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# Photochemical iron reduction and iron bioavailability in seawater

by William L. Miller[1,2] and Dana Kester[1]

## ABSTRACT

Photochemical redox cycling of iron in natural seawater may affect the availability of iron to phytoplankton for cellular growth. Fe(III) colloids that support only limited phytoplankton growth were added to coastal seawater at pH 8 and exposed to simulated solar irradiation for varied time intervals. Photo-production of Fe(II) in the seawater samples was measured during each irradiation. Following irradiation, the marine diatom, *Skeletonema costatum,* was inoculated into each treatment and cell densities were monitored in batch cultures over the next ten days. Increases in steady state concentrations of Fe(II) were observed during irradiation and subsequent elevated phytoplankton growth was observed in most solutions exposed for 60 minutes or more. The photo-production of Fe(II) in seawater results in the transfer of iron between chemical states which differ in their ability to supply iron on a time scale required for phytoplankton growth.

## 1. Introduction

Iron is an essential element for plant growth and is used in various redox enzymes, iron-sulfur proteins critical for photosynthetic electron transport, chlorophyll, and other respiratory pigments (Lehninger, 1975). The chemical form of iron in natural waters controls iron accumulation by phytoplankton for use in cellular metabolism (Anderson and Morel, 1982). Because of the limited solubility of Fe(III) hydroxides in seawater (Byrne and Kester, 1976), the bioavailability of iron in colloids is a central question in the study of iron uptake by phytoplankton. This question has been addressed by various researchers resulting in disparate conclusions (Goldberg, 1952; Hayward, 1968; Davies, 1970; Anderson and Morel, 1982; Wells *et al.,* 1983; Rich and Morel, 1990; Wells *et al.,* 1991). Recent studies using marine phytoplankton species conclude that growth depends on soluble iron species, either Fe(II) or Fe(III) (Davies, 1970; Anderson and Morel, 1982; Brand *et al.,* 1983; Morel, 1987; Rich and Morel, 1990). Rich and Morel (1990) looked for iron-stimulated growth in *Thalassio-*

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*sira weissflogii* using three well-defined Fe(III) colloids and saw no evidence for direct uptake of iron from colloids by this coastal marine diatom.

Various mechanisms have been suggested to explain increases in marine phytoplankton growth following additions of various forms of particulate Fe(III) oxides. These include thermal dissolution (Wells *et al.,* 1983; Rich and Morel, 1990), reduction of Fe(III) by organic reductants (Finden *et al.,* 1984) and photo-reductive dissolution of Fe(III) oxides both with and without organic ligands (Finden *et al.,* 1984; Wells *et al.,* 1987, 1991; Rich and Morel, 1990). Simply stated, any process which controls soluble iron concentrations in natural waters provides a mechanism to increase iron availability to phytoplankton for support of cellular growth and primary production. Ultimately, the rate at which soluble iron becomes available, the rate of iron binding to uptake ligands, and the rate of iron incorporation into cellular components will dictate cellular responses to iron (Hudson and Morel, 1990; Morel *et al.,* 1991).

Colloidal Fe(III) matures in seawater by dehydration to become much less soluble and subsequently less available for biological uptake. Photochemical reduction of Fe(III) from these "mature" particles can result in soluble Fe(II) release to the dissolved phase. Oxidation of the Fe(II) and subsequent formation of colloidal hydrous ferric oxides can place the iron in a form that is more reactive and bioavailable than the "mature" particles from which it was derived. Cycling of iron between particulate and dissolved forms in oceanic ecosystems is critical for sustained plant growth. Photochemical reduction may be a key process in sustaining this cycle.

The question explored here is this: Does the photochemical production of Fe(II) in seawater repartition iron from a form that is not biologically available to one that is available for use in cell growth? Evidence for photo-production of Fe(II) in seawater (Waite and Morel, 1984; Hong and Kester, 1986; King *et al.,* 1991; Miller, 1990; O'Sullivan *et al.,* 1991; Kuma *et al.,* 1992) along with arguments for the biological importance of iron dissolution, might suggest that this question has been answered. It is possible, however, given the paucity of knowledge on the source material for Fe(II) photo-production in natural waters, that the Fe(II) observed in seawater is generated from a pool of iron that is already available for biological uptake. If this is the case, photo-production of Fe(II) will not increase the amount of biologically available iron; it will merely recycle the iron that is already available.

The strongest evidence to date that increased iron availability results from the irradiation of iron in seawater comes from the work of Wells, Mayer, and colleagues (Wells *et al.,* 1983, 1991; Wells and Mayer, 1991, 1992). Using the complexing agent 8-hydroxyquinoline for evaluation of labile iron in seawater, they have shown correlations between the chemical lability of iron and phytoplankton growth. They have also shown that chemical lability of iron increases due to irradiation of various iron colloids in seawater. But as pointed out by Wells and Mayer (1992), without

direct measurements of reduced iron this increased chemical lability can only be assumed to reflect iron photolysis in seawater at pH 8.

Using novel analytical techniques developed in our laboratory, we measured Fe(II) resulting from sunlight exposure of iron colloids in seawater and evaluated growth changes in the marine diatom, *Skeletonema costatum.* A batch culture experimental approach was used to test the hypothesis that solar irradiation of seawater can stimulate phytoplankton growth due to an increase in the bioavailability of iron resulting from a photo-reductive dissolution involving the formation of Fe(II).

## 2. Material and methods

*a. General methods.* All glassware and plasticware used for this study were cleaned with detergent, rinsed with 18 megohm $cm^{-1}$ Milli-Q water (MQ) (Millipore, Corp.), soaked for 2 days in 6 N HCl, rinsed with MQ, and soaked in MQ for 2 days prior to first use. Subsequent cleaning included a detergent wash, MQ rinse, 2 N HCl soak, and three rinses with MQ. FEP teflon was used for storage of all stock solutions and all trace metal work was done in a class-100 laminar flow clean bench. Glassware was only used in preparation of stock solutions and contact time with trace metal solutions was minimized.

Catalase (44,200 Units $mg^{-1}$), peroxidase (Type II, 200 purpurogallin Units $mg^{-1}$) and hydrogen peroxide (30% w/w) were obtained from Sigma Chemical Company and used as received. Crystalline p-hydroxyphenylacetic acid (Sigma Chemical) was further purified by recrystallization in warm water. All other chemicals were reagent grade.

Seawater for the experiments was collected from Narragansett Bay, RI, in polyethylene containers and filtered sequentially through two 142 mm diameter all-teflon Millipore® filter holders containing 0.4 μm and 0.2 μm pore size acid cleaned polycarbonate Nuclepore® filters. Measurements of pH were made using an Orion Ross® 8102 combination electrode calibrated on the free hydrogen ion scale using Tris-seawater buffers as described by Millero (1986).

*b. Laboratory irradiation methods.* Experiments used a Kratos Solar Simulator system equipped with a 1000 Watt xenon arc lamp, quartz focusing optics, air mass filters, a sunlens diffuser, infrared filter, and 90° light tube (Fig. 1). Actinometry studies show that light from this system is similar to sunlight both in intensity and in its spectral qualities (Miller, 1990). Resulting output was focused into a 15.24 cm diameter irradiation field incident on the surface of our uncovered 4 liter sample. Dye studies showed that the polycarbonate reaction vessel with a teflon stir bar and plexiglass cross-mixer was completely mixed in less than 30 seconds. The temperature in the reaction vessel, measured throughout the experiments, was maintained with a plexiglass thermal jacket connected to a Lauda thermocirculator.

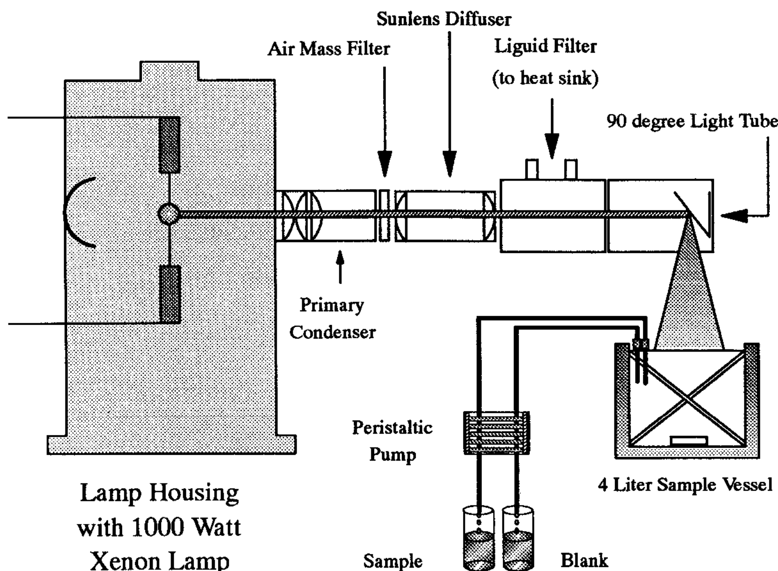


Figure 1. Diagram of the solar simulation apparatus used in experiments 1 and 2.

*c. Chemical methods.* Total iron concentrations were determined using a Perkin Elmer Model 5000 atomic absorption spectrophotometer (AAS) equipped with an HGA-500 graphite furnace and an AS-40 auto sampler. The cobalt-APDC preconcentration method of Boyle and Edmond (1975) was used as modified by Huizenga (1981) using filtration to separate the precipitate from the seawater. The reported detection limit for the 250 ml samples used here is 0.2 nmol/kg with a relative standard deviation less than 10%.

Fe(II) analysis was performed using the analytical methods described by King *et al.* (1991). The method employs the immobilization of a highly selective ferrous iron reagent, ferrozine (FZ) ([3-(2-pyridyl)-5,6 bis(4-phenylsulfonic acid)-1,2,4-triazine]), onto a reverse phase C-18 Sep-Pak cartridge (Waters, Inc.) pretreated with 10 ml of methanol, a 20 ml MQ rinse, and a 5 ml rinse with 0.7 molal NaCl/0.005 molal $NaHCO_3$. FZ was loaded onto the Sep-Pak using 2 ml of 0.0040 molar FZ in 0.7 molal NaCl/0.005 molal $NaHCO_3$ followed by a 5 ml rinse of 0.7 molal NaCl/0.005 molal $NaHCO_3$ to remove excess FZ. Fe(II) was quantitatively retained from seawater as the Fe(II)-ferrozine complex, $Fe(FZ)_3$, by passing a seawater sample through the cartridge. $Fe(FZ)_3$ is eluted from the cartridge with 10 ml of methanol and its concentration determined colorimetrically using a 10-cm quartz cell with a Model 260 Shimadzu UV/VIS recording spectrophotometer. This procedure results in a 10-fold $Fe(FZ)_3$ preconcentration and a limit of detection of 0.2 nmol/kg using a flow rate of 15 ml $min^{-1}$ and a 100 ml sample volume. Precision is approximately 2%.

Hydrogen peroxide was measured using the method of Miller and Kester (1988) which employs the peroxidase catalyzed dimerization of p-hydroxyphenylacetic acid

Table 1. Composition of MM1 medium for phytoplankton growth experiments. The medium concentrations listed here result from the addition of stock solutions to sterile filtered (0.2 μm pore size) Narragansett Bay water treated with a Chelex-100 column resulting in a total iron concentration of 3 nmol/kg and a pH of 8.2.

| Component | Concentration |
|---|---|
| Nutrients: | |
| $NaNO_3$ | 100 μM |
| $NaH_2PO_4$ | 10 μM |
| $Na_2SiO_3$ | 40 μM |
| Trace Metals: | |
| Zn (granular) | 100 nM |
| $MnSO_4$ | 100 nM |
| $Na_2MoO_4$ | 10 nM |
| $Co(NO_3)_2$ | 10 nM |
| $CuSO_4$ | 10 nM |
| Vitamins: | |
| Thiamin-HCl | 10 μg $L^{-1}$ |
| Biotin | 50 ng $L^{-1}$ |
| Vitamin $B_{12}$ | 50 ng $L^{-1}$ |

and formation of a stable fluorescent product in the presence of $H_2O_2$. Fluorescence was measured using a Perkin Elmer Model MPF-2A spectrofluorometer. Additional details for use of this method in seawater can also be found in Miller (1990).

*d. Biological methods.* The growth media used in these experiments, designated MM1 media for purposes of this discussion, was based on seawater collected from Narragansett Bay (NBSW) and supplemented to achieve the nutrient and trace metal concentrations used by Brand *et al.* (1983) for iron limitation studies of a variety of coastal and open ocean phytoplankton species. The concentrations of nutrients and metals in the MM1 media resulting from additions of three separate stocks are presented in Table 1. After filtration as described above, the water (salinity = 32.1) was passed through a 50–100 mesh Chelex-100 column (5 ml $min^{-1}$) prepared as described by Davey *et al.* (1970) for use with phytoplankton cultures and then sterile filtered through an acid cleaned, sterile 0.2 μm Gelman mini capsule filter. Studies by Davey *et al.* (1970) using sensitive phytoplankton species and metal complexation titrations show no evidence that resin leachates occur when the column is carefully prepared in this manner. The nutrient stock was also passed through the Chelex-100 column to remove trace metal contamination prior to sterile filtration. The total iron concentration was measured for the chelexed NBSW and for separate additions of the vitamin stock, trace metal stock, and nutrient stock to MQ to test for iron contributions from reagents. The results for chelexed NBSW and the vitamin stock addition were 3.5 ± 0.8 nmol/kg ($n$ = 2) and 0.7 nmol/kg ($n$ = 1)

respectively. Both the nutrient and trace metal stock additions contributed no measurable iron ( < 0.35 nmol/kg ($n$ = 1)).

The two sources for iron uptake used in this study were prepared to be equivalent to those used by Wells and Mayer (1992). The first was freshly precipitated ferric hydroxide (FPFH) made by adding 450 μM $FeCl_3$ to unbuffered MQ water. The second source (90°-FERR) was made by heating a subsample of FPFH for 5 minutes at 90°C. The physical form of iron resulting from these two treatments is discussed by Wells *et al.* (1983). Briefly, the FPFH stock results in polynuclear ferric colloids which attain an estimated size of 2–16 nm after 24 hours at room temperature. Heating FPFH results in further dehydration and greater thermodynamic stability due to replacement of Fe-$(OH)_2$-Fe bridges within the colloid by Fe-O-Fe bonds.

The phytoplankton species used for this study was *Skeletonema costatum* Cleve (clone SK6 isolated from Narragansett Bay by T. J. Smayda, April 1966) obtained from the culture collection of Dr. Smayda. The cultures were not axenic but all plasticware was microwave pasteurized to minimize external contamination. Both stock and experimental cultures were maintained in 50 ml polycarbonate centrifuge tubes. Stock cultures were acclimated to the light and temperature regime used in the experimental treatments for a minimum of two weeks prior to the experiments. Media for *S. costatum* cultures, described in Table 1, was prepared in a 500 ml acid cleaned black FEP teflon bottle. Iron was added at a concentration of $4.5 \times 10^{-7}$ M Fe to each individual vial as required. The stock cultures were transferred to new media once a week to prevent nutrient depletion and new FPFH was made weekly to maintain the iron in a form available for growth. Studies by Wells *et al.* (1983) have demonstrated maximal growth with FPFH and no decreased growth for FPFH aged 1 week at room temperature.

Both stock and experimental cultures were continuously irradiated with two 15 watt GTE Cool-White fluorescent tubes at a level of 50 $\mu E\ m^{-2}\ sec^{-1}$ photosynthetically available radiation (PAR) as measured by a LiCor, Inc. Model LI-185B Quantum Radiometer with a Model LI-193SB Spherical Quantum Sensor. The maximum growth rate for this clone of *S. costatum* occurs at an irradiance above about 200 $\mu E\ m^{-2}\ sec^{-1}$ (Langdon, 1987). The low irradiance here was chosen to minimize the photochemical reduction of iron within the culture tubes as has been observed previously for various iron colloids irradiated at 120 $\mu E\ m^{-2}\ sec^{-1}$ (Rich and Morel, 1990). Fe(II) analysis of culture media with no added cells and added 90°-FERR exposed to 50 $\mu E\ m^{-2}\ sec^{-1}$ for one hour showed no measurable Fe(II) ( < 1 nmol/kg). The cultures were maintained at room temperature (23 ± 4°C) over the length of the study.

Changes in *in vivo* fluorescence were monitored by inserting the centrifuge tube containing the culture directly into a Turner Designs Model 10 Series fluorometer (10-045 blue lamp, 5-60 excitation filter, and 2-64 emission filter). These arbitrary fluorescent values were used for comparison of growth results between experimental

treatments. Microscopic examination of cell samples preserved with Lugol's fixative were used to confirm the vitality of the culture and to estimate the cell numbers present at various stages during the experimental treatments. Cell estimates from microscopic counts were good to approximately 20%. The result of 10 separate determinations of the fluorescence to cell count (cells $ml^{-1}$) ratio was 7.5 ($\pm$0.2) $\times$ $10^{-6}$.

*e. Experimental procedures.* Two irradiation experiments and two chemical control experiments were done for this study. Each experiment consisted of a photochemical or chemical treatment followed by evaluation of phytoplankton cell growth subsequent to each treatment. Batch culture experiments were begun following each treatment with a 100 to 500 $\mu$L inoculation from a single SK6 stock growing exponentially in 20 ml of MM1 media containing 450 nM FPFH iron. Starting cell densities were between 500 and 1500 cells $ml^{-1}$. Cells were not exposed to the solar simulator lamp at any time. For comparison between experimental treatments, two sets of triplicate cell cultures, one having no added iron and one having 450 nM added FPFH iron, were run concurrently with each experiment. The two irradiation experiments (1 and 2) involved placement of the specific media under the solar simulator and removing triplicate 20 ml aliquots at specified irradiation intervals. In experiment 1, four (4) liters of MM1 media containing 450 nM 90°-FERR was irradiated for 240 minutes. Experiment 2 used four (4) liters of filtered Narragansett Bay water (salinity = 29.0) with no added iron. Total iron concentration was 13 nmol/kg as measured by atomic absorption spectrometry as described earlier. At each time point, triplicate 20 ml aliquots from the irradiated solutions were mixed with 1 ml of stock nutrient and trace metal mixture directly in the incubation tubes followed by inoculation with phytoplankton. The two control experiments did not involve irradiation of the culture media but were used to examine the effect of $H_2O_2$ and added Fe(II) on culture growth. Duplicate $H_2O_2$ additions (experiment 3) were performed in MM1 media with 450 nM added 90°-FERR. The Fe(II) additions (experiment 4) were done in triplicate to MM1 media with no other added iron.

## 3. Results

Both $H_2O_2$ and Fe(II) were measured throughout the two irradiation experiments (1 and 2). The results from experiments 1 and 2 are shown in Figures 2 and 3 respectively. Experiment 1 exhibited a significant increase in Fe(II) within the first 30 minutes of irradiation which rapidly dropped to below detection limits for the portion of the experiment beyond 1 hour. The $H_2O_2$ accumulation rate over 180 minutes of irradiation was 49.8 $\pm$ 0.2 nM $hr^{-1}$ ($r^2$ = 0.994). Measurements made at 30 minutes after the lamp was turned off showed a continued increase of $H_2O_2$ in the dark. Experiment 2 showed a rapid increase of Fe(II) to about 0.35 nmol/kg where it

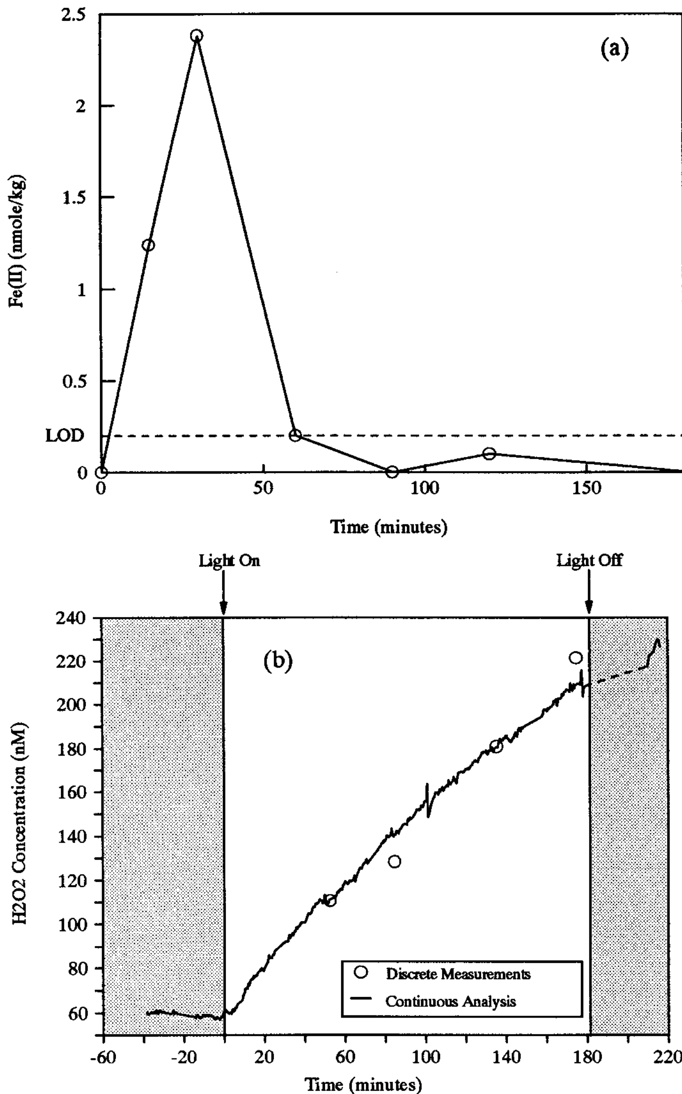


Figure 2. Fe(II) (a) and $H_2O_2$ (b) results from irradiation of MM1 presented as experiment 1. LOD is limit of detection (0.2 nmol/kg) and shaded panel is dark period after irradiation.

remained until the light was turned off. The $H_2O_2$ accumulation rate for experiment 2 was 54 ± 5 nM $hr^{-1}$ ($r^2 = 0.959$).

The addition of 450 nM 90°-FERR does not support the same magnitude of cell growth as observed in 450 nM additions of FPFH. A typical result for the difference in growth between stock cultures with 450 nM added as FPFH and 90°-FERR is shown in Figure 4. Experiment 1 (Fig. 5) also shows limited growth on 90°-FERR compared to FPFH. Additionally, when all other components are added to the

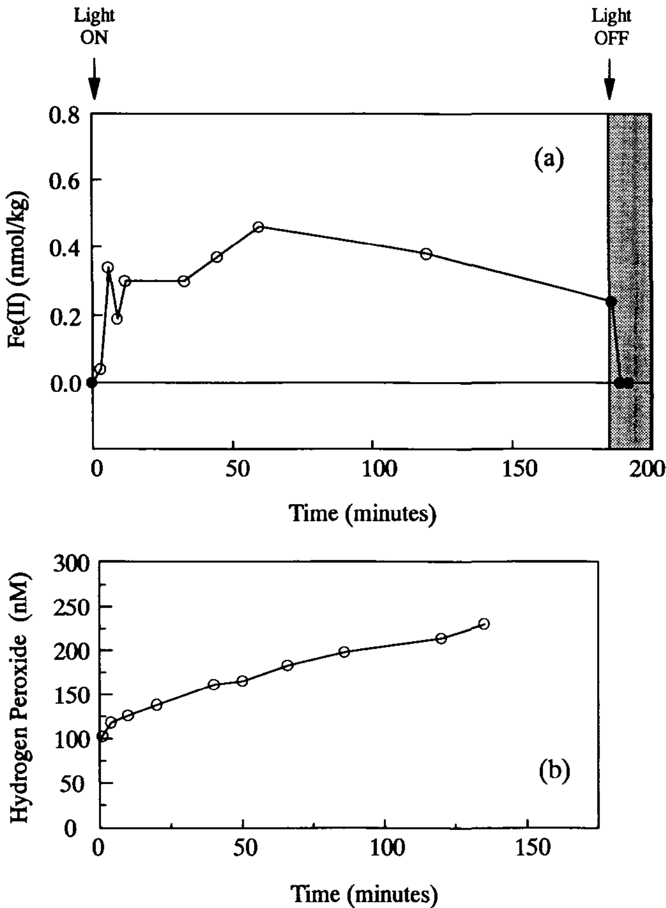


Figure 3. Fe(II) (a) and $H_2O_2$ (b) results from irradiation of Narragansett Bay seawater (NBSW) presented as experiment 2. Shaded panel in (a) is the dark period after irradiation for which Fe(II) measurements were made.

culture in MM1 media without added iron of any form, cell yield as measured by fluorescence is also much lower than when FPFH is added. The lowest cell yield observed at 8 days for any treatment was in experiment 2 (Fig. 6) where cells were inoculated into 0.2 μm filtered NBSW with no addition of nutrients, vitamins, trace metals, or iron. Without any additions at all, filtered NBSW sustained exponential cell growth for approximately 3 days.

A summary of the cell growth results from our four experiments is presented in Table 2. The symbol $h\nu(x)$ in the table denotes irradiation for $x$ minutes. The comparison between various experimental treatments is designated "FL Ratio," defined as the mean fluorescence for each individual treatment on the last measurement prior to day 8 divided by the mean fluorescence for cultures with 450 nM FPFH

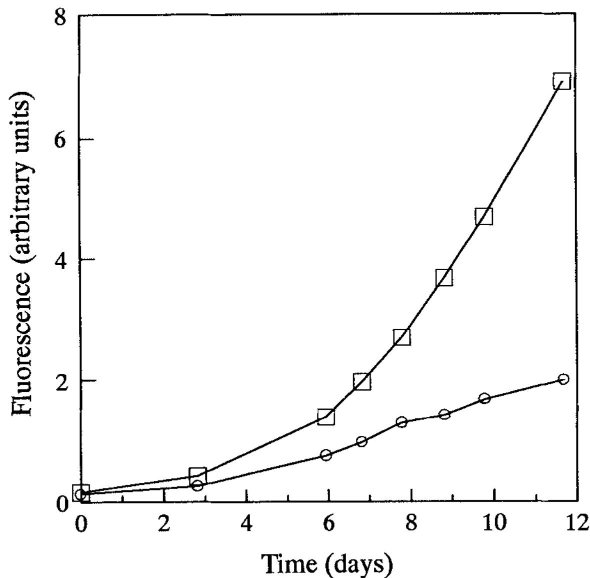


Figure 4. Comparison of fluorescence data for *Skeletonema costatum* cell growth on the two sources of iron used in this study. Squares and circles are for the 450 nM FPFH and 90°-FERR iron sources (as defined in the text) respectively.

measured at the same time. In experiment 1, we made the comparison at $t = 6.4$ days because fluorescence for the FPFH cultures after that day was beyond the range of our fluorometer. Since cultures generally did not reach a plateau in fluorescence by 8 days the FL Ratio combines information on both cell yield and growth rates. This ratio provides a comparison of growth in our various treatments relative to growth in cultures without iron limitation (ex. FL Ratio = 0.25 = cell growth 25% that of an equivalent culture with no iron limitation). Statistical analysis was carried out using methods presented by Natrella (1963) for comparison of mean values and observed proportions based on normal and $\chi^2$ distributions respectively. For all cases where changes in the mean fluorescent value at day 8 proved statistically significant, the calculated FL Ratio also proved to be significant. Consequently, values marked with (+) in Table 2 represent statistical variation at the $\alpha = 0.05$ significance level for both mean fluorescence and FL Ratio. In experiment 1 and 2, results were compared to $h\nu(0)$ for evaluation of significance. In the two experiments without irradiation, results were compared to the lowest addition, 38 nM $H_2O_2$ and 50 nM Fe(II) in experiments 3 and 4 respectively. Initial growth rates, determined by linear regression of log linearized plots of fluorescence against time in days, showed no significant difference between treatments. This probably reflects the internal cellular iron reserves and usable iron carried over from the initial cell transfer.

Additions of $H_2O_2$ and Fe(II) in experiments 3 and 4 were done to isolate growth effects resulting from the presence of these two photochemical products from other

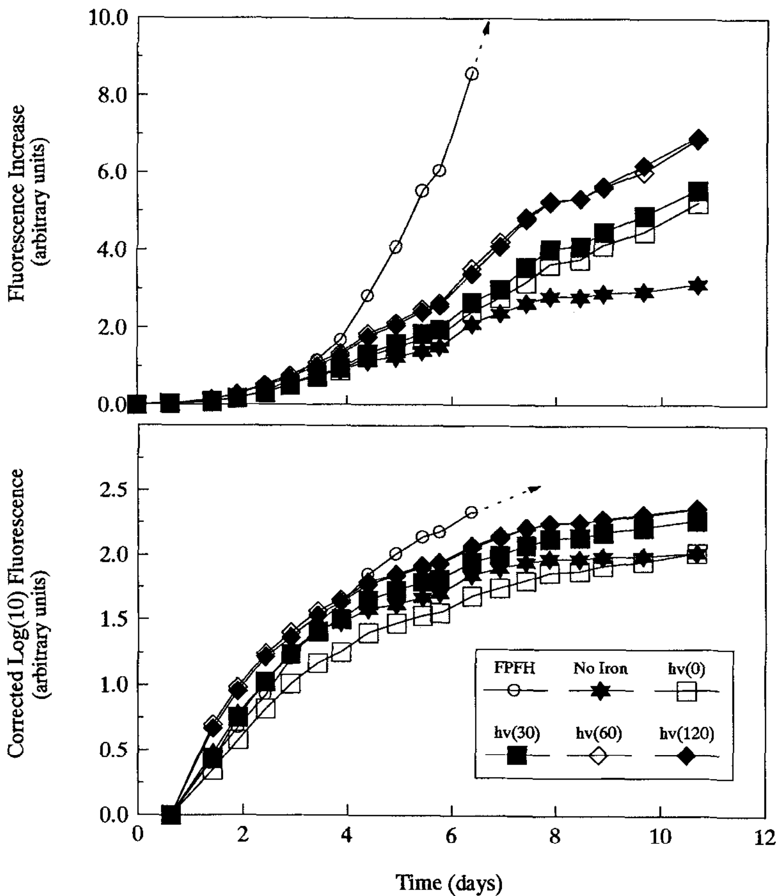


Figure 5. Cell culture fluorescence results for experiment 1 following irradiation of MM1 medium. Fluorescence Increase is the difference from $t = 0$. For clear comparison, all "log(10) fluorescence" values in the lower panel were zeroed at 0.63 days when exponential growth was clearly evident. Symbols are as indicated on the figure and treatments are described in the text. $h\nu(x)$ denotes irradiation for $x$ minutes. All treatments contain added 90°-FERR except the FPFH treatment.

potential effects due to irradiation of the media. The presence of $H_2O_2$ in concentrations ranging from 38 nM to 289 nM with 90°-FERR as an iron source had no significant effect on FL Ratio (Table 2). Additions of Fe(II) as the iron source in concentrations from 50 nM to 450 nM produced fluorescence responses similar to those resulting from 450 nM FPFH addition.

Cultures grown on both irradiated MM1 media and NBSW exhibited statistically significant increases in cell densities over cultures grown in identical media that had not been irradiated. In the MM1 media experiment, the increase was significant when the media had been irradiated for 60 minutes or longer. In one treatment

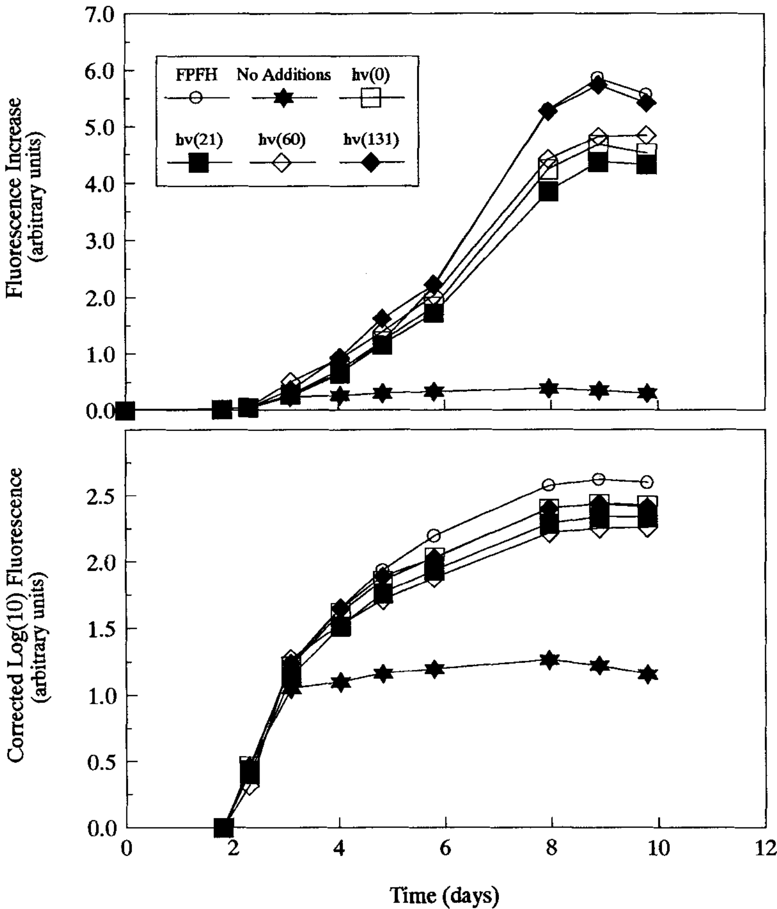


Figure 6. Cell culture fluorescence results for experiment 2 following irradiation of Narragansett Bay water (NBSW). Fluorescence Increase is the difference from $t = 0$. For clear comparison, all "log(10) fluorescence" values in the lower panel were zeroed at 1.8 days when exponential growth was clearly evident. Symbols are as indicated on the figure and treatments are defined in the text. $h\nu(x)$ denotes irradiation for $x$ minutes. "No Additions" indicates unamended NBSW. All other treatments contained added nutrients and trace elements as described in the text. No iron was added to any treatment except the FPFH treatment.

which was irradiated for 3 hours (experiment 1, Table 2) and left in the dark for 1 hour prior to inoculation, the results were not significantly different from the $h\nu(120)$ treatment which was not placed in the dark prior to inoculation. For NBSW in experiment 2, the increase in cell yield was observed only in the sample irradiated for 2 hours. All of the treatments in experiment 2 displayed larger increases in growth relative to cells grown in NBSW + FPFH than was observed for MM1 media with added 90°-FERR (FL Ratio $\approx$ 0.8 compared to 0.3).

Table 2. Summary of results from batch experiments performed in this study. FL Ratio was calculated as described in the text. Treatments marked (+) are significantly different from the treatment to which it is compared, marked (*), at the $\alpha = 0.05$ significance level while those marked (−) are not. $h\nu(x)$ denotes irradiation for $x$ minutes.

| Experiment Description | Treatment | FL Ratio |
|---|---|---|
| 1 | 450 nM FPFH | 1 |
| | No Added Iron | 0.25 |
| | $h\nu(0)$ | 0.28 (*) |
| *Irradiation of MM1* | $h\nu(30)$ | 0.31 (−) |
| *Media with added* | $h\nu(60)$ | 0.41 (+) |
| *90°-FERR* | $h\nu(120)$ | 0.40 (+) |
| | $h\nu(240)$ | 0.38 (+) |
| 2 | 450 nM FPFH | 1 |
| | No Additions | 0.07 |
| | $h\nu(0)$ | 0.80 (*) |
| *Irradiation of NBSW* | $h\nu(21)$ | 0.73 (−) |
| *with no added iron* | $h\nu(60)$ | 0.83 (−) |
| | $h\nu(131)$ | 1.00 (+) |
| 3 | 450 nM FPFH | 1 |
| | No Iron Additions | 0.10 |
| | 38 nM | 0.30 (*) |
| *$H_2O_2$ Additions* | 123 nM | 0.36 (−) |
| *(All with 90°-FERR)* | 207 nM | 0.29 (−) |
| | 289 nM | 0.30 (−) |
| 4 | 400 nM FPFH | 1 |
| | No Iron Additions | 0.11 |
| | 50 nM | 0.93 (*) |
| | 150 nM | 0.91 (−) |
| *Fe(II) Additions* | 250 nM | 0.84 (−) |
| | 350 nM | 0.77 (−) |
| | 450 nM | 0.80 (−) |

## 4. Discussion

This study confirms that freshly precipitated iron hydroxides in seawater can provide iron for cell growth. In the absence of photochemistry, this process occurs by thermal dissolution (Wells *et al.,* 1983; Rich and Morel, 1990; Hudson and Morel, 1990). The degree of Fe(II) complexation to cell surface ligands will be a result of competition for those sites with dissolved Fe(III) (Hudson and Morel, 1990). In our cultures, direct complexation of Fe(II) is assumed to be an insignificant source of iron for *S. costatum* since the cultures are grown in low light and Fe(II) created by solar simulator irradiation of the media is oxidized prior to cell inoculations. Without a continual source for Fe(II), uptake in pH 8 seawater is not favored since the oxidative loss of Fe(II) is faster than the turnover rate of the cell surface ligand

(Hudson and Morel, 1990). In natural irradiated water, elevated steady state concentrations of Fe(II) resulting from photoreduction may allow Fe(II) to effectively compete with Fe(III) for cell surface ligands. Once complexed, Fe(II) may be stabilized against oxidation. Previous measurements of unfiltered samples of Narragansett Bay water, using the same method employed in this study, have shown high Fe(II) concentrations relative to filtered samples (King, 1988; King *et al.,* 1991), possibly resulting from measurement of Fe(II) bound to cell surfaces and other particles.

Recent work done in F.M.M. Morel's laboratory (Rich and Morel, 1990; Hudson and Morel, 1990) has provided good evidence that the rate of growth in batch cultures for the marine diatom *Thalasiosira weissflogii* depends on complex kinetic interactions involving cell surface ligand exchange rates and rates of iron dissolution. Any experimental treatment which results in either a change in the dissolution rate of the iron present or transfers iron between two forms with different solubilities has the potential to alter cell growth. Under our experimental conditions, increased growth observed after irradiation of coastal seawater is consistent with increased iron availability to *Skeletonema costatum.* Control experiments demonstrate that the observed change does not result from photochemically produced $H_2O_2$ (experiment 3) or other photochemical transients with a residence time shorter than 1 hour (experiment 1). Growth in all experiments using MM1 media is significantly depressed without some addition of iron. Intensified growth for batch cultures in irradiated media cannot be explained by other known light initiated processes such as decomposition of vitamin $B_{12}$ or the loss of phosphate or essential trace metals due to co-precipitation with iron since these processes would result in decreased growth.

Cultures grown on MM1 media irradiated for longer than 60 minutes (120 and 240 minutes) showed no further increases in cell growth. This suggests that photochemical reactions occurring beyond about 1 hour either do not make a further contribution to the available iron pool or attain a steady state condition between labile iron production and loss. This result is strikingly similar to that of Wells and Mayer (1992) who found decreased rates of photo-conversion of 90°-FERR to chemically labile iron with continued exposure to light. Their irradiation of 90°-FERR in Sargasso seawater showed a rapid increase in labile iron from 30 nM to 60 nM in the first hour with no further increase over the next four hours of irradiation.

The sudden decrease in Fe(II) concentrations to below detection limits in samples taken at 60 minutes and beyond in irradiated MM1 media is unlike the Fe(II) steady state produced in the filtered Narragansett Bay water irradiated in experiment 2. Possible explanations for this result include the depletion of source material for Fe(II) photo-production and/or an increased iron oxidation rate due to elevated radical populations from a photochemical source such as nitrate photolysis (Zepp *et al.,* 1987; Kotzias *et al.,* 1987). The addition of nutrients to irradiated seawater may result in an increased steady state concentration of hydroxyl radical and/or superox-

ide that would cause increased oxidation rates for Fe(II). If this is the case, Fe(II) formed by photochemical reduction of 90°-FERR will oxidize at an accelerated rate causing the steady state Fe(II) concentration to fall below detectible limits. The source(s) for Fe(II) production might also be depleted with a similar result. An alternative explanation is the modification or elimination of natural organic material capable of Fe(II) complexation and stabilization against oxidation. Fe(II) oxidation in NBSW may be slowed by organic complexation. Loss of complexing compounds during MM1 media construction may result in faster oxidation rates than observed in unaltered NBSW with a full complement of natural ligands.

Much of the 13 nmol/kg total iron present in the filtered NBSW used for experiment 2 appears to already exist in a form that is available to *S. costatum.* NBSW, when supplemented with nutrients, trace metals, vitamins, and no additional iron, supported cell densities that were about 80% of those observed for 450 nM FPFH additions (Table 2). This compares to only 28% in the unirradiated MM1 media with 450 nM added 90°-FERR, even though both solutions had similar carryover of available iron from the cell inoculation. Only after about 2 hours of irradiation was there a statistically significant increase in cell densities for cultures grown in irradiated NBSW. It appears that only a portion of the iron originally present in the filtered sample was altered to a more biologically labile form by irradiation. Since this sample was collected from sunlit surface water and used for experiment 2 on the same day, filtration at 0.2 μm, while significantly decreasing total iron in the sample, may have isolated the dissolved species and colloidal iron that are most bioavailable. This would leave little capacity for further conversion to more biologically labile forms of iron.

Ideally, one would hope for fully characterized, identical iron chemistries in both our MM1 and NBSW irradiations for purposes of comparison. EDTA has been used quite successfully in the past to establish a "well defined medium" with regards to iron chemistry for phytoplankton studies and was considered for use here. In full sunlight, however, the EDTA-Fe(III) complex photo-decarboxylates to form Fe(II), $CO_2$, and formaldehyde (Budac and Wan, 1992). This clearly does not reproduce the photochemical iron reduction involving natural organic ligands in seawater since it magnifies Fe(II) production and increases formaldehyde concentration. Without nutrient, trace metal, and vitamin additions, exponential growth in NBSW is maintained for only about 3 days (experiment 2). MM1 medium was formulated both to maintain the natural organic ligands critical to seawater photochemistry and to supplement nutrients for maximal growth when iron was available. Without direct measurements of Fe(II) during the irradiation portion of these experiments one might erroneously assume that MM1 *did* mimic NBSW for iron photochemistry. Judging from our Fe(II) data, the irradiation of MM1 media may produce an oxidation environment for Fe(II) that is different from unaltered seawater. Consequently, extrapolation of the magnitude of these results to natural water measure-

ments should be done with care. Nevertheless, correlation between Fe(II) production and the consequent stimulation of cell growth suggests that the photo-reduction of Fe(III) to Fe(II) is indeed important to changes in iron bioavailability.

Fe(II) measured during irradiation might be expected to provide an upper limit for the amount of iron cycled by photochemical reduction into fresh ferric hydroxides with the potential to support phytoplankton growth by dissolution. The solutions used for the irradiation experiments were well mixed and did not experience the attenuation of UV light that is found within the oceanic mixed layer. By assuming steady state oxidation-reduction conditions for Fe(II), photoproduction rates can be calculated using published oxidation rates for Fe(II) in seawater (Millero *et al.,* 1987; Millero and Sotolongo, 1989). For the MM1 irradiation, an Fe(II) steady state was not maintained and this approach could not be applied. For experiment 2, average concentrations of Fe(II) and $H_2O_2$ (0.35 nmol/kg and 150 nM respectively) and measured salinity, pH, and temperature (29.0, 7.90, and 23.0°C respectively) were used to calculate an apparent oxidation rate of 0.435 nmol/kg/min. Based on this calculated oxidation rate of Fe(II), the steady-state concentration of Fe(II) (0.35 nmol/kg) must be turning over with a time scale of minutes.

Taking a geochemical approach, this oxidation rate suggests that all of the colloidal and dissolved Fe(III) pool in our NBSW sample (13 nmol/kg) could be photochemically cycled within about 30 minutes. Our data, however, indicate that this approach may not be valid since not all of the iron seems to be available for photochemical transfer into a more biologically labile pool. This is suggested by experiment 1 with added 90°-FERR where irradiations longer than about an hour do not cause further increases in cell growth. Cell growth results from experiment 2 with NBSW indicate that much of the iron is available prior to experimental irradiation. A significant portion of the Fe(II) measured in our irradiation of NBSW must represent iron which is photochemically recycled from freshly precipitated colloidal iron or dissolved Fe(III). If particulate ferric hydroxides already exist in a form that is soluble on the time scale required for phytoplankton uptake and growth (i.e., freshly precipitated colloidal ferric hydroxides), further irradiation may enhance particle solubility but would not be reflected as an increase in bioavailable iron in a study such as this one. If only a subset of the "dissolved" iron pool cycles by way of photochemistry, the process must be very rapid indeed.

There is a marked difference in the phytoplankton growth when FPFH and 90°-FERR were used as the source for iron (Fig. 4). The iron dissolution rates present in our irradiation experiments probably result from a continuum of particle types rather than two distinct pools such as FPFH and 90°-FERR with very different solubility kinetics. Mature particles may become coated with freshly precipitated ferric hydroxide of varying thickness. Some particulate phases may not support maximal cell growth, but rather supply iron at a rate which allows continued slow cell growth throughout a batch experiment. The supply rate of bioavailable iron may be

more important in determining phytoplankton growth rates than the total quantity of iron that can be made bioavailable.

## 5. Summary and conclusions

This study presents direct evidence for the photoproduction of Fe(II) in seawater at pH 8. It appears that some part of the Fe(II) produced from the irradiation of seawater containing Fe(III) colloids represents the re-partition of iron between forms with different capabilities to support cell growth. Iron is transferred by photochemical processes from forms which support only limited cell populations into a form that is soluble on the time scale required to supply iron for cellular growth. Photoproduction of Fe(II) appears to be central to this transfer. The consequence of this photochemical production of Fe(II) in the marine environment could be substantial. Photodissolution and precipitation of ferric hydroxides will counteract the maturation of iron colloids and maintain an iron pool capable of supporting phytoplankton growth.

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