Marine Chemistry 128–129 (2012) 1–12

Contents lists available at SciVerse ScienceDirect

Marine Chemistry

journal homepage: www.elsevier.com/locate/marchem

Quasi-Lagrangian drifter studies of iron speciation and cycling off Point Conception, California

Andrew L. King *, Kristen N. Buck [1], Katherine A. Barbeau

Geosciences Research Division, Scripps Institution of Oceanography, University of California San Diego, 9500 Gilman Dr., La Jolla, CA 92093-0218, USA

ARTICLE INFO

Article history:
Received 12 May 2011
Received in revised form 3 November 2011
Accepted 7 November 2011
Available online 23 November 2011

Keywords:
Phytoplankton
Iron
Trace metals
Oceanography
Biogeochemistry
Chemical speciation
Ligands

ABSTRACT

The distribution and speciation of dissolved Fe (dFe) were measured during four quasi-Lagrangian drogued drifter studies (~4 d duration each) that were conducted in the southern California Current System in May 2006 and April 2007. Three of the four drifter studies were within the coastal upwelling regime and one drifter study was in a warm-core anticyclonic eddy. Incubation bottle experiments were also conducted to determine the degree of phytoplankton Fe limitation and to assess changes in the concentration of Fe-binding ligands. In the coastal upwelling drifter studies, *in situ* dFe (1.4–1.8 nM) and macronutrients were initially high and declined over time. Fe addition incubation experiments indicated that the phytoplankton community was not Fe limited at the beginning of the coastal upwelling drifter experiments (when μM nitrate:nM dFe ratios were ~7–8). By the end of two of the three drifter studies (when μM nitrate:nM dFe ratios were ~12–19), Fe addition resulted in larger nitrate and silicic acid drawdown, and larger accumulations in chlorophyll a, particulate organic carbon and nitrogen, and diatom and dinoflagellate-specific carotenoid pigments. Fe speciation was measured *in situ* in three of the four drifter studies with stronger L_1-type ligands found to be present in excess of dFe in all samples. In Fe speciation incubation experiments, L_1-type ligand production was observed in conjunction with phytoplankton growth under Fe-limiting conditions. The results presented here support and add a quasi-Lagrangian perspective to previous observations of dFe and macronutrient cycling over space and time within the California coastal upwelling regime, including Fe limitation within the phytoplankton community in this region and the biological production of Fe-binding ligands concomitant with Fe limitation.

1. Introduction

The supply of nutrients to the euphotic zone via coastal upwelling supports highly productive phytoplankton blooms in eastern boundary current upwelling regimes (Chavez and Barber, 1987; Toggweiler and Carson, 1995). Various field studies over the last decade have highlighted the importance of the micronutrient Fe, in addition to the macronutrients nitrate, phosphate, and silicic acid, for phytoplankton growth and community structure in eastern boundary current upwelling regimes, such as the California Current System (CCS) (Hutchins et al., 1998; Bruland et al., 2001; Johnson et al., 2001; Fitzwater et al., 2003) and off the coast of Peru (Bruland et al., 2005) and Mauritania (Schlosser and Croot, 2009). In coastal waters off central and northern California, Fe has been shown to control phytoplankton growth during high nutrient, low chlorophyll (HNLC) conditions (Hutchins et al., 1998; Firme et al., 2003), similar to Fe limitation observations in open ocean HNLC regimes (Martin and Fitzwater, 1988; de Baar et al., 2005; Boyd et al., 2007). In the southern CCS region, Fe limitation has been observed both in the coastal upwelling nearshore (Firme et al., 2003) and offshore in the wind stress curl-driven upwelling region (King and Barbeau, 2007).

Coastal upwelling systems like the CCS are inherently heterogeneous, especially during times of active upwelling, making them difficult to study due to large spatial and temporal variability in physical and biogeochemical features. Traditional fixed-station observations and experiments in coastal upwelling systems can only provide pseudo-synoptic data that do not afford observations pertaining to the biogeochemical "evolution" of an upwelling water mass. The use of drogued drifters or conservative tracers, such as SF_6, offers quasi-Lagrangian perspectives and more coherent spatio-temporal observations of biogeochemically-relevant processes when compared to fixed-station studies (Dugdale and Wilkerson, 1989; Alvarez-Salgado et al., 2001; Boyd et al., 2005). While drogued drifter studies are considerably more time-consuming to conduct, they can yield a valuable understanding of upwelling processes, such as the dynamic relationship between phytoplankton growth and nutrient availability over the timescale of days. Fixed-station data over a similar distance

* Corresponding author at: National Oceanic and Atmospheric Administration, Northeast Fisheries Science Center, 212 Rogers Ave., Milford, CT 06460, USA. Tel.: +1 203 882 6520; fax: +1 203 882 6570.
E-mail address: andrew.king@gmail.com (A.L. King).
[1] Present address: Bermuda Institute of Ocean Sciences, 17 Biological Station, Ferry Reach, St. George's GE 01, Bermuda.

0304-4203/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.marchem.2011.11.001

or time could be biased by complex circulation and otherwise misinterpreted, as waters sampled at each station may have originated from water masses with different biogeochemical histories.

As part of a larger California Current Ecosystem Long-Term Ecological Research (CCE-LTER) field program (Landry et al., 2009; http://cce.lternet.edu/), data on mixed layer dissolved Fe (dFe) and Fe-binding ligand concentrations are presented here in the context of a suite of biogeochemical measurements from four-day long "process drifter studies" that followed drogued drifter arrays. Also included are the results from shipboard bottle experiments assessing phytoplankton Fe limitation and biologically-induced changes in Fe speciation. In total, we describe and discuss Fe biogeochemistry in four drifter studies — one conducted in May 2006 and three in April 2007. Three of the four drifter studies began in recently upwelled water masses where we observed declines in macronutrients and dFe over time. The relative availability of nutrients at different times during the evolution of these water masses, particularly the relative availabilities of nitrate and dFe, was found to be associated with phytoplankton Fe stress as supported by results from shipboard Fe addition bottle experiments. The speciation of dFe in the shipboard experiments reflected the apparent Fe stress observed, consistent with previous Fe speciation measurements in an incubation experiment (Buck et al., 2010) and field observations (van den Berg, 1995; Boye et al., 2001; Croot et al., 2004; Buck and Bruland, 2007; Gerringa et al., 2008). Our results compliment and expand upon previous fixed-station studies of phytoplankton Fe limitation in the coastal upwelling CCS: although relatively close to continental sources of Fe, dFe concentrations decrease rapidly with distance from the continental shelf, and the low availability of Fe can affect phytoplankton growth and community structure (Hutchins et al., 1998; Firme et al., 2003; King and Barbeau, 2007, 2011). Our combined field and incubation studies allow additional insight into the interactive influences of biota on iron speciation in a dynamic eastern boundary coastal upwelling regime.

2. Sampling and methods

2.1. Cruises and sampling protocol

The CCE-LTER process cruises utilized multiple four-day quasi-Lagrangian experimental cycles that tracked and studied water masses with drogued drift arrays. The experimental studies, the drogued drift array, and related experimental theory are described in detail in Landry et al. (2009). The drift array consisted of a satellite-tracked holey sock drogue centered at 15 m attached to a low-profile float to minimize the effect of wind on transport. Data presented in this article are from four experimental cycles: one drifter study in May 2006 on the R/V Knorr, and three drifter studies in April 2007 on the R/V Thompson (Table 1). Drifter paths and surrounding California Cooperative Fisheries Investigations (CalCOFI) station locations are shown in Fig. 1 for geographical reference.

Conventional sampling was conducted with Niskin bottles and a rosette sampling system — discrete seawater samples were collected from Niskin bottles for analysis of chlorophyll a (hereafter referred to as "chl") and the macronutrients nitrate + nitrite ("nitrate"), silicic acid, and phosphate.

Trace metal clean seawater samples from the mixed-layer were collected using either a custom-made trace metal clean Teflon pumping system via Teflon tubing connected to an air-driven diaphragm pump (Cole-Parmer), or GO Flo bottles (General Oceanics) attached to a synthetic line (New England Ropes) tripped with lead messengers coated with epoxy-based paint (General Oceanics). Seawater was dispensed from the GO Flo bottles using overpressure with ultra-high purity N_2.

Trace metal clean seawater samples were processed under Class 100 laminar flow hoods in positive-pressure clean areas. Operationally defined dissolved seawater fractions were collected by vacuum filtering (<500 mm Hg) samples through acid-cleaned 0.4 μm polycarbonate track-etched membrane filters (Millipore) on a Teflon filtration rig (Savillex). Dissolved Fe samples were stored in acid-cleaned 250 mL low density polyethylene (LDPE; Nalgene) bottles — each bottle was rinsed with the filtrate, filled, and acidified to pH ~1.8 by adding ultrapure HCl to a final concentration of 0.025 M. Dissolved Fe speciation samples were collected in acid-cleaned 500 mL fluorinated polyethylene (FLPE; Nalgene) bottles that were filled with ultrapure water (>18 MΩ cm^{-1}; Milli-Q, Millipore) to condition for at least two weeks prior to rinsing and filling with sample. All samples were frozen at −20 °C and returned to the lab for analysis.

2.2. Shipboard incubation experiments

Fe addition grow-out experiments were conducted for a ~3–4 d incubation period at the beginning (t = 1 d) and end (t = 4 d) of drifter studies 1, 2, and 4. Only one grow-out experiment was conducted during drifter study 3 (t = 1 d). Trace metal clean seawater collected from the mixed layer via Teflon pump or GO Flo bottles was dispensed into acid-cleaned 2.7 L polycarbonate bottles (Nalgene). For each grow-out experiment, duplicate bottles of Fe amended (+5 nM ferric chloride) and unamended controls were placed in deck-board incubators cooled by flow-through seawater at 35% incident light with two layers of neutral density screening. Integrated mixed layer photosynthetically active radiation during the drifter studies ranged between 25 and 33% of surface light. The setup and sampling of experiments were carried out under Class 100 laminar flow hoods to avoid contamination. Samples were collected daily for chl and macronutrients. Samples for high performance liquid chromatography (HPLC) and particulate organic carbon/nitrogen (POC/N) were collected at the initial and final time point of the experiments.

To assess biological influences on dFe speciation, shipboard Fe speciation incubation studies were conducted with unamended trace metal clean seawater collected during drifter study 2 (t = 3 d) and drifter study 3 (t = 2 d) from the mixed layer using consecutive deployment of 30 L GO-Flo bottles. Collected seawater was gently homogenized in an acid cleaned and ultrapure water-conditioned 50 L carboy before disbursement into similarly acid-cleaned and ultrapure water-conditioned 2.7 L polycarbonate bottles (Buck et al., 2010). Incubation bottles were placed in on-deck flow through incubators for a period of 7 d; three replicates were incubated at 35% incident light and three replicates were placed in heavy-duty (4 mil) black garbage bags for dark reference controls. Replicate incubation bottles were chosen at random to sample for dFe and Fe speciation, macronutrients, and chl throughout the experiment. Due to limitations in sample volume, one incubation bottle from each treatment was sampled at every time point, and every bottle was sampled 2–3 times over the course of the experiment (Buck et al., 2010). Setup and sampling were conducted in Class 100 laminar flow hoods with care taken to prevent contamination.

2.3. Dissolved Fe analysis

DFe was measured using an FeLume flow injection analysis system (Waterville Analytical), with an Fe(II) sulfite reduction chemiluminescent (CL) flow injection analysis (FIA) method, described previously in King and Barbeau (2007, 2011). Here additional details of the method and interference testing are provided. The method was modified from Powell et al. (1995) and Bowie et al. (1998). The most significant modifications were the use of the commercially available nitriloacetic acid (NTA) Superflow chelating resin (Qiagen) for the Fe preconcentration step (Lohan et al., 2005), and the use of pH 1.8 ultrapure water and pH 1.8 trace metal-free ultraviolet-irradiated seawater (UVSW; obtained from K. Bruland) for analytical blank measurements.

Table 1
In situ hydrographic and biogeochemical conditions for drifter studies 1, 2, 3, and 4, including date, time, drifter study day number (d), latitude (lat), longitude (long), temperature (temp, °C), salinity (sal, psu), density (dens, σ_t), mixed layer depth (mld, m), chlorophyll a (chl, μg L^{-1}), mean specific growth rate (μ) and standard deviation (sd), phosphate (PO_4, μM), nitrate (NO_3, μM), silicic acid (Si, μM), dissolved iron (dFe, nM), L_1 ligand (L_1, nM), conditional stability constant of ligand L_1 (log K_1), L_2 ligand (L_2, nM), conditional stability constant of ligand L_2 (log K_2), excess L_1 ligand (eL_1, nM), nitrate:dissolved iron ratio (N:Fe), excess silicic acid (Si_{ex}), and community structure as counted by microscopy for diatoms (dia), autotrophic dinoflagellates (dino), and other unidentified eukaryotic phytoplankton (other). Also included are the distance traveled during each drifter study and the concentration and percent change of chl, PO_4, NO_3, Si, and dFe. 'nda.' refers to 'no data available' and 'nd.' refers to 'not detected'.

	time	d	lat	long	temp	sal	dens	mld	chl	μ	sd	PO_4	NO_3	Si	dFe	L_1	log K_1	L_2	log K_2	eL_1	N:Fe	Si_{ex}	dia	dino	Other
Drifter 1																									
11 May 2006	0400	1	34.33	−120.80	11.3	33.64	25.69	30	2.8	0.51	0.14	1.1	13.6	15.8	1.8	nda	nda	nda	nda	nda	8	2.2	0.23	0.42	0.35
14 May 2006	0430	4	34.27	−121.06	12.3	33.59	25.46	40	6.2			0.6	6.5	10.2	1.1	nda	nda	nda	nda	nda	6	3.7	0.27	0.42	0.30
Distance			35 km				Change		+3.4			−0.5	−7.1	−5.6	−0.7										
							% Change		126%			−46%	−52%	−35%	−39%										
Drifter 2																									
4 April 2007	0200	1	34.26	−120.84	12.2	33.60	25.49	40	2.2	0.65	0.33	0.7	7.6	10.2	1.1	1.5	13.0	0.23	11.7	0.40	7	2.6	0.01	0.41	0.58
6 April 2007	0730	3	34.36	−120.99	12.4	33.50	25.35	20	1.0			0.6	5.2	6.6	0.33	0.72	12.8	0.71	11.5	0.39	16	1.3	0.05	0.47	0.48
7 April 2007	1900	4	34.35	−121.10	12.5	33.47	25.34	20	1.2			0.5	4.7	5.9	0.25	nda	nda	nda	nda	nda	19	1.2	0.05	0.34	0.64
Distance			40 km				Change		−1.0			−0.2	−2.9	−4.3	−0.9										
							% Change		−44%			−28%	−38%	−42%	−77%										
Drifter 3																									
10 April 2007	0400	1	33.54	−123.16	14.3	33.18	24.72	50	0.17	0.34	0.24	0.2	0.1	2.1	0.47	0.89	13.0	0.58	11.8	0.32	0	2.0	<0.01	0.31	0.69
11 April 2007	1900	2	33.38	−123.38	14.2	33.17	24.73	55	0.21			0.2	0.1	2.1	0.54	0.97	12.7	nd	nd	0.40	0	2.0	<0.01	0.30	0.70
13 April 2007	900	4	33.21	−123.71	14.1	33.18	24.77	70	0.26			0.2	0.0	2.3	nda	nda	nda	nda	nda	nda	nda	2.3	<0.01	0.23	0.77
Distance			70 km				Change		+0.04			0.0	−0.1	+0.2	+0.07										
							% Change		+24%			−4%	−76%	10%	+15%										
Drifter 4																									
16 April 2007	0300	1	34.23	−121.19	11.8	33.70	25.64	30	1.0	0.50	0.16	0.9	11.1	14.3	1.4	nda	nda	nda	nda	nda	8	3.2	0.14	0.19	0.67
18 April 2007	1900	3	33.77	−121.02	12.2	33.61	25.48	35	1.0			0.7	7.9	9.0	0.36	0.60	12.9	0.45	10.6	0.24	22	1.2	0.23	0.15	0.61
19 April 2007	0530	4	33.76	−120.97	12.5	33.44	25.32	40	0.68			0.5	4.4	5.4	0.36	nda	nda	nda	nda	nda	12	1.0	0.09	0.22	0.69
Distance			50 km				Change		−0.36			−0.4	−6.7	−8.9	−1.0										
							% Change		−35%			−46%	−60%	−62%	−74%										

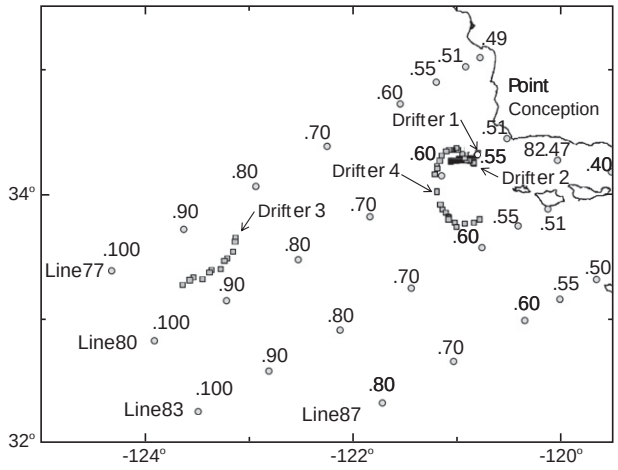

Fig. 1. Map of study location with tracks of drifter studies (squares) and CalCOFI station locations (circles). Drifter study 1 originated near station 80.55 in May 2006 (dark gray squares). Drifter studies 2, 3, and 4 originated near station 80.55, 80.80, and 80.60, respectively, in April 2007 (light gray squares). Drifter origination locations are denoted by the arrows.

DFe in pH 1.8 seawater samples was reduced to Fe(II) quantitatively over 12 h after addition of sodium sulfite to a final concentration of 2 μM. Prior to sample loading, the NTA column was rinsed with 0.15 M ultrapure HCl carrier solution. The seawater sample was then buffered in-line to pH 6 with the addition of 2 M ammonium acetate prior to preconcentration of Fe(II) on the NTA column at a rate of 2.4 mL/min for 30 s (a longer load time can be used to further improve sensitivity). Fe(II) recovery has been shown previously to be near 100% at pH 6 on NTA, and quantitative above pH 5.5 (Lohan et al., 2005). This was confirmed in our work for Fe(II) additions to seawater samples (data not shown). Following sample loading, the column was then rinsed for 5 s with ultrapure water and Fe(II) was eluted with 0.15 M HCl carrier solution and mixed in the reaction coil with the luminol–NH_3OH buffer. The final pH of the carrier/luminol–NH_3OH mixture was ~9.0. Radical intermediates, primarily superoxide and hydroxyl, produced by the oxidation of Fe(II) then oxidize luminol and produce light at 425 nm (Rose and Waite, 2001 and references therein) which is detected by the PMT.

The base system was designed and assembled by Waterville Analytical (King et al., 1995), including stream select and injection valves (Valco Instruments), a peristaltic pump (Rainin Instruments), and a photomultiplier tube (PMT; Hamamatsu Photonics HC-135-11, 875 V). We used a 1 cm long, conically-shaped mini-column with an 85 μL internal volume (Global FIA) that was fitted with non-metallic frits (Global FIA) that was filled with NTA resin (Superflow, Qiagen) (Lohan et al., 2005) and cleaned with 1.5 M HCl prior to use. Samples and reagents were placed in a class-100 laminar flow bench (AirClean 600 PCR Workstation, AirClean Systems), while the instrument was under normal laboratory conditions.

As an analytical blank measurement, we used a pH 1.8 ultrapure water and pH 1.8 trace metal-free ultraviolet-irradiated seawater (UVSW; obtained from K. Bruland; Donat and Bruland, 1988) — the summed measurement of the contribution of Fe(II) from the instrumental blank (tubing, valves and other wetted parts within apparatus) and a reagent blank (addition of sulfite, ammonium acetate, HCl). This method for blank correction was checked and supported with Fe concentrations from certified reference materials and seawater samples with previously assigned dFe concentrations. The use of pH 1.8 ultrapure water and UVSW resulted in a mean blank value of 0.126 nM (n = 27) with a detection limit of 0.02 nM (three times the standard deviation of blank). Because the method is dependent on the pH of the sample/reagent mixture, we were unable to ascertain the contributions of individual reagents to the blank signal. The reported blank value is within range of other reported blank values from a variety of Fe analysis methods (*e.g.*, Bowie et al., 2003; Lohan et al., 2005).

Generally, Fe(III) standard additions were run several times during an analysis period and used to generate a standard curve. Fe concentration was determined by average peak height and subtracting out blank and baseline signals. Standard additions usually resulted in curvilinear responses — there was a larger ratio of signal to Fe added with higher Fe concentrations (Fig. 2). The increased sensitivity with higher Fe concentrations is consistent with modeled Fe(II)–luminol interactions (Rose and Waite, 2001 and references therein). A quadratic polynomial regression was fit to the standard addition curve and used to calculate the concentration of the sample and determine Fe concentration of subsequent samples (regressions varied <5% during analysis periods).

We tested potential interferences from reduced V species and desferrioxamine B (DFOB) on Fe(II) determination. In a non-separation and 8-HQ separation Fe(II) luminol CL techniques for seawater, V(III) and V(IV) were reported to initiate the luminol CL reaction (Hopkinson and Barbeau, 2007; Ussher et al., 2009). A 30 nM V(IV) addition (both before and after reduction) to a 0.50 nM Fe sample resulted in an indiscernible change in CL. This may be due to inherent differences in V retention between the NTA and 8-HQ resin. We also investigated the effect of a strong Fe-binding ligand, desferrioxamine B (DFOB; Sigma-Aldrich), on the recovery of Fe(II) on NTA. The DFOB additions were also undetectable indicating that Fe(II) remained reduced at pH 6 and was unaffected by the addition of a strong Fe(III)-binding ligand.

The average relative standard deviation of the method for dFe samples ranging from 0.1 to 8 nM was 3.9 ± 4.7% (mean and 1 standard deviation; n = 183). Using this method, seawater reference samples from the Sampling and Analysis of Fe (SAFe) intercomparison cruise in October 2004 were measured to be 0.10 ± 0.02 nM Fe (surface bottle 279, mean ± 1 SD, n = 4) and 0.92 ± 0.03 nM Fe (deep2 bottle 285, mean ± 1 SD, n = 4). The consensus value of the surface reference sample is 0.097 ± 0.043 (n = 140) nM Fe and the deep2 reference sample is 0.91 ± 0.17 nM Fe (n = 168; Johnson et al., 2007).

2.4. Dissolved Fe speciation analysis

Samples for *in situ* dFe speciation were collected during drifter study 2 (t = 1 and 3 d), drifter study 3 (t = 1 and 2 d), and drifter study 4 (t = 3 d). Additional samples were collected from the shipboard Fe speciation incubation experiments. All dFe speciation samples were stored frozen at −20 °C until returned to shore for analyses. An established competitive ligand exchange-adsorptive cathodic stripping voltammetry (CLE-ACSV) method (Rue and Bruland, 1995; Buck et al.,

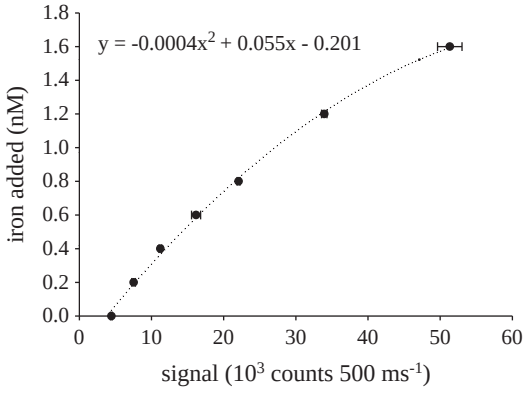

Fig. 2. Example standard addition curve showing the relation between photomultiplier tube signal (10^3 counts 500 ms^{-1}) and Fe added (nM). The sample's initial dissolved Fe concentration was ~0.2 nM. Error bars represent 1 standard deviation (n = 3).

2007) was used to measure the organic complexation of dFe in these samples.

Back in the lab, frozen samples were thawed and shaken vigorously prior to analyses at room temperature. Speciation sample aliquots (10 mL) were buffered with 50 μL of a 1.5 M ammonium–borate buffer (99.99% boric acid in 0.4 M Ultrex ammonium hydroxide; Fisher: Ellwood and van den Berg, 2000) to a final pH of 8.2 (Buck et al., 2007). Dissolved Fe (1000 ppm AA standard, diluted in 0.024 M Ultrex HCl; Fisher) additions of 0, 0, 0.2, 0.5, 1, 1.5, 2.5, 4, 7.5, and 10 nM were made to ten aliquots and allowed to equilibrate for at least two hours (Rue and Bruland, 1995; Buck et al., 2007). Following equilibration of Fe additions, a final concentration of 25 μM salicylaldoxime (from 5 mM stock in LC-MS grade methanol; Fisher) was added to each aliquot and equilibrated for an additional 15 min prior to analyses (Rue and Bruland, 1995). Analyses were performed on a BioAnalytical Systems (BASi) controlled growth mercury electrode (CGME) on static drop (SMDE) setting (drop size: 3.34 mm^2), coupled to an Epsilon E2 (BASi) electrochemical analyzer. The use of a borate buffer, combined with this instrumentation, provides high sensitivity for this method (average S from this work: 140 nA nM^{-1} min^{-1}) and allowed deposition times of 3 min for these analyses (Buck et al., 2007, 2010).

Dissolved Fe speciation data was interpreted using combined Ruzic/van den Berg and Scatchard linearization techniques (Ruzic, 1982; van den Berg, 1984; Scatchard, 1949) applied to titration data for the average dFe concentrations measured in each sample. The use of both Ruzic/van den Berg and Scatchard techniques for each titration dataset allows more insight into variability within the raw data, as well as whether the system is better characterized by one or two ligand classes (Buck et al., 2010). The distinction between the stronger L_1 and weaker L_2 ligand classes is based on breaks in the Scatchard linearization fits (Rue and Bruland, 1995; Buck et al., 2007), reflecting different average conditional stability constants for the ligand classes. All Fe speciation results include the averaged output from both linearization techniques (Buck et al., 2010) and are calculated using an $\alpha_{Fe'}$ of 10^{10}, as described by Rue and Bruland (1995). The theory, development, and application of this method are detailed further elsewhere (Rue and Bruland, 1995; Buck et al., 2007).

2.5. Ancillary data analysis

Samples for carotenoid pigments were filtered onto 0.7 μm GF/F filters (Whatman), stored in liquid N_2, separated via reverse phase HPLC, and detected with an ultraviolet/visible spectrophotometer (Goericke and Montoya, 1998). Samples for macronutrients were frozen and concentrations of nitrate ($NO_2^- + NO_3^-$), phosphate (PO_4^{2-}), and silicic acid ($Si(OH)_4$) were determined colorimetrically by an automated analyzer (Parsons et al., 1984; Oceanographic Data Facility, La Jolla, California and Marine Science Institute, Santa Barbara, California). Chl was measured using a 90% acetone extraction/acidification method with a Turner Designs fluorometer (Parsons et al., 1984). POC and PON samples were vacuum-filtered onto combusted 0.7 μm Whatman GF/F filters and measured using a carbon–hydrogen–nitrogen (CHN) analyzer (Parsons et al., 1984; Scripps Institution of Oceanography Analytical Facility, La Jolla, California).

3. Results

3.1. Biogeochemical observations in situ

Mixed layer (5–10 m) data from drogue drift array experiments for temperature, salinity, density (σ_t), chl, nitrate, phosphate, silicic acid, and dFe from 11 to 14 May 2006 (drifter study 1), 4 and 7 April 2007 (drifter study 2), 10 and 13 April 2007 (drifter study 3), and 16 and 19 April 2007 (drifter study 4) are presented in Table 1.

In drifter studies 1, 2, and 4, the mixed layer conditions were typical of wind-driven coastal upwelling (Hayward and Venrick, 1998), with initially low temperature (11.3–12.2 °C), shallow mixed layer (~20–40 m), and elevated salinity (33.60–33.70) and density (25.49–25.69 σ_t). Phytoplankton biomass (1.04–2.76 μg/L chl), macronutrients (7.6–13.6 μM nitrate, 0.7–1.1 μM phosphate, 10.2–15.8 μM silicic acid), and dFe (1.2–2.0 nM) were initially relatively high. Over the span of four days in these drifter studies, temperature increased by 0.3–1 °C and salinity decreased by ~0.05–0.26.

In drifter study 1, chl increased (+126%) and the phytoplankton community was composed of ~25% diatoms and ~42% autotrophic dinoflagellates throughout the study as derived by microscopy-based biomass estimates (Landry et al., 2009), while macronutrients and dFe decreased 35–52%. In drifter studies 2 and 4, on the other hand, chl decreased by 44% and 35%, respectively. The phytoplankton community in drifter study 2 was dominated by unidentified (non-diatom/non-dinoflagellate) eukaryotic autotrophs (~60% of biomass) and autotrophic dinoflagellates (~34–41%), both at the beginning and end of the drifter study (Landry et al., 2009), while macronutrients decreased 28–42% and dFe decreased by 77%. In drifter study 4, the phytoplankton community ranged from ~9–14% diatoms, ~19–22% autotrophic dinoflagellates, and ~67–69% unidentified autotrophs (Landry et al., 2009). Macronutrients in drifter study 4 decreased by 46–62% and dFe decreased by 74%.

In these three drifter studies in the coastal upwelling region (1, 2, 4), the absolute decrease in macronutrients ranged from 2.9–7.1 μM nitrate, 0.2–0.5 μM phosphate, 4.3–8.9 μM silicic acid, and 0.7–1.0 nM dFe (Table 1). Due to differences in the relative decrease of nitrate and dFe, the ratio of μM nitrate to nM dFe (μM nitrate:nM dFe) increased from 7 to 19 in drifter study 2, and from 8 to 22, then falling to 12, in drifter study 4. In drifter study 1 (May 2006), μM nitrate:nM Fe decreased slightly from 8 to 6 (Table 1).

Drifter study 3 (10–13 April 2007) was situated in a warm-core anticyclonic eddy with a relatively deep mixed layer and nitracline depth (~50–70 m). In comparison to the other drifter studies within coastal upwelling waters, drifter study 3 was more representative of an oceanic locale — temperature was relatively high (~14.2–14.3 °C), salinity and density relatively low (33.17–33.18 and 24.72–24.77 σ_t), as well as chl (0.17–0.26 μg L^{-1}), macronutrients (0.2 μM phosphate, 0–0.1 μM nitrate, and 2.1–2.3 μM silicic acid), and dFe (0.47–0.54 nM) (Table 1). The concentration of macronutrients and dFe, and the μM nitrate:nM dFe ratio were relatively low, and did not change appreciably between day 1 and day 2 (Table 1). By microscopy-derived biomass, the phytoplankton community was composed of ~70–77% unidentified autotrophs and ~23–31% autotrophic dinoflagellates (Landry et al., 2009).

Mixed layer Fe speciation data from drifter studies 2, 3, and 4 are also presented in Table 1. Two ligand classes, L_1 and L_2, were detected during these drifter studies. The stronger L_1-type ligands were tightly coupled to dFe, with L_1 concentrations in excess of dFe in all samples (L_1-dFe ranged from 0.24 to 0.40 nM, Table 1). The $K^{cond}_{FeL_1,Fe'}$ for the L_1 ligand class measured during the three drifter studies averaged 12.9 ± 0.1 (n = 5), while log $K^{cond}_{FeL_2,Fe'}$ for the L_2 ligand class averaged 11.4 ± 0.6 (n = 4), where detected (Table 1). The concentrations of weaker L_2 ligands were, where detected, generally lower than L_1 (Table 1). Offshore drifter study 3 was dominated by the stronger L_1 ligand with no L_2 ligands detected. In contrast, L_2 ligands were present in both coastal upwelling drifter studies 2 and 4. During drifter studies 2 and 4, L_1 concentrations decreased with dFe over the four-day sampling period. In drifter study 2, dissolved L_2 ligand concentrations increased as L_1 ligand concentrations declined (Table 1).

3.2. Shipboard bottle incubation experiments

In shipboard Fe addition bottle experiments from waters with *in situ* dFe >1 nM (experiments initiated at drifter study 1 t = 1 d and

4 d; drifter study 2 t = 1 d; drifter study 4 t = 1 d), there appeared to be little effect of Fe addition on phytoplankton growth and depletion of macronutrients (Figs. 3A, B, 4A, 5A). In these experiments, chl increased to similar concentrations in both unamended and Fe-added replicates (as high as ~20 μg chl L^{-1}; *e.g.,* experiment initiated at drifter study 1 t = 1 d, Fig. 3A). While chl accumulation was somewhat greater in Fe-added replicates from drifter study 1 t = 4 d (Fig. 3B) and drifter study 2 t = 1 d (Fig. 4A), there was little difference in nitrate depletion in those experiments.

In contrast, for Fe addition experiments that were initiated on t = 4 d of drifter study 2 and t = 4 d of drifter study 4, when *in situ* dFe had decreased to <0.4 nM, Fe addition resulted in a significantly larger accumulation of chl and a larger decrease in macronutrients over the course of the experiments (*t*-test, $p<0.05$;Table 2). Phytoplankton chl in Fe-added replicates of these experiments increased 3.2–6.7×, nitrate decreased 1.9–2.1×, and silicic acid decreased 2.1–2.4×, relative to unamended control replicates (Figs. 4B and 5B; Table 2). In the same Fe-amended bottles, POC concentrations increased 1.8–2.9× and PON concentrations increased 2.2–3.9×, relative to control experiments. The addition of Fe also supported some substantial changes to the phytoplankton community as represented by carotenoid pigments. Notably, there were ~3–4× increases in fucoxanthin and diadinoxanthin, representative of phytoplankton taxa including diatoms and dinoflagellates, respectively (Table 2).

Fe speciation incubation experiments were conducted with unamended waters collected from drifter studies 2 and 3; macronutrients, chl, and dFe speciation data for these experiments are detailed in Table 3. Measured ligand concentrations and conditional stability constants are given as the average and standard deviation of the output from both linearization techniques employed (Table 3). The incubation experiment begun during coastal upwelling drifter study 2 (initiated on t = 3 d) was relatively high in macronutrients (~6 μM nitrate, ~0.6 μM phosphate, ~5 μM silicic acid) and chl (~1.2 μg L^{-1}), and moderate in dFe (~0.7 nM) at the outset. The Fe speciation incubation conducted during offshore drifter study 3 (initiated on t = 2 d), on the other hand, was initially low in macronutrients (~0.5 μM nitrate, ~0.2 μM phosphate, ~1.7 μM silicic acid) and chl (~0.2 μg L^{-1}), and moderate in dFe (~1 nM). These incubation studies may have been marginally contaminated with dFe, based on comparison with *in situ* dFe data from drifter studies 2 and 3 (Table 1), although initial L_1 concentrations were still in excess of dFe concentrations (Table 3, Fig. 6B, D).

Dark replicates were used in these experiments to provide a reference for any changes in Fe speciation observed in the light bottles. In the incubation experiments from both drifter studies, dark replicates behaved similarly: macronutrients changed very little, chl decreased to near zero, and dFe and L_1 concentrations remained coupled, decreasing by nearly half over the course of the incubations (Table 3).

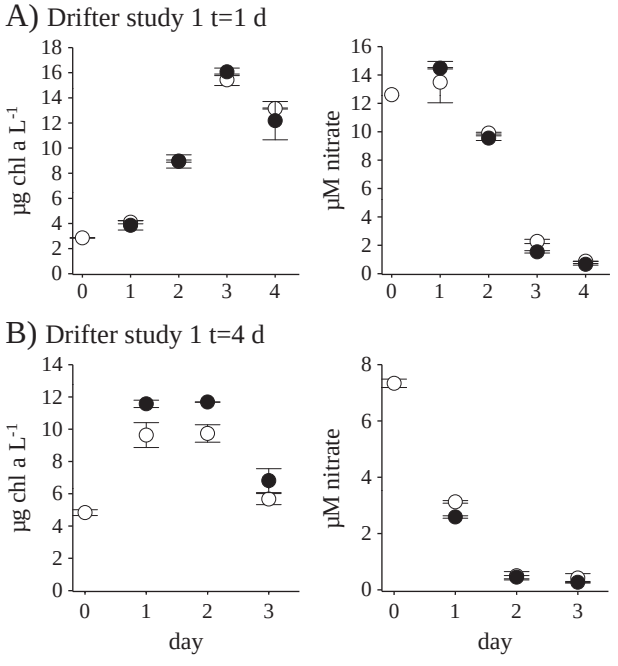

Fig. 3. Chl (μg L^{-1}) and nitrate (μM) for Fe addition bottle experiments that were initiated during drifter study 1 on A) t = 1 d and B) t = 4 d. Open circles are from unamended control replicates and closed circles are +5 nM Fe replicates. Error bars represent 1 standard deviation (n = 3).

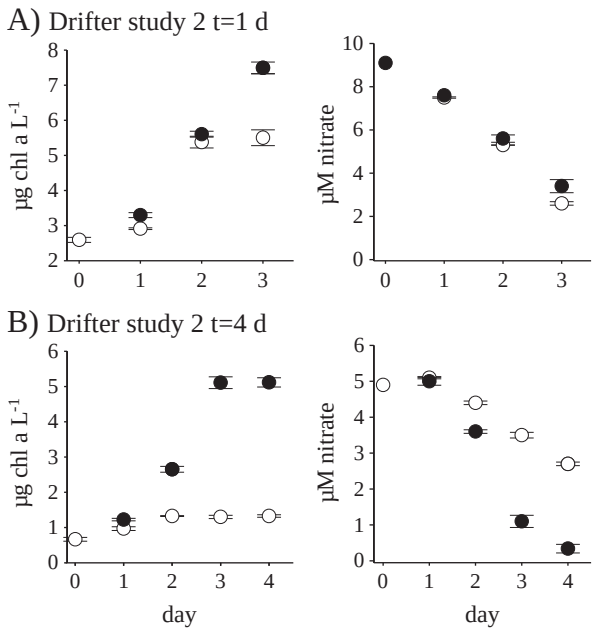

Fig. 4. Chl (μg L^{-1}) and nitrate (μM) for Fe addition bottle experiments that were initiated during drifter study 2 on A) t = 1 d and B) t = 4 d. Symbols and error bars are the same as described for Fig. 3.

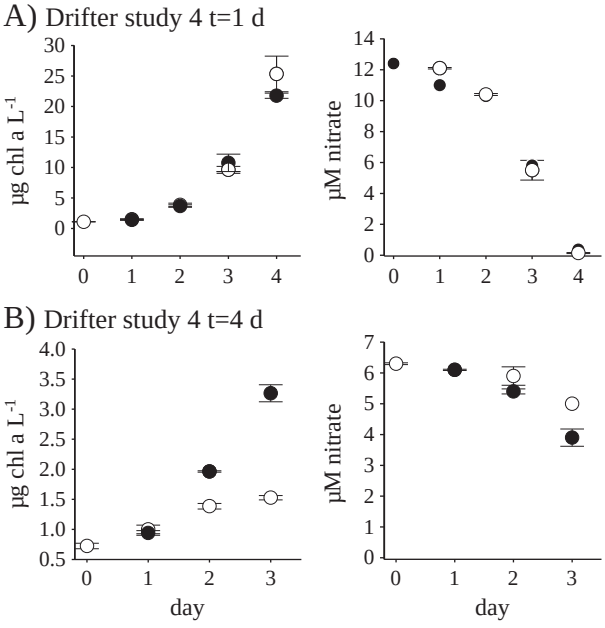

Fig. 5. Chl (μg L^{-1}) and nitrate (μM) for Fe addition bottle experiments that were initiated during drifter study 4 on A) t = 1 d and B) t = 4 d. Symbols and error bars are the same as described for Fig. 3.

Table 2
Normalized changes between initial and t = 4 d of Fe addition bottle experiments that were initiated at the beginning and end of drifter studies 2 and 4 (April 2007) for chlorophyll a (chl), phosphate (PO_4), nitrate (NO_3), silicic acid (Si), particulate organic nitrogen (PON) and carbon (POC), and pigments chlorophyll c (chl c), 19′-butanoyloxyfucoxanthin (19 but), fucoxanthin (fuc), 19′-hexanoylfucoxanthin (19 hex), diadinoxanthin (dd), and chlorophyll b (chl b). Values in bold indicate $p<0.05$ using paired t-tests. 'nd' refers to 'not detected'.

Drifter	d	chl	PO_4	NO_3	Si	PON	POC	chl c	19but	fuc	19hex	dd	chl b
2	1	**1.7**	**1.2**	1.1	1.1	1.1	1.1	1.2	nd	1.3	0.9	1.4	1.1
2	4	**6.7**	**1.7**	**2.1**	**2.4**	**3.9**	**2.9**	**5.2**	**1.4**	**4.3**	**3.6**	**2.8**	**1.6**
4	1	0.9	1.0	1.0	0.9	1.0	0.9	1.1	1.0	1.0	0.9	0.8	1.0
4	4	**3.2**	1.0	**1.9**	**2.1**	**2.2**	**1.8**	**3.6**	1.1	**3.7**	1.0	3.7	1.7

In the light replicates from drifter study 2, chl increased to 6 μg L^{-1} over the seven-day incubation period, while nitrate and dFe were drawn down to 0.4 μM and 0.13 nM, respectively (Table 3). Pigment data indicated significant growth of diatoms in this experiment, based on increasing fucoxanthin concentrations (>5×). Only one Fe-binding ligand class, L_1, was detected in the incubation, with an average log $K^{cond}_{FeL_1,Fe'}$ of 12.2 ± 0.2 (n = 11). Dissolved L_1 concentrations decreased in conjunction with dFe over the first three days of the experiment from 1.3 to 0.89 nM, and then increased markedly over the last four days to a final concentration of 1.8 nM as chl nearly tripled (2.2 μg L^{-1} to 6 μg L^{-1}) and dFe fell below 0.2 nM (Table 3, Fig. 6A).

In the incubation experiment from offshore drifter study 3, very little difference was observed between light and dark bottles (Table 3), although dFe was depleted slightly more in the light replicates (Fig. 6C). In all treatments, little change in macronutrients was measured and chl decreased to near zero (Table 3). In both light and dark bottle replicates, stronger L_1 concentrations remained closely coupled to dFe concentrations, declining from an initial L_1 concentration of 1.8 nM to 0.82 nM in the dark bottles, and to 0.9 nM in the light bottles (Fig. 6C). Only one ligand class was observed throughout this experiment, with an average log $K^{cond}_{FeL_1,Fe'}$ of 12.0 ± 0.3 (n = 5).

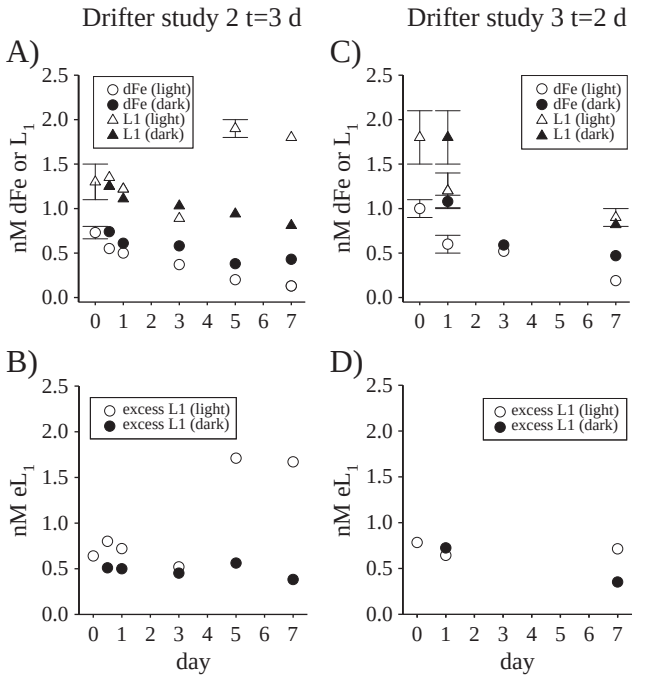

Fig. 6. Dissolved Fe (nM), ligand L_1 (nM), and excess L_1 ($[L_1]-[dFe]$; eL_1) concentrations during Fe speciation incubation experiments initiated during drifter study 2 (t = 3 d) (panels A and B) and drifter study 3 (t = 2 d) (panels C and D) under light (open symbols) and dark (closed symbols) experimental conditions. Error bars represent 1 standard deviation (n = 3); standard deviations less than symbol height were excluded for visual clarity.

4. Discussion

4.1. In situ observations from coastal upwelling drifter studies

The waters in the vicinity of a drogued drifter are typically not uniform and it is difficult to ascertain whether or not the drifter studies tracked a singular water mass. We acknowledge that the movement and mixing of biogeochemical constituents in seawater is a complex physical process at several scales (basin-, meso-, and micro-scales) that can result in horizontal and vertical variability that would affect the apparent continuity of the water mass observations during the quasi-Lagrangian studies. The observed decrease in salinity of 0.13 and 0.26 in drifter studies 2 and 4, respectively, suggest that mixing with less saline, offshore California Current water masses may have occurred and could have contributed to observed decreases in macro- and micronutrient concentrations. Additionally, many organisms are capable of moving horizontally and vertically in the water column, further confounding a singular water mass scenario. However, biogeochemical conditions observed over the 4 d experimental time-span were relatively consistent with an aging coastally-upwelled water mass (*e.g.*, increase in temperature and decrease in macronutrients in drifter studies 1, 2, and 4). Furthermore, there was a strong correlation between observed (*in situ*) and experimentally-estimated net growth of chl a (the difference between chl a specific growth and grazing

Table 3
Fe speciation incubation experiments initiated during drifter study 2 (t = 3 d) and drifter study 3 (t = 2 d). Data presented from drifter studies on various days of incubation (t = 0 to 7 d), the light/dark condition of incubation (l/d), dissolved Fe (nM), ligand L_1 (nM), conditional stability constant of ligand L_1 (log K_1), chlorophyll a (chl, μg L^{-1}), phosphate (PO_4, μM), nitrate (NO_3, μM), silicic acid (Si, μM), and excess ligand L_1 (eL_1). 'nda' refers to 'no data available'.

Drifter	d	l/d	dFe	L_1	log K_1	chl	PO_4	NO_3	Si	eL_1
2	0		0.78 ± 0.02	1.42 ± 0.02	12.0	1.24 ± 0.09	0.58	6.2	5.1	0.64
	0.5	Light	0.55 ± 0.02	1.35 ± 0.02	12.0	nda	nda	nda	nda	0.80
		Dark	0.74 ± 0.02	1.25 ± 0.01	12.0	nda	nda	nda	nda	0.51
	3	Light	0.37 ± 0.02	0.89 ± 0.01	12.2	2.2 ± 0.2	0.52	5	5.2	0.52
		Dark	0.58 ± 0.03	1.03 ± 0.01	12.3	0.41 ± 0.05	0.6	6.2	5.1	0.45
	5	Light	0.20 ± 0.04	1.9 ± 0.1	12.5	4.9 ± 0.4	0.26	1.7	0.8	1.71
		Dark	0.38 ± 0.04	0.94 ± 0.02	12.5	0.13 ± 0.02	0.65	7.4	6.2	0.56
	7	Light	0.13 ± 0.02	1.80 ± 0.01	12.6	6.0 ± 1.0	0.15	0.36	0.2	1.67
		Dark	0.43 ± 0.01	0.81 ± 0.01	12.6	0.05 ± 0.02	0.51	5.6	4.6	0.38
3	0		1.0 ± 0.1	1.8 ± 0.3	12.1	0.17	0.21	0.53	1.75	0.78
	1	Light	0.56 ± 0.13	1.2 ± 0.2	12.1	nda	nda	nda	nda	0.64
		Dark	1.1 ± 0.1	1.8 ± 0.3	12.3	nda	nda	nda	nda	0.72
	7	Light	0.19 ± 0.02	0.9 ± 0.1	11.6	0.05 ± 0.01	0.22	0.34	2	0.71
		Dark	0.47 ± 0.04	0.82 ± 0.02	11.8	<0.01 ± n.a.	0.22	0.35	1.8	0.35

rates) at eight similarly conducted drifter studies in May 2006 and April 2007 in the sCCS off Point Conception ($r^2 = 0.86$) (Landry et al., 2009), of which the four drifter studies described in this article were a subset of. The following discussion is based upon the assumption that the drogued drifters tracked singular and relatively coherent water masses.

Initial dFe concentrations from the three "upwelling" drifter studies (1, 2, and 4) ranged between 1.4 and 1.8 nM (Table 1), which is in agreement with previously observed dFe measurements in the region (Bruland et al., 2001; Firme et al., 2003; King and Barbeau, 2011). In our drifter studies, dFe decreased by ~0.7–1.0 nM over the course of the four days and a distance of ~50 km, equivalent to declines of 39%, 77%, and 74% in drifter studies 1, 2, and 4, respectively (Table 1). While we have not found comparable dFe data from Lagrangian-type studies in coastal upwelling systems in the literature, the large decrease in dFe has been observed in several pseudo-synoptic studies. In a region about 70 km north of the drifter studies, King and Barbeau (2011) observed a similar decrease in dFe from 1.9 nM to 0.38 nM (−80%) over the span of 14 km from CalCOFI station 77.49 to 77.51 in April 2003 and from 3.5 to 0.28 nM dFe (−92%) over the same two stations in April 2004 (see Fig. 1 station plan). In coastal waters off central California near Monterey Bay, CA, Bruland et al. (2001) reported a decrease in dFe of ~84% over the distance of ~50 km (~2.5 nM to ~0.4 nM). A similar trend was observed off Monterey Bay, CA, in total dissolvable Fe (dissolvable (unfiltered and acidified samples) v. dissolved (filtered and acidified samples)), with the mean decrease in dissolvable Fe between July and October 1999 of ~55% over a ~50 km transect (Johnson et al., 2001). Over the span of 10s of km off Año Nuevo (Central California), total dissolvable Fe was observed to decrease from 6 nM to <1 nM (Fitzwater et al., 2003). Pseudo-synoptic studies in strong upwelling eastern boundary current systems off the northwest United States (Oregon) and Peru also observed similar patterns in macronutrient and dFe distributions, albeit with much higher nearshore concentrations (Chase et al., 2002; Bruland et al., 2005).

The observed ~1 nM decrease in dFe concentration during the three coastal upwelling drifter studies was presumably due to primary loss processes in seawater: scavenging and biological uptake. Using chl-based intrinsic growth rates (grazing-corrected with dilution experiments; Landry et al., 2009), *in situ* POC:chl ratios (g:g), and a range of phytoplankton Fe:C ratios (Sunda and Huntsman, 1995), we calculated that with μmol Fe:mol C ratios ranging from 10–30 it is plausible that phytoplankton growth and utilization could account for the observed drawdown in dFe in all three coastal upwelling drifter studies (Table 4). During the drifter studies, rates of phytoplankton growth, zooplankton grazing, and carbon export at depth were found to be relatively coupled (Landry et al., 2009), indicating that grazing and subsequent sinking of fecal pellets were significant processes affecting export out of the euphotic zone, and the same likely applies to Fe export (*e.g.*, Firme et al., 2003).

Table 4
Estimated *in situ* biological uptake of dFe from May 2006 and April 2007 drifter studies. The following parameters are listed: change in dFe from t = 0 d and t = 4 d (ΔdFe), chl mean specific growth rate (μ) over the 4 d study as determined by dilution experiments (Landry et al., 2009), initial μg chl L^{-1} (chl_{init}) and expected chl (chl_{exp}) based on a natural log growth equation using μ, mean μg C:μg chl ratio over the 4 d study based on POC (C:chl), expected increase in μM C based on C:chl ratio (C_{exp}), and nM dFe drawdown based on μmol Fe:mol C (Fe:C) ratios from 10 to 40 (Sunda and Huntsman, 1995; Quigg et al., 2003). Values in bold indicate the closest match to observed drawdown (ΔdFe). Calculations predict phytoplankton Fe:C ratios to range between 10–30.

drifter	ΔdFe	μ	chl_{init}	chl_{exp}	avg C:chl	C_{exp}	nM dFe drawdown				
							10	15	20	30	40
1	−0.7	0.51	2.8	14	64	59	**0.6**	0.9	1.2	1.8	2.4
2	−0.9	0.65	2.2	12	82	70	0.7	**1.0**	1.4	2.1	2.8
4	−1.0	0.5	1.0	4.9	93	31	0.3	0.5	0.6	**0.9**	1.2

Beyond scavenging and biological uptake, the observed decrease in dFe may also be explained in part by mixing with low Fe water masses. For instance, we used mean salinity and dFe from recently upwelled water masses (drifter studies 2 and 4 of this study), data from offshore water masses in the region during April 2003 and April 2004 (mean salinity of 33.0 and 0.23 nM dFe, n = 16; data from King and Barbeau, 2011) to perform a simple end-member calculation. This indicates that mixing could explain a decline of ~0.2 nM and ~0.4 nM dFe in drifter studies 2 and 4, respectively. Deepening of the mixed layer, as observed during drifter study 4 (from 30 m to 40 m), could potentially increase dFe concentrations and salinity — although the observed net change in salinity during the study was negative.

In the drifter studies where Fe speciation was measured (drifter studies 2, 3, 4), strong L_1 ligands exceeded the concentration of dFe in all samples (Table 1, Fig. 7A). The stronger L_1 ligand concentrations averaged ~1 nM (0.94 ± 0.35 nM, $n = 6$, Table 1) in the mixed layer samples, with an average log $K^{cond}_{FeL_1,Fe'}$ of 12.88 ± 0.13 ($n = 6$, Table 1). The concentrations and conditional stability constants of the stronger L_1 ligand class measured in these samples are consistent with the measurements of other speciation studies (Rue and Bruland, 1995; Croot et al., 2004, 2007; Kondo et al., 2007; Gerringa et al., 2008; Kondo et al., 2008; Rijkenberg et al., 2008a). A weaker L_2-type ligand class was identified in all but one of the samples from the drifter studies (Table 1). The weaker L_2 ligand concentrations were much more variable between samples than [L_1] (Table 1), averaging 0.49 ± 0.20 nM ($n = 5$, Table 1). The average logarithmic conditional stability constant for the weaker L_2 ligand class was 11.40 ± 0.55 ($n = 5$, Table 1).

Over the course of drifter study 2 in the coastal upwelling region, stronger L_1-type ligand concentrations decreased while the weaker L_2 ligands increased (Table 1). In drifter study 3, however, no L_2-type ligands were measured *in situ*, and L_1 concentrations increased over time, as the mixed layer deepened. The coupled increase in L_2 with a decline in L_1 observed as the mixed layer shallowed in drifter study 2 (Table 1), may indicate loss of L_1 from surface waters by photochemical (*e.g.*, Barbeau, 2006) or biological uptake (Maldonado and Price, 1999) processes, although our data cannot discern between these processes. Photochemical reactions of FeL_1 may result in the L_2 increase observed during drifter study 2 (Barbeau, 2006); additional sources of L_2 may also include bacterial remineralization of particles (Boyd et al., 2010) and viral lysis of cells (Poorvin et al., 2011). On the other hand, the increase in L_1 with a deepening mixed layer in drifter study 3 may alternatively indicate a source of L_1 below the mixed layer from ligand production in subsurface chlorophyll maxima (Croot et al., 2004; Gerringa et al., 2008), or from cell lysis and organic remineralization processes (Gerringa et al., 2006; Croot et al., 2007; Sato et al., 2007).

4.2. Phytoplankton Fe limitation in bottle experiments

Shipboard Fe addition experiments initiated on day 4 of both drifter studies 2 and 4 provided evidence for Fe limitation within the phytoplankton community. On the other hand, Fe addition to incubation experiments initiated during both t = 1 and 4 d drifter study 1, t = 1 d of drifter study 2, t = 1 d of drifter study 4 (Fig. 3), and drifter study 3 (conducted on 10 April 2007, data not shown) did not have apparent effects on phytoplankton growth. In drifter studies 2 and 4, conditions transitioned from Fe-replete to Fe-limited over the distance of <50 km and three days. Based on pigment analyses, the net increase in phytoplankton chl in response to Fe addition appeared to be primarily manifested through increases in diatom and dinoflagellate populations. While bottle experiments are subject to enclosure effects that affect naturally-occurring processes (such as mixing, grazer interaction, light/temperature variability), our findings are supported by *in situ* observations of declining Fe availability. Similar observations of phytoplankton Fe limitation have been made during high nitrate, low dFe

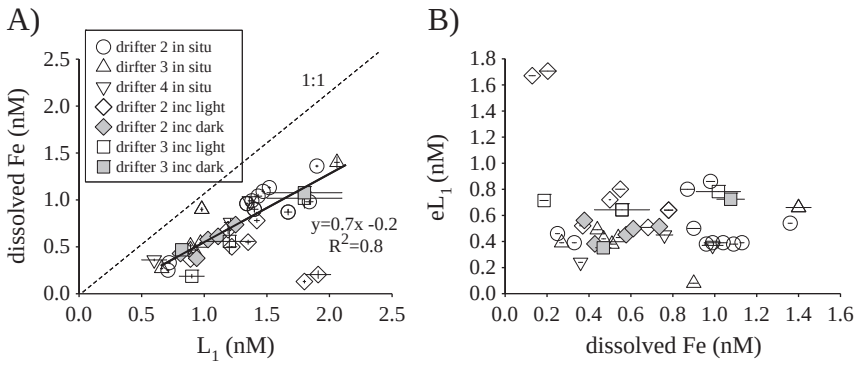

Fig. 7. Combined data from *in situ* observations and Fe speciation incubation experiments on the relationship between A) L_1 (nM) and dFe (nM) and B) dFe (nM) and excess L_1 ($[L_1]-[dFe]$; eL_1). Error bars represent 1 standard deviation (n = 3).

upwelling conditions in strong coastal upwelling regimes off central and northern California and Peru (Hutchins et al., 1998; Firme et al., 2003).

Fe limitation of diatoms and dinoflagellates was apparent in bottle experiments in which initial μM nitrate:nM dFe ratios were 19 and 12 (*in situ* measurements from drifter study 2 t = 4 d and drifter study 4 t = 4 d, respectively; Table 1). Fe addition did not appear to have a measurable effect on phytoplankton-related parameters in experiments where μM nitrate:nM dFe was <8. The low μM nitrate:nM dFe ratios were due to high nitrate coupled with high dFe (*e.g.*, drifter study 1 t = 1 d) and low nitrate with low dFe (*e.g.*, drifter study 3 t = 2 d) (Table 1). Quotas for a variety of phytoplankton at maximum growth rates have been estimated to range from 16 μM nitrate: ~2–30 nM Fe (Bruland et al., 1991; Sunda and Huntsman, 1995; Quigg et al., 2003), equivalent to a requirement of μM nitrate:nM dFe at ratios of ~0.5–8. While phytoplankton did not appear to be Fe-limited in water masses with μM nitrate:nM dFe <8 in this study, previous work from the sCCS transition zone (50–350 km offshore) during July 2003 and July 2004 documented Fe limitation in water masses with μM nitrate:nM dFe as low as ~5 (King and Barbeau, 2007).

The observation of phytoplankton Fe limitation due to the decline in dFe and rise of *in situ* μM nitrate:nM dFe during drifter studies 2 and 4 is further supported by the observed *in situ* decrease of silicic acid relative to nitrate. Under nutrient-replete conditions, diatoms use silicic acid and nitrate in relatively equal proportions and when Fe-limited, diatoms have been observed to take up more silicic acid relative to nitrate (Brzezinski, 1985; Hutchins and Bruland, 1998). An *in situ* excess silicic acid (Si_{ex}) calculation ($Si_{ex} = [Si(OH)_4] - [NO_3^-]$) can therefore be used as a proxy of the *in situ* Fe limitation status of diatoms (King and Barbeau, 2011). In drifter studies 2 and 4, in which phytoplankton were Fe-limited by the end of each study, Si_{ex} decreased *in situ* by 1.4 and 2.2 over 4 d, respectively (Table 5). It should be noted that although diatoms did respond to Fe addition in drifter study 2, they were not a significant fraction of the *in situ* phytoplankton community as measured by biomass. Si_{ex} increased by 1.5 and was relatively unchanged in drifter studies 1 and 3, respectively, in which Fe limitation was not observed (Table 5).

Table 5
Change in *in situ* Si_{ex} from the beginning and end of drifter studies 1, 2, 3, and 4.

Drifter	d	ΔSi_{ex}
1	3.0	+1.5
2	4.7	−1.4
3	3.2	+0.3
4	3.1	−2.2

4.3. Fe speciation in bottle experiments

There were two primary features evident in the Fe speciation data from the incubation experiments conducted during drifter studies 2 and 3: 1) the coupled decline in dFe and L_1 concentrations in the dark bottles of both experiments, and 2) the distinct difference in L_1 behavior between the light replicates of the two experiments, with the observed production of strong L_1-type Fe-binding ligands in the drifter study 2 incubation.

In the dark bottle replicates of both Fe speciation incubations, dFe and L_1 concentrations were tightly coupled and decreased over the seven-day course of the experiments, with the bulk of the decrease occurring within the first three days (Table 3, Fig. 6A, C). In the drifter study 2 dark bottles, dFe declined from 0.73 nM to 0.43 nM and L_1 declined from 1.3 nM to 0.81 nM, a loss of 41% dFe and 38% L_1. Similarly, dFe and L_1 in the drifter study 3 dark bottles declined from 1.0 nM to 0.47 nM (−53%) and 1.8 nM to 0.82 nM (−54%), respectively (Table 3, Fig. 6A, C). The loss of dFe and L_1 in the dark bottles of both experiments is likely due to wall loss effects. Previous studies have shown that polycarbonate bottles adsorb Fe over time (Fitzwater et al., 1982; Fischer et al., 2007), although this loss term for dFe is lower in polycarbonate bottles than other plastics tested (Fischer et al., 2007). Given the similar percentages of dFe and L_1 decreasing in the dark bottles of the incubations and the overwhelming complexation of dFe by L_1, our data indicates that the wall loss was primarily due to adsorption of the FeL_1 complex. This is also consistent with previous incubation experiment observations (Buck et al., 2010), where dFe and L_1 declined by ~50% in dark bottles over the first three days of an incubation.

A similar trend of decreasing dFe and L_1 was also seen in the light bottles of the drifter study 3 Fe speciation incubation experiment (Table 3, Fig. 6C). Photochemical degradation of FeL_1 or L_1 is not expected in the incubation experiments, as the polycarbonate bottles employed for the incubations are not transparent to UV. We expect that the wall loss effect observed in the dark bottles was also occurring in light bottles, and this would account for some of the loss in dFe and L_1 observed in the light bottles. In this incubation, dFe declined by 81%, from 1.0 nM to 0.19 nM, while L_1 concentrations decreased 50% from 1.8 nM to 0.9 nM. Thus, a larger % of dFe compared to L_1 was lost in the light bottles of this experiment, possibly due to uptake by bacteria, as chl also decreased overall (Table 3). While macronutrients in this incubation were largely unchanged, nitrate was slightly depleted over the course of the experiment, supporting the conclusion of some growth during the incubation. There was no evidence for ligand production in the drifter study 3 experiment, with [Fe], $[L_1]$ and $K^{cond}_{FeL_1,Fe'}$ all decreasing over the seven days of the incubation. The ratio of nitrate to dFe (μM nitrate: nM dFe) was low and generally less than 1 throughout the

experiment (Table 3). In combination with observed macronutrient and dFe data, and the results from the Fe addition bottle experiments during drifter study 3, these results indicate that this experiment was not Fe-limited.

The Fe speciation incubation begun during drifter study 2, on the other hand, was initially relatively high in macronutrients (*e.g.*, ~6 μM nitrate; Table 3) and chl (~1.2 μg/L), and moderate in dFe (~0.7 nM). The ratio of nitrate to dFe at the beginning of this incubation was ~8.5 μmol: nmol (from Table 3), consistent with the Fe-limited conditions observed during drifter study 2 and the response to Fe additions in the shipboard bottle experiments of this study. There was little change in macronutrients or chl over the first three days of the incubation (Table 3); dFe and L_1 concentrations were coupled and decreased 49% and 31%, respectively, during this period (Fig. 6A), similar to the wall loss effects observed in the dark bottles and drifter study 3 incubation. As a result, by day 3, the ratio of nitrate to dFe had increased to ~13.5 μmol: nmol.

After day 3 in the drifter study 2 incubation experiment, chl increased dramatically (+123% days 3 to 5) in light bottles, with a concomitant sharp decrease in macronutrients and dFe (Table 3). With this increase in chl, dFe and L_1 became uncoupled, and L_1 concentrations increased markedly (+113% days 3 to 5) while dFe continued to decline (Table 3, Fig. 6A). The continued decrease in dFe with growth indicates that the strongly complexed Fe was bioavailable to the ambient phytoplankton community. With respect to $K^{cond}_{FeL_1,Fe'}$ values, there was no distinction between light and dark bottles of the drifter study 2 experiment. Light bottles had an average log $K^{cond}_{FeL_1,Fe'}$ of 12.2 ± 0.2 (n = 6), dark bottles 12.3 ± 0.3 (n = 5), and all bottles together 12.2 ± 0.2 (n = 11). Instead, over the course of the experiment in both light and dark bottles, ligands measured in the last two days had higher log $K^{cond}_{FeL_1,Fe'}$ values (12.55 ± 0.06, n = 2) than those from the first three days (12.1 ± 0.1, n = 4). As the log $K^{cond}_{FeL_1,Fe'}$ values increased in both light and dark bottles in this experiment, and L_1 increases were only observed in the light bottles, the increase in $K^{cond}_{FeL_1,Fe'}$ values cannot be attributed to new ligand release alone.

The increase in L_1 over dFe observed in the drifter study 2 incubation was clearly tied to phytoplankton growth (Table 3). Previous field studies have shown a positive link between elevated excess L_1 concentrations and phytoplankton biomass in the water column (van den Berg, 1995; Boye et al., 2001; Croot et al., 2004; Gerringa et al., 2006; Buck and Bruland, 2007). There are several possible explanations for this relationship, including inadvertent release of L_1 via grazing and cell lysis (Gerringa et al., 2006; Sato et al., 2007; Kondo et al., 2008) and active production of L_1 by microorganisms growing under Fe-limiting conditions (Boye et al., 2001; Croot et al., 2004; Buck and Bruland, 2007; Gerringa et al., 2008; Buck et al., 2010). Our data from the drifter study 2 incubation cannot delineate between these sources, as time and incubator space limitation prohibited multiple treatments of the bottles. However, when our incubation data is put into the context of previous incubation results, we find that active L_1 production is a more likely explanation for the increase in L_1 observed in the drifter study 2 incubation experiment.

The production of strong Fe-binding ligands in a shipboard incubation experiment has recently been described by Buck et al., (2010). In that case, both unamended and Fe-amended seawater from the Southern Ocean was incubated for fifteen days. While significant growth occurred in all light treatment bottles, L_1 increases in that study were only observed in the unamended, and presumably Fe-limited, light bottles (Buck et al., 2010). Similar to the drifter study 2 experiment discussed here, the Southern Ocean incubation bottles also demonstrated a lag time where dFe and L_1 concentrations were coupled and decreasing until phytoplankton growth was apparent, at which point dFe and L_1 decoupled in the unamended, Fe-limited bottles only, with the production of L_1 as dFe declined (Buck et al., 2010). Thus, even though there was roughly 30% more phytoplankton biomass in the Fe-amended incubation bottles of that study, L_1 concentrations only increased beyond dFe with phytoplankton growth in the unamended bottles (Buck et al., 2010). This indicates that the increase in L_1 observed in these incubation experiments is not strictly related to growth, and passive release of ligands via grazing or cell lysis, while certainly important, was not evident in the Fe speciation data, possibly because the ligands released by grazing/cell lysis fell outside of the detection window employed here. Together, the Fe speciation incubation experiments of Buck et al., (2010) and of this work suggest that strong Fe-binding (L_1-type) ligand production in excess of dFe occurs in conjunction with active phytoplankton growth under Fe-limiting conditions.

These observations are consistent with compiled field observations of dFe speciation from the published literature (*e.g.*, Fig. 5 in Buck and Bruland, 2007), which have shown a generally good correlation between dFe and L_1 concentrations until dFe falls below ~0.2 nM, when large and variable excesses of L_1 are common. In the drifter study 2 incubation experiment, ligand production was observed at low dFe, ≤0.2 nM (Table 3, Fig. 6A, C). As depicted in Fig. 7A, the field and incubation data from this study (Tables 1, 3, and samples collected from >100 m; unpubl.) largely fall along the best-fit trend line applied by Buck and Bruland (2007) for assembled published data from several ocean basins. The last two sampling points (days 5, 7) from the drifter study 2 incubation experiment deviate from the trend line, exhibiting large excesses of L_1 at low dFe (Fig. 7A, B).

In Buck and Bruland (2007), the authors suggested that large and variable excesses of L_1 at dFe <0.2 nM may be due to either ligand production under Fe-limiting conditions or to residual L_1 persisting after uptake of dFe from the FeL_1 complexes (Buck and Bruland, 2007). The incubation data we report here supports the first of the two hypotheses put forward by Buck and Bruland (2007) — that L_1 excesses observed at low dFe *in situ* may be a result of active ligand production under Fe limitation. Furthermore, given the results of Buck et al., (2010), this feature of ligand production under Fe-limiting conditions is not restricted to low dFe waters. While the identity of the L_1 measured is beyond the scope of this work, recent studies have identified the presence of siderophores in natural unamended seawater with a similar range in dFe to the work described here (Mawji et al., 2008; Velasquez et al., 2011).

It should be noted, however, that in both sets of incubation experiments, ligand production was observed concomitant with phytoplankton growth under Fe-limiting conditions (Buck et al., 2010; this work), and not simply with either the growth of phytoplankton or the status of Fe-limitation. This is analogous to the field observations of Buck and Bruland (2007), where the largest excesses of L_1 were observed in the highly productive, but still Fe-limited, "Green Belt" of the Bering Sea (Aguilar-Islas et al., 2007). This feature of large excesses in L_1 over dFe associated with high phytoplankton biomass and low Fe or Fe limited regions of the ocean is also evident in several other field studies (Rue and Bruland, 1995; Boye et al., 2001; Croot et al., 2004; Gerringa et al., 2008). These particular phytoplankton communities are often dominated by Fe stressed diatom populations; as diatoms have previously been shown to increase the reactive pool of Fe (Rijkenberg et al., 2008b) and diatom biomass has been linked to Fe-binding ligand concentrations in the field (Gerringa et al., 2006), these eukaryotic communities may play a more substantial role in the cycling of Fe-binding ligands than previously considered.

5. Summary and conclusions

The quasi-Lagrangian drifter studies described here were conducted during springtime off Point Conception, California, and illustrate the differing biogeochemical processes of coastal upwelling regimes and oligotrophic waters offshore. In one case, drifter study 1, both macronutrients and dFe were initially high and remained high enough

through the end of the drifter study to not limit phytoplankton growth. In drifter studies 2 and 4, the relative decrease in dFe was about two times that of macronutrients, increasing μM nitrate:nM Fe to 19 and 12 — a biogeochemical combination of nitrate and dFe at which phytoplankton such as diatoms and dinoflagellates become Fe-stressed. In drifter study 3, situated within a warm core anti-cyclonic eddy, phytoplankton biomass, macronutrients, and dFe were relatively low and remained relatively unchanged throughout the cycle. The absolute decline in dFe was similar for the three coastal upwelling drifter studies (1, 2 and 4), but residual dFe remained substantial at the end of drifter study 1 due the higher initial concentration of dFe. Phytoplankton uptake of dFe appears to be consistent with previously published Fe:C ratios.

In drifter studies 2 and 3, the combination of quasi-Lagrangian observations of *in situ* dFe and Fe speciation, as well as incubation experiments assessing Fe limitation and changes in Fe speciation, provided a unique perspective of dFe dynamics in evolving coastal water masses. The dramatic increase in strong Fe-binding ligands observed concomitant with phytoplankton growth under Fe-limiting conditions in the drifter study 2 Fe speciation incubation is particularly insightful toward understanding the natural distributions of Fe and Fe-binding ligands in the marine environment. Drifter study 3 was in an offshore, low nutrient, and low Fe warm-core eddy and provided a contrast to the more biogeochemically-dynamic coastal upwelling locale. Although close to continental sources, low dFe availability in coastal waters can play a significant role in influencing phytoplankton community structure via Fe limitation, and the associated cycling of major nutrient elements C,N, P, and Si.

Despite the difficulties involved with tracking a singular water mass in a biogeochemically- and physically-heterogeneous environment, such as a coastal upwelling regime, quasi-Lagrangian studies provided a unique experimental platform for investigating Fe biogeochemistry. While the distribution of dFe and other macronutrients in the drifter studies described in this article reaffirms the marked decrease in dFe observed in fixed-station observations over similar distances (~35–50 km), the use of drogued drifters offers a temporal aspect that is difficult to resolve when using fixed-station approaches. The observed spatio-temporal decrease in dFe, among other biogeochemical parameters, during drifter studies is useful for understanding and interpreting phytoplankton bloom dynamics and community succession, in addition to constraining rates for biogeochemical models.

Acknowledgments

We thank B. Hopkinson, C. Dupont, J. Leblond, and the CCE-LTER research group, especially M. Landry and the officers and crew of the R/V Thompson and Knorr, for assistance in collecting and analyzing the data. This research was funded by NSF OCE-0550302 and LTER NSF/OCE-0417616. This manuscript constitutes BIOS contribution number 2012 for co-author Buck. Buck was funded by a Scripps Institution of Oceanography Earth Section postdoctoral fellowship. We also thank three anonymous reviewers for their helpful comments and suggestions.

References

Aguilar-Islas, A.M., Hurst, M.P., Buck, K.N., Sohst, B., Smith, G.J., Lohan, M.C., Bruland, K.W., 2007. Micro- and macronutrients in the southeastern Bering Sea: insight into iron-replete and iron-depleted regimes. Prog. Oceanogr. 73, 99–126.

Alvarez-Salgado, X.A., Doval, M.D., Borges, A.V., Joint, I., Frankignoulle, M., Woodward, M., Figueiras, F.G., 2001. Off-shelf fluxes of labile materials by an upwelling filament in the NW Iberian upwelling system. Prog. Oceanogr. 51, 321–337.

Barbeau, K., 2006. Photochemistry of organic iron(III) complexing ligands in oceanic systems. Photochem. Photobiol. 82, 1505–1516.

Bowie, A.R., Achterberg, E.P., Mantoura, R.F.C., Worsfold, P.J., 1998. Determination of sub-nanomolar levels of iron in seawater using flow injection with chemiluminescence detection. Anal. Chim. Acta 361, 189–200.

Bowie, A.R., Achterberg, E.P., Blain, S., Boye, M., Croot, P.L., de Baar, H.J.W., Laan, P., Sarthou, G., Worsfold, P.J., 2003. Shipboard analytical intercomparison of dissolved iron in surface waters along a north–south transect of the Atlantic Ocean. Mar. Chem. 84, 19–34.

Boyd, P.W., Law, C.L., Hutchins, D.A., Abraham, E., Croot, P.L., Ellwood, M., Frew, R.D., Hall, J., Handy, S., Hare, C., Higgins, J., Hill, P., Hunter, K.A., LeBlanc, K., Maldonado, M.T., McKay, R.M., Mioni, C., Oliver, M., Pickmere, S., Safi, K., Sander, S., Sanudo-Wilhelmy, S.A., Smith, M., Strzepek, R., Tovar-Sanchez, A., Wilhelm, S.W., 2005. FeCycle: attempting an iron biogeochemical budget from a mesoscale SF_6 tracer experiment in unperturbed low iron waters. Global Biogeochem. Cycles 19, GB4S26. doi:10.1029/2005GB002494.

Boyd, P.W., Jickells, T., Law, C.S., Blain, S., Boyle, E.A., Buesseler, K.O., Coale, K.H., Cullen, J.J., de Baar, H.J.W., Follows, M., Harvey, M., Lancelot, C., Levasseur, M., Owens, N.P.J., Pollard, R., Rivkin, R.B., Sarmiento, J., Schoemann, V., Smetacek, V., Takeda, S., Tsuda, A., Turner, S., Watson, A.J., 2007. Mesoscale iron enrichment experiments 1993–2005: synthesis and future directions. Science 315, 612–617.

Boyd, P.W., Ibisanmi, E., Sander, S.G., Hunter, K.A., Jackson, G.A., 2010. Remineralization of upper ocean particles: implications for iron biogeochemistry. Limnol. Oceanogr. 55, 1271–1288.

Boye, M., Van Den Berg, C.M.G., De Jong, J.T.M., Leach, H., Croot, P., De Baar, H.J.W., 2001. Organic complexation of iron in the Southern Ocean. Deep Sea Res. Part I 48, 1477–1497.

Bruland, K.W., Donat, J.R., Hutchins, D.A., 1991. Interactive influences of bioactive trace metals on biological production in oceanic waters. Limnol. Oceanogr. 36, 1555–1577.

Bruland, K.W., Rue, E.L., Smith, G.J., 2001. Iron and macronutrients in California coastal upwelling regimes: implications for diatom blooms. Limnol. Oceanogr. 46, 1661–1674.

Bruland, K.W., Rue, E.L., Smith, G.J., DiTullio, G.R., 2005. Iron, macronutrients and diatom blooms in the Peru upwelling regime: brown and blue waters of Peru. Mar. Chem. 93, 81–103.

Brzezinski, M.A., 1985. The Si–C–N ratio of marine diatoms — interspecific variability and the effect of some environmental variables. J. Phycol. 21, 347–357.

Buck, K.N., Bruland, K.W., 2007. The physicochemical speciation of dissolved iron in the Bering Sea, Alaska. Limnol. Oceanogr. 52, 1800–1808.

Buck, K.N., Lohan, M.C., Berger, C.J.M., Bruland, K.W., 2007. Dissolved iron speciation in two distinct river plumes and an estuary: implications for riverine iron supply. Limnol. Oceanogr. 52, 843–855.

Buck, K.N., Selph, K.E., Barbeau, K.A., 2010. Iron-binding ligand production and copper speciation in an incubation experiment of Antarctic Peninsula shelf waters from the Bransfield Strait, Southern Ocean. Mar. Chem. 122, 148–159.

Chase, Z., van Geen, A., Kosro, P.M., Marra, J., Wheeler, P.A., 2002. Iron, nutrient, and phytoplankton distributions in Oregon coastal waters. J. Geophys. Res. 107 (C10), 3174. doi:10.1029/2001JC000987.

Chavez, F.P., Barber, R.T., 1987. An estimate of new production in the equatorial Pacific. Deep Sea Res. Part A 34, 1229–1243.

Croot, P.L., Andersson, K., Öztürk, M., Turner, D.R., 2004. The distribution and speciation of iron along 6°E in the Southern Ocean. Deep Sea Res. Part II 51, 2857–2879.

Croot, P.L., Frew, R.D., Sander, S., Hunter, K.A., Ellwood, M.J., Pickmere, S.E., Abraham, E.R., Law, C.S., Smith, M.J., Boyd, P.W., 2007. Physical mixing effects on iron biogeochemical cycling: FeCycle experiment. J. Geophys. Res. 112, C06015.

De Baar, H.J.W., Boyd, P.W., Coale, K.H., Landry, M.R., Tsuda, A., Assmy, P., Bakker, D.C.E., Bozec, Y., Barber, R.T., Brzezinski, M.A., Buesseler, K.O., Boye, M., Croot, P.L., Gevais, F., Gorbunov, M.Y., Harrison, P.J., Hiscoock, W.T., Laan, P., Lancelot, C., Law, C.S., Levasseur, M., Marchetti, A., Millero, F.J., Nishioka, J., Nojiri, Y., van Oijen, T., Riebesell, U., Rijkenberg, M.J.A., Saito, H., Takeda, S., Timmermans, K.R., Veldhuis, M.J.W., Waite, A.M., Wong, C.-S., 2005. Synthesis of iron fertilization experiments: from the iron age in the age of enlightenment. J. Geophys. Res. 110. doi:10.1029/2004JC002601.

Donat, J.R., Bruland, K.W., 1988. Direct determination of dissolved cobalt and nickel in seawater by differential pulse cathodic stripping voltammetry preceded by adsorptive collection of cyclohexane-1,2-dione dioxime complexes. Anal. Chem. 60, 240–244.

Dugdale, R.C., Wilkerson, F.P., 1989. New production in the upwelling center at Point Conception, California — temporal and spatial patterns. Deep Sea Res. Part A 36, 985–1007.

Ellwood, M.J., van den Berg, C.M.G., 2000. Zinc speciation in the Northeastern Atlantic Ocean. Mar. Chem. 68, 295–306.

Firme, G.F., Rue, E.L., Weeks, D.A., Bruland, K.W., Hutchins, D.A., 2003. Spatial and temporal variability in phytoplankton iron limitation along the California coast and consequences for Si, N, and C biogeochemistry. Global Biogeochem. Cycles 17, 1016. doi:10.1029/2001GB001824.

Fischer, A.C., Kroon, J.J., Verburg, T.G., Teunissen, T., Wolterbeek, H.T., 2007. On the relevance of iron adsorption to container materials in small-volume experiments on iron marine chemistry: ^{55}Fe-aided assessment of capacity, affinity and kinetics. Mar. Chem. 107, 533–546.

Fitzwater, S.E., Knauer, G.A., Martin, J.H., 1982. Metal contamination and its effect on primary production measurements. Limnol. Oceanogr. 27, 544–551.

Fitzwater, S.E., Johnson, K.S., Elrod, V.A., Ryan, J.P., Coletti, L.J., Tanner, S.J., Gordon, R.M., Chavez, F.P., 2003. Iron, nutrient and phytoplankton biomass relationships in upwelled waters of the California coastal system. Cont. Shelf Res. 23, 1523–1544.

Gerringa, L.J.A., Veldhuis, M.J.W., Timmermans, K.R., Sarthou, G., de Baar, H.J.W., 2006. Co-variance of dissolved Fe-binding ligands with phytoplankton characteristics in the Canary Basin. Mar. Chem. 102, 276–290.

Gerringa, L.J.A., Blain, S., Laan, P., Sarthou, G., Veldhuis, M.J.W., Brussaard, C.P.D., Viollier, E., Timmermans, K.R., 2008. Fe-binding dissolved organic ligands near

the Kerguelen Archipelago in the Southern Ocean (Indian sector). Deep Sea Res. Part I 55, 606–621.
Goericke, R., Montoya, J.P., 1998. Estimating the contribution of microalgal taxa to chlorophyll a in the field — variations of pigment ratios under nutrient- and light-limited growth. Mar. Ecol. Prog. Ser. 169, 97–112.
Hayward, T.L., Venrick, E.L., 1998. Nearsurface pattern in the California Current: coupling between physical and biological structure. Deep Sea Res. Part II 45, 1617–1638.
Hopkinson, B.M., Barbeau, K.A., 2007. Organic and redox speciation of iron in the eastern tropical North Pacific suboxic zone. Mar. Chem. 106, 2–17.
Hutchins, D.A., Bruland, K.W., 1998. Iron-limited diatom growth and Si:N uptake ratios in a coastal upwelling regime. Nature 393, 561–564.
Hutchins, D.A., DiTullio, G.R., Zhang, Y., Bruland, K.W., 1998. An iron limitation mosaic in the California upwelling regime. Limnol. Oceanogr. 43, 1037–1054.
Johnson, K.S., Chavez, F.P., Elrod, V.A., Fitzwater, S.E., Pennington, J.T., Buck, K.R., Walz, P.M., 2001. The annual cycle of iron and the biological response in central California coastal waters. Geophys. Res. Lett. 28, 1247–1250.
Johnson, K.S., Boyle, E., Bruland, K., Coale, K., Measures, C., Moffett, J., Aguilar-Islas, A., Barbeau, K., Bergquist, B., Bowie, A., Buck, K., Cai, Y., Chase, Z., Cullen, J., Doi, T., Elrod, V., Fitzwater, S., Gordon, M., King, A., Laan, P., Laglera-Baquer, L., Landing, W., Lohan, M., Mendez, J., Milne, A., Obata, H., Ossiander, L., Plant, J., Sarthou, G., Sedwick, P., Smith, G.J., Sohst, B., Tanner, S., van den Berg, S., Wu, J., 2007. The SAFe iron intercomparison cruise: an international collaboration to develop dissolved iron in seawater standards: EOS Trans. AGU, 88, pp. 131–132.
King, A.L., Barbeau, K., 2007. Evidence for phytoplankton iron limitation in the southern California Current System. Mar. Ecol. Prog. Ser. 342, 91–103.
King, A.L., Barbeau, K., 2011. Dissolved iron and macronutrient distributions in the southern California Current System. J. Geophys. Res. Oceans 116, C03018.
King, D.W., Lounsbury, H.A., Millero, F.J., 1995. Rates and mechanism of Fe(II) oxidation at nanomolar total iron concentrations. Environ. Sci. Technol. 29, 818–824.
Kondo, Y., Takeda, S., Furuya, K., 2007. Distribution and speciation of dissolved iron in the Sulu Sea and its adjacent waters. Deep Sea Res. Part II 54, 60–80.
Kondo, Y., Takeda, S., Nishioka, J., Obata, H., Furuya, K., Johnson, W.K., Wong, C.S., 2008. Organic iron(III) complexing ligands during an iron enrichment experiment in the western subarctic North Pacific. Geophys. Res. Lett. 35 Art. No. L12601.
Landry, M.R., Ohman, M.D., Goericke, R., Stukel, M.R., Tsyrklevich, K., 2009. Lagrangian studies of phytoplankton growth and grazing relationships in a coastal upwelling ecosystem off Southern California. Prog. Oceanogr. 83, 208–216.
Lohan, M.C., Aguilar-Islas, A.M., Franks, R.P., Bruland, K.W., 2005. Determination of iron and copper in seawater at pH 1.7 with a new commercially available chelating resin, NTA Superflow. Anal. Chim. Acta 530, 121–129.
Maldonado, M.T., Price, N.M., 1999. Utilization of iron bound to strong organic ligands by plankton communities in the subarctic Pacific Ocean. Deep Sea Res. Part II 46, 2447–2473.
Martin, J.H., Fitzwater, S.E., 1988. Iron-deficiency limits phytoplankton growth in the northeast Pacific Subarctic. Nature 331, 341–343.
Mawji, E., Gledhill, M., Milton, J.A., Tarran, G.A., Ussher, S., Thompson, A., Wolff, G.A., Worsfold, P.J., Achterberg, E.P., 2008. Hydroxamate siderophores: occurrence and importance in the Atlantic Ocean. Environ. Sci. Technol. 42, 8675–8680.
Parsons, T.R., Maita, Y., Lalli, C.M., 1984. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergammon Press.
Poorvin, L., Sander, S.G., Velasquez, I., Ibisanmi, E., Lecleir, G.R., Wilhelm, S.W., 2011. A comparison of Fe bioavailability and binding of a catecholate siderophore with virus-mediated lysates from the marine bacterium *Vibrio alginolyticus* PWH3a. J. Exp. Mar. Biol. Ecol. 399, 43–47.
Powell, R.T., King, D.W., Landing, W.M., 1995. Iron distributions in surface waters of the South-Atlantic. Mar. Chem. 50, 13–20.
Quigg, A., Finkel, Z.V., Irwin, A.J., Rosenthal, Y., Ho, T.Y., Reinfelder, J.R., Schofield, O., Morel, F.M.M., Falkowski, P.G., 2003. The evolutionary inheritance of elemental stoichiometry in marine phytoplankton. Nature 425, 291–294.
Rijkenberg, M.J.A., Powell, C.F., Dall'osto, M., Nielsdottir, M.C., Patey, M.D., Hill, P.G., Baker, A.R., Jickells, T.D., Harrison, R.M., Achterberg, E.P., 2008a. Changes in iron speciation following a Saharan dust event in the tropical North Atlantic Ocean. Mar. Chem. 110, 56–67.
Rijkenberg, M.J.A., Gerringa, L.J.A., Timmermans, K.R., Fischer, A.C., Kroon, K.J., Buma, A.G.J., Wolterbeek, H.T., de Baar, H.J.W., 2008b. Enhancement of the reactive iron pool by marine diatoms. Mar. Chem. 109, 29–44.
Rose, A.L., Waite, T.D., 2001. Chemiluminescence of luminol in the presence of iron(II) and oxygen: oxidation mechanism and implications for its analytical use. Anal. Chem. 73, 5909–5920.
Rue, E.L., Bruland, K.W., 1995. Complexation of iron(III) by natural organic-ligands in the central North Pacific as determined by a new competitive ligand equilibration adsorptive cathodic stripping voltammetric method. Mar. Chem. 50, 117–138.
Ruzic, I., 1982. Waters and its information for trace metal speciation. Anal. Chim. Acta 140, 99–113.
Sato, M., Takeda, S., Furuya, K., 2007. Iron regeneration and organic iron(III)-binding ligand production during *in situ* zooplankton grazing experiment. Mar. Chem. 106, 471–488.
Scatchard, G., 1949. The attractions of proteins for small molecules and ions. Ann. N. Y. Acad. Sci. 51, 660–672.
Schlosser, C., Croot, P.L., 2009. Controls on seawater Fe(III) solubility in the Mauritanian upwelling zone. Geophys. Res. Lett. 36, L18606. doi:10.1029/2009GL038963.
Sunda, W.G., Huntsman, S.A., 1995. Iron uptake and growth limitation in oceanic and coastal phytoplankton. Mar. Chem. 50, 189–206.
Toggweiler, J.R., Carson, S., 1995. What are upwelling systems contributing to the ocean's carbon and nutrient budgets? Upwelling in the Ocean: Modern Processes and Ancient Records. John Wiley & Sons, Chichester, UK, pp. 337–360.
Ussher, S.J., Milne, A., Landing, W.M., Attiq-ur-Rehman, K., Seguret, M.J.M., Holland, T., Achterberg, E.P., Nabi, A., Worsfold, P.J., 2009. Investigation of iron(III) reduction and trace metal interferences in the determination of dissolved iron in seawater using flow injection with luminol chemiluminescence detection. Anal. Chim. Acta 652, 259–265.
van den Berg, C.M.G., 1984. Determination of the complexing capacity and conditional stability constants of complexes of copper(II) with natural organic ligands in seawater by cathodic stripping voltammetry of copper–catechol complex ions. Mar. Chem. 15, 1–18.
van den Berg, C.M.G., 1995. Evidence for organic complexation of iron in seawater. Mar. Chem. 50, 139–157.
Velasquez, I., Nunn, B.L., Ibisanmi, E., Goodlett, D.R., Hunter, K.A., Sander, S.G., 2011. Detection of hydroxamate siderophores in coastal and sub-Antarctic waters off the South Eastern Coast of New Zealand. Mar. Chem. 126, 97–107.