Effects of Iron and Chelators on Primary Production and Nitrogen New Production in the Equatorial Pacific Upwelling System

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적도 태평양 용승계에서 철과 킬레이트 화합물이 일차생산과 질소 신생산에 미치는 영향

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Effects of iron and/or chelator addition on primary production in the equatorial upwelling system were studied during the TOGA(Tropical Oceans and Global Atmosphere) and EPOCS (Equatorial Pacific Ocean Climate Studies) cruises in June and November-December of 1989. Changes in the phytoplankton biomass and the degree of iron stress were estimated using the changes in *in vivo* fluorescence before and after the addition of DCMU, which is an inhibitor of photosynthetic electron transport system. Nitrate uptake was measured using ^{15}N labeled KNO_3 to estimate the new production.

When samples were taken from the upwelling area where nitrate concentrationwas higher than 5 µM, there were significant differences between the control and chelated iron treatments in *in vivo* fluorescence and in nitrate uptake capacity. However, CFC (Cellular fluorescence capacity) did not show any significant difference between the control and treatments until nutrient limitation becomes severe and cells become shifted-down. Outside of the upwelling area where surface nitrate concentration was low (below 0.5 µM), there was no significant difference between the control and treatments in *in vivo* fluorescence and CFC.

It is evident that primary and new production in the equatorial Pacific upwelling region are limited by the availability of iron. However, the physiology of phytoplankton indigenous to this region does not appear to be iron stressed judging from CFC values.

철과 킬레이트 화합물이 적도용승계에서 일차생산에 미치는 영향이 1989년 TOGA와 EPOCS 항해 기간 중 연구되었다. 식물플랑크톤 현존량의 변화와 철 결핍으로 인한 영향은 광합성 전자전달계의 저해제인 DCMU에 의한 생체형광의 변화를 이용하여 추정하였다. 질소 신생산은 안정동위원소인 ^{15}N KNO_3의 흡수를 이용하여 측정하였다.

표층 질산염의 농도가 5 µM 이상인 용승해역에서는 대조군과 킬레이트화된 철 처리군 사이에 생체형광과 질산염 흡수능력에 유의성 있는 차이를 보였다. 그러나 CFC(세포형광용량)에 있어서는 영양염에 의한 제한이 대조군과 처리군 사이에 유의성 있는 차이가 나타나지 않았다. 표층 질산염 농도가 낮은 (0.5 µM 이하) 용승해역 바깥은 대조군과 처리군 사이에 생체형광과 CFC 값에 유의성 있는 차이가 보이지 않았다.

적도용승계에서 1차생산과 질소 신생산은 철의 가용도에 의해 제한을 받는 것이 자명하다. 그러나 CFC 값에 의하면 이 해역에 자생하는 식물플랑크톤의 생리작용은 철 결핍에 의한 영향을 받지않는 것으로 사료된다.

INTRODUCTION

Iron is one of the vital trace metals in plant metabolism, being involved in the synthesis of photosynthetic pigments, and an essential element of the electron transport and the nitrate assimilation systems. Harvey (1937) first discussed the role of iron as a limiting factor for primary production in the oceanic environment. Subsequently, Barber and Ryther (1969) performed enrichment experiments with iron, other trace metals, and chelators in the eastern equatorial Pacific at 92°W and found chelation to be an important factor in determining the productivity of freshly upwelled waters. Iron has been suggested as a possible factor limiting primary production in the equatorial Pacific, Antarctic Ocean, and Northern Pacific where atmospheric and terrestrial input is small (Martin and Fitzwater, 1988; Martin, 1990). Iron is highly surface-active in seawater (active surface scavenging), has a relatively short oceanic residence time (77 years) and becomes firmly fixed (strongly refractory) onto particles that fall through the sea to the sediment (Broecker and Peng, 1982; Chester, 1990). The chemistry of iron dissolution and its subsequent utilization by organisms are complex, and most of the iron present in sea water is not biologically available (iron stoichiometry versus kinetics; Anderson and Morel, 1982; Wells and Mayer, 1991b; Wells et al., 1991; for review see Morel et al., 1991). Even the dissolved form of iron (conventionally defined as the fraction which passes through a 0.45 µM pore filter) may be up to 75% non-labile and unavailable to phytoplankton (Wells and Mayer, 1991b). However, Moore et al. (1984) reported the potential for biological mobilization of trace elements from aeolian dust in the ocean and its importance for iron.

The equatorial Pacific is remote from landmasses and Martin et al. (1989) have suggested the relatively low productivity of that area is due to the lack of input of iron from atmospheric sources. In the open ocean, atmospheric transport is the most important pathway for the long-range transport of particulate material (Chester, 1990). Young et al. (1991) found a good correlation bet-

ween the wind-driven dust input and primary production in the north Pacific (26°N, 155°W) and concluded that atmospheric input was the most important source of iron in that area. They reported that productivity in that region was enhanced by iron-rich (10-15%) atmospheric dust input related to the jet stream in the upper atmosphere and to seasons and storm events in the Asian continent. Zhuang et al. (1990) measured the dissolution of atmospheric iron in surface seawater in the open ocean and concluded that this source was sufficient to meet the needs of primary production. Olivarez et al. (1991) reported that in the northeast Pacific the flux of the aeolian dust was greater during the glacial period and showed a maximum between 35°N and 42°N. However, they reported that aeolian fluxes were greatest during interglacial periods in the equatorial Pacific. The equatorial Pacific upwelling system was more productive during the interglacial period than the glacial period (Pisias and Lyle, 1988). Even though upwelling areas of the equatorial Pacific is one of the major sources of CO_2 to tht atmosphere and net sinks of CO_2 are the subduction zones of Antarctic and Arctic areas, the equatorial Pacific could act as an important control site on the global CO_2 budget through changes in primary production.

Trace metals are important as selective forces on marine phytoplankton populations and communities and bacterial populations may compete with phytoplankton for iron and other trace metals. Brand et al. (1983) found that neritic phytoplankton species have higher zinc (Zn), manganese (Mn), and iron (Fe) requirements than oceanic species. Murphy et al. (1976) reported that the competition for iron between cyanobacteria and other phytoplankton could be the reason for the dominance of cyanobacteria which produce hydroxamate chelators under iron limited conditions. Cyanobacteria apparently excrete siderophores specific to them which allow them to use iron and make it unavailable for other organisms (Kerry et al., 1988). The excretion of siderophores by some species of marine phytoplankton has been discovered (Trick et al., 1983). The heterotrophic marine bacterium *Vibrio aglinolyticus* was found

to produce extracellular copper-binding compounds actively when exposed to copper in a seawater medium (Schreiber et al., 1990). Because of this biological modification, seawater cannot be treated as a simple reagent without regard to the physiological state of the phytoplankton population (Zhou et al., 1989). The growth cycle stage and type of phytoplankton present need to be known to interpret analytical results that depend upon the organic material present in the sample.

In the natural environment it is very difficult to define the trace metal activity accurately enough to address the problem of iron limitation. The increase in photosynthetic activity following upwelling may be due to decreased heavy metal toxicity (e.g., Cu^{2+}) and/or increased iron or other trace metal availability due to chelation (Barber and Ryther, 1969). Use of a controlled system under defined trace metal conditions (e.g., Aquil media) in laboratory experiments is indispensable to address the problem adequately (Morel et al. 1979). Huntsman and Barber (1975) observed that the lag phase for the growth of fresh phytoplankton stock was shortened by adding exudate from 3 day old exponentially growing phytoplankton populations, which they suggested was due to the conditioning of water by exudates from exponentially growing phytoplankton. Wangersky (1986) suggested that the distribution of trace metals in seawater was controlled by biological activity and demonstrated this using a mesocosm (Wangersky et al., 1989), which was monitored over periods of one or a few days. Even if iron contamination problems are addressed adequately using trace metal clean techniques according to Fitzwater et al. (1982), there are controversies whether the increased phytoplankton biomass in iron enrichment experiments is due to an actual increase in phytoplankton growth rate or due to a shift in phytoplankton species composition which influences grazing in the incubation bottle (Banse, 1990; Martin et al., 1990; Dugdale and Wilkerson, 1990; Banse, 1991a).

The purpose of this paper is to describe experiments designed to examine the effect of iron and chelator treatments on the growth of phytoplankton and on nitrogen new production (by ^{15}N-labe-

led nitrate uptake) in the equatorial Pacific upwelling system. New production, which was introduced by Dugdale and Goering (1967), is the fraction of the nitrogen requirement of the total primary production derived from newly available forms of nitrogen such as NO_3-N (provided by upwelling, vertical mixing, or eddy diffusion) or N_2-N (dinitrogen fixation; e.g., by nitrogen fixing bacteria). It also gives a measure of the organic matter which can be exported from the euphotic zone without the production system running down, and as such, is considered equivalent to the downward vertical flux of organic nitrogen (Eppley and Peterson, 1979). By measuring in vivo fluorescence and ^{15}N nitrate uptake, I hoped to find out whether iron and/or chelation capacity is an important controlling factor for new production in the study area.

Hypothesis to be tested are;

Hypothesis 1: Ho: Iron is not a limiting factor for primary and new production in the equatorial Pacific.

To test for the iron limitation of primary production in the field, experiments with the addition of iron and chelators (EDTA, DTPA, and CDTA) were performed and in vivo fluorescence and ^{15}N nitrate uptake rates were measured. The results of these experiments may be difficult to interpret because of many complicating factors (i.e., the history of water mass, unknown concentration of available trace metal due to the complex behavior of metals and natural chelators, and differences in the physiological state and species composition of phytoplankton community, etc.). However, some general trends can be obtained from this approach.

Hypothesis 2: Ho: The history of the water mass is not important in determining the response of primary production to the addition of iron and chelators.

To test this hypothesis, water samples from different locations in the equatorial Pacific with different biological histories which have different physical and chemical conditions were collected and treated with chelators and iron to modify the level of "conditioning" (Barber and Ryther, 1969). If samples from the upwelling center and off the center show different responses to iron and/or chelator

enrichment, the null hypothesis can be rejected.

MATERIALS AND METHODS

Water samples were collected with Niskin bottles retrofitted to reduce trace metal contamination (Price et al., 1986; Chavez et al., 1990) or plastic buckets with all the metal parts replaced, immediately after the ship arrived on station to reduce contamination from the ship. Sea water samples were distributed into 2 liter polycarbonate bottles. In order to examine the effect of the addition of iron or chelator on ^{15}N nitrate uptake and phytoplankton photosynthetic capacity and growth as measured by in vivo fluorescence, iron (as $FeCl_3$) and/or chelators (EDTA; Ethylene-diamine-tetraacetic acid, DTPA; Diethylene-triamine-pentaacetic acid, CDTA; ($\pm$)-trans-1,2-Diamino-cyclohexane-N,N,N',N ! -tetra-acetic acid) were added in 0.01, 1, or 10 μM concentrations to water samples. Incubations were done from 1 to 10 days in 250 ml polycarbonate bottles subsampled from the 2 liter polycarbonate bottles. To eliminate the pseudo-replication problem, replicates of three or four 250 ml bottles for each iron/chelator treatment were subsampled from different 2 liter polycarbonate bottles. At the end of each day, aliquots of 5 ml were removed from each incubation bottle and in vivo fluorescence was measured before and after the addition of 5 μM DCMU using a Turner Designs fluorometer to monitor the changes in the photosynthetic capacity and the degree of stress of phytoplankton to iron and chelator addition. At the end of the long term (up to 3-10 days) incubation, PON concentration and ^{15}N nitrate uptake were measured. For some experiments, time series measurements were performed to monitor the changes in PON (particulate organic nitrogen) and nitrate uptake capacity. Samples for ^{15}N and PON analyses were filtered onto precombusted (4 hours at 450°C) GF/F filters and the filters were dried at 60°C until analyses with Drierite as a dessicant. ^{15}N enrichment in the particulate fraction was measured using a Europa Roboprep-Tracermass GC/MS (Gas Chromatography-Mass Spectrometer) system (Owens, 1988). PON

was also determinded with the mass spectrometer using pre-weighed $(NH_4)_2SO_4$ granules as standards. Nutrients were determined using on-board Technicon AutoAnalyzerII according to Whitledge et al. (1981).

In vivo fluorescence was used to monitor the changes in biomass and physiological status of the phytoplankton. Samuelson and Öquist (1976) reported a good correlation between photosynthesis and DCMU-enhanced fluorescence in four species of unicellular green algae. DCMU [3-(3',4'-dichlorophenyl)-1,1-dimethylurea] is an inhibitor of the electron transport system which blocks electron transport from Q_A^- (quinone-type electron acceptor of photosystem II) to PQ (plastoquinone), probably binding the Q_B site of the D1 protein (Krause and Weis, 1991). Falkowski and Kiefer (1985) discussed the merits and pitfalls of the fluorescence for monitoring photosynthesis. Rueter and Ades (1987) described the enhancement of in vivo fluorescence by DCMU addition (F_d) in controlled trace metal conditions, and concluded that this enhancement of in vivo fluorescence could be used as an indicator to measure iron stress in phytoplankton cell physiology.

RESULTS

Effect of iron and chelators on the growth of phytoplankton

Fig. 1 shows the study area which is located in the mid-eastern equatorial Pacific, occupied during the TOGA (Tropical Oceans and Global Atmosphere) and EPOCS (Equatorial Pacific Ocean Climate Studies) cruises during June (EP89A) and November-December (EP89B) of 1989. Sampling date, time, and location along with SST (Sea surface temperature), nitrate and silicate concentrations are summarized in Table 1. During EP89A, at the equator, 155°W (ST29; SST 26.62°C, NO_3 4.38 μM, and $Si(OH)_4$ 2.56 μM), the addition of iron alone to surface water did not accompany increases in in vivo fluorescence or biomass unless it was accompanied by chelators (Fig. 2A and B). Increases in in vivo fluorescence and PON occur-

red after an initial lag of several days (at least 2 days). Fig. 3A to D show experiments carried out at the equator, 150°W (ST30; SST 26.14°C, NO_3 5.66 μM, $Si(OH)_4$ 2.88 μM). There was no significant difference (at 95% confidence level) in F_d on Day 3 between control and treatments with either iron (0.2 μM or 1 μM) or EDTA (1 μM) added alone (Table 2). When iron and EDTA were added together (0.2 μM or 1 μM EDTA and 1 μM Fe), there was a significant increase in DCMU enhanced fluorescence (F_d). When 4 μM ^{15}N nitrate was added at the start of the incubation, the result was similar except that 1 μM EDTA addition also showed a significant increase at the 95% confidence level. Nitrate transport rate measured by ^{15}N uptake showed the same tendency as that
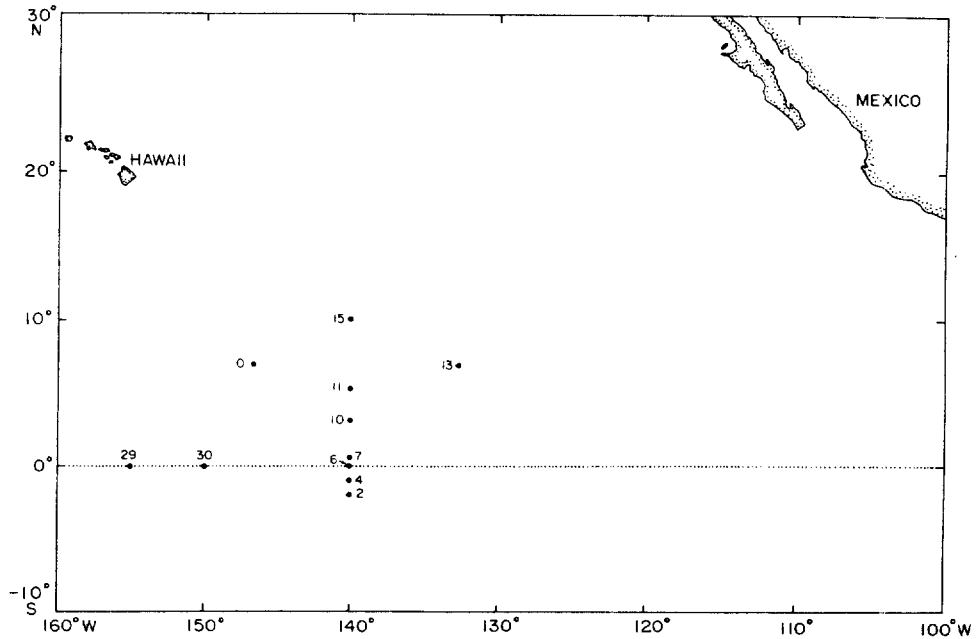

Fig. 1. Map of sampling stations for iron/chelator enrichment experiments in the equatorial Pacific Ocean during 1989 TOGA and EPOCS cruises.

Table 1. Sampling date, location, sea surface temperature (SST) and surface nutrient concentration for iron/;chelator experiments during EP89A and EP89B cruises

Cruise I.D.	Stn. No.	Date	Time(Local)	Lat.(°N)	Long.(°W)	SST(°C)	NO_3(μM)	$Si(OH)_4$(μM)
EP89A	29	6/23/89	4 : 00	0.0	155.0	26.62	4.38	2.56
EP89A	30	6/24/89	5 : 00	0.0	150.0	26.14	5.66	2.88
EP89B	0	11/23/89	7 : 15	7.0	147.5	27.41	0.37	1.64
EP89B	2	11/26/89	10 : 05	−2.0	140.0	25.48	6.52	4.28
EP89B	4	11/27/89	14 : 50	−1.0	140.0	25.43	6.63	4.45
EP89B	6	11/29/89	21 : 25	0.0	140.0	24.69	6.92	4.74
EP89B	7	11/29/89	1 : 27	0.5	140.0	25.90	6.23	5.08
EP89B	10	11/29/89	21 : 15	3.0	140.0	26.47	2.27	2.57
EP89B	11	11/30/89	20 : 45	5.0	140.0	26.63	2.51	2.61
EP89B	13	12/6/89	1 : 30	6.9	132.4	27.07	0.40	2.07
EP89B	15	12/8/89	0 : 45	10.0	140.0	26.46	0.10	1.74

(A) ST29 (0°, 155°W) on Day 3
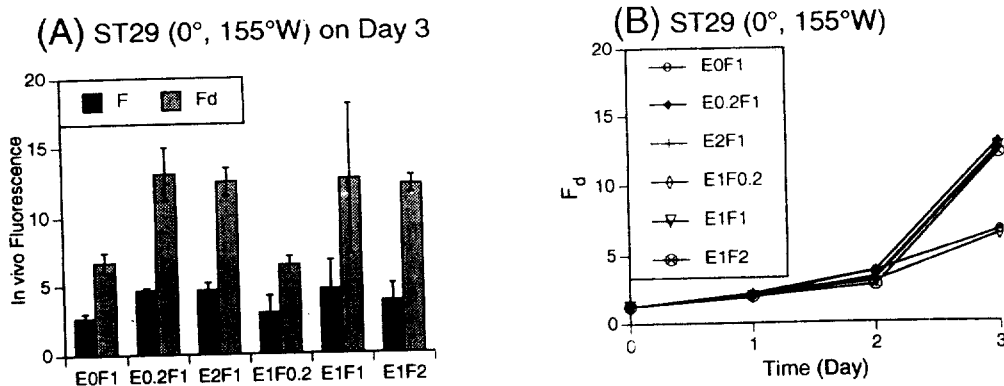

(B) ST29 (0°, 155°W)

Fig. 2. *In vivo* fluorescence (A) on Day 3, and (B) during 3 day incubation before and after the addition of DCMU at different concentrations of iron (F) and EDTA (E) treatment during EP89A at ST29 (155°W, Equator). Iron and chelator concentrations are in μM. Error bars are standard errors of the mean. n=4.

(A)
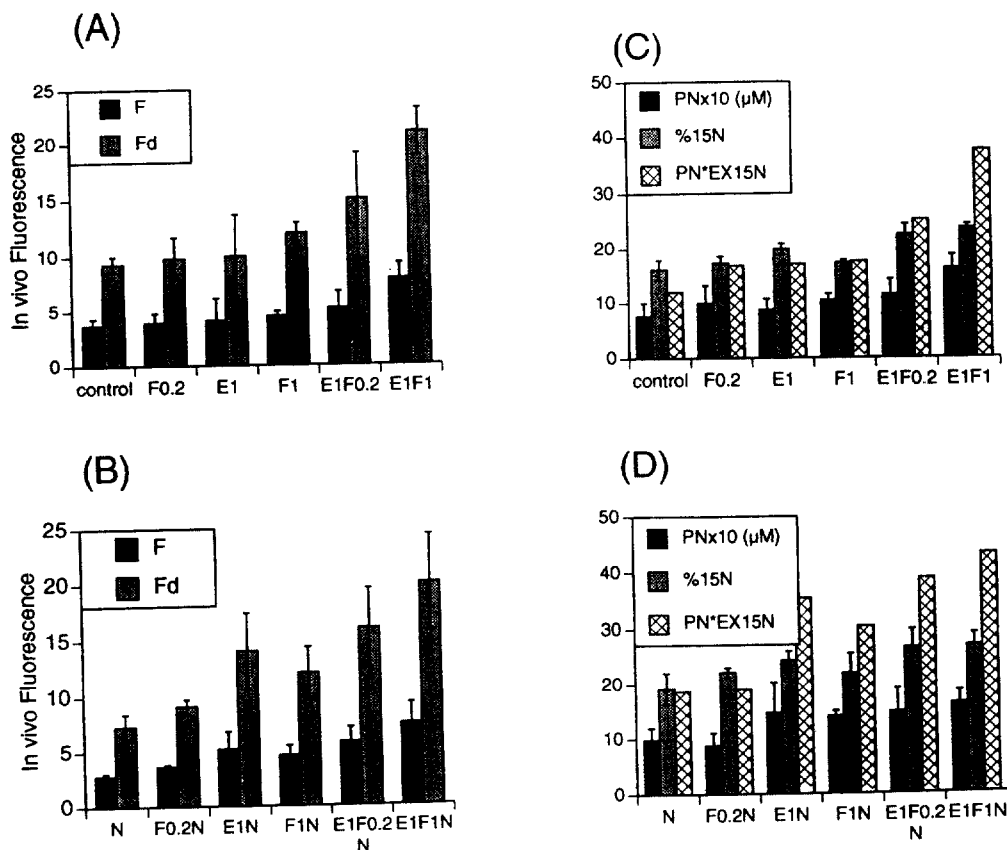

(B)

(C)

(D)

Fig. 3. *In vivo* fluorescence before and after the addition of DCMU, particulate organic nitrogen content (PON), and nitrate transport rate after 3 day treatment with different concentrations of iron (F) and EDTA (E) and with and without nitrate enrichment during EP89A at ST30 (150°W, Equator). (A) fluorescence without nitrate enrichment, (B) fluorescence with 5 μM ^{14}N nitrate enrichment. (C) PON and nitrate transport rate (PN*EX^{15}N) without nitrate enrichment, (D) PON and (PN*EX^{15}N) with 5 μM ^{14}N nitrate enrichment. Iron and chelator concentrations are in μM. Error bars are standard errors of the mean. n=4.

Table 2. Statistical table of iron/chelation experiment at station 30 (0°, 150°W) showing the mean and standard deviation of DCMU enhanced fluorescence (F_d) and DCMU enhancement ratio (CFC; $(F_r\text{-}F)/F_d$) at day 3

	F_d	Stdev	$(F_r\text{-}F)/F_d$	Stdev
control	9.28	0.71	0.607	0.037
Fe0.2	9.83	1.86(n.s.)	0.601	0.027(n.s)
E1	10.30	3.63(n.s.)	0.613	0.068(n.s)
Fe1	11.67	0.90(n.s.)	0.616	0.010(n.s)
E1Fe0.2	15.13	4.09(**)	0.657	0.038(n.s.)
E1Fe1	20.88	2.15(****)	0.622	0.034(n.s.)
N	7.25	1.16(n.s.)	0.614	0.039(n.s.)
Fe0.2+N	9.05	0.61(n.s.)	0.603	0.020(n.s.)
E1+N	14.20	3.40(*)	0.642	0.040(n.s.)
Fe1+N	11.73	2.34(n.s.)	0.605	0.013(n.s.)
E1Fe0.2+N	15.75	3.56(***)	0.634	0.036(n.s.)
E1Fe1+N	20.40	4.32(****)	0.636	0.014(n.s.)

Fe: iron treatment. Numbers are concentrations in μM.
E: EDTA treatment. Numbers are concentrations in μM.
N: 5 μM nitrate enrichment.
Fluorescence values are in arbitrary units
Significant at 95%(*), 99%(*), 99.5%(***), 99.9(****) level.

Fig. 4. DCMU enhanced fluorescence (F_d) during (A) 4 day, and (B) 6 day incubation with different concentrations of iron (F) and EDTA (E) treatments during EP89B at ST0 (7°N 147.5°W). 5 M ^{14}N nitrate was added at the beginning of day 5. Iron and chelator concentrations are in μM. n=4.

of fluorescence (Fig. 3 C and D). PON concentrations also increased in chelated iron treatments.

The effect of iron and chelator enrichments varied with locations. When samples were taken in oligotrophic areas outside of the upwelling area (EP89B ST0, at 7°N, 147.5°W), there was no significant difference in F_d between the control and iron/chelator treatments (Fig. 4A). At this station, surface nitrate concentration was very low (0.37 μM) and iron addition had little effect on F_d compared to limitation by nitrate. When additional nitrate (5 μM) was added later, fluorescence increased immediately (Fig. 4B), confirming that nitrate is the limiting factor at this station. At station 2 (EP89B, 2°S, 140°W), which was near the center of upwelling, as indicated by low surface temperature and relatively high surface nitrate concentration during the cruise (25.48°C and 6.52 μM, respectively), adding low concentrations (0.01 μM) of EDTA or EDTA chelated iron did not show any significant change in DCMU enhanced fluorescence compared to the control (Fig. 5 and Table 3). When 1 μM of different chelators (EDTA, CDTA, DTPA) were added, fluorescence increased signifi-
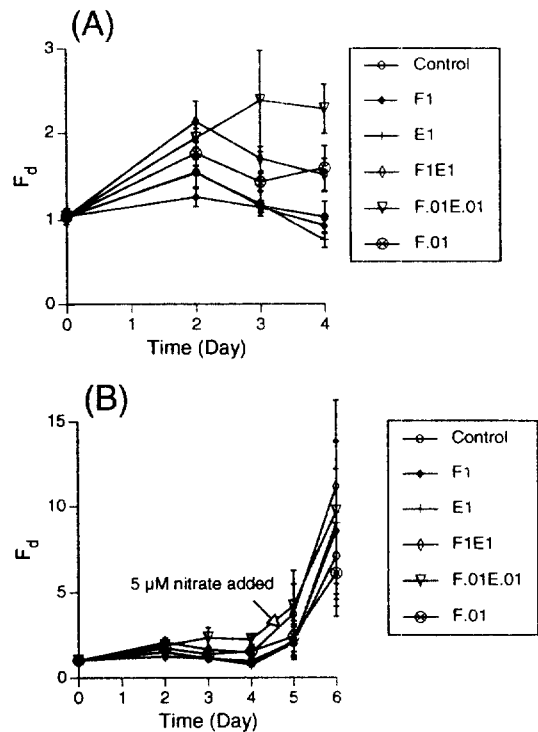
cantly by Day 3 and it became more conspicuous on Day 4 (at 99.9% confidence level). By Day 5, the fluorescence increase in 1 μM chelator treatments declined significantly and the final fluorescence yield at Day 5 was the same as for the control or low concentration (0.01 μM) of EDTA or EDTA/iron treatments. There was no significant difference in the response by three different chelators given at 1 μM concentration. Treatments with chelators reduced the lag period of the fluorescence (as F_d) increase but did not affect the final yield significantly. At station 4 (EP89B, 1°S, 140°W), physico-chemical conditions were similar to the previous station with SST of 25.43°C, nitrate concentration of 6.63 μM, and silicate concentration of 4.45 μM. However, there was no difference between the control and iron and/or EDTA treatments. All showed a 2 day lag period and increa-
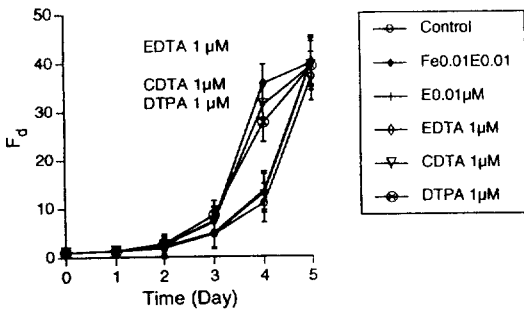
Fig. 5. DCMU enhanced fluorescence (F_d) during 5 day incubation with different concentrations of iron, EDTA, CDTA, and DTPA during EP89B at ST2 (2°S 140°W). Iron and chelator concentrations are in μM. n=4.
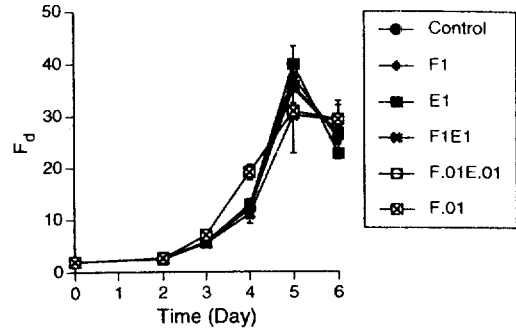

Fig. 6. DCMU enhanced fluorescence (F_d) during 6 day incubation with different concentrations of iron (F) and EDTA (E) treatment during EP89B at ST4 (1°S 140°W). Iron and chelator concentrations are in μM. n=4.

Table 3. Statistical table of iron/chelation experiment at station 2 (2°S, 140°W) showing the mean and standard deviation of DCMU enhanced fluorescence (F_d) and DCMU enhancement ratio (CFC; (F_d-F)/F_d) at day 4

	F_d	Stdev	(F_d-F)/F_d	Stdev
control	10.95	2.59	0.561	0.036
Fe0.01E0.01	12.95	2.03(n.s.)	0.574	0.024(n.s.)
E0.01	13.50	2.23(n.s.)	0.568	0.022(n.s)
E1	35.63	4.01(***)	0.582	0.016(n.s.)
CDTA1	31.28	7.54(***)	0.603	0.022(n.s.)
DTPA1	27.58	2.97(***)	0.557	0.016(n.s.)

Fe: iron treatment. Numbers are concentrations in μM.
E: EDTA treatment.(μM).
D: DTPA treatment.(μM).
Fluorescence values are in arbitrary units
***: Significant at 99.9% confidence level.

ses in DCMU enhanced fluorescence until Day 5. Fluorescence (F_d) decreased at Day 6 (Fig. 6). Neither iron nor the chelation capacity seemed to be limiting phytoplankton growth at this station.

At station 6 (0°, 140°W) during EP89B, treatments with EDTA alone (1 μM and 0.01 μM) or EDTA chelated iron (0.01 μM) resulted in higher fluorescence yield (i.e., F_d) than the control by Day 3 (Fig. 7A; Table 4). However, treatment with a high concentration (10 μM) of DTPA, which is a stronger chelator of iron than EDTA, significantly (at 99.9% significance level) decreased F_d which never showed any sign of recovery during 6 day incubation (Fig. 7B). Addition of 1 μM DTPA re-
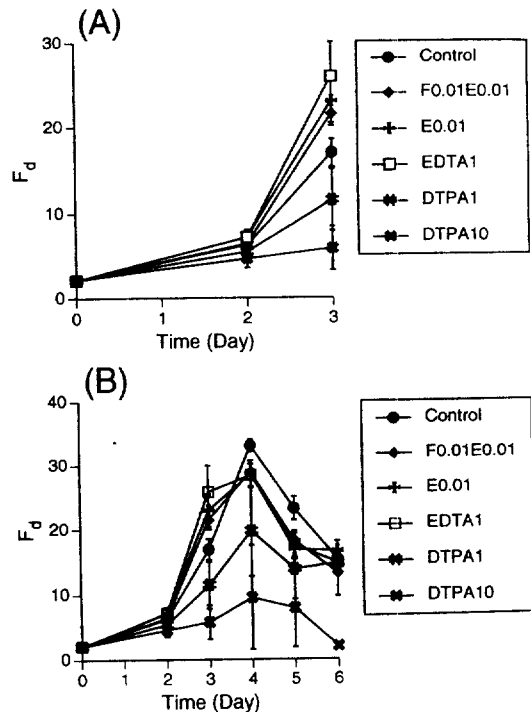

Fig. 7. DCMU enhanced fluorescence (F_d) with different concentrations of iron, EDTA, and DTPA treatment during EP89B at ST6 (Equator 140°W). (A) during 3 day incubation, and (B) during 6 day incubation. Iron and chelator concentrations are in μM. n=4.

sulted in a lower F_d than control but higher than 10 μM DTPA treatment between Day 3 and Day 5. However, by Day 6, the 1 μM DTPA treatment

Table 4. Statistical table of iron/chelation experiment at ST6 (0°, 140°W) showing the mean and standard deviation of DCMU enhanced fluorescence and DCMU enhancement ratio (CFC; $(F_d$-F)/F_d)

	Day3		Day4		Day5	
	F_d	Stdev	F_d	Stdev	F_d	Stdev
Control	16.90	3.35	33.15	1.34	23.23	3.21
Fe0.01+E0.01	21.43	2.94(n.s.)	28.93	2.02(*)	18.25	2.06(*)
E0.01	22.93	3.42(*)	28.60	4.42(*)	16.88	1.96(**)
E1	25.78	8.78(**)	28.45	3.10(*)	17.58	4.54(**)
DTPA1	14.05	1.48(n.s.)	26.78	4.00(**)	19.15	4.25(*)
DTPA10	3.25	0.91(****)	2.53	0.50(****)	2.64	0.59(****)
	$(F_d$-F)/F_d	Stdev	$(F_d$-F)/F_d	Stdev	$(F_d$-F)/F_d	Stdev
Control	0.60	0.02	0.54	0.03	0.46	0.07
Fe0.01+E0.01	0.61	0.01(n.s.)	0.52	0.01(n.s.)	0.46	0.04(n.s.)
E0.01	0.61	0.00(n.s.)	0.52	0.08(n.s.)	0.46	0.03(n.s.)
E1	0.63	0.01(n.s.)	0.53	0.01(n.s.)	0.44	0.02(n.s.)
DTPA1	0.59	0.01(n.s.)	0.53	0.05(n.s.)	0.45	0.01(n.s.)
DTPA10	0.46	0.06(****)	0.49	0.03(n.s)	0.37	0.06(*)

Fe: iron treatment numbers are concentrations in μM. E: EDTA treatment (μM). D: DTPA treatment (μM). Fluorescence values are in arbitrary units. Significant at 95%(*), 99%(**), 99.5%(***), 99.9%(****) level.
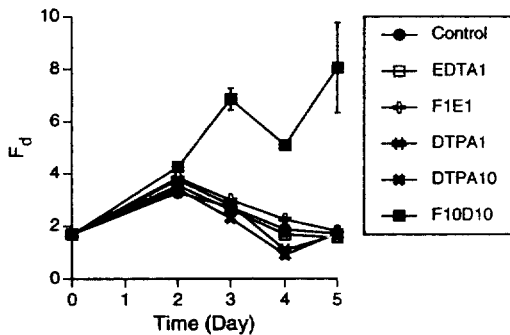

Fig. 8. DCMU enhanced fluorescence (F_d) during 5 day incubation with different concentrations of iron, EDTA, and DTPA treatment during EP89B at ST13 (7°N 132.4°W). Iron and chelator concentrations are in μM. n=4.

showed a similar F_d yield to the control. The effect of high concentrations of DTPA treatment was not due to a direct toxic effect of DTPA on phytoplankton. This is evident in another experiment at station 13 (Fig. 8), in which this inhibition was completely reversed and significantly higher fluorescence was observed in the treatment with 10 μM iron/DTPA than the control or with addition of DTPA alone. The DTPA treatment appears to scavenge available iron and other trace metals.

The relationship between ^{15}N nitrate transport rate (PON*(Excess ^{15}N)) and increase in DCMU enhanced fluorescence (F_d-F) showed a strong linear relationship ($R^2=0.752$) with a close to zero Y intercept (Fig. 9A). This implies that the enhancement of in vivo fluorescence after DCMU addition could be used to estimate the magnitude of nitrate transport rate by phytoplankton. (PON* (Excess ^{15}N)) versus F_d showed a similar pattern with R^2 of 0.752 (Fig. 9B). There was also a linear relationship between PON and F_d with a positive intercept on the PON axis (Fig. 9C), suggesting a significant contribution to the total PON content by detrital fraction (non-fluorescing particulate matter including bacteria). The regression of %^{15}N and (F_d-F) gave an R^2 of 0.573 (Fig. 9D). The Y intercept had a high value of 13.9 atom % ^{15}N nitrogen. The highest R^2 value between (PON* (Excess^{15}N)) and (F_d-F), and close to zero intercept implies that the (F_d-F) value can be a good indicator of nitrate transport rate. Fluorescence measurements are instantaneous and less prone to manipulation error than nitrate uptake rate which needs incubation time (up to several hours) with ^{15}N nitrate enrichment which may perturb the nitrate uptake system.
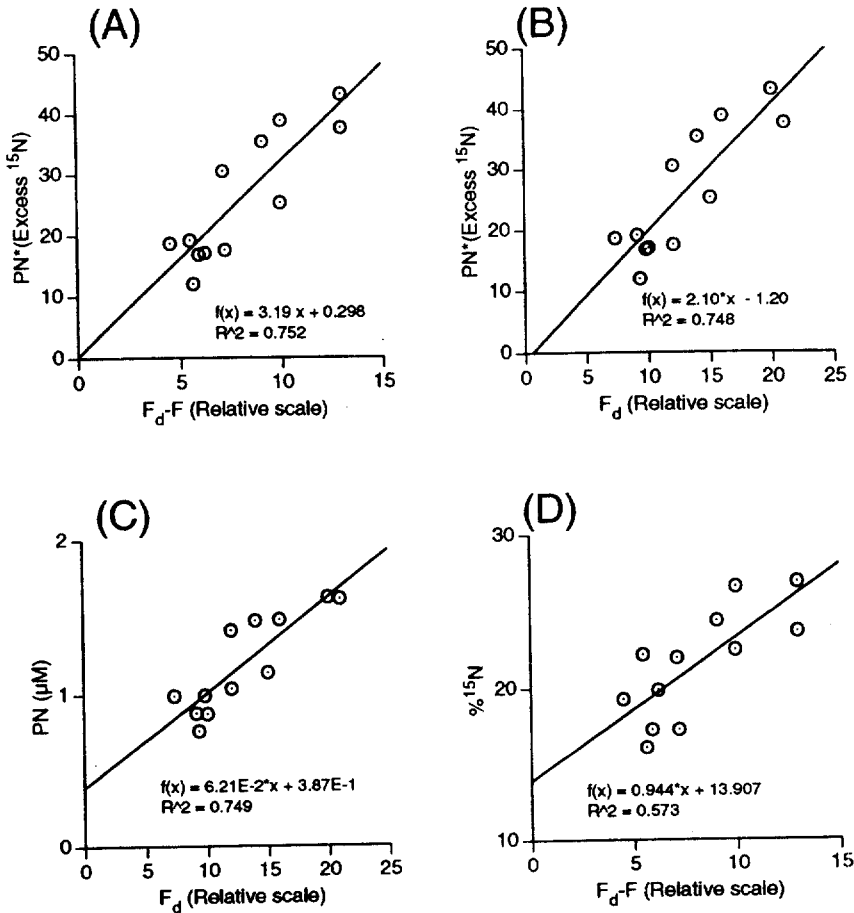
Fig. 9. Relationship between *in vivo* fluorescence [F, F_d, and $(F_d\text{-}F)$], particulate organic nitrogen (PN), %[15]N, and nitrate transport rate (PN*Excess[15]N) during EP89A cruise at 150W equator. (A) $(F_d\text{-}F)$ versus (PN*Excess[15]N). (B) (PN*Excess[15]N) versus F_d. (C) F_d versus PN. (D) %[15]N versus $(F_d\text{-}F)$.

Changes in the ratio of DCMU enhanced fluorescence (CFC; Cellular Fluorescence Capacity)

The ratio of *in vivo* fluorescence before and after the addition of photosynthetic electron transport inhibitor DCMU can be used as a diagnostic tool to monitor the iron stress on the phytoplankton cell (Rueter and Ades, 1987). They reported that when phytoplankton cultures were severely iron stressed by adding strong chelators, DCMU enhanced fluorescence (F_d) and the $(F_d\text{-}F)/F_d$ ratio were less than in the control. This ratio [the $(F_d\text{-}F)/F_d$] ratio which is also called the CFC; cellular fluorescence capacity; Vincent, 1980] was higher (a large increase in DCMU enhanced fluorescence) when

iron stress was removed by adding chelated iron. However, the experiment performed at ST30 during EP89A did not show any significant difference in the CFC in the control or EDTA and/or iron enrichment, even though the mean values for EDTA and iron treatments were a little higher than the control (Table 2). During the EP89B cruise, the result was the same at ST2 with no significant difference in CFC between the control and treatments even though there were significant differences in the magnitude of fluorescence (Table 3). At ST6 (Table 4), no significant difference in CFC was found between the control and treatments other than 10 μM DTPA treatment, which resulted in a significantly low CFC value (0.46 co-

Table 5. Statistical table of iron/chelation experiment at station 7 (0.5°N, 140°W) showing the mean and standard deviation of DCMU enhanced fluorescence and DCMU enhancement ratio (CFC; $(F_d\text{-}F)/F_d$).

| | Day3 | | Day4 | | Day5 | |
	F_d	Stdev	F_d	Stdev	F_d	Stdev
Control	22.68	2.07	30.88	1.77	18.55	1.84
Fe1	28.80	5.76(*)	27.63	3.47(n.s.)	19.05	2.08(n.s.)
E1	23.90	9.67(n.s.)	27.65	7.83(n.s.)	16.10	5.07(n.s.)
Fe1+E1	33.48	2.73(*)	26.33	3.30(n.s.)	15.30	2.80(n.s.)
Fe0.01+E0.01	23.45	1.32(n.s.)	35.48	10.15(n.s.)	23.50	9.96(n.s.)
Fe0.01	24.05	9.45(n.s.)	30.28	3.96(n.s.)	20.58	2.71(n.s.)
	$(F_d\text{-}F)/F_d$	Stdev	$(F_d\text{-}F)/F_d$	Stdev	$(F_d\text{-}F)/F_d$	Stdev
Control	0.59	0.03	0.48	0.05	0.49	0.03
Fe1	0.63	0.01(n.s.0	0.51	0.05(n.s.)	0.47	0.01(n.s.)
E1	0.58	0.03(n.s)	0.45	0.02(n.s.)	0.41	0.05(**)
Fe1+E1	0.63	0.01(n.s.)	0.56	0.01(*)	0.45	0.00(n.s.)
Fe0.01+E0.01	0.61	0.04(n.s.)	0.48	0.06(n.s.)	0.43	0.07(*)
Fe0.01	0.63	0.03(n.s.)	0.55	0.02(*)	0.47	0.01(n.s.)

Fe: iron treatment. Numbers are concentrations in µM. E: EDTA treatment. (µM). Fluorescence values are in arbitrary units. Significant at 95%(*), 99%(**) level.

Table 6. Statistical table of iron/chelation experiment at station 10(3°N, 140°W) showing the mean and standard deviation of DCMU enhanced fluorescence and DCMU enhancement ratio (CFC; $(F_d\text{-}F)/F_d$)

| | Day3 | | Day4 | | Day5 | |
	F_d	Stdev	F_d	Stdev	F_d	Stdev
Control	4.31	0.73	8.19	0.25	8.41	0.94
Fe1	5.01	0.34(n.s.)	10.05	0.87(n.s.)	6.73	1.04(n.s.)
E1	4.66	0.42(n.s.)	7.45	3.27(n.s.)	5.41	1.50(*)
Fe1+E1	5.46	0.53(**)	10.53	0.79(n.s.)	6.68	0.83(*)
Fe0.01+E0.01	5.14	0.73(*)	9.60	1.51(n.s.)	7.25	1.26(n.s.)
Fe0.01	3.98	0.34(n.s.)	7.10	0.98(n.s.)	7.61	1.17(n.s.)
	$(F_d\text{-}F)/F_d$	Stdev	$(F_d\text{-}F)/F_d$	Stdev	$(F_d\text{-}F)/F_d$	Stdev
Control	0.67	0.04	0.62	0.02	0.59	0.03
Fe1	0.65	0.05(n.s.)	0.59	0.01(n.s.)	0.53	0.02(n.s.)
E1	0.68	0.04(n.s.)	0.58	0.03(*)	0.47	0.04(****)
Fe1+E1	0.67	0.01(n.s.)	0.61	0.03(n.s.)	0.51	0.03(***)
Fe0.01+E0.01	0.65	0.05(n.s.)	0.60	0.01(n.s.)	0.51	0.06(***)
Fe0.01	0.68	0.03(n.s.)	0.62	0.03(n.s.)	0.56	0.01(n.s.)

Fe: iron treatment. Numbers are concentrations in µM. E: EDTA treatment. (µM). Fluorescence values are in arbitrary units. Significant at 95%(*), 99%(**), 99.5%(***), 99.9%(****) level.

mpared to the control of 0.60; significant at 99.9% confidence level) at Day 3. At ST7 (Table 5), there was no significant difference in CFC values between the control and treatments on Day 3. At ST10 (Table 6), EDTA treatments with or without iron showed no difference compared to the control on Day 3, but resulted in significantly low CFC values on Day 5. However, this appears to be due to major nutrient (nitrate or silicate) limitation not due to iron limitation, because by Day 5 there were significant decreases in DCMU enhanced fluorescence in treatments, suggesting that phytoplankton were in the process of shifting down after the depletion of nutrients. Also at ST11, ST13, and

Table 7. Statistical table of iron/chelation experiment at station 11(5°N. 140°W) showing the mean and standard deviation of DCMU enhanced fluorescence and DCMU enhancement ratio (CFC: $(F_d\text{-}F)/F_d$)

	Day3		Day4		Day5	
	F_d	Stdev	F_d	Stdev	F_d	Stdev
Control	11.78	2.07	11.70	1.40	7.66	2.48
Fe1	12.68	2.50(n.s.)	12.83	1.51(n.s.)	8.63	1.17(n.s.)
E1	13.18	3.59(n.s.)	11.83	1.40(n.s.)	8.20	1.57(n.s.)
Fe1+E1	18.85	5.77(**)	14.18	5.56(n.s.)	8.41	3.55(n.s.)
Fe0.01+E0.01	18.30	2.85(*)	12.45	2.66(n.s.)	6.93	1.70(n.s.)
Fe0.01	16.45	2.76(n.s.)	13.50	3.22(n.s.)	8.40	2.28(n.s.)
	$(F_d\text{-}F)/F_d$	Stdev	$(F_d\text{-}F)/F_d$	Stdev	$(F_d\text{-}F)/F_d$	Stdev
Control	0.65	0.04	0.56	0.02	0.51	0.02
Fe1	0.61	0.04(n.s.)	0.56	0.04(n.s.)	0.44	0.05(n.s.)
E1	0.62	0.04(n.s.)	0.56	0.01(n.s.)	0.49	0.01(n.s.)
Fe1+E1	0.63	0.01(n.s.)	0.56	0.04(n.s.)	0.48	0.06(n.s.)
Fe0.01+E0.01	0.61	0.02(n.s.)	0.54	0.03(n.s.)	0.48	0.03(n.s.)
Fe0.01	0.60	0.02(n.s.)	0.50	0.06(n.s.)	0.43	0.06(*)

Fe: iron treatment. Numbers are concentrations in μM. E: EDTA treatment. (μM). Fluorescence values are in arbitrary units. Significant at 95%(*), 99%(**) level.

Table 8. Statistical table of iron/chelation experiment at station 13(6.9°N, 132.4°W) showing the mean and standard deviation of DCMU enhanced fluorescence and DCMU enhancement ratio (CFC; $(F_d\text{-}F)/F_d$)

	Day3		Day4		Day5	
	F_d	Stdev	F_d	Stdev	F_d	Stdev
Control	2.70	0.06	1.86	0.11	1.70	0.06
EDTA1	2.60	0.40(n.s.)	1.66	0.16(n.s.)	1.55	0.17(n.s.)
Fe1+E1	2.98	0.03(n.s.)	2.23	0.18(n.s.)	1.80	0.46(n.s.)
DTPA1	2.78	0.03(n.s.)	1.08	0.07(*)	1.65	0.23(n.s.)
DTPA10	2.28	0.14(n.s.)	0.90	0.31(*)	1.70	0.23(n.s.)
Fe10+D10	6.85	1.04(****)	5.08	0.32(****)	8.05	4.21(*)
	$(F_d\text{-}F)/F_d$	Stdev	$(F_d\text{-}F)/F_d$	Stdev	$(F_d\text{-}F)/F_d$	Stdev
Control	0.53	0.04	0.62	0.00	0.61	0.00
EDTA1	0.56	0.06(n.s.)	0.76	0.12(n.s.)	0.59	0.02(n.s.)
Fe1+E1	0.58	0.00(n.s.)	0.57	0.04(n.s.)	0.59	0.06(n.s.)
DTPA1	0.59	0.01(n.s.)	0.58	0.04(n.s.)	0.60	0.02(n.s.)
DTPA10	0.59	0.01(n.s.)	0.58	0.12(n.s.)	0.63	0.12(n.s.)
Fe10+D10	0.61	0.01(n.s.)	0.52	0.02(n.s.)	0.50	0.02(n.s.)

Fe: iron treatment. numbers are concentrations in μM. E: EDTA treatment (μM). D: DTPA treatment (μM). Fluorescence values are in arbitrary units. Significant at 95%(*), 99%(**), 99.5%(***), 99.9%(****) level.

ST15 (Tables 7, 8 and 9), there were no significant differences in CFC values between the control and treatments. However, at ST15 (Table 9) when 5 M nitrate was added at the beginning of the experiment (day 1) the enhancement ratio in the DTPA treatment (1 and 10 μM) showed significantly lower values compared to the control, indicating that when nitrate is low (0.10 μM initial concentration) depriving iron by adding a strong chelator (DTPA) does not affect the physiology of phytoplankton. When additional nitrate was added, iron limitation seems to be affecting the DCMU enhanced fluorescence.

Overall, there is a trend of gradual decrease in

Table 9. Statistical table of iron/chelation experiment at station 15(10°N, 140°W) showing the mean and standard deviation of DCMU enhanced fluorescence and DCMU enhancement ratio (CFC; $(F_d\text{-}F)/F_d$)

	Day3		Day4		Day5	
	F_d	Stdev	F_d	Stdev	F_d	Stdev
Control	0.81	0.70	0.27	0.02	0.43	0.09
EDTA1	0.86	0.73(n.s.)	0.45	0.06(***)	0.43	0.09(n.s.)
Fe1+E1	1.08	0.98(n.s.)	0.45	0.06(*)	0.45	0.12(n.s.)
DTPA1	0.99	0.85(n.s.)	0.53	0.03(***)	0.40	0.00(n.s.)
DTPA10	0.63	0.15(n.s.)	0.55	0.06(***)	0.60	0.06(n.s.)
Fe10+D10	0.72	0.31(n.s.)	0.50	0.00(***)	0.45	0.00(n.s.)
	$(F_d\text{-}F)/F_d$	Stdev	$(F_d\text{-}F)/F_d$	Stdev	$(F_d\text{-}F)/F_d$	Stdev
Control	0.50	0.15	0.43	0.04	0.39	0.12
EDTA1	0.38	0.14(n.s.)	0.55	0.06(n.s.)	0.29	0.01(n.s.)
Fe1+E1	0.44	0.18(n.s.)	0.63	0.14(n.s.)	0.18	0.21(n.s.)
DTPA1	0.43	0.12(n.s.)	0.55	0.05(n.s.)	0.38	0.14(n.s.)
DTPA10	0.63	0.07(n.s.)	0.59	0.01(n.s.)	0.58	0.04(n.s)
Fe10+D10	0.51	0.02(n.s.)	0.55	0.06(n.s.)	0.44	0.13(n.s.)
	F_d	Stdev	F_d	Stdev	F_d	Stdev
Control	1.80	0.06	2.35	0.06	3.45	0.29
Fe1+N5	2.00	0.06(n.s.)	2.90	0.06(n.s.)	4.73	0.14(*)
EDTA1+N5	2.35	0.23(*)	3.70	0.46(***)	5.98	0.32(***)
Fe1+E1+N5	2.10	0.17(n.s.)	3.68	0.20(***)	6.55	0.75(****)
DTPA1+N5	0.48	0.03(****)	0.53	0.09(****)	0.55	0.06(****)
DTPA10+N5	1.30	0.17(*)	1.03	0.03(***)	0.88	0.09(***)
	$(F_d\text{-}F)/F_d$	Stdev	$(F_d\text{-}F)/F_d$	Stdev	$(F_d\text{-}F)/F_d$	Stdev
Control	0.68	0.01	0.60	0.04	0.62	0.01
Fe1+N5	0.62	0.04(n.s.)	0.65	0.04(n.s.)	0.65	0.01(n.s.)
EDTA1+N5	0.62	0.04(n.s.)	0.65	0.03(n.s.)	0.66	0.02(n.s.)
Fe1+E1+N5	0.63	0.03(n.s.)	0.65	0.00(n.s.)	0.66	0.01(n.s.)
DTPA1+N5	0.68	0.02(n.s.)	0.61	0.06(n.s.)	0.37	0.04(***)
DTPA10+N5	0.54	0.02(**)	0.46	0.18(n.s.)	0.41	0.11(**)

Fe: iron treatment. numbers are concentrations in μM. E: EDTA treatment. (μM). D: DTPA treatment (μM). N5: 5 μM nitrate enrichment.
Fluorescence values are in arbitrary units. Significant at 95%(*), 99%(**), 99.5%(***), 99.9%(****) level.

CFC values with time as nutrients become limiting and phytoplankton goes into the shift-down stage. The CFC values indicate that phytoplankton in this area, at least in incubation bottles, were not under severe iron stress. Alternatively, CFC may not be a good indicator for diagnosis of iron stress in the equatorial Pacific, even though there are some positive indications. Also changes in CFC values may not be significant until iron limitation becomes extreme, and so this does not entirely eliminate the possibility of iron limitation on growth rate *in situ*.

DISCUSSION

Bottle incubation experiments can be of some help in diagnosing iron limitation symptoms, but cannot be used as a definitive key to solving the problem of whether iron is limiting the primary production *in situ*. Iron experiments using bottle incubations may provide misleading results compared to what is occurring under natural environments, since enclosure or confinement could lead to significant changes in the availability of iron. The lack of UV photochemistry (organic-depen-

dent Fe photoreduction; Wells and Mayer, 1991a) in the incubation bottle, removal from the continuous input of atmospheric fall out, and the adsorption of iron to walls of the incubation bottle (which is more significant under low iron concentrations) may reduce the availability of iron for phytoplankton growth.

Contamination during the sampling and handling of water samples is difficult to address in conducting iron enrichment experiments. Cullen *et al.* (1992) carried out EDTA and iron enrichment experiments in the equatorial Pacific (150°W) during the WEC88 cruise and did not see any significant difference in photosynthesis versus irradiance (P vs. I) curves in treatment or control using the ^{14}C uptake method (short term uptake measurement) between 15 minutes and 48 hours after enrichment. They concluded that iron contamination or other trace metal (divalent ions) toxicity may not be a problem at least during 48 hours. Martin *et al.* (1989) also reported no difference in ^{14}C productivity measurement (<24 hour incubation) between control and iron enriched samples in the northeast Pacific. These are consistent with my results which showed no difference on the first day after enrichment (Yang, 1992); i.e., iron or other trace metal contamination will not affect productivity measurements in the ocean if some precautions are heeded. Results of iron/chelator enrichment experiments in the equatorial Pacific showed different responses depending on the location. Outside of the upwelling area where nitrate concentrations were low (<0.2 μM), iron and/or chelator treatments did not have any significant effect on *in vivo* fluorescence or CFC, and phytoplankton appeared to be nitrate rather than iron limited. At equatorial upwelling stations, addition of EDTA or chelated iron showed significantly higher *in vivo* fluorescence than the control by Day 3. Addition of DTPA, a stronger chelator of iron than EDTA, showed a lower yield. Final yield was the same for the control and all treatments except under extreme iron stress (as occurred with 10 μM DTPA addition). Near the center of the upwelling where the age (time elapsed after the upwelling) of the phytoplankton population may

be younger. 1 μM addition of all chelators showed shorter lag periods of increases in fluorescence compared to controls. The effect of iron and/or chelator treatments on *in vivo* fluorescence and CFC values indicates that equatorial phytoplankton populations are not severely iron limited *in situ*. Iron may be limiting the growth rate of phytoplankton to a certain degree but its impact is not severe enough to adversely affect cell physiology detected using DCMU enhanced fluorescence. However, Falkowski (1991, ASLO 91 Symposium) reported that iron limitation in the equatorial Pacific may be occurring and documented using the *in situ* double flash fluorometer.

It is evident that iron/chelator enrichments do not affect the final fluorescence yield in most of the EP89 experiments, but that the lag period before the response is reduced. This reduction in the lag period can significantly affect productivity and hence the utilization of nitrate even if the control may have been contaminated with trace metal. Whether grazing is tightly controlling the biomass, or diel vertical mixing and upwelling play important roles in keeping the residence time of phytoplankton population at the surface relatively short, shortening of the lag phase of growth will lead to significant accumulation of biomass that should cause the depletion of nutrients in this region.

The effect of iron on grazing may be insignificant in the equatorial Pacific (Price *et al.*, 1991). Grazers can excrete ammonia and inhibit nitrate uptake or shift-up of the nitrate utilization system (Wheeler and Kokkinakis, 1990; Wheeler, 1991), and at the same time any increase in grazers can dilute PON and decrease the apparent V_{NO_3} (nitrogen specific nitrate uptake), which may be happening in the equatorial Pacific. Price *et al.* (1991) reported that iron enrichment increased nitrate uptake without affecting ammonia uptake in the equatorial Pacific. However, Banse (1991a) suggested that bottle experiments may eliminate large grazers leading to changes in the size composition of phytoplankton and the production of large sinking particle is not evaluated. The only way to solve the problem of eliminating large grazers and

also the containment effect on the iron photoche-mistry and supply appears to be large scale *in situ* experiments as proposed by Martin (1992).

Iron/chelator addition experiments showed no significant increase in PON or nitrate uptake when either was added alone, but showed significant increase when iron and chelators were added together. The important factor to be considered for enrichment experiments is to ensure that iron is made available for phytoplankton to utilize, because iron present in seawater does not necessarily indicate what is available for phytoplankton (Wells and Mayer, 1991b; Wells *et al.*, 1991). Iron enrichment experiments are still controversial, and to address the problem adequately further knowledge of iron speciation in the natural environment, of its utilization by organisms, and of the supply rate is needed. Even though the chemistry of iron and the physiology of phytoplankton iron metabolism is understood, the effect of iron enrichment at the ecosystem level is still unpredictable. Rueter and Ades (1987) reported that iron-limited cultures fixed about twice as much carbon to protein relative to the total carbon fixed than iron-replete cultures. If carbon metabolism does not increase at the same rate as nitrogen metabolism does, then large scale iron enrichment experiments could decrease the total carbon dioxide consumption per unit nitrate and will eventually increase the input of CO_2 to the atmosphere. This is just the opposite of what is expected to be accomplished with iron fertilization, which is to reduce the CO_2 flux from the ocean to the atmosphere (Martin, 1992).

CONCLUSION

1. There was a significant increase in the DCMU enhanced fluorescence when iron was added with a chelator (EDTA) in the upwelling area where nitrate concentrations were higher than 5 μM. The lag period for the increase in fluorescence was significantly reduced by the addition of chelated iron. The availability of iron appears to be an important factor controlling the increase in phytoplankton biomass.

2. In the oligotrophic areas where initial nitrate

concentrations were low (below 0.5 μM), addition of iron and/or chelators did not increased the DCMU enhanced fluorescence significantly, suggesting that iron is not the primary limiting factor in that area.

3. There was no significant difference in CFC values [$(F_d-F)/F_d$: DCMU enhanced fluorescence ratio] between the control and iron and/or chelator treatments on Day 3. However, with 10 μM DTPA treatment, there was a significant decrease in the ratio, suggesting that the strong chelator caused severe iron stress. The CFC values decreased after several days incubation especially in chelated iron treatments. However, this decrease may have been due to the limitation by major nutrients (nitrate and/or silicate) not by iron.

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