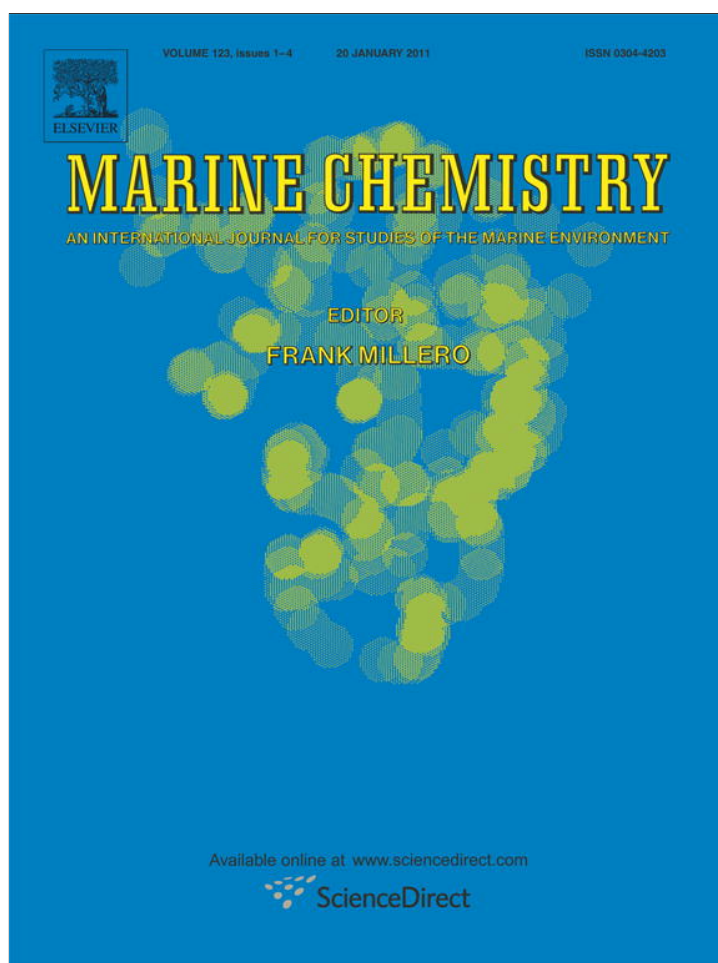


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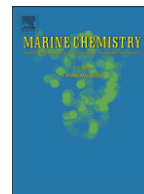
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Evidence for regulation of Fe(II) oxidation by organic complexing ligands in the Eastern Subarctic Pacific

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ABSTRACT

Redox cycling of iron in natural seawater is an important process that can affect iron availability to marine phytoplankton. In this work, luminol chemiluminescence was used to measure picomolar Fe(II) oxidation rate constants in continental shelf waters and oceanic surface (upper 200 m) waters of the iron-limited eastern subarctic Pacific. In both cases, Fe(II) oxidation rate constants were faster within the chlorophyll maximum than in UV oxidized seawater (UVOS), while rate constants were comparable to UVOS rate constants in waters from below the mixed layer. The larger Fe(II) oxidation rate constants in surface waters converged with UVOS rate constants upon stepwise additions of either Fe(II) or Fe(III), while Fe titrations did not affect Fe(II) oxidation rate constants in waters from below the mixed layer. Direct measurements of hydrogen peroxide, a common Fe(II) oxidant in surface waters, do not explain the high Fe(II) oxidation rate constants. We hypothesize that excess concentrations of strong Fe(III)-complexing organic ligands measured in surface seawater increase Fe(II) oxidation, and that titration of these ligands with added Fe explains the observed slowing of Fe(II) oxidation rates. Given that Fe(III)-complexing ligands are found in both surface and deep waters, and higher Fe(II) oxidation rate constants were measured only near the chlorophyll maximum, we suggest that the chemical nature and perhaps origin of natural Fe(III)-complexing organic ligands differ between in surface and deep waters.

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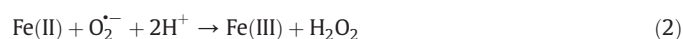
1. Introduction

Marine primary production by phytoplankton is an important factor regulating net carbon dioxide flux across the air-sea interface, and thus affects atmospheric CO₂ concentrations. It now is well established that phytoplankton production is limited by the trace element iron in up to 40% of the world's ocean, including the subarctic Pacific, equatorial Pacific, and Southern Ocean, even though total dissolved iron concentrations in these surface waters (~50–100 pM Fe) in principle are sufficient to support a diatom bloom (Wells, 2003; and references therein). The bulk of dissolved Fe appears then to be poorly available to at least diatoms and other large eukaryotic phytoplankton. We still lack fundamental insights into the control and dynamics of the chemical speciation of iron in seawater, and how these restrict or enhance iron availability to marine phytoplankton.

It is known that the marine chemistry of Fe, at equilibrium, is controlled by strong organic Fe(III) chelators (Gledhill and Van den Berg, 1994; Rue and Bruland, 1995; Wu and Luther, 1995) but there is a growing body of evidence that Fe speciation in surface seawater deviates from equilibrium conditions, with Fe(II) becoming quantitatively significant in oceanic surface waters (Hansard et al., 2009; Johnson et

al., 1994; Rose and Waite, 2003b; Roy et al., 2008). These dynamics are important because Fe uptake models for marine phytoplankton predict that Fe(II) is a biologically active species (Salmon et al., 2006; Shaked et al., 2005). However, our ability to explain Fe acquisition by marine eukaryotic phytoplankton is challenged by our lack of understanding of Fe redox dynamics in seawater.

Fe(II) is generated photochemically in surface waters either by direct photolysis of organic complexes and colloids (Barbeau et al., 2001; Wells et al., 1991), or indirectly through reduction by photo-produced superoxide (Rose et al., 2005a; Rose and Waite, 2005; Voelker and Sedlak, 1995). Based on the action spectra of these photochemical reactions, Fe(II) can be produced deep in the photic zone (Laglera and Van den Berg, 2007; Wells et al., 1991). In addition to photochemical production, Fe(II) is thought to be generated through biological processes, by released superoxide (Kustka et al., 2005; Rose et al., 2005b) cell surface reductases (Maldonado and Price, 2001; Wells et al., 2005), and thermal reduction of organically complexed Fe(III) (Hansard et al., 2009). Once formed, the ephemeral Fe(II) is reoxidized inorganically in oxic seawater by the four-step Haber-Weiss process:



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Superoxide and hydrogen peroxide can accumulate in the sample when Fe(II) is at high nanomolar and micromolar concentrations, producing a 4:1 product stoichiometry (Fe:O_x). But Fe(II) concentrations in most oceanic surface waters are too low to generate significant concentrations of O₂⁻, and hydrogen peroxide (H₂O₂) concentrations also are low (<200 nM), so dissolved oxygen (O₂) becomes the dominant oxidant for Fe(II) (Santana-Casiano et al., 2006).

Past studies have shown that solution composition has a profound effect on Fe(II) oxidation rate constants (King, 1998; Millero, 1989; Santana-Casiano et al., 2005). The overall Fe(II) oxidation rate in seawater is well described as the sum of the product of distribution coefficients (α_i) and second order rate constants for each Fe(II) species (k_i).

$$\frac{d\text{Fe(II)}}{dt} = -[\text{Fe(II)}][\text{O}_2] \sum_i \alpha_i k_i \quad (5)$$

Although the traditional assessment from these measurements is that Fe(II) is a transient species in seawater at pH 8.0 (King et al., 1995) this perception has been challenged recently, where oxidation kinetics have been slower than expected in some regions of the ocean (Croot et al., 2001; Croot and Laan, 2002; Hansard et al., 2009; Roy et al., 2008). Complexation by Fe(II) binding organic ligands has been proposed in some cases as an underlying mechanism for these lowered Fe(II) oxidation rates (Roy et al., 2008; Kieber et al., 2003).

While organic Fe(II) complexing ligands contribute to Fe redox dynamics in rainwater (Kieber et al., 2001, 2005; Willey et al., 2008) and freshwater (Emmenegger et al., 1998; Theis and Singer, 1974), their existence in seawater remains speculative. The potential effects of model organic ligands on Fe(II) oxidation rates have been demonstrated in well-defined laboratory conditions at high Fe(II) concentrations (Rose and Waite, 2003a; Santana-Casiano et al., 2000). But it is difficult to extrapolate these findings to natural waters where Fe(II) and ligand concentrations are orders of magnitude lower than tested in the laboratory.

Here we show evidence that Fe(II) oxidation rate constants in the eastern subarctic Pacific are markedly higher than rate constants measured in UV oxidized seawater near the chlorophyll maximum, and agree with model predictions below the surface mixed layer. The higher than expected Fe(II) oxidation rate constants converged with to rate constants measured in UV oxidized seawater with stepwise Fe additions. In this manuscript, we consider the possibility that these results are due to the influence of Fe(III) complexing ligands, which are found in ubiquitous excess in surface seawater.

2. Materials and methods

2.1. Sampling

Seawater samples were collected while underway during two cruises to the eastern subarctic Pacific Ocean on the R/V Thomas G. Thompson using a trace metal clean pumping and tow fish system described elsewhere (Roy et al., 2008). Samples for Fe(II) oxidation studies were collected at two stations: Ocean Station PAPA (50°N 145°W) on 13 Jun 2006 and 25 May 2007 (hereafter referred to as OSP 2006, OSP 2007) and from a continental shelf station north of Vancouver Island, British Columbia, Canada (51° 15.3' N 129° 01.7' W) on 14 May 2007 (hereafter referred to as Shelf 2007). On the 2006 cruise, all deep (>5 m) trace metal clean samples were collected by attaching the tow fish to a 150 m spool of 1 cm inner diameter acid-cleaned high density polyethylene (HDPE) tubing, lowering the fish to depth, and pumping the sample directly to a shipboard clean room. The tubing was allowed

to flush for 25 min at each depth and the time delay between depth and the deckboard instrument was ~1.5 min. On the 2007 cruise, deep trace metal samples were collected using a 5 L, Teflon-coated Go Flo bottle (General Oceanics) on a Kevlar line, tripped using a Teflon messenger. Once back on deck, the Go Flo bottle was sampled under a high-efficiency particle air (HEPA) filter, inside the shipboard fabricated clean room. The time between closing the Go Flo bottle and sample introduction to the instrument was slightly longer (≤3 min.) for deeper samples using this approach. Samples for oxidation studies were filtered through a 0.2 μm polyethersulfone capsule (PCI Membrane Systems, Inc.) or 0.45 μm Teflon capsule filters (Sterlitech — Go Flo samples) at low pressure (<70 kPa) and collected in rigorously cleaned fluorinated polyethylene (FPE) bottles. Additional filtered and unfiltered samples were pulled from the Go Flo bottle for later Fe(II) oxidation studies, that probe the effect of sample filtration. Samples for H₂O₂ analysis were collected in black HDPE bottles from Niskin bottles on the sampling rosette.

2.1.1. Reagents

All solutions were prepared using >18 MΩ water from a Barnstead Nanopure Diamond Lab Water System. Chemicals were purchased immediately prior to each cruise and used as received; luminol (5-Amino-2,3-dihydro-1,4-phthalazinedione) (Fluka), ferrous ammonium sulfate hexahydrate, ferric iron atomic absorption standard, potassium carbonate, and sodium sulfite were purchased from Sigma. Catalase, concentrated hydrochloric acid (Optima), ammonia (Optima), and glacial acetic acid (Optima) were purchased from Fisher. Acridinium ester was a gift from D. Whitney King. The luminol reagent and carrier solutions for Fe analysis are described elsewhere (Roy et al., 2008) and were stored in acid-cleaned HDPE bottles. The reagents for H₂O₂ analysis by acridinium ester chemiluminescence were prepared according to Miller et al. (2005) and stored in black HDPE bottles.

2.2. Flow injection analysis for total dissolved Fe, Fe(II), and H₂O₂

An automated flow injection-based FeLume system (Waterville Analytical) was used for analysis of Total Dissolved Fe, Fe(II), and H₂O₂. The optimized analytical methods and steps taken to minimize contamination for Fe(II) are described elsewhere in detail (King et al., 1995; Roy et al., 2008), and for H₂O₂ (Miller et al., 2005). Total dissolved Fe was measured by reducing all Fe to Fe(II) in filtered seawater samples using sulfite prior to chemiluminescent detection (Lannuzel et al., 2006). We also found that the reproducibility of the reduction step was improved by first removing oxygen from the Total Fe samples by bubbling with nitrogen for 30 min prior to adding the reducing agent. For all reaction chemistries, a seawater sample mixes with a chemiluminescent reagent (luminol for Total Dissolved Fe and Fe(II), acridinium ester for H₂O₂) in a reaction spiral seated beneath a photomultiplier tube (PMT). The FIA system, reagents, and samples to be analyzed were kept in a separate HEPA-filtered air bench surrounded by a heavy black plastic curtain to minimize light interference with the analyses. Detection limits (3 standard deviations of Chelex-treated seawater blank solutions) for Fe(II) ranged between 3 and 11 pM, 8–22 pM for Total Dissolved Fe, and between 2 and 4 nM for H₂O₂.

2.3. Fe(II) oxidation experiments

The FeLume system was adapted to determine apparent Fe(II) oxidation rate constants by bypassing the injection valve, thereby allowing a continuous stream of sample and luminol through the plexiglas reaction coil. Plumbed in this way, the system enabled uninterrupted measurement of Fe(II) over time. All samples (both filtered and unfiltered) used in the Fe(II) oxidation studies were stored at room temperature in the dark for 24 h to allow for complete decay of any Fe(II), O₂⁻, and other radicals present at the time of sample

collection (Hansard et al., 2010; Rose et al., 2010). After dark aging, samples were brought to 25.0 °C by immersing the bottle in a temperature controlled bath (Neslab) while still in the dark and saturated with O₂ ([O₂] = 200–210 μM) by bubbling with air that had been passed through a potassium permanganate solution to minimize the addition of H₂O₂ from laboratory air. The pH of the sample then was measured on the free ion scale (Millero, 1986) with an Accumet AP62 electrode and adjusted to 8.00 at 25.0 °C using dilute ammonia or HCl. Samples were spiked with Fe(II) to an initial concentration of 100 pM and then pumped directly into the reaction spiral while the PMT signal was recorded continuously. The decay of the Fe(II) signal was measured at 1 Hz for 10 min, in triplicate for each sample. Output from a typical Fe(II) oxidation experiment is shown in Fig. 1A. Napierian log transformation of the chemiluminescence signal yielded linear fits to the first 1.5 half lives of the decay curve (Fig. 1B), indicating pseudo-first-order Fe(II) oxidation with rate constant k_{ox} . For consistency, data beyond 1.5 half lives were not used for estimating the rate constants because the signal to noise ratio became unsatisfactory in some samples. The flow cell was rinsed with 0.01 M HCl between replicates to remove any mineral precipitates that can form at the high pH in the reaction spiral. The measured Fe(II) oxidation rate constants were compared to rate constants measured in UVOS (irradiated 48 h) collected from ocean station PAPA in spring of 2006.

Additional experiments were conducted to assess whether Fe(II) oxidation rate constants changed as a function of Fe added to the sample. Small (~200 pM) stepwise Fe(II) additions on were done to samples collected from OSP and the shelf station during the May–June 2007 cruise and their sequential oxidation rate constants measured. Water for these experiments was collected from three depths at each station: surface (5 m), the chlorophyll maximum, and below the thermocline (180 m). All samples used in the stepwise Fe(II) addition experiments were filtered prior to 24 h dark aging, and received the same

temperature, pH adjustment, and bubbling treatment as described above. Each sample received 7 sequential Fe(II) spikes, allowing 75 min between spike additions for complete oxidation of Fe(II), resulting in a cumulative added iron concentration of 1.3 nM. To evaluate whether the observed changes in Fe(II) oxidation rate constants with increasing Fe(II) additions were related to the inorganic Fe(III) generated, stepwise Fe(II) additions were interspersed with equivalent spikes of Fe(III) in a separate experiment (i.e., Fe(II) spike, Fe(III) spike, Fe(II) spike, etc.).

3. Results

3.1. Fe(II) concentrations in surface seawater

Fe(II) concentrations in surface and subsurface waters were determined in samples collected at mid-day from a number of stations in the eastern subarctic Pacific and coastal shelf waters (Table 1), but Fe(II) was not detectable at any location (Table 1).

3.2. Vertical profiles of H₂O₂

Open ocean and coastal H₂O₂ concentrations were measured to a depth of 150 m at the following stations: OSP 2006, OSP 2007, and Shelf 2007. H₂O₂ concentrations were highest (~40 nM for OSP 2006, OSP 2007, ~110 nM at Shelf 2007) in the surface mixed layer, and decreased to less than 10 nM below the mixed layer. The summary of H₂O₂ measurements during these cruises and for UVOS experiments is shown in Table 1.

3.3. Fe(II) oxidation rate constants as a function of depth in amended samples

Fe(II) oxidation rate constants (Fe(II)₀ = 100 pM pH 8.00, 25 °C, O₂ = saturated) measured in filtered samples varied as a function of depth at OSP 2006, and OSP 2007 (Fig. 2A, B). The surface (5 m) Fe(II) oxidation rate constant for filtered samples collected at OSP

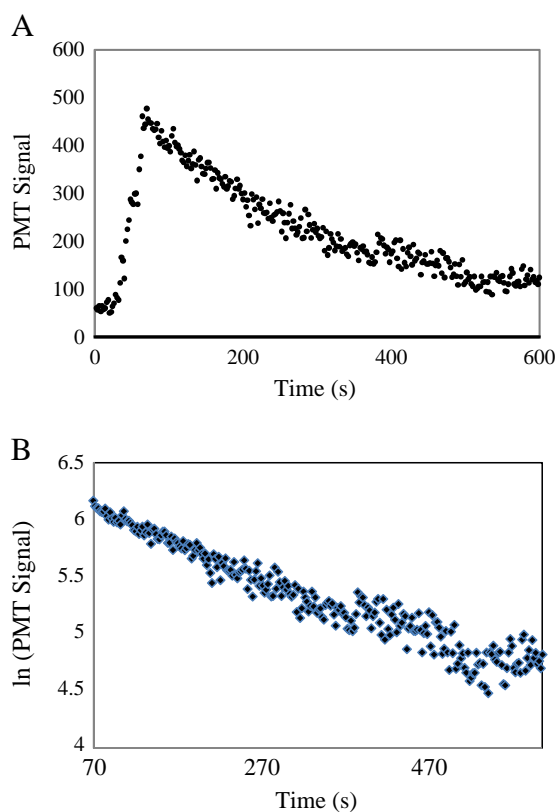


Fig. 1. Example of (A) PMT output during an Fe(II) oxidation rate experiment (B) Napierian log transformation of the decay data shown in Fig. 1A. For clarity, every 5th data point is shown.

Table 1
Concentrations of Fe(II), Total Dissolved Fe, and H₂O₂ from this study.

Date	Station	Depth (m)	[Fe(II)] (pM)	[Total Fe] (pM)	+/- (1 SD)	[H ₂ O ₂] (nM)	+/- (1 SD)
13 Jun 2006	OSP 2006	5	*	58	17	43	2
		20	*	71	12	41	3
		30	*	76	14	35	4
		45	*	88	13	37	3
		60	*	104	12	38	4
		80	*	99	21	14	3
		100	*	127	9	4	2
		120	*	111	14	3	3
		150	*	124	17	****	4
		180	*	204	22	****	2
25 May 2007	OSP 2007	5	**	78	8	52	2
		15	**	63	9	47	3
		20	**	76	10	44	2
		30	**	80	11	46	4
		45	**	69	20	43	2
		60	**	91	14	35	2
		75	**	87	12	16	3
		100	**	102	20	5	4
		150	**	109	12	6	1
180	**	210	30	*****	2		
14 May 2007	Shelf 2007	5	***	1900	240	109	6
		10	***	1300	90	114	7
		180	***	3200	410	6	7
23 April 2007	UVOS	7	*	84	8	17	4

* Below detection limit (3 pM).

** Below detection limit (11 pM).

*** Below detection limit (8 pM).

**** Below detection limit (2 nM).

***** Below detection limit (3 nM).

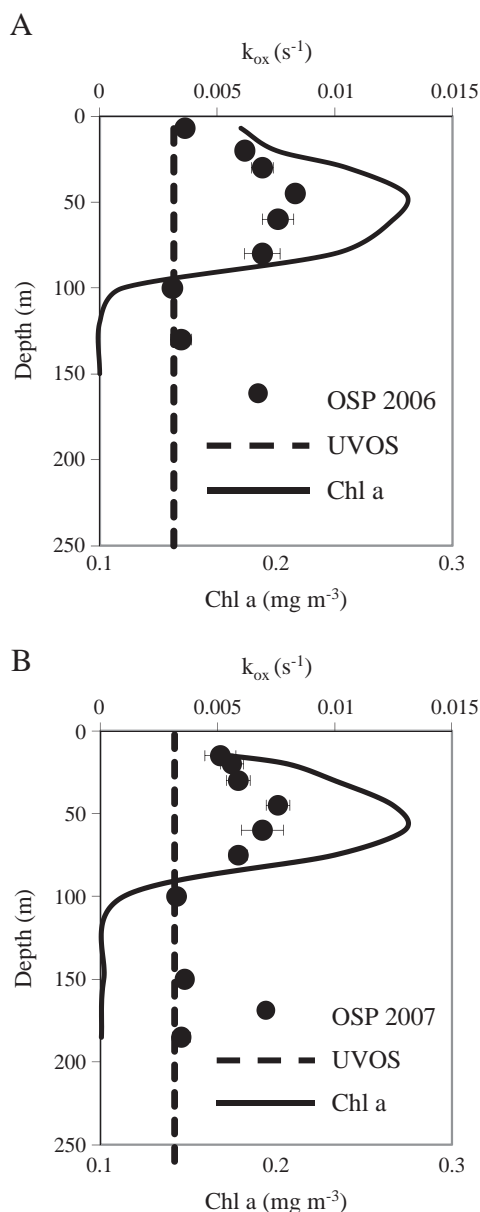


Fig. 2. Pseudo-first-order Fe(II) oxidation rate constants (25 °C, pH 8.0) as a function of depth for Ocean Station Papa (50 °N, 145 °W) in (A) 2006 and (B) 2007. Data points (closed circles) represent the average of triplicate measurements, ± 1 standard deviation. The dashed line indicates the Fe(II) oxidation rate constant in UV oxidized seawater (UVOS) (Roy et al., 2008). The solid trace shows in vivo chlorophyll *a* concentrations collected on the morning of each sampling day.

2006 was $0.00361 (\pm 0.0002) s^{-1}$, rose to a maximum of $0.00832 (\pm 0.0002) s^{-1}$ at 45 m, and decreased to $0.00309 (\pm 0.0001) s^{-1}$ at 100 m and $0.00346 (\pm 0.0005) s^{-1}$ at 130 m. For OSP 2007, surface (5 m) Fe(II) oxidation rate constants were $0.00529 s^{-1} (\pm 0.0006) s^{-1}$, increased to a maximum of $0.00786 (\pm 0.0005) s^{-1}$ by 45 m, and decreased to $0.00326 (\pm 0.0002) s^{-1}$ at 100 m. In general, the Fe(II) oxidation rate constants measured in filtered samples were inversely proportional with chlorophyll biomass in both years, shown here as in vivo fluorescence in Fig. 2A, B. Fe(II) oxidation rate constants below the chlorophyll maximum in both years were in good agreement with UVOS rate constants reported by Roy et al. (2008). A complete list of all measured Fe(II) oxidation rate constants are shown in Table 2.

Fe(II) oxidation rate constants ($Fe(II)_0 = 100 \text{ pM}$ pH 8.00, 25 °C, $O_2 = \text{saturated}$) measured in unfiltered samples followed the same

Table 2
Measured Fe(II) oxidation rate constants (25 °C, pH 8.0) from Ocean Station Papa as a function of depth.

Station	Depth (m)	Filtered Samples		Unfiltered Samples	
		$\log k_{ox}$ (s^{-1})	\pm (1 SD)	$\log k_{ox}$ (s^{-1})	\pm (1 SD)
OSP 2006	5	-2.44	0.02	-2.42	0.03
	20	-2.21	0.02	-2.24	0.05
	30	-2.16	0.03	-2.14	0.03
	45	-2.08	0.01	-2.06	0.05
	60	-2.12	0.04	-2.13	0.03
	80	-2.16	0.05	-2.17	0.02
	100	-2.51	0.02	-2.43	0.10
	130	-2.46	0.06	-2.45	0.07
OSP 2007	5	-2.32	0.06	-2.30	0.06
	15	-2.29	0.06	-2.24	0.05
	20	-2.25	0.04	-2.23	0.08
	30	-2.23	0.04	-2.20	0.05
	45	-2.12	0.03	-2.10	0.09
	60	-2.16	0.06	-2.21	0.07
	75	-2.23	0.03	-2.19	0.10
	100	-2.49	0.02	-2.55	0.16
	150	-2.50	0.02	-2.51	0.03
	185	-2.46	0.05	-2.44	0.05
	250	-2.52	0.01	-2.44	0.09

general pattern as observed in filtered samples. The surface (5 m) Fe(II) oxidation rate constant for unfiltered samples collected at OSP 2006 was $0.0038 (\pm 0.0002) s^{-1}$, increased to a maximum of $0.00871 (\pm 0.0009) s^{-1}$ at 45 m, and decreased to $0.00372 (\pm 0.0008) s^{-1}$ at 100 m and $0.00302 (\pm 0.0005) s^{-1}$ at 130 m. For unfiltered samples collected at OSP 2007, surface (5 m) Fe(II) oxidation rate constants were $0.00501 s^{-1} (\pm 0.0006) s^{-1}$, increased to a maximum of $0.00743 (\pm 0.0015) s^{-1}$ by 45 m, and decreased to $0.002818 (\pm 0.0009) s^{-1}$ at 100 m. As was the case for filtered samples, measured Fe(II) oxidation rate constants in unfiltered samples were inversely proportional with chlorophyll biomass in both years (chlorophyll data in Fig. 2A, B). Fe(II) oxidation rate constants below the chlorophyll maximum in both years were in good agreement with UVOS rates reported by Roy et al. (2008). A complete list of all measured Fe(II) oxidation rate constants in unfiltered samples are listed in Table 2.

3.4. Fe(II) oxidation rate constants as a function of added Fe

To investigate the nature of the larger-than-anticipated Fe(II) oxidation rate constants measured in the euphotic zone, stepped spike additions of Fe(II) were added to samples from three depths at OSP 2007 (Fig. 3) and Shelf 2007 (Fig. 4): surface (5 m), the chlorophyll maximum, and deep waters (180 m). In the first series of experiments Fe(II) was added incrementally, re-measuring the oxidation rate constants each time, to determine if the rate constants converged with those measured in UVOS. In both years, oxidation rate constants were significantly higher in surface (5 m) waters [$0.00478 (\pm 0.0006) s^{-1}$ for OSP 2007; $0.00724 (\pm 0.0008) s^{-1}$ for Shelf 2007] than rate constants measured in UVOS ($0.00316 s^{-1}$), in reasonable agreement with those measured in the vertical profiles (Figs. 3A, B; 4A, B). Oxidation rate constants decreased progressively with each spike addition of Fe(II) to agreement with UVOS rate constants after a total of 0.6–0.8 nM Fe(II) had been added. Results were similar for the chlorophyll maximum depth (Figs. 3B and 4B), where the initial Fe(II) oxidation rate constants [$0.0076 (\pm 0.0005) s^{-1}$ for OSP 2007; $0.0079 (\pm 0.0007) s^{-1}$ for Shelf 2007], slowed to converge with UVOS rate constants after ~0.8 nM Fe had been added to the sample. When these experiments were repeated in UVOS, the Fe(II) oxidation rate constant pattern observed

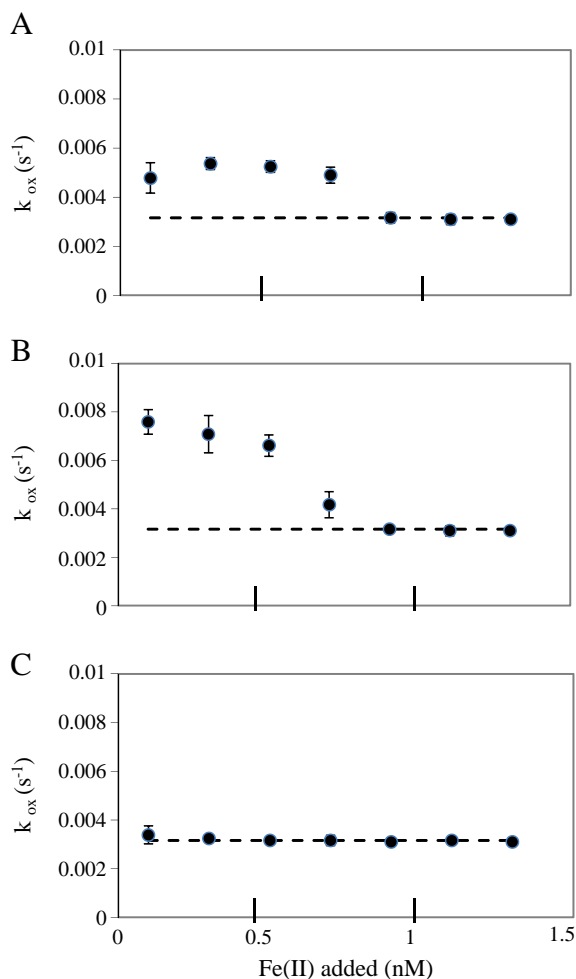


Fig. 3. Pseudo-first-order Fe(II) oxidation rate constants (25 °C, pH 8.0) as a function of the total amount of Fe(II) added for OSP 2007 at (A) 5 m (B) chlorophyll maximum and (C) below the surface mixed layer (180 m). Data points (closed circles) represent the average of three replicate measurements \pm 1 standard deviation. The dashed line indicates the Fe(II) oxidation rate constant measured in UV oxidized seawater (UVOS) (Roy et al., 2008).

at the surface and at the chlorophyll was not observed (rate constants ranged from 0.00372 s^{-1} to 0.00295 s^{-1}) as the total amount of Fe (II) increased (Table 3).

To determine whether the decrease in Fe(II) oxidation rate constants were related to the increasing concentration of Fe(III) in the sample, the experiment was repeated but this time the Fe(II) spikes were alternated with equivalent Fe(III) additions. The results yielded the same pattern of decreasing Fe(II) oxidation rate constants with Fe addition; i.e., Fe(II) oxidation rate constants changed in synchrony with the total amount of Fe added (Table 3). In other words, the changes in Fe(II) oxidation rate constants in waters from the euphotic zone were dependent on the amount of Fe(III) in the sample and not the amount of Fe(II) added. In contrast, there were no changes observed in Fe(II) oxidation rate constant with these stepwise alternating Fe(II)/Fe(III) additions in deeper waters (180 m) (Figs. 3C, 4C; Table 3). Fe(II) oxidation rate constants in waters below the euphotic zone were uniform in both offshore and shelf waters, and were in good agreement with Fe(II) oxidation rate constants measured in UVOS (Roy et al., 2008). When these experiments were repeated in UVOS, the results were similar to those measured with increasing Fe (II) concentrations; no pattern was observed and rate constants ranged from 0.00295 s^{-1} to 0.00347 s^{-1} .

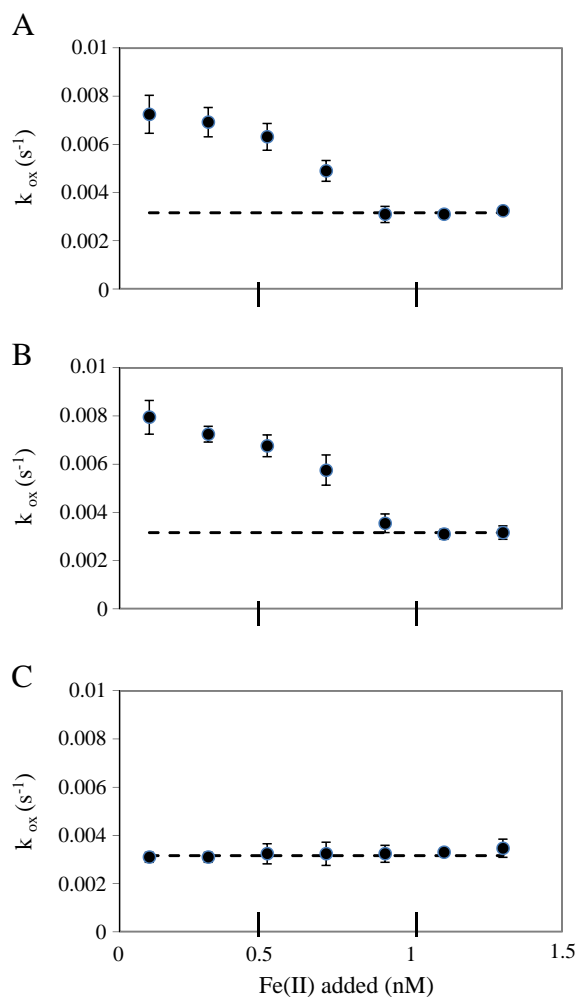


Fig. 4. Pseudo-first-order Fe(II) oxidation rate constants (25 °C, pH 8.0) as a function of the total amount of Fe(II) added for Shelf 2007 at (A) 5 m (B) chlorophyll maximum and (C) below the surface mixed layer (180 m). Data points (closed circles) represent the average of three replicate measurements \pm 1 standard deviation. The dashed line indicates the Fe(II) oxidation rate constant measured in UV oxidized seawater (UVOS) (Roy et al., 2008).

4. Discussion

Advancements in trace metal analytical techniques enabling measurements of picomolar concentrations of Fe(II) in seawater have provided oceanographers new insights to the natural chemical speciation of Fe in seawater. Fe(II) can comprise a substantial fraction of the total dissolved Fe during daylight hours in surface waters (e.g. Croot et al., 2008; Hansard et al., 2009; Roy et al., 2008), due in large part to slowed oxidation kinetics of photoproducted Fe(II) (Croot et al., 2008; Roy et al., 2008). In contrast from the WSAP, no Fe(II) was detected here in surface waters at OSP during either 2006 or 2007. A marked difference from rate constants measured in the WSAP, Fe(II) oxidation rate constants in ESAP surface waters were larger than constants measured in UVOS (Fig. 2A, B), although rate constants converged with those in UVOS below the euphotic zone in both studies. Fe(II) oxidation rate constants were fastest at the chlorophyll maximum at OSP in both years indicating that some factor associated with phytoplankton production was enhancing Fe (II) oxidation. Given that identical sampling and analytical procedures were used in both studies (Roy et al. 2008) these opposing results show real differences in iron oxidation dynamics between these Fe-limited regimes.

Table 3
Fe(II) oxidation rate constants as a function of total added Fe(II) and Fe(III) (25 °C, pH 8.0).

Station	Depth (m)	All Fe(II) additions			Alternating Fe(III) additions	
		Total Fe added (nM)	log k_{ox} (s^{-1})	+/- (1 SD)	log k_{ox} (s^{-1})	+/- (1 SD)
OSP2007	5	0.1	-2.32	0.06	-2.30	0.03
		0.3	-2.27	0.02		
		0.5	-2.28	0.02	-2.26	0.04
		0.7	-2.31	0.03		
		0.9	-2.50	0.03	-2.48	0.03
		1.1	-2.51	0.01		
		1.3	-2.51	0.01	-2.54	0.06
OSP2007	45*	0.1	-2.12	0.03	-2.13	0.07
		0.3	-2.15	0.05		
		0.5	-2.18	0.02	-2.15	0.02
		0.7	-2.38	0.06		
		0.9	-2.50	0.02	-2.47	0.05
		1.1	-2.51	0.03		
		1.3	-2.50	0.01	-2.53	0.04
OSP 2007	180	0.1	-2.47	0.05	-2.44	0.08
		0.3	-2.49	0.02		
		0.5	-2.50	0.02	-2.55	0.04
		0.7	-2.50	0.03		
		0.9	-2.51	0.01	-2.48	0.07
		1.1	-2.50	0.01		
		1.3	-2.51	0.01	-2.44	0.06
Shelf 2007	5	0.1	-2.14	0.05	-2.11	0.09
		0.3	-2.16	0.04		
		0.5	-2.20	0.04	-2.14	0.08
		0.7	-2.31	0.05		
		0.9	-2.51	0.02	-2.51	0.04
		1.1	-2.51	0.02		
		1.3	-2.49	0.03	-2.40	0.09
Shelf 2007	10*	0.1	-2.10	0.04	-2.04	0.08
		0.3	-2.14	0.02		
		0.5	-2.17	0.03	-2.09	0.10
		0.7	-2.24	0.05		
		0.9	-2.45	0.05	-2.37	0.07
		1.1	-2.51	0.03		
		1.3	-2.50	0.04	-2.62	0.11
Shelf 2007	180	0.1	-2.51	0.03	-2.47	0.08
		0.3	-2.49	0.04		
		0.5	-2.51	0.01	-2.44	0.06
		0.7	-2.49	0.03		
		0.9	-2.50	0.03	-2.40	0.12
		1.1	-2.48	0.02		
		1.3	-2.46	0.05	-2.44	0.11
UVOS		0.1	-2.51	0.04	-2.47	0.09
		0.3	-2.46	0.11		
		0.5	-2.50	0.08	-2.46	0.12
		0.7	-2.43	0.20		
		0.9	-2.53	0.07	-2.52	0.10
		1.1	-2.48	0.05		
		1.3	-2.50	0.08	-2.53	0.04

* Indicates approximate depth of chlorophyll maximum.

Overall Fe(II) oxidation rates are influenced by several factors in seawater. Small differences in pH will sharply affect Fe(II) oxidation rates but in this case all samples were adjusted to pH 8.00 +/- 0.01 prior to measurement. Biologically mediated oxidation also can occur at cell surfaces (Salmon et al., 2006; Shaked et al., 2005). But this effect was not significant in our study because sample filtration had no effect on the observed patterns of Fe(II) oxidation rate constants (Table 2), so dissolved factors were responsible for increasing Fe(II) oxidation rate constants. Dissolved oxygen is a major oxidant of Fe(II) in surface waters, but oxygen was in gross excess of dissolved Fe(II) in these experiments and small vertical changes in concentrations O₂ had no relationship to measured Fe(II) oxidation rate constants. Hydrogen peroxide is also known to oxidize Fe(II), but if H₂O₂ were responsible for increasing Fe(II) oxidation rate constants in this work, oxidation rate constants would not change drastically

with sequential subnanomolar Fe additions (Figs. 3A, B and 4A, B). The results of these stepwise Fe addition experiments suggest that the substance responsible for increasing measured Fe(II) oxidation rate constants is consumed or deactivated after roughly 0.5–1 nM of additional Fe is added to the system. Production of reactive oxygen species via Eqs. (2)–(4) would be negligible at the very low (100 pM) Fe(II) concentrations used, and the influence from photo-produced and biological O₂⁻ production before filtration and storage (Kustka et al., 2005; Rose et al., 2005b, 2008) was negligible, given that measured oxidation rate constants were unchanged when cells were removed through filtration. Some unidentified inorganic oxidant might have been responsible for increasing Fe(II) oxidation but if so it would have to be unreactive enough to persist through 24 h of dark aging only to react quickly with spike additions of Fe(II). It also would have to be destroyed or inactivated by UV light, because Fe(II) oxidation rate constants decreased after UV irradiation, and it must occur only in surface waters associated with the chlorophyll maximum. Barring such a persistent inorganic oxidant species, the findings here are better explained by the presence of Fe(II) reactive organic compounds.

How feasible is it that organic constituents might reduce Fe(II) oxidation rate constants in some regions (Roy et al. 2008), but increase Fe(II) oxidation rate constants in other regions? In the fundamental framework of Eq. (5), addition of an Fe(II)-organic complexing species would decrease α_i for inorganic Fe(II), causing overall Fe(II) oxidation rates to decrease if the complex is less reactive to oxidation, or increase if the complex acts to facilitate Fe(II) oxidation. We proposed that picomolar Fe(II) oxidation rates in surface seawaters of the WSAP are regulated by naturally occurring organic ligands (Roy et al., 2008), and the findings here suggest that the k_{ox} of these Fe(II)L complexes can vary spatially in the subarctic Pacific ocean.

We can examine the likelihood that Fe(II)L organic complexes regulate Fe(II) oxidation by using the free energy (ΔG°) of Fe(II) oxidation to estimate the relative conditional stability constant of the purported Fe(II) organic complexes. Marcus Theory has been used to describe the oxidation of Fe(II) complexed by inorganic ligands (King and Farlow, 2000) and model organic ligands (Rose and Waite, 2003a) in aquatic systems. In a derivation of the Marcus equation (Eq. 6), the second order rate constants (k_{ox}) can be estimated if the free energy (ΔG°) for the one electron transfer is known (Tratnyek and Hoigne, 1994), which this case is the reaction shown in Eq. (1).

$$k_{ox} = \frac{k_d}{1 + \frac{k_{dZ}}{K_{dZ}} + \exp\left[\frac{\lambda}{4} \left(1 + \frac{\Delta G^\circ}{\lambda}\right)^2 / (RT)\right]} \quad (6)$$

where k_d is the diffusion-controlled limit ($10^{10} \text{ mol}^{-1} \text{ L}$), $\frac{k_{dZ}}{K_{dZ}} = 0.1$ (King and Farlow, 2000), $\lambda = 103 \text{ kJ mol}^{-1}$ (King and Farlow, 2000) as a fitting parameter related to the energy required to reorganize the transition state, R is the universal gas constant, and T is temperature in Kelvin. Eq. (6) provides a convenient means of estimating ΔG° for the Fe(II) oxidation rate constants measured in our field samples.

The initial k_{ox} in the chlorophyll maximum at OSP (2007) was $35.5 \text{ mol}^{-1} \text{ L s}^{-1}$, which was calculated by dividing the measured pseudo-first-order rate constant by the oxygen concentration, which corresponds to a $\Delta G^\circ \sim 46 \text{ kJ mol}^{-1}$ (Eq. 6). The free energy change (ΔG°) for the oxidation of a FeL complex by oxygen is defined as:

$$\Delta G^\circ = -F(E_{O_2 \rightarrow O_2^-}^\circ - E_{FeL}^\circ) \quad (7)$$

where F is the Faraday constant, $E_{O_2 \rightarrow O_2^-}^\circ = -0.16 \text{ V}$, and E_{FeL}° is the half-reaction potential for reduction of the FeL complex (Eq. 8).



From Eq. (7) we find that E_{FeL}° is 0.32 V for ambient Fe(II) oxidation of the 100 pM Fe(II) added at the chlorophyll maximum of OSP 2007. Using E_{FeL}° in the Nernst equation provides an estimate of the equilibrium constant ratio ($K_{Fe(III)L}/K_{Fe(II)L}$) for the purported Fe(II)L and Fe(III)L complexes:

$$E_{FeL}^{\circ} = E_{Fe}^{\circ} - 0.059 \log \left(\frac{K_{Fe(III)L}}{K_{Fe(II)L}} \right) \quad (9)$$

Using a standard half-reaction potential of $E_{Fe}^{\circ} = 0.77$ V and the estimated $E_{FeL}^{\circ} = 0.32$ V yields a $K_{Fe(III)L}/K_{Fe(II)L}$ ratio of $\sim 10^7$ to 10^8 ; the difference in relative Fe(III)/Fe(II) affinities of a number of known siderophores (Boukhalfa and Crumbliss, 2002). Siderophore production by marine microbes is well-established (e.g. Martin et al., 2006; Martinez et al., 2003; Reid et al., 1993) and characteristic siderophore molecular functionalities have been measured in seawater (Macrellis et al., 2001). Moreover, direct measurement of hydroxamate siderophores in surface waters confirms the existence of intact molecules (Mawji et al., 2008). It appears feasible then that enhanced release of siderophores (or siderophore-like Fe(III) complexing ligands) within the chlorophyll maximum might be responsible for the increasing the measured Fe(II) oxidation rate constants at OSP.

Although intriguing, this simplistic assessment is based on several assumptions. For example, it depends on all Fe(II) being bound by an excess of organic ligands during the oxidation experiments, which may be reasonable given that Fe^{2+} is the dominant inorganic Fe(II) species (Millero et al., 1995) and thus readily available to form complexes. But if complexation depends upon metal exchange the kinetics can be slow in some cases, particularly for multidentate ligands (Hering and Morel, 1989). The model also assumes only a single ligand (within the strong ligand class) is responsible, but measured oxidation rate constants decreased slightly during the first few stepwise Fe(II) additions (Fig. 3A, B and Fig. 4A, B), indicating either that the ligand may not have been in gross excess or the distribution coefficient for the Fe(II)L complexes changed slightly with each Fe addition. We also assume that the affinity ratio for Fe(III)/Fe(II) for thermodynamic constants $K_{Fe(II)L}$ and $K_{Fe(III)L}$ hold for the corresponding conditional constants for seawater, which awaits testing.

Barring any catalytic mechanism, the higher than expected Fe(II) oxidation rate constants decreased with progressive Fe(II) additions, ultimately approaching the constants measured in UVOS. This effect was observed in both surface and chlorophyll maximum waters at OSP 2007 and Shelf 2007, with rate constants converging with UVOS rate constants with the stepwise additions of ~ 0.8 nM Fe(II) (Figs. 3 and 4). Although this does not rule out the effect of some unexpected inorganic oxidant, the concentrations are within an order of magnitude of voltammetrically determined organic ligand concentrations in many offshore surface waters (e.g. Buck and Bruland, 2007; Rue and Bruland, 1995; Sato et al., 2007). It is important to note that these free ligand measurements are made after several hours of equilibration time, instead of the short time scale (< 1 h) used here, severely limiting the ability for direct quantitative comparisons. But even so, it is a striking qualitative agreement between the transition points for Fe(II) oxidation rate constants (Figs. 3A, B, 4A, B) and the anticipated free Fe(III) complexing ligand concentrations.

In contrast, Fe(II) oxidation rate constants measured in deep (180 m) samples agreed closely with UVOS rate constants and were independent of Fe addition (Fig. 3C, 4C). Although excess concentrations of Fe(III) complexing ligands are reported at all depths in the water column (Gerringa et al., 2006; Rue and Bruland, 1997; Witter et al., 2000), the stronger ligand class believed to represent siderophores is present mainly in surface waters (e.g., Rue and Bruland, 1995; Cullen et al. 2006), while weaker ligands of perhaps humic-type composition predominant below the euphotic zone (Laglera and van den Berg, 2009). The difference measured here in Fe(II)

oxidation rate constants between surface and deep waters provide additional evidence for dissimilar compositions of these organic ligand pools.

If Fe(II) complexing ligands indeed can regulate overall Fe(II) oxidation rates, why then might this effect differ between studies in the WSAP and ESAP. The sluggish Fe(II) oxidation rate constants measured in sunlit WSAP surface waters are provisionally explained by the presence of an Fe(II) complexing organic ligand class having a conditional stability constant of $\sim 10^8$ – 10^9 with respect to Fe(II)' (Roy et al., 2008). A $K_{Fe(III)L}/K_{Fe(II)L}$ of $\sim 10^7$ to 10^8 at OSP with a conditional constant for Fe(III)L of $\sim 10^{12}$ – $10^{13} M^{-1}$ with respect to Fe(III)' (Buck and Bruland, 2007; Gledhill and Van den Berg, 1994; Rue and Bruland, 1995), yields a $K_{Fe(II)L}$ of only $\sim 10^5$ – $10^6 M^{-1}$, or 3–4 orders of magnitude weaker than that estimated in the WSAP (Roy et al., 2008). This difference might be related to the relative degree of Fe stress of each region, or perhaps the seasonal timing of the respective studies. ESAP surface waters suffer greater Fe limitation than the WSAP (Harrison et al., 1999), presumably due to lower aeolian Fe inputs (Moore and Braucher, 2008), and so it may be expected that there may be a greater release of siderophores, with their 'hard', oxygen containing hydroxamate and catecholate functional groups (Boukhalfa and Crumbliss, 2002; Martel and Smith, 1982) that have a preferential affinity for Fe(III) than Fe(II) and would be expected to increase overall Fe(II) oxidation rates. Alternatively, the WSAP study was conducted during summer when mesozooplankton grazing was substantial (Tsuda et al., 2007), with an enhanced release of intracellular Fe complexing ligands (Sato et al., 2007). This phytoplankton debris would have contained many relatively 'soft' ligands with π -donor coordination sites that favor Fe(II) over Fe(III) and potentially slow Fe(II) oxidation kinetics. In any case, the findings here show heterogeneous ligand characteristics within otherwise "uniform" oceanographic HNLC regimes.

In summary, our findings show that kinetics of Fe redox cycling in surface waters are more complicated than previously realized. In contrast to recent work showing that Fe(II) accumulates in surface waters of the WSAP because of slow oxidation kinetics (Roy et al., 2008), there was no Fe(II) accumulation in surface waters of the eastern subarctic Pacific, and measured Fe(II) oxidation rate constants were markedly higher. These differences did not exist below the euphotic zone, where Fe(II) oxidation rate constants were consistent with laboratory studies at higher Fe(II) concentrations and in UVOS at picomolar Fe(II) additions. The disparity in Fe(II) oxidation rate constants appears to result from naturally occurring organic ligands, and the relationship between Fe(II) oxidation enhancement and phytoplankton biomass indicate that these ligands have specific biological origins. Measurements of Fe(II) oxidation kinetics may offer a new window into the nature of Fe complexing ligands in seawater, and the ecological processes that regulate their abundance.

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