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The redox and complexation chemistry of iron within freshwater sources to the ocean: rivers, rain and glaciers

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ABSTRACT

FACULTY OF NATURAL AND ENVIRONMENTAL SCIENCES

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THE REDOX AND COMPLEXATION CHEMISTRY OF IRON WITHIN FRESHWATER SOURCES TO THE OCEAN: RIVERS, RAIN AND GLACIERS

by Mark James Hopwood

In Fe-limited or Fe-stressed waters Fe(II) cycling is an important feature of Fe biogeochemistry because processes favouring Fe(II) production or stability always expand the pool of Fe available to aquatic microorganisms. However almost nothing is known about Fe(II) organic speciation in natural waters and this limits our understanding of how this metal ion may interact with biological Fe uptake systems. Therefore I investigate how organic material affects Fe(II) biogeochemistry in rainwater, riverwater and in suspensions of aged glacial particulates. We compare Fe(II) concentrations (12-3600 nM) and, by adapting a ferrozine based reverse titration technique, ligand binding constants ($\log K_{Fe(II)}$ <5.5-11) in 5 temperate and sub-tropical river-estuary systems with varying dissolved organic carbon (DOC 200-1300 µM), labile dissolved Fe (Fe <0.2µm available to ferrozine after reduction with ascorbic acid 100 nM-20 µM) and pH (5.5-8.5). In riverwater we identify a natural class of humic Fe(II) ligands with weak Fe(II) binding constants ($\log K_{Fe(II)}$ <8) and concentrations in excess of Fe(II). Stronger ligands ($\log K_{Fe(II)}$ 11) were found only in the Itchen (Hampshire, UK) and may be associated with anthropogenic waste inputs. Similarly, the previously identified stabilisation of Fe(II) within North Carolina rainwater may be associated with anthropogenic emissions. Fe(II) concentrations in Wilmington (North Carolina, USA) rainwater have decreased from a high of 52 nM (and a 1:1 ratio of Fe(II):Fe(III)) in summer 2000 to a record low of 3 nM (and a 0.23:1 ratio of Fe(II):Fe(III)) in summer 2013 concurrently with improving air quality.

The weak ($\log K_{Fe(II)}$ <8), terrestrially derived ligands we report in 4 temperate/sub-tropical river systems are likely to be found in most surface freshwater systems, but their impact in higher salinity coastal seawater will be minimal. Stronger Fe(II) ligands ($\log K_{Fe(II)}$ 11) associated with effluent emissions however may exert a stabilising effect on Fe(II) concentrations in the natural environment. This may result in localised increases in bioavailable Fe concentrations and have implications for aquatic ecosystems in industrialised areas.

Contents

List of figures/tables/illustrations

1.0 Marine Fe Biogeochemistry

2.0 Dissolved Fe(II) in a river-estuary system rich in dissolved organic matter

3.0 Dissolved iron(II) ligands in river and estuarine water

4.0 Glacial flours as a potential source of Fe(II) and Fe(III) to polar waters

5.0 Fe(II) in rainwater

Academic Thesis: Declaration Of Authorship

I, Mark James Hopwood declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

The redox and complexation chemistry of iron within freshwater sources to the ocean: rivers, rain and glaciers

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;

2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;

3. Where I have consulted the published work of others, this is always clearly attributed;

4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;

5. I have acknowledged all main sources of help;

6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;

7. Parts of this work have been published as:

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Hopwood MJ, Statham PJ, Milani A, (2014) Dissolved Fe(II) in a river-estuary system rich in dissolved organic matter, Estuarine and Coastal Shelf Science, 151, 1-9, 10.1016/j.ecss.2014.09.015

Hopwood MJ, Statham PJ, Tranter M, Wadham J, (2014) Glacial flours as a potential source of Fe(II) and Fe(III) to polar waters, Biogeochemistry, 118(1), 443-452, 10.1007/s10533-013-9945-y

Willey JD, Kieber RJ, Humphreys JJ, Rice BC, ***Hopwood MJ****, Brooks Avery G and Mead RN, The role of fossil fuel combustion on the stability of dissolved iron in rainwater (Submitted to Global Biogeochemical Cycles)*

Signed: M J Hopwood
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Specific contributions towards individual chapters are acknowledged within the text. Where chapters have been published as complete articles (as listed in the declaration of authorship), M.J. Hopwood was in all cases the principal author. All listed authors contributed to the design of each study, where applicable assisted with fieldwork and provided comments on draft manuscripts during the writing process. M.J. Hopwood was principally responsible for all analytical laboratory work, the interpretation of results and the drafting of all manuscripts.

The insitu prototype Fe sensor described in ‘Dissolved Fe(II) in a river-estuary system rich in dissolved organic matter’ was designed, maintained and operated entirely by A. Milani.

Some results from the chapter ‘Fe(II) in rainwater’ are included as a small component of a larger manuscript (submitted for publication at the time of writing). The chapter itself however was entirely written by M.J. Hopwood prior to the production of this [submitted] manuscript.

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1. Marine Fe Biogeochemistry

1.1 Prologue

Several parts of this thesis, listed in the declaration of authorship, have been published (or submitted for publication). I have therefore opted to structure chapters around these manuscripts. The format (particularly section numbers) of some work has therefore changed slightly from its published version. To maintain consistency with the online versions of these manuscripts, the numbering of figures and tables is not changed but a letter is added to distinguish figures and tables within each chapter. An overview explaining the rationale of individual chapters and their significance in terms of the overall aims of this thesis has also been added.

The rationale for focussing this thesis on the biogeochemistry of Fe(II) in the estuarine environment was a dataset collected shortly before I commenced my studies. This dataset (Figure 1A) raises several questions about how dissolved Fe(II) speciation changes as it crosses the salinity gradient in the estuarine environment.

Figure 1A shows Fe(II) concentrations in the River Itchen (Southampton, UK) collected by Yitzhak Jacobson in July 2011. Fe(II) appears to have almost perfect conservative behaviour along the full salinity gradient (i.e. the concentration of Fe(II) present at any point is linearly proportional to salinity, R^2 0.84). This conservative behaviour is entirely unexpected given our present understanding of dissolved Fe chemistry in the estuarine environment. The rapid, non-conservative loss of dissolved Fe at low salinities is well established in almost all estuaries (see section 1.7) and thus anything other than rapid removal of Fe(II) at low salinities is anomalous.

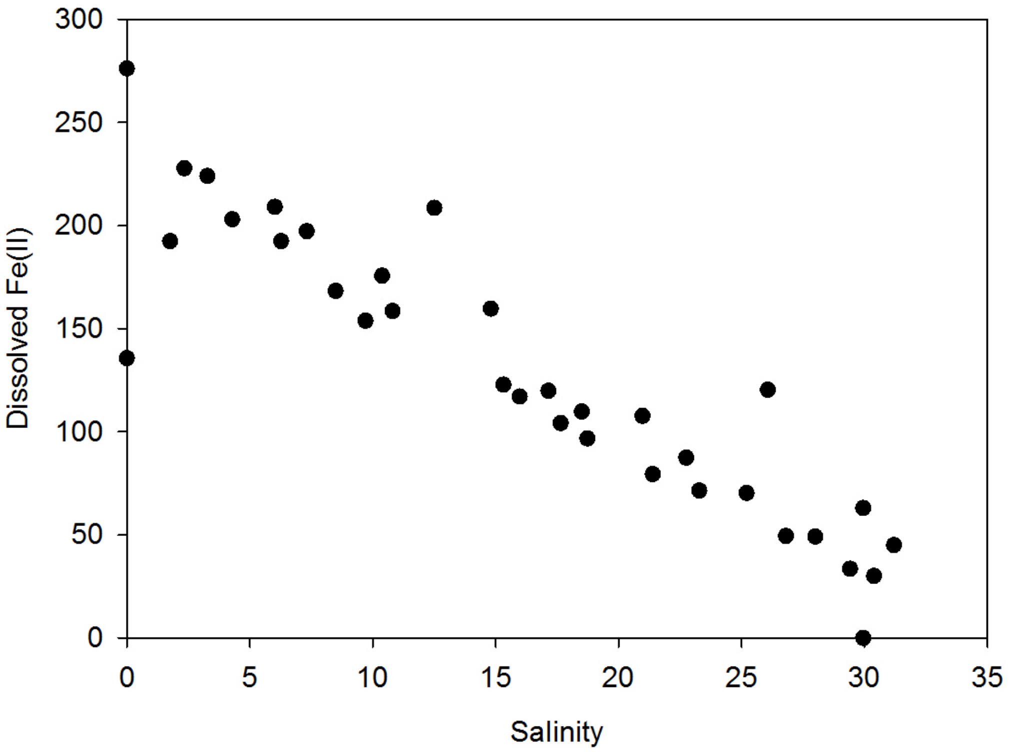

Figure 1A Dissolved (0.45 µm) Fe(II) concentration (nM) in the River Itchen (Southampton, UK), July 21 2011. Data collected and provided by Yitzhak Jacobson and Peter Statham.

Most work on estuarine Fe has neglected to consider the chemistry of Fe(II) and Fe(III) separately. Therefore it is possible that some aspect of aqueous Fe(II) chemistry in the natural environment, presently unknown, means that the estuarine mixing of Fe(II) is fundamentally different to that of Fe(III). One aim of this thesis is therefore to investigate the estuarine biogeochemistry of Fe(II) and to determine why conservative mixing of Fe(II) has been observed in the River Itchen. It is of course possible that the data shown in Figure 1A are the result of experimental artefacts, possibly associated with the ferrozine-based method used (Statham et al., 2012) and this possibility will be considered. It is also possible that aspects of the River Itchen's biogeochemistry are particularly unusual and thus the observed conservative Fe(II) mixing may not be representative of most estuaries. Equally this possibility will be explored.

1.2 Introduction

Fe is an essential requirement for primary metabolism and therefore a necessary nutrient for phytoplankton growth (Geider and Laroche, 1994; Greene et al., 1991; Harrison and Morel, 1986; Raven, 1990; Rueter and Ades, 1987; Rueter et al., 1990; Sunda, 2001). Despite the relatively high Fe content of Earth's crust (Wedepohl, 1995), Fe has a very low dissolved concentration across the surface of the open ocean, typically between 0.02 and 1 nM (de Baar and de Jong, 2001; Johnson et al., 1997b; Turner et al., 2001). For comparison, a concentration of 0.3-1.4 nM is usually found in deep waters, 1-10 nM in coastal seas, 10-400 nM in estuaries and 300-3000 nM in suboxic or anoxic marine basins (de Baar and de Jong, 2001). Low dissolved Fe concentrations in surface waters arise because of the present composition of Earth's atmosphere, specifically its high oxygen content (Turner and Hunter, 2001). Under oxic conditions Fe exists in the Fe(III) redox state. Fe(III) is only sparingly soluble in seawater with the dominant dissolved inorganic Fe(III) species, $Fe(OH)_3$, readily precipitating from solution (Liu and Millero, 1999; Liu and Millero, 2002; Millero, 1998; Millero et al., 1995; Waite, 2001).

In the modern day ocean, low dissolved Fe concentrations can act as a constraint on primary production and therefore also on the associated flux of carbon dioxide from the atmosphere to the surface ocean (Boyd et al., 2007; Kolber et al., 1994; Martin et al., 1991; Pollard et al., 2009). Nitrate was previously thought to limit surface primary production throughout the ocean, however both mesoscale Fe artificial enrichment experiments and in situ observations of natural phytoplankton blooms have shown conclusively that over 25% of the surface ocean is, as a direct result of low Fe concentrations, permanently high-nitrate, low-chlorophyll (HNLC, often synonymous with high-nutrient, low chlorophyll) (Boyd et al., 2007; de Baar et al., 2005). The three large permanent HNLC zones are: the Southern Ocean, the subarctic northeast Pacific and the equatorial Pacific (Behrenfeld et al., 1996; Behrenfeld and Kolber, 1999; Boyd et al., 2007; Coale et al., 1996; de Baar et al., 1990; Martin et al., 1994; Martin and Fitzwater, 1988). Across these vast swathes of the surface ocean, bioavailable Fe is the dominant limiting factor constraining primary production. Whilst bioavailable Fe is the main limiting factor in these zones, it is unlikely to be the sole limitation on the activity of all primary producers at all times. Grazing pressure, light, dissolved silicon, Zn and Cu are all potential co-limiting factors in HNLC waters (Coale, 1991; Morel et al., 1994; Price et al., 1991; Watson, 2001).

Outside HNLC zones Fe is by no means in abundant supply as it may still sporadically limit or co-limit primary production in other marine waters (Firme et al., 2003). For example, some parts of the North Atlantic may become Fe limited or co-limited for a short time period after the spring bloom in some years (Nielsdottir et al., 2009; Ryan-Keogh et al., 2013). Furthermore, in low nitrate waters the process of di-nitrogen fixation can be controlled by Fe availability as a result of the high Fe requirement of the nitrogenase enzyme (Moore et al., 2009; Moore et al., 2001). Stress induced by low bioavailable Fe concentrations may in fact be a continuous scale as even in coastal waters higher Fe concentrations than the open ocean are offset by much greater cellular Fe requirements (Hutchins and Bruland, 1998; Hutchins et al., 1998; Strzepek and Harrison, 2004; Watson, 2001).

The limiting role of Fe has led to suggestions that variations in bioavailable Fe input to the ocean on glacial-interglacial timescales may lead to significant changes in atmospheric carbon dioxide uptake by the oceans (Maher et al., 2010; Martin, 1990; Watson et al., 2000). Larger bioavailable Fe fluxes to HNLC areas of the ocean would, provided other nutrients and conditions remained non-limiting, increase primary production and thereby also increase the flux of carbon dioxide from the atmosphere to the surface ocean (and vice versa if bioavailable Fe fluxes to HNLC areas were to decrease) (de Baar et al., 1995; Martin, 1990). Enhanced primary productivity could in turn lead to enhanced carbon export from surface waters to the deep ocean which would decrease atmospheric carbon dioxide concentration (Martin et al., 1990b). This hypothesis, that variation in the scale of HNLC zones may affect atmospheric carbon dioxide concentrations, has led to the argument that artificial addition of bioavailable Fe to HNLC areas of the ocean, or ‘Fe seeding’, may increase carbon dioxide uptake by phytoplankton and thereby ultimately increase the flux of carbon to marine sediments (Buesseler et al., 2008; Lampitt et al., 2008). This form of geoengineering would have the suppositional effect of sequestering excess anthropogenic carbon dioxide from the atmosphere (Martin et al., 1990a). However, several assumptions about the efficiency of this process and the absence of significant perturbations to other biogeochemical systems are made in this argument (Boyd, 2008; Buesseler et al., 2008; Lampitt et al., 2008; Meyers, 2007; Turner et al., 2004). The main assumption is that a significant proportion of the carbon taken up by induced phytoplankton blooms at the ocean's surface would ultimately be exported to deep waters, as if this is not the case the argument is fatally flawed (Shepherd et al., 2007). Studies to date have had varying degrees of success in demonstrating increased carbon fluxes to deep waters following Fe induced phytoplankton blooms, with some experiments reporting only limited increases in export to the deep ocean (Boyd et al., 2004; de Baar et al., 2008; Pollard et al., 2009; Smetacek et al., 2012).

The status of dissolved Fe as a limiting nutrient in much of the open ocean means that atmospheric dust has been the subject of much attention because of its ability to supply Fe to waters far removed from landmasses (Duce and Tindale, 1991; Jickells et al., 2005; Johnson, 2001; Mahowald et al., 2005; Zhuang et al., 1990). Whilst dust is one of many Fe sources to the ocean, most other terrestrially derived inputs are deposited solely in coastal waters and shelf seas. Sources other than aeolian deposition are therefore very limited in their capacity to supply Fe further offshore. The decline in Fe concentrations away from terrestrial sources is illustrated by the island effect which is well documented around small, isolated landmasses surrounded by open ocean such as the Crozet, Galapagos, Falkland, Kerguelen islands and South Georgia (Chever et al., 2010; Chisholm and Morel, 1991; Planquette et al., 2011; Planquette et al., 2007; Watson, 2001). Close to these islands, terrestrial inputs of dissolved and particulate Fe allow phytoplankton blooms to develop in what would otherwise be HNLC waters. Fe sources which are primarily limited to coastal waters or shelf seas include rivers (see section 1.7) (Chase et al., 2007; Wetz et al., 2006), re-suspension and lateral advection of shelf sediments (Elrod et al., 2004; Johnson et al., 1999; Lam and Bishop, 2008; Lam et al., 2006; Lam et al., 2012; Moore and Braucher, 2008), hypoxic 'dead zones' (Lohan and Bruland, 2008) and glacial melt water and ice bergs (Bhatia et al., 2013; Gerringa et al., 2012; Lin et al., 2011; Raiswell et al., 2008; Shaw et al., 2011; Statham et al., 2008). Upwelling of relatively Fe-rich waters, hydrothermal vents and rainfall are notable exceptions to the list of Fe sources that are mainly, or solely, delivered into coastal waters (Hawkes et al., 2013a; Kieber et al., 2001; Saito et al., 2013).

‘Wet’ and ‘dry’ aeolian depositions (in simple terms dust and rain respectively) of Fe to the surface ocean are often distinguished because of the processing of solid Fe phases that can occur naturally within rain droplets. Photochemistry, relatively low pH and the organic components within rainwater mean that wet deposition events (which deposit both rainwater dissolved components and associated particles) deliver Fe to the oceans in a more soluble form than dry deposition (Paris and Desboeufs, 2013). Wet deposition therefore provides a significant source of Fe to the open ocean distinct from aerosol dusts (Kieber et al., 2003a; Kieber et al., 2001; Willey et al., 2008).

The impact of point sources such as hydrothermal vents upon surface Fe concentrations is difficult to determine due to both rapid dilution with seawater and the precipitation of Fe minerals close to the vent site. However, both in situ measurements and modelling approaches suggest that these vents are certainly a significant source of dissolved and particulate Fe to the deep ocean (Bennett et al., 2008; Hawkes et al., 2013a; Saito et al., 2013; Sander and Koschinsky, 2011; Tagliabue et al., 2010; Yucel et al., 2011).

1.2.1 Bioavailable Fe

‘Bioavailability’ is a fundamental concept in the biogeochemical cycling of Fe. However, despite the term being used widely, it is unsatisfactorily defined (Shaked and Lis, 2012). Qualitatively the bioavailability of a specific Fe containing compound is a description of the relative ease with which a specific biological species may utilize it for metabolic purposes. The application of this definition to global models, or even conceptual models of single ecosystems, is however fraught with difficulty. In all natural terrestrial and marine environments Fe exists heterogeneously as a multitude of different compounds with substantially different chemical and physical properties (Moffett, 2001; Raiswell and Canfield, 2012; Rickard and Luther, 2007). Furthermore Fe speciation is dynamic and subject to changes on timescales varying from seconds to years. Similarly at any given time a variety of biological species may be simultaneously taking up Fe from the same source. As they do so, biological processes will induce physical and chemical changes in the chemistry of Fe (Bruland et al., 1991). The biological uptake of Fe itself thereby affects the bioavailability of the Fe source (Barbeau et al., 2001; Gobler et al., 2002; Hunter and Boyd, 2007; Kranzler et al., 2011;

Rubin et al., 2011; Strzepek et al., 2005; Tang et al., 2011). Bioavailability is therefore a complicated concept that cannot be defined by simply considering either the chemistry of Fe, the ambient environmental conditions, or the physiology of individual biological species in isolation (Shaked and Lis, 2012).

Broad generalisations can however be made about the relative bioavailability of different Fe pools. Dissolved Fe is often regarded as much more bioavailable than the particulate or colloidal pools (Sunda, 2001), but there is evidence that colloids are widely bioavailable and that some forms of dissolved iron are not (Wells et al., 1983). For particulate Fe phases, freshly precipitated Fe oxyhydroxides are among the most bioavailable. The aging of freshly formed Fe particulate phases results in decreased bioavailability as more crystalline iron minerals are progressively less soluble in seawater (Raiswell, 2011a; Raiswell, 2011b; Rich and Morel, 1990; Wells et al., 1983; Yoshida et al., 2006). Processes that favour the dissolution of Fe, even if the dissolved Fe formed is then rapidly re-deposited as Fe oxyhydroxides, are therefore a positive influence on Fe bioavailability (Sunda, 2001).

1.3 Dissolved Fe speciation

‘Dissolved’ Fe is defined in marine chemistry operationally as Fe passing through a 0.20 μm (or, typically in older literature, a 0.45 μm) filter. This size fraction will include the Fe(III) ion bound to inorganic ligands, Fe(III) complexed with natural organic ligands, small colloids and nanoparticles (Bruland and Rue, 2001; Raiswell and Canfield, 2012). These different Fe species are difficult to separate via filtration as their size ranges overlap. For this reason the conventional approach of using filtration to define Fe species as ‘dissolved’ is unsatisfactory (Bruland and Rue, 2001). The size distribution of Fe(II) is yet to be described. If Fe(II) exists only as the free (hydrated) ion then it would be expected that all Fe(II) would pass through a 0.20 or 0.01 μm filter. However there is some evidence of Fe(II) existing in non-dissolved phases, for example in sulphur-rich colloids (Yucel et al., 2011).

The low solubility of Fe(III) in seawater means that the concentration of the truly dissolved inorganic Fe(III) species is incredibly low and accounts for <1% of the dissolved Fe measured in the surface ocean (Bruland and Rue, 2001; Waite, 2001). The vast majority of dissolved Fe(III), >99.9% (Gledhill and Buck, 2012), is associated with organic material. However, as will be discussed later, it is important to note that organically associated 'dissolved Fe' includes both organic complexes and colloids (Gledhill and Van Den Berg, 1994; Raiswell and Canfield, 2012; Rue and Bruland, 1995; Wu and Luther III, 1995).

Whilst Fe(II) is much more soluble than Fe(III), Fe(II) is not stable in the presence of oxygen (Millero et al., 1995). Thus the solubility of Fe increases by several orders of magnitude under reducing conditions and this explains the extremely high concentrations of dissolved Fe that can be observed in suboxic or anoxic waters. Under reducing conditions in porewaters dissolved Fe can be on the order of 100 μM and in hydrothermal vent fluids millimolar dissolved Fe concentrations have been reported (Burdige, 1993; de Baar and de Jong, 2001; Douville et al., 2002; Sarradin et al., 2005). On geological timescales, the increased solubility of Fe(II) compared to Fe(III) has resulted in marked changes in the chemical composition of the ocean. Before oxygen was present as a major component in the atmosphere, dissolved Fe concentrations in the open ocean were several orders of magnitude higher than they are at present (Hunter and Boyd, 2007; Saito et al., 2003). The appearance and rise of oxygen concentrations in Earth's oceans and atmosphere is recorded geologically in the deposition of Fe rich 'banded iron formations' in Precambrian marine sediments (Cloud, 1973; Raiswell and Canfield, 2012).

1.3.1 Natural Fe(III) ligands

Natural Fe(III) ligands have been detected throughout the ocean and are a mechanism used to explain how dissolved Fe can exist at much higher concentrations than Fe(III)'s limited solubility in oxic seawater (0.08-0.2 nM) (Hunter and Boyd, 2007; Johnson et al., 1997a; Kuma et al., 1996; Liu and Millero, 2002; Powell and Donat, 2001; Wu et al., 2001). The binding efficiency, or stability constant, of a ligand binding site[1] for a particular trace metal ion (such as Fe(II) or Fe(III)) is defined as K ([Fe-L]/([L]×[Fe^{3+}])), often presented as logK, with larger values of logK representing stronger ligands. Modelling suggests that wherever bioavailable Fe ligands are present in surface marine waters increasing logK, increasing ligand concentration, or reducing ligand photolability will always result in an increased fraction of ‘dissolved’ Fe being bioavailable (Tagliabue et al., 2009). The strength of metal ion-ligand interaction is determined by a number of factors including the charge on the metal ion. The organic interaction of Fe(II) and Fe(III) in natural waters is likely therefore to differ as strong Fe(III) ligand binding sites are likely to be much poorer Fe(II) binding sites and vice versa (Harrington and Crumbliss, 2009).

In the ocean two distinct classes of Fe(III) ligand appear to exist; L_1 and L_2 (Rue and Bruland, 1995). Some ligands, such as siderophores, appear to be produced by open-ocean microorganisms specifically for the purpose of enhancing Fe sequestration and exhibit particularly high affinities for Fe(III) (Butler, 2005; Haygood et al., 1993; Hopkinson and Morel, 2009; Kraemer, 2004; Reid and Butler, 1991; Trick, 1989; Trick et al., 1983). Several organisms incapable of specific siderophore synthesis appear to be equipped to uptake such siderophore bound Fe (Granger and Price, 1999; Hutchins et al., 1999; Maldonado and Price, 1999; Sunda, 2001). L_1 ligands have high Fe(III) stability constants similar to those of siderophores and, consistent with their suggested biological origin, are distributed across the surface ocean. L_2 ligands are weaker, but appear throughout the water column rather than just in surface waters (Cullen et al., 2006; Rue and Bruland, 1995; Rue and Bruland, 1997). Weaker ligands, such as the L_2 class, are less likely to have been synthesized specifically as Fe chelators and may be better considered as incidental Fe ligands. These ligands could be released into the water column through processes such as the grazing of cells, photochemical decomposition of stronger ligands or more generally from decay of dissolved organic material (DOM) (Boyd and Ellwood, 2010).

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[1] Ligands, especially large ligands, may be multidentate. That is that one ligand molecule has multiple binding sites that may have different binding strengths for a specific trace metal.

Whilst microorganisms in the surface ocean can recycle Fe very efficiently (Strzepek et al., 2005), Fe is ultimately removed from the water column predominantly by scavenging onto sinking particles (Boyd and Ellwood, 2010). The particulate Fe pool, whilst less available than dissolved Fe to most organisms, is very large compared to the dissolved pool in the surface ocean (Raiswell, 2011b). Upon the introduction of particles into a water mass, dissolved Fe concentrations are generally subject to a rapid decrease due to scavenging and then steadily increase back to equilibrium concentration (Homoky et al., 2012). Particulate Fe in a water column can therefore be considered to behave as a buffer of dissolved Fe concentrations. Ligands, photochemical mediated dissolution, reductive dissolution and biological activity mediate supply of dissolved Fe from particulate forms (Boyd et al., 2010; Siffert and Sulzberger, 1991; Stumm and Sulzberger, 1992).

1.4 Nanoparticulate and colloidal Fe

When considering the behaviour of ‘dissolved’ Fe in natural waters it is worth noting that much ‘dissolved’ Fe (defined as the Fe present after filtration at 0.45 or 0.2 μm) is in fact colloidal and therefore behaves very differently from truly dissolved metal species.

Despite their similar elemental composition, true complexes and colloids have distinct structures: a complex is a metal centre surrounded by one or more organic ligands[2] which, depending on the stability of the metal-ligand interaction, can be exchanged for other ligands. Unlike complexes, colloids are particles with a distinct surface such that trace metal ions or organic molecules may have very different reactivity at the surface relative to the centre of the colloid (Raiswell, 2011b; Raiswell and Canfield, 2012). This is illustrated by the non-lability of marine colloidal Fe towards competitive ligand exchange, a feature that would not be exhibited by complexed Fe (Fitzsimmons and Boyle, 2014). Size-fractionated studies show that nanoparticulate Fe (small colloids less

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[2] Natural DOM ligands are quite large compared to metal ions therefore, whilst multi-ligand complexes are possible, a 1:1 ligand:metal ratio is generally a reasonable assumption for natural complexes.

than 100 nm in diameter) is in fact more prevalent than organically complexed Fe in marine surface waters (Bergquist et al., 2007; Boye et al., 2010; Wu and Luther III, 1994; Wu and Luther III, 1995; Wu and Luther III, 1996). Ligands are also found in multiple size fractions. L_1 and L_2 ligands are found within both truly dissolved and colloidal size ranges (Boyd and Ellwood, 2010; Cullen et al., 2006; Wu et al., 2001). Hassler et al. (2011) suggest that saccharides, a class of organics with binding constants within the L_2 range, are in fact the dominant organic component in marine Fe(III) colloids. Similarly, in fresh waters Fe(III) appears to exist as a mixture of organic complexes and larger Fe rich colloids which may have surface coatings of DOM (Lofts et al., 2008; Lyven et al., 2003). The enhanced residence time of Fe <0.02 μm compared to 0.02-0.4 μm Fe in surface waters and variation in the depth profiles of these two fractions demonstrates the difference between colloid and complex biogeochemistry (Cullen et al., 2006; Fitzsimmons and Boyle, 2014; Raiswell and Canfield, 2012; Wu et al., 2001; Wu and Luther III, 1994).

The resistance of colloids towards ligand exchange is problematic when conducting titrations to determine Fe ligand concentration and logK values. The difficulty in distinguishing colloidal Fe from complexed Fe may mean that many titration derived ligand concentrations and binding constants are in fact overestimates (Bruland and Rue, 2001; Gledhill and Buck, 2012).

Numerous mechanisms exist that interconvert the particulate, colloidal and truly dissolved pools of Fe in marine waters (Boyd and Ellwood, 2010). Furthermore, similar mechanisms may also transfer Fe ligands between different phases (Boyd et al., 2010). Dissolution of particulate Fe most likely results largely in colloidal, rather than complexed or truly dissolved, Fe species (Wu and Boyle, 2002). However details of the structure and reactivity of colloidal Fe are largely unknown. Organic matter may provide a quasi-uniform coating around nanoparticulate iron oxyhydroxides, or alternatively organic compounds may be intertwined with Fe oxyhydroxides throughout the colloid. The nature of this organic distribution, which may be varied, is the key to determining the surface reactivity of colloids. This is particularly important if Fe(II) exists in a colloidal form (which it certainly does with sulphur and possibly also more generally with DOM) (Rickard and Luther, 2006; Yucel et al., 2011). A surface layer of DOM could protect Fe(II) within a colloid from oxidation or more generally inhibit dissolution of Fe. The possible widespread existence of Fe(II) ligands also affects our understanding of Fe(II) colloid stability. Ligands are generally perceived as a stabilisation mechanism allowing elevated dissolved Fe(III) concentrations to exist (Hunter and Boyd, 2007). However, if Fe(II) ligands exist then they could destabilise Fe(II)-S colloids by favouring the dissociation of Fe(II) from the colloid. Depending on the relative oxidation rates of Fe(II) species, this could be a destabilising process resulting in faster Fe(II) oxidation.

The solid state speciation of Fe in the natural environment is equally as diverse as its dissolved speciation. A series of different mineral phases exist in the natural environment with the mineral ferrihydrite formed first upon the aging of freshly precipitated iron oxyhydroxides. Further crystallisation and aging of natural Fe-rich particulates generally lowers the bioavailability and lability of the Fe present (Raiswell, 2011b; Raiswell et al., 2010). An interesting hypothesis concerns the oxidation state of Fe within the solid phase. The Fe(II) content of the solid phase can be used to identify the source of particulate Fe, but it has also been suggested that it may influence the solubility and bioavailability of particulate Fe (Schroth et al., 2009; von der Heyden et al., 2012). A distinction should intuitively be drawn here between Fe(II) on, or close to, the surface of particles and Fe(II) within the solid mineral matrix. Both instances of Fe(II) may indicate the origin of the particle but, under ambient conditions, only surface Fe(II) would be expected to exert any influence on dissolution processes in lithogenic material.

1.5 The existence of Fe(II) under oxic conditions

The rate of Fe(II) oxidation is dependent primarily on dissolved oxygen concentration, pH and the concentration of other oxidising species such as H_2O_2. Temperature and salinity also have a small effect (Davison and Seed, 1983; Millero, 1989; Millero et al., 1987). Oxidation is rapid in oxic seawater whereas lower pH and suboxic conditions favour higher Fe(II) concentrations. Under anoxic conditions Fe(II) is removed from solution through reaction with sulfide to form FeS_2 and under oxic conditions Fe(II) should rapidly oxidise to Fe(III). High Fe concentrations in low pH, or low oxygen, waters such as porewaters, hydrothermal vent fluids, sub-glacial waters and hypoxic dead zones can be explained by inorganic processes favouring the Fe(II) oxidation state (Landing and Westerlund, 1988; Rickard and Luther, 2007). Fe(II)'s increased solubility thereby allows elevated concentrations of dissolved Fe to occur (Lohan and Bruland, 2008). To some extent we can therefore circumvent discussions about how bioavailable Fe(II) is compared to Fe(III). Increasing total dissolved Fe concentrations through formation of Fe(II) is almost certain to expand the bioavailable Fe pool in surface waters as formation of Fe(II) from any Fe(III) phase rejuvenates labile Fe reversing the processes that lead to precipitation and net removal (Croot et al., 2001; Emmenegger et al., 2001).

Trace amounts of Fe(II) have been measured in surface seawater (Hansard et al., 2009; O'Sullivan et al., 1991; Sarthou et al., 2011). In addition to considerations about Fe(II) oxidation rate, the rate of formation of Fe(II) via several different processes must also be considered in order to explain the observed natural distribution of Fe(II). Photochemistry can produce Fe(II) via a number of distinct pathways (Barbeau, 2006; Garg et al., 2013; Rose and Waite, 2005). In marine surface water photochemistry is the dominant source of Fe(II) with other processes such as biological release (from extracellular reduction, grazing or remineralisation) relatively small, or negligible, by comparison (Croot et al., 2008; Johnson et al., 1994; O'Sullivan et al., 1991; Roy et al., 2008). Modelling suggests that regardless of how bioavailable Fe is characterised, there is a consistent positive correlation between the fraction of dissolved Fe that is bioavailable and irradiance. This is consistent with considering Fe(II) formation to be a solubilisation process (Tagliabue et al., 2009).

In precipitation and other aerosols, low pH and a high photon flux (relative to standing surface water) combine to produce very high dissolved Fe(II) concentrations (Behra and Sigg, 1990; Kieber et al., 2003a; Kieber et al., 2001; Willey et al., 2000; Willey et al., 2005; Zhuang et al., 1995). Sinking particles provide a source of Fe(II) throughout the water column as diffusion of Fe(II) from reducing micro-zones, such as the centres of large carbon-rich particles, has been demonstrated (Alldredge and Cohen, 1987; Balzano et al., 2009). Similarly Fe(II) release from the low pH digestive tracts of organisms is a viable process (Tang et al., 2011). In bottom waters Fe(II) can diffuse from porewaters to overlying waters with rates largely dependent on the dissolved oxygen gradient and biological activity (Homoky et al., 2012). The flux of trace metals from sediments into overlying water can be considered as a mixture of a very slow background rate plus a more intense flux associated with re-suspension events and bioturbation (Aquilina et al., 2014; Roberts, 2012).

Whilst sources of Fe(II) such as photochemistry, biological reduction and diffusion from reducing micro-zones can explain the presence of Fe(II) under oxic, alkaline conditions (such as surface seawater); the measured properties of Fe(II) in such environments have often been found to be inconsistent with that of the Fe(II) free ion. Fe(II) oxidation rates and Fe(II) temporal and spatial distributions hint at the existence of Fe(II) ligands in both fresh and seawater (Breitbarth et al., 2009; Kieber et al., 2005; Okada et al., 2005; Roy and Wells, 2011; Roy et al., 2008; Sarthou et al., 2011; Willey et al., 2008; Willey et al., 2005; Zhuang et al., 1995). The widespread existence of natural Fe(II) organic ligands is highly probable given the non-specificity of bulk natural organic material for particular trace metal ions (Mantoura et al., 1978).

To date, despite the wealth of knowledge about Fe(III) ligands in the marine environment, natural Fe(II) ligands are poorly characterised. Fe(II) ligands' natural concentrations, lifetimes, binding constants and their net effect upon Fe(II) oxidation rate are largely unknown- especially in the marine environment. Determining the speciation of Fe(II) in natural waters is an important task because Fe(II) is perceived as being amongst the most bioavailable forms of Fe (Sunda, 2001).

One key unknown, which at present severely limits our understanding of Fe(II) biogeochemistry, is what the various methods for measuring 'Fe(II) concentration' are actually measuring. Three main analytical approaches have been used to date: chemiluminescence (Croot and Laan, 2002; Jones et al., 2013; King et al., 1995; Ussher et al., 2005), spectrometry using synthetic Fe(II) ligands (King et al., 1991; Stookey, 1970; Waterbury et al., 1997) and a modification of the cathodic stripping voltammetry method used to measure Fe(III) concentrations (Aldrich and van den Berg, 1998; Boye et al., 2003; Gledhill and Van Den Berg, 1994). A three, or even two, method comparison of these different methodologies has yet to be conducted. Given the limitations of each method and the inherent difficulties of trying to determine the in-situ concentration of a transient redox species, it is highly likely that the method used (and the specific set up of a particular investigation with respect to the time delay between sampling and measurement, filtration and sample manipulation) has some influence on reported Fe(II) concentrations. A degree of care should therefore be taken in interpreting any work on Fe(II), particularly where a careful consideration of the methodological limitations is not made.

1.6 Fe(II) Ligands

Organic ligands are widespread in marine waters and exert a large influence on the biogeochemical cycling of Fe and other trace metals (Hunter and Boyd, 2007). Their characterisation has identified a broad range of natural ligands (Gledhill and Buck, 2012; Vraspir and Butler, 2009). The hypothesis that some of these compounds are synthesised specifically to bind Fe(III) is supported by the very high Fe(III) affinities observed for some ligands, such as siderophores, and the confinement of these stronger ligands to surface waters (Hunter and Boyd, 2007). The binding strength of only one such siderophore has been characterised for both Fe(II) and Fe(III) (Brandon et al., 2003). As expected,[3] the siderophore binds Fe(III) much more effectively than Fe(II). One reason why Fe(II) ligands are of specific interest is because of their ability to moderate the oxidation rate of Fe(II). Where this rate of oxidation is slowed (or the rate of formation of Fe(II) is increased), the overall effect is to increase observed dissolved Fe concentrations and thereby to increase the proportion of total Fe that is bioavailable.

LogK values for Fe(II) complexes have been determined for the humic and fulvic fractions of terrestrially derived plant material (Rose and Waite, 2003a; Theis and Singer, 1974). These terrestrially derived ligands have a broad range of ligand binding strengths (Table 1A). There are insufficient data in the literature to discuss the distribution of Fe(II) ligands, but ligands detected in the River Itchen (Southampton, UK) are within the range of logK values quoted for ligands derived from terrestrial plant material (Rose and Waite, 2003b; Statham et al., 2012). Existing data also supports the concept of a decrease in binding constants upon aging of natural material. Whilst a natural reaction (i.e. a chemical change) could in theory either increase or decrease the binding capacity of a ligand for a trace metal ion, the vast majority of changes are likely to result in a lowering of logK. This is because the reactive groups most available to

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[3] A change in the charge on a metal ion alters the strength of metal-ligand binding.

natural biochemical reactions, for example carboxylic acids and amines, are also those that are most able to bind trace metals. Therefore aging of ligands in the natural environment is most likely to lead to a decrease in binding efficiency (Cuss and Gueguen, 2012). Over short time periods the microbial decay of Cu ligands from a vegetative source display this expected overall decrease in logK (Cuss and Gueguen, 2012).

Source	**Log K**	**Ligand origin**
Theis and Singer (1974)	4.0	Tannic acid
Bao and Yu (1987)	3.0-4.9 4.0-8.0	Anaerobically decomposed plant material Soil solutions
Brandon et al. (2003)	12	Fe(III) siderophore
Rose and Waite (2003b)	6.6-10.2 7.5	Leachate from six vegetation types Riverine fulvic acid
Yamamoto et al. (2010)	5.8 4.4	Humic acid compost extract Fulvic acid compost extract
Statham et al. (2012)	7.8-8.8	River Itchen, Southampton
Iawi et al. (2013)	5.9-6.2	Compost extract

Table 1A Natural Fe(II) ligands characterised (in terms of logK) to date. Note the definition of logK varies slightly between studies.

Kranzler et al. (2011) suggest that Fe(III) reduction is an essential part of Fe uptake during Fe acquisition by cells. Such a strategy is thought to be effective when acquiring organically bound Fe(III) (Harrington and Crumbliss, 2009; Hudson and Morel, 1990; Kranzler et al., 2011; Maldonado and Price, 2001). This is consistent with strong Fe(III) ligands having lower affinities for Fe(II) as reduction would result a priori in a weaker organic ligand-Fe bond so that Fe would be more easily assimilated. The same study assumes however that Fe reduction in the bulk medium cannot contribute significantly towards Fe uptake given the short residence time of Fe(II) in an oxic environment and this is inconsistent with the speculated existence of Fe(II) stabilising ligands in seawater (Breitbarth et al., 2009; Kranzler et al., 2011; Okada et al., 2005; Roy and Wells, 2011; Roy et al., 2008; Sarthou et al., 2011; Zhuang et al., 1995). A widespread Fe(III) reductive uptake system for Fe acquisition in marine biota would raise questions about the bioavailability of Fe(II) organic species, as acquisition of such complexes may not be feasible via an Fe(III) reductive uptake system. However, whether or not Fe(II) complexes are available to specific Fe acquisition mechanisms, the stabilisation of Fe(II) itself expands the dissolved pool of Fe and therefore Fe(II) ligands may serve to increase the pool of bioavailable dissolved Fe.

1.7 Estuarine behaviour of dissolved Fe

Whilst dissolved Fe concentrations in freshwater are very high compared to seawater, much of this dissolved Fe is removed during estuarine mixing (Boyle et al., 1977). As salinity increases along an estuary, the repulsion between like-charged Fe containing colloids and complexes in the water column becomes less effective at preventing particle aggregation. Charged particles therefore begin to aggregate and progressively scavenge more Fe from solution resulting in the removal of the vast majority (typically 90-99%) of dissolved Fe from the water mass (Boyle et al., 1977; Mayer, 1982a; Sholkovitz, 1978). This removal process will affect the magnitude of any fresh water dissolved Fe flux into saline waters. The aggregation process has two stages. In the first stage dissolved Fe (i.e. small Fe containing colloids and complexes) and organic carbon rapidly (on a timescale of minutes) aggregate to form large colloids. In the second stage, which is slower and proceeds over several hours, colloids aggregate to form larger particles (Mayer, 1982a). In most temperate and sub-tropical estuaries 90-99% of all dissolved Fe is removed from solution across the estuarine salinity gradient (Table 2). Some experimental factors such as filter size and seasonality may affect the exact dissolved Fe removal factor for a given estuary (Moore et al., 1979; Sholkovitz, 1978; Sholkovitz et al., 1978).[4] A strong temperature dependence on the second stage of colloidal aggregation has been proposed to explain variation between 76 and 98% dissolved Fe removal in the Saco estuary with greater removal in summer than winter (Mayer, 1982a). Similarly, particle size may be a factor in determining the extent of aggregation with smaller size fractions reported to behave much more conservatively

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[4] Older dissolved Fe data are subject to uncertainty due to potential Fe contamination. The effect of low (nM) systematic Fe contamination in samples across the full salinity gradient of an estuary would be to underestimate Fe removal.

than total ‘dissolved’ Fe in several estuaries (Dai and Martin, 1995; Figueres et al., 1978). This may be due to differing behaviour between complexed and colloidal Fe (Krachler et al., 2010).

River/estuary	**Filter size µm**	**Estuarine removal %**[5]	**Reference**
Congo (Zaire), Congo	1.2 0.45 0.22 0.05 0.025	85 75 65 55 50	(Figueres et al., 1978)
Beaulieu, UK	0.45	85	(Holliday and Liss, 1976)
Saco, USA	0.50	76-98	(Mayer, 1982a)
Connecticut, USA	0.4 0.1	94 99	(Hong and Kester, 1985)
Connecticut, USA	1.2	71	(Boyle et al., 1977)
Merrimack, USA	1.2	54-71	
Parker, USA	1.2	66	
Mullica, USA	0.7	96	
Rappahannock, USA	1.2	91	
Rhine, The Netherlands	0.45	94	(Duinker and Nolting, 1976)
Amazon, Brazil	0.45	> 95	(Sholkovitz et al., 1978)
Lena, Russia	0.40	80	(Guieu et al., 1996)
Peconic, USA	0.45	> 90	(Wilke and Dayal, 1982)

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[5] Where estuarine removal is not calculated in the cited source, coastal seawater of S 35.0 and 1.0 nM total dissolved Fe is assumed to be the theoretical estuarine end member.

Ob, Russia	0.40	> 80	(Dai and Martin, 1995)
Yenisey, Russia	0.40	> 80	
Copper River, USA	0.45	85	(Schroth et al., 2014)

Table 2A Estuarine removal of dissolved Fe in different river systems.

The strong non-conservative behaviour of Fe has been observed in many estuaries (Table 2A) and rapid removal is normally evident even at very low salinity (<1) (Holliday and Liss, 1976; Wilke and Dayal, 1982). As a result of this removal process, rivers account for only a small fraction (<3%) of the total filterable (0.45 μm) Fe delivered to the oceans (Raiswell and Canfield, 2012). There are a few reports of estuaries where dissolved Fe behaviour is more conservative (Shiller and Boyle, 1991). It is unclear exactly why dissolved Fe may behave more conservatively in these estuaries, but two plausible explanations are that removal is less efficient under some physical conditions (for example with low initial dissolved and colloidal river water trace metal concentrations or low temperature) or that the exact nature of the organic constituents may be responsible (Gustafsson et al., 2000; Statham et al., 2012). Isolated reports of conservative behaviour do not affect our understanding of river systems delivering dissolved Fe to the ocean as dissolved Fe behaviour in large, moderate to high Fe river systems has been studied at most latitudes and is therefore relatively well understood.

Lowering the concentration of dissolved Fe does not necessarily remove Fe from the water column as in flowing estuarine waters much of the aggregated Fe particulate may remain in suspension (Gustafsson et al., 2000; Hong and Kester, 1985; Mayer, 1982b). The total flux of estuarine Fe into costal waters may therefore be relatively unaffected by flocculation, even if the size distribution of Fe is shifted to larger particle sizes. Either through transport of a fraction of this material laterally offshore (Lam and Bishop, 2008), or re-suspension of sediments, significant quantities of labile particulate Fe of estuarine origin may be delivered to the ocean (Jones et al., 2011). Particulate Fe, although to some extent bioavailable (Hyacinthe et al., 2006), is considered to be directly bioavailable to only a minority of microorganisms (Nodwell and Price, 2001; Sunda, 2001). Suspended particulate does however provide a pool of Fe that is acted on by processes such as microbial activity and photochemistry to release small quantities of dissolved Fe into the water column (Boyd et al., 2010; Stumm and Sulzberger, 1992).

The particulate Fe pool can therefore be considered to act as a buffer of dissolved Fe concentrations: at high dissolved Fe concentrations scavenging of dissolved Fe from solution occurs whereas at very low dissolved Fe concentrations particles are a net source of dissolved Fe (Homoky et al., 2012). Through acting as a source of dissolved Fe, particulate Fe is indirectly bioavailable to species that cannot directly access solid Fe phases.

River water is generally rich in terrestrially derived DOM and this acts as a significant source of trace metal ligands in natural waters (Jirsa et al., 2013; Mantoura et al., 1978; Perdue et al., 1976; Rose and Waite, 2003a). The terms ‘fulvic’ and ‘humic’ acid are often used to distinguish between the fractions of DOM that are soluble and insoluble in water at pH 1 respectively. Terrestrially derived humic and fulvic acids are known to bind different trace metals with varying logK values (Mantoura et al., 1978). Ligand stability constants for Fe(III) are comparable to those of Ca, Mg, Mn, Co, Zn and Cd ions. Only Cu and Hg have markedly stronger interactions with natural DOM (Mantoura et al., 1978). Whilst most DOM behaves conservatively during estuarine mixing, 60-80% of the humic acids are removed via flocculation (Sholkovitz et al., 1978). This may constitute removal of a large fraction of Fe ligands. Humic material however constitutes only a small fraction (3-6%) of total dissolved organic carbon (DOC) and hence DOC behaviour appears to be almost conservative in estuaries (Sholkovitz et al., 1978).

1.8 Summary

The presence of Fe(II) across many fresh and surface marine waters cannot be explained by inorganic processes alone. The apparent stability of Fe(II) concentrations under oxic conditions in some aquatic systems, unless it can be attributed to widespread overestimation of Fe(II) concentrations, strongly suggests the presence of organic Fe(II) binding ligands (Breitbarth et al., 2009; Kieber et al., 2001; Okada et al., 2005; Roy and Wells, 2011; Sarthou et al., 2011; Statham et al., 2012; Willey et al., 2008; Willey et al., 2005; Zhuang et al., 1995). Despite several observations of Fe(II) stability in natural waters (Hansard et al., 2009; Lofts et al., 2008; Sarthou et al., 2011), data on Fe(II) distribution and speciation is lacking compared to Fe(III) or total dissolved Fe. Very few natural Fe(II) ligands have been characterised (Table 1A).

Fresh water sources of Fe to the ocean are disregarded as a significant source of dissolved Fe because of the high efficiency with which flocculation removes dissolved Fe upon mixing between fresh water and seawater. Whilst this process minimises the effect that riverine dissolved Fe inputs have even into coastal waters, other fresh water sources such as rainwater and glacial systems may still be significant sources of bioavailable Fe to shelf seas and possibly the open ocean (Kieber et al., 2001; Lin et al., 2011; Raiswell et al., 2008; Raiswell et al., 2006). Fe(II) speciation in these systems is almost entirely unknown, although the presence of stable relatively strong Fe(II) ligands in rainwater has been suggested (Kieber et al., 2005; Paris and Desboeufs, 2013; Willey et al., 2008) .

DOM is known to affect the solubility of Fe in riverine, estuarine and coastal waters (Batchelli et al., 2010; Chen et al., 2004; Jirsa et al., 2013; Laglera and van den Berg, 2009; Nishimura et al., 2012). Similar natural ligands may be present in other freshwater sources (rain and glacial melt water). Given the ability of DOM to bind many trace metal ions and significantly affect their natural speciation (Cuss and Gueguen, 2012; Mantoura et al., 1978; Nimmo et al., 1989; Perdue et al., 1976; Rose and Waite, 2003a; Sholkovitz and Copland, 1981), DOM is a good candidate to investigate for its ability to bind Fe(II). If terrestrially derived DOM is shown to be a widespread source of Fe(II) ligands then; in addition to the natural concentration and binding capacities of these ligands; their lifetime, distribution and behaviour during estuarine mixing must be determined in order to assess their likely impact on the marine Fe cycle.

1.9 Aims and objectives

The impact of Fe(II) complexation in natural water systems remains, as indicated above, largely unknown. The overarching aim of this thesis is thus to improve our knowledge of this potentially important form of Fe. In addition to providing data on Fe(II) concentrations in natural waters, specific objectives are to:

- Further evaluate the ability of a ligand reverse titration approach to describe Fe(II) speciation in natural waters.

- Determine the concentration and speciation of Fe(II) in fresh water of varying origin and natural DOM content in order to establish whether Fe(II) ligands are present.

- Determine the behaviour of Fe(II) during salinity induced flocculation and thereby assess whether fresh water sources are likely to deliver significant quantities of Fe(II) to marine waters.

- Investigate dissolved Fe and Fe(II) concentration and speciation in rainwater and glacial melt water in order to compare how Fe in these freshwater sources behaves during mixing with seawater.

- Investigate the use of ferrozine as an Fe(II) specific leaching agent for natural sedimentary material.

1.10 Chapter outlines

A brief explanation of why the work in each chapter was conducted and how it contributes to the overall aims of the thesis.

1.10.1 Dissolved Fe(II) in a river-estuary system rich in dissolved organic matter

Almost no data at all exists on freshwater Fe(II) concentrations. Similarly, the estuarine mixing of Fe(II) has not been studied in great detail despite the estuarine mixing of Fe(III) and total dissolved Fe receiving much attention. One of the main reasons for the lack of information on Fe(II) concentrations in natural waters is the lack of any preservation technique. Fe(II) concentrations must be determined shortly after samples are collected and, unlike samples for total dissolved trace metals, cannot be acidified and stored for later analysis. The development of a sensor based upon the ferrozine method for in situ Fe(II) measurements would solve this logistical problem. One of the aims of this work was to test a prototype for such a sensor (Milani, 2013) in a high dissolved organic material (DOM) environment to see how well the sensor would function.

In addition to this, a high DOM environment is an obvious place to look for evidence of Fe(II) ligands. If terrestrial DOM has any impact upon Fe(II) biogeochemistry, then evidence of this should be found in river systems with high dissolved organic carbon (DOC) and in the associated estuaries. The change in Fe(II) concentration with increasing salinity along an estuary is of particular interest in order to determine whether or not this terrestrially derived material continues to affect Fe(II) biogeochemistry in coastal waters as other authors has suggested it does (Breitbarth et al., 2009; Okada et al., 2005; Rose and Waite, 2003a; Zhuang et al., 1995). This location (on the south coast of the UK) is particularly useful as corresponding offshore Fe(II) concentrations are available for coastal seawater (Boye et al., 2003; Ussher et al., 2007).

1.10.2 Dissolved iron(II) ligands in river and estuarine water

Whilst Fe(III)-ligand interactions have been relatively well explored in surface marine waters (Croot and Johansson, 2000; Rue and Bruland, 1995; Van Den Berg, 1995), only one study has attempted to characterise Fe(II) ligands in natural waters (Statham et al., 2012). The ferrozine based titration method used in this study however was flawed (it failed to account for the production of Fe(II) after the addition of ferrozine) and therefore we present a modification to the original method. Furthermore the River Itchen (site of the original study) has an atypically low Fe concentration, a low DOC concentration and the estuary has a very high effluent input which mean that it is not appropriate to extrapolate the earlier results of Statham et al. (2012) elsewhere. We therefore apply our amended ferrozine titration method to a range of temperate and sub-tropical natural river systems in order to determine whether or not there is any strong complexation of Fe(II) in more typical river and estuarine waters.

1.10.3 Glacial flours as a potential source of Fe(II) and Fe(III) to polar waters

Glacial fluxes of Fe are known to be important contributors to the global marine Fe cycle, particularly in the Southern Ocean where in some locations glaciers may be the dominant source of ‘new’ Fe to marine waters (Gerringa et al., 2012; Raiswell et al., 2008). The flux of particulate Fe to the surface marine environment is an order of magnitude greater than the corresponding flux of dissolved Fe. Processes that transfer Fe between the particulate and dissolved pools are therefore important in determining the concentration of dissolved Fe in the surface ocean.

Dissolution of particulate Fe is promoted under acidic conditions, in the presence of light, in the presence of high Fe-ligand concentrations and also in the absence of oxygen. Estimates of the solubility of Fe within natural terrestrial aerosol particulates, the dominant source of particulate Fe to the open ocean, vary widely (Cwiertny et al., 2008; Jickells et al., 2005). A variety of factors contribute to this variability in Fe solubility (Baker and Croot, 2010), but dust source appears to be the most critical factor, particularly where aerosols are affected by anthropogenic emissions.

The release of Fe(II) from biogenic particulate material has been demonstrated (Balzano et al., 2009). Two features of biogenic particles that promote this release are the possibility of an oxygen gradient and the presence of ligands. When the particle size of carbon rich material is large enough to allow a gradient in oxygen concentration to develop between the particle surface and centre, reducing micro-zones can form. This creates a low oxygen, high organic microenvironment within which Fe dissolution is much more favourable than the surrounding seawater. Diffusion of dissolved Fe from these micro-zones can then act as a measurable source of Fe into the water column (Alldredge and Cohen, 1987). A similar process of Fe dissolution can occur from the low pH environment within the digestive tracts of microorganisms (Tang et al., 2011). Similarly, the presence of ligands (derived from the same carbon-rich material) could promote this release.

Release of Fe(II) from lithogenic particles with low organic carbon content would be more surprising, but could be explained by the presence of concentrated areas of organic carbon on the particle surface. Glacial flour is fine particulate material delivered to the ocean at high latitudes both via meltwater plumes and as an aerosol. Aerosol fluxes of this particulate material are particularly of interest in south Alaska and Iceland as movement of such dust plumes offshore from these regions could deliver Fe to the Fe limited water of the sub Arctic North Pacific and the seasonally Fe stressed North Atlantic respectively (Bullard, 2013; Crusius et al., 2011; Prospero et al., 2012). Several dissolution processes, specifically reductive dissolution and photochemical reduction, involve the formation of dissolved Fe(II) from colloidal or surface particulate Fe(III). In oxic surface seawater it is widely assumed that this Fe(II) exists only for a very short period of time (seconds) before oxidation to Fe(III) occurs. Our experiments here essentially probe whether or not the composition of glacially derived particulate material affects the rate at which dissolution occurs in the presence of an Fe(II) binding ligand.

1.10.4 Fe(II) in rainwater

Work presented in this chapter was conducted with the MACRL (Marine and Atmospheric Chemistry Research Laboratory) research group at the Department of Chemistry and Biochemistry, University of North Carolina Wilmington, USA. The MACRL group has an on-going continuous program of rainwater collection and analysis for a range of chemical parameters. All analytical work conducted was therefore done in accordance with standard procedures used within the group to maintain comparability with the significant (>20 year) historical record available for the MACRL Wilmington rainwater collection site. This historical record is a useful asset for assessing the effect of changing air quality upon trace metal concentrations and behaviour within rainwater.

Kieber et al. (2005) have suggested that some organic components within rainwater are capable of retarding Fe(II) oxidation. This stabilisation has implications for the delivery of Fe into marine surface waters as stabilising the Fe(II) present within rainwater, even after mixing with seawater (Kieber et al., 2001; Willey et al., 2008; Willey et al., 2000), impedes the precipitation of Fe as Fe(III) oxyhydroxides. Rainfall thereby becomes a very efficient delivery system of bioavailable Fe to surface waters. An important question in order for the effect of this stabilisation mechanism to be assessed is why this stabilisation appears to occur only in some, rather than all, rainstorms at this location. In addition to expanding the historical record of Fe rainwater concentrations at this location, the main aim of this work was to determine whether any evidence of Fe(II) stabilisation was still present in North Carolina rainwater.

2. Dissolved Fe(II) in a river-estuary system rich in dissolved organic matter

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2.1 Abstract

Reduced iron, Fe(II), accounts for a significant fraction of dissolved Fe across many natural surface waters despite its rapid oxidation under oxic conditions. Here we investigate the temporal and spatial variation in dissolved Fe redox state in a high dissolved organic matter (DOM) estuarine system, the River Beaulieu (Hampshire, UK). We couple manual sample collection with the deployment of an autonomous in situ analyser, designed to simultaneously measure dissolved Fe(II) and total dissolved Fe, in order to investigate processes operating on the diurnal timescale and to evaluate the performance of the analyser in a high DOM environment.

Concentrations of dissolved Fe available to the ligand ferrozine are elevated (relative to other temperate rivers) throughout the estuary (up to 21 μM in fresh water) and notably higher than those previously reported likely due to seasonal variation. Fe(II) is observed to account for a large, varying fraction of the dissolved Fe available to ferrozine (25.5 ± 12.5%) and this fraction decreases with increasing salinity. We demonstrate that the very high DOM concentration in this environment and association of this DOM with dissolved Fe, prevents the accurate measurement of dissolved Fe concentrations in situ using a sensor reliant on rapid competitive ligand exchange.

2.2 Introduction

Fe(II) has been reported to account for a large fraction of dissolved Fe in a range of both fresh and marine waters (Hong and Kester, 1985; Landing and Westerlund, 1988; Sarthou et al., 2011). The dominant source of Fe(II) in surface waters is normally photochemical (Collienne, 1983; Croot et al., 2008; Roy et al., 2008). Despite its presence in marine and fresh surface waters, and its greater solubility than Fe(III) (Shaked and Lis, 2012), little information is available on the distribution and speciation of dissolved Fe(II). As formation of Fe(II) is generally represented as a poorly characterised solubilisation process (Emmenegger et al., 2001), this mechanistic gap in our knowledge may impede the development of better global ocean models (Tagliabue and Voelker, 2011).

During estuarine mixing, dissolved Fe is known to be strongly non-conservative (Boyle et al., 1977). However, few studies have distinguished between the estuarine mixing of dissolved Fe(II) and total dissolved Fe. Hong and Kester (1985) demonstrated that oxidation of Fe(II) is not a significant contributor to dissolved Fe removal at low salinities. Given the importance of humic materials in initiating flocculation and the significant difference in the metal-ligand binding preferences of the two redox states of Fe (Sholkovitz et al., 1978), the question is therefore raised as to whether the ratio of dissolved Fe(II):Fe(III) present in riverine waters is maintained during estuarine mixing and delivery to the ocean. If so, estuaries would be a source of Fe(II) to coastal waters.

Measuring the concentration of Fe(II) during estuarine mixing in a high DOM estuary will establish whether or not Fe(II) is removed similarly to Fe(III) in the presence of high concentrations of natural organic compounds. Coastal waters are known to contain organic material that can stabilise higher Fe(II) concentrations than would be possible inorganically under oxic conditions (Okada et al., 2005; Rose and Waite, 2003a; Zhuang et al., 1995). If estuaries are a source of such material we would expect to see a relatively high fraction of dissolved Fe present as Fe(II) throughout estuarine waters. The main objective here was therefore to use the Beaulieu (Hampshire, UK) as a model of a high DOM river to investigate the forms of dissolved Fe present and to see how Fe(II) concentration changes during estuarine mixing.

2.3 Sample collection and methods

2.3.1 The River Beaulieu

The River Beaulieu drains the New Forest (Hampshire, UK) and thus contains water primarily from exposed heathland and some mixed coniferous/deciduous forest. The river water has a near neutral pH (6.5-7.8), high dissolved organic carbon (DOC, 250-1800 μM) and high total dissolved (< 0.45 μm) Fe (1-8 μM) content (Fang, 1995; Holliday and Liss, 1976; Jones, 1993; Moore et al., 1979; Smith, 1995).[6]

2.3.2 Sample collection

All sampling apparatus and containers for trace metal work were filled with 3 M HCl (reagent grade), submerged in a 3 M HCl acid bath for in excess of one week, rinsed three times with deionised water (MilliQ, 18.2 MΩ·cm) and then stored in sealed plastic bags (rinsed with deionised water) until required. Filters were rinsed with 0.1 M HCl, followed by deionised water prior to use. Laboratory sample handling was conducted under a class 100 laminar flow hood.

All river water samples (and the estuarine 0.0 salinity (S) end member) were collected from King’s Hat footbridge on the River Beaulieu (50° 50’ 46” N, 001° 27’ 17” W). A diurnal study on river water at King’s Hat footbridge was conducted on 17 December 2012 and an estuary transect was conducted on 15 January 2013 from 50° 46’ 43” N, 001° 23’ 43” W to 50° 49’ 9” N, 001° 27’ 2” W covering the salinity range 0.0-32.0. Estuarine samples of S > 15 were collected on-board RV Bill Conway. Seawater was pumped through a peristaltic pump continuously along 10 m of acid washed LDPE tubing into a 1 L container (LDPE, Nalgene), in which salinity and temperature were continuously measured, and sub-sampled as necessary. Samples S < 15 were collected

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[6] See supplementary information for a summary of historical dissolved Fe values.

from a small boat by submerging a 1 L container (LDPE, Nalgene) upstream of the boat and then sub-sampling as necessary. River water samples were collected by hand with a 0.5 L container (LDPE, Nalgene) mounted in a plastic holder on a metal-free 2 m long pole. All water samples were filtered at the sampling site (0.2 μm). Samples for Fe(II) analysis were then immediately put into Al foil wrapped vials (to exclude light) containing ferrozine and mixed by shaking. Filtered water was retained for DOC analysis in combusted glass vials and acidified by the addition of 100 μL 6 M HCl (reagent grade) per 20 mL. Porewater samples from the riverbank at King’s Hat footbridge were collected in situ using Rhizon samplers (Rhizosphere) with a mean 0.15 μm pore size. After extraction porewater was immediately dropped into pre-prepared vials containing ferrozine to minimise any sample oxidation. Dissolved oxygen samples were collected by gently submerging Winkler bottles under the water surface, and then storing them submerged after reagent addition and mixing. 1.0 L bulk water samples were returned to the laboratory (within 4 hours of collection) for pH measurement.

A clean, open HDPE 10 L container was gently submerged and partly filled (10 cm depth) with surface river water at King’s Hat. This water was then left to stand from sunrise for 4 hours exposed to full sun in an unsheltered location adjacent to the riverbank (approximately 40 m from the main river channel). Temperature, irradiance and pH were recorded. 5 mL aliquots were taken at circa 10 minute intervals using a syringe for dissolved Fe and dissolved Fe(II) determination using ferrozine.

2.3.3 Experimental methods

Except where stated, all chemicals were used as purchased from Sigma Aldrich. Ligand available reduced iron (Fe(II)) concentrations were determined in 10 mL filtered (0.2 μm) water samples after the addition of 100 μL freshly prepared ferrozine reagent consisting of 10 mM ferrozine (‘for spectrochemical determination of Fe’) in 0.1 M ammonium acetate buffer (prepared from a stock made by bubbling electronic grade (> 99.9995% purity) ammonia gas through Romil SpA grade glacial acetic acid). Absorbance was measured at 562 nm as described by Waterbury et al. (1997), but with a longer sample/reagent equilibration time of 7-8 hours (see Section 2.4). Corrections were made to account for post-reagent addition Fe(III) reduction (see Section 2.4) and also for background absorbance at 562 nm as described by Waterbury et al. (1997). Interference from dissolved Cu and Co can lead to overestimation of Fe concentrations using ferrozine (Stookey, 1970). The concentration of these metals was not measured here, but their concentration in the River Beaulieu and its estuary has previously been shown to be consistently low compared to the Fe concentrations reported here (data shown in supplementary material). To determine total ligand available dissolved Fe (DFe), 100 µL ferrozine reagent and 500 µL of 10 mM ascorbic acid (trace metal grade) were added to 10 mL samples followed by storage overnight. For low DFe concentration waters (S > 24) absorbance was measured with a 250 cm 3000 Series (World Precision Instruments) Liquid Waveguide Capillary Cell (LWCC) connected (Fig. 1B) to a USB4000 Fiber-optic Spectrometer (Ocean Optics) and a LS-1 tungsten halogen light source (Ocean Optics) (Waterbury et al., 1997). This apparatus was assembled and used under a class 100 laminar flow hood.[7]

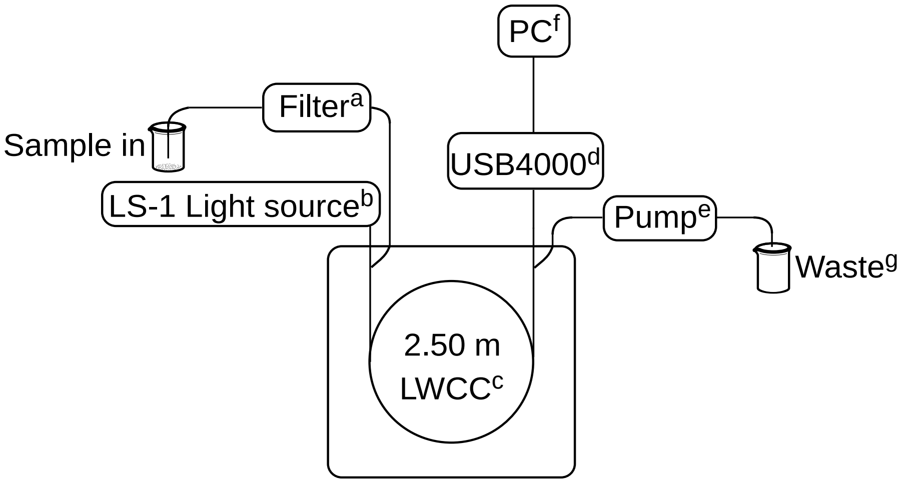

Fig. 1B. Liquid Waveguide Capillary Cell (LWCC) used to measure dissolved Fe(II) concentrations, (a) 0.2 µm filter (Millex-GS 33 mm), (b) LS-1 tungsten halogen light source, (c) 3000 Series LWCC, (d) Fiber-optic Spectrometer, (e) peristaltic pump, (f) SpectraSuite software (Ocean Optics) and (g) waste container.

A 1 mM Fe(II) stock was prepared by dissolving ammonium Fe(II) sulphate hexahydrate (trace metal grade) in 0.1 M hydrochloric acid (trace metal grade) containing 10 µM hydrogen sulphite (laboratory grade). This was diluted to give working standards. The LWCC produced a linear response to dissolved Fe standards

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[7] Specific operational details describing the set up and use of the LWCC are included in the supplementary material.

(n = 11) between 15 and 200 nM ($R^2 > 0.997$). Samples with a higher absorbance than the linear response range of the spectrometers were diluted by the addition of deionised water. For high DFe concentrations (S < 24) a 1 cm glass cuvette in a U-2800 UV-Vis spectrophotometer was used. The U-2800 produced a linear response ($R^2 > 0.999$) to dissolved Fe standards (n = 10) between 0.4 and 70 μM. A subset of 10 mL samples, 12 standards, procedural blanks and a certified reference material (SLRS-5) for inductively coupled plasma mass spectrometry (ICP-MS) analysis were acidified after filtration by the addition of 1 mL 6 M HNO_3 (trace metal grade), double bagged and then refrigerated at 3 °C for one month prior to analysis on an Agilent 7500cx series ICP-MS with an Agilent auto-sampler. Dissolved oxygen was determined via Winkler titration (Carpenter, 1965). DOC was determined by high temperature combustion analysis on a TOC 5000-A (Shimadzu) as per Farmer and Hansell (2007). Irradiance was recorded in situ at ground level using a LI-210SA photometric sensor attached to a LI-1400 Datalogger (Licor). The pH of river water (and standard solutions) was measured using a glass pH electrode (Unitrode 6.0258.010 with PT1000 temperature sensor, Metrohm) after 1 L water samples were returned to the laboratory. Half-lives of Fe(II) with respect to oxidation were estimated in aerated Al foil wrapped vials of filtered river water, or Fe(II) spiked deionised water. Fe(II) concentration was monitored using the ferrozine method on aliquots collected at time intervals (beginning with 0.5 minute intervals and increasing to 15 minute intervals) over a 3 hour period.

Dissolved Fe in standard river water reference material SLRS-5 was measured at 1.53 ± 0.02 μM (ferrozine method) and 1.57 ± 0.004 μM (ICP-MS) compared to a certified value of 1.63 ± 0.10 μM. Triplicate procedural blanks were found to have 2.1 ± 2.7 nM dissolved Fe (ICP-MS) and were below the detection limit (4.0 nM) of the LWCC (ferrozine method).

2.3.4 Determination of Fe(II) and DFe using ferrozine

One problem with the use of ferrozine and a reducing agent to quantify dissolved Fe is the potential for incomplete reduction of strong organic-Fe complexes or colloidal Fe (Luther et al., 1996). DFe determined after addition of ferrozine and reductant is therefore likely to be less than total dissolved Fe determined after acidification in most natural waters, particularly those with high DOM (Box, 1984).

A further issue is that the measurement of Fe(II) concentrations in natural waters is particularly difficult because of the rapid oxidation of Fe(II) at ambient pH and the potential for post-sampling artefacts (Croot and Hunter, 2000; Shapiro, 1966). As with DFe, if Fe(II) is present as strong organic complexes or colloids, estimates of Fe(II) concentration obtained via the ferrozine method are also likely to be underestimates. Acidification (which would improve recovery of Fe from colloidal and complexed phases) is not appropriate for Fe(II) analysis as it leads to overestimation of Fe(II) by favouring Fe(III) reduction (Pullin and Cabaniss, 2001; Shapiro, 1966). Here, we therefore opted to present Fe(II) data alongside DFe data also determined via ferrozine to ensure a consistent analytical approach. Both Fe(II) and ligand available dissolved Fe concentrations are therefore likely to exclude some colloidal and/or strongly complexed Fe in Beaulieu waters.

The main potential artefact when measuring dissolved Fe(II) at river water pH (circa 6 in the Beaulieu) using ferrozine is the overestimation of Fe(II) concentrations due to some reduction of Fe(III) after the addition of reagents (Cowart et al., 1993; Murray and Gill, 1978; Waterbury et al., 1997). If dissolved Fe(II) in a natural water sample were present only as ‘free’ Fe(II), the time required for complete complexation by ferrozine would be minimal (seconds-minutes) and therefore a slow Fe(III) reduction process, occurring after ferrozine addition, would not produce a significant artefact in measured Fe(II) concentration. Natural organic Fe(II) ligands may be present in seawater, but appear to be either too weak, or too low in concentration to affect the recovery of Fe(II) standard additions via a complexometric method (Croot et al., 2007; Croot and Hunter, 2000).

In DOM rich natural waters, such as the River Beaulieu and its estuary, Fe(II) may be present as a range of organic complexes or colloids that will equilibrate more slowly with ferrozine and result in asymptote graphs of absorbance (proportional to ferrozine complexed Fe(II)) against time (Box, 1984; Rose and Waite, 2003b). There is therefore a compromise to be made between allowing time for ferrozine to complex Fe(II) associated with colloids/complexes and minimising the extent of undesired Fe(III) reduction. A short mixing and equilibration time is only likely to bind ‘free’, or weakly complexed, Fe(II) and thus underestimate the true concentration of Fe(II).

After the addition of ferrozine reagent, ‘undesired’ Fe(III) reduction to Fe(II) is known to be faster when samples are exposed to light and at lower pH (Box, 1984). Two necessary practical precautions are therefore the storage of samples in the dark after collection and the maintenance of a near-neutral pH (6-8) (Pullin and Cabaniss, 2003). Ferrozine concentration may also be an important factor. Shaked et al. (2004) suggest that the rate of Fe(III) reduction becomes significant above around 400 μM ferrozine- only 100 μM is used here. Post-reagent Fe(III) reduction however has been found to be more pronounced in the presence of organic material and therefore, in DOM rich Beaulieu water, 100 μM ferrozine may still be sufficient to induce significant Fe(III) reduction (Hudson et al., 1992; Langford et al., 1977). To estimate the extent of post-reagent Fe(III) reduction in River Beaulieu water, a series of tests were conducted to monitor the change in measured Fe(II) concentration with time after reagent (ferrozine) addition. Trials were conducted where ferrozine concentration and salinity were varied. The mechanism of ferrozine induced reduction is not clear. We assume that the concentrations of ferrozine and any other species that may affect the rate of ferrozine induced Fe reduction, such as reducible Fe(III), are not depleted significantly over several hours after reagent addition and also that any increase in the concentration of Fe(II) measured after 150 minutes is solely due to ongoing reduction of Fe(III).

Across all trials (n = 16) the increase in Fe(II) concentration with time (determined between 150 and 500 minutes after adding ferrozine reagent, where absorbance increased linearly with $R^2 > 0.98$ for all graphs) varied from 0.2-1.8 nM minute^{-1}. Slightly increasing the ferrozine concentration, to 125 or 150 μM, did not result in a significantly increased accumulation of Fe(II). Seven trials (all using 100 μM ferrozine) with varying salinity (between 0.0 and 15.8) showed no significant variation with a mean Fe(II) concentration increase of of 1.6 ± 0.2 nM minute^{-1}. A lower accumulation of Fe(II) with time both above this salinity range and in a fresh water sample (S 0.0) with low, < 500 nM, DFe (0.3 nM minute^{-1}) was likely due to lower Fe(III) activities.

Using 100 μM ferrozine in high DFe (μM) River Beaulieu water we would therefore expect Fe(III) reduction to cause an overestimation of ferrozine labile Fe(II) by approximately 2 nM after 1 minute, 20 nM after 10 minutes and 100 nM after 1 hour. There are some limitations to this approximation. Some of the Fe(III) species present as a heterogeneous mixture in river water (colloids, complexes and dissolved species) are likely to be more readily reduced to Fe(II) than others. If some Fe(III) species are reduced much more rapidly than the mean rate of reduction for all reducible Fe(III) species, our estimates of post reagent Fe(II) accumulation will be an underestimate. Error bars in reported Fe(II) concentrations therefore allow for a 20% error in the estimated quantity of Fe(II) accumulated from Fe(III) reduction that we use to correct raw Fe(II) concentrations.

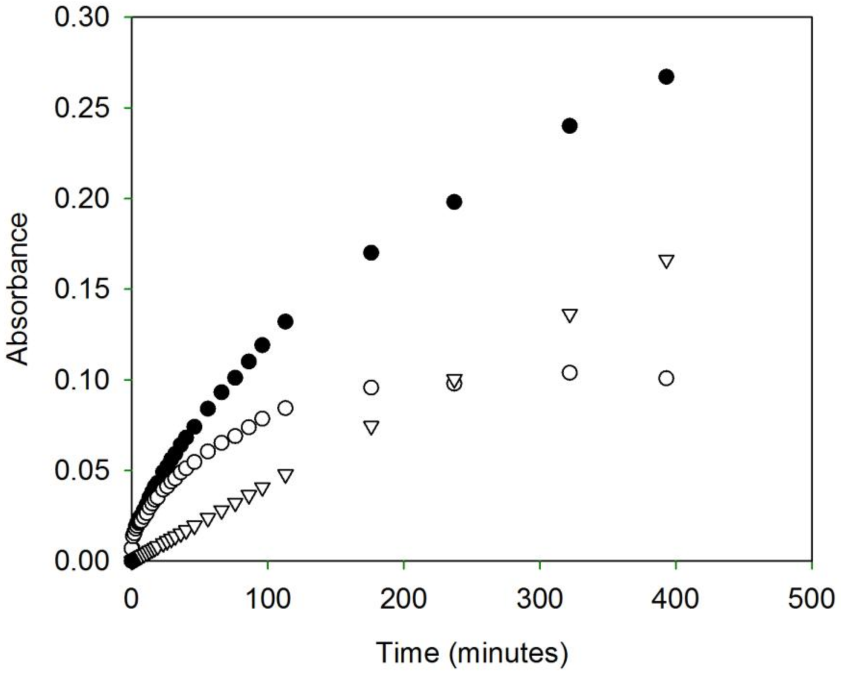

Fig. 2B. The increase in absorbance, relative to a ferrozine free control, after ferrozine reagent is added to a filtered River Beaulieu water sample (filled circles). By determining the extent of ferrozine induced Fe(III) reduction to Fe(II), the absorbance at any time can be split into the estimated absorbance arising from ferrozine reduced Fe(III) (open triangles) and that from complexation of Fe(II) already present within the sample (open circles).

The balance of Fe(III) reduction by ferrozine and the complexation of Fe(II) by ferrozine in the presence of natural complexing organics is shown in Fig. 2B. Deducting the accumulated Fe(II), produced via ferrozine induced reduction of Fe(III), from the measured Fe(II) concentration allows calculation of the ‘true’ ferrozine labile Fe(II) concentration in the natural sample (Fig. 2B). We have therefore corrected our raw measured Fe(II) concentrations by deducting our estimate of Fe(II) accumulated from the reduction of Fe(III) after reagent addition. The mean difference between raw measured Fe(II) and corrected Fe(II) for all estuarine samples is 13%.

2.3.5 In situ analyser[8]

In order to investigate short term temporal changes in dissolved Fe concentration, a recently developed in situ analyser for the simultaneous detection of dissolved Fe(II) and DFe in natural waters was deployed (Milani, 2013).[9] The autonomous analyser is a portable device (15 cm x 15 cm x 50 cm) based on lab-on-a-chip technology. It uses microfluidic architecture and an inbuilt spectrometer (a similar model has been developed for nitrate/nitrite analysis by Beaton et al. (2012)). The design minimises reagent volumes and power consumption. The sensor is mounted in a sealed, anodised Al container that can be completely submerged in fresh or salt water. Its Li ion battery (Lipo 11.1 V 10 Ah 31207-01, Tenergy Corporation, USA) can power the device for over 24 hours of autonomous operation.

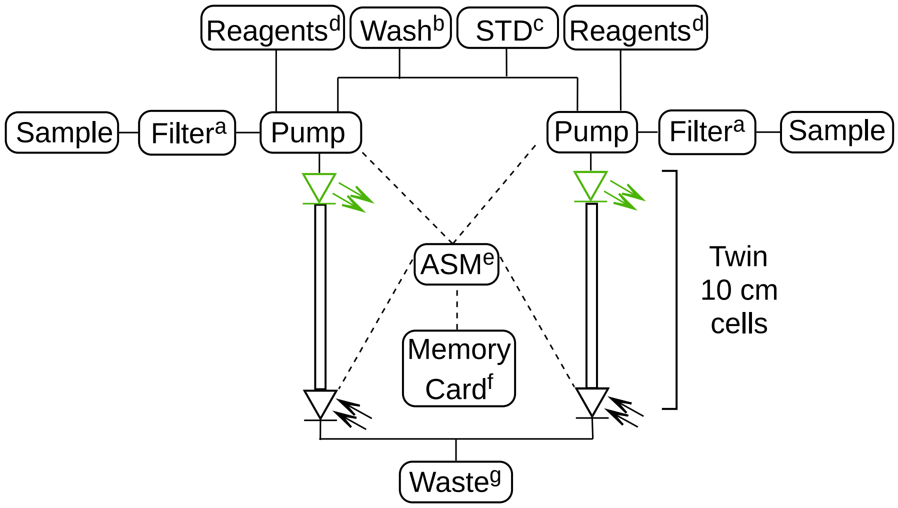

Fig. 3B. Dissolved Fe sensor, (a) 0.2 µm filters (Millex-GS 33 mm), (b) deionised water, (c) 2 standard solutions, (d) independent reagent lines to each 10 cm cell (one for

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[8] A. Milani was entirely responsible for all aspects of the sensor design, deployment and the collection of data using it.

[9] More detailed technical specifications are included in the supplementary material.

ferrozine only, the other for ferrozine with reductant), (e) Autonomous State Machine operating a micro-controller, (f) 2 GB flash memory card and (g) retained waste.

The analyser (Fig. 3B) miniaturises the colorimetric ferrozine method for the detection of dissolved Fe(II) and DFe (via reduction with 0.1 M ascorbic acid) in a similar approach to that used by Sarradin et al. (2005) and Vuillemin et al. (2009). In the microfluidic chip samples and reagents are injected by a custom-made pump, mixed in the optical cell channels and finally the absorbance of the resulting colour at 562 nm is measured in a miniaturised 10 cm long, 600 μm wide cell engraved into the chip. The chip has two independent manifolds for reagent/sample mixing, each with separate reagent lines and 10 cm cells, such that dissolved Fe(II) and DFe can be measured simultaneously. The total internal volume of the entire chip system is 50 μL. This minimises reagent use and increases the number of samples that can be collected on a single deployment without changing the inlet filter or waste bag. Each engraved 10 cm cell has its own green light emitting diode (B5b-433-20, Roithner LaserTechnik, Austria), with peak emission at 572 nm, and photodioide detector (TSL257, TAOS Inc., USA), capable of internal amplification. The analyser is controlled by a custom-made electronics package. Low-level operation software allows the user to define the sampling routine and to control in real time the sensor operations. Collected data is stored on a fitted memory card.

One channel, for dissolved Fe(II), was connected to mixed ferrozine/buffer reagent. The other channel, for DFe, was connected to ferrozine/buffer reagent with ascorbic acid added. The two reagent solutions (10 mM ferrozine in 2 M acetate buffer with, and without, 0.1 M ascorbic acid added), two Fe standard solutions and waste were all stored in 150 mL Flexboy bags (Satorius, UK, ethylene-vinyl-acetate/ethylene-vinyl-alcohol) held on a Ti bracket outside the Al sensor housing. The analyser was set to perform an in situ calibration from on-board standards once deployed and subsequently to collect both DFe and Fe(II) samples every 5 minutes. A filter (0.2 μm, Millex-GS 33 mm) was fitted to each intake line. After every 10 river water measurements, deionised water was injected into the manifold to assess the 100% transmission signal and thus compensate for any drift in the system. A background absorbance measurement was also made (and automatically corrected for), by injecting filtered water into the 10 cm cells without ferrozine reagent.

The operational range of the sensor depends on the injection routine. Decreasing the sample to reagent ratio shifts both the upper and lower detection limits to higher Fe concentrations. The carrying of on-board standards allows rapid re-calibration following changes to the injection routine. If standards were allowed to fill the 10 cm cells (maximising the signal peak with a sample:reagent ratio of 10:1) then the limits of detection were 27 nM for Fe(II) and 35 nM for DFe with a linear response of $R^2 = 0.951$ (n = 5) from 30 nM to 2.0 μM. The reproducibility of standard peaks was excellent: 50 deionised water readings produced a raw peak of 3.1256 ± 0.0004 V, 50 readings of an on-board 1.0 μM Fe standard produced a mean peak of 1.1102 ± 0.0035 V (a lower voltage corresponds to greater absorbance and higher dissolved Fe concentrations). For the injection routine used here, a linear calibration ($R^2 > 0.966$, n = 3) was achieved using on-board standards and deionised water. The upper limit of the operational range tested with dissolved Fe standards was in excess of 50 μM and the limit of detection below 750 nM. However, the upper limit of the operational range may be subject to limitations in high DOM environments where some dissolved (0.2 μm) Fe is not readily complexed by ferrozine (see Section 4.1).

2.4 Results

2.4.1 Temporal and spatial variation in riverine water

Dissolved oxygen in 10 °C river water averaged 370 ± 20 μM (calculated saturation at 10.0 °C is 352 μM). Diurnal changes in irradiance, DFe and Fe(II) concentrations (17 December 2012), as determined manually and using an in situ analyser, are shown in Fig. 4B. DFe and Fe(II) concentrations determined manually throughout the day had mean values (and standard deviations) of 20.1 ± 0.40 μM and 3.96 ± 0.87 μM respectively. Over the sampling period, pH increased from 6.4 to 6.6 and water level fell by 10 cm. Irradiance peaked around midday, but was very irregular due to the partially shaded woodland location. A small decrease on the order of 1 μM over the eight-hour sampling period was evident for manually measured values of DFe (Fig. 4B). The manually measured concentration of Fe(II) decreased more significantly (by > 3 μM). A similar trend is evident in the sensor data, though the concentrations of both DFe and Fe(II) reported by the sensor[10] are consistently much lower (Fig. 4B).

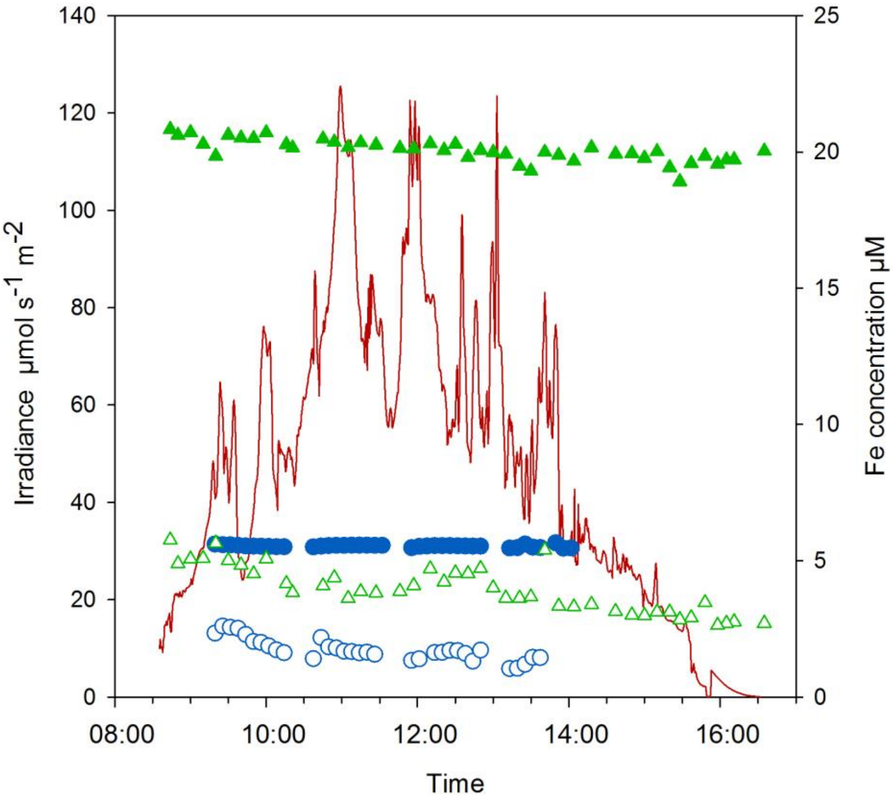

Fig. 4B. Diurnal changes in riverine DFe (filled shapes) and Fe(II) (open shapes) measured by manual sample collection (triangles) and deployed sensor (circles). Irradiance, measured adjacent to the riverbank, is shown in red. Data collected on 17 December 2012 at King's Hat footbridge.

Changes in Fe(II) concentration for an open container of river water exposed to ambient light, close to the river water collection site, are shown in Fig. 5B. Under these non-dynamic conditions the depth of water (10 cm) was sufficiently shallow that incident light would not be heavily attenuated. An increase in Fe(II) concentration, from 4.3 to 5.4 µM over two hours, was measured in this isolated river water. A small change in temperature (3.6 °C at 07:56 decreasing to 2.6 °C at 08:51, then rising to 3.9 °C by 10:32 with solar heating) occurred over the same time period, whilst pH remained constant at 6.4. Water collected at the same time, but stored in the dark in an Al foil wrapped container (1 L LDPE Nalgene), showed a decrease in Fe(II) concentration of 0.9 µM over 2 hours. DFe was subject to some variation (light mean 12.5 ± 0.6 µM,

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[10] This data was collected and formatted as a time series entirely by A. Milani.

n = 24; dark mean 12.6 ± 0.4 µM, n = 6), likely due to the lack of mixing, but did not show a significant trend.

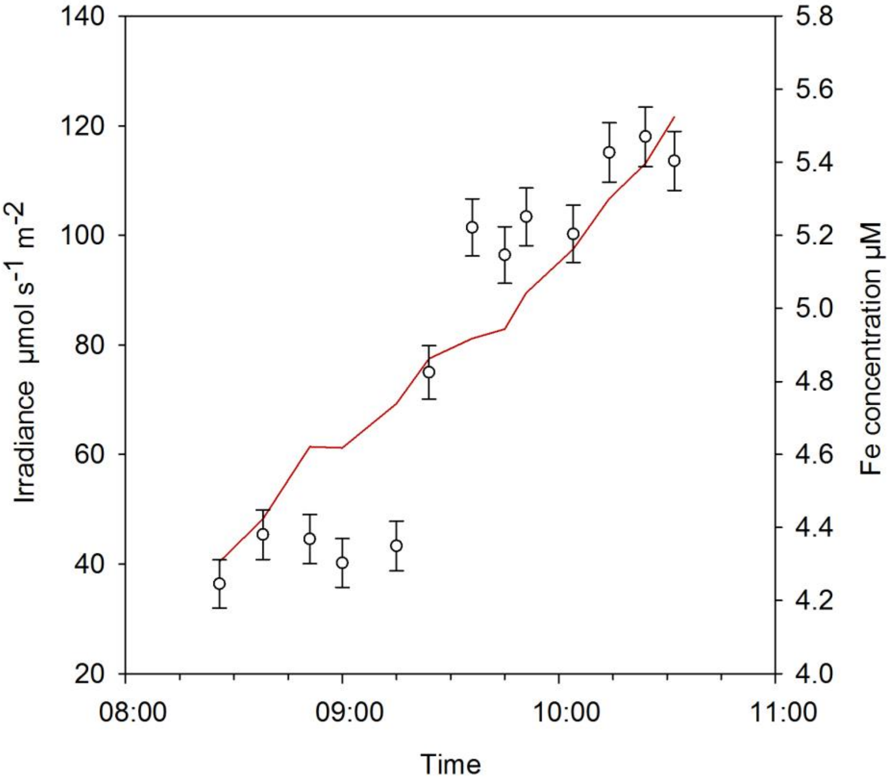

Fig. 5B. Fe(II) concentration (open circles) in an open HDPE container (10 L), filled 10 cm deep with surface river water, upon exposure to sunlight after sunrise (07:44) on 28 January 2013. Irradiance, measured adjacent to the container, is also shown (red line). Error bars include the standard deviation of triplicate measurements (circa 3%) and the maximum plausible error (20%) in the deducted quantity of Fe(II) to correct the measured Fe(II) concentrations for ferrozine induced Fe(III) reduction.

Surface water samples across a 100 m line, running at right angles to the river at King’s Hat footbridge, had DFe from 11.4 to 20.5 µM and Fe(II) from 2.9 to 14.0 µM (Table 1). Dissolved oxygen varied from 220 to 380 µM. Pore water samples (n = 5), collected from surface sediment on the same riverbank site at King’s Hat, averaged 727 ± 292 µM Fe(II).

The half-life of Fe(II) in a spiked deionised water solution was determined to be 10 minutes at pH 7.2 (oxygen saturated), close to the calculated value of 14 minutes at pH 7.2 (Davison and Seed, 1983). Fe(II) in 0.2 µm filtered River Beaulieu water (pH 7.6), subject to oxygen saturation, exhibited an apparent half-life of 370 minutes, much longer than the predicted half-life of 2.3 minutes at pH 7.6 (Davison and Seed, 1983).

Distance from centre of river (m)	DFe (μM)	Fe(II) (μM)	Dissolved oxygen (μM)	DOC (μM)
30 W	13.3	5.7	*ND*[b]	2900
10 W	12.7	7.5	220	1300
2 W	17.6	3.2	360	860
2 E	20.5	2.9	380 ± 30[a]	810 ± 40[c]
8 E	17.0	4.1	290	1700
10 E	19.7	3.4	260	1700
45 E	15.9	14.0	*ND*[b]	1600
50 E	13.9	7.1	*ND*[b]	2200
50 E	11.4	5.6	*ND*[b]	4300

Table 1B. Surface water samples collected along a line perpendicular to the River Beaulieu at King's Hat footbridge, 11:00 28 January 2013. Distance from centre of main river channel denoted metres west (W) or east (E). [a] n = 5, [b] ND 'not determined' due to shallow (< 5 cm) depth of water, [c] n = 3.

2.4.2 Estuarine mixing

DOC in the river end member (15 January 2013) was 2480 ± 60 μM. The observed DFe concentrations for both our cruise end member (15 μM, Fig. 6B) and diurnal deployment (20 μM, 17 December 2012, Fig. 4B) are high compared to previously reported values (Fang, 1995; Holliday and Liss, 1976; Moore et al., 1979). Of 21 DFe concentrations in the River Beaulieu (mean 10 μM, range 1-21 μM), measured between November 2012 and November 2013, the highest values were in late December and early January corresponding to the highest observed water levels.[1] For previously reported Beaulieu freshwater DFe concentrations (February 1974 [7.6 μM]; February 1976 [3.5 μM]; March [2.7 μM], May [2.0 μM], July [14.0 μM] and September [2.0 μM] 1994), the highest reported values for freshwater DFe were in July.

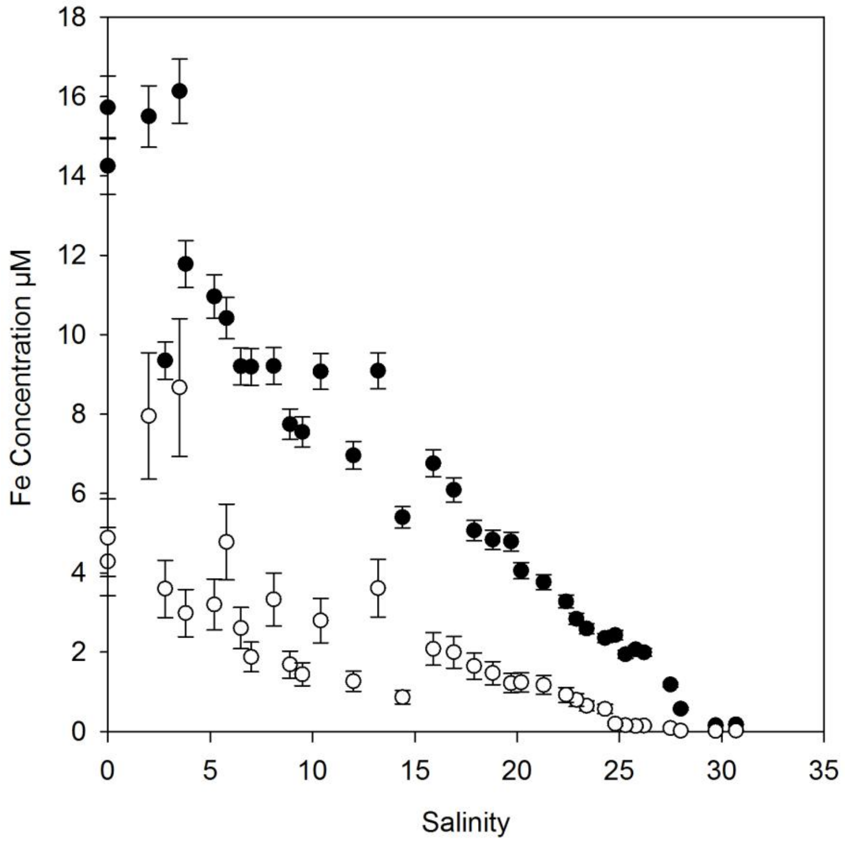

Fig. 6B. DFe (filled circles) and Fe(II) (open circles) along the Beaulieu estuary 15 January 2013. Error bars include the standard deviation of triplicate measurements (circa 5%) and, for Fe(II) only, the maximum plausible error (20%) in the deducted quantity of Fe(II) to correct the measured Fe(II) concentrations for ferrozine induced Fe(III) reduction.

2.5 Discussion

2.5.1 Temporal and spatial variation at King’s Hat

Two features of Fig. 4B. are particularly striking. First, there is a very large difference between reported dissolved Fe(II) and DFe using the two sampling and analysis methods. Second, there is an apparent absence of a diurnal sinuous trend in Fe(II). Such a trend has been reported in both fresh and marine surface waters (Emmenegger et al., 2001; Johnson et al., 1994).

Although the trend (Fig. 4B) observed throughout the day by both measurement methods is similar, manually collected samples report significantly higher values for both DFe and Fe(II) measurements than the in situ sensor. Before and after deployment, sensor determined concentrations of Fe(II) and Fe(III) standards in the laboratory, encompassing the observed range in Beaulieu water, produced concentrations using the sensor within ± 5.0% of the values reported via the LWCC and U-2800 UV-Vis spectrophotometer. Therefore the stark difference between sensor values and manually collected samples cannot be attributable to a malfunction or contamination. No such difference between manual and sensor reported concentrations was previously found when the same sensor was deployed in the River Itchen (Southampton, UK), where both DFe and DOC concentrations are much lower (see supplementary material), or with SLRS-5 reference material (Milani, 2013). Furthermore, a similar ferrozine based sensor with a shorter (3 cm) cell has been reported to work satisfactorily within the vicinity of hydrothermal vents at Fe concentrations in excess of 250 μM (Sarradin et al., 2005), much higher than those detected here.

We assume therefore that equilibration in the sensor between the added ferrozine reagent and DFe is not reached and thus mixing time is the crucial difference between the two datasets. In the sensor there is sufficient time for ferrozine to mix with the river water (300 seconds). However, for Fe(II) measurements, this time is apparently insufficient for kinetic equilibrium to be achieved with strongly complexed or small colloidal Fe(II). Similarly for DFe measurements, this mixing time is insufficient for the combined process of reduction (of Fe(III) by ascorbic acid) and complexation (of Fe(II) by ferrozine). This leads to gross underestimation of Fe(II) and DFe concentrations (Rose and Waite, 2003b; Suzuki et al., 1992). The magnitude of the difference between sensor and manual readings (Fig. 4B) is surprising given the similarity previously found with River Itchen (Southampton, UK) water and with standard solutions (see supplementary material). DFe is much lower in the River Itchen, but the cause of sensor DFe and Fe(II) underestimation in the River Beaulieu does not appear to simply be because of saturation at high DFe concentrations. The sensor's underestimation of DFe is consistent with terrestrial DOM interacting with both DFe and Fe(II) to form small colloids or complexes < 0.2 μm (Breitbarth et al., 2009; Meunier et al., 2005; Rose and Waite, 2003b). In lower DOM environments, such as surface marine waters, dissolved Fe complexation by added ligands proceeds to completion more rapidly, such that only a short time delay between mixing and the measurement of absorbance is required (Croot and Hunter, 2000). In very high DOM waters, as here, the mixing time (300 seconds) within the sensor is inadequate to allow equilibrium to be achieved between reagents and natural Fe species.

The fraction of Fe(II) measured by the sensor here can therefore be considered kinetically labile Fe(II): that is the fraction of Fe(II) that is either free Fe(II) or only weakly complexed by DOM, rather than colloidal or strongly complexed Fe(II). Manual measurements of Fe(II) and DFe are much higher because there was more adequate time for both ligand exchange (7-8 hours) and reduction (DFe vials were left to stand overnight after the addition of ascorbic acid) than with the in situ analyser (Fig. 4B).

The absence of a clear trend between irradiance and Fe(II) (Fig. 4B) suggests that either 1) photochemistry is not the dominant source of Fe(II) in the river, 2) variations in Fe(II) concentration in the different water masses being sampled throughout the day are much greater than photochemically induced variation in Fe(II), or 3) that the half-life of Fe(II) with respect to oxidation in the river is sufficiently long (~12 hours or greater) such that there is no significant decay in Fe(II) concentration overnight. The estimated half-life of Fe(II) in the river water is longer than expected for free Fe(II) at the given pH and temperature. This may be because of numerous organic or inorganic species interacting with Fe(II), but regardless of the cause is clearly not sufficiently long to ensure that Fe(II) concentrations would remain stable overnight.

The effect of natural light on a constant body of still surface river water (Fig. 5B) strongly suggests a photochemical source of Fe(II). Irradiance on both experiment days (17 December 2012 and 28 January 2013) was very similar, peaking between 125 and 130 µmol s^{-1} m^{-2}. The effectiveness of this photochemical source may however be more limited in the river itself. When the water level is high in the River Beaulieu, turbidity tends to be great enough to limit light penetration. If light penetration is sufficiently limited by turbidity (and the water column is not stratified), then photochemical production of Fe(II) may be impeded. Assuming the cross sectional shape of a section of the river can be approximated to half of an ellipse, we can perform an exploratory calculation to determine the fractional volume that is exposed to sunlight. For a section of river with length 1.0 m, width 7.4 m, water depth 1.2 m, light penetration depth 0.4 m (estimated on the survey date as the depth at which a submerged white disk could no longer be observed) and integrated daily irradiance in the shade as a fraction of integrated daily irradiance in full sunlight of 0.7; the exposure of the river water to sunlight would be < 15% of that in the microcosm experiment (Fig. 5B). A clear diurnal Fe(II) cycle may therefore be absent in turbid water bodies simply because the exposure of river water to sunlight is markedly limited. A similar absence has been noted in a high DOM, turbulent mountain stream by Borman et al. (2010), but attributed to a thermal or photochemical oxidation process.

The small decrease in DFe and Fe(II) throughout the day observed by both the in situ sensor and manual sampling (Fig. 4B) is probably related to a change in the water mass being sampled. The increase in pH lowers Fe solubility and favours more rapid Fe(II) oxidation which is consistent with the decrease in both DFe and Fe(II).

Dissolved Fe concentrations in the River Beaulieu clearly vary significantly throughout the year (see supplementary material). As in other rivers, many variables including water properties (e.g. temperature and pH), hydrological factors (e.g. rainfall intensity and river flow rate) and biogeochemical processes (e.g. biological activity and photochemistry) influence dissolved Fe concentrations. Attributing changes in dissolved Fe concentration to specific environmental changes is therefore difficult. The freshwater values reported here (Figs. 4B and 6B) are at the upper end of the reported range (data shown in supplementary material). The DFe content of surface porewater collected from the riverbank is very high. Similarly, surface water in pools adjacent to the River Beaulieu has higher DOC, lower dissolved O_2 and higher dissolved Fe(II) concentration than flowing river water (Table 1). Therefore, when the ground is saturated following prolonged rainfall (as is normally the case for much of winter encompassing the dates of our transect and diurnal study), lateral inputs of water into the river may be responsible for the particularly high DFe concentrations. Conversely, the lowest two DFe concentrations we report, August and October 2013 (see supplementary material), corresponded to particularly low water levels.

2.5.2 Estuarine mixing

To date sparse data are available on the redox speciation of dissolved Fe in estuaries, and on the behaviour of reduced Fe species during estuarine mixing. A summer transect in the low DOC and relatively high pH River Itchen showed, in the few data points presented, a decrease in the ratio of dissolved Fe(II):Fe(III) from 28% at S 0.3 to 23% at S 31.2 (Statham et al., 2012). Dissolved Fe behaviour in the Itchen however is almost conservative compared to the strong, non-conservative removal at low salinities observed in most other estuaries. Conservative behaviour of dissolved Fe during estuarine mixing has only ever been reported in atypical rivers with very low DOM and therefore the behaviour of Fe(II) in the Itchen is unlikely to be representative of the majority of rivers (Shiller and Boyle, 1991).

90% loss of dissolved Fe is reported for many estuaries during the mixing between river water and seawater (Boyle et al., 1977). There are several ways of calculating the loss of dissolved Fe during estuarine mixing but, regardless of how it is calculated, the estuarine mixing observed here (Fig. 6B) does not appear to show the sharp loss of DFe normally observed at low salinities. Integrating the area under the curve (Fig. 6B) and deducting this from a conservative mixing line suggests that only a very limited quantity of DFe, 20%, is removed between a salinity of 0.0 and 30.7. Similarly, extrapolating DFe in Fig. 6B linearly backwards from high salinity waters indicates removal of only 20% (23% if linear extrapolation occurs backwards from S 20.2-30.7 and 20% if from S 24.8-30.7).

As river water dissolved Fe concentrations can vary significantly both spatially (see Table 1) and temporally on short time scales (hours-days), it is possible our river end members are low. If we assume a higher true freshwater end member (a value of 21 μM, compared to the observed 16 μM, is the highest recorded DFe for the Beaulieu) for the freshwater already in the estuary, then the percentage of DFe removed could be as high as 40-60%, with the exact value depending on the method of calculation.

Even with this consideration however, DFe removal is still significantly below the 90% removal often quoted. Earlier work on this estuary has observed DFe removal factors of approximately 90% (March 1994), 80% (May 1994), 60% (September 1994) and 90%

(February 1974) (Fang, 1995; Holliday and Liss, 1976). The exact salinity ranges, filtration size and analytical methods used in these studies differ. However, the three 1994 values were produced using the same method and indicate quite large temporal variation. Therefore, whilst methodological differences (such as filter size) have some influence on reported DFe removal (Figueres et al., 1978; Hong and Kester, 1985), these are not likely to be the dominant reason for the observed variation here. In other estuaries, whilst an estuarine removal factor of 90 ± 10% covers the majority of reported values, there are examples of 50-80% DFe removal (Boyle et al., 1977), but < 60% in a high DFe river is anomalously low.

Both seasonal variation and spatial variation in Fe concentration and speciation may contribute to the range of DFe removal factors reported (Mayer, 1982a), but the exact reasons why such a low removal factor is observed here are unclear. We can speculate that underestimating freshwater DFe values may have led to some underestimation of DFe removal. However, even a large underestimation (of up to 5 μM) would still only suggest a modest DFe removal factor of at most 60%. We cannot identify the cause of this low removal factor, but it appears to be an unaccounted for addition of DFe to the estuary. Intermittent discharge from the sewage treatment works at Beaulieu (which outflows within 200 m of the low salinity transect end point) may be a contributing factor. This could also explain the very high Fe(II) concentrations close to this location (Fig. 6B).

Fe(II) as a fraction of DFe (Fig. 7B) generally decreases as salinity increases (linear R^2 0.46). Ussher et al. (2007) report dissolved Fe concentrations of ~1 nM in the English Channel with Fe(II) constituting a relatively constant 5.0 ± 2.7% of total dissolved Fe. In the Beaulieu estuary Fe(II) is a much more variable fraction of dissolved Fe (25.5 ± 12.5%, Fig. 7B), but the fraction of Fe(II) in higher salinity waters (S > 25) approaches that previously reported for offshore waters (Fig. 7B). Variability in Fe(II) values is most pronounced in the shallow (< 1 m depth, S 2-10 on survey date) upper estuary. A much smoother trend in DFe and Fe(II) was found in deeper, high salinity (S > 15) waters (Fig. 6B).

At low salinities (< 5) in the shallow upper estuary there is possibly some addition of dissolved Fe to the water column (Fig. 6B). Two data points, at salinities of 2.0 and 3.5, show slight addition of DFe above what would be expected during conservative mixing (105 and 114% respectively). Both of these points have anomalous high Fe(II) fractions (51 and 54% respectively). As noted above, this addition may be exaggerated if the reported river fresh water end member is not representative. Furthermore, both of these points are within 200 m of several storm drainage outflow pipes.

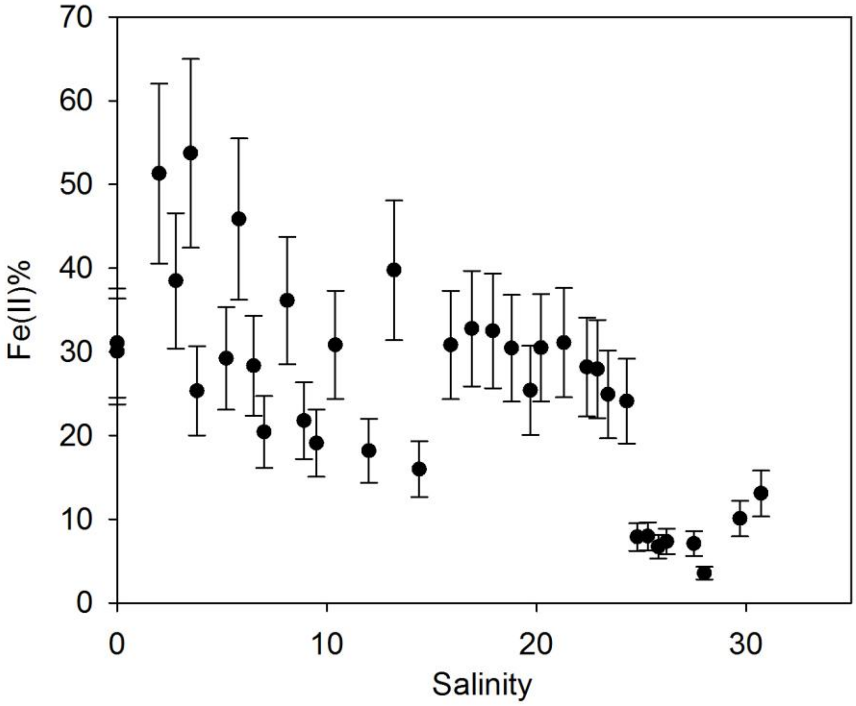

Fig. 7B. Fe(II) as a fraction of DFe along the Beaulieu estuary 15 January 2013.

Significant Fe(II) inputs from groundwater (see Table 1B) or outflow pipes, coupled with localised resuspension of sedimentary material, could therefore explain the much greater spatial variability in DFe and Fe(II) in the shallow (< 1 m) upper estuary (Fig. 6B). Furthermore, if other dissolved Fe inputs into the estuary are significant compared to the natural River Beaulieu input, this could be a contributing factor as to why the estuarine DFe removal appears anomalously low.

Riverine Fe speciation may be subject to temporal variations due to seasonal changes in temperature, pH, DOM or water flow and this may explain large differences in estimated particle and dissolved Fe export (Heikkinen, 1990; Raiswell, 2006; Wang et al., 2012). Peak flow in many rivers, including the Beaulieu, occurs during large floods in winter or spring. Here, winter riverine discharge appears to have very high DFe content compared to other times of year.

2.6 Conclusions

Five transects measuring dissolved Fe are now reported for the Beaulieu (15 January 2013; 5 February 1974 (Holliday and Liss, 1976); 17 March, 13 May and 22 September 1994 (Fang, 1995)) providing snapshot images of an estuarine Fe distribution that varies hourly and only the transect reported here distinguishes between Fe(II) and DFe. We demonstrate that Fe(II) decreases as a fraction of DFe with increasing salinity. A range of DFe removal factors are reported for the Beaulieu estuary (60-90%), with the < 60% removal found here anomalously low.

High resolution sampling of estuaries for nutrient concentrations reveals significant short timescale variability that is missed by discrete sampling (Statham, 2012) and therefore the deployment of Fe sensors within estuaries could provide important information which is logistically impossible to collect manually. However, in ligand based Fe detection systems, designed for rapid in situ spectrophotometric DFe concentration determination, natural DOM associated Fe may lead to severe underestimation of dissolved Fe. This is especially the case in natural waters where Fe-binding DOM concentrations are high compared to DFe concentrations. Our data (Fig. 4B) demonstrate the incompatibility of the present design of ferrozine (or similar ligand) based Fe detection systems with a high DOM environment.

The unexpected absence of a diurnal photochemical Fe(II) cycle in the River Beaulieu, and other similar DOM rich rivers with shallow light penetration, appears to result from continuous mixing of surface waters and limited light exposure that impedes photochemical Fe(II) formation.

In rainwater there is strong evidence for two distinct pools of Fe(II). One pool is photochemically produced Fe(II) that is readily oxidised; the other is a much more stable form that most likely exists as an organic complex (Kieber et al., 2005; Willey et al., 2005). A similar situation may exist in rivers and estuaries where a fraction of Fe(II) in the dissolved phase appears to be associated with DOM.

2.6.1 Acknowledgements

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2.7 Supplementary material

2.7.1 Manual measurements using a LWCC

The LWCC was assembled (as shown Fig. 1B) beneath a class 100 laminar flow hood with 0.25 mm diameter silicone pump tubing (Gradko International) throughout, except for the inlet line between the sample and LWCC which was 0.38 mm PTFE (Gradko International). Optical fibres (Laboratory-grade Patch Cord, Ocean Optics) connected the LS-1 lamp (b) to the LWCC (c) and the LWCC to the USB4000 spectrometer (d). The LWCC was cleaned daily with 0.01 M HCl and a dilute detergent followed by prolonged (> 30 minutes) pumping of deionised water (MilliQ, 18.2 MΩ·cm) through the cell. When not in use the LWCC was stored full of deionised water and sealed to minimise evaporation. The LS-1 and PC were switched on whilst deionised water was continuously pumped through the LWCC for at least one hour before commencing measurements to allow a stable baseline to be achieved. A 0.2 μm filter (a) was permanently mounted on the inlet line to protect the LWCC.

Light intensity was monitored continuously in SpectraSuite at 562 nm and 700 nm with the 562 nm intensity used to calculate absorbance and the 700 nm (non Fe-Ferrozine absorbing wavelength) monitored to check for baseline drift. Deionised water was used as a baseline in all experiments with the background absorbance of filtered samples (no reagent additions) measured and manually corrected for.

Three dissolved working standards (10 nM, 100 nM and 200 nM) were run at the start of every sample batch to check the linear response and stability of the LWCC. A single working standard was then re-run after every 10 samples. The pump rate (non-variable, approximately 0.3 mL minute^{-1}), combined with the total length of the LWCC plus connecting tubing, resulted in a time delay of 2 minutes between allowing a sample to be drawn into the inlet and filling the LWCC. A total time of 6 minutes (including post sample flushing with deionised water) was therefore required per sample measurement. A sample volume of approximately 2 mL was required per reading.

Interference from air bubbles was minimised by regular cleaning, maintaining the LWCC in an upright position and by switching off the pump when moving the inlet line between vials.

The sensitivity of the software and the exact range of linear relationship between absorbance and dissolved Fe concentration could be manipulated in several ways. SpectraSuite requires selection of an ‘integration time’ (akin to shutter speed), ‘scans to average’ and ‘boxcar width’ (akin to a smoothing function). The intensity peak from a source increases with increasing shutter speed and becomes smoother with increased averaging or boxcar width. Intensity could also be lowered by limiting the input of light from the source (LS-1) into the cell. Our standard settings were: Integration time 10 ms, scans to average 20 and boxcar width 30.

2.7.2 Sensor design and assembly

The sensor is built around a microfluidic chip manufactured, using a LPKF Protomat S100 micromill, in 8.0 mm thick tinted polymethylmethacrylate (PMMA) with a matching 3.0 mm thick lid. The microchannels are 300 μm deep, 400 μm wide. The two identical, independent spectrophotometric cells are 600 μm wide and 100 mm long. Reagent lines to the two cells are entirely independent such that two separate variables can be measured simultaneously. Solenoid valves (Lee products 300 series, USA) are used throughout to direct fluid through the manifold.

The housing is a cylinder of anodised Al and is sealed full of mineral oil prior to deployment. PTFE tubing connects the sensor to 150 mL Flexboy reservoir bags (Satorius, UK, ethylene-vinyl-acetate/ethylene-vinyl-alcohol) held on a Ti bracket outside the Al housing. These bags hold reagents, standards and waste. A total waste volume of 1.25 mL is created per sample (including flushing with deionised water).

Custom-designed electronic control of the sensor includes a temperature measure and a real time clock. In continuous use the system can operate autonomously for over 24 hours on battery power collecting a dissolved Fe(II) and total dissolved Fe sample every 10 minutes. Loading of one or two standards into the Flexboy bags allows the system to run its own calibrations in situ which means that the system can correct for drift. This also means that the system can rapidly be recalibrated following changes to sampling procedure.

All sensor components exposed to sample water were checked for potential dissolved Fe contamination by measuring the total dissolved Fe concentration after components were soaked in undiluted ferrozine and ascorbic acid reagent for > 12 hours. Several problematic components on the predecessor to this model (‘prototype 1’(Milani, 2013)) were identified in this way and substituted.

The waste and memory card capacity are vastly in excess of battery life. Whilst the sensor is completely autonomous once programmed for a deployment, when deployed in shallow water (as here) it can also be connected to a laptop PC such that the performance of the sensor can be monitored in real time.

All information concerning the technical specifications and development of the sensor is adapted from Milani (2013) Chapters 4-6 where the system is referred to as ‘Microfluidic Analyser Prototype 2 (P2)’.

2.7.3 Sensor performance

Environment	DFe concentration	Number of data points	Mean Fe(II) compared to manual measurements
River Itchen diurnal deployment 25/10/12	60-120 nM	10	108%
Standard run post Beaulieu deployment 18/12/13	6.0 μM	3 (triplicate analysis)	94%
SLRS-5 reference material 18/12/13	1.63 μM	3 (triplicate analysis)	95%

Supplementary Table 1B Performance of the Fe(II) sensor compared to manual measurements under varied conditions.

Prior to the work presented here the sensor was deployed in the low dissolved Fe River Itchen (Southampton, UK). A comparison of manually measured Fe(II) and sensor reported Fe(II) concentrations is shown (Supplementary Table 1B). Data points for comparison of sensor Fe(II) performance were, for river water samples, collected within 1 minute of the sensor measurement. Sensor performance, compared to manually measured values, was relatively good with standards and in river water with low dissolved Fe and DOC.

2.8 River Beaulieu historical data

In addition to studies on River Beaulieu water by Moore et al (1979) and Holliday and Liss (1976), two further sources with appropriate sampling/analytical procedures detailed are available for historical water composition. Where available, dissolved Cu and Co concentrations are shown as high concentrations of these metals can lead to overestimation of Fe concentrations using ferrozine (Stookey, 1970). Copies of both of the theses referred to are held by The University of Southampton. Additional data collected as part of this study (methods as described in the manuscript) is also included. Data from other sources are presented without any modification from the original form.

2.8.1 Methods

J. Smith (1995)
Study of the composition of the River Beaulieu with particular reference to the processes affecting trace element concentrations.

Dissolved organic carbon (DOC) samples were preserved with 2-3 drops of 1% mercury chloride upon collection and then filtered (0.4 µm) upon return to the laboratory. DOC was determined as per Statham and Williams (1983). pH was measured using a Whatman PHA 300 meter. Trace metal samples were collected in HNO_3 cleaned 1 L PTFE containers, filtered (0.4 µm) upon return to the laboratory and then acidified to 0.02 M HNO_3. Dissolved Fe was determined via inductively coupled plasma atomic emission spectroscopy (ICP-AES) with a 1.07 µM detection limit. Other trace metal concentrations were determined via ICP-mass spectroscopy (Co and Cu have 9 and 16 nM detection limits respectively). The British Geological Survey conducted both ICP analyses. 25% of all samples submitted for ICP analysis were duplicates. Triplicate analysis of filtration blanks were below detection limit for Fe, Co and Cu.

T. H. Fang (1995)
Studies of the behavior of trace metals during mixing in some estuaries of the Solent region

Trace metal samples were filtered (0.4 µm) upon return to the laboratory and 1 mL distilled HNO_3 added per litre of sample. DOC was determined as per Statham and Williams (1983). Trace metal concentrations in estuarine water were determined using graphite furnace atomic absorption spectrophotometry (GFAAS) on a Perkin-Elmer

1100B AAS after application of a APDC (ammonium pyrrolidine dithiocarbamate) - DDDC (diethylammonium diethyldithiocarbonamate) / Freon TF (1,1,2-trichloro-1,2,2-trifluoroethane) preconcentration method as described by Althaus (1992) and Hall (1993). Detection limits (DL) and certified reference materials shown in Supplementary Table 2.

Metal	Blank (n = 12 nM)	DL (nM)	CASS-2 measured (nM)	CASS-2 certified (nM)
Co	ND	0.075	0.44 ± 0.05	0.42 ± 0.10
Cu	ND	0.47	10.70 ± 0.39	10.62 ± 0.61
Fe	ND	3.57	22.57 ± 1.92	21.49 ± 2.15

Supplementary Table 2B Blank, detection limit (DL) and certified reference values for trace metals measured via GFAAS after preconcentration. ‘ND’ non detectable.

2.8.2 Data

DOC (μM)	Fe (μM)	pH	Co (nM)	Cu (nM)
472	15.41	7.5	17	ND
431	9.98	7.5	15	ND
	16.68	7.3	20	ND

Supplementary Table 3B Properties of filtered (0.4 μm) River Beaulieu water collected 22 July 1994 from within 500 m of King’s Hat Footbridge site used in this study. ‘DOC’ dissolved organic carbon, ‘ND’ non detectable (Smith, 1995).

S	DOC (μM)	Co (nM)	Cu (nM)	Fe (nM)
32.99	153	0.58	4.56	12.6
32.90	167	1.30	7.72	92.3
32.85	145	1.66	9.36	27.4
32.42	178	1.17	6.85	13.5
32.20	231	1.76	8.34	16.8
31.37	239	3.56	9.67	22.1
30.85	193	3.94	10.54	24.6

29.64	258	5.55	12.91	30.8
27.33	323	8.07	17.54	37.2
26.26	355	7.87	12.14	48.8
24.83	323	7.97	25.29	65.4
23.94	328	8.25	17.69	75.8
22.73	338	8.58	19.25	121.2
22.16	342	16.03	19.93	128.4
21.01	379	15.67	19.94	142.7
15.24	513	16.22	15.00	195.1
12.64	516	20.26	15.21	300.6
11.70	505	20.07	14.49	349.3
9.79	546	21.32	16.60	526.2
5.06	564	23.24	13.05	1245
0.35	601	20.17	7.81	2750
0.13	648	18.69	4.92	2695

Supplementary Table 4B Filtered (0.4 µm) water DOC and trace metal content in the Beaulieu estuary. Transect conducted 17 March 1994 (Fang, 1995). ‘S’ salinity, ‘DOC’ dissolved organic carbon.

S	DOC (µM)	Co (nM)	Cu (nM)	Fe (nM)
33.09		1.23	10.76	18
32.84		1.51	12.13	20
32.75		1.62	10.92	366
31.78		3.76	12.91	605
30.22		3.01	23.08	151
28.87		5.70	26.98	585
27.94	291	4.57	34.56	24
26.15	350	4.44	31.81	279
25.51	353	3.63	25.98	33
23.97	419	3.76	26.25	60
23.78	383	5.87	33.33	75
23.32	387	6.44	39.87	100
16.43	544	8.82	24.84	207

15.89	486	8.61	28.23	107
9.35	627	7.95	17.63	246
7.47	618	10.42	21.02	196
4.99	664	9.66	14.12	1385
4.61	698	9.67	12.89	1395
4.53	689	10.61	13.49	1332
4.47	669	10.69	13.07	1840
4.16		8.90	11.80	1930
3.55	694	9.63	10.73	2120
1.22	706	9.42	9.73	1906
0.33		9.77	8.92	1992

Supplementary Table 5B Filtered (0.4 μm) water DOC and trace metal content in the Beaulieu estuary. Transect conducted 13 May 1994 (Fang, 1995). 'S' salinity, 'DOC' dissolved organic carbon.

S	DOC (μM)	Co (nM)	Cu (nM)	Fe (nM)
34.08	160	0.57	7.34	21.9
34.04	164	0.75	6.28	14.7
32.96	152	1.50	10.43	30.8
31.59	180	2.29	14.20	31.2
30.19	206	2.62	19.96	25.1
28.79	256	2.81	21.82	84.2
27.65	264	3.97	29.10	37.3
26.08	296	4.09	30.24	68.7
25.28	304	3.88	30.98	62.1
21.76	360	4.17	24.01	68.7
25.06	315	5.09	32.89	97.0
19.45	394	7.24	23.43	241.4
18.21	416	5.26	19.63	92.3
16.70	437	4.88	16.89	83.5
15.73	464	4.96	17.89	105.6
11.99	496	5.55	13.37	164.0
8.16	536	6.56	15.73	357.6

5.17	552	6.94	9.15	581.6
5.87	576	6.65	11.12	539.4
4.89	596	5.84	9.77	806.9
2.87	640	6.33	9.46	837
1.45	604	10.38	7.43	1248
0.24	680	9.31	6.55	1713
0.13	696	6.05	6.98	2030

Supplementary Table 6B Filtered (0.4 µm) water DOC and trace metal content in the Beaulieu estuary. Transect conducted 22 September 1994 (Fang, 1995). ‘S’ salinity, ‘DOC’ dissolved organic carbon.

Date	DFe (µM)	T	pH
26/11/2012	11700	9.0	6.3
06/12/2012	18600	2.5	7.5
17/12/2012	20100	5.0	6.5
14/01/2013	20800	4.6	7.2
14/01/2013	18600	4.6	6.7
28/01/2013	13000	4.2	6.5
04/04/2013	6980	3.2	6.9
15/04/2013	6740	6.5	7.0
15/08/2013	1430	15.3	7.6
01/09/2013	5910	14.0	7.7
03/09/2013	6350	14.0	7.0
17/09/2013	9420	10.5	7.4
22/09/2013	5740	14.0	7.4
28/09/2013	8260	14.0	7.5
12/10/2013	3110	11.0	6.8
24/10/2013	7590	11.5	7.8
28/10/2013	10150	13.0	6.9
29/10/2013	9640	10.5	7.3
03/11/2013	5890	10.0	5.6
16/11/2013	14260	5.5	5.7
19/11/2013	12270	6.5	5.6

Supplementary Table 7B DFe (0.2 μm) collected as described in methods (main paper) at King's Hat.

3. Dissolved iron(II) ligands in river and estuarine water

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3.1 Abstract

We present the first evidence of Fe(II) complexation by natural organic ligands in estuarine waters. Across five diverse river/estuary systems we find evidence of terrestrially derived ligands with binding constants (log $K_{Fe(II)L}$) mainly in the range 6-8. These Fe(II) ligands were stable over short time periods (1-2 days), generally equivalent to, or in excess of, ambient freshwater Fe(II) concentrations (which ranged from 12 to 3600 nM) and had similar binding constants to ligands that were leached by water from vegetation and detritus (log $K_{Fe(II)L}$ 7-8). A class of terrestrially derived ligands may therefore be important in stabilising Fe(II) concentrations in freshwater systems. However, in coastal seawater the impact of these ligands upon Fe(II) speciation is likely to be diminished due to a combination of dilution, loss of humic material during flocculation and increased ionic strength.

The temperate and sub-tropical river systems studied included the Beaulieu (England), Itchen (England), Cape Fear (North Carolina, USA), Winyah Bay (South Carolina, USA) and Loch Etive (Scotland). Freshwaters in each system possessed a broad range of dissolved organic carbon (DOC, 200-1300 μM), labile dissolved Fe (LDFe, Fe <0.2 μm available to ferrozine after reduction with ascorbic acid, 100 nM-20 μM) and pH (5.5-8.5). In the Itchen estuary, where anthropogenic discharge constitutes >10% of freshwater input, ligand binding constants were elevated (up to log $K_{Fe(II)L}$ 11) and the expected decrease in LDFe with increasing salinity along the estuary was not observed (LDFe and DOC both peaked at a salinity of 7) due to effluent inputs.

3.2 Introduction

Dissolved Fe(II) in oxic seawater arises from photochemical production, biological reduction and diffusion from reducing micro-zones (Balzano et al., 2009; Barbeau et al., 2001; Hansard et al., 2009). However observed Fe(II) concentrations, distributions and oxidation rates in marine waters are sometimes inconsistent with that of the expected Fe(II) inorganic speciation (Croot et al., 2008; Croot et al., 2007; Roy et al., 2008). The observed chemical properties of Fe(II) in some marine and fresh waters hint at the widespread distribution of Fe(II) organic ligands (Breitbarth et al., 2009; Kieber et al., 2005; Okada et al., 2005; Roy and Wells, 2011; Willey et al., 2008; Zhuang et al., 1995). Such Fe(II) ligands are highly probable given the non-specificity of the complexing capacity of much natural organic material for trace metal ions and the observed binding of many other trace metal ions by natural organic ligands (Mantoura et al., 1978; Vraspir and Butler, 2009).

Knowledge of Fe(II) concentrations in surface marine waters is limited, although existing data indicate large variability in both Fe(II) concentration and the fraction of total dissolved Fe present as Fe(II). Bowie et al. (2002) report surface Fe(II) concentrations in the subtropical Atlantic varying diurnally from 45 to <12 pM with Fe(II) accounting for up to 40% of total dissolved Fe. Slightly lower Fe(II) concentrations, with a maximum of 29 pM (up to 13% of total dissolved Fe), were found in the Southern Ocean (Bowie et al., 2002), whilst Sarthou et al. (2011) measured surface Fe(II) concentrations from 16-120 pM (5-65% of dissolved Fe) along a transect of the South Atlantic and Southern Ocean. Moving from the North Atlantic to the coastal waters of the English Channel, Boye et al. (2003) measured an increase in dissolved Fe(II) from <0.16 nM to 1.8 nM (up to 80% of dissolved Fe). An increase in Fe(II) concentrations from <12 pM to 130 pM was measured along a similar transect by Ussher (2007), but Fe(II) never exceeded 11% of total dissolved Fe. Hansard et al. (2009) present one of the most extensive marine Fe(II) datasets available and find a relatively constant median Fe(II) concentration of 25-30 pM (12% of total dissolved Fe) across the central Pacific with more elevated Fe(II), up to 260 nM (30% of total dissolved Fe), in coastal waters. Differences in both analytical methods and sample handling are likely to account for some of the observed variation between these studies

(Hansard et al., 2009), but Fe(II) concentrations do appear to be consistently elevated in shelf and coastal waters compared to the open ocean. This may be partly due to benthic fluxes of soluble Fe (Elrod et al., 2004) in addition to photochemical and micro-reducing zone processes.

Despite the wealth of knowledge about Fe(III) ligands and their strong influence on Fe biogeochemistry in the marine environment (Boyd and Ellwood, 2010; Gledhill and Buck, 2012; Hunter and Boyd, 2007), natural Fe(II) ligands have not received significant attention. Fe(II) speciation is of specific interest because natural redox cycling of Fe is a mechanism by which elevated dissolved Fe concentrations are retained in surface waters (Croot et al., 2001; Emmenegger et al., 2001). Ligands potentially moderate both the photochemical production (Barbeau et al., 2001; Kuma et al., 1992) and the oxidation rate (Gonzalez et al., 2014; Rose and Waite, 2003a; Santana-Casiano et al., 2000) of Fe(II) species. The interaction of Fe(II) with biota is of particular interest as, whilst Fe(II) is thought to be bioavailable (Sunda, 2001), it is not entirely clear how Fe(II) interacts with biological Fe uptake systems (Croot et al., 2008; Shaked and Lis, 2012).

Here we investigate Fe(II) complexation in estuarine waters. We apply an adapted reverse titration technique to waters with varying dissolved Fe and DOC concentrations in order to characterise any Fe(II) ligands present. We assess the limitations of this technique and discuss the likely impact of Fe(II) ligands on Fe biogeochemistry in estuarine and coastal waters.

3.2.1 Measuring Fe(II) concentrations

The transient nature of Fe(II) in warm marine waters makes quantifying its natural concentrations a formidable task. Three main analytical approaches have been developed for measuring dissolved Fe(II) concentrations: spectrophotometry using synthetic Fe(II) ligands; chemiluminescence using luminol; and catalytic cathodic stripping voltammetry (CSV) using an Fe(II) masking agent. Spectrophotometric methods were the first to be developed. The ligands 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine (ferrozine) (Stookey, 1970), 3-(2-pyridyl)-5,6-bis(5-furyl)-1,2,4-triazine (ferene) (Hennessy et al., 1984) and 4,7-diphenyl-1,10-phenanthroline (bathophenanthroline) (Smith et al., 1952) produce intensely coloured magenta, blue and red complexes respectively with Fe(II). Use of long capillary cells produces detection limits as low as 0.1 nM (Waterbury et al., 1997). However, attention must be paid to interferences from other trace metal ions and some forms of colloidal or organically bound Fe(II) may not be complexed by the added ligand (Kieber et al., 2005; Stookey, 1970). An additional concern is that adding synthetic Fe(II) ligands favours Fe(III) reduction such that Fe(II) concentrations may be overestimated (Cowart et al., 1993; Hudson et al., 1992; Murray and Gill, 1978).

Chemiluminescence has proved to be the most readily adaptable method for determining Fe(II) concentrations at sea (Bowie et al., 2002; Croot and Laan, 2002). Luminol is subject to interferences from some Fe(III) species and natural organic compounds. However in seawater, due to a combination of higher pH, lower DOM and lower total dissolved Fe concentrations than in freshwater, these interferences with the Fe(II) signal are relatively small (Ussher et al., 2005). The principal advantage of chemiluminescence is the rapid nature of the reaction between luminol and Fe(II) which minimises the time for Fe(II) oxidation between sample collection and analysis (King et al., 1995). Mean reported detection limits, without preconcentration, are as low as 10 pM (Ussher et al., 2007). Furthermore, continuous Fe(II) determination can be coupled to a similar luminol based H_2O_2 detection system (Yuan and Shiller, 1999).

CSV is widely used to measure Fe(III) concentrations and to probe dissolved Fe(III) interactions with organic ligands (Gledhill and Buck, 2012). Although not widely used to quantify Fe(II), CSV has been adapted for such a purpose (Gledhill and Van Den Berg, 1995). Dissolved Fe concentrations are measured with and without an Fe(II) ligand, 2,2-dipyridyl, which masks the voltammetric response of Fe(II). This technique has been used in both freshwater and marine systems (Aldrich and van den Berg, 1998; Boye et al., 2003). Detection limits of 0.12 and 0.16 nM, reported by Boye et al. (2003) and Gledhill and van den Berg (1995) respectively, are similar to those reported for spectrochemical techniques.

Whilst total dissolved Fe measurements in seawater can now be made with good reproducibility (Cutter and Bruland, 2012; Fitzsimmons and Boyle, 2012), Fe(II) concentrations are operationally defined. Differences in analytical procedures with respect to filtration, pH buffering, temperature control and the accounting for oxidation during analysis exist between different studies. Furthermore, extensive Fe(II) databases are few in number which further impedes an assessment of the inter-comparability between different techniques.

3.2.2 Fe(II) oxidation rates

The oxidation rate of dissolved Fe(II) is represented by Equation 1 where k (k=[Fe(II)-L]/([L] × [Fe(II)])) is affected by salinity and temperature (Davison and Seed, 1983; Millero et al., 1987). Changes in pH, salinity and temperature in the dynamic estuarine environment will therefore affect the oxidation rate of Fe(II). At fixed pH and in excess oxygen, Equation 1 can be simplified to Equation 2. The mechanism of inorganic Fe(II) oxidation is descried by Weiss (2014).

$$-\frac{d[Fe(II)]}{dt} = k[Fe(II)][O_2][OH^-]^2 \quad \text{Equation 1}$$

$$-\frac{d[Fe(II)]}{dt} = k_1[Fe(II)] \quad \text{Equation 2}$$

Two other factors that may be important influences on Fe(II) concentration and oxidation rate specifically in the estuarine environment are turbidity and DOM. Adsorption of Fe(II) onto particle surfaces can accelerate the observed oxidation rate (Stumm, 1997). Similarly, complexation of dissolved Fe by DOM may either accelerate or retard Fe(II) oxidation rates (see Section 1.3).

3.2.3 DOM interaction with Fe(II)

Organic ligands strongly influence the marine chemistry of Fe(III) in both the truly dissolved and colloidal phases (Boyd and Ellwood, 2010). Fe(II) ligands may be actively released by some microorganisms (Gonzalez et al., 2014; Santana-Casiano et al., 2014), but the distribution of these ligands in the marine environment and their relationship to Fe(III) ligands is not well defined. Complexation of Fe(III) by organic material can make the metal ion more susceptible to photoreduction and thus enhance formation of Fe(II) (Barbeau et al., 2001; Kuma et al., 1992). Complexation also has a direct effect upon Fe redox potential. Relative to the standard redox potential of the hydrated ion (+ 770 mV), strong Fe(II) ligands shift the redox potential to higher values, whereas strong Fe(III) ligands shift it to lower values (Boukhalfa and Crumbliss, 2002; Harrington and Crumbliss, 2009).

The effect of Fe-ligand interactions in the estuarine environment is complicated by changing salinity (Miller et al., 2012). Along an estuary the activity coefficients of dissolved species, concentrations of competing ions and formation constants of DOM-Fe complexes all change appreciably. Additionally, the aggregation of Fe and humic species at low salinities to form large colloids may, as well as removing approximately 90-99% of dissolved Fe, remove some terrestrial ligands from solution (Boyle et al., 1977; Holliday and Liss, 1976; Sholkovitz et al., 1978).

3.3 Methods and materials

3.3.1 Sample collection

Water samples were filtered upon collection (0.22 μm Millipore) using portable hand pumps or vacuum filtration and then stored in Al foil wrapped containers in cool boxes out of direct sunlight. Upon return to the laboratory, water samples were refrigerated at 4 °C. The Cape Fear estuary (North Carolina, USA) was sampled over two days onboard RV Cape Fear (10 and 12 June 2013) from 34° 15.545’ N 077° 59.148’ W to 33° 52.611’ N 078° 0.278’ W. The Winyah Bay estuary (South Carolina, USA) was sampled using a small flat-bottomed motorboat (28 June 2013) from 33° 20.876’ N 079° 16.573’ W to 33° 18.667’ N 079° 9.355’ W. Both of these systems in the southeastern USA are organic-rich with DOC derived from blackwater sources (Goni et al., 2003; Shank et al., 2004). The River Awe (Argyl, UK) was sampled 21 March 2013. The River Beaulieu (New Forest, UK) and River Itchen (Hampshire, UK) were sampled at intervals from October 2012 - February 2014. Water samples were collected by submerging a 1 L LDPE container attached to a plastic coated 2 m wood pole or by continuous pumping through LDPE tubing mounted on a metal-free pole and held approximately 20 cm below the water surface.

3.3.2 Analytical methods

All apparatus for Fe sample collection was cleaned (a mild detergent wash, a 3 M HNO_3 (laboratory grade) acid bath for one week and finally three rinses with deionised water), then stored in sealed plastic bags until required. Filters were rinsed with 0.1 M HCl (laboratory grade) followed by deionised water. Sample handling in the laboratory was conducted under a class 100 laminar flow hood. Deionised water (18.2 MΩ·cm) was obtained from a Milli-Q system. 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-*p,p′*-disulfonic acid monosodium salt hydrate ‘for spectrochemical determination of Fe’, ascorbic acid (trace metal grade) and ammonium Fe(II) sulphate hexahydrate (ACS grade) were obtained from Sigma Aldrich. Reagents were used as received.

DOC (<0.2 μm) samples were collected in combusted 20 mL glass vials with Al foil lined lids and acidified by addition of 100 μL 6 M HCl. DOC was determined by high temperature combustion analysis on a Shimadzu 5000-A Total Organic Carbon Analyzer as per Farmer and Hansell (2007) and verified against a potassium hydrogen phthalate (reagent grade) calibration curve. Chromophoric dissolved organic material (CDOM) samples were collected in combusted tinted glass vials. Excitation/emission spectra with excitation wavelengths from 250-500 nm (at 4 nm intervals) were obtained from a Jobin Yvon SPEX Fluoromax-3 scanning fluorometer equipped with a 150 W arc lamp and R928P detector. Raw data processing was conducted exactly as described by Kieber et al. (2006). Peaks for the four broad DOM groups (A, C, M and T) were determined from excitation/emission plots by integrating the sum of data points within the defined areas of each peak estimated by Coble (1996). Dissolved (<0.2 μm) nitrate and phosphate were determined according to United States Environmental Protection Agency (USEPA) analytical protocol via continuous flow analysis on a Bran Luebbe Autoanalyzer 3 with a digital colorimeter (Lawson et al., 2014; Mernild et al., 2006).

Labile dissolved Fe(II) concentration (LDFe(II), Fe(II) available to ferrozine) was determined by measuring the absorbance of river water at 562 nm as described by Waterbury et al. (1997) after the addition of 100 μL 10 mM freshly prepared ferrozine and 0.1 M ammonium acetate buffer to 10 mL filtered (<0.2 μm) water samples. Ammonium acetate buffer (pH 6) was made from a stock solution of ammonium acetate, made by bubbling ammonia gas (electronic grade) through glacial acetic acid (Romil SpA grade), and ammonium hydroxide (trace metal grade) (Stookey, 1970).

LDFe(II) concentration was determined after samples had equilibrated with ferrozine for 7-8 hours. The increase in absorbance from the Fe(II)-Ferrozine complex due to ferrozine induced Fe(III) reduction was accounted for by measuring the increase in absorbance with time and extrapolating back to the time of reagent addition (Nielsdottir et al., 2012). LDFe was measured using the same method but with the addition of 500 μL 10 mM ascorbic acid to 10 mL samples followed by storage overnight. Dissolved Fe standards from 1.0 nM to 1.0 mM were prepared with a tenfold excess of ferrozine from a stock solution of ammonium Fe(II) sulphate hexahydrate in 10 mM ascorbic acid. For low Fe concentrations (<500 nM) absorbance was measured with a 3000 Series (World Precision Instruments) 250 cm Liquid Waveguide Capillary Cell (LWCC) using a USB4000 Fiber Optic Spectrometer (Ocean Optics) and a LS-1 Tungsten lamp light source (Ocean Optics). The LWCC was used under a class 100 laminar flow hood and detection limits for both LDFe and LDFe(II) were 4.0 nM or lower. For higher Fe concentrations (>500 nM) a 1 or 10 cm glass cell in a U-2800 UV-Vis spectrophotometer was used with detection limits of 350 nM and 100 nM respectively. A range of dissolved Fe samples were duplicated, acidified with HNO_3 (trace metal grade) to a final concentration of 0.6 M, allowed to stand for one month and then analysed for total dissolved Fe by inductively coupled plasma optical emission spectroscopy (ICP-OES) on a Perkin Elmer Optima 2100 DV. ICP-OES calibration curves from 0.0-20 μM were linear (R^2 >0.999). Blank (filtered deionised water) LDFe(II) and LDFe measurements were consistently below the detection limit, defined as three times the standard deviation of blank measurements, (<4.0 nM) of the LWCC.

3.3.3 Reverse titration procedure for Fe(II)

The theoretical aspects of using reverse titrations to estimate ligand properties are described in detail elsewhere (Hawkes et al., 2013b; Van Den Berg, 1984). A reverse titration procedure previously adapted for Fe(II) was used for the River Itchen and River Awe (Statham et al., 2012). This titration incrementally increases the concentration of ferrozine ligand added to aliquots of a natural water sample. The fraction (X) of Fe(II) chelated by ferrozine, defined by Equation 3, increases from 0 to 1 with increasing ferrozine concentration such that at X=1 maximum absorbance from the $Fe(II)Fz_3$ complex is obtained. L represents a natural organic ligand. $C_{Fe(II)}$ and C_L are the total concentrations of Fe(II) (strictly speaking this is LDFe(II)- Fe(II) available to ferrozine rather than total Fe(II)) and ligand respectively.

$$X = \frac{[Fe(II)Fz_3]}{C_{Fe(II)}} \qquad \text{Equation 3}$$

$$C_{Fe(II)} = [Fe(II)Fz_3] + [Fe(II)'] + [Fe(II)L] \qquad \text{Equation 4}$$

X can also be expressed in terms of the α-coefficients of Fe(II) species (defined by Equations 5-7) and the conditional stability constant of natural ligands present (Equation 9) (Nuester and van den Berg, 2005). Fe(II)' represents all inorganic Fe(II) species.

$$[Fe(II)'] = \alpha_{Fe(II)}[Fe(II)] \qquad \text{Equation 5}$$

$$[Fe(II)L] = \alpha_{Fe(II)L}[Fe(II)] \qquad \text{Equation 6}$$

$$[FeFz_3] = \alpha_{FeFz_3}[Fe(II)] \qquad \text{Equation 7}$$

$$[Fe(II)L] = \frac{K_{Fe(II)L}[Fe(II)]C_L}{1 + K_{Fe(II)L}[Fe(II)]} \qquad \text{Equation 8}$$

$$X = \frac{\alpha_{FeFz_3}}{\alpha_{Fe(II)} + \alpha_{FeFz_3} + \frac{K_{Fe(II)L}C_L}{1 + K_{Fe(II)L}[Fe(II)]}} \qquad \text{Equation 9}$$

Titrations were conducted on filtered (<0.2 µm) water samples at room temperature. Varying ferrozine concentration was added to 18×10 mL subsamples such that, after equilibrating for 24 hours, the subsamples produced a range of absorbances between $X = 0$ and $X = 1$. The absorbance of Cape Fear River water subsamples was found to be still increasing 12 hours after reagent addition, but no further increase was observed after 24 hours so this equilibration period was used in all titrations. Absorbance at 562 nm for the titration sub-samples was measured exactly as per LDFe(II) concentrations above. 0.1 M ammonium acetate buffer was used to maintain a pH of 6 in all samples. The total addition of reagents to every 10 mL sample aliquot was made up to exactly 2.0 mL with deionised water. Reverse titrations were conducted on Fe(II) spiked solutions of International Humic Substances Society (IHSS) Suwannee River humic acid standard I and Suwannee River fulvic acid standard II. IHSS standards were used as received. Stock solutions of 100 mg L^{-1} were made up in deionised water and stirred overnight. Final concentrations of 13.3 mgL^{-1} and 12.9 mgL^{-1}, humic and fulvic acid respectively, (corresponding to approximately 500 μM DOC, http://www.humicsubstances.org/elements.html) with 1 μM Fe(II) spikes were titrated as per natural DOC rich river water samples.

X was plotted against (log) ferrozine concentration. $K_{Fe(II)L}$ and C_L were then determined from Equation 9 using a curve fitting procedure exactly as described by Statham et al (2012). Briefly, the measured values of *X* were deducted from modelled data plotted using an estimate of $K_{Fe(II)L}$ and C_L. The difference at every datapoint was squared, summed and then minimised by adjusting $K_{Fe(II)L}$ and C_L by least-squares regression (Nuester and van den Berg, 2005). The above method assumes that $C_{Fe(II)}$ remains constant throughout the titration procedure. As already outlined, one problem with the use of spectrophotometric methods to quantify Fe(II) concentration is that the addition of a strong Fe(II) ligand may lead to accumulation of Fe(II) with time from Fe(III) reduction. The mechanism of this process is not clear, but it has been widely observed (Box, 1984; Cowart et al., 1993; Waterbury et al., 1997). This artefact was not previously reported to interfere with Fe(II)-ferrozine reverse titrations conducted in the River Itchen (Statham et al., 2012) possibly because of low DOC concentrations, low Fe(II) concentrations (<240 nM) and the low ferrozine concentration required to complete the titrations. In higher DOM waters (the Beaulieu, Winyah Bay and Cape Fear) it was found that Fe(II) concentrations accumulated linearly with time for several hours after ferrozine additions rendering the titration as described previously erroneous. As no other titration method is available to investigate Fe(II) speciation, a solution was developed that would not affect the existing experimental design. 500 μL of 10 mM ascorbic acid was added to every 10 mL aliquot in order to reduce all labile dissolved Fe to Fe(II). This amended method was used to conduct all Beaulieu, Cape Fear and Winyah Bay titrations. The effect of the ascorbic acid itself upon absorbance and the shape of the titration curve is negligible (Figure 1C), consistent with previous work by Iwai et al. (2013). However this modification does affect our interpretation of the results (see section 3.3.3).

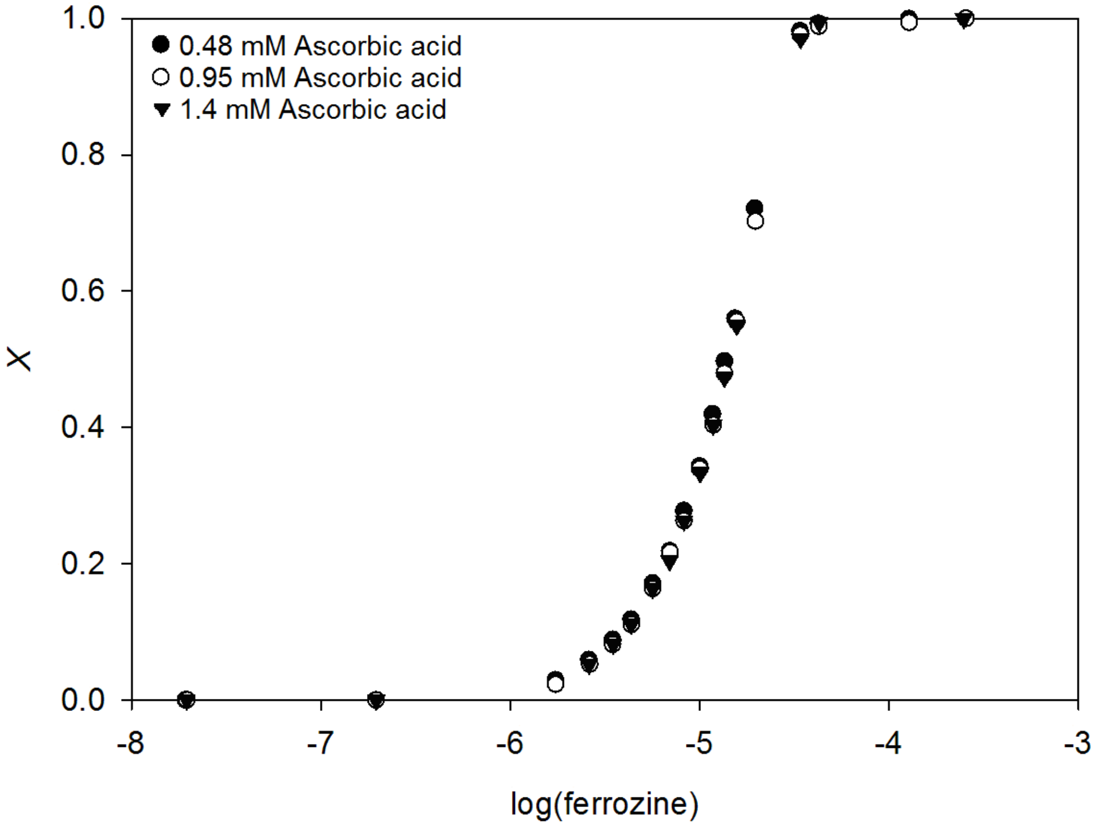

Figure 1C Three titration curves using the same River Beaulieu water sample with varying ascorbic acid concentration (100, 150 and 200 % of the spike added to Beaulieu, Cape Fear and Winyah Bay titrations) demonstrate that use of the reducing agent does not affect the shape of the titration curve.

Duplicate independently collected and analysed reverse titrations on River Beaulieu water demonstrate the consistency and reproducibility of the technique (Table 1C and Figure 2C). The slight decline in LDFe concentration between independent titrations 1 and 4 likely reflects on-going precipitation and container surface removal of dissolved Fe (Lewin and Chen, 1973) which is inevitable given the period of time required to filter and subsample the water samples.

N	LDFe (µM)	C_L (µM)	Log $K_{Fe(II)L}$
1	7.72	7.15	6.41
2	7.56	6.91	6.44
3	7.36	7.26	6.40
4	7.30	7.21	6.42

Table 1C Four entirely independent reverse titrations of separate River Beaulieu water samples collected in rapid succession: LDFe, labile dissolved (<0.2 µm) Fe, C_L, modelled ligand concentration; log $K_{Fe(II)L}$, modelled binding constant. The standard deviation of triplicate LDFe measurements was <2%.

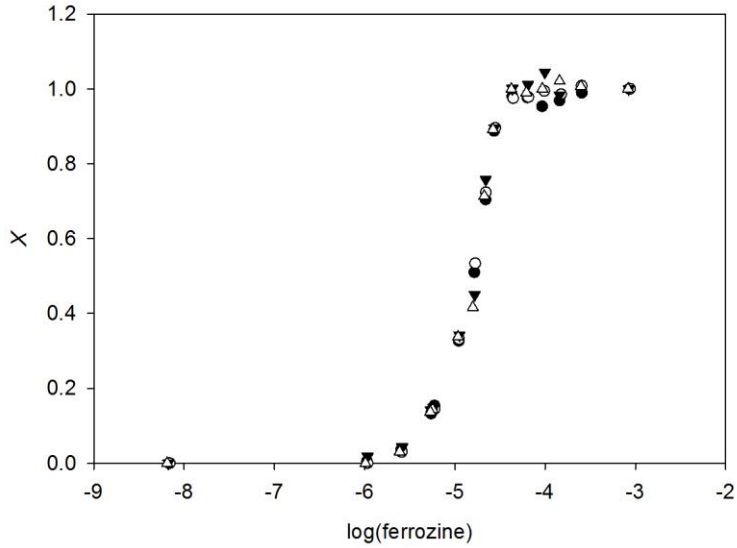

Figure 2C Four independent titrations of four River Beaulieu water samples collected in quick succession. Fitted titration data in Table 1C: 1 open circle, 2 closed circle, 3 closed triangles and 4 open triangles.

3.3.4 Analytical constraints on determination of Fe(II) speciation using a ferrozine based reverse titration

Our use of ascorbic acid to reduce all LDFe(III) to LDFe(II) increases the LDFe(II) concentration approximately 5 fold. Fe(III) ligands are also likely to be released into solution. If an excess of ligands with varying log $K_{Fe(II)L}$ values are present then, at equilibrium, Fe(II) ions will be bound to those ligands with the highest log $K_{Fe(II)L}$ values. Natural ligands with a high affinity for Fe(III) are likely to have a much lower binding constant with Fe(II) (Ussher et al., 2005). Therefore increasing LDFe(II) concentration and liberating Fe(III) ligands, which may weakly bind Fe(II), is likely to slightly lower log $K_{Fe(II)L}$ and increase C_L.

Determining a ‘correct’ combination of log $K_{Fe(II)L}$ and C_L from the shape of a titration curve when multiple combinations are possible is an issue discussed at length elsewhere as it affects all ligand titration approaches (Hawkes et al., 2013b; Sander et al., 2011). Natural organic ligands, especially in the estuarine environment, are heterogeneous and likely to display a broad range of properties (Harden et al., 2014). Even ligands from a comparable source may show a broad range of binding constants (Rose and Waite, 2003b). Inorganically stabilised Fe(II), such as sulphur rich species, may also exist alongside organic species and these cannot be separated from organic complexes via filtration (Luther and Rickard, 2005). We have opted to present reverse titration data as a single ligand class. It is not mathematically valid to argue that the ligand log $K_{Fe(II)L}$ and C_L values produced are mean or modal values. They are best considered as a representation of the net effect of natural Fe(II) binding ligands in a water sample. The detection window of the technique in terms of log $K_{Fe(II)L}$ is approximately 5.5-16. Ligands with log $K_{Fe(II)L}>16$ are too strong for ferrozine to compete. Weak ligands below the detection window cannot be characterised as they do not sufficiently complex Fe(II) (Ussher et al., 2005) and thus produce a titration curve indistinguishable from the aqueous Fe(II) ion. It is certainly possible that stronger or weaker ligands exist outside our detection window. To date strong Fe(II) ligands (log $K_{Fe(II)L}>16$) have not been identified in natural waters, although Kieber et al. (2005) have speculated that they may exist in rainwater. Weak Fe(II) ligands, where log $K_{Fe(II)L}<5.5$, almost certainly exist in relatively large quantities in natural waters as continuous reaction of natural dissolved organic material, for example through biological and photochemical decomposition (Cuss and Gueguen, 2012; Helms et al., 2013), will always result in a range of weaker ligands existing under natural conditions wherever stronger ligands are present. The direct influence of such weak ligands upon Fe(II) speciation will however be minimal (Ussher et al., 2005).

Our log $K_{Fe(II)L}$ values (quoted throughout at pH 6) are within the range of known values for Fe(II) humic interactions (Bao and Yu, 1987; Rose and Waite, 2003b; Yamamoto et al., 2010) and similar to those quoted for the interaction between humic material and other transition metal ions (Mantoura et al., 1978; Vraspir and Butler, 2009).

3.4 Results

3.4.1 Fe(II) organic interaction

A summary of analyses of LDFe, LDFe(II), the concentration and log $K_{Fe(II)L}$ values of Fe(II)-complexing ligands, and ancillary measurements for study sites in the UK and USA are shown in Table 2C. Any Fe(II) binding ligands present in solutions containing IHSS humic and fulvic acid standards spiked with Fe(II) were below the detection window of the technique (log $K_{Fe(II)L}$ 5.5). Non-ferrozine labile Fe ranged from 0-57% of total dissolved Fe determined via ICP (mean 38% for 30 estuarine samples from the Beaulieu, Cape Fear and Winyah Bay).

Sample location	Type	N	LDFe (nM)	pH	T (°C)	LDFe(II) (nM)	log $K_{Fe(II)L}$	C_L (nM)
River Beaulieu, Hampshire, England	River water	12	1400-19000	6.9-7.7	2-15	180-3600	6.1-8.3	370-7600
	Oak leaf detritus	3					7.0-8.1	
River Itchen, Hampshire, England	River water	5	79-450	7.6-8.5	5-12	12-230	8.5-11	14-200
	Diurnal sampling	4	240-480	6.3	12	26-81	11	18-77
Cape Fear, North Carolina, USA	River water	1	5200	5.5	22	770	8.5	910
	Transect	12	38-5000	5.6[a]	27	46-700	6.2-Bdw	2900-Bdw
Winyah Bay, South Carolina, USA	Transect	4	92-6200	5.6[a]	27	30-1900	6.5-Bdw	4000-Bdw
River Awe, Argyl, Scotland	River water	1	580	8.2	8	170	8.6	150

Table 2C LDFe(II) concentration, modelled ligand properties and complementary data for sampled river water and leachates: n, number of different samples titrated; LDFe, labile dissolved (<0.2 μm) Fe; LDFe(II), labile dissolved Fe(II); log $K_{Fe(II)L}$, modelled binding constant; C_L, modelled ligand concentration; bdw, complexation below detection window. [a] pH refers to fresh water.

Surface detritus collected from the New Forest (within the vicinity of the River Beaulieu) and suspended in deionised water for 24 hours released Fe(II) binding ligands with log $K_{Fe(II)L}$ values of 7.0-8.1, similar to those isolated from the river itself (Table 2C) and comparable to the range (log $K_{Fe(II)L}$ 6.6-10.2) obtained from six different vegetation types using a similar method (Rose and Waite, 2003b). Unfiltered River Beaulieu water stored for 1-2 days showed marked changes in the ligand binding constants obtained via reverse titration (Table 3C). The change in filtered samples stored under the same conditions was much less (an initial log $K_{Fe(II)L}$ value of 7.3 increased to 7.4 after 2 days).

Aging and storage conditions	log $K_{Fe(II)L}$	C_L (μM)	LDFe (μM)	LDFe(II) (μM)
Initial titration	7.3	0.57	5.7	0.95
2 days at 20° C	7.7	1.3	11	1.4
2 days at 3° C	7.7	2.0	13	2.3

Table 3C Changes observed in labile dissolved Fe and Fe(II) concentration and binding capacity of unfiltered river water after storage: LDFe, labile dissolved Fe; LDFe(II), labile dissolved Fe(II) present in water sample prior to titration; C_L, modelled ligand concentration; log $K_{Fe(II)L}$, modelled binding constant. The standard deviation of triplicate LDFe and LDFe(II) measurements was <5%.

Intra-day variation in ligands was monitored over a diurnal cycle for the River Itchen and the River Beaulieu. The greatest variation in both LDFe concentration and ligand properties was observed in the Itchen (Table 4C), although the absolute change was quite small. Water temperature (12°C) and pH (6.3) remained constant throughout the day from before sunrise at 07:52 until after sunset at 17:51.

Time	Log $K_{Fe(II)L}$	C_L (nM)	LDFe (nM)	LDFe(II) (nM)
07:20	11	77	400	81
11:20	11	18	360	44

15:20	11	59	180	55
18:30	11	25	400	26

Table 4C Intra-day variation in labile dissolved Fe(II) complexation in the River Itchen (24 October 2012, sunrise 07:52 sunset 17:51): C_L, modelled ligand concentration; LDFe(II) labile dissolved Fe(II); log $K_{Fe(II)L}$, modelled binding constant; LDFe, labile dissolved Fe. The standard deviation of triplicate LDFe and LDFe(II) measurements was <9%.

3.4.2 Estuarine mixing of Fe

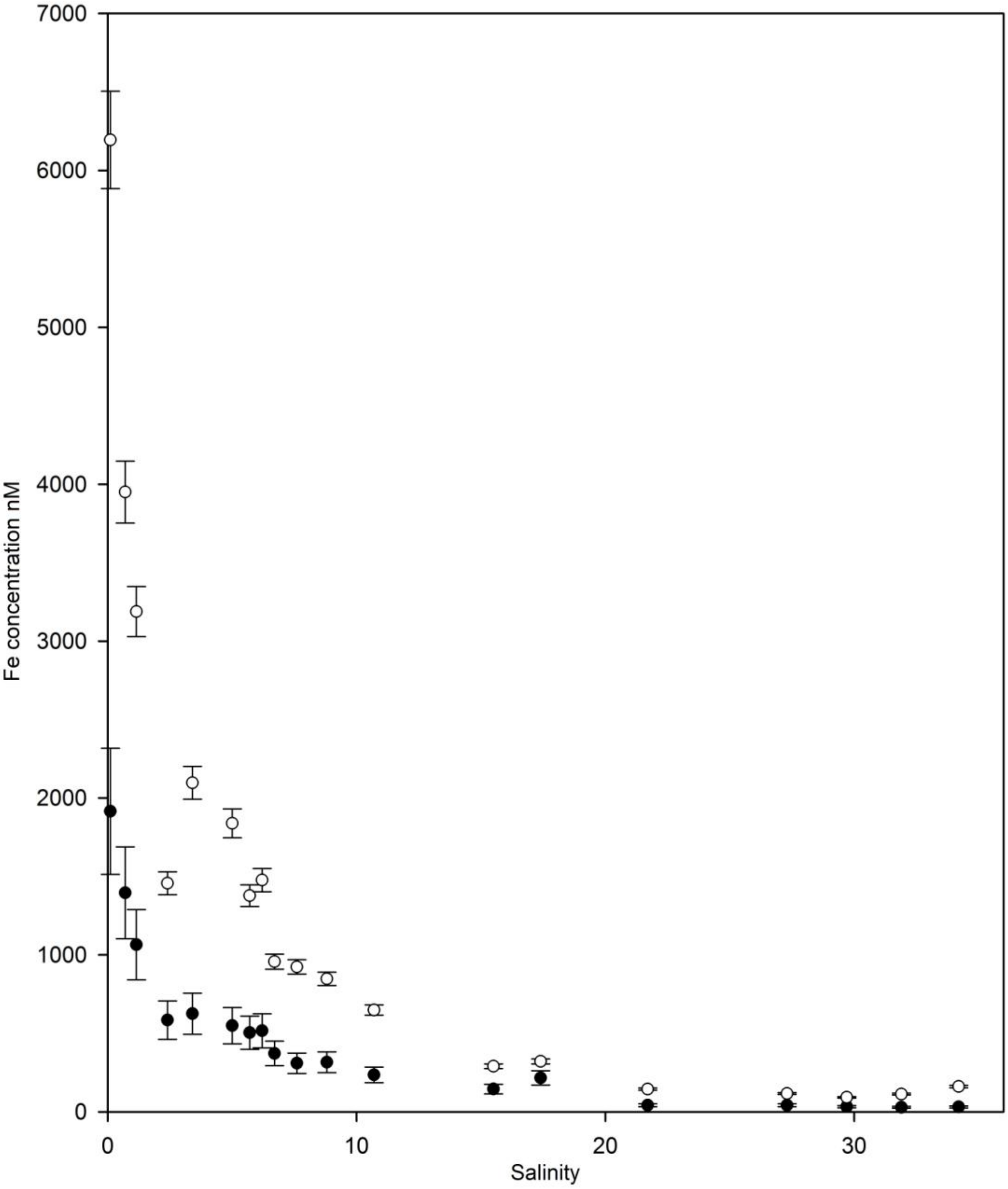

Figure 3C Estuarine mixing of labile dissolved (<0.2 μm) Fe (open circles) and Fe(II) (closed circles) in Winyah Bay 28 June 2013.

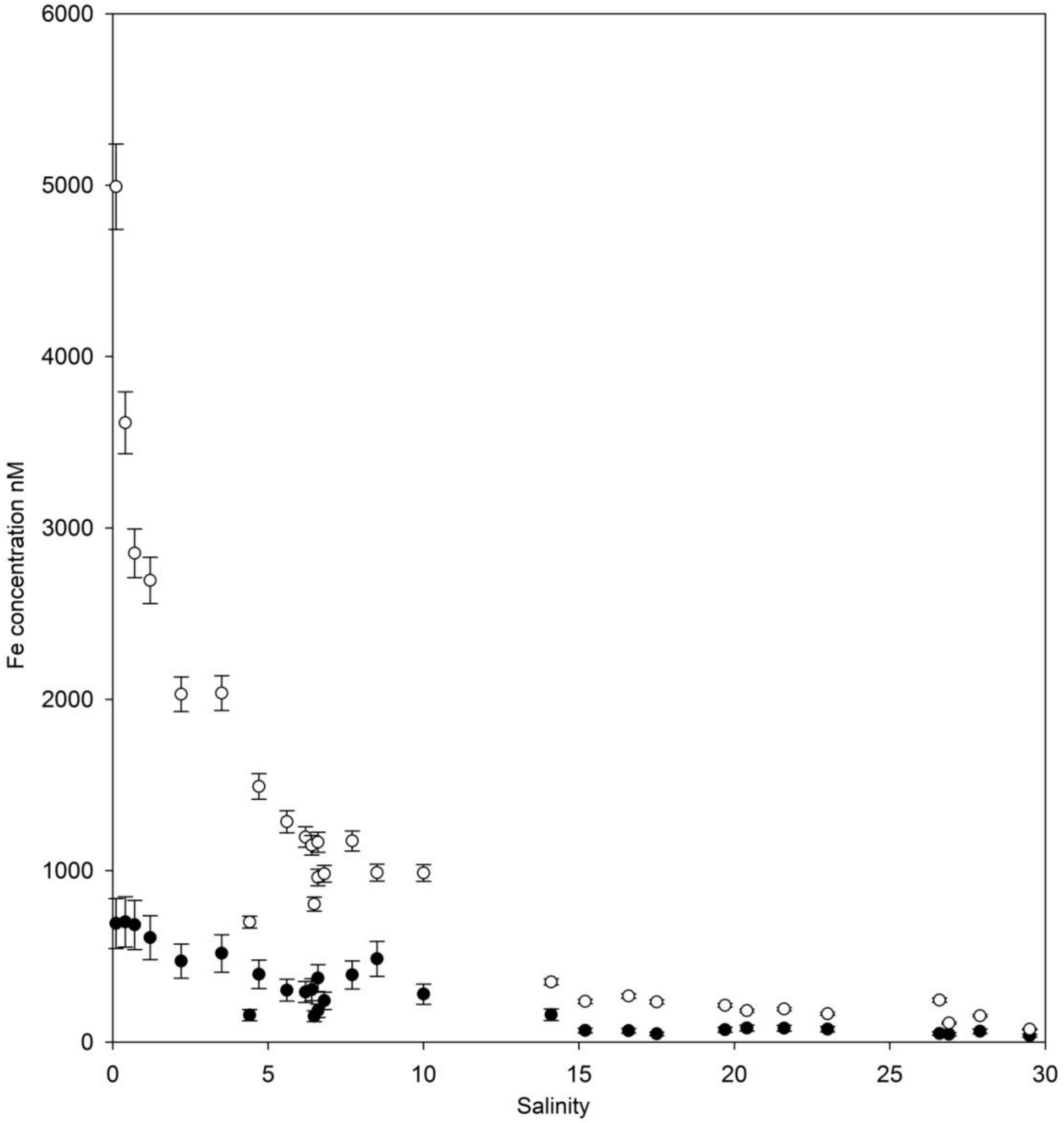

Figure 4C Estuarine mixing of labile dissolved (<0.2 μm) Fe (open circles) and Fe(II) (closed circles) in the Cape Fear estuary. Data collected 10 and 12 June 2013

In Winyah Bay (Figure 3C) and the River Beaulieu (Nielsdottir et al., 2012) labile dissolved Fe(II) is removed in a similar fashion to Fe. Particularly rapid removal at low salinities (<3) for both LDFe and LDFe(II) is evident in Winyah Bay as observed for dissolved Fe in many other river systems (Boyle et al., 1977; Mayer, 1982a; Sholkovitz, 1978). The Cape Fear (Figure 4C), which has very similar dissolved Fe and DOC concentrations to Winyah Bay, shows a similar pattern of estuarine LDFe mixing but with more conservative mixing of Fe(II). LDFe concentrations along the Itchen estuary (25 January 2014) are anomalous showing an apparent increase with salinity (Table 5C). A pronounced peak in both LDFe and DOC is evident at a salinity of 7.0.

Distance along estuary (km)	Salinity	LDFe (nM)	DOC (µM)
0.0	0	110 ± 13	200
0.5	0.1	140 ± 13	250
1.0	0.3	210 ± 40	250
5.5	7.8	190 ± 6	220
6.0	8.8	120 ± 28	220
7.0	7.0*	17000 ± 4600	450
7.5	22.8	190 ± 33	220

Table 5C Labile dissolved Fe concentrations (triplicate mean ± standard deviation) along the Itchen estuary (25 January 2014). *Collected within 200 m of a large effluent influx into the estuary.

Reverse titrations conducted along the salinity gradient of the Cape Fear and Winyah Bay (Table 6C) suggested a decrease of both binding constant and ligand concentration with increasing salinity. The binding constants at the lowest measured salinities were however close to the lowest measurable value that could be distinguished from uncomplexed Fe(II) in both estuaries. As C_L and log $K_{Fe(II)}$ fell with increasing salinity, Fe(II) complexation could not be characterised above a salinity of 5 in either estuary. Small additions of salt water to River Beaulieu water (final salinity of <0.5) under laboratory conditions similarly showed a decrease in C_L and log $K_{Fe(II)}$ with increasing salinity.

DOC shows generally conservative mixing across the salinity gradient from from 1270 (S 0.0) to 150 µM (S 34) in Winyah Bay (Figure 5C) and from 990 (S 0.0) to 280 µM (S 28) in the Cape Fear (Figure 6C), but mixing is anomalous in the Itchen (Table 5C). A decrease in CDOM of terrestrial origin (A and C) is seen with increasing salinity in both Winyah Bay (Figure 5C) and Cape Fear. In the Winyah Bay system an almost linear decrease in both DOC and terrestrially derived CDOM is clear with a slight change of gradient at a salinity of between 10 and 15. This gradient change may reflect the mixing of different water masses as five distinct major rivers merge in the Winyah Bay system. In the Cape Fear there is more significant deviation in both DOC and CDOM from conservative mixing.

River	Salinity	C_L (μM)	Log $K_{Fe(II)L}$
Cape Fear	0.05	1.9	6.2
	0.14	1.5	6.2
	0.50	0.84	6.2
	1.00	2.9	5.9
	1.32	Bdw	
Winyah Bay	0.14	4.0	6.5
	5.00	Bdw	
Beaulieu	0.00	16	6.8
	0.06	16	6.7
	0.10	16	6.7
	0.16	14	6.6
	0.38	14	6.6

Table 6C Changes in Fe(II) ligand behaviour derived from reverse titrations along the respective estuaries. Further titrations (7 more for the Cape Fear, 2 more for Winyah Bay) at higher salinities were all below the detection window ‘bdw’. To investigate ligand removal at very low salinities, filtered Beaulieu water (<0.2 μm, S 0) was mixed with small aliquots of aged, filtered seawater (<0.2 μm, S 35) and then allowed to stand overnight (at 20 °C in the dark) prior to filtration (<0.2 μm). C_L, ligand concentration and log $K_{Fe(II)}$, Fe(II) binding constant.

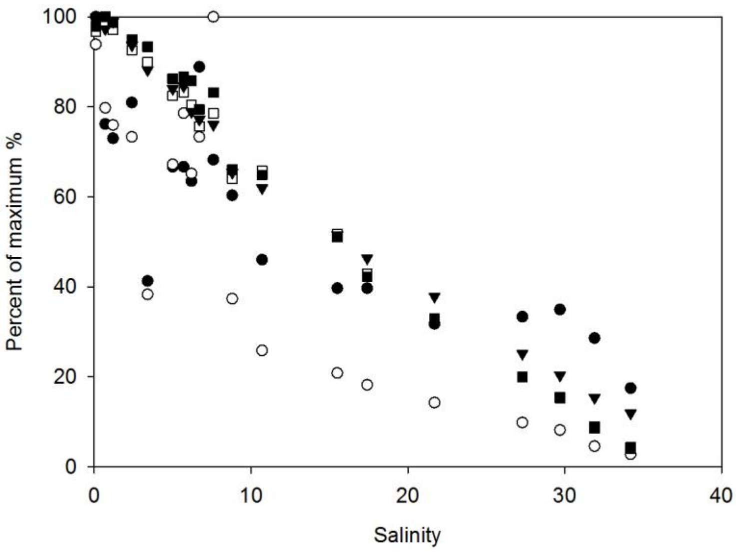

Figure 5C Winyah Bay water dissolved (<0.2 µm) components during estuarine mixing 28 June 2013: DOC (closed triangles), phosphate (closed circles), nitrate (open circles), CDOM A (open squares) and CDOM C (closed squares). Maximum values were 1.26 µM phosphate, 16.7 µM nitrate and 1270 µM DOC.

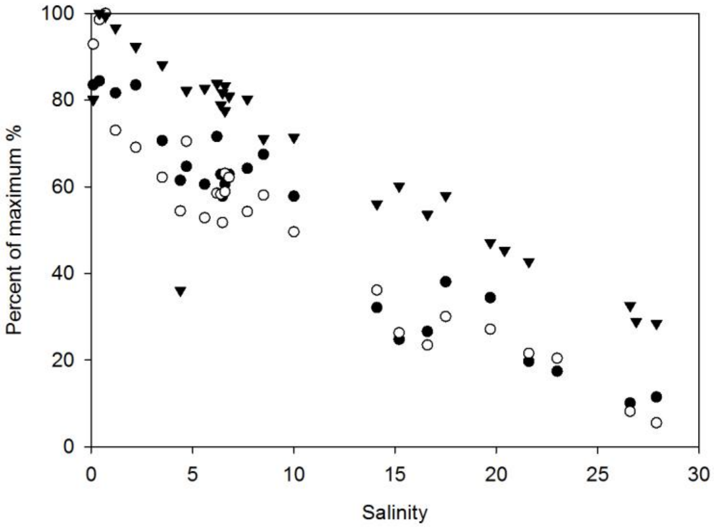

Figure 6C Cape Fear dissolved (<0.2 µm) components during estuarine mixing: DOC (closed triangles), phosphate (closed circles) and nitrate (open circles). Data collected 10 and 12 June 2013. Maximum values were 2.18 µM phosphate, 45.8 µM nitrate and 990 µM DOC.

3.5 Discussion

3.5.1 Fe(II) ligand properties and distribution

Ligand properties in the Beaulieu, Cape Fear and Winyah Bay are broadly similar with an average log $K_{Fe(II)L}$ of 6-7 consistent with moderately weak binding of Fe(II) by natural organic material as is observed with other metal ions (Mantoura et al., 1978). A loss of binding capacity during estuarine mixing beyond that expected solely because of dilution is found at low salinity (Table 6C) consistent with some removal of ligands from the dissolved phase during Fe flocculation (Mayer, 1982a; Sholkovitz et al., 1978). Several processes however are simultaneously affecting the concentration and effect of Fe(II) binding ligands along our estuarine transects. As salinity increases complex formation between metal ions and organic ligands is impeded due to increasing ionic strength, terrestrially derived humics are being diluted and to some extent flocculated, pH is increasing and the concentration of ions that may compete with Fe(II) for ligands (especially Ca^{2+} and Mg^{2+}) is also increasing (Miller et al., 2012). Attributing the decline in apparent C_L and log $K_{Fe(II)}$ to specific factors is therefore difficult with natural samples. Our results however suggest that Fe(II) ligands in the Itchen are much stronger than those observed elsewhere.

The Fe(II) ligands we detect in the Beaulieu, Cape Fear and Winyah Bay are almost certainly derived from natural detritus as indicated by the similarity of ligands leached from vegetation (Table 2C) and consistent with studies focusing on DOC cycling in freshwater (Harden et al., 2014; Rose and Waite, 2003a). There is insufficient data in the literature to discuss the global distribution of Fe(II) ligands in natural waters, but existing data do support the concept of a net decrease in binding constants upon prolonged aging of natural material (Bao and Yu, 1987; Rose and Waite, 2003a; Yamamoto et al., 2010). Whilst a natural reaction (i.e. a chemical change) to a ligand could in theory either increase or decrease its binding capacity for a trace metal ion, the vast majority of changes are likely to result in a lowering of the conditional binding constant through the destruction of functional groups that effectively bind a range of trace metals. This trend likely explains the generally weaker complexation estimated for decomposed material compared to fresher vegetation leachate (Bao and Yu, 1987; Cuss and Gueguen, 2012; Rose and Waite, 2003b).

Dissolved iron(II) ligands in river and estuarine water

Ligands in filtered river water were not found to change significantly over short (<2 day) time periods when stored post-filtration in the dark either at room temperature or at 3°C. Unfiltered samples however showed relatively large changes in both ligand properties (an increase in C_L and log $K_{Fe(II)L}$) and LDFe (Table 3C). This indicates that ongoing biogeochemical processes are likely to affect the ligand concentration and binding capacity in a natural water body. The increase in LDFe in isolated samples must be derived from large colloidal or particulate phases so increasing ligand concentration may have a similar source. Work on Cu ligands using incubated vegetation and the associated natural microorganisms shows a general decrease in the binding constant for Cu with time, although increases were observed for some organic components during short incubations (Cuss and Gueguen, 2012). If humic ligands are present in colloids, especially after estuarine flocculation where a fraction of humic compounds are known to be largely removed from solution (Sholkovitz et al., 1978), then a key factor controlling Fe(II) ligand activity in coastal waters may be the extent to which ligands are released from particulate or sedimentary phases. Release of Cu ligands from sediments has been observed in estuaries (Shank et al., 2004; Skrabal et al., 1997), but the extent of Fe ligand release from particles has yet to be thoroughly assessed.

There are limitations to how we interpret our estuarine results as our conditional binding constants are determined at fixed pH and our titration method artificially increases the concentration of Fe(II) at the expense of Fe(III). However it is clear that:

- Natural DOM is a source of relatively weak Fe(II) ligands to estuaries.
- The conditional binding constant of the majority of Fe(II) ligands in freshwater appears to be only just sufficient to complex Fe(II).
- Fe(II) complexation becomes weaker with increasing salinity and this cannot be explained by dilution alone.
- If strong Fe(II) ligands (log $K_{Fe(II)}$>>6) are present in the Cape Fear, Winyah Bay or the Beaulieu, then they can only account for a small fraction of the total ligand pool.

3.5.2 Is there an anthropogenic impact on estuarine Fe(II)?

Unlike other estuaries, Fe(II) ligand concentrations are measurable at a salinity of over 21 in the Itchen (Statham et al., 2012) and have consistently high binding constants (8.5-11.3). The increase in LDFe along the Itchen (Table 5C) is a complete contrast to the expected mixing pattern (Boyle et al., 1977).

There is strong evidence for the existence of relatively stable dissolved polysulphide species binding Fe(II) and other trace metal ions in many watersheds affected by sewage effluent (Kramer et al., 2007; Rozan et al., 2000). The River Itchen certainly falls into this category with 10% of freshwater input into the combined Test/Itchen estuary estimated to be sewage effluent (Webber, 1980).[11] This high fraction of wastewater and low LDFe concentrations in the natural river end member mean that the Itchen is likely to be particularly sensitive to anthropogenic Fe inputs. Anomalously high LDFe concentrations (Table 5C), particularly strong Fe(II) ligands (Table 2C) and the maintenance of Fe(II) ligand binding capacity at high ($S > 21$) salinity (Statham et al., 2012), are almost certainly therefore a direct consequence of anthropogenic waste input into the estuary.

The Cape Fear also suffers from significant inputs of effluent and organic-rich wastewater from concentrated animal operations. We estimate that this accounts for typically 2% of freshwater input (based on US Geological Survey flow data http://waterdata.usgs.gov/nc/nwis/sw and a recent estimate of wastewater discharge (Mallin et al., 2013)).[12] Effluent or other waste inputs may be responsible for some of the observed scatter in LDFe and LDFe(II) between salinities of 5-12 (collected within the vicinity of Wilmington, North Carolina), but the overall effect of 2% effluent by

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[11] This likely means that sewage is much greater than 10% by volume of the freshwater input into the Itchen itself as the River Itchen has a lower average freshwater discharge and a higher effluent input that the neighboring River Test.

[12] Considering a range of waste discharge volumes from 0 up to the maximum volume permitted, and the range of natural freshwater river discharge, sewage could constitute 0-10.5% of Cape Fear River water by volume.

volume in the Cape Fear is much less than it would be in the Itchen because LDFe and DOC concentrations in natural river water are an order of magnitude greater.

Like the Itchen, the Beaulieu is an estuary with a relatively small freshwater discharge. Several transects reported for the Beaulieu estuary have shown slightly lower than expected (<60%) estuarine removal of LDFe (Nielsdottir et al., 2012). Effluent discharge into the Beaulieu is episodic and no accurate data exist regarding the contribution these discharges make to the total freshwater input. However it does appear reasonable to suggest that effluent discharge into small estuaries is likely to significantly affect the concentrations and apparent removal of LDFe.

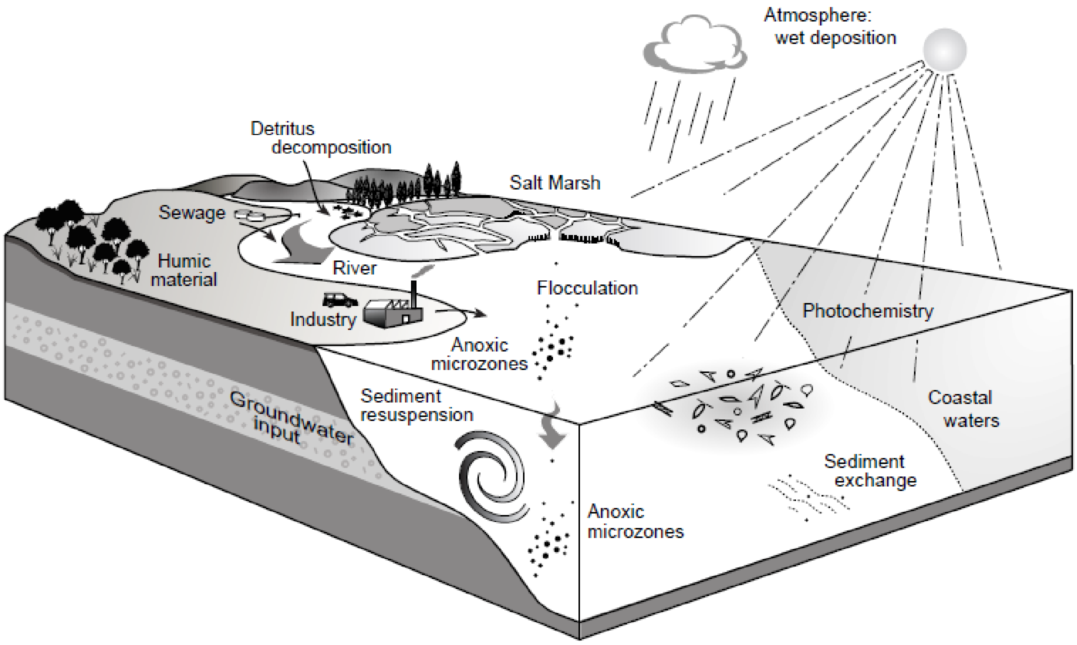

Figure 7C Conceptual illustration of fluxes and biogeochemical processes likely to be significant in controlling Fe(II) speciation in estuaries.

3.6 Conclusions

LDFe(II) forms a significant fraction of LDFe (7-30% at S 0) in five diverse estuarine systems with varying latitude, water temperature, DOC content, Fe concentrations and pH. Fe(II) humic ligands with a log $K_{Fe(II)L}$ of 8.6 or less (comparable to that for other transition metal ions) were found in natural waters with moderate to high DOC content.

Our data suggest that in freshwater these relatively weak Fe(II) ligands are normally equivalent to, or in excess of, ambient Fe(II) concentrations.

If strong Fe(II) ligands (log $K_{Fe(II)}$ >>9) exist in these high DOC estuaries, they cannot constitute more than a small minority of the ligands present. Fe(II) ligands leached from vegetation occur with an overlapping but broader range of binding constants compared to those in river water. A decline in the concentration, greater than that expected from dilution alone, and conditional binding constants of ligands along estuaries suggests that these ligands are less effective at binding Fe(II) in coastal seawater. These terrestrially derived Fe(II) ligands may be exported to seawater in both the aqueous and particulate phases (Figure 7C), however their impact upon Fe biogeochemistry in coastal seawater may be limited by their very low conditional binding constants.

It has been suggested that changing land use can strongly influence estuarine Fe concentrations and bioavailability by changing DOM inputs into adjacent waters (Kawaguchi et al., 1997; Lewitus et al., 2004). We find evidence of another anthropogenic impact on estuarine Fe biogeochemistry. Effluent introduces Fe(II) and Fe species into estuarine water. In high DOC, high LDFe rivers where wastewater constitutes a small (<10%) fraction of freshwater input, the impact of this additional Fe is not likely to be significant. However, in small estuaries with low natural DOC and LDFe concentrations (typically alkaline chalk streams) Fe input may be dominated by wastewater. In these small estuaries, where dissolved Fe concentrations would otherwise be much lower, this increase in Fe supply may have implications for estuarine ecosystems (Lewitus et al., 2004).

3.6.1 Acknowledgements

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4. Glacial flours as a potential source of Fe(II) and Fe(III) to polar waters

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4.1 Abstract

Supply of Fe to High Nitrate Low Chlorophyll (HNLC) waters, such as the Southern Ocean, is the principal limiting factor for primary production and the associated carbon uptake. Glacial meltwaters have recently been shown to provide a globally substantial input of Fe to both Antarctic and Arctic polar waters. The particulate Fe flux in glacial runoff is several orders of magnitude greater than the dissolved Fe flux and yet little is known, to date, about the potential bioavailability of this particulate Fe. A fraction of particulate Fe may be bioavailable to organisms and thus have a significant impact on biota in HNLC zones. Here, we describe in detail the composition and speciation of Fe released from four different glacial flours. We show that the fraction of labile Fe varies markedly between different flours and is not proportional to total Fe. Furthermore a small fraction of the particulate Fe is available to the Fe(II) selective ligand ferrozine, which indicates active redox cycling on the surface of the flour.

4.2 Introduction

In polar systems, glacial run off and icebergs deliver significant quantities of Fe and other nutrients into adjacent coastal waters (Apolloni, 1973; Dierssen et al., 2002; Gerringa et al., 2012; Statham et al., 2008; Wadham et al., 2010). Hart (1934) noted the pronounced beneficial effect on phytoplankton growth that small traces of Fe may provide and speculated that there may be a link with the large number of debris-rich icebergs in the Weddell Sea, where there were large phytoplankton populations. By inducing changes in the flux of bioavailable Fe into coastal waters and thus modifying carbon dioxide uptake, the glacial Fe cycle in the Southern Ocean may have played an important role in regulating glacial/interglacial cycles (Byrne et al., 2011; Raiswell et al., 2006; Wolff et al., 2006).

The magnitude of glacial Fe inputs to the ocean, whilst recognised as potentially important, has been derived from only a few datasets, and some aspects of the Fe flux, in particular the release of dissolved Fe from glacial sediment, have yet to be fully characterised. Polar seas are becoming rapidly impacted by a warming climate, (Overland et al., 2008) and a better knowledge of the particulate Fe that will increasingly be released is of importance for our ability to predict any likely biogeochemical impacts.

4.2.1 Origin and impact of glacial particulate Fe

The terrestrial origin of Fe in polar seas is intimately linked to ice-water-rock interactions. Erosion of bedrock by ice and subsequent re-freezing of pore waters leads to high particle loading in the basal layers of glaciers, observed as visibly darkened layers (Lin et al., 2011; Shaw et al., 2011). This comminuted bedrock, or glacial flour, can reach the ocean via two direct routes, in meltwater as suspended particles and as debris frozen within icebergs (Raiswell et al., 2008). It can be found also across proglacial plains having been deposited from glacial streams, or as melt out debris from the glacial margins. The majority of glacial particles and associated Fe carried by meltwater are likely to be removed from the water column at low salinities due to settling and estuarine mixing processes (Boyle et al., 1977). Off the Antarctic Peninsula turbidity and total acid leachable Fe in surface waters both decrease rapidly (within a few km) offshore (Dierssen et al., 2002; Gerringa et al., 2012). Re-suspension in shallow shelf areas may, however, redistribute some sedimentary material into the water column (Fitzwater et al., 2000) and lateral advection may carry a small fraction of this material away from the continental shelf over long distances (Lam and Bishop, 2008). Glacially derived particles may therefore act as a significant source of bioavailable Fe to coastal polar waters (Raiswell and Canfield, 2012; Smith et al., 2007; Smith and Gordon, 1997).

To date there has been little discussion concerning the availability of glacial particulate for complexation with ligands, despite the known importance of ligands in Fe biogeochemical cycling (Boyd et al., 2010; Hunter and Boyd, 2007). Another important question of current interest is exactly how “bioavailable” the various particulate Fe phases are (Planquette et al., 2007; Raiswell and Canfield, 2012).

4.2.2 Meltwaters as a source of dissolved Fe

Low concentrations of dissolved Fe arise throughout icebergs (and other ice) from aerosol deposited debris, whereas higher dissolved Fe concentrations are associated with basal ice rich in terrestrially associated debris (Lin et al., 2011). ‘Dissolved’ Fe in meltwater will in fact include nanoparticles that have been identified in this type of ice (Raiswell, 2011a; Shaw et al., 2011). A mounting body of data supports melting glaciers as a significant source of dissolved Fe to coastal waters in Antarctica. For example, Gerringa et al. (2012) measured elevated surface dissolved concentrations > 100 km from the mouth of Pine Island Glacier, which they attributed to an enhanced supply of Fe from meltwater. Similarly, Fitzwater et al. (2000) report elevated dissolved Fe concentrations in the inshore waters of the Ross Sea. They observe that intense blooms in Antarctic waters are generally limited to shallow shelf areas along receding ice margins, where we would expect a high bioavailable Fe input from meltwater enriched in dissolved Fe.

Previous studies of meltwaters have not distinguished between the two redox states of Fe in the dissolved phase (Bhatia et al., 2013; Gerringa et al., 2012; Lin et al., 2011; Statham et al., 2008). Under oxic conditions dissolved Fe(II) should rapidly oxidise unless stabilised by natural ligands, or a steady state flux from biological or photochemical sources. However in spite of this Fe(II) concentrations in the Southern Ocean have been found to constitute up to 63% of surface dissolved Fe (Sarthou et al., 2011).

Glacial Fe inputs are clearly significant and may dominate supply of this biologically essential element to the Southern Ocean (Raiswell and Canfield, 2012). De Jong et al. (2012) calculate that horizontal advection of dissolved Fe from shelf sediments is the largest source of ‘new’ Fe (54%) to the Atlantic section of the Southern Ocean indicating that glacially derived sediments play an important role in supplying Fe to the largest HNLC region. The flux of particulate Fe from glaciers is several orders of magnitude greater than that of dissolved Fe. However there are few studies exploring the chemical lability of this particulate fraction, which is of critical importance in determining its likely bioavailability and therefore its likely impact on primary productivity in polar seas (Raiswell et al., 2008). Through a detailed characterisation of Fe speciation we will estimate the fraction of Fe in glacial flours that may be bioavailable and determine whether this fraction varies significantly between different flours. We will then attempt to estimate the flux of bioavailable particulate Fe to polar seas and determine whether processes such as reductive dissolution of glacial particulate provide a significant source of Fe(II) to polar waters.

4.3 Experimental

4.3.1 Sample collection and handling

Comminuted rock material was collected from the proglacial plain directly in front of the glacier terminus (Table 1D). All samples are estimated to have been deposited no more than 3 months prior to the time of collection as the collection sites would have been below the glacier throughout the prior winter.

Glacier	Location	Bed rock geology
Greenland	South west Greenland	Archean metamorphics
Bodalsbreen	Norway	Granite
Les Bossons	Alps	Granite

Signy Island	Antarctica	Mica schists and phyllites

Table 1D Provenances of the four glacial flours.

All sampling, storage and pre-treatment methods can impact the surface properties of solid phases to some extent. For example, aging, freezing and drying may reduce the reactivity of amorphous phases (Raiswell et al. 2010), and wet storage may promote microbial processes on grain surfaces, which could result in sulphide oxidation and the formation of reactive Fe oxyhydroxides. We chose to store the four glacial flour samples without drying, without freezing and in the dark, since we felt these conditions best reflect sediment just below the surface of the proglacial plain in summer. Small quantities were freeze dried to constant mass as and when required. For the leaching experiments the freeze dried glacial flours were separated into 5 fractions using 63, 106, 180 and 500 μm nylon mesh and plastic contamination-free sieves, with the 500 μm sieve used to remove occasional large particles. Leaches were begun immediately after the size separation. All apparatus was soaked in 3 M HCl (trace metal grade) and rinsed three times with deionized water (MQ 18.2 MΩ·cm) before use. Sample handling was conducted under a class 100 laminar flow hood.

4.3.2 Methods for determining different fractions of Fe

In order to examine the Fe phase associations in the flours, four different chemical leaches were applied: 1) prolonged dissolution in either deionised or coastal sea water, 2) ascorbic acid, 3) citrate dithionite, and 4) an Fe II selective ligand leach. Total Fe was also measured. Previously, concentrations of Fe determined by each of the leachates have been operationally defined. When determining water soluble Fe, leaching times, particle size and particle load have varied from study to study in previous work on other particulates (Aguilar-Islas et al., 2010; Chen et al., 2006). Natural variability in the Fe content and association of particles from different environments is high however and thus differences in methods are argued to be relatively small in comparison (Aguilar-Islas et al., 2010; Schroth et al., 2009; von der Heyden et al., 2012). Here, glacial particles were suspended in either deionised water ('DW', MilliQ, 18.2 MΩcm) or UV-treated coastal seawater (salinity 25.9, Southampton Water), for 30 days. The total dissolved (<0.2 μm) Fe in solution was then measured providing information on the steady state Fe concentration that can be maintained in solution for an extended period of time.

Chemical leaches of particulates, such as 2) and 3) are better defined, because of greater knowledge about what specific Fe minerals are targeted by different leaching conditions (Poulton and Canfield, 2005; Poulton and Raiswell, 2005). An ascorbic acid leach specifically dissolves only the labile oxyhydroxide ferrihydrite, which is frequently present as nanoparticles (Raiswell et al., 2010). Hyacinthe et al. (2006) showed that 65% of the Fe extracted via this method is microbially available to *Shewanella putrefaciens*. A more reducing dithionite-citrate leach has been shown to bring into solution more crystalline Fe oxides (Poulton and Canfield, 2005).

We acknowledge that the determination of an absolute value for the “soluble” fraction of Fe present in any sample is difficult, but contend that the systematic use of different leaching approaches can provide important information on the relative availability of the Fe present in glacial flour, as it has in studies on other particulate matter (Aguilar-Islas et al., 2010; Cwiertny et al., 2008; Schroth et al., 2009; Spolaor et al., 2013).

4.3.3 Use of ferrozine as a leaching agent and mimic of natural Fe(II) ligands in solution

Whilst high structural Fe(II) content has been observed in both glacial flour and particles collected from the Weddell Sea gyre (Schroth et al., 2009; von der Heyden et al., 2012), thus far, no ligand leaching approach has been used to estimate ‘available’ Fe(II) in glacial flour. Here, the Fe(II) selective reagent, ferrozine, was used to leach Fe(II) from glacial flour as a mimic of ligands which may be present in natural waters. Several experiments were run to assess the impact of particle loading, temperature, light, and agitation on the leaching process, and to aid selection of optimal experimental conditions. Tests were run with particle loadings between 0.25 and 2.5 g L^{-1}, establishing that there was substantial variability between duplicate experiments (likely due to the heterogeneous composition of the flours) at particle loadings <1.0 g L^{-1}. A series of leaches at different temperatures to the standard conditions (21 ± 1°C) showed a 30-60% decrease in measured Fe(II) at 3°C and a 35-130% increase at 35°C. Various authors have reported that photochemistry increases the release of Fe from minerals, particularly in the presence of organic complexes (Miller et al., 1995; Stumm and Sulzberger, 1992; Wells and Mayer, 1991). Therefore, all ferrozine leaches were conducted inside Al foil wrapped vials to prevent photochemical enhancement of Fe(II) release. Ferrozine leach replicates exposed to light always showed an increase in measured Fe(II). The average dissolved (0.45 µm) Fe(II) concentration after 10 days of a 100 µM ferrozine leach in deionized water was 2 times greater if left exposed to ambient light. For sea water, the increase was slightly less. A small decline in the measured concentration of Fe(II) in an Fe(II)-ferrozine standard of the order 2-3% week^{-1} was measured under the experimental conditions used. This was attributed to oxidation of the ferrozine complexed Fe(II) and removal of the dissolved Fe(III) from solution.

4.3.4 Methods

Scanning electron microscopy (SEM) micrographs were obtained from 200 mg of each freeze dried flour, mounted in electron grade resin and coated with 20 nm carbon. Backscattered images of sample fragments were obtained on a LEO 1450VP SEM (Carl Zeiss SMT Ltd), equipped with a Princeton Gamma Technology light element dispersive microanalysis system. X-ray diffraction (XRD) was conducted using a Philips X'Pert Pro with a Cu X-ray tube. The four samples were loaded on a silicon substrate and minerals were identified using the Joint Committee for Power Diffraction Studies database. All chemicals were trace metal grade and used "as purchased" from Sigma Aldrich. Total Fe and Al (TFe and TAl) were measured by ICP-MS after HF/HNO_3 digestion. Triplicate analysis of BCSS-1 certified reference material, gave TFe of 576 ± 17 µmol g^{-1} and TAl 2.23 ± 0.049 mmol g^{-1} (certified values are 587 ± 25 µmol g^{-1} and 2.32 ± 0.040 mmol g^{-1}). Total organic carbon (TOC) and nitrogen were measured using a Carlo Erba EA 1108, after acid treatment of the samples and verified against an internal standard. Ascorbic acid and buffered dithionite solutions were prepared as per Raiswell (2011a). Fe concentration in the ascorbic acid (FeAsc) and dithionite (FeDi) leaches was determined by flame atomic absorption spectroscopy (Varian Spectra AA55). Ferrozine leaches were conducted by adding 20 mg of flour to 20 mL of deionized water containing 100 µM ferrozine, then mixing in Al foil wrapped vials for 24 hours. A Fe(II) stock standard was prepared from ammonium Fe(II) sulphate hexahydrate in 0.3 M hydrochloric acid and 0.08 M sodium sulphite, and diluted to give working standards. Fe concentrations were determined using UV/Vis spectroscopy with an Ocean Optics SB4000 Fiber Optic spectrometer and a 3000 series 250 cm World Precision Instruments liquid waveguide capillary cell. The system detection limit was 4.0 nM. Deionized water and coastal seawater leaches were conducted by adding 20 mg of glacial flour to 20 mL water in Al foil wrapped vials on a rotating table, allowing equilibrium with the atmosphere to prevent the depletion of oxygen in solution. Measurement of dissolved Fe was conducted spectrophotometrically after adding ascorbic acid (final concentration 0.5 mM) and ferrozine (final concentration 0.12 mM) to filtered (0.45 μm) samples

4.4 Results

4.4.1 Solid phase Fe speciation

TFe of the glacial flours varied between 2.6 and 8.2 (wt%), with the different grain size suites of each flour having broadly similar TFe values. When all flour fractions are combined using the appropriate fractions of total mass for each size suite, TFe of the flours decreases in the order Signy Island (1200 μmol g^{-1}) > Les Bossons (660 μmol g^{-1}) > Greenland (570 μmol g^{-1}) > Bodalsbreen (390 μmol g^{-1}) (see supplementary table for full dataset). TFe/TAl ratios across all grain-size suites [Signy (0.54-0.81), Les Bossons (0.40-0.56), Greenland (0.32-0.48) and Bodalsbreen (0.26-0.39)] were close to the range of 0.35-1.61, as previously found in four glacial meltwater sediments (Poulton and Raiswell, 2005).

Scanning electron micrographs (Fig. 1D) show a heterogeneous distribution of Fe across all four glacial flours with surface Fe on Bodalsbreen significantly greater than that of Les Bossons and Greenland. XRD detected the Fe bearing minerals, riebeckite, and an unidentified amphibole in all glacial flours except Les Bossons, and clinochlore in all flours except Greenland. TOC differs by over two orders of magnitude between the glacial flours. Greenland has a particularly low TOC content (<30 μmol g^{-1} across all size fractions) and the larger size fractions of Bodalsbreen flour have particularly high TOC content (90-180 μm 900 μmol g^{-1} and 180-500 μm 2020 μmol g^{-1}). Nitrogen (data not shown) was below detection in all samples, apart from two Bodalsbreen fractions. A significant linear correlation between FeAsc and the TOC content of flours (R^2 0.63 compared to 0.03 between TFe and TOC) is found.

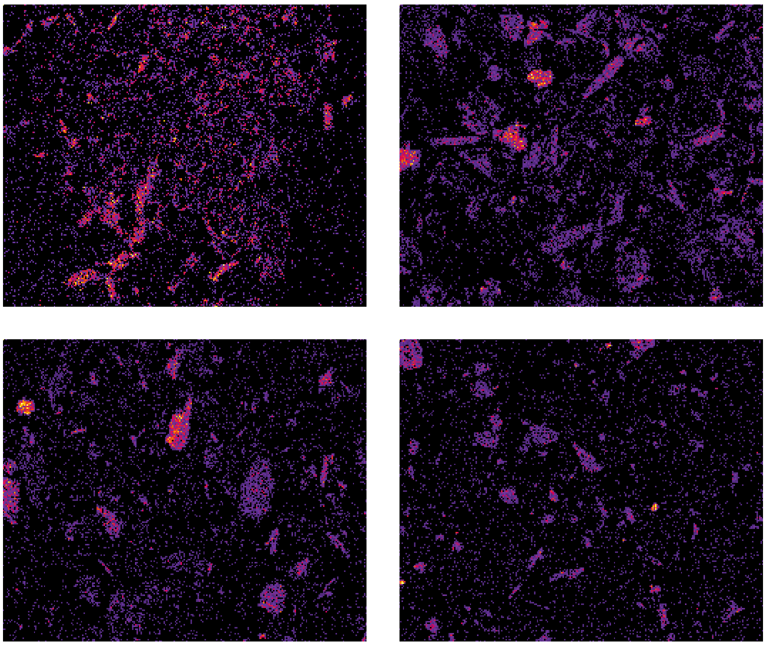

Fig.1D SEM images under 400 times magnification highlighted to show Fe in purple and Fe hotspots in orange. Top left clockwise: Bodalsbreen, Signy Island, Greenland and Les Bossons.

All size fractions of each flour had similar FeDi, displaying a mean of 60 μmol g^{-1} (n=16), compared to a mean TFe of 740 μmol g^{-1}. This compares well with average values of 84 μmol g^{-1} (FeDi) and 756 μmol g^{-1} (TFe) for 36 sediment samples collected from a range of glacial meltwater streams by Poulton and Raiswell (2002). Similar average values of 100 μmol g^{-1} (FeDi) and 590 μmol g^{-1} (TFe) have been reported from two ice rafted debris samples collected from Weddell Sea icebergs (Shaw et al., 2011).

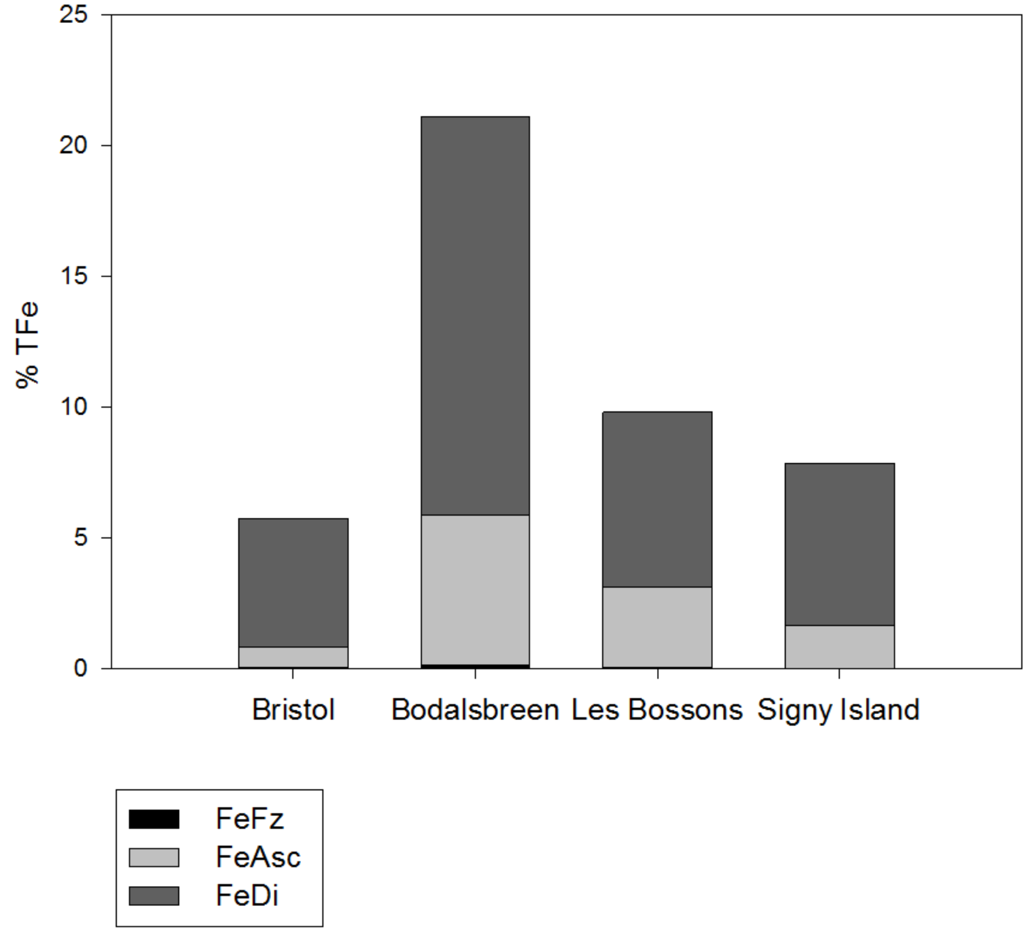

Fig. 2D FeFz, FeAsc and FeDi expressed as a fraction of TFe for < 63 µm flour fractions.

FeAsc, which reflects concentrations of bioavailable ferrihydrite, displays greatest concentrations in the finer grain size fraction (i.e. < 63 um, Fig. 2D) for all flours except Bodalsbreen. The Bodalsbreen flour is notable for its high proportion of ascorbic acid soluble Fe (4.7-11.1% for the four Bodalsbreen fractions compared with 0.3-2.4% for other flours). A similar range of 1.1-14.3% is reported for sediments collected from two Weddell Sea icebergs (Shaw et al., 2011).

4.4.2 Dissolved phase Fe

The total dissolved Fe (Fe(II) plus Fe(III)) released into DW and SW ranged between 0.012 (Les Bossons) and 0.699 (Greenland) µmol g^{-1}. There is no relationship between total Fe and the quantity of Fe extractable by deionized water (R^2 0.00), coastal seawater (R^2 0.09), or ferrozine (R^2 0.23) in our dataset.

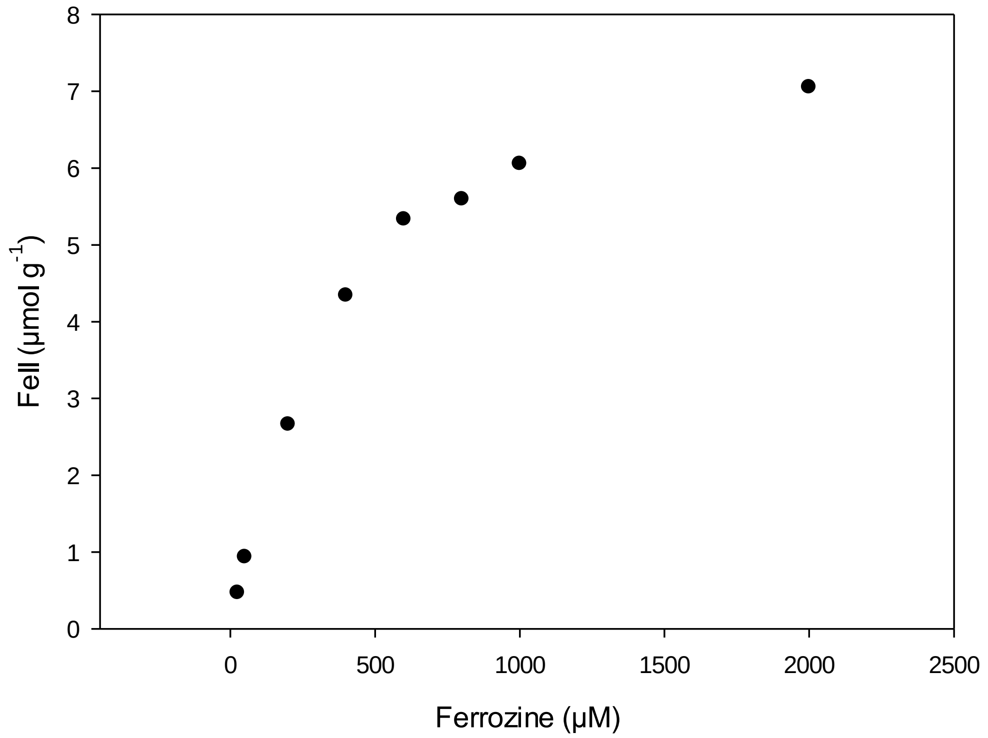

Fig. 3D Ferrozine leachable Fe(II) measured as a function of Ferrozine concentration for Greenland < 63 µm flour.[13]

The amount of Fe leached in the presence of ferrozine depended on the concentration of the ligand (Fig. 3D). When ferrozine concentration was fixed at 100 µM, leachable Fe ranged between 0.08 and 0.52 µmol g^{-1}, which is a very small fraction of total Fe compared to the FeAsc leach (Fig. 2D). At higher concentrations over longer time periods however, ferrozine was capable of releasing more Fe than a FeAsc leach (Fig. 3D). Ferrozine leaches left for prolonged periods of time (>2 weeks) produced an almost linear relationship between Fe(II) and Ferrozine concentration, up to around 0.50 mM of the complexing ligand (Fig. 3D). Beyond this, there was only a limited increase in measured Fe(II) with increasing ligand concentration. The concentration of leached Fe(II) decreased to an average of 64% of that initially measured if the leach was repeated using the same flour. Fe fractions leached by deionised water, seawater and 100 µM Ferrozine correlated better with FeAsc than with TFe, producing linear R^2 values of 0.31, 0.28 and 0.42 respectively.

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[13] The ferrozine leach removed more Fe(II) here at 100 µM than observed on a separate batch of the same material (Supplemental Data). A lack of homogenisation of the bulk flour may explain this difference.

4.5 Discussion

4.5.1 Fe fractions in the solid phase

FeAsc and FeDi vary significantly between different flours and are not directly proportional to TFe (Fig. 2D). 85-97% of TFe in all size suites is unavailable to a dithionite leach and therefore unlikely to be mobilised by any natural process (supplementary table). Cwiertny et al. (2008) conducted acid leaches on five mineral dusts and similarly found no correlation between TFe and the fraction of leachable Fe.

The large discrepancy between TFe and SEM observed surface Fe may be explained by the formation of surface Fe oxyhydr(oxide) nanoparticles which are a source of labile surface Fe that will be partially included in the FeAsc fraction (Raiswell, 2011a; Raiswell et al., 2006). A significant correlation exists between FeAsc and the TOC content of flours (R^2 0.63 compared to 0.03 between TFe and TOC) as reported previously elsewhere (Poulton and Raiswell, 2005). TOC surface distribution is highly heterogeneous occurring as localised smears rather than a uniform coating (Poulton and Raiswell, 2005). The correlation between TOC and FeAsc may therefore result from either enhanced development of localised anoxia in organic carbon rich glacial sedimentary environments, leading to the reductive release of Fe(II) followed by ferrihydrite formation; or from TOC facilitating the adhesion of nanoparticles to the mineral surface and thereby allowing reactive surface Fe to accumulate. A similar accumulation of Fe nanoparticles has been observed on the edge of clay minerals (Poulton and Raiswell, 2005).

The difference between SEM observed surface Fe and TFe, determined by acid digestion, is in fact greatest for the Bodalsbreen flour which also has both the highest TOC content and the highest FeAsc fraction of TFe. Surface Fe determined by SEM is significantly greater for Bodalsbreen than for Les Bossons or Greenland despite their higher TFe content and this is consistent with their much lower TOC and FeAsc. Of our four flours, Bodalsbreen contains the most potentially bioavailable Fe by sediment mass, despite having the lowest TFe and a similar mineralogy to the other three flours. Bodalsbreen glacier had recently advanced before this glacial flour was collected therefore the particularly high levels of organic rich material may originate from vegetation in proglacial sediment. As all of the flours had aged to some degree since they were collected, the Fe present may have become more crystalline and therefore less available to chemical leaches and dissolution (Raiswell et al., 2010). We therefore expect our estimates for FeAsc and FeDi to be underestimates of labile Fe.

4.5.2 Water soluble Fe

The pH, temperature, salinity and oxygen content of solutions influences the quantity and speciation of Fe released into solution (Liu and Millero, 1999; Millero et al., 1995). Temperature and oxygen content were constant across all samples. At the end of the prolonged dissolution experiments, the seawater pH (initially 7.9) was between 6.2 and 7.6. The deionized water, in the absence of natural buffering, was more sensitive to changes in pH upon addition of glacial flour. After one month in deionized water (pH=6.9), the Les Bossons pH averaged 7.0, Greenland 7.1, Signy Island 8.3 and Bodalsbreen 8.7, thus showing a move towards more basic conditions over the experiments. These pH shifts may mirror initial changes in porewater chemistry that would occur over significant time periods (months to years) in fluvially deposited glacial sediments on proglacial plains, in the absence of other confounding factors.

Any Fe(II) released from particles into these oxygen containing solutions would be expected to oxidise very rapidly. The total dissolved Fe remaining in 0.2 µm filtrate must be stabilised, either as small colloids or complexes, as the reported concentrations are well above expected solubility values with respect to oxide phases (Liu and Millero, 1999; Millero, 1998). There are marked differences in the dynamics of Fe acquisition by the solution between different grain size fractions from the same flour. The measured Fe in solution arises from two competing effects, firstly the release of dissolved or colloidal Fe into solution, and secondly, the removal of Fe from solution by precipitation or adhesion to the surface of mineral grains.

The absence of a relationship between TFe and dissolved Fe is consistent with a large fraction of TFe being inert. Fe fractions leached by deionised water and seawater correlated better with FeAsc, producing linear R^2 values of 0.31 and 0.28 respectively. These correlations are poor and coupled with no significant correlation between dissolved Fe and TOC suggest that a range of factors affect the concentration of Fe obtained in solution with the solid phase Fe speciation one of multiple considerations.

4.5.3 Leaching of Fe(II) from the solid phase

The ferrozine extraction detects solely Fe(II). This could include Fe(III) that may have been reduced under the experimental conditions. It is possible that some of the Fe(II) observed may be of biological origin as glacial flours are known to be populated with active microbial communities (Skidmore et al., 2000; Tranter et al., 2005). The Fe(II) leached by ferrozine increases with ferrozine concentration (Fig. 3D), but even with 100 μM ferrozine in solution the Fe leached after 24 hours is still tiny compared to the quantity of bioavailable Fe present in the solid phase (Fig. 2D). The levelling off of Fe concentrations at higher Ferrozine concentrations most probably reflects depletion of available Fe from the surface of the flour. This suggests that ferrozine leaches a specific ligand-available surface Fe fraction, which probably overlaps, to some extent, with FeAsc; but the generally poor correlation (R^2 0.42) suggests that the ligand available Fe(II) is not directly proportional to the labile FeAsc present. TOC may contain chemical groups that effectively bind Fe(II) or provide micro zones of anoxia from where Fe(III) can be reduced and bind to ferrozine, but there is no correlation between ferrozine leached Fe(II) and TOC therefore this is not likely to be a major source of Fe(II) into solution.

Ferrozine has been used previously to efficiently extract surface adsorbed Fe(II) from marine sediments where high Fe(II) concentrations exist because of reducing conditions (van der Zee et al., 2002). Surface adsorbed Fe^{2+} is rapidly complexed by ferrozine and therefore only a short exposure time of 5 minutes was used. Structural Fe(II) is likely to be present in the solid Fe phase of the four glacial flours as this has been previously demonstrated in both glacial flour and Weddell Sea particulate using X-ray absorption spectroscopy (Schroth et al., 2009; von der Heyden et al., 2012). Structural Fe(II) is unlikely to be present at the mineral surface as Fe(II) rapidly oxidises under oxic conditions and therefore should not be directly accessible to ferrozine. The only viable mechanism via which measurable quantities of Fe(II) could exist at the surface on these particles is by stabilisation with TOC, but the absence of a correlation between FeFZ and TOC (R^2 of 0.12) indicates that this is not the case. In this study using 100 μM ferrozine, leached Fe(II) was still increasing after 10 days of leaching in the dark and therefore the ferrozine complexed Fe(II) must either be strongly bound to the surface of an iron mineral, or be formed continuously. Continuous formation of Fe(II) via reduction is more likely given the oxic conditions, therefore the ferrozine leach may be indicating active surface reduction of labile Fe mediated by ferrozine itself and possibly other natural ligands (Stumm and Sulzberger, 1992), or active biological reduction.

4.5.4 Potential environmental impacts of Fe in glacial flours

The experiments here indicate that glacial flours have the potential to be significant sources of particulate Fe to biota in coastal and oceanic systems (Byrne et al., 2011; Raiswell, 2013; Statham et al., 2008). The FeAsc fraction, which has been shown to be largely bioavailable to some organisms (Hyacinthe et al., 2006) constitutes a varying fraction of glacial flour TFe with values here between 0.34-11%. Freshly deposited glacial flours may exhibit higher FeAsc fractions due to the effects of aging making Fe more crystalline and less labile. Few other glacial particulate FeAsc values are available but Shaw et al. (2011) found FeAsc constituted 1-14% of TFe in four particulate fractions collected from Weddell Sea icebergs which indicates that our values are likely to be representative.

Estimated global sediment fluxes to coastal waters are 140 Tg yr^{-1} from meltwaters (inclusive of a 90% removal factor at low salinities) and 1500 Tg yr^{-1} from icebergs (Raiswell et al., 2006). We can therefore construct estimates of bioavailable Fe delivered to the ocean. The mean FeAsc content of our 16 glacial flour fractions is 15 µmol g^{-1} which produces FeAsc fluxes of 2.1×10^6 mol yr^{-1} from meltwater and 22×10^6 mol yr^{-1} from icebergs. For comparison the total annual dissolved (<0.4 µm) Fe content of glacial meltwater is estimated to be 75×10^6 mol yr^{-1} (Statham et al., 2008). This flux will be subject to some removal during estuarine mixing and, assuming that the 90% removal factor derived from rivers can be applied to glaciers, will result in a flux of 7.5×10^6 mol yr^{-1} to coastal waters. Particulate glacial Fe fluxes are therefore still significantly greater than dissolved Fe glacial fluxes, even when only the most bioavailable fraction is considered.

4.6 Conclusions

The Fe speciation within a range of glacial flour samples has been shown to be quite diverse with variation in labile, ascorbic acid soluble Fe over an order of magnitude both in absolute terms as a fraction of total Fe. Despite the vast majority of Fe in all particles being unreactive and significant removal of glacial particulate in low salinity waters, the glacial inputs of particulate labile Fe to polar seas are still greater than dissolved glacial Fe inputs. The absence of a clear relationship between total and bioavailable Fe is problematic for modelling the global Fe cycle. However the ascorbic acid leach used here and elsewhere may provide a useful standard estimate of bioavailable particulate Fe input from glacial and other particulate sources.

The release of dissolved and small colloidal Fe from glacial flours into solution is not directly proportional to either the labile Fe, total Fe or TOC content of the particulate and is likely to be influenced by multiple factors including particle surface area and the extent of adhesion between nanoparticulate Fe and the particle surface. The Fe(II) selective ligand ferrozine acquired only limited quantities of dissolved Fe(II) in solution at concentrations much higher than the nM levels exhibited by natural ligands. Therefore processes akin to reductive dissolution of glacial flour are unlikely to be a measurable source of dissolved Fe(II) into the water column unless a class of presently unknown ligands exists in seawater with a much higher affinity for Fe(II) than ferrozine.

Lippiatt et al. (2010) concluded that glacial-derived particulate Fe input into the Gulf of Alaska is much greater than can possibly be solubilized by natural organic ligands. Similarly, Thuróczy et al. (2012) found that natural organic ligands were close to saturation with Fe near Pine Island Glacier, Antarctica. Our results show that particles from a broad range of glaciers contain significant quantities of labile Fe and therefore the capacity of natural Fe ligands is likely to be saturated within the vicinity of any glacial discharge into the ocean. For most phytoplankton particulate Fe is inaccessible directly and must first enter solution before it can be taken up (Rich and Morel, 1990; Wells et al., 1991). The impact of glacial particulate Fe inputs on biota may therefore be limited by natural ligand concentrations in polar waters as no natural ligand concentration could ever solubilise more than a small fraction of the labile Fe available in near-shore waters enriched with glacial flour.

4.6.1 Acknowledgements

SEM was conducted by Mr Gordan Thomas and Dr Richard Pearce. XRD was conducted by Mr Ross Williams. TOC measured with assistance from Mr Shir Akbari and ICP-MS measurements were made by Dr Matthew Cooper.

Glacial flour	Size / µm	% of total mass	TFe / µmol g^{-1}	FeDi / µmol g^{-1}	FeAsc / µmol g^{-1}	TAl / µmol g^{-1}	TOC / µmol g^{-1}
Greenland	< 63	32.6	661	32.3	5.3	2830	16
	63-90	15.3	560	23.6	3.1	2820	12
	90-180	28.4	527	32.6	2.3	3220	29
	180-500	23.8	507	17	1.7	3230	22
Bodalsbreen	< 63	72	427	53.1	20	3400	181
	63-90	10.4	506	50.9	26.8	3420	308
	90-180	10.5	481	56.5	53.5	2730	900
	180-500	7	515	59.7	47.9	2730	2020
Les Bossons	< 63	43	720	37.6	17.2	2570	122
	63-90	11.7	718	47.9	5.9	3270	127
	90-180	20.3	778	60.6	4	3940	247
	180-500	25	692	62.8	4	3610	104
Signy island	< 63	11.5	1470	90.7	24.6	3760	277
	63-90	8.4	1200	116	8.8	3950	236
	90-180	34.9	1050	92.1	7.6	4000	245
	180-500	45.2	1270	139	8.5	4640	255

Supplemental Data: Composition of glacial flours, and leachable Fe fractions for each size fraction analysed. TFe- total particulate Fe, FeDi-sodium dithionite extractable Fe, FeAsc- ascorbic acid extractable Fe, TAl-total particulate Al and TOC- total organic carbon.

Glacial flour	Size / µm	DW / µmol g^{-1}	SW/ µmol g^{-1}	FZ / µmol g^{-1}	FeAsc / TFe %	FeDi / TFe %
Greenland	< 63	0.134	0.207	0.382	0.8	4.88
	63-90	0.228	0.699	0.204	0.56	4.22
	90-180	0.122	0.102	0.132	0.44	6.18
	180-500	0.129	0.075	0.139	0.34	3.35
Bodalsbreen	< 63	0.519	0.194	0.521	4.69	15.2
	63-90	0.267	0.302	0.376	5.29	10.1
	90-180	0.296	0.623	0.473	11.1	11.7
	180-500	0.321	0.397	0.349	9.31	11.6
Les Bossons	< 63	0.051	0.322	0.319	2.38	6.69
	63-90	0.012	0.164	0.163	0.83	6.68
	90-180	0.069	0.125	0.169	0.52	7.79
	180-500	0.019	0.09	0.234	0.58	9.08
Signy island	< 63	0.441	0.156	0.181	1.67	6.17
	63-90	0.227	0.213	0.222	0.73	9.68
	90-180	0.214	0.119	0.08	0.73	8.78
	180-500	0.143	0.216	0.187	0.67	11.0

Supplemental Data. Composition of glacial flours, and leachable Fe fractions for each size fraction analysed. DW deionised water released Fe, SW- Coastal seawater released Fe and FZ ferrozine leached Fe(II). 'Total mass' for % calculations excludes particles > 500 µm.

5. Fe(II) in rainwater

5.1 Introduction

Dry and wet deposition events both deliver dust to the surface ocean, but they are often distinguished with good reason. Rainwater provides favourable conditions for the dissolution of solid phase Fe into dissolved (or possibly colloidal) species and thereby facilitates the transfer of Fe from less, to more labile pools. Wet deposition therefore delivers Fe to the surface ocean in a more labile form than dry deposition. Fe(II) is known to account for a high fraction of dissolved Fe within atmospheric waters because of the high photon flux in the atmosphere (compared to surface waters) and relatively low pH of rainwater (Behra and Sigg, 1990; Zhuang et al., 1995). Furthermore some organic components within rainwater that promote dissolution of the solid phase, particularly oxalate, may also increase dissolved Fe(II) concentrations through reductive dissolution (Paris and Desboeufs, 2013).

The speciation of dissolved Fe(II) in rainwater is not known. However, there is evidence of slower than expected Fe(II) oxidation rates within rainwater (Kieber et al., 2005; Willey et al., 2005). Curiously these slow Fe(II) oxidation rates also seem to be observed even after mixing between rainwater and surface seawater which suggests that the speciation of Fe(II), rather than the concentration of other rainwater components, is responsible (Kieber et al., 2003b; Willey et al., 2008). Rainwater deposition could therefore be a particularly efficient method of bioavailable Fe delivery to the ocean. The exact mechanism behind this delayed oxidation is unclear, but the disappearance of Fe(II) stabilisation after exposure to intense UV light suggests that organic compounds are responsible (Kieber et al., 2005).

The use of ferrozine to measure Fe(II) will exclude any Fe(II) bound to strong ligands ($\log K_{Fe(II)}$ >16 at pH 6) from detection. Dissolved Fe measured by ferrozine in Wilmington rainwater normally shows a significant (mean 25% from 17 rain events) increase after UV irradiation (Kieber et al., 2005). This indicates release of dissolved Fe from a phase that was previously inaccessible to ferrozine. This phase could be either strong Fe-organic complexes or Fe and C containing colloids, both of which may be destabilised by the oxidation of organic constituents (Parazols et al., 2006).

If Fe (or other trace metal) ligands are present within rainwater then their origin is an important question. Variation in the observed diurnal behavior of Fe(II) in the atmosphere is reported. For example, Kieber et al. (2003b) demonstrated a diurnal cycle of Fe(II) and Fe(III) in Wilmington rainwater, whereas Parazols et al. (2006) find no such cycle in cloud water collected at the Puy de Dôme station (Central Massif Region, France). Varying origin and nature of DOC in atmospheric waters may contribute to this spatial and temporal change in rainwater Fe(II) chemistry. Natural organic compounds within rainwater have been demonstrated to vary with both season and air mass back trajectory (Kieber et al., 2006; Seaton et al., 2013). In addition to the natural organic components in rainwater, anthropogenic emissions are also now a source of DOC in the northeast USA. The products of fossil fuel combustion constitute 1/3 of the DOC detected in Wilmington rainwater (Avery et al., 2013). Vehicle exhaust fumes have been demonstrated to contain compounds that impede Fe(II) oxidation (Humphreys, 2013). Furthermore, dusts produced from fossil fuel combustion (fly ash) contain a high fraction of soluble Fe compared to natural Fe minerals (Schroth et al., 2009; Sholkovitz et al., 2009). Therefore there may be an anthropogenic source of both Fe and Fe(II) ligands to rainwater large enough in magnitude to be comparable to natural sources.

Determining the precise origin of Fe(II) ligands, or more specifically the mechanism that has been observed to impede rainwater Fe(II) oxidation, is important because of an observed change in Wilmington since 2001. Mean rainwater Fe(II) concentrations in summer (July, August and September) 2002, 2008, 2010 and 2011 were significantly lower than previously recorded values in 1997, 1998, 2000 and 2001 (Willey et al., 2009). Furthermore, Fe(II) in summer 2008 was found to be less stable than in summer 2002 and 2003. This change was not correlated with pH or H_2O_2 concentration (H_2O_2 is the main oxidant in rainwater) (Mullaugh et al., 2011; Willey et al., 2012). However, the Fe(II) oxidation rate constant did appear to correlate with fluorescence suggesting that changes in CDOM (Chromophoric Dissolved Organic Material) are linked to changes in Fe(II) oxidation rate (Willey et al., 2009). A decline in the abundance of rainwater CDOM, specifically terrestrially originating CDOM, which appears to be a better Fe(II) stabilizer than marine CDOM, may therefore have led to the observed decrease in Fe(II) concentration and stability (Willey et al., 2009).

An interesting hypothesis is that previously observed (prior to 2001) high Fe(II) concentrations and unexpected slow Fe(II) oxidation rates may have been a result of anthropogenic organic emissions (Willey et al., 2009). A fall in such emissions in the northeast USA from power plants and vehicle emissions may have led to lower rainwater Fe(II) concentrations since 2001. Multi-annual changes in rainwater composition due to improving air quality have been recorded in Wilmington rainwater with pH increasing between 1985 and 2005, and DOC concentration falling between 1995 and 2005 (Willey et al., 2012). The decrease in H^+ and DOC concentration appears to continue through to 2013 (unpublished data) although, as values of these two components appear to be declining towards natural levels, this decline cannot continue indefinitely. Due to decreasing SO_2 emissions, an increase in H_2O_2 concentration has also been reported over the decade to 2011 (Mullaugh et al., 2011). All three of these changes lead to lower Fe(II) concentrations and could contribute to a long term decrease in mean Fe(II) concentration. However, such changes would not explain the change in Fe(II) stability per se.

5.1.1 Objectives

- To monitor dissolved Fe(II) and Fe concentrations over the duration of a three month period in order to further expand the existing database of summer Wilmington rainwater Fe concentrations.

- To determine the chemical lability of dissolved Fe in rainwater at this site and to perform mixing experiments with seawater to see how Fe speciation changes immediately after simulated precipitation in seawater.

5.2 Experimental

5.2.1 Collection of rainwater and complementary data

The MACRL (Marine and Atmospheric Chemistry Research Laboratory) rainwater collection site in Wilmington North Carolina (34° 13.9’ N 77° 52.7’ W) is in the centre of an open hectare of land surrounded by mixed deciduous/coniferous woodland approximately 8.5 km from the North Atlantic coast (Kieber et al., 2006). The site consists of three Aerochem Metrics model 301 automatic sensing wet/dry precipitation collectors. The design of these collectors includes a sensor that automatically opens the containers for rain collection at the start of, and similarly closes them after, a rain event. Rainwater is therefore stored in the dark at ambient temperature between deposition and collection.

One collector was fitted with 3 trace metal clean PTFE containers to collect samples solely for Fe analysis. The remaining collectors contained combusted glass beakers to collect samples for measurement of DOC, CDOM and other complementary chemical parameters. Collection and transport of samples between the collection site and laboratory took less than 10 minutes. All rain events are numbered continuously by MACRL and this is denoted ‘event number’. Prolonged rainfall events (over an hour) with sufficiently large volumes of rainfall are sampled multiple times and are this is denoted with letters (eg event 1315 and event 1315B). Complementary data (quantity and origin of rainfall, pH, H_2O_2, DOC, CDOM, Cl^-, NO_3^- and SO_4^{2-}) was collected/measured by all students of the MACRL group and is available upon request.

5.2.2 Trace metal cleaning procedures

Standard procedures used by the MACRL group were adopted for all rainwater chemical analysis. PTFE containers were used throughout. All apparatus for trace metal sampling was allowed to stand in 3 M HNO_3 acid baths, designated for rainwater containers only, for one week. This was followed by three washes with deionised water (Milli-Q 18.2 MΩ·cm). Sampling containers were then twice filled with deionised water and allowed to stand overnight prior to another four washes with deionised water. All sample handling and trace metal analysis was conducted under a class 100 laminar flow hood. An operational blank measurement was made with every batch of samples by pouring deionised water into clean rainwater collection bottles and then sampling this deionised water in the same way as fresh rainwater.

5.2.3 Rainwater dissolved Fe, Fe(II) and total acid leachable Fe

Dissolved (0.2 µm) Fe sample handling and reagent addition was conducted exactly as described by Willey et al. (2000). Fe(II) and dissolved Fe concentrations were measured via absorbance at 562 nm on a USB4000 Fiber Optic Spectrometer (Ocean Optics) with a 250 cm 3000 series Liquid Waveguide Capillary Cell (LWCC, World Precision Instruments). All rainwater samples were run in triplicate with the mean and standard deviation (SD) reported. A deionised water baseline was used and the background absorbance of rainwater components was deducted from the measured absorbance at 562 nm (Waterbury et al., 1997). The three rainwater samples used to determine each triplicate mean were taken from three separate PTFE containers deployed within the same rain collector. Fe(III) concentration was calculated by deducting Fe(II) from total dissolved Fe.

To determine total acid leachable Fe, 120 µL conc HNO_3 (trace metal grade) was added to 2.00 mL unfiltered rainwater. After standing for one week, the sample pH was raised to 5.5 by the addition of approximately 163 µL conc NaOH (trace metal grade) and 500 µL pH 5.5 ammonium acetate buffer. 1 mL ascorbic acid (10 mM, trace metal grade) and 100 µL ferrozine (10 mM, ‘for spectrochemical determination of Fe’) were then added immediately and the sample left to stand for one hour before absorbance at 562 nm was measured. A 0.2 µm inline filter was used between the sample vial and 2.5 m LWCC to remove any residual particles.

5.2.4 Rainwater incubation and mixing experiments

In addition to measuring the dissolved Fe concentration of every rain event, a series of experiments was designed to investigate specific aspects of rainwater Fe chemistry that are of relevance to the efficiency with which dissolved Fe may be delivered to the surface ocean. There is potential for changes in Fe speciation to occur rapidly after a rain event and therefore all of these experiments were conducted immediately after a sufficient volume of freshly deposited rainwater had been collected (rather than using stored rainwater).

It is not known how Fe in rainwater is distributed across different particle size fractions or how labile the solid Fe-bearing material within rainwater is. It has been demonstrated that aerosol dust origin is a key factor in determining what fraction of Fe is soluble (Schroth et al., 2009), but many other chemical and physical factors also simultaneously play a role (Baker and Croot, 2010). Filter size was varied after a selection of rain events to see whether, as with seawater (Fitzsimmons and Boyle, 2014), colloids account for a significant fraction of the ‘dissolved’ Fe present. An ascorbic acid leach, which targets specifically only the most bioavailable particulate Fe phases (Hyacinthe et al., 2006), was adapted to probe the lability of rainwater particulate Fe. To minimise speciation changes, rather than filtering rainwater to collect the small amount of particles present (<0.5 g L^{-1}), ascorbic acid and hydrochloric acid leaches were conducted directly on unfiltered rainwater. Subtracting dissolved Fe, as determined after filtration, from total leached Fe in these samples allows the determination of Fe leached from solid particles.

To investigate the stability of rainwater Fe(II) upon mixing with seawater, mixing experiments were conducted using both freshly collected (<24 hours between collection and commencing experiments) coastal seawater and aged (stored for over one year in the dark) surface North Atlantic seawater. Although this was not quantified, the coastal seawater is likely to contain a low concentration (nM) of natural trace metal ligands, whereas the aged North Atlantic water is unlikely to contain any ligands. To simulate natural daylight a solar lamp (Spectral Energy Corp LH 153 lamp housing, 1-kW Xe arc lamp, LPS 256 SM power supply, AM1 filter) was used (Kieber et al., 2003a). Duplicate dark experiments were conducted wrapped in Al foil inside the solar lamp housing. Other dark experiments were conducted inside an Al foil lined cupboard.

Experiment 1: Investigating the stability of Fe(II) in collected rainwater

Fe(II) in rainwater

Collected, filtered rainwater was stored in the dark at 3°C. Dissolved Fe was then measured after several hours of storage. Times are denoted since the rain event ceased, rather than since when the rainwater was collected or the experiments started.

Experiment 2: The effect of prolonged solar irradiation upon dissolved Fe concentration

Filtered rainwater was irradiated under simulated solar irradiation and dissolved Fe measured at regular time intervals. Times are denoted since the rain event ceased. After 0.45 hours dissolved Fe was measured immediately before the simulator was switched on. After 22 hours, solar irradiation ceased. The rainwater vials were then wrapped in Al foil and stored in the dark.

Experiment 3: Simulated deposition of rainwater in surface seawater

Freshly collected coastal seawater (S 25.3) was filtered (0.2 μm) and stored in the dark. Seawater, rainwater, and a 75% seawater: 25% unfiltered rainwater water mixture were then irradiated under the solar lamp. 75% seawater: 25% filtered rainwater was also prepared and wrapped in Al foil. Sub samples were extracted and Fe(II) concentration was measured at regular time intervals. After 10 hours, solar irradiation was stopped and all vials were stored in the dark.

Experiment 4: Mixing of rainwater/seawater in the dark

Filtered, aged Gulf Stream seawater (S 36.2 stored for over a year at 3°C in the dark) and filtered, aged coastal water (S 29.5) were used to conduct mixing experiments with 25% rainwater and 75% seawater. All mixtures were stored in the dark at room temperature and Fe(II) measured at increasing time intervals.

Experiment 5: Ascorbic acid and ferrozine leaches

Ascorbic acid leaches and concentrated ferrozine leaches were conducted on unfiltered rainwater. Given the small quantity of particles present within rainwater and the potential for contamination during filtration, these leaches were conducted by adding reagents directly to unfiltered rainwater samples. 500 μL 10 mM ascorbic acid solution was added to 3 mL freshly collected unfiltered rainwater and allowed to stand for 24 hours before dissolved Fe concentration was determined using the ferrozine method. 100 μL 100 mM ferrozine solution was added to 3 mL freshly collected unfiltered rainwater. Leached Fe was then measured after the vials had stood in the dark for 4 days.

Experiment 6: Sequential filtration of rainwater

3 filter sizes (0.45, 0.20 and 0.01 μm) were used sequentially to establish whether the Fe(II) and Fe(III) measured in rainwater is truly dissolved or includes a significant colloidal fraction. 0.45 and 0.20 μm filters are widely used to removed particulate Fe from solution, but do not distinguish between colloidal and truly dissolved Fe in seawater (Fitzsimmons and Boyle, 2014). 0.01 μm filters are easily clogged and thus the exact cut off size is difficult to determine, but these filters should remove all large colloidal material from solution and leave only truly dissolved species and small nanoparticles.

Experiment 7: Effect of diluting rainwater with deionised water

Unfiltered rainwater was mixed with deionised water in 50:50 and 75:25 (deionised water: rain) ratios to see if dilution, without the addition of extra natural dissolved components such as those found in coastal seawater, affects the rate at which dissolved Fe is removed from solution.

Experiment 8: The effect of UV irradiation upon dissolved Fe concentration

Filtered rainwater was split into two triplicate sets. One triplicate set was stored in the dark whilst the other was irradiated under a UV lamp overnight. Samples (both UV and non–UV irradiated) were then subject to solar irradiation for 3 hours. After 3 hours the vials were stored in the dark. Fe(II) was measured at regular time intervals during and after solar irradiation.

Experiment 9: The effect of decreasing pH upon dissolved Fe in rainwater

Unfiltered rain was subject to differing degrees of acidification using dilute HNO_3 (trace metal grade diluted with deionised water). Rainwater vials were stored in the dark at room temperature. Dissolved Fe and pH were then measured 72 hours after the addition of acid.

5.3 Results

The detection limit of the LWCC (defined as three times the standard deviation of four blank measurements) was 0.94 nM. A summary of all rain events collected over a 3-month period (07 May-20 July 2013) is shown (Table 1E). The mean rain event (and blank) Fe(II), Fe(III) and total acid leachable Fe concentrations over this period are also shown (Table 2E). The standard deviation for Fe(III) is higher than for Fe(II) because Fe(III) was calculated by deducting Fe(II) from total dissolved Fe rather than being measured directly.

Date	Event	Dissolved Fe(II) / nM	SD / nM	dissolved Fe(III) / nM	SD / nM	TDM Fe / nM	SD / nM
20/05/2013	1307	9.9	6.0	4.5	7.8	318	9.8
21/05/2013	1309	7.2	4.0	10.9	2.6	286	17.4
22/05/2013	1310	8.5	1.0	9.3	4.1	302	12.3
23/05/2013	1311	6.1	0.8	6.3	1.3	298	11.6
06/03/2013	1312	16	0.3	7.2	0.7	2060	152
06/03/2013	1313	4.2	0.4	9.7	2.7	1620	215
06/06/2013	1315	5.0	1.0	63.5	4.5	904	19.1
06/06/2013	1315 B	31.6	8.3	52.3	8.7	470	6.7
06/10/2013	1316	5.8	0.3	12.4	4.2	191	21.5
14/6/2013	1317	16.7	1.1	20.5	3.0	436	19.4
18/6/2013	1318	10.6	1.0	52.6	0.5	366	26.1
19/6/2013	1319	4.2	1.1	12.4	0.4	148	7.3
19/6/2013	1320	5.5	0.6	13.6	1.2	131	6.5
22/06/2013	1321	3.7	2.7	10.2	0.9	453	24.8
22/06/2013	1322	3.4	0.2	9.4	4.4	392	4.0
23/06/2013	1323	3.7	0.5	11.0	7.3	1010	32.0

24/06/2013	1324	60.1	11.1	94.2	30.7	104	28.6
27/06/2013	1326	5.8	0.3	22.3	0.9	63.2	9.8
28/06/2013	1327	8.7	1.3	22.5	6.1	355	11.4
30/06/2013	1328	64.2	2.3	39.8	2.1	228	171
30/06/2013	1328 B	41.5	6.0	74.1	5.6	157	74.3
01/07/2013	1329	3.3	0.5	31.8	11.0	368	7.1
02/07/2013	1330	ND	ND	ND	ND	269	10
11/07/2013	1331	3.5	0.2	9.3	0.9	1170	175
12/07/2013	1332A	2.7	0.6	10.8	1.4	626	385
12/07/2013	1332B	4.4	0.2	15.7	0.3	1150	54.4
13/7/2013	1334	3.3	0.4	5.0	1.2	1480	23.7
13/7/2013	1335	3.8	0.1	6.3	1.3	1860	61.3
14/7/2013	1336	3.4	0.3	8.1	0.5	138	65.0

Table 1E Fe in rainwater for all rain events 07/05/13-20/07/13, missing event numbers provided insufficient volume to measure DFe, SD ‘standard deviation’, ND ‘not determined’ for logistical reasons. TDM total acid leachable Fe.

	Mean nM	Standard deviation %	Mean blank nM
Dissolved Fe(II)	12.4	17.0	1.2
Dissolved Fe(III)	23.1	24.8	1.4
Acid leachable Fe	598	13.5	1.7

Table 2E Simple mean rain event Fe concentrations (no volume weighting of different rain events is applied to produce these values) over the observed period and the mean Fe content of blank (de-ionised water) samples.

Where mean values are presented with standard deviations, data points are mean triplicate values (from three independent, identical vials). Table 3E demonstrates the relative stability of Fe(II) concentration after a low Fe(II) rain event (event 1311 was an overnight event). No significant change is evident in Fe(II) between 5 and 10 hours after deposition. A small unexpected increase in Fe(II) is however observed between 5 and 29 hours after deposition. 7 rain events collected between November 2012 and July 2012 with similar Fe(II) concentration (1-8 nM) all either showed a decrease or no significant change in Fe(II) concentration between 5 and 24 hours after collection (Rice, 2013). Between 0 and 4 hours after collection a decrease was always observed (Rice, 2013).

Time / hrs	Fe(II) / nM	SD / nM	Fe(III) / nM	SD / nM
5	6.1	0.76	4.6	1.3
10	7.1	0.67	NA	NA
29	8.5	0.39	4.7	0.47

Table 3E Experiment 1, dissolved Fe in rainwater (event 1311) stored in the dark after collection, ‘NA’ not analysed.

The effect of solar irradiation upon rainwater (event 1313, Figure 1E) was a sharp increase in Fe(II) from 5.9 to 9.5 nM within 1 hour which then levelled off and showed no further increase over 20 hours of simulated irradiation. 6 hours of irradiation is equivalent to one day of solar irradiation at sub-tropical latitudes. After irradiation ceased Fe(II) was remarkably stable in this sample with concentrations still elevated above initial Fe(II) after 72 hours of storage in the dark (Figure 1E).

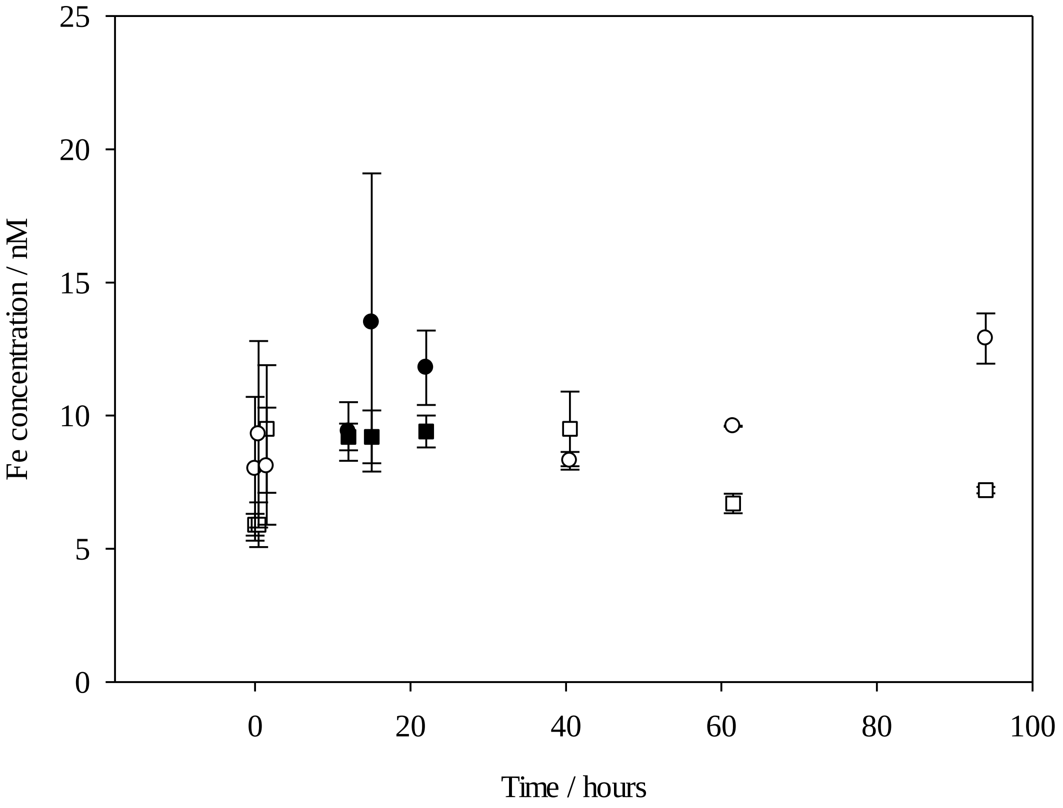

Figure 1E Experiment 2, dissolved Fe(II) (squares) and Fe(III) (circles) in aged rainwater (event 1313) exposed to solar irradiation, shaded values are reported during simulated solar irradiation (irradiation commenced immediately after the 0.45 hours measurement and ceased immediately after the 22 hours measurement)

Storage of filtered natural seawater in the dark should result in a lowering of dissolved Fe with time. This is initially due to the oxidation of any Fe(II) present, which results in the formation, and subsequent precipitation, of the less soluble Fe(III). Surface removal of Fe then becomes the dominant removal process resulting, unless the water is acidified, in the precipitation of most dissolved Fe present (Lewin and Chen, 1973). Irradiation of the coastal seawater (S 25.3) used in experiment 3 resulted in no significant increase in the dissolved Fe(II) present (Table 4E). This coastal seawater almost certainly contains Fe(II) ligands otherwise such a high dissolved Fe(II) concentration would decay rapidly after irradiation ceased (between 10 and 24 hours there is no significant decay). In contrast to the rainwater, the lack of an increase in Fe(II) between 0 and 10 hours suggests that there is no reducible Fe(III) present that can be converted to Fe(II) species via solar irradiance (Table 4E). Some evidence of Fe(II) stability is noted in both seawater and rainwater after irradiation stops. In the dark, the

Fe(II) in rainwater

Fe(II) in mixed seawater and rainwater decays, albeit relatively slowly. Unfiltered rainwater mixed with seawater shows a rapid increase in Fe(II) upon irradiation and enhanced Fe(II) stability after irradiation was stopped (Table 4E).

	Dissolved Fe(II) / nM			
Time / hrs	SW f	25% SW:75% f RW dark	25% SW:75% uf RW light	RW f
0	33.0	26.4	33.0	5.0
4	33.9	24.4	46.5	9.2
10	32.8	24.8	44.8	9.0
24	31.7	22.3	40.4	7.2

Table 4E Experiment 3; Fe(II) measured in rainwater (event 1315), seawater and mixtures of the two before, after and whilst subject to simulated solar irradiation; ‘SW’ coastal seawater (S 25.3); ‘uf’ unfiltered; ‘f’ filtered (0.20 μm); ‘RW’ rainwater; shaded values are reported during simulated solar irradiation which commenced after the 0 hours measurement and ceased immediately after the 10 hours measurement.

Mixing experiments conducted in the dark initially show the expected decrease in Fe(II) in all solutions (Figure 2E). Fe(II) in unmixed seawater (both S 29.5 coastal seawater and S 36.2 Gulf Stream seawater) remained below detection (1 nM) throughout the experiment demonstrating that contamination was not a significant experimental artefact (Figure 2E). Ferrozine extracted more Fe(II) from unfiltered rainwater than filtered rainwater, possibly indicating the presence of some surface Fe(II) or readily reducible Fe(III) on particles. This Fe(II) present in the unfiltered rainwater was also much more stable, declining over 41 hours much more slowly than Fe(II) in filtered rainwater. Between 0 and 41 hours Fe(II) concentrations declined in all solutions above the detection limit (Figure 2E). Fe(II) behaviour was very similar when rainwater was mixed with either Gulf Stream seawater or coastal seawater. Between 41 and 89 hours there was an unexpected increase in Fe(II) in all solutions, apart from the unfiltered rainwater (Figure 2E).

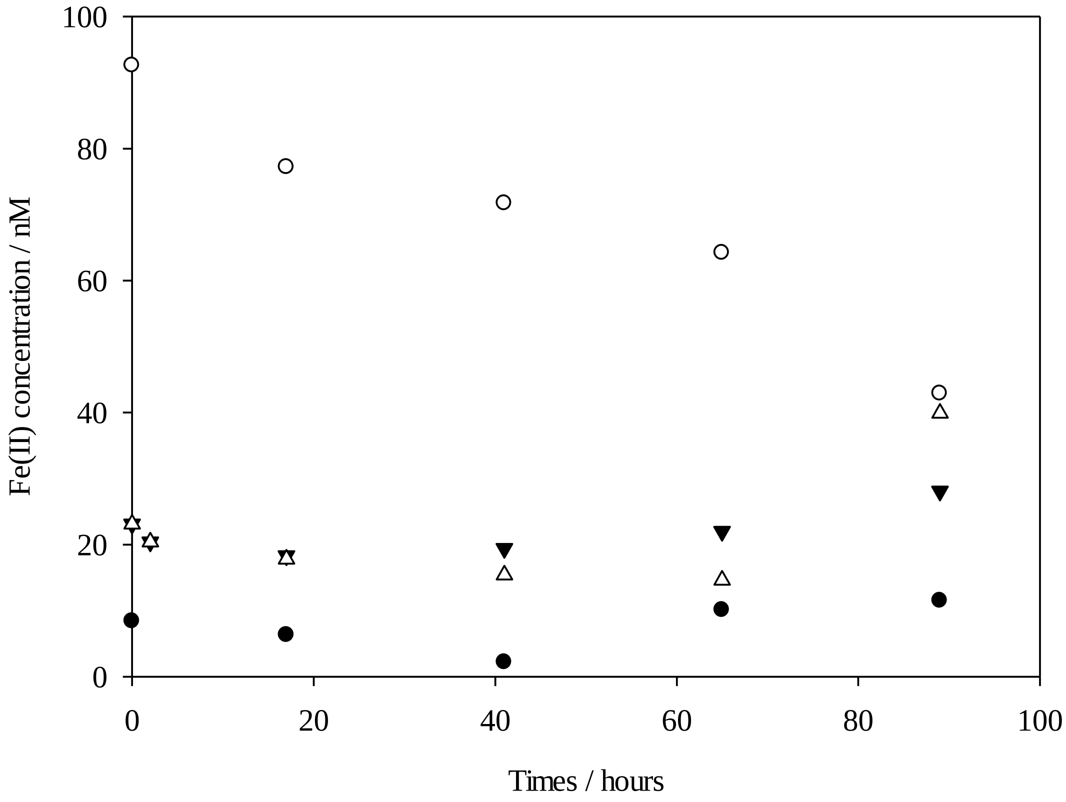

Figure 2E Experiment 4, dissolved Fe(II) measured in filtered rainwater (filled circles), unfiltered rainwater (open circles), 25:75 rain: Gulf Stream seawater (filled triangles) and 25:75 rain: coastal seawater (open triangles). Rainwater from event 1317 used throughout, Fe(II) concentrations in both Gulf Stream and coastal seawater (aged in the dark) were consistently below the detection limit (1 nM).

An adapted ascorbic acid leach on four rain events (Table 5E) demonstrated that the fraction of particulate Fe that is most soluble and bioavailable varies markedly from 8.0-60% of total acid leachable Fe. The ascorbic acid soluble Fe content within rain is a much larger pool of Fe than the dissolved Fe present in all events. A ferrozine leach demonstrates that a small, but significant, fraction of the acid leachable Fe present (0.5-27%) is directly available to a ligand.

Event	[Fe] Fz / nM	SD / nM	[Fe] Asc / nM	SD / nM	% Asc PFe	% Fz PFe
1315	73.4	14.7	273	1.2	22.6	0.5
1321	138	10.0	253	52.1	52.8	27.3
1322	96.2	10.5	248	8.1	60.0	21.3

Fe(II) in rainwater

1330	59.6	3.0	64.5	18.8	8.0	6.6

Table 5E Experiment 5, ascorbic acid and ferrozine leachable particulate Fe, both leaches are expressed as a fraction of acid leached particulate Fe (PFe) (calculated as total acid leachable Fe minus dissolved Fe), ‘Fz’ ferrozine leachable Fe, ‘Asc’ ascorbic acid leachable Fe.

Sequential filtering of 2 rain events through 0.45, 0.20 and 0.01 μm filters did not show a decrease in dissolved Fe(II) or Fe(III) concentrations (Table 6E). However, both of these events had below average dissolved Fe concentrations.

Event	Size / μm	Fe(II) / nM	SD / nM	Fe(III) / nM	SD / nM
1319	0.45	3.5	0.5	11.0	2.1
	0.2	4.2	1.1	12.4	0.4
	0.01	4.0	1.0	12.3	2.5
1321	0.45	5.4	0.7	16.6	3.6
	0.2	3.7	2.7	10.2	0.9
	0.01	3.8	0.6	15.6	4.9

Table 6E Experiment 6, Fe(II) and dissolved Fe(III) concentrations after sequential filtering of fresh rainwater samples through 0.45, 0.22 and 0.01 μm filters.

One further mixing experiment was done using deionised water, rather than seawater, to dilute rainwater. Fe(II) decayed in both 50:50 and 25:75 (rainwater:deionised water) mixtures which, after 20.5 hours, had the same Fe(II) concentration (Table 7E).

	Dissolved Fe(II) / nM	
Time / hrs	50 % RW:50 % de-ionised water	25 % RW:75 % de-ionised water
2.5	22.5	20.6
11.5	18.5	16.4
20.5	16.4	16.3

Table 7E Experiment 7, mixing and aging of unfiltered rainwater (RW) and de-ionised water in ratios of 50:50 and 25:75 whilst monitoring dissolved Fe(II) concentration.

The effect of prolonged exposure to intense UV light is to oxidise most DOC in solution to CO_2 which thereby removes any organic ligands present. Batches of rainwater (event 1330), both with and without overnight exposure to a UV lamp, were subject to solar irradiation. During irradiation total dissolved Fe remained constant and was identical in magnitude for both UV and non-UV exposed batches (Figure 3E). After irradiation ceased, Fe(II) in both samples decayed at rates not significantly different.

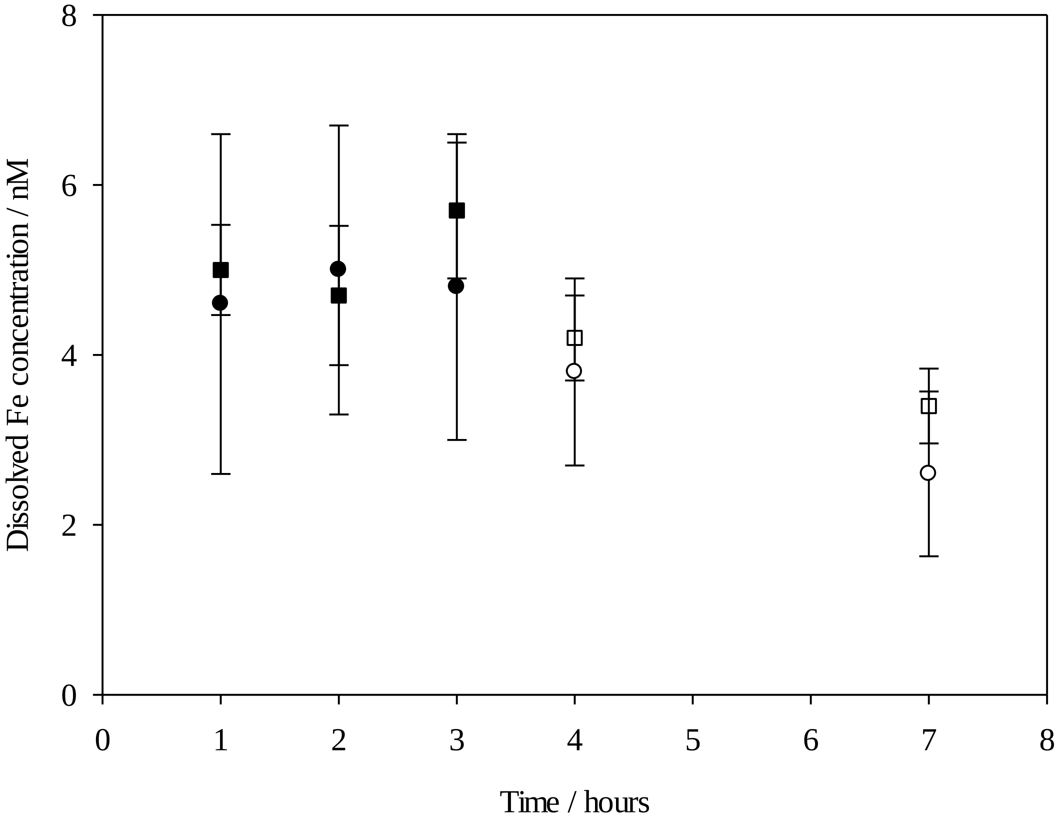

Figure 3E Experiment 8, rainwater (event 1330) with (circles) and without (squares) prior exposure to a high intensity UV lamp exposed to simulated solar irradiation and aged, shaded values are reported during simulated solar irradiation, irradiation ceased immediately after the 3 hour measurement.

The lowering of pH in unfiltered rainwater (event 1332A) by the addition of dilute HNO_3 resulted in the expected increase in dissolved Fe concentration (Figure 4E).

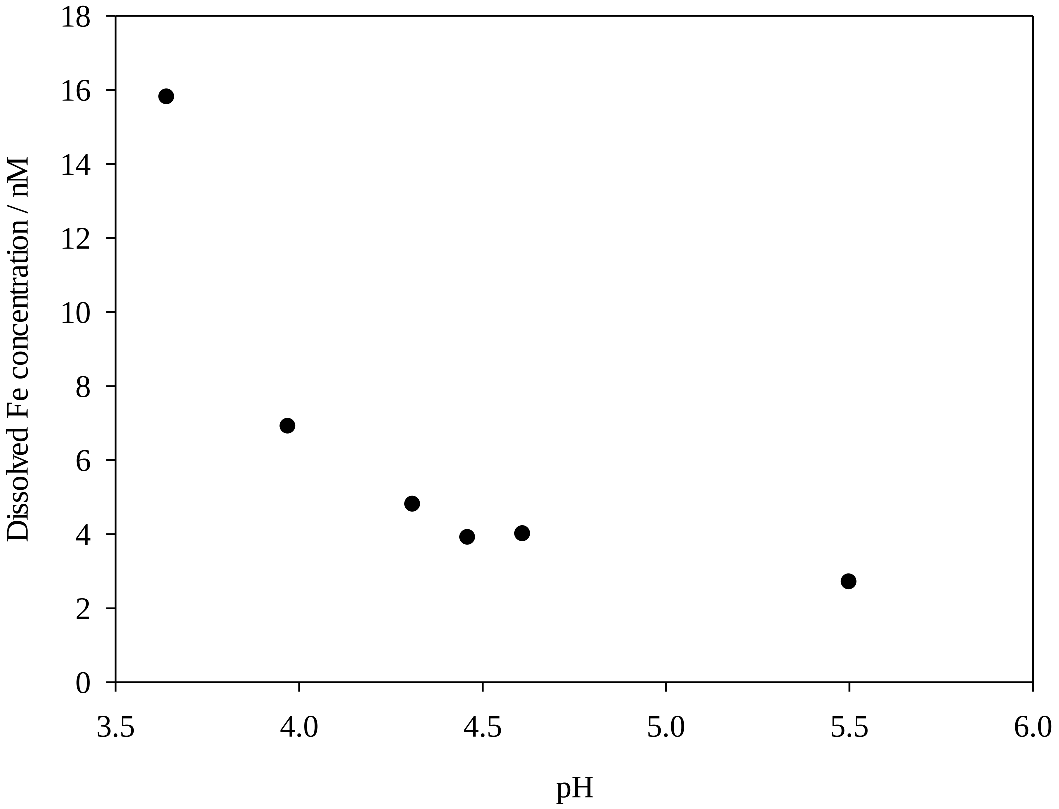

Figure 4E Experiment 9, the pH of a rainwater sample (event 1332A) was adjusted using dilute HNO_3, total dissolved Fe was determined after solutions had stood at room temperature for 72 hours. The initial pH of this sample was 4.84.[14]

5.4 Discussion

Experiment 1 demonstrated that there was no measurable increase or decrease in measured Fe over intermediate storage periods using methods previously adopted by the MACRL group (Table 3E). This indicates that cleaning procedures were adequate to prevent detectable release of Fe into solution from containers. The adequacy of trace metal procedures adopted in this work is also supported by the consistent measurement of below detection (< 1 nM) dissolved Fe concentrations in aged seawater (Figure 2E)

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[14] The increase in pH during storage observed with the unacidified sample (from 4.84 to 5.50) is not an anomalous measurement. pH increases in rainwater stored at room temperature have been observed previously (personal communication, Dr J. D. Willey). If no preservative is added, decay of organic acids (primarily by bacteria) allows the pH to increase beyond that measured immediately after sample collection.

and the consistently small standard deviations of triplicate measurements throughout (Table 1E).

A summary of previously collected data on Wilmington rainwater Fe concentration is shown (Table 8E). The most extensive available dataset for historical dissolved Fe in rainwater is for summer concentrations in Wilmington (Table 9E). Values measured here (Table 1E) have been used to compile the summer mean volume weighted Fe concentrations for 2013.[15] Volume weighting accounts for the bias that rainfall intensity exerts upon dissolved Fe concentrations (high volume rainfall events tend to have lower Fe concentrations due to depletion of particles in the atmosphere and vice versa for small volume rainfall events); hence there is a difference between these values (Table 9E) and the mean event Fe concentrations (Table 2E).

Year	N	Fe(II) / nM	Fe(III) / nM
1998-1999	48	42	33
2001	54	32	48
2008	53	10	11
2011-2012	26	4	12

Table 8E Annual mean dissolved Fe(II) and dissolved Fe(III) in Wilmington rainwater, Fe concentrations are rainfall volume weighted (Willey et al., 2000; Willey et al., 2009; Willey et al., 2012; Willey et al., 2005), ‘N’ number of rain events sampled.

Summer dissolved Fe(II), dissolved Fe and total Fe concentrations in Wilmington rainwater measured here are comparable to those measured in previous years since 2001 (Table 8E) (Willey et al., 2009). There is no doubt that Fe(II) concentrations have decreased substantially since 1997. A smaller decrease is notable for Fe(III). Dissolved Fe(II):Fe(III) ratios in summer rainwater fall significantly from 1.16 in 1997 to 0.25 in 2013 (linear against time with R^2 0.85). Total Fe (for which there is less data available) appears to be much more variable. The decrease in Fe(II) concentration cannot be associated with decreasing total Fe concentrations because the particulate fraction (total Fe minus dissolved Fe) has not decreased appreciably.

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[15] Volume weighting of the Fe concentrations shown in Table 1E was conducted by Dr J. D. Willey.

Year	Fe(II)	Fe(III)	Fe total (nM)	Dissolved Fe(II):Fe(III)
1997	36	31	358	1.2
1998	44	31	306	1.4
2000	52	46	NA	1.1
2001	30	28	183	1.1
2002	7	11	NA	0.64
2008	10	13	225	0.77
2011	5	17	NA	0.29
2013*	3	12	568	0.23

Table 9E Summer dissolved Fe(II), dissolved Fe(III) and total Fe concentrations in Wilmington rainwater (Willey et al., 2008; Willey et al., 2000; Willey et al., 2009; Willey et al., 2012; Willey et al., 2005). Fe concentrations are rainfall volume weighted, * shaded values are from data shown in Table 1E, 'summer' dates for 2013 were slightly earlier than previous years (07 May – 20 July), 'NA' not analysed.

Similarly, the decline in Fe(II) and Fe(III) is not likely to be due to limited lability of the particulate Fe present within rainwater. All of the 4 rain events subject to chemical leaches had some ascorbic acid and ferrozine available Fe (Table 5E). If we deduct the dissolved concentration of Fe from total acid leached Fe (thereby getting an estimate of particulate Fe), between 8 and 60% of the particulate Fe present was labile (ascorbic acid leachable) and 1-27% was available to the ferrozine ligand- a very high fraction compared to some lithogenic material. The mineralogy of particulate within rain does not therefore presently appear to limit the concentration of dissolved Fe measured in solution.

The slight increase in Fe(II) observed after 29 hours of storage in the dark (Table 3E) and the similar rise between 41 and 89 hours (Figure 2E) during mixing experiments is entirely unexpected. Incubations longer than several days under such conditions have not been reported previously. There are several possible explanations for an increase in the measured DFe or Fe(II) concentration after a prolonged period of storage. Other than contamination (an increase in Fe due to leaching from the container), plausible causes are:

1. Photochemical or biological production of Fe(II).
2. Dissolution of solid material within the rain releasing more Fe into solution (this assumes that the particulate and dissolved phases are not in equilibrium upon deposition).
3. Lowering of pH favouring the dissolution of more Fe into solution (demonstrated in Figure 4E).
4. Dissociation of Fe from strong ligands or colloids that were initially undetectable by ferrozine, but becomes ferrozine complexable with increased storage time, perhaps due to bacterial decomposition.

Storage in the dark (in Al foil wrapped containers in a dark box) and filtration of rainwater at 0.2 μm should be adequate to minimise the first two of these mechanisms of Fe(II) production. Furthermore, unfiltered rainwater showed no such increase in Fe(II) concentration after prolonged storage (Figure 2E) which suggests that biological production was not responsible.

Whilst no direct measurements of pH changes were made (due to the small volume of rainwater available), possibility 3 can also be discounted. The rebound in DFe concentrations (Figure 2E) was observed in mixtures of rainwater and seawater. These mixtures would have had a higher pH than the pure rainwater alone and yet the rebound effect was more pronounced in these mixtures than it was in unmixed rainwater. Furthermore, it is only at relatively low pH (approximately 4 or less, Figure 4E) that small decreases in pH have a large effect upon DFe solubility and in the few experiments where pH changes have been monitored after prolonged storage, pH increased rather than decreased with time (which would favour lower Fe(II) concentrations).

Dissolution of Fe from solid material would be unexpected after prolonged storage of a natural water sample unless dissolved O_2 concentrations or pH decline markedly. The dominant effect on a stored solution is normally scavenging (a term applied generically to all forms of DFe removal from solution), in this case primarily due to container surface removal of Fe (Lewin and Chen, 1973). Unfiltered rainwater behaved as expected, with a steady decay in DFe (Figure 2E). It was mixtures of unfiltered rainwater and filtered seawater that demonstrated the most pronounced evidence of a rebound in DFe.

Possibility 4, essentially that the change is an artefact of the ferrozine method, has been briefly discussed before. Kieber et al. (2005) noted that particularly strong Fe(II) ligands in rainwater may be above the detection window of the ferrozine complexing agent used here. All natural Fe(II) ligands characterised to date are reported to have relatively low binding constants compared to ferrozine. The possibility remains however that the Fe(II) ligands within rainwater are (as will be discussed shortly) distinctly different from those observed to date (all of which are various extracts of natural organic material). It is therefore possible that the apparent increase in DFe (Figures 1E and 2E) could be due to the dissociation of Fe from strong colloids/complexes that initially prevented complexation of Fe by ferrozine (Willey et al., 2008).

A possible artefact throughout much of this work is that the samples in these experiments are possibly subject to an increase in DOC content during storage and analysis. Working under trace metal clean conditions involves substituting the combusted glassware, normally used to handle DOC and CDOM samples in order to prevent contamination, with PTFE. Whilst evidently an excellent material for minimising Fe contamination, plastics are less ideal for preventing DOC contamination. Similarly, working under a class 100 laminar flow hood is adequate to prevent dissolved Fe contamination, but exposure of the rainwater samples to both natural and laboratory organic components in the atmosphere is inevitable. When a freshly collected sample of rainwater was sequentially filtered three times through the trace metal clean apparatus used here (rather than the combusted glassware normally used for DOC samples), an increase in DOC was noted (from 29.6 ± 0.70, to 43.4 ± 1.2, to 46.4 ± 1.4 µM). This suggests that, whilst there is no measurable contamination of Fe(II) or Fe(III), a limitation of this work is the introduction into rainwater samples of DOC present in the laboratory atmosphere, or on apparatus surfaces. This added DOC is increasing in the rainwater solutions whilst they are handled. After prolonged handling of rainwater samples in the laboratory (under trace metal clean conditions) or storage in PTFE containers, the DOC present within samples could be markedly higher in concentration and chemically distinguishable from that initially present. This change in DOC concentration and composition could trigger changes in Fe speciation, however the ‘added’ DOC may be relatively unreactive (it is widely assumed that this is the case as trace metal ligand titrations to determine ligand properties normally ignore this artefact- Personal communication Dr M. Gledhill). Even so, if this artefact is a major contributor to the observed increase in Fe(II) (Figure 2E), it is still not clear how the Fe(II) is formed or why it resurges rather suddenly after 41 hours given its previous steady decay. This increase in DOC therefore is probably not the major reason for such a sudden resurgence of Fe(II).

One other experiment, (Table 7E) does suggest a further possibility that could explain the apparent increase in Fe(II) after prolonged storage in the dark. It has not been shown to date whether colloids account for much of the ‘dissolved’ (> 0.2 μm) Fe present within rainwater. In seawater, colloids account for much of both the ‘dissolved’ Fe and the dissolved organic ligands present (Fitzsimmons and Boyle, 2014; Hassler et al., 2011). Rainwater has a very different composition to seawater, but similarly the 0.2 μm filtration used to operationally define ‘dissolved’ components could contain a colloidal fraction. When a 0.01 μm filter (which unlike a 0.20 or 0.45 μm should remove all particulate material except small nanoparticles from solutions) was used on two rain events, no significant decline in ‘dissolved’ Fe concentration was noted (Table 6E). Both of the rain events where this sequential filtration was conducted however had a very low dissolved Fe concentration.

One of the most intriguing results is the difference between 25:75 and 50:50 mixing ratios of rainwater with seawater or deionised water. It is counter intuitive that the mixing ratio affects the rate at which Fe(II) decays. A 50% rainwater: 50% de-ionised water solution initially had a higher dissolved Fe(II) concentration than a 25% rainwater: 75% de-ionised water solution (Table 7E) as expected. However, the difference was not as large as would be expected based on simple dilution. Furthermore the concentration of Fe(II) decayed to a similar value for both mixtures within 20 hours, despite the difference in composition (Table 7E). A possible explanation of this phenomenon is that the particle density in the solution is responsible. Greater dilution of unfiltered rainwater lowers the particle density and may therefore lower scavenging rates of Fe from solution. Dissolved Fe may therefore be removed from solution faster in a 50:50 rainwater:de-ionised water mixture than in a 25:75 mixture. Scavenging may also explain why unfiltered rainwater does not exhibit the increase in Fe(II) apparent in filtered rainwater (and filtered rainwater/seawater mixtures, Figure 2E).

Solar irradiation of rainwater resulted in an initial rise in Fe(II), but this plateaued and then the concentration remained very stable (Tables 4E and 5E, Figure 3E) suggesting a steady state concentration had been achieved. The trend in Fe(III) concentration is less clear due to large error bars relative to the concentration. After the light source was removed, Fe(II) showed prolonged stability and an elevated concentration was still present 72 hours after irradiation ceased (Figure 1E). Upon irradiation seawater showed no significant change in Fe(II) concentration (Table 4E), other than a very small decay after several hours which would be expected due to container surface removal of Fe during storage (Lewin and Chen, 1973). The rainwater behaviour under simulated solar irradiation is as has been reported before although, as has already been demonstrated, the Fe(II) concentrations are low compared to most historical values (Table 9E). An increase of around 80% in Fe(II) concentration is measured after irradiation and this then slowly decayed with elevated Fe(II) still present over a day after irradiated ceased (Table 4E). A seawater and rainwater mixture showed a small rate of Fe(II) decrease similar to that observed in the seawater alone (Table 4E). An irradiated seawater (75%): rainwater (25%) mixture demonstrated that rainwater can act as a significant source of Fe(II) to surface waters, as there was over a 40% increase in Fe(II) concentration upon irradiation of this mixture (Table 4E). This is greater than the equivalent increase observed in filtered rainwater and therefore there must be a net release of Fe(II) from the particulate phases present in the unfiltered rainwater.

Both UV irradiated and non-UV irradiated samples initially showed consistently high dissolved Fe(II) with no significant difference between the two sample types after between 1 and 3 hours of solar irradiation. After irradiation ceased, Fe(II) in the UV treated sample did not decay significantly faster. Dissolved Fe(II) concentration in this rainwater was however very low (even compared to the raw mean 12 nM, Table 2E) such that the error in Fe(II) concentration is sufficient to mask any potential difference between UV and non-UV treated behaviour. Thus if Fe(II) ligands (or another chemical species capable of impeding Fe(II) oxidation) are still present within rainwater, as has been observed previously, their concentration must have decreased appreciably.

When pH was varied in unfiltered rainwater, dissolved Fe increased with decreasing pH due to dissociation of Fe from particulate phases and the increasing stability of the dissolved phase under more acidic conditions (Figure 4E).

5.5 Conclusions

One of the aims of this work was to characterise Fe(II) ligands present in rainwater in order to determine whether they are similar to the humic derived ligands extractable from compost and river water, or fundamentally different. However, the very low Fe(II) concentrations now present in Wilmington rain water (the summer 2013 season reported here has the lowest volume weighted Fe(II) concentration on record, Table 9E) prevents the characterisation of any ligands that may be present. The reverse titration procedure to characterise Fe(II) ligands at present requires a minimum Fe concentration of 40 nM and a solution volume of at least 40 mL. Whilst it might have been possible to characterise the ligands thought to be present in Wilmington rainwater prior to 2001 (Table 8E), Fe(II) concentration is now far below this threshold (Table 2E).

Summer rainwater dissolved Fe(II), dissolved Fe(III) and total Fe have been determined and added to an on-going dataset of Wilmington (North Carolina, USA) rainwater composition (Table 9E). A clear decrease in dissolved Fe(II) and dissolved Fe(III) is evident in summer rainwater since 2001. Furthermore, the ratio of dissolved Fe(II):Fe(III) has also decreased sharply (Table 9E). Changes in rainwater pH cannot explain the magnitude of the decrease in Fe(II) concentration, or the change in Fe(II) behaviour when rainwater is mixed with seawater. A decrease in the pH of rainwater from 5.5 to 3.6 (an 80 fold increase in H^+ concentration) produced a 6 fold rise in rainwater Fe(II) concentration under laboratory conditions (Figure 4E). However, since 1985 Wilmington rainwater mean H^+ concentration has ‘only’ fallen by approximately half and yet summer Fe(II) concentrations have been subject to a 10 fold decrease (Table 9E).

Recent work has demonstrated that 20% of DOC in Wilmington rainwater has an anthropogenic origin and that both water soluble components of vehicle exhaust fumes and combusted coal fumes can effectively stabilise Fe(II) against oxidation (Avery et al., 2006; Humphreys, 2013). The decrease in Fe(II) concentration in Wilmington rainwater appears therefore not simply to be because of inorganic changes to the chemistry of rainwater (for example the increase in pH recorded since 1985, or the decrease in H_2O_2 recorded since 2001). A two part change in the terrestrial input of DOC is likely to have been a significant factor in the marked decrease in Fe(II) since 2001. First air quality has improved decreasing the supply of combustion product material that stabilizes Fe(II) against oxidation to rainwater, second the terrestrial component of DOC in rainwater over Wilmington has decreased in favour of more marine material (which inevitably contains much less of an anthropogenic signature than terrestrial material) due to changing weather patterns (Willey et al., 2009).

A strong anthropogenic impact upon aerosol Fe deposition to the ocean has been reported before through the release into the atmosphere of both highly soluble Fe in fly ash and acidic salts that promote aerosol particulate Fe dissolution (Schroth et al., 2009; Sedwick et al., 2007; Sholkovitz et al., 2009). The Wilmington rainwater record presents evidence of a further impact. The strong stabilisation of Fe(II) that was previously observed (and can still be detected in some samples although with much lower Fe(II) concentrations, Figure 1E and Table 4E) and has since largely disappeared is almost certainly a direct result of anthropogenic emissions. No other explanation would explain both the change in concentration and behaviour of Fe(II) since 2001.

The effect of anthropogenic emissions on the delivery of aeolian Fe to the open ocean has been studied in the Sargasso Sea and at four North Atlantic island sites (Tenerife, Barbados, Bermuda and Ireland) (Sedwick et al., 2007; Sholkovitz et al., 2009). Aerosol origin was found to be the dominant control on iron solubility with air masses originating from the North American continent having the highest operational solubility of aerosol Fe (Sholkovitz et al., 2009). The main reason for this elevated Fe solubility was demonstrated to be the presence of oil combustion products. Furthermore, in Bermuda and Ireland it was estimated that anthropogenic aerosol Fe accounted for a greater quantity of Fe deposition to surface marine waters than natural Fe (Sholkovitz et al., 2009). If oil combustion products can have such a measurable effect upon dry aerosol deposition, it is plausible that a similar effect, the delivery of Fe(II) stabilising ligands, may be widespread.

Due to improving air quality, Fe delivery to coastal waters downwind of Wilmington may therefore now be less efficient than it was prior to 2001. What are the consequences of this? Fe stress in microorganisms is thought to be widespread, even in coastal waters where dissolved Fe concentrations an order of magnitude greater than the open ocean are offset by greater cellular Fe requirements (Hutchins et al., 1998). Direct Fe limitation of primary production is however largely confined to the permanent HNLC zones and in the North Atlantic is only temporarily observed in some years in the Icelandic and Irminger Basins (Nielsdottir et al., 2009; Ryan-Keogh et al., 2013). The appearance and, now disappearance, of an anthropogenic class of Fe(II) stabilising compounds in rainwater may therefore have acted to alleviate Fe stress in offshore areas of the northwest Atlantic, but is unlikely to have affected primary production rates in any direct way as dissolved Fe is not thought to be a limiting nutrient in these waters.

Could anthropogenic emissions of Fe(II) stabilising compounds from fossil fuel combustion affect primary production rates in any permanently Fe limited waters? The three large, permanent HNLC zones are Fe limited because of low atmospheric dust deposition (from terrestrial sources) (Jickells and Spokes, 2001). An anthropogenic Fe(II) stabilisation phenomenon, as observed in Wilmington rainwater, is likely to be confined to areas of terrestrial dust deposition and therefore unlikely to have any measurable impact in those parts of the ocean where dust deposition is minimal. However, if combustion of fossil fuels is considered the direct source of such Fe(II) stabilisation, there is the possibility that marine shipping may be acting as a source of Fe(II) stabilising compounds into the marine environment. Emissions from marine traffic are only sporadic in two of the three major HNLC zones, the Southern Ocean and the equatorial Pacific, but more intense around the sub-Arctic Pacific (Corbett and Fischbeck, 1997). It is therefore plausible that increased emissions from marine traffic in some parts of the ocean could increase Fe bioavailability via the addition of Fe(II) stabilising compounds and thereby partially alleviate the extent of Fe limitation.

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