9*	***0.7***	***1.6***	1.1
σ_{PSII}	1.0	*1.0*	1.1	*1.1*	0.9	0.9	1.1	1.2	*1.1*	*1.2*	*1.3*	1.0	1.1	1.1	1.0	*1.2*	1.0	0.9
Chl *a*	1.0	0.9	1.0	1.0	1.7	1.0	*1.3*	2.6	*2.3*	*2.0*	2.4	1.2	*1.4*	*4.8*	***5.4***	***12***	***8.0***	*1.6*
Pennates	1.0	1.0	1.0	1.0	0.7	1.0	9.9	77	*27*	*67*	*24*	*4.7*	*5.0*	*86*	*102*	*153*	*16*	***15***
Photo.Euks.	1.0	1.0	0.9	1.0	*0.4*	0.8	*2.1*	2.2	*2.1*	*1.9*	*2.6*	*1.5*	*2.8*	2.6	*2.9*	*1.7*	***3.4***	***2.2***
Synechococcus	1.0	0.4	1.0	1.0	1.3	1.0	3.4	0.7	3.4	1.6	*2.4*	1.0	*5.2*	0.9	*1.5*	1.1	*3.1*	1.0
Prochlorococcus	1.0	1.0	1.0	1.0	0.8	0.9	*2.0*	1.0	*3.1*	1.1	0.9	0.6	***2.8***	1.0	1.5	*1.2*	0.9	*0.7*
eMIT9312	1.0	1.0	0.9	1.1	0.5	0.8	2.3	6.0	*7.9*	1.4	2.6	0.4	*2.5*	4.0	4.7	1.4	2.0	1.3
eMED4	1.0	1.0	2.3	0.1	2.7	0.9	*3.1*	—	*5.3*	0.8	9.1	2.4	***4.7***	4.5	4.8	1.2	8.0	1.5
eNATL2A	0.7	0.7	1.0	0.8	0.6	0.6	1.8	1.2	1.1	0.8	2.8	1.4	1.6	1.0	0.9	0.9	0.8	1.1
eMIT9313	1.0	1.1	1.0	1.0	0.6	1.3	1.0	1.0	2.8	1.0	5.9	0.9	1.0	1.0	2.6	1.0	0.3	0.1

duration of the grow-out experiment, perhaps due to changes in community structure (Behrenfeld *et al.,* 1996; Suggett *et al.,* 2009). Overall, except for Sta. D where there were dramatic changes in Fv/Fm over the duration of the experiment and some small decreases in the +Light treatment at other stations, there were only relatively minor changes over the course of the experiments in Fv/Fm (and σ_{PSII}), regardless of treatment or control.

b. Phytoplankton biomass

Unlike photophysiology, total chlorophyll *a* (Chl *a*) biomass showed significant changes in the different treatments at all of the stations sampled (Fig. 4, Table 2). Sta. A had significant increases in Chl *a* in the +Light and +Fe +Light treatments that peaked on day 3, while control and +Fe bottles remained constant through the experiment. Sta. B–D also had large increases in the +Light and +Fe +Light bottles, but these responses peaked on the final day of the experiment. For Sta. C, D the control and +Fe bottles remained constant over the five days, whereas for Sta. B the control and +Fe bottles decreased slightly, but steadily over the duration of the experiment. Like for the other four sampling locations, Chl *a* response from Sta. E and F increased dramatically for the +Light and +Fe +Light bottles, peaking on the final day of the experiments. However, for these stations the control and +Fe bottles also increased during the experiment albeit less than the +Light and +Fe +Light bottles. Overall, the general trend for Chl *a* biomass in all of these grow-out experiments is that the +Light and +Fe +Light bottles increased during the experiment, whereas the control and +Fe bottles were similar. The timing and magnitude of the peak in Chl *a* and the trend in the control and +Fe bottles varied depending on location. All of these trends in Chl *a* were dominated by the increase in cell concentration with less dramatic changes in chlorophyll per cell as assessed by flow cytometry (red fluorescence per cell).

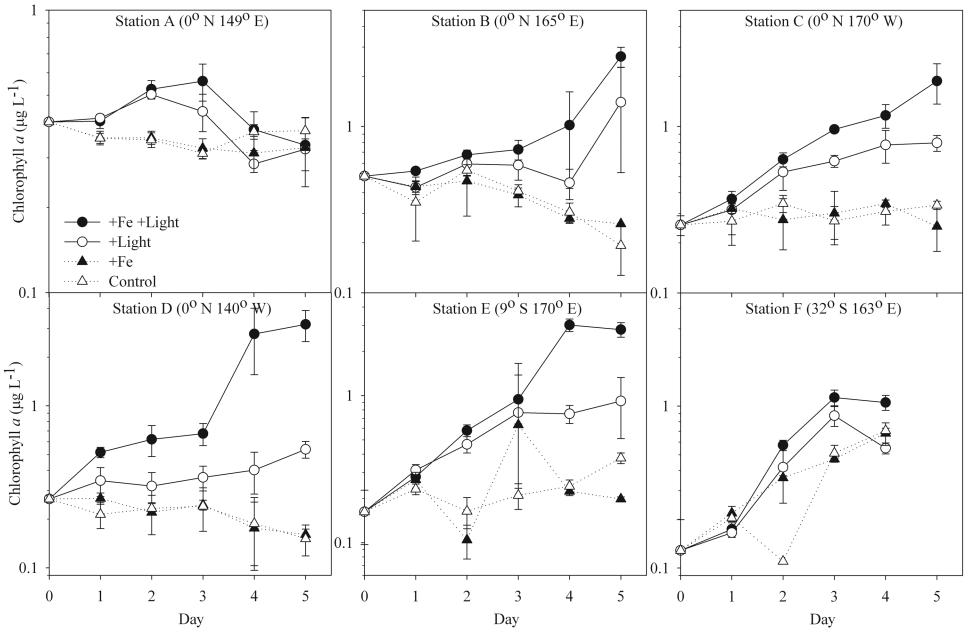

Figure 4. Chl *a* from six incubations. Error bars as in Figure 3.

c. Phytoplankton community structure

Increases in total phytoplankton biomass (Chl *a*) were further teased apart into component populations using flow cytometry. Of these populations, pennate diatoms had the greatest response (Fig. 5, Table 2); the +Light and +Fe +Light bottles had dramatic increases in the pennate populations consistently across the six stations sampled. In all but Sta. A, pennate populations increased approximately 100 fold in response to light addition over controls, which is about 10X more than the Chl *a* response in the same bottles. Sta. A showed the same general pattern, but the +Light and +Fe +Light bottles only increased 10X over controls. At all stations the response was almost entirely driven by the addition of light as the +Light and +Fe +Light were statistically indistinguishable (except for a slight increase in +Fe +Light over +Light in Sta. F on the final day of sampling) (Table 2). In Sta. A–D the pennate populations from control and +Fe bottles were constant over the duration of the experiment (and were not statistically distinguishable from each other). Sta. E, F showed increases in both the pennate populations in the control and +Fe bottles although these populations were also not statistically different from each other. Increases in phytoplankton populations in control bottles have previously been observed (Martin *et al.,* 1990; Venrick *et al.,* 1977), but here the overall trends in the responses from the +Light and +Fe +Light bottles are still significantly different from control bottles regardless of any "bottle effect."

Although pennate diatoms had the greatest increase in biomass of any phytoplankton

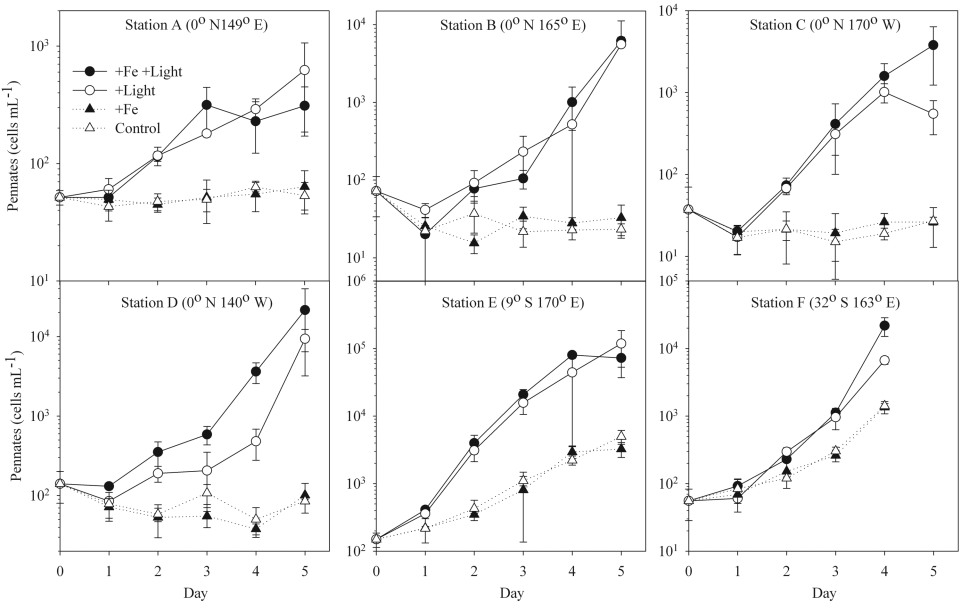

Figure 5. Pennate diatom concentrations from six incubations. Error bars as in Figure 3.

group measured here, other populations increased in some treatments relative to control bottles. For example, photosynthetic eukaryotes also all increased in response to +Light and +Fe +Light (Table 2). On average, the maximum response during the experiment was 2.1X and 2.5X for the +Light and +Fe +Light, respectively, over the controls (Table 2). Like for pennate diatoms, this response was largely due to the addition of light; only Sta. E, F had slight increases in the +Fe +Light over the +Light bottles. Thus, although the magnitude of the response was muted in relation to the pennate diatoms, the small photosynthetic eukaryotes showed the same general trends.

Synechococcus populations, had initial concentrations that ranged from 130 (Sta. C) to 11,600 cells mL^{-1} (Sta. B) and did not comprise a majority fraction of the total phytoplankton cell concentrations at any of the stations sampled, increased in response to +Light at Sta. C and in response to +Fe and +Light at Sta. A, C, and E (Table 2). Overall, the magnitude of increase in the +Light and +Fe +Light bottles averaged 2.4X and 3.3X, which is roughly consistent with the similarly sized photosynthetic eukaryotes. As with pennate diatoms and photosynthetic eukaryotes, there were no differences in the measured *Synechococcus* populations between +Fe and control bottles. Although the initial population size at some stations was small, the overall proportional responses are generally consistent with those observed for photosynthetic eukaryotes.

Prochlorococcus was the most numerically dominant ambient phytoplankton group found at each of the stations. Initial concentrations from the DCM ranged from 3.5×10^4 (Sta. F) to 1.4×10^5 (Sta. B) cells mL^{-1} although the population concentrations at the

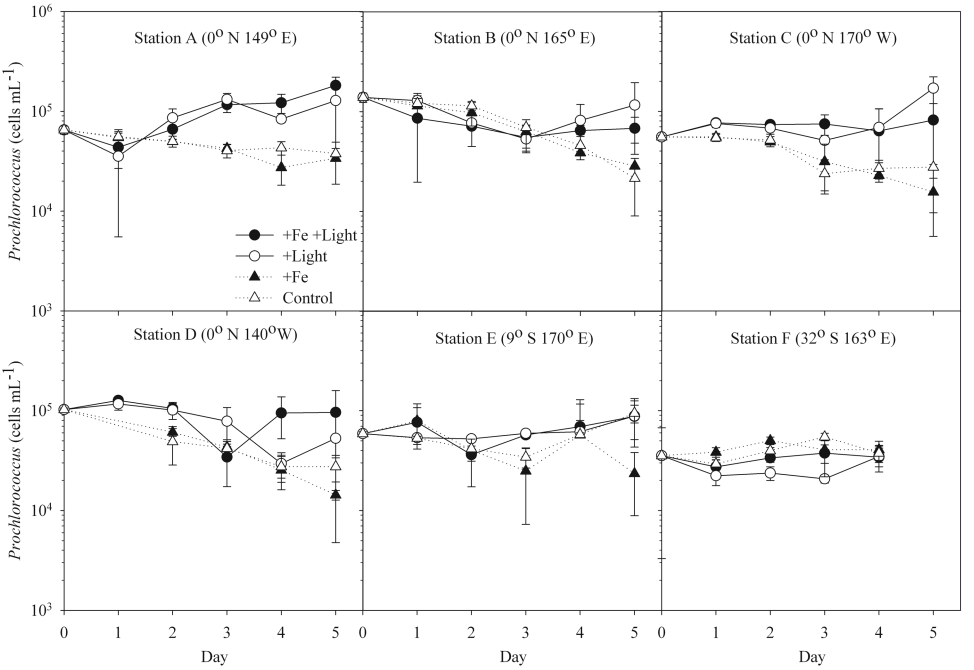

Figure 6. *Prochlorococcus* concentrations from six incubations. Error bars as in Figure 3.

DCM were not necessarily the maximum within each respective water column. In spite of their initial dominance among the major phytoplankton groups assessed, *Prochlorococcus* had the most muted response to any of the treatments (Fig. 6). Sta. A, C had minor increases in the +Light bottles over controls, while +Fe +Light bottles from Sta. A, D had increases. As with the other phytoplankton populations, there were no differences between +Fe and control bottles for *Prochlorococcus.* The direction of these responses is generally consistent with other phytoplankton populations even though the magnitude of the responses is greatly reduced.

d. Prochlorococcus ecotypes

In addition to assessing total *Prochlorococcus* populations as defined by flow cytometry, the four major genetic clades, including eMIT9312 (HL-II), eMIT9313 (LL-IV), eMED4 (HL-I), and eNATL2A (LL-I) as defined in (Zinser *et al.,* 2006), were also tracked over the duration of the grow-out experiments. The other clades that can be monitored using our qPCR-based approach (eSS120 and eMIT9211) were not found in concentrations above 10 cells mL^{-1} in ambient populations at any of the stations and therefore their populations were not further monitored. Although these genetic clades were assessed using a quantitative PCR approach that has a relatively low level of precision compared to other techniques such as flow cytometry (Zinser *et al.,* 2006) some differences in responses were apparent

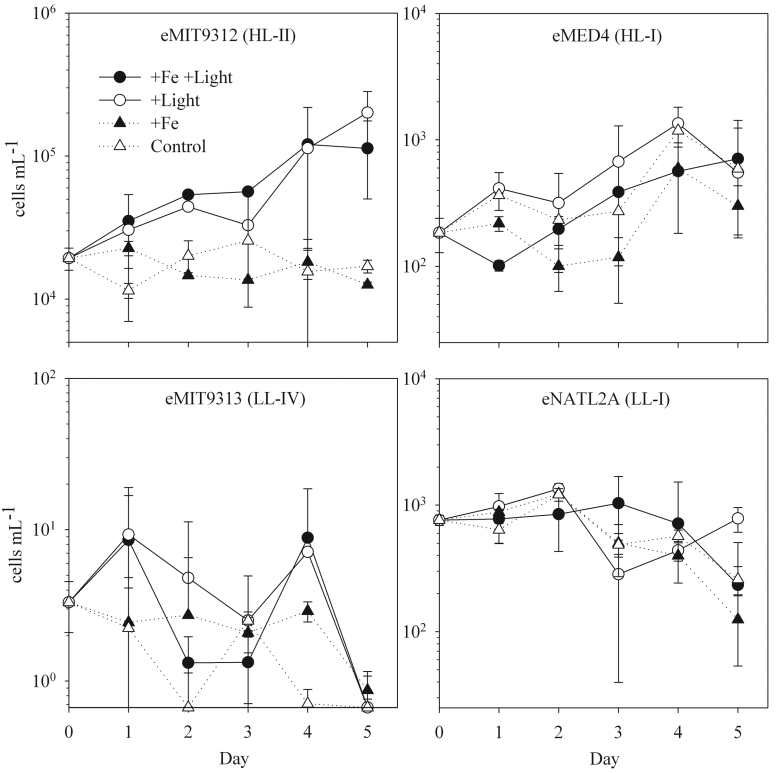

Figure 7. Typical responses of major ecotypes of *Prochlorococcus* to +Light, +Fe, or +Light +Fe at a representative station (Sta. C: 0°N 170°W). Error bars as in Figure 3.

among the different clades. For example, using Sta. C as a representative station (Fig. 7), there are increases in eMIT9312 in the +Light and +Fe +Light bottles relative to control (and +Fe) bottles. At this station, eMED4 also has statistically significant increases in the +Light bottles relative to control, while the other two clades of *Prochlorococcus* did not differ among the three treatments and control bottles. Among the six stations, the +Light bottles had increases in eMIT9312 only at Sta. C and eMED4 at Sta. A, C (Table 2). For the +Fe +Light bottles, there were increases in eMIT9312 and eMED4 only at Sta. A. At many of the other stations, there also were increases in the mean values of eMIT9312 and eMED4 populations relative to those of control bottles, but because of the high variability among samples due to the low precision of this technique, these changes were not statistically significant. The other two clades of *Prochlorococcus* (eMIT9313 and eNATL2A) did not show any differences among any of the treatments relative to the controls. Taken together, these results show that some deep populations of eMIT9312 and eMED4 from the DCM can be stimulated by light addition, but eNATL2A and eMIT9313 do not respond to additions.

4. Discussion

Previous studies of Fe and light co-limitation of the DCM in the eastern Pacific Ocean have demonstrated that at some locations there can be a significant stimulation of the deep phytoplankton community by the addition of both Fe and light (Hopkinson and Barbeau, 2008) and that this light limitation significantly influences the community structure at these depths (Selph *et al.,* 2010). In particular, this community response leads to a dramatic drawdown of nitrate and increases in total Chl *a* biomass. These responses are largely driven by the large increases in diatom populations and especially *Pseudonitzchia* (Hopkinson and Barbeau, 2008). These previous experiments are notable because they are different from "classic" Fe addition experiments (e.g. Martin *et al.,* 1989) in that the phytoplankton populations are co-limited by Fe and light—Fe alone has little to no affect.

It is well-established that some areas of the Equatorial Pacific Ocean, surface mixed layer populations of phytoplankton are Fe-limited (Coale *et al.,* 1996b; Lindley *et al.,* 1995; Martin *et al.,* 1994), but there are relatively few studies on the corresponding deep populations. The results of the experiments described here generally provide a contrasting picture to those in the surface ocean (Brzezinski *et al.,* 2008; Coale *et al.,* 1996b; Marchetti *et al.,* 2010), namely that Fe addition alone does not stimulate any of the phytoplankton populations from any of the locations examined. In addition, our results are somewhat different from some others done on deep-populations in the eastern North Pacific Ocean (Hopkinson and Barbeau, 2008) and suggest that light is the proximal regulator of phytoplankton populations from the DCM in the regions we examined and that Fe plays an important, but additive role. Thus, there are likely differences between the surface and deep phytoplankton populations in this region of study as well as differences between the deep-populations in the western Pacific and some of those examined by others in the eastern North Pacific Ocean.

a. Limitation of the DCM

Although Fe has been shown to regulate phytoplankton populations in a variety of oceanographic regions, in our study we found that Fe alone had a less pronounced effect on deep phytoplankton populations in the western and central equatorial and southern western Pacific. In particular, Fv/Fm does not change significantly relative to controls at any of the stations. Although most of the initial Fv/Fm values were moderately high (~0.50–0.55), they are similar to previous DCM enrichment studies, but they did not further increase in +Fe bottles as has been observed in other regions (Hopkinson and Barbeau, 2008). At Sta. C, D, where initial Fv/Fm values were lower and more consistent with presumed Fe limitation (~0.25–0.30), values did increase over the duration of the experiment, but were not different from control bottles. We cannot exclude the possibility that the increases in Fv/Fm in the control bottles from 170°W and 140°W are due to Fe contamination. However, we did not see associated increases in the phytoplankton populations (Chl *a*) in the control bottles suggesting that Fe contamination is not the explanation for the increase in Fv/Fm. Total dissolved Fe in the eastern equatorial Pacific Ocean near the deep-

chlorophyll maxima (Mackey *et al.,* 2002; Slemons *et al.,* 2009; 2010) are typically above the putative half saturation for the phytoplankton population growth rate (~0.12 nmols L^{-1}) (Coale *et al.,* 1996a) also suggesting that these populations may not be chronically Fe limited.

However, light appears to play a strong role in limiting phytoplankton populations of the DCM in our study areas. All of the stations investigated showed significant increases in biomass upon exposure to higher light (Fig. 4, Table 2) and this response is consistent with previous results (Hopkinson and Barbeau, 2008). Surprisingly this response is remarkably consistent across the locations sampled in spite of a broad range of depths of the DCM (<40 m to >110 m) (Table 1), although the isolumes associated with the DCMs are remarkably constant (Table 1).

Although light appears to be a significant limiting resource at most of the locations sampled, there is an additional enhancement by Fe in some of phytoplankton population characteristics. For example, many of the stations sampled showed the strongest phytoplankton community response when both Fe and light were added (Table 2). Further, although all stations had an increase in the chlorophyll biomass with the addition of light, only the +Fe +Light bottles had statistically significant increases at all stations and these mean increases in total biomass were always greater than with light alone. This effect was most pronounced for the total phytoplankton community (Chl *a*), but is also observed for some stations for pennate diatoms and other photosynthetic eukaryotes (Table 2, Fig. 5). Thus, although there may be nutrient fluxes and in particular Fe fluxes associated with deep water mixing and the equatorial undercurrent, these fluxes do not appear to be adequate to sustain an enhanced level of phytoplankton when additional light is supplied—additional Fe is required.

b. Differential community response

Although there was a universal response of the phytoplankton population as a whole to Fe and light addition, the responses among the phytoplankton taxa varied similarly to other amendment experiments (Eldridge *et al.,* 2004). For example, pennate diatoms responded most dramatically, increasing over two orders of magnitude in cell concentrations at some stations over the course of the five day experiment (Table 2, Fig. 5). Increases in the pennate populations were predominantly driven by the increase in light, however like for chlorophyll biomass, Fe enhanced this growth at some stations. Previous grow-out experiments in the Equatorial Pacific Ocean have shown that pennate diatoms are the "first responders" to a variety of stimuli including Fe (Martin *et al.,* 1994) and silicate (Brzezinski *et al.,* 2008; Marchetti *et al.,* 2010). Thus, as with other nutrients, pennate diatoms are the most capable of quickly utilizing the increase in light energy, once available, but a full response is dependent on an adequate Fe supply.

For Fe, the preferential stimulation of these larger pennate diatom cells has been suggested to be in part related to their cell size; faster growing large cells that utilize available nitrate have higher Fe requirements and once Fe-limitation is relieved, it is these

larger cells that outcompete their slower growing competitors (Landry *et al.,* 1997; Price *et al.,* 1994). Smaller cells such *Prochlorococcus* and *Synechococcus* dominate when Fe concentrations are low, in part because of faster uptake kinetics from higher surface to volume ratios enable them to more effectively compete when Fe is scarce. Rapid population increases in a large-size class phytoplankton-community are also observed when silicate is added to limited populations (Brzezinski *et al.,* 2008; Marchetti *et al.,* 2010). In this case, most smaller cells have only trivial (if any) silicate requirements. Although light is an energy resource, rather than substrate, here it appears to be acting in the same way as silicate or Fe limitation: under limiting conditions the smaller cells, many with low maximal growth rates (Moore and Chisholm, 1999), dominate whereas the pennate diatoms, which have higher maximal growth rates, are able to grow faster and increase their population when supplied with additional energy. Because these cells are large and have higher Fe requirements, in some cases the addition of Fe leads to more substantial increases in biomass, even though initially these larger cells were proximally light limited. In other oceanographic regions, Fe and light co-limitation has also been shown to influence the taxonomic composition of the diatom assemblage (Timmermans *et al.,* 2001). In addition, there is significant evidence that in the eastern equatorial Pacific the natural supply of Fe to the base of the euphotic zone from the equatorial undercurrent is not fully utilized by diatoms and other large phytoplankton because they are light limited (Selph *et al.,* 2010). The smaller phytoplankton that dominate these deep depths such as *Prochlorococcus* can take advantage of these fluxes and remove the newly available Fe before it ever reaches the larger phytoplankton located shallower in the water column.

Pennate diatoms had the most dramatic response of any of the taxonomic groups, but other groups responded to the various treatments as well. Photosynthetic eukaryotes also had increases in their population size after the addition of light, although these increases were not as great (Table 2). As with pennate diatoms, these populations had larger increases when both Fe and light were added, but Fe alone was not sufficient to stimulate the population.

Intriguingly, the small-celled prokaryotic populations including *Synechococcus* and *Prochlorococcus* also had some degree of light stimulation (Table 2, Fig. 6). However, the increase was not observed at all of the stations, and when observed it was not as great as for other taxa. These small cells, particularly *Prochlorococcus,* may dominate the deeper DCM because of inherent optical efficiencies associated with their small size (Johnson *et al.,* 1999; Morel *et al.,* 1993) including less "packaging" of pigments leading to more efficient light absorption or because their pigment compliment is more tuned to the blue wavelengths that dominate the spectra at the depths of the DCM (Moore *et al.,* 1995). Although there was a minor degree of stimulation of the total *Prochlorococcus* in response to light relative to the control bottles, data from the four dominant ecotypes that can currently be measured and that comprise between 18 and 120% of the initial population demonstrates that this response may be more complex and may differ between strains. For example, at some stations, both the eMIT9312 and eMED4 clades of *Prochlorococcus*

have an enhancement with the addition of light whereas the eNATL2A and eMIT9313 never show such an enhancement. eMIT9312 and eMED4 represent the so-called "high-light" clade of *Prochlorococcus* and are adapted to higher light environments whereas eNATL2A and eMIT9313 are adapted to low light environments (Moore and Chisholm, 1999). The natural abundance of these clades within the water column is consistent with these adaptations—eMIT9312 and eMED4 typically dominate the surface waters of the open ocean, whereas eMIT9313 and eNATL2A dominate deeper in the water column (Johnson *et al.,* 2006; Zinser *et al.,* 2006; Zinser *et al.,* 2007). *Prochlorococcus* populations associated with the DCM and its low light environment can represent a mixture of both "high" and "low"-light adapted clades, possibly because of previous mixing events or other aspects of their niche, but only the high-light adapted strains respond to the addition of light. Thus, data from the +Light incubations provide field corroboration to data from strain isolates that eMIT9312 and eMED4 are high-light adapted clades whereas the eMIT9313 and eNATL2A clades are low-light adapted clades.

Because of their small size, *Prochlorococcus* and *Synechococcus* are not expected to be Fe-limited (Morel *et al.,* 1991), although there is some evidence that even these smallest phytoplankton could be stimulated by Fe addition in the upper ocean (Barber and Hiscock, 2006; Eldridge *et al.,* 2004; Mann and Chisholm, 2000). Our results generally support that these small cell populations are not Fe limited in the DCM since neither the aggregate *Synechococcus* and *Prochlorococcus* populations nor specific sub-clades of *Prochlorococcus* were enhanced by the addition of Fe. The +Fe +Light treatments also did not show a substantial increase for the total or sub-clades over +Light alone also suggesting that light proximal regulator of these populations. These results are also consistent with the recent discovery of new clades of *Prochlorococcus* that are found in low-iron regions of the equatorial Pacific Ocean that appear to have adaptations for low-iron environments (Rusch *et al.,* 2010).

c. Variability among locations

Although each of the six experiments had the same overall patterns with respect to Fe and light limitation, there is variability in DCM phytoplankton communities and their response among the locations. The first line of evidence for these differences comes from the initial photophysiological state. Initial profiles of Fv/Fm of the ambient phytoplankton community generally fall into three categories: moderate and constant with depth (Sta. A, B), low and increasing with depth (Sta. C, D) and high, but decreased at the DCM (Sta. E, F). The moderate, but constant Fv/Fm profiles suggest that phytoplankton are not chronically Fe limited anywhere in the water column. The stations with low Fv/Fm at the surface that increases with depth suggest that Fe limits the surface populations of phytoplankton but that deeper within the water column Fe flux is sufficient to maintain functional reaction centers. Both moderate surface values at Sta. A, B and low surface values at Sta. C, D are consistent with previous Fv/Fm observations from these areas (Behrenfeld *et al.,* 2006) and corresponding Fe concentrations (Brzezinski *et al.,* 2008).

The decreased values at the DCM at Sta. E and F suggest that there may be some degree of localized Fe-limitation of the ambient community. However, these values are still relatively high (~0.5) indicating that this limitation is not chronic. It is important to note that Fv/Fm is influenced by many variables, and is not necessarily under the direct control of Fe, thus the patterns observed are likely controlled by other variables as well (Suggett *et al.,* 2009).

Grow-out experiments from different locations also have different responses in Fv/Fm to the various treatments further suggesting that the photophysiology of the ambient communities is regulated by different environmental variables. In particular, Fv/Fm was relatively constant over the duration of experiments from Sta. A, B suggesting that neither light nor Fe were limiting photosynthetic efficiency in these regions. Conversely, the Fv/Fm at Sta. C, D increases over the duration of the experiment in all treatments except for the +Light, whereas Fv/Fm decreases at Sta. E, F for most of the treatments over the course of the experiment. Differences in Fv/Fm between the stations (and over the course of the experiment) could be due to initial differences in phytoplankton community composition or changes in composition over time (Suggett *et al.,* 2009). However, the patterns in Fv/Fm are most consistent with changes in the photophysiology and together these differences suggest that each of the ambient communities was differentially poised. Indeed, decreases from high ambient Fv/Fm at Sta. E, F on day 2 resulting from photoacclimation, photoinhibition, or some other initial incubation shock decreased the photosynthetic competency after initial handling suggesting that these phytoplankton populations were initially more photophysiologically sensitive to our incubation processing.

Although light was the proximal regulator among the stations, the duration and magnitude of the response was significantly different between the stations. For example, the pennate diatoms at Sta. B–F increase by approximately 100-fold whereas at Sta. A this increase is only 10-fold. More striking are the differences in the duration of the increases. At Sta. B, D, and E the peak response in chlorophyll biomass occurs on the final day of the experiment (day 5), whereas at Sta. A, C, and F the peak response occurs on day 3 or 4 (Fig. 4). The differences in the timing of the peak response are likely related to the initial macronutrient concentration; Sta. A, C, and F have the lowest initial nitrate concentrations (Table 1) suggesting that the response is terminated by the drawdown of macronutrients. Similarly, although Fe may not initially limit the phytoplankton community or specific taxa, when differences are present between the +Light and +Light +Fe treatments they occur on the final days of the experiment (Figs. 4, 5) and suggest that initial Fe concentrations are not sufficient for the levels of macronutrients present to further increase phytoplankton biomass. This highlights the importance of Fe plays in modulating the phytoplankton community response to light availability.

Finally, at Sta. E, F there are significant increases in the total phytoplankton (Chl *a*) and pennate diatom biomass (Figs. 4 and 5) in control populations. Although the +Light and +Fe +Light treatments are still significantly higher than the controls at these stations,

these patterns suggest that conditions may have changed in control bottles that resulted in shifts from the ambient phytoplankton community distributions. We cannot exclude the possibility that these stations were contaminated by Fe during sampling or processing and the phytoplankton populations may have been particularly sensitive in part because ambient Fe concentrations were the lowest measured among the six stations samples (Table 1). However, there are no significant increases in Fv/Fm in control or +Fe bottles over the duration of the incubation suggesting that Fe is not proximally limiting these populations. Others have also observed increases in larger phytoplankton (including diatoms) in "control" incubations and when care has been to taken to simulate the in situ light environment these changes have often been attributed to large grazer exclusion (Brzezinski *et al.,* 2008; Martin and Fitzwater, 1988; Venrick *et al.,* 1977). This is also consistent with no significant increases in the *Prochlorococcus* populations in these bottles over time and this is likely because this population is already tightly regulated by grazers and that the grazers are able to respond to increases in cell growth on the same time scales as the phytoplankton populations (Landry *et al.,* 1997). Although this hypothesized grazer exclusion appears to have most dramatically affected the incubations from Sta. E, F (the only stations off the equator), nevertheless the proximal regulation of the DCM by light is still apparent. Thus, although there are differences in the ambient phytoplankton communities, macronutrient concentrations, or grazer control, these differences do not appear to influence the general trends observed at other stations.

In conclusion, results from this study demonstrate that light is the proximal resource regulating phytoplankton populations in the DCM of the Western Pacific Ocean with a critically important additive role for Fe confirming the important coupling between Fe and light in natural phytoplankton populations (Sunda and Huntsman, 1998). These patterns were generally consistent across multiple stations representative of this vast region. Nevertheless, because of substantial variability in the chemistry and movement of waters in this region, it likely that there is variability in the regulation of these populations and that at certain times or locations Fe alone may be limiting. The results observed here are also generally consistent with those found by others for other oceanic regions (Hopkinson and Barbeau, 2008; Selph *et al.,* 2010) with the exception of one unique station that was hypothesized to be different due to a recent nutrient injection event (Hopkinson and Barbeau, 2008). Releasing the ambient phytoplankton populations from light or Fe and light co-limitation leads to dramatic shifts in the community composition, highlighting the role that these limiting resources play in structuring the ambient community. In particular, pennate diatoms appear to be most sensitive to this control. Finally, these results emphasize the importance of vertical variability of Fe-limitation and that putatively "Fe-limited" oceanographic regions are not necessarily Fe-limited deeper in the euphotic zone.

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