



Disassembling iron availability to phytoplankton

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The bioavailability of iron to microorganisms and its underlying mechanisms have far reaching repercussions to many natural systems and diverse fields of research, including ocean biogeochemistry, carbon cycling and climate, harmful algal blooms, soil and plant research, bioremediation, pathogenesis, and medicine. Within the framework of ocean sciences, short supply and restricted bioavailability of Fe to phytoplankton is thought to limit primary production and curtail atmospheric CO₂ drawdown in vast ocean regions. Yet a clear-cut definition of bioavailability remains elusive, with elements of iron speciation and kinetics, phytoplankton physiology, light, temperature, and microbial interactions, to name a few, all intricately intertwined into this concept. Here, in a synthesis of published and new data, we attempt to disassemble the complex concept of iron bioavailability to phytoplankton by individually exploring some of its facets. We distinguish between the fundamentals of bioavailability – the acquisition of Fe-substrate by phytoplankton – and added levels of complexity involving interactions among organisms, iron, and ecosystem processes. We first examine how phytoplankton acquire free and organically bound iron, drawing attention to the pervasiveness of the reductive uptake pathway in both prokaryotic and eukaryotic autotrophs. Turning to acquisition rates, we propose to view the availability of various Fe-substrates to phytoplankton as a spectrum rather than an absolute “all or nothing.” We then demonstrate the use of uptake rate constants to make comparisons across different studies, organisms, Fe-compounds, and environments, and for gaging the contribution of various Fe-substrates to phytoplankton growth *in situ*. Last, we describe the influence of aquatic microorganisms on iron chemistry and fate by way of organic complexation and bio-mediated redox transformations and examine the bioavailability of these bio-modified Fe species.

Keywords: iron, bioavailability, uptake, phytoplankton, speciation, redox reactions, biogeochemistry, organic complexation

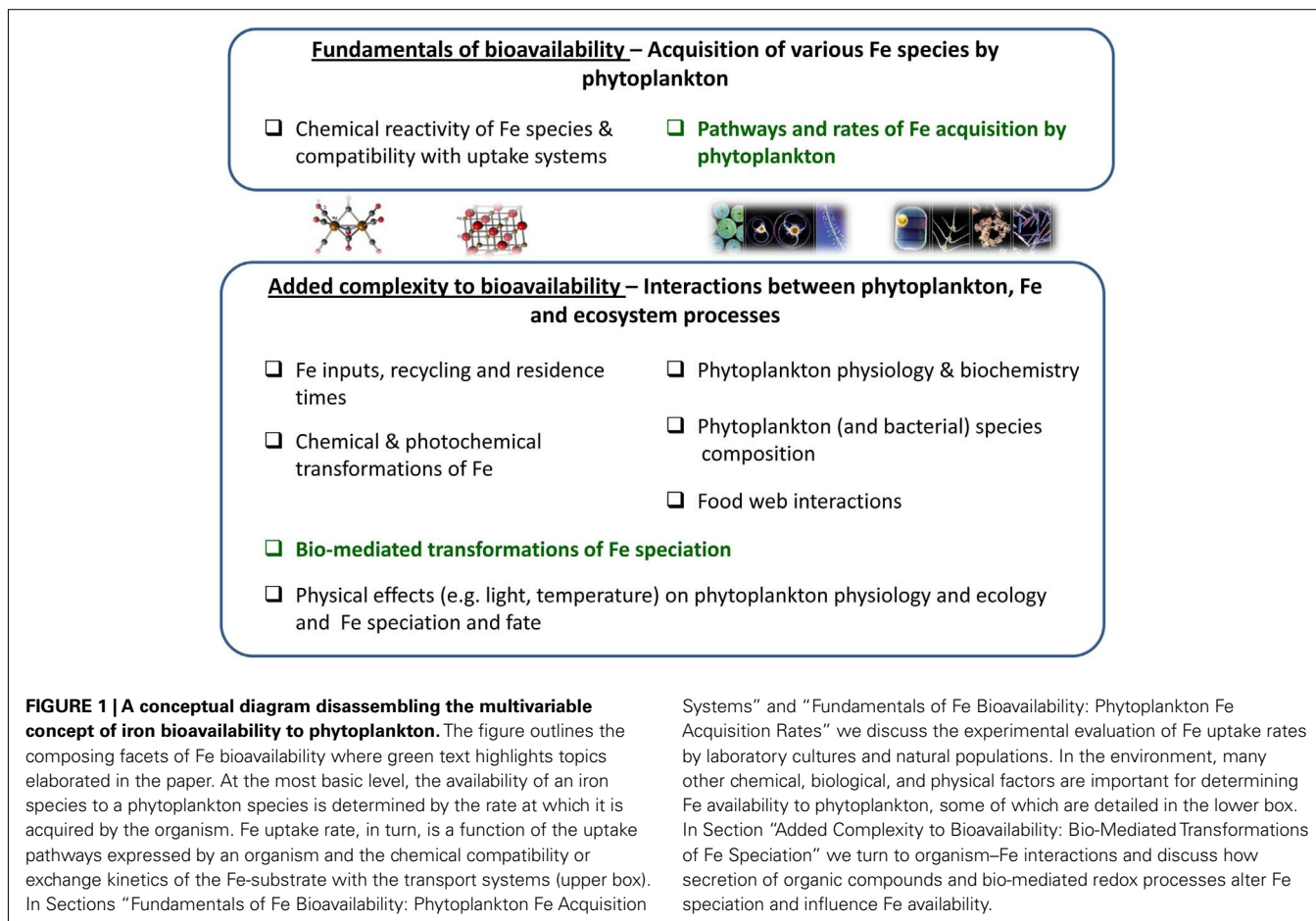
INTRODUCTION

By virtue of its flexible redox chemistry, iron (Fe) plays an integral role in many biological processes such as photosynthesis, respiration, processing of reactive oxygen species, and nutrient acquisition. In view of these functions, it is not surprising that iron inputs and bioavailability in aquatic environments have far reaching repercussions for many natural systems and diverse areas of study as briefly outlined in **Box 1**. At the basis of these lies the role of iron in controlling phytoplankton growth. Photosynthetic life on Earth originated in reduced, low oxygen aquatic environments where the soluble ferrous iron – Fe(II) – was abundant and freely available. The rise of oxygenic photosynthesis favored the oxidized ferric form – Fe(III) – which rapidly precipitates out of oxic solutions as iron oxides or hydroxides. Modern day oceans and lakes thus cater poorly to the Fe requirements of phytoplankton with surface waters bearing picomolar to nanomolar concentrations of dissolved unchelated inorganic iron, Fe²⁺ (Johnson et al., 1997), the most readily available form of Fe to phytoplankton, be it in ferrous Fe(II)²⁺ or ferric form Fe(III)³⁺ (Morel et al., 2008).

Extensive research on iron bioavailability to phytoplankton has been conducted over recent decades, yet a clear-cut definition of this term remains elusive. Bioavailability may be defined as the

degree to which a certain compound can be accessed and utilized by an organism. However this definition may be oversimplistic as elements of iron speciation and kinetics, phytoplankton physiology, light, temperature, and microbial interactions, to name a few, are all intricately intertwined into what we term “bioavailability” (Wells et al., 1995; Worms et al., 2006). Given the complex and interdisciplinary nature of Fe bioavailability, progress in understanding this concept depends on addressing its sub-aspects by means of well-defined questions and multiple analytical techniques. In this contribution, rather than seeking a definition capable of encompassing the multiple aspects and scales of Fe bioavailability to phytoplankton, we attempt to disassemble this concept into its composing facets and explore them further.

At a fundamental level, cellular Fe acquisition or uptake rates are indicative of the availability of any single Fe-substrate to a specific phytoplankton species (**Figure 1**). Fe uptake rate, in turn, is a function of the uptake pathways expressed by an organism and the chemical compatibility or exchange kinetics of the Fe-substrate with the transport systems (**Figure 1**). Rates of Fe acquisition can be determined experimentally using model or naturally occurring phytoplankton and Fe-substrates. In the next two sections we discuss the experimental evaluation of Fe uptake



pathways and rates and suggest the use of uptake rate constants as a means of comparing between organisms, Fe species, and environments, and gaging the relative contribution of specific Fe-compounds to phytoplankton in a natural setting. Needless to say, the availability of Fe to natural phytoplankton assemblages in oceans and lakes is influenced by many chemical, biological, and physical factors outside the experimental beaker (see **Figure 1** for an outline of some of these factors). For example, both Fe speciation and phytoplankton physiology are dynamic in time and space and to complicate matters even further, these two factors are interconnected. Moreover, interactions among the various organisms in the ecosystem, in addition to a host of environmental variables, can strongly impact the ability of phytoplankton to meet their Fe requirements. While a complete description of this added complexity to bioavailability is beyond the scope of this contribution, in the last section we describe how aquatic microorganisms influence iron chemistry and fate by way of organic complexation and bio-mediated redox transformations, emphasizing the resulting effects on Fe bioavailability to phytoplankton.

FUNDAMENTALS OF Fe BIOAVAILABILITY: PHYTOPLANKTON Fe ACQUISITION SYSTEMS

In order to determine the effects of Fe inputs on phytoplankton productivity it is essential to identify the relationship between

the concentration of various Fe species and their uptake by phytoplankton. As such, phytoplankton iron transport systems, uptake strategies, and rates are important for our understanding of Fe availability. In the next two sections we tackle the question of bioavailability at the organism level and look at mechanistic studies of iron acquisition pathways and rates under controlled conditions – an approach which has provided much insight into Fe bioavailability in natural systems (Hudson and Morel, 1993; Sunda and Huntsman, 1995; Hutchins et al., 1999; Maldonado and Price, 1999; Maldonado et al., 1999; Shaked et al., 2005; Morel et al., 2008). In Section “Uptake Pathways of Aquatic Phytoplankton” we explore two well-studied iron uptake pathways in aquatic phytoplankton – siderophore mediated and reductive Fe uptake – looking at the environmental relevance of each strategy. Since our focus is phytoplankton, we will not cover Fe uptake pathways of heterotrophic bacteria. More on this subject can be found in a recent report by Hopkinson and Barbeau (2012). In Section “Behavioral Patterns of Iron Mining” we briefly mention some behavioral patterns amongst phytoplankton which may be relevant to Fe acquisition from seawater.

UPTAKE PATHWAYS OF AQUATIC PHYTOPLANKTON

Several uptake pathways for free inorganic iron (Fe^{\prime}) and organically bound iron (FeL) have been described amongst aquatic phytoplankton. While Fe^{\prime} is clearly an important iron source,

Box 1 | Scope of iron influence on natural systems and research fields.

Aquatic iron biogeochemistry has been in the limelight over the past three decades with numerous studies linking Fe to carbon cycling and global climate (Martin et al., 1990; Watson et al., 2000; Blain et al., 2007; Martinez-Garcia et al., 2011). A particular emphasis has been placed on Fe availability to phytoplankton since over 45% of global photosynthesis occurs in aquatic environments (Falkowski et al., 1998) and photosynthetic systems are heavily dependent on iron (e.g., Raven, 1990; Greene et al., 1991). It is now well established that limited iron availability lowers phytoplankton pigment content and light harvesting capabilities, hinders photosynthesis and growth rates, and subsequently diminishes the production of organic matter and biogenic minerals (CaCO₃ and opal) and curtails CO₂ drawdown in vast ocean regions (Figure 2; Boyd et al., 2007). Many biogenic gases other than CO₂ are important determinants of atmospheric chemistry and climate, but far less is known about the controls iron exerts on the sea-atmosphere fluxes of such gases (Figure 2; Liss, 2007; Buesseler et al., 2008). What is known however, is that phytoplankton growth, death, and decomposition, all of which may be controlled by Fe availability, result in emissions of dimethylsulfide (DMS) and isoprene (cloud formation promoters), N₂O and CH₄ (potent greenhouse gases), and CO and OH (reactive species influencing the atmosphere oxidation potential; Law and Ling, 2001; Meskhidze and Nenes, 2006). The combined effects of these emissions on atmospheric radiative forcing remain largely unknown (Lampitt et al., 2008).

By controlling phytoplankton standing stocks, Fe availability may also influence the surface ocean light field, and subsequently

play a role in the surface ocean heat budget (Figure 2; Manizza et al., 2005). In addition to constraining primary productivity, iron deficiency impedes biogenic element cycling since phytoplankton cannot synthesize the enzymes required for utilizing major nutrients such as nitrate and N₂ (Figure 2; Milligan and Harrison, 2000; Kustka et al., 2002; Sohm et al., 2011). Low iron availability may also alter ecosystem structure and function: under Fe limitation smaller phytoplankton are favored, resulting in rapid carbon regeneration and lowered carbon export flux to the deep ocean (Figure 2; Price et al., 1994; Finkel et al., 2010). As limited Fe availability alters nutrient assimilation ratios and phytoplankton species composition, it bares implications for the reconstruction of ocean paleo-productivity and paleo-nutrient distributions. Examples include the intensively studied sedimentary records of diatoms, whose abundance, morphology, and composition is strongly regulated by Fe (Figure 2; Strzepek and Harrison, 2004; Marchetti et al., 2006; Marchetti and Cassar, 2009). An additional, less explored example, is the recently reported effect of Fe limitation on cadmium (Cd) drawdown from seawater by phytoplankton (Lane et al., 2009). Subsequently, Fe limitation may alter seawater Cd:P ratios (Cullen et al., 1999) and thus bias past reconstruction of PO₄³⁻ distributions which is based on seawater Cd:P ratios (Figure 2; Boyle, 1988; Elderfield and Rickaby, 2000). Recent attention has also been drawn to the effect of iron inputs and availability on toxic algal species occurrence and toxin production in oceans and lakes (Figure 2; e.g., Trick et al., 2010; Alexova et al., 2011).

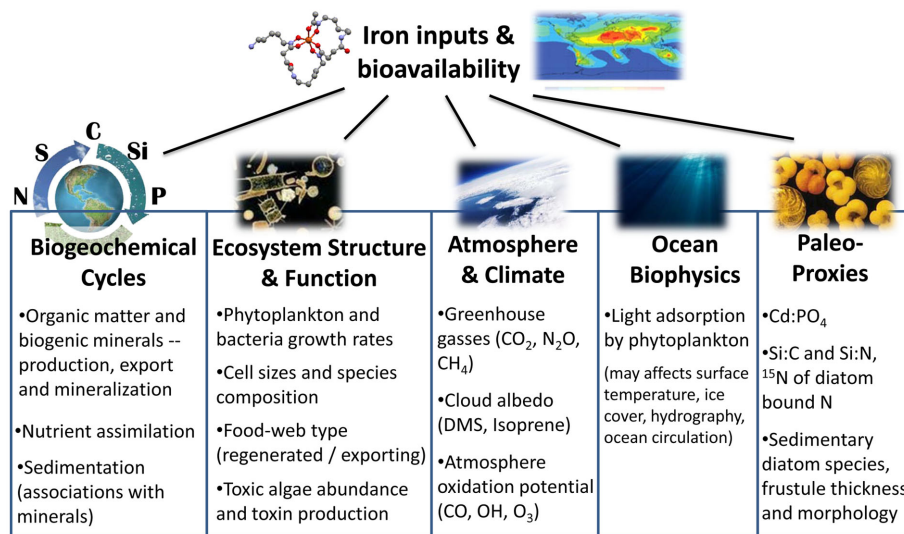


FIGURE 2 | Summary of processes, systems, and research fields which are influenced by iron inputs and bioavailability (see text for details).

we focus on FeL, where the transport machinery an organism employs will dictate the accessibility of a specific compound (Morel et al., 2008). To date, two major FeL uptake pathways have been described for phytoplankton: siderophore mediated Fe acquisition (e.g., Goldman et al., 1983; Soria-Dengg et al., 2001) and the reductive iron uptake pathway (Allnut and Bonner, 1987; Eckhardt and Buckhout, 1998; Maldonado and Price, 2001; Shaked et al., 2005). According to a prevalent paradigm shared by many oceanographers, prokaryotic phytoplankton

adopt siderophore-based Fe uptake systems while eukaryotes utilize a reductive strategy (e.g., Hutchins et al., 1999). However, genetic evidence (Webb et al., 2001; Hopkinson and Morel, 2009) as well as results of short term iron uptake experiments (Rose et al., 2005; Lis and Shaked, 2009; Fujii et al., 2010; Kranzler et al., 2011), contradict this paradigm. We propose that the occurrence of siderophore vs. reductive iron uptake can be put down to environmental rather than taxonomic considerations and that reductive iron uptake is a prevalent Fe

acquisition strategy amongst phytoplankton (**Figure 3; Table A1** in Appendix).

Siderophore mediated Fe uptake involves the synthesis and secretion of ferric iron chelators which are capable of solubilizing, capturing, and delivering Fe(III) to the cell (Kraemer, 2004). The efficiency of this uptake pathway depends on: (a) the probability of the siderophore finding an Fe-substrate and (b) the probability of the ferrisiderophore complex finding its way back to the secreting cell. The build-up and maintenance of an Fe-siderophore diffusion gradient bringing iron back to the host cell is an essential feature of this strategy (Hutchins et al., 1991; Völker and Wolf-Gladrow, 1999). Therefore, siderophore production works best at high cell densities and in quiet waters or contained environments where turbulent disruption is unlikely (e.g., within biogenic aggregates or dense algal colonies). Consequently, siderophore production would be impractical in open waters, with high turbulence, and low cell densities (Hutchins et al., 1991). Indeed, a conspicuous lack of siderophore synthesis or uptake genes amongst open ocean cyanobacteria and eukaryotic phytoplankton (Hopkinson and Morel, 2009) suggests that this strategy may be ill suited to free-living aquatic phototrophs. Given the limitations of siderophore mediated iron uptake in dilute media, particularly open ocean waters, we turn our attention to an alternative strategy better suited to these conditions – Fe acquisition by means of reduction.

Reductive iron uptake offers a practical alternative to siderophore production as demonstrated in the numerical model constructed by Völker and Wolf-Gladrow (1999) comparing the

efficiency of these two strategies in the marine environment. Several studies, as well as our own data, support the prevalence of reductive iron uptake amongst a variety of representative phytoplankton from both eukaryotic and prokaryotic taxa as well as in natural phytoplankton communities in high Fe and low Fe environments (**Figure 3; Table A1** in Appendix). Reduction operates on both Fe' and FeL where it involves the dissociation of Fe from its chelating ligand followed by transport of free iron into the cell (Atkinson and Guerinot, 2011). A key feature of this uptake pathway is the formation of an Fe(II) intermediate and thus experimental assays for reductive Fe uptake employ a ferrous iron binding ligand such as ferrozine or bathophenanthrolinedisulfonic acid (BPDS) which competes with the cell for Fe(II) and inhibits Fe uptake (see **Figures A1** and **A2** in Appendix; Shaked et al., 2004, 2005). On a genetic level, cell surface ferric reductases, similar to those in the yeast *Saccharomyces cerevisiae*, have been found in green algae (Allen et al., 2007) and ocean dwelling diatoms (Kustka et al., 2007; Bowler et al., 2010). These reductases may operate in conjunction with permease-oxidase complexes which reoxidize the Fe(II) as it is transported into the cell (Maldonado et al., 2006; Chen et al., 2008; Terzulli and Kosman, 2010). Not much is known about the reductive processes only recently shown to exist in aquatic cyanobacteria (Rose et al., 2005; Lis and Shaked, 2009; Kranzler et al., 2011).

The greatest advantage of the reductive strategy is its potential to operate not only on Fe' but also across a range of organically bound iron complexes, even Fe bound to strong siderophores

Laboratory cultures	
Eukaryotes	Prokaryotes
<p>Green Algae</p> <p><i>Chlamydomonas reinhardtii</i>^{a-b}</p> <p><i>Dunaliella bardawil</i>^c</p> <p><i>Chlorella</i> spp.^{d-e}</p> <p><i>Tetraselmis suecica</i>[†]</p> <p><i>Chlorococcum littorale</i>^f</p> <p>Dinoflagellates</p> <p><i>Peridinium gatunense</i>^g</p>	<p>Diatoms</p> <p><i>Thalassiosira</i> spp.^{h-j}</p> <p><i>Cylindrotheca fusiformis</i>[‡]</p> <p><i>Phaeodactylum tricornutum</i>^k</p> <p>Haptophytes</p> <p><i>Emiliania huxleyi</i>[‡]</p>
<p>Cyanophytes</p> <p><i>Synechocystis</i> PCC6803^l</p> <p><i>Trichodesmium erythraeum</i>[‡]</p> <p><i>Synechococcus</i> WH8102[‡]</p> <p><i>Synechococcus</i> WH7803[‡]</p> <p><i>Microcystis aeruginosa</i>^m</p> <p><i>Lyngbya majuscula</i>ⁿ</p>	
Natural environments	
Low Fe waters (HNLC)	High Fe waters
<p>Southern Ocean^o</p> <p>Subarctic Pacific (> 3μm)^p</p> <p>Bering Sea (> 5μm)^q</p>	<p>Gulf of Aqaba (> 0.2μm)^r</p> <p>Loch Scridain (> 20μm)[‡]</p> <p>Lake Kinneret^g</p>

FIGURE 3 | Prevalence of the reductive iron uptake pathway amongst phytoplankton. Listed are laboratory cultures and natural environments for which inhibition of uptake by Fe(II) binding ligands (Ferrozine/BPDS) was observed experimentally and taken to indicate the formation of an Fe(II) intermediate during iron transport. For some species, genomic and proteomic research identified various components of the reductive iron uptake pathway including ferrireductases and multicopper oxidases. See Appendix for supporting data and methodology (**Tables A1** and **A2** and **Figures A1** and **A2** in Appendix). Note on locations: The Gulf of Aqaba is located at the northern

tip of the Red Sea, Loch Scridain is a sea loch located on the Atlantic coastline of the island of Mull, Scotland, and Lake Kinneret (Sea of Galilee) is a fresh water lake in the north of Israel. References: ^aEckhardt and Buckhout (1998), ^bWeger (1999), ^cKeshtacher et al. (1999), ^dAllnutt and Bonner (1987), ^eMiddlemiss et al. (2001), ^fSasaki et al. (1998), ^gShaked et al. (2002), ^hShaked et al. (2005), ⁱJones and Morel (1988), ^jMaldonado and Price (2001), ^kSoria-Dengg and Horstmann (1995), ^lKranzler et al. (2011), ^mFujii et al. (2010), ⁿRose et al. (2005), ^oMaldonado et al. (2005), ^pMaldonado and Price (1999), ^qShaked et al. (2004), ^rLis and Shaked (2009), [‡]Lis and Shaked, in preparation.

such as ferrioxamine B (FeDFB; Maldonado et al., 2005; Shaked et al., 2005; Lis and Shaked, 2009; Shi et al., 2010; Kranzler et al., 2011). Phytoplankton equipped with this strategy would be able to integrate iron from a variety of sources, giving them an obvious competitive advantage in Fe acquisition. While we advance the idea of reduction as a prevalent Fe uptake strategy in aquatic systems, we by no means claim it to be exclusive. A single organism may possess both direct FeL uptake pathways (e.g., ferrisiderophore transporters) as well as iron reductases, a classic example being baker's yeast (Kosman, 2003). Moreover, siderophore mediated and reductive iron uptake do not discount the existence of other Fe uptake pathways, be they under investigation (e.g., Stintzi et al., 2000; Pick et al., 2008; Sutak et al., 2010; Wirtz et al., 2010) or undiscovered.

BEHAVIORAL PATTERNS OF IRON MINING

The data covered thus far clearly demonstrates that phytoplankton are not passive in their quest for iron. A major energetic investment is clearly required in order to accumulate intracellular Fe concentrations that are four to six orders of magnitude greater than those in their surrounding environment (Morel and Price, 2003). Some phytoplankton take this a step further and exhibit behavioral patterns which aid in the active mining of iron from the environment. The most striking example is the collection and processing of iron-rich dust particles by colonies of the globally important N₂ fixing cyanobacterium *Trichodesmium* spp. (Rueter et al., 1992; Rubin et al., 2011). The positively buoyant *Trichodesmium* forms massive blooms at the sea surface where it is likely to encounter dust deposits. Recently, we reaffirmed previous observations on efficient capturing and retention of dust by natural *Trichodesmium* colonies and documented a specialized ability to actively shuffle dust and iron oxides from the colony periphery to its core (Rubin et al., 2011). Packaging of dust in the colony interior can minimize its detachment and loss and also facilitate its chemical processing within a semi-enclosed microenvironment (Rubin et al., 2011). We found that *Trichodesmium* colonies were able to mediate dust dissolution, most likely via reduction (Rubin et al., 2011; Shaked, unpublished). Our mechanistic study complements several field observations documenting the ability of *Trichodesmium* to utilize iron from dust (Moore et al., 2009; Chen et al., 2011). Additionally, large diatoms (such as *Rhizosolenia* spp. and *Ethmodiscus* spp.) and dinoflagellates (such as *Alexandrium* spp. and *Gymnodinium* spp.) are known to migrate vertically to the nutricline to stock up on nutrients (Villareal et al., 1999; Ralston et al., 2007). While vertical migration was repeatedly proven efficient in nitrogen accumulation (Singler and Villareal, 2005), no clear evidence for iron mining at depth is present (McKay et al., 2000).

FUNDAMENTALS OF Fe BIOAVAILABILITY: PHYTOPLANKTON Fe ACQUISITION RATES

The discussion so far has centered on the mechanisms phytoplankton employ for acquiring Fe, knowledge which is crucial for analyzing and predicting their ability to access iron from various Fe pools in the environment (Shaked et al., 2005; Morel et al., 2008). We now focus on a more common approach to determining Fe availability: rate of transport. In this line of research the bioavailability of an Fe-substrate is ascertained by means of

growth or short term iron uptake experiments. Although this approach is very promising, it faces significant challenges in terms of quantitative extrapolation to systems outside the experimental framework (be it a beaker or a grow-out incubation). In Section "Experimental Probing of Availability" we discuss the importance of experimental design, stressing the use of well-defined experimental media and organisms in the pursuit of unambiguous data regarding the accessibility of different Fe species to various phytoplankton. However, even when high quality data are obtained, it is very difficult to reach a consensus regarding the availability of any one Fe-compound. The same Fe-substrate, for example, may be available to one organism but not to another, making bioavailability not only a question of "what?" but also of "to whom?". Moreover, the same organism may employ additional transport pathways upon Fe limitation, further extending the question to "when?" and "where?" in natural environments. In Section "Computing Availability Using Uptake Constants," we describe the use of uptake rate constants for comparing between studies, organisms, compounds, and environments as well as for gauging the contribution of specific Fe species to phytoplankton growth *in situ*. We propose that the availability of Fe species to phytoplankton can be viewed as a spectrum rather than an absolute "all or nothing" and establish a relative scale of bioavailability for a range of Fe species and various phytoplankton cultures and natural populations.

EXPERIMENTAL PROBING OF AVAILABILITY

Two common experimental approaches for probing the bioavailability of a given Fe-compound are: (1) long term or steady state iron uptake experiments which follow growth rates and/or intracellular Fe quotas of cells grown on a certain Fe-substrate, and (2) short term uptake experiments where the change in intracellular iron is followed over several hours. In order to enable inter- and intra-study comparisons, common grounds in methodology must be established by means of a robust experimental design in which medium and/or organism are well-defined.

Due to the fast hydrolysis and precipitation of Fe(III), an artificial or natural Fe complexing agent is required to keep dissolved iron in solution during experiments. For the study of free inorganic iron (Fe'), EDTA (ethylenediaminetetraacetic acid), and other carboxylic acid compounds are typically used (e.g., Anderson and Morel, 1982). The FeEDTA complex itself is membrane impermeable and not bioavailable (Shaked et al., 2005) and EDTA buffers an easily calculated pool of unchelated iron or Fe' in the medium (Sunda et al., 2005). There has been some criticism of the applicability of EDTA based studies to natural systems (Gerringa et al., 2000) but the alternative of using uncomplexed FeCl₃ when trying to measure free inorganic iron uptake rates has serious pitfalls. When spiking experimental media with FeCl₃, the iron is found in two pools – dissolved Fe and freshly precipitated colloids – whose relative proportions and bioavailability fluctuate over time (Wells et al., 1983; Kuma et al., 1996). When examining the bioavailability of organically bound iron (FeL, where L is an organic ligand) many chemical factors such as ligand strength, metal to ligand ratios, FeL equilibration time, and photolability should be taken into consideration. Sufficiently high FeL complex stability and/or a sufficient excess of the ligand compared to Fe are important in order to prevent iron precipitation. On the other hand, high

concentrations of free ligand (L) were shown to slow Fe uptake rates down due to competition with the cells for unchelated Fe which is formed during reductive uptake (Maldonado and Price, 2001; Shaked et al., 2005; Lis and Shaked, 2009). Experiments probing the bioavailability of partially complexed, particulate, or colloidal iron require careful support measurements and/or use of chemical speciation modeling software which establish Fe speciation in the experimental medium (e.g., Nodwell and Price, 2001; Rijkenberg et al., 2006, 2008; Hassler et al., 2011a). The concentration of Fe used in short term uptake experiments is often a compromise between environmentally relevant low concentrations and those required for adequate signal. However, it must be noted that in order to compare between experiments Fe concentrations must be sub-saturating (see Computing Availability Using Uptake Constants).

Characterization of the experimental organism is no less important than defining Fe speciation, as physiological status and growth phase can greatly affect experimental outcomes. Iron limitation, for example, is known to cause the upregulation of high affinity Fe acquisition systems (Maldonado and Price, 1999, 2001; Maldonado et al., 2006; Kustka et al., 2007; Allen et al., 2008), allowing access to previously unavailable Fe pools. This high affinity acquisition system allows iron limited diatoms to acquire iron bound to the xenosiderophore ferrioxamine B (FeDFB), whereas this complex is non-accessible to iron replete diatoms (see **Figure 5A**; Maldonado and Price, 2001). Growth phase may also affect Fe uptake since various transport pathways may be inactivated during different growth phases (Lis and Shaked, unpublished). In addition, the presence of bacteria in uptake assays using non-axenic cultures or natural assemblages may influence the availability of specific compounds to the studied algae or bias the uptake signal through bacterial acquisition of compounds unavailable to the studied organism (Soria-Dengg et al., 2001; Roe et al., 2011). Experimental choices regarding cell density, illumination, temperature, use of pH buffers, and experiment duration, to name a few, may alter uptake rates by influencing both algal physiology and Fe chemistry, and should be carefully evaluated in preliminary experiments. As a rule of thumb, cell density should be kept as low as possible to minimize bio-mediated changes in Fe chemistry (e.g., through the secretion of Fe-binding compounds into the medium), and experimental length should be kept short to avoid changes in the transport systems (Sunda et al., 2005). Short experiments (4–12 h) are preferably conducted in the dark or under red light (which prevents Fe photochemistry while supplying photo-synthetically active irradiation (Kranzler et al., 2011)). Many organic pH buffers bind Fe and their effect on uptake rates should be carefully examined (Shi et al., 2009). Whenever possible, data points should be collected several times throughout the experiment rather than at its beginning and end. This ensures the measurement of meaningful rates that can be extrapolated further.

Once answers to fundamental questions of bioavailability have been established using model phytoplankton species and Fe-substrates, the next level of complexity may be added by conducting experiments with either natural aquatic communities or natural ligands (e.g., Maldonado et al., 2005; Shi et al., 2010). This combination of basic laboratory based research and field work has proven to be a powerful tool in unraveling the complexities of

natural systems. Experiments of this kind are analytically challenging and involve considerable planning and careful determination of the desired goals. Examples include studying the effect of photochemistry on the availability of model and natural Fe complexes to size fractionated natural phytoplankton communities (Maldonado et al., 2005) and the use of well-studied model organisms to report on changes in the availability of natural Fe-compounds due to variations in pH (Shi et al., 2010).

COMPUTING AVAILABILITY USING UPTAKE CONSTANTS

As stated above, uptake data are experiment-specific and thus often hard to extrapolate to other organisms or environments. Here we propose a relatively straight forward approach using the uptake rate constant – k_{up} – for comparing between experiments conducted with strongly bound organically complexed Fe (FeL) or unchelated Fe (Fe'). When an Fe-substrate is applied at sub-saturating concentrations, its rate of cellular uptake (ρ) is proportional to its concentration (Eq. 1, **Figure 4**):

$$\rho = k_{up} \times [Fe] \quad (1)$$

where ρ is cellular Fe uptake rate ($\text{mol Fe cell}^{-1} \text{ day}^{-1}$) and $[Fe]$ is Fe-substrate concentration (mol L^{-1}). Under these conditions (linear range in **Figure 4**), the dependency of cellular uptake rate on Fe concentration is described by the uptake constant – k_{up} with units of $\text{L cell}^{-1} \text{ day}^{-1}$ (different units may be used in accordance with the units of uptake rate). Unlike cellular uptake rate (ρ), which varies with Fe concentration, k_{up} is a more faithful representation of the ability of an organism to internalize the iron. In order to convert uptake rates to uptake constants, an action which can also be regarded as normalization, we need to define the concentration of Fe that serves as a substrate for uptake. In experiments probing the uptake of strongly complexed FeL, where Fe' concentrations are negligible, the substrate for uptake equals the total Fe added to the experiment as precomplexed FeL (**Figure 4**). In contrast, when probing Fe' uptake in the presence of the metal buffer EDTA, the total Fe is present predominantly as FeEDTA which is biologically unavailable, while Fe' – the substrate for uptake is found at minute concentrations (Shaked et al., 2005). The k_{up} calculation cannot be performed for weak FeL complexes as the experimental media contains both Fe' and FeL. Experiments using FeCl_3 are also hard to analyze due to Fe precipitation, unless Fe is added at concentrations lower than its solubility limits in seawater (0.2–0.5 nM; Liu and Millero, 2002). Despite its limitations, the uptake rate constant, k_{up} , is highly useful in comparing the “relative bioavailability” of different Fe-substrates to various phytoplankton, both in the laboratory or in natural environments. Moreover, we can use k_{up} to predict if a specific Fe-compound can support phytoplankton Fe demands *in situ*. These two applications are described below and in **Figures 4** and **5** and **Table 1**.

Relative bioavailability scale as a means of comparing organisms, Fe species, and environments

We first demonstrate the use of k_{up} in comparing the bioavailability of Fe' and FeDFB (where DFB is the strong siderophore ferrioxamine B) to Fe-limited cultures of the open ocean coccolithophore *Emiliania huxleyi* (**Figure 4**). In these experiments we determined

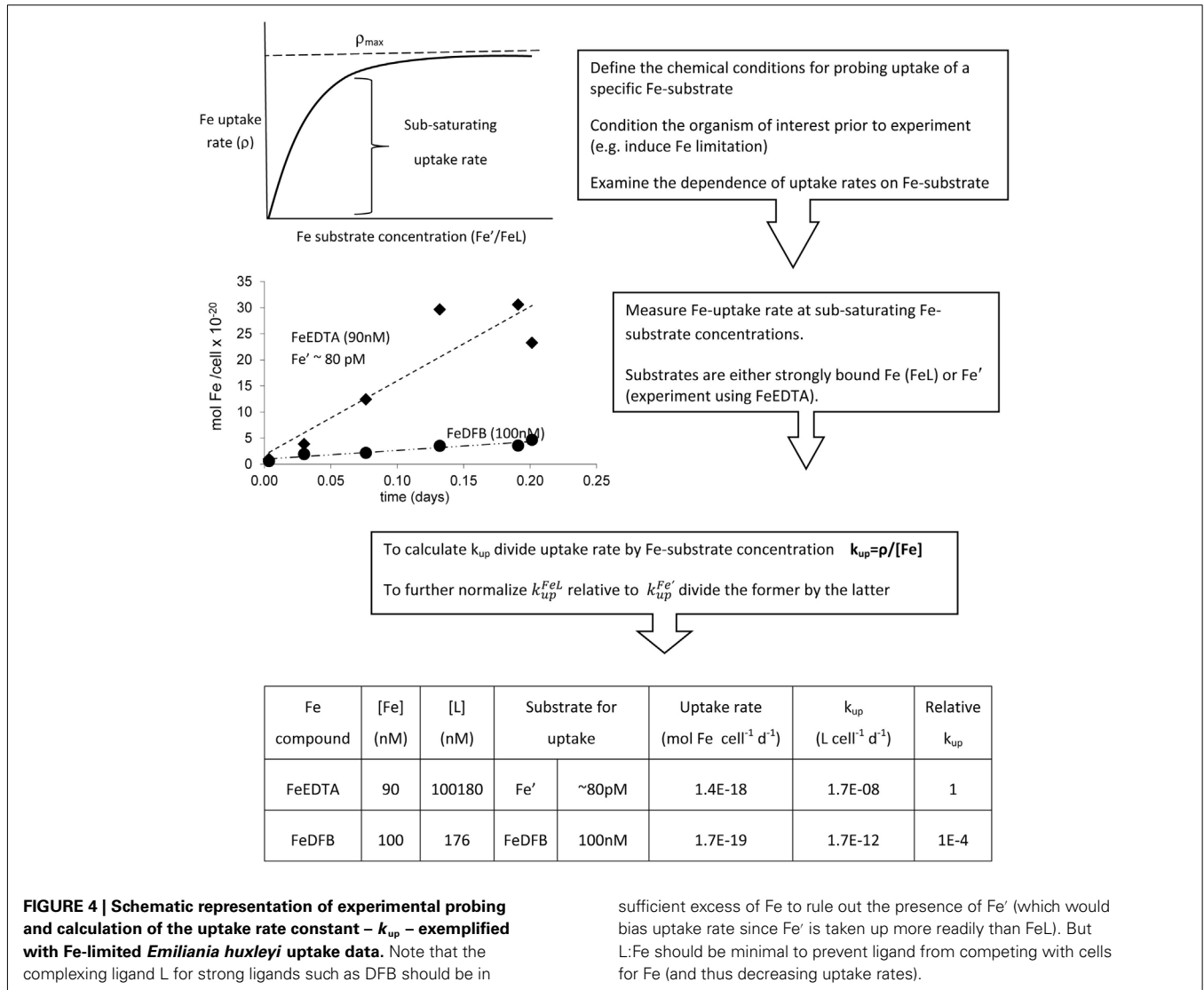


Table 1 | A comparison between the minimal daily Fe requirements and the daily FeDFB uptake capacity of *Synechococcus* spp.

Daily FeDFB uptake capacity: $k_{up} \times [Fe]$		Minimal daily Fe requirement: $Q \times \mu$	
k_{up} (DFB)–	[Fe]–	Q–	μ –
Uptake rate constant for FeDFB ^a	Fe-substrate concentration ^b	intracellular Fe quota for <i>Synechococcus</i> spp. ^c	Fe-limited growth rate ^d
$\sim 3 \times 10^{-13}$ L cell ⁻¹ day ⁻¹	$\sim 4 \times 10^{-9}$ mol L ⁻¹	$\sim 10^{-18}$ mol cell ⁻¹	~ 0.4 day ⁻¹
$\sim 1.2 \times 10^{-21}$ mol Fe cell ⁻¹ day ⁻¹		$\sim 4 \times 10^{-18}$ mol Fe cell ⁻¹ day ⁻¹	

Minimal daily Fe requirements are calculated by multiplying intracellular Fe quota (Q) by Fe-limited growth rates (μ). Uptake capacity is calculated by multiplying k_{up} by the concentration of Fe-substrate [Fe] in the environment. Calculating for FeDFB, we take a generous estimate of dissolved iron concentrations in open ocean waters and assume this pool it is made up entirely of FeDFB-like compounds.

^a See **Figure A1**; in Appendix, $\rho = 2.6 \times 10^{-20} \pm 8.2 \times 10^{-22}$ mol Fe cell⁻¹ day⁻¹; FeDFB = 90 nM.

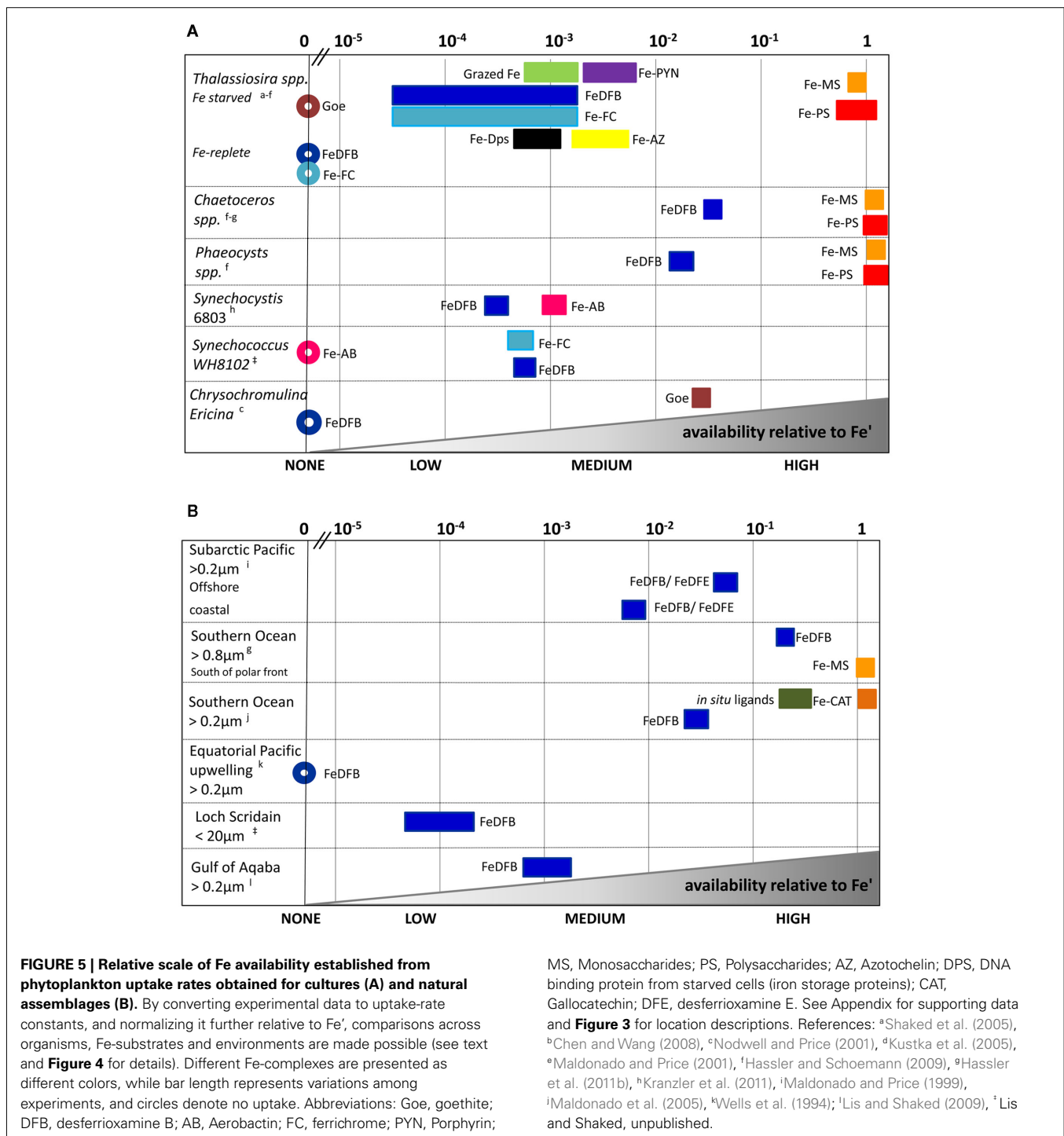
^b Hypothetical dissolved iron value in open ocean waters. We assume all dissolved iron is FeDFB.

^c Henley and Yin (1998).

^d Based on Kudo and Harrison (1997) and Brand (1991).

Fe uptake rates of *E. huxleyi* from 100 nM radiolabeled FeDFB (Fe:DFB ratio 1:1.1) to 90 nM radiolabeled FeEDTA (Fe:EDTA ratio 1:1111; Fe' ~80 pM). While uptake rate of Fe' (in the presence of EDTA) is only about twice as fast as for FeDFB, the uptake

constants span four orders of magnitude with $k_{up}^{Fe'} = 1.7 \times 10^{-8}$ mol Fe cell⁻¹ day⁻¹ and $k_{up}^{FeDFB} = 1.7 \times 10^{-12}$ mol Fe cell⁻¹ day⁻¹ (**Figure 4**). This marked difference in k_{up} stems from the much lower substrate concentration of Fe' as compared to FeDFB. While



browsing through published uptake data it becomes apparent that k_{up} values for any specific Fe-compound varies among organisms in accordance with their sizes and degree of Fe limitation (Sunda and Huntsman, 1995; Shaked et al., 2005). Seeking a way to present data on many Fe-compounds and many phytoplankton species simultaneously, we chose to establish a relative bioavailability scale which is illustrated in Figure 5. Here, we divide the uptake constants of various Fe complexes by the uptake constant of Fe' where both constants are obtained from a single study. The resulting ratio

represents the availability of model Fe-compounds relative to Fe', shown on a logarithmic scale for a variety of cultured phytoplankton in Figure 5A. A similar calculation was conducted for natural phytoplankton populations as shown in Figure 5B.

The data presented in Figure 5 clearly show that both in the laboratory and natural environment Fe' is highly bioavailable as compared to most organically bound iron forms and colloids, as previously suggested by Morel et al. (2008). A notable exception to this is the recently reported high availability of Fe bound to

saccharides, tested using model mono- and polysaccharides with several cultured species, and natural phytoplankton (Figures 5A,B and references therein). Iron bound to siderophores such as ferriochrome (FC), ferrioxamine B (DFB), aerobactin (AB), and azotochelin (AZ) is accessible to several cultured phytoplankton at a low to intermediate degree as compared to Fe'. However, when grown under Fe-replete conditions, diatoms from the genus *Thalassiosira* (and we estimate that this is probably true for other diatoms) are unable to access siderophore bound Fe (Figure 5A). Similarly, ferrioxamine complexes are more accessible to natural phytoplankton assemblages in low Fe than high Fe waters (e.g., Southern Ocean vs. Loch Scridain or offshore vs. coastal stations in the subarctic Pacific, Figure 5B). While centric *Thalassiosira* spp. diatoms acquire Fe from a wide range of organic substrates, they are unable to internalize stable Fe oxides (Rich and Morel, 1990). On the other hand, mixotrophic dinoflagellates capable of consuming particles (e.g., *Chrysochromulina ericina*) are able to utilize goethite but not FeDFB (Maranger et al., 1998; Nodwell and Price, 2001). It is important to keep in mind that the source of variation between phytoplankton species and Fe-compounds in Figure 5 may also be attributed to experimental conditions. For example, the relative bioavailability FeDFB may change depending on the amount of excess free DFB present in the experimental medium, where a higher excess makes FeDFB seem less bioavailable. Nonetheless, we find this method of comparison illuminating when considering the bioavailability of different Fe-substrates to aquatic phytoplankton. In this respect, the use of EDTA to buffer known Fe' concentrations is indispensable since Fe' is easily calculated despite differences in experimental set-ups.

Gauging the contribution of Fe-substrates to meeting phytoplankton Fe demand in situ

Experimentally obtained uptake rate constants can help examine the availability of various Fe species in natural settings. This is done by multiplying k_{up} by measured or predicted concentrations of a specific Fe-compound. For example, multiplying typical HNLC Fe concentrations of 70 pM chelated Fe (FeL) and 0.07 pM unchelated Fe (Fe', Rue and Bruland, 1995) by representative uptake constants- $k_{up}^{FeL} \sim 2 \times 10^{-9} \text{ L cell}^{-1} \text{ day}^{-1}$ and $k_{up}^{Fe'} \sim 1 \times 10^{-6} \text{ L cell}^{-1} \text{ day}^{-1}$ (Shaked et al., 2005), results in FeL uptake rate of $\sim 1.4 \times 10^{-19} \text{ mol Fe cell}^{-1} \text{ day}^{-1}$ and Fe' uptake rate of $\sim 7 \times 10^{-20} \text{ mol Fe cell}^{-1} \text{ day}^{-1}$. The overall uptake rate of $2 \times 10^{-19} \text{ mol Fe cell}^{-1} \text{ day}^{-1}$, contributed 2/3 by FeL and 1/3 by Fe', is well within the estimated steady state uptake of natural phytoplankton of 2×10^{-20} – $4 \times 10^{-18} \text{ mol Fe cell}^{-1} \text{ day}^{-1}$ (e.g., Strzepek et al., 2005). Since the k_{up} of most FeL complexes is much lower than that of Fe' (Figure 5A), 1 pM Fe' in the ocean can be equated to 100–10000 pM of organically bound Fe, making Fe' a potentially important Fe source, despite its low concentrations. Therefore, Fe' formed by processes such as photoreductive dissolution of Fe oxides (Waite and Morel, 1984; Wells et al., 1991; Barbeau and Moffett, 2000) or the degradation of photolabile Fe complexes (Barbeau et al., 2001; Rijkenberg et al., 2006; Amin et al., 2009; Steigenberger et al., 2010) may contribute to a transient yet significant Fe pool which caters, at least partially, to phytoplankton iron requirements in surface waters (Morel et al., 2008).

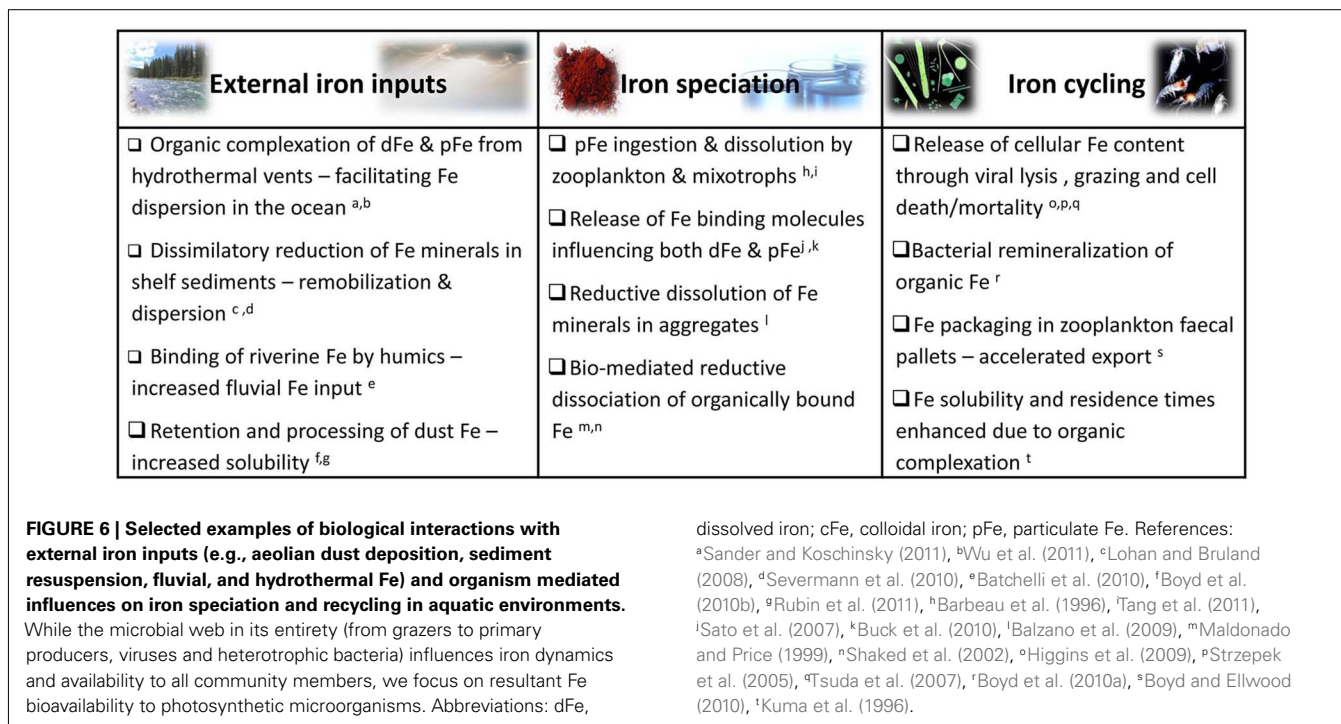
Such calculations can also be used to examine if a specific compound is likely to support *in situ* growth of certain phytoplankton species by comparison to the minimal daily Fe requirements. As an example we examine the ability of FeDFB to support *Synechococcus* spp. growth. Table 1 details the calculation and shows that the minimal theoretical daily Fe requirement of *Synechococcus* is three orders of magnitude greater than its FeDFB uptake capacity, even given an overshoot in the estimated concentration of FeDFB-like Fe complexes in natural waters. Therefore while *Synechococcus* is capable of transporting DFB bound iron (i.e., FeDFB can be termed bioavailable should uptake be the single criterion for bioavailability), FeDFB or similar compounds alone are insufficient in meeting the Fe demands of this organism.

Needless to say, care should be taken in the use of k_{up} as a tool of comparison: factors such as experimental conditions and environmental constraints should be taken into account. When synthesizing laboratory and field data it is important to bear in mind that the degree to which a specific organic Fe complex (FeL) supports phytoplankton growth *in situ* is influenced by factors other than its direct uptake rate. The residence time of FeL in surface water and its tendency to undergo chemical and physical transformations all influence its final availability, an issue we further explore in the next section.

ADDED COMPLEXITY TO BIOAVAILABILITY: BIO-MEDIATED TRANSFORMATIONS OF Fe SPECIATION

Having discussed the fundamentals of bioavailability in the form of uptake pathways and rates – we now turn our attention to the added complexity of Fe–organism interactions (Figure 1). While iron inputs and availability are widely recognized as factors shaping the biology of marine and fresh water environments, biological activities, in turn, exert strong control on iron speciation and cycling in aquatic ecosystems, and may even influence its inputs (Figure 6). Here we explore how Fe–organism interactions can affect iron bioavailability in complex and sometimes unexpected ways due to the many players and intricacies involved in natural systems. We endeavor to address some of these complexities in the present section.

Basic life processes significantly impact the inputs, speciation and fate of iron in aquatic ecosystems (Figure 6). Secretion of exopolymers and siderophores, bacterial Fe-oxide respiration, and food web interactions involving bacteria, phytoplankton, grazers, and viruses may all alter iron chemistry and resulting bioavailability (Figure 6; e.g., Poorvin et al., 2004; Boye et al., 2005; Sarthou et al., 2008; Schlosser and Croot, 2009). Thus, far from being slaves to Fe, microorganisms are able to manipulate iron speciation in their surroundings to some extent. Having said this, it should be noted that very few microbial processes influencing iron chemistry are clearly intended to serve iron acquisition purposes and more importantly, not all biological modifications of iron speciation influence its bioavailability favorably. In this section we classify a number of major pathways by which microbial communities alter iron speciation in aquatic environments. Microorganisms are able to mediate Fe redox transformations in their immediate surroundings while biological production and release of Fe-binding ligands are imperative to keeping iron in solution. No less important is the role microbial food web interactions play in the rapid



recycling of iron in surface waters. Iron regeneration has received some recent attention (Strzepek et al., 2005; Boyd et al., 2010a) and will not be discussed in detail here. Rather, we will focus on the two former processes and their impact on the Fe pool available to phytoplankton.

BIOLOGICAL CONTROL ON Fe REDOX TRANSFORMATIONS

Iron has two oxidation states of importance to its aquatic chemistry – Fe(II) and Fe(III). Processes of Fe oxidation and reduction, known as redox reactions, take place throughout the water column, across chemical gradients, in sediments, and microenvironments. Redox reactions are central in determining the physical and chemical form of iron and its subsequent chemical and biological reactivity (Figure 7; Table 2). While both abiotic and biotic processes regulate iron redox transformations, we focus on the role biology plays in mediating iron redox cycling and the resulting effects on Fe availability to phytoplankton. We also attempt to distinguish between redox reactions of dissolved inorganic, dissolved organic and colloidal/particulate iron, as the governing factors and the ensuing changes in Fe bioavailability are likely to vary between the different iron species (Figure 7; Table 2).

Speciation and reactions

In oxygenated, neutral pH surface waters iron persists predominantly in its oxidized form – Fe(III), as fluxes of the thermodynamically unstable Fe(II) will undergo prompt oxidation by oxygen and hydrogen peroxide (Figure 7; Table 2; Gonzalez-Davila et al., 2005; Santana-Casiano et al., 2005). Fe(II) oxidation rates are not only regulated by the abiotic factors of temperature, pH, and salinity (Santana-Casiano et al., 2006), but also by biology. Bio-generated redox reactive species, oxygen consumption and release via respiration, as well as photosynthesis are able to influence oxidizing agent type and concentration as well as local pH conditions. In environments characterized by high biomass, low turbulence,

poor ventilation, or low alkalinity (e.g., marine aggregates, coastal waters, oxygen minimum zones, and lakes) significant biological modifications of the reactants and/or conditions involved in Fe(II) oxidation have been reported (Emmenegger et al., 2001; Shaked et al., 2002; Moffett et al., 2007; Lohan and Bruland, 2008). In pelagic, low biomass surface ocean waters, biology is thought to exert minor controls on Fe(II) oxidation rates (e.g., Miller et al., 1995; Shaked, 2008). However, this view may require reassessment given the biological production of superoxide and hydrogen peroxide recently observed in several new open ocean studies (Rose et al., 2008; Hansard et al., 2010; Vermilyea et al., 2010).

In order for Fe(II) to persist at measurable concentrations in oxygen rich water, continuous Fe(II) production and/or Fe(II) supply must take place (Figure 7; Table 2). Multiple Fe reduction pathways were studied and suggested to operate at varying degrees in surface waters, including direct photochemical reduction, reduction by superoxide of photochemical or biological origin, thermal reduction, reduction by phytoplankton cell surface enzymes, and microbial reduction in isolated suboxic and anoxic microenvironments such as settling fecal pellets and aggregates (Table 2; Alldredge and Cohen, 1987; Kuma et al., 1992; Johnson et al., 1994; Voelker and Sedlak, 1995; Maldonado and Price, 2001; Shaked et al., 2002; Kustka et al., 2005; Rose et al., 2005; Barbeau, 2006; Rijkenberg et al., 2006; Balzano et al., 2009; Roy and Wells, 2011). Fe(II) may be supplied from external sources such as sediments, anoxic or suboxic water, hydrothermal vents, rain and aerosols, or originate from the *in situ* recycling of cellular iron through grazing and viral lysis (Kieber et al., 2001; Croot et al., 2005; Statham et al., 2005; Buck et al., 2006; Breitbarth et al., 2009). In addition, retardation of Fe(II) oxidation by low temperature, low pH, or complexation by Fe(II) stabilizing organic ligands are thought to contribute to the maintenance of measurable Fe(II) concentrations (Croot et al., 2001, 2008; Roy et al., 2008).

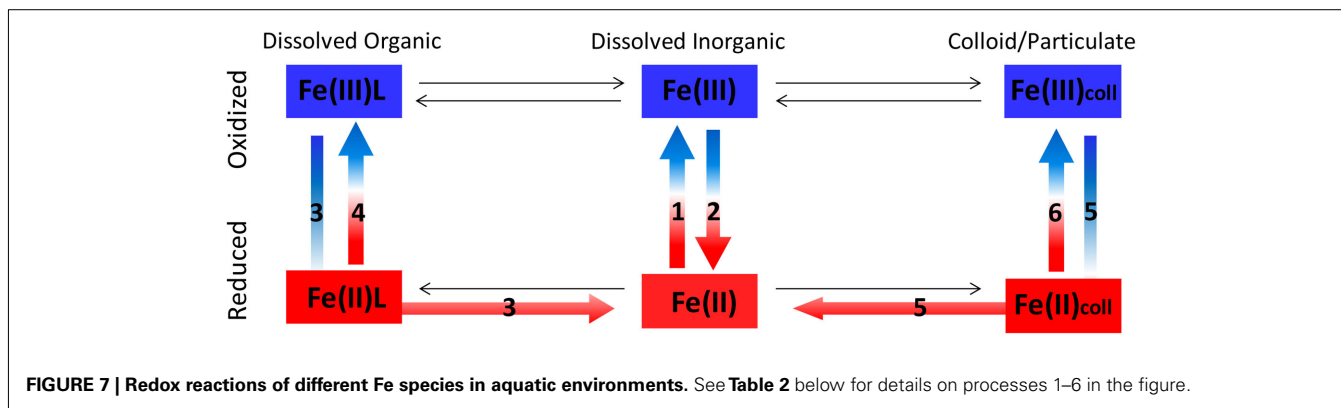


Table 2 | Redox reactions of different Fe species in aquatic environments, their governing factors, and potential influence on Fe speciation and bioavailability (this table accompanies Figure 7).

Mediators and governing factors	Effects on speciation and bioavailability	References
1. Fe(III)' OXIDATION		
<ul style="list-style-type: none"> • O_2, O_2^-, H_2O_2 • Regulated by pH, temp, salinity 	<ul style="list-style-type: none"> • Fe(III) formation and subsequent hydrolysis and precipitation, decreases bioavailability 	a–c
2. Fe(III)' REDUCTION		
<ul style="list-style-type: none"> • Enzymatic– phytoplankton (assimilatory) & bacteria (dissimilatory – low oxygen) • Direct photolysis or reduction by photo-produced reactive transients • O_2^- (photochemical/biological origin) • Humic and fulvic acids 	<ul style="list-style-type: none"> • Elevated [Fe(II)s.s] and maintenance of active redox cycle, both of which slow down Fe(III) hydrolysis and precipitation • Availability is enhanced when [Fe'] increases (as Fe(III) and Fe(II) are equally bioavailable) 	d–g
3. Fe(III)L REDUCTION AND DISSOCIATION		
<ul style="list-style-type: none"> • Enzymatic (as above) • Photochemical (as above) • Superoxide (as above) 	<ul style="list-style-type: none"> • Weaker ligand binding to Fe(II) (compared to Fe(III)L) may favor dissociation of free Fe(II) and increase in [Fe'] and bioavailability • If Fe(II) remains complexed – its oxidation may be stalled/accelerated compared to Fe(II). Bioavailability of Fe(II)L – unknown 	h–i
4. Fe(II)L OXIDATION		
<ul style="list-style-type: none"> • O_2, O_2^-, H_2O_2 • Ligands (L)– some stabilize the complex as Fe(II)L, some accelerate its oxidation 	<ul style="list-style-type: none"> • Rates are slower/faster than Fe(II) oxidation resulting in elevated/lowered [Fe(II)Ls.s] • Bioavailability of Fe(II)L – unknown 	j–k
5. REDUCTIVE DISSOLUTION OF SOLID PHASE IRON		
<ul style="list-style-type: none"> • Bacterial dissimilatory reduction in aggregates • Ingested colloids (zooplankton) • Direct photolysis and light enhanced siderophore mediated dissolution 	<ul style="list-style-type: none"> • Elevated [Fe'] due to increased Fe(II) flux • Elevated FeL (when ligands are at play) • Dissolution enhances bioavailability as solid phase iron is largely inaccessible 	l–m
6. OXIDATION OF SURFACE ADSORBED Fe(II)		
<ul style="list-style-type: none"> • O_2 and mineral surfaces (O_2 oxidizes surface bound Fe(II) much faster than dissolved Fe(II)) 	<ul style="list-style-type: none"> • If oxidation precedes detachment of Fe(II): decreased flux of Fe(II) • Following oxidation, the newly precipitated Fe is more reactive than the original mineral 	n

[Fe(II)s.s] , steady state Fe(II) concentrations.

References: a. Santana-Casiano et al. (2005), b. Gonzalez-Davila et al. (2005), c. King et al. (1995), d. Shaked et al. (2002), e. Kustka et al. (2005), f. Voelker and Sedlak (1995), g. Miller et al. (1995), h. Barbeau et al. (2001), i. Rijkenberg et al. (2006), j. Rose and Waite (2002), k. Rose and Waite (2003), l. Balzano et al. (2009), m. Barbeau et al. (1996), n. Stumm and Sulzberger (1992).

Thanks to the development of rapid and sensitive flow injection based chemiluminescence techniques (FI-CL), Fe(II) datasets in the oxygenated upper waters of oceans and lakes have recently begun to accumulate (Emmenegger et al., 2001; Shaked et al., 2002; Ussher et al., 2007; Shaked, 2008; Hansard et al., 2009; Sarthou et al., 2011). Some of these measurements point to a strong biological control on Fe(II) formation as attested to by the co-variance of chlorophyll and Fe(II) concentrations as well as measurable nighttime Fe(II) levels. Commonly, the presence of non-photochemical Fe(II) in the photic zone is suggested to reflect Fe reduction by assimilatory cell surface enzymes of phytoplankton or by biologically produced superoxide (e.g., Sarthou et al., 2011). Assimilatory Fe reduction by cell surface enzymes is not likely to generate high fluxes of Fe(II), as the Fe(II) is probably generated within an enclosed protein complex and subsequently internalized by an adjacent transport protein (Kustka et al., 2005; Shaked et al., 2005). Rather, superoxide, a diffusible reducing agent which has recently been shown to be generated non-photochemically throughout the photic zone (Rose et al., 2008, 2010; Hansard et al., 2010), may be a central player in Fe(II) formation. Similarly, experimental observations of phytoplankton mediated Fe reduction in flow through systems [where Fe(II) is detected downstream of membrane-mounted cells], may be caused by superoxide released from the cells (Milne et al., 2009; Saragosti et al., 2010) in addition to or rather than cell surface enzymes. Despite emerging evidence for non-photochemical Fe(II) formation, photochemistry should not be underestimated as a strong mediator of Fe redox reactions. Currently, it is analytically challenging to detect photochemically produced Fe(II) as it oxidizes completely by the time the water is retrieved and analyzed. Minimizing collection time with high throughput pumps, Shaked (2008) observed a highly active photo-induced redox cycle in the surface waters of the Gulf of Aqaba.

Bioavailability

As Fe(II) is far more soluble than Fe(III), reductive processes generating Fe(II) and/or the occurrence of the thermodynamically unstable Fe(II) in surface waters are commonly linked to enhanced iron bioavailability (e.g., Sunda, 2001). This often justified notion merits careful consideration as some Fe(II) species may not be available and since not all reductive processes increase Fe availability. Aided with **Table 2** and **Figure 7**, we outline the effects of several reductive processes on Fe bioavailability below.

While Fe(II) is potentially the more bioavailable redox state of Fe, several factors will determine whether iron availability is indeed increased by reductive processes. These include the characteristics of the Fe species undergoing reduction, the reductive pathway, the presence of other ligands and oxidants in the immediate surroundings, and ultimately the uptake machinery of the phytoplankton utilizing this iron. As a rule of thumb, redox processes that increase the concentrations of dissolved inorganic Fe (be it Fe(II)' or Fe(III)'), will generally tend to boost the bioavailability of iron. With this in mind, we discuss the potential for Fe' release and/or changes to Fe lability occurring in different reductive processes.

Reductive dissolution of solid phase Fe. As solid phase Fe is considered unavailable to phytoplankton (Rich and Morel, 1990; Wells et al., 1991), reductive dissolution of mineral Fe or

surface adsorbed Fe generate Fe', and thus enhance Fe availability (**Figure 7**; **Table 2**). Even when the Fe' formed through reductive dissolution exceeds its solubility limit, it will hydrolyze and form fresh hydroxides which serve as a better iron source than their parent minerals (Stumm and Sulzberger, 1992; Yoshida et al., 2006). When reductive dissolution of mineral Fe is mediated, assisted, or occurs in the presence of organic ligands, it results in FeL (organically bound Fe) rather than Fe' (Kraemer, 2004; Borer et al., 2005). As stated previously, Fe' is acquired at faster rates than most studied dissolved organic Fe-complexes (see section "Phytoplankton Fe Acquisition Rates", **Figure 5**), and hence the presence of organics may slow uptake down. On the other hand, organic complexation may sustain dissolved Fe in surface waters for longer and at concentrations exceeding its solubility limit (see next section "Biological Production and Release of Iron Binding Ligands"), subsequently providing phytoplankton with Fe over a longer duration.

Reduction of organically bound Fe(III). As reasoned above, reductive release of Fe' from organic complexes is likely to enhance uptake, at least over short time scales (**Figure 7**; **Table 2**). When the ligand (L) undergoes photo-destruction, free, unchelated Fe(II) is liberated and Fe' increases, as has been extensively demonstrated for Fe(III)EDTA (Hudson and Morel, 1993; Sunda and Huntsman, 1997). In other cases, Fe(II) may remain complexed as Fe(II)L, undergo reoxidation to Fe(III)L or dissociate as unchelated Fe(II) due to reduced ligand affinity for Fe(II) (Harrington and Crumbliss, 2009). Hence, some of these redox transformations will ultimately not alter Fe speciation, but others may temporarily shift Fe(III) from strong complexes into weak complexes, possibly enhancing Fe bioavailability.

Reduction of Fe(III)'. Unlike the former reductive processes which enhance Fe' concentrations, the total unchelated iron pool remains unchanged when ferric Fe' transforms into ferrous Fe(II)' (**Figure 7**; **Table 2**). Nonetheless, this reaction mediated by enzymes on the cell surface, is central to the acquisition of Fe' through the reductive uptake pathway described in Section "Uptake Pathways of Aquatic Phytoplankton," at least for eukaryotes (Shaked et al., 2005). Fe(III)' may alternatively undergo reduction by superoxide in the bulk solution (Voelker and Sedlak, 1995), but the effect of this process on Fe availability is disputable. Kustka et al. (2005) found that Fe' uptake by diatoms remained unaffected by superoxide mediated reduction of Fe(III)' in the experimental medium, while Rose et al. (2005) reported that superoxide enhanced Fe' uptake by cyanobacteria. More work is required to evaluate the importance of bulk solution Fe' reduction in increasing Fe availability.

Thus far we examined the effect of reductive processes on Fe availability and now we turn to the chemical nature and bioavailability of Fe(II), where increasing reports suggest that some of the measured Fe(II) in the ocean is organically bound (Croot et al., 2008; Roy et al., 2008). The existence of Fe(II) binding ligands is deduced from deviations in Fe(II) oxidation kinetics in some natural samples as compared to seawater free of organic matter (Roy et al., 2008). Currently, neither the identity nor the bioavailability of these complexes is known and hence elevated Fe(II) levels are not necessarily indicative of enhanced bioavailability. Indeed,

phytoplankton iron stress was not alleviated in Fe fertilized areas where Fe(II) accounted for a significant fraction of the dissolved iron pool (Croot et al., 2008; Roy et al., 2008). Moreover, as the iron in Fe(II)L complexes is already reduced, phytoplankton cannot employ reductive pathways to liberate it from the complex prior to transport as they do for Fe(III)L (see Uptake Pathways of Aquatic Phytoplankton). Thus, if these Fe(II)L are slow to oxidize, we are faced with yet another challenge in determining the transport pathways of Fe(II)L.

BIOLOGICAL PRODUCTION AND RELEASE OF IRON BINDING LIGANDS

Over the course of their short life span, aquatic microbes release copious amounts of biomolecules into their immediate environments. These diverse secretions and exudates include waste products, secondary metabolites, sugars, and proteins as well as cellular contents released via grazing, viral attack or programmed cell death (Bidle and Falkowski, 2004; Poorvin et al., 2004; Strzepak et al., 2005; Dalbec and Twining, 2009; Boyd et al., 2010a). While the minority of microbial secretions are synthesized and released with the explicit purpose of binding iron, many do possess an Fe-binding capacity of some kind (Rijkenberg et al., 2008; Hassler et al., 2011a; Levy et al., 2011). It is now well established that the overwhelming majority of dissolved (and in some cases colloidal) iron in most aquatic environments is found as organic complexes (Hunter and Boyd, 2007; Vraspir and Butler, 2009). Research efforts to elucidate the nature and origin of these Fe-binding organic ligands have yielded ambiguous results, reflecting the fact that (a) organic ligands may be introduced into aquatic systems via multiple pathways within and outside the water column and (b) organically bound iron (FeL) may be subjected to further biological and chemical transformations (Figure 7; Table 3). Not all organic Fe complexes are available to all phytoplankton, and even those FeL which are utilized are most likely less available than dissolved inorganic iron (Fe'; Figure 5; Morel et al., 2008). Nonetheless, iron binding ligands are vital for the maintenance of dissolved iron concentrations well above the Fe solubility limit and for retarding Fe aggregation and loss from surface waters (Kuma et al., 1996). Here we provide an abridged overview of some of the organic Fe complexes thought to exist in seawater, highlighting the ways in which organic complexation affects Fe speciation and bioavailability.

Speciation

Organic ligands capable of binding iron (and other metals), were detected in pelagic and coastal ocean waters, estuaries, lakes, and rivers, often in excess of dissolved Fe concentrations (Boye et al., 2001; Nagai et al., 2007; Duckworth et al., 2009; Hassler et al., 2009; Laglera and van den Berg, 2009; Buck et al., 2010). Using competitive ligand exchange (CLE) techniques, researchers have classified two major organic Fe ligand groups based on their stability constants with regards to Fe³⁺ – the strong L1 and weaker L2 class (e.g., Gledhill and van den Berg, 1994; Ibisani et al., 2011). A common view is that the strong L1 ligand class consists of siderophore-like molecules, while the less strongly complexing L2 ligands consist of cellular degradation products. However, the picture is probably more complex and there are additional weaker natural Fe ligands, such as saccharides, overlooked by CLE

Table 3 | The influence of siderophore and saccharide release on iron chemistry and availability in aquatic environments.

Siderophores	Saccharides (EPS, TEP)
RESULTING IRON SPECIES	
Strongly bound organic dFe & cFe Fe(III)L	Organically bound/adsorbed dFe, cFe, & pFe
IRON SPECIES ACTED UPON	
dFe, cFe, & pFe	dFe & cFe
IMPACT	
<ul style="list-style-type: none"> • Maintain Fe in solution • Fe proximity to cell (amphiphilic) • Particulate Fe dissolution 	<ul style="list-style-type: none"> • Maintain Fe in solution or promote its aggregation and export from photic zone • Fe proximity to cell
SUBSEQUENT TRANSFORMATIONS	
Photochemical reduction	<ul style="list-style-type: none"> • Photochemical reduction • Ligand exchange • Adsorption/desorption of dFe
ORGANISMS AT PLAY	
Heterotrophic bacteria, fresh water phytoplankton	Phytoplankton, Bacteria, Viruses, Zooplankton
BIOAVAILABILITY	
Varies between complexes and organisms but probably lower compared to Fe'	As high as Fe' for all studied organisms
REFERENCE	
a–f	g–m

dFe, dissolved iron; cFe, colloidal iron; pFe, particulate Fe.

References: a. Sandy and Butler (2009), b. Vraspir and Butler (2009), c. Holmén and Casey (1996), d. Martinez et al. (2000), e. Kraemer et al. (2005), f. Barbeau et al. (2003), g. Hassler et al. (2011a), h. Hassler and Schoemann (2009), i. Steigenberger et al. (2010), j. Passow (2002), k. Strmecki et al. (2010), l. Berman-Frank et al. (2007), m. Decho (1990).

methods (Town and Filella, 2000; Hunter and Boyd, 2007; Boyd and Ellwood, 2010).

The most studied microbial Fe-binding exudates are siderophores, compounds which have been isolated from both freshwater and seawater (Table 3; Macrellis et al., 2001; Mawji et al., 2008; Velasquez et al., 2011). Typified by an exceptionally high Fe-binding capacity and low molecular weight, siderophores are produced under iron limitation by marine heterotrophic bacteria and some fresh water cyanobacteria (e.g., Haygood et al., 1993; Vraspir and Butler, 2009), while production by marine cyanobacteria remains controversial (Hopkinson and Morel, 2009). Less studied, but widely spread microbial exudates capable of binding iron are exopolymer substances (EPS) and their transparent exopolymer particles (TEP) derivatives (Table 3). These high molecular weight saccharide-rich exopolymers are secreted by most microorganisms, including phytoplankton, bacteria, and zooplankton (Decho, 1990; Passow, 2002; Wotton, 2004; Croot et al., 2007). EPS are thought to weakly bind iron compared to siderophores based on recent data from several model saccharides (Hassler et al., 2011a). TEP, on the other hand, have been shown to have a high affinity for Fe (Quigley et al., 2002). Many other compounds secreted or released due to grazing and lysis contribute to

the “Fe ligand soup” in aquatic environments (Boye et al., 2005; Tsuda et al., 2007; Strmecki et al., 2010; Poorvin et al., 2011). The release of iron binding ligands in cultures supplemented with Fe was reported for the marine haptophyte *E. huxleyi* and two diatom species, qualifying these as non-siderophore Fe-binding ligands (Boye and van den Berg, 2000; Rijkenberg et al., 2008). Some toxins, capable of binding Fe (but not as strongly as siderophores) are known to be synthesized by harmful bloom forming phytoplankton upon Fe limitation (Rue and Bruland, 2001). Secretion of both the neurotoxin domoic acid (a water soluble amino acid produced by *Pseudo-nitzschia* spp.) and the hepatotoxin microcystin (a peptide produced by *Microcystis aeruginosa*) were shown to improve growth under low Fe conditions (Maldonado et al., 2002; Wells et al., 2005; Alexova et al., 2011). Interestingly, these toxins do not directly provide the cell with Fe, but rather increase copper supply for *Pseudo-nitzschia* spp. enabling the synthesis of copper containing high affinity iron uptake proteins (Maldonado et al., 2002; Wells et al., 2005), and probably aid *M. aeruginosa* in oxidative damage protection (Alexova et al., 2011). In addition, terrestrial humics have recently been suggested to be important iron binding ligands in open ocean waters (van den Berg, 1995; Laglera and van den Berg, 2009; Laglera et al., 2011).

Bioavailability

At the cellular level, acquisition of a specific compound depends to a large degree on the uptake machinery possessed by an organism (see Phytoplankton Fe Acquisition Systems). In the natural environment, additional parameters such as the compound residence time in surface waters and its tendency to undergo biological and chemical transformation influence its ability to support growth (Figure 1). In this section we briefly examine the effect of environmental and chemical factors on phytoplankton Fe acquisition from EPS and siderophore bound iron.

At the organism level, recent studies have found that iron bound to model saccharides is highly available to diatoms and natural phytoplankton assemblages (Figure 5; Table 3; Hassler and Schoemann, 2009; Hassler et al., 2011a,b). This was accounted for by the ability of saccharides to stabilize iron in the dissolved and colloidal form and increase the labile Fe pool (Hassler et al., 2011b). In the natural environment, the effect of saccharides on Fe solubility and residence time in the surface water is less clear. EPS and TEP were shown to promote aggregate formation and particle export (Decho, 1990; Passow, 2002), thus possibly shortening the residence time of saccharide bound Fe in surface waters (Berman-Frank et al., 2007). Additionally, iron bound to saccharides may be subjected to chemical and photochemical transformations, further altering its fate and accessibility to phytoplankton. Iron is weakly bound to saccharides and may be exchanged with stronger ligands such as siderophores (Hunter and Boyd, 2007). Photochemical Fe(II) production from saccharide bound iron was recently reported (Steigenberger et al., 2010), but its environmental repercussions are unexplored as yet. Lastly, EPS often create protective microenvironments around microbial consortia, single cells, and cell aggregates (Decho, 1990; Passow, 2002). The formation of such microhabitats impacts all concentration dependent processes and, as such, EPS surrounded cells may be diffusion limited when it comes to acquiring dissolved Fe from the surrounding

waters. However, EPS confers two significant advantages on cells: firstly, EPS are highly adsorbent allowing for the storage and easier processing of iron and secondly the confined microenvironment provides protection from diffusive losses of Fe associated with the EPS (Sunda, 2001; Hassler et al., 2011a).

The role of siderophores in supporting phytoplankton growth *in situ* has received more attention than any other Fe chelator, but in turn raised many new questions (Table 3; Maldonado et al., 2005; Pickell et al., 2009). As the acquisition of siderophores was detailed in Section “Phytoplankton Fe Acquisition Systems,” we briefly discuss two chemical features of siderophores with potential repercussions for iron fate and bioavailability. Firstly, many of the Fe-siderophore complexes isolated from marine bacteria are photolabile (Barbeau et al., 2001, 2003; Vraspir and Butler, 2009). However, due to the fast oxidation of Fe(II) within the complex and since the photoproduct still binds Fe with high affinity, this photo-reactivity does not seem to confer any clear biological advantage (Vraspir and Butler, 2009). This is not always the case however: recently, photo-reduction of iron bound to a newly identified siderophore, vibrioferrin, and the subsequent release of Fe⁺ was reported to enhance iron uptake by dinoflagellates (Amin et al., 2009). Vibrioferrin undergoes photo-degradation and does not retain significant Fe-binding capacity, thus releasing Fe⁺. Secondly, many marine siderophore isolates also tend to be amphiphilic and closely associate with bacterial membranes, possibly preventing their loss by diffusion in the marine environment (Vraspir and Butler, 2009). Although the study of amphiphilic siderophore partitioning to membranes is at a very early stage, it may bear interesting implications for the function of siderophores in iron acquisition in the ocean.

SUMMATION

The topic of iron bioavailability has garnered wide spread interest from the scientific community, yet due to its intrinsic complexity a well-rounded understanding of this concept is lacking. In this contribution we have attempted to disassemble bioavailability and address some of its more biologically orientated facets. Of the many topics covered in this synthesis, we conclude with a summary of the principal arguments and perhaps unorthodox perspectives which we hope lend some insights into Fe bioavailability in the aquatic environment.

- Iron acquisition by means of reduction is a widespread Fe uptake strategy in the ocean, common to both eukaryotic and prokaryotic phytoplankton. Both experimental and genomic data challenge the prevalent paradigm of siderophore-based Fe uptake as an exclusive iron acquisition pathway amongst marine cyanobacteria. We propose that the occurrence of siderophore vs. reductive iron uptake can be put down to environmental rather than taxonomic considerations. Organisms residing in densely populated, low turbulence environments (e.g., fecal pellets, marine snow, or colony-consortiums) will favor siderophore-based Fe acquisition, while pelagic phytoplankton will favor the non-specific reductive strategy enabling them to access the dilute, heterogeneous Fe pool.
- The availability of various Fe-substrates to phytoplankton can be viewed as a spectrum rather than an absolute “all or nothing.”

A bioavailability scale can be established by comparing substrate normalized uptake rates (by means of the uptake rate constant – k_{up}). We suggest that a further normalization of FeL uptake constants relative to the Fe' uptake constant is highly useful in the comparison of different studies, organisms and environments. Hence, in order to establish a baseline for the determination of relative bioavailability, we urge for the use of EDTA in order to better regulate Fe' instead of or in addition to ligand-free FeCl₃. Looking beyond short term uptake, k_{up} can be used to gage if a specific compound is likely to be a significant contributor to the Fe requirements of phytoplankton in the environment. This same approach can be extended to natural systems where k_{up} values of natural ligands by known organisms may be compared to model ligands while the k_{up} values of known Fe-substrates by natural communities may be contrasted with those of model organisms.

- For a fuller understanding of iron bioavailability we look beyond phytoplankton iron uptake pathways and rates. Microorganisms are not only influenced by their environment but are themselves agents of change when it comes to Fe bioavailability. Bio-mediated redox transformations, microbial exudates and secretions as well as food web interactions impact iron speciation, residence time in surface waters and ultimate accessibility to phytoplankton be it in a positive or negative manner.

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APPENDIX

DATA IN SUPPORT OF THE PREVALENCE OF REDUCTIVE IRON UPTAKE AMONGST PHYTOPLANKTON (FIGURE 3)

The laboratory cultures (Table A1) and field populations (Table A2) included in Figure 3 were taken from various studies which demonstrate the presence of a reductive mechanism by one or more of the following techniques:

- Colorimetric detection of cell-mediated Fe reduction – measured as accumulation of Fe(II) over time in the presence of cells and a ferrous trapping ligand that binds Fe(II) prior to its oxidation by oxygen or transport. Commonly, cell-mediated Fe reduction is measured via colorimetric methods in the presence of Ferrozine (Fz) or Bathophenanthroline-disulfonic acid (BPDS). This method requires relatively high micromolar iron concentrations due to its low sensitivity. Shaked et al. (2004) developed a sensitive assay for cell-mediated Fe' reduction using radiolabeled iron. This reduction assay is conducted simultaneously with Fe' uptake at physiologically relevant nanomolar iron concentrations, and it was applied for many of the data presented here.
- Genetic studies which find components of the reductive uptake system.
- EPR – Electron paramagnetic resonance showing Fe reduction.
- Inhibition of uptake by Fz/BPDS – Comparing uptake rates in the presence and absence of the membrane impermeable Fe(II) ligands, Ferrozine or BPDS. Here, Fe(II) formed by cell-mediated reduction is trapped and made unavailable for uptake by the cell, hence inhibiting iron uptake by cells employing this strategy. An explanatory illustration of this procedure is found in Figure A1 while Figure A2 provides an example of experimental data using this method.

DATA SUPPORTING THE RELATIVE BIOAVAILABILITY SCALE (FIGURE 5)

Tables A3 and A4, outline the data used for constructing Figure 5.

Uptake rates obtained from different sources were first normalized to Fe-substrate concentration (either Fe' for FeEDTA or to FeL for iron bound to ligand L, providing ligand L complexed iron strongly and/or was present in sufficient excess to rule out Fe precipitation) in order to calculate k_{up} according to Eq. 1:

$$k_{up} = \rho/[Fe]. \quad (A1)$$

where ρ is Fe uptake rate [$\text{mol Fe cell}^{-1} \text{h}^{-1}$], [Fe] is Fe-substrate concentration [mol L^{-1}]. The calculation applies only to *sub-saturating* uptake rates. The k_{up} obtained for the different Fe complexes – k_{up}^{FeL} were further normalized by dividing it by $k_{up}^{\text{Fe}'}$.

Note on Fe-substrates

Goe – goethite is an Fe-oxide mineral (FeOOH); DFB – desferrioxamine B is a trihydroxamate siderophore; AB – Aerobactin is hydroxamate photolabile siderophore; FC – ferrichrome is a trihydroxamate siderophore; PYN – Porphyrin; MS – Monosaccharides; PS – Polysaccharides; AZ – Azotochelin is a *bis*-catechol siderophore produced by nitrogen-fixing soil bacterium *Azotobacter vinelandii*; DPS – DNA binding protein from starved cells (iron storage proteins); CAT – Galocatechin is a polyhydroxybenzene catechol derivative found in green tea; DFE-desferrioxamine E is a trihydroxamate siderophore.

Note on the saccharides uptake data

In the saccharides uptake experiments no Fe' uptake data was reported. Since we view these as potentially important experiments we chose to normalize the saccharide uptake constant to that of FeCl_3 added at concentration of 1 nM. Given a solubility limit of 0.2–0.5 nM (Liu and Millero, 2002), it is possible that some of the Fe precipitated in the experiments. This was demonstrated in control measurements – roughly 30% of the FeCl_3 remained in the dissolved fraction, about 12% was in colloidal form and the remainder in the particulate fraction. To calculate $k_{up}^{\text{Fe}'}$ we related to the dissolved fraction as the Fe-substrate concentration.

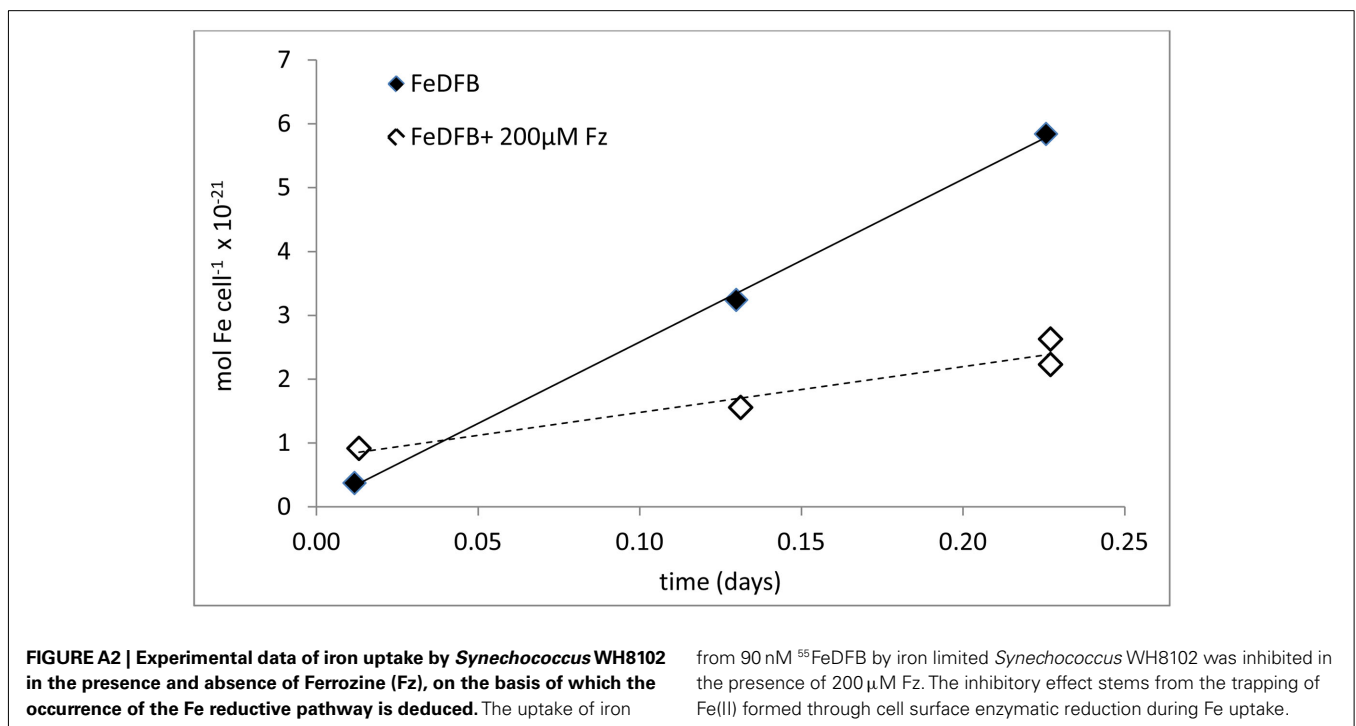
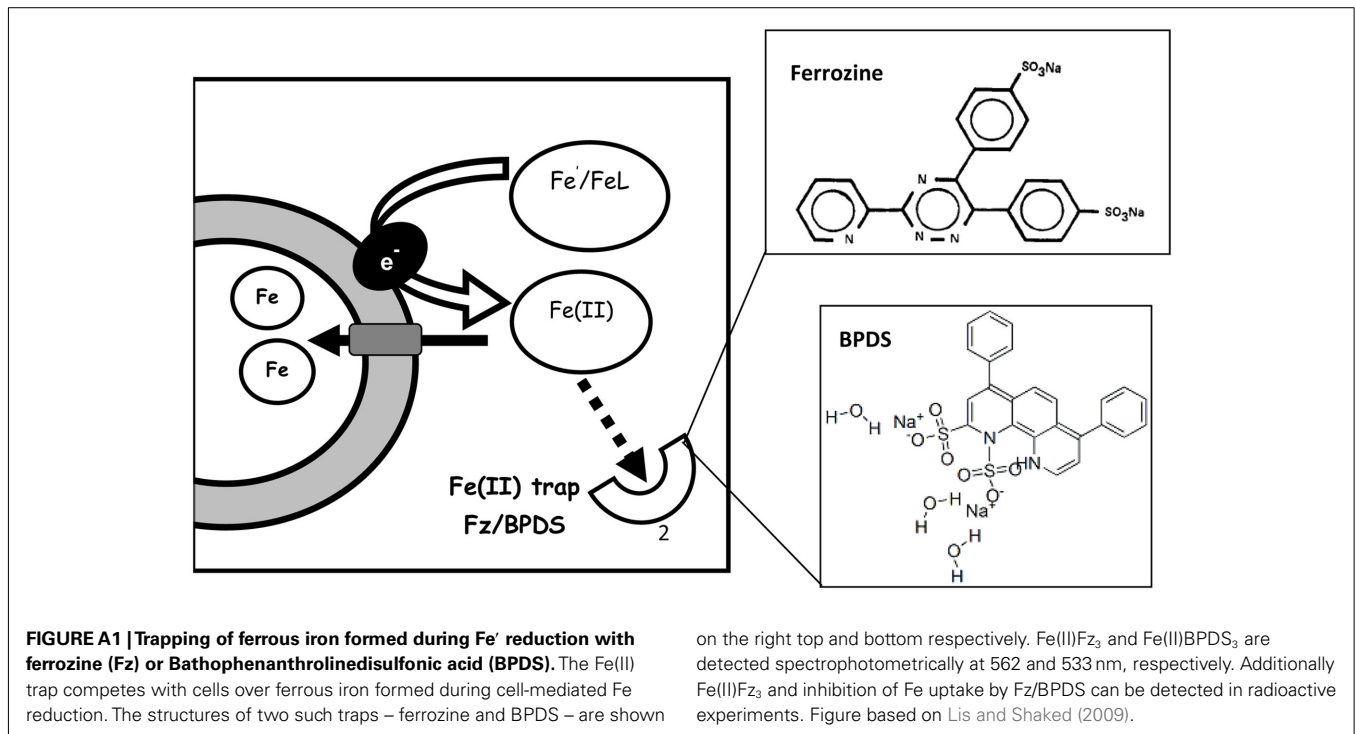


Table A1 | Experimental evidence showing the existence of an iron reductive uptake pathway in laboratory cultures.

Organism	Type of evidence	Reference
<i>Thalassiosira pseudonana</i>	FZ/BPDS inhibits uptake, Fe reduction Genetic – Fe reductase, multi-Cu oxidase	Kustka et al. (2005), Shaked et al. (2005), Maldonado et al. (2006)
<i>Thalassiosira weissflogii</i>	FZ/BPDS inhibits uptake; Fe reduction	Jones et al. (1987), Shaked et al. (2005)
<i>Thalassiosira oceanica</i>	FZ/BPDS inhibits uptake; Fe reduction Cu induced Fe uptake inhibition multi-Cu oxidase	Maldonado and Price (2001), Maldonado et al. (2006)
<i>Cylindrotheca fusiformis</i>	FZ/BPDS inhibits uptake; Fe reduction	Lis and Shaked (in preparation)
<i>Phaeodactylum tricornutum</i>	FZ/BPDS inhibits uptake; Fe reduction	Soria-Dengg and Horstmann (1995)
<i>Peridinium gatunense</i>	FZ/BPDS inhibits uptake; Fe reduction	Shaked et al. (2002)
<i>Tetraselmis suecica</i>	FZ/BPDS inhibits uptake	Lis and Shaked (in preparation)
<i>Chlamydomonas reinhardtii</i>	Fe reduction; Genetic – Fe reductase, multi-Cu oxidase	Eckhardt and Buckhout (1998), Weger (1999), Allen et al. (2007), Chen et al. (2008)
<i>Dunaliella bardawil</i>	Fe reduction; Genetic – Fe reductase	Keshtacher et al. (1999), Paz et al. (2007)
<i>Chlorella vulgaris</i>	FZ/BPDS inhibits uptake; Fe reduction EPR	Allnut and Bonner (1987)
<i>Chlorella kessleri</i>	Fe reduction	Middlemiss et al. (2001)
<i>Chlorococcum littorale</i>	Fe reduction	Sasaki et al. (1998)
<i>Emiliana huxleyi</i>	FZ/BPDS inhibits uptake; Fe reduction	Lis and Shaked (in preparation)
<i>Synechococcus</i> WH7803	FZ/BPDS inhibits uptake; Fe reduction	Lis and Shaked (in preparation)
<i>Synechococcus</i> WH8102	FZ/BPDS inhibits uptake; Fe reduction	Lis and Shaked (in preparation)
<i>Synechocystis</i> PCC6803	FZ/BPDS inhibits uptake; Fe reduction	Kranzler et al. (2011)
<i>Microcystis aeruginosa</i>	FZ/BPDS inhibits uptake; Fe reduction	Fujii et al. (2010)
<i>Lyngbya majuscula</i>	FZ/BPDS inhibits uptake; Fe reduction	Rose et al. (2005)
<i>Trichodesmium erythraeum</i>	FZ/BPDS inhibits uptake; Fe reduction	Shaked (unpublished)

Table A2 | Experimental evidence showing the existence of the reductive iron uptake pathway in field populations.

Field location	Type of evidence	Reference
Southern ocean	Calculation	Maldonado et al. (2005)
Subarctic Pacific (>3 μm)	Uptake	Maldonado and Price (1999)
Bering Sea (>5 μm)	Uptake	Shaked et al. (2004)
Gulf of Aqaba (>0.2 μm)	Uptake	Lis and Shaked (2009)
Loch Scridain (>20 μm)	Uptake	Lis and Shaked (in preparation)
Lake Kinneret	Uptake	Shaked et al. (2002)

Table A3 | Data used in the calculation of relative bioavailability of laboratory cultures (Figure 5A).

Organism	Fe Ligand	Avg k_{up}	Units	Rel. bioavail	Reference	Notes
THALASSIOSIRA PSEUDONANA						
Fe-limited	Fe'	4.6E-09	L ⁻¹ cell ⁻¹ h ⁻¹		Shaked et al. (2005)	Fe(diss) estimated at 0.1 nM
	Fe'	2.9E-07	L ⁻¹ μm ⁻² h ⁻¹		Chen and Wang (2008)	Based on growth rates
	Fe'	3.7E+07	L ⁻¹ mol ⁻¹ day ⁻¹		Nodwell and Price (2001)	
	DFB	6.0E-13	L ⁻¹ cell ⁻¹ h ⁻¹	1.3E-04	Shaked et al. (2005)	Fe:DFB (1:5)
	DFB	7.2E-10	L ⁻¹ μm ⁻² h ⁻¹	2.5E-03	Chen and Wang (2008)	Fe:DFB (1:1.1)
	Ferrichrome	1.2E-10	L ⁻¹ μm ⁻² h ⁻¹	4.1E-04	Chen and Wang (2008)	Fe:FC (1:200)
Fe-replete	Goethite	0		0	Nodwell and Price (2001)	Based on growth rates
	DFB	0		0	Chen and Wang (2008)	Fe:DFB (1:5)
	Ferrichrome	0		0	Chen and Wang (2008)	Fe:FC (1:200)
SYNECHOCYSTIS PCC6803						
	Fe'	12.9E-10	L ⁻¹ cell ⁻¹ h ⁻¹		Kranzler et al. (2011)	
	DFB	6.6E-14	L ⁻¹ cell ⁻¹ h ⁻¹	3.4E-04	Kranzler et al. (2011)	Fe:DFB (1:1.3)
	Aerobactin	2.3E-13	L ⁻¹ cell ⁻¹ h ⁻¹	1.2E-03	Kranzler et al. (2011)	Fe:AB (1:2.3)
PHAEOCYSTIS spp						
	Fe'	4.1E-09	L ⁻¹ cell ⁻¹ h ⁻¹		Hassler and Schoemann (2009)	~30% of FeCl ₃ in dissolved phase
	DFB	6.10E-11	L ⁻¹ cell ⁻¹ h ⁻¹	1.5E-02	Hassler and Schoemann (2009)	Fe:DFB (1:15)
	Monosaccharides	4.8E-09	L ⁻¹ cell ⁻¹ h ⁻¹	1.2	Hassler and Schoemann (2009)	Fe:MS (1:15)
	Polysaccharides	6.2E-09	L ⁻¹ cell ⁻¹ h ⁻¹	1.53	Hassler and Schoemann (2009)	Fe:PS (1:15)
SYNECHOCOCCUS spp						
	Fe'	4.2E-08	L ⁻¹ μm ⁻² h ⁻¹		Chen and Wang (2008)	Fe(diss) estimated at 0.1 nM
	Fe'	2.2E-11	L ⁻¹ cell ⁻¹ h ⁻¹		Lis and Shaked (in preparation)	
	DFB	2.8E-11	L ⁻¹ μm ⁻² h ⁻¹	6.7E-04	Chen and Wang (2008)	Fe:DFB (1:5)
	DFB	1.2E-14	L ⁻¹ cell ⁻¹ h ⁻¹	5.4E-04	Lis and Shaked (in preparation)	Fe:DFB (1:2)
	Ferrichrome	2.0E-11	L ⁻¹ μm ⁻² h ⁻¹	4.8E-04	Chen and Wang (2008)	Fe:FC (1:200)
THALASSIOSIRA WEISSFLOGII						
	Fe'	6.00E-08	L ⁻¹ cell ⁻¹ h ⁻¹		Shaked et al. (2005)	Fe' from Shaked et al. (2005) used for all T.w ratio calculations
	DFB	2.90E-11	L ⁻¹ cell ⁻¹ h ⁻¹	4.8E-04	Shaked et al. (2005)	Fe:DFB (1:1.1)
	DFB	2.56E-11	L ⁻¹ cell ⁻¹ h ⁻¹	4.3E-04	Shi et al. (2010)	Fe:DFB (1:2)
	Ferrichrome	1.6E-10	L ⁻¹ cell ⁻¹ h ⁻¹	2.7E-03	Shaked et al. (2005)	
	Deuteroporphyrin	1.9E-10	L ⁻¹ cell ⁻¹ h ⁻¹	3.2E-03	Kustka et al. (2005)	
	Grazed Fe	3.4E-11	L ⁻¹ cell ⁻¹ h ⁻¹	5.6E-04	Shi et al. (2010)	
	Fe-Azotochelin	1.6E-10	L ⁻¹ cell ⁻¹ h ⁻¹	2.7E-03	Shi et al. (2010)	
	Fresh ferrihydrite	3.3E-10	L ⁻¹ cell ⁻¹ h ⁻¹	5.6E-03	Shi et al. (2010)	
	Fe-dPS	4.5E-11	L ⁻¹ cell ⁻¹ h ⁻¹	7.5E-04	Shi et al. (2010)	
THALASSIOSIRA OCEANICA						
	Fe'	2.56E-08	L ⁻¹ cell ⁻¹ h ⁻¹		Maldonado and Price (1996)	
	DFB	4.0E-12	L ⁻¹ cell ⁻¹ h ⁻¹	1.6E-04	Maldonado and Price (2001)	Fe:DFB (1:10)
CHRYSOCHROMULINA ERICINA						
	Fe'	2.0E+07	L ⁻¹ mol ⁻¹ day ⁻¹		Nodwell and Price (2001)	Based on growth rates
	DFB	0	L ⁻¹ mol ⁻¹ day ⁻¹	0	Nodwell and Price (2001)	Based on growth rates
	Goethite	5.0E+05	L ⁻¹ mol ⁻¹ day ⁻¹	2.53E-02	Nodwell and Price (2001)	Based on growth rates

(Continued)

Table A3 | Continued

Organism	Fe Ligand	Avg k_{up}	Units	Rel. bioavail	Reference	Notes
CHAETOCEROS spp						
	Fe'	2.0E-09	L ⁻¹ cell ⁻¹ h ⁻¹		Hassler and Schoemann (2009)	~30% of FeCl ₃ in dissolved phase
	DFB	6.1E-11	L ⁻¹ cell ⁻¹ h ⁻¹	3.1E-02	Hassler and Schoemann (2009)	Fe:DFB (1:15)
	Monosaccharides	2.6E-09	L ⁻¹ cell ⁻¹ h ⁻¹	1.3E+00	Hassler and Schoemann (2009)	Fe:MS (1:15)
	Polysaccharides	3.5E-09	L ⁻¹ cell ⁻¹ h ⁻¹	1.8E+00	Hassler and Schoemann (2009)	Fe:PS (1:15)
THALASSIOSIRA ANTARCTICA						
	Fe'	3.67E-08	L ⁻¹ cell ⁻¹ h ⁻¹		Hassler and Schoemann (2009)	~30% of FeCl ₃ in dissolved phase
	DFB	6.1E-11	L ⁻¹ cell ⁻¹ h ⁻¹	1.7E-03	Hassler and Schoemann (2009)	Fe:DFB (1:15)
	Monosaccharides	2.9E-08	L ⁻¹ cell ⁻¹ h ⁻¹	7.9E-01	Hassler and Schoemann (2009)	Fe:MS (1:15)
	Polysaccharides	3.5E-08	L ⁻¹ cell ⁻¹ h ⁻¹	9.6E-01	Hassler and Schoemann (2009)	Fe:PS (1:15)

Table A4 | Data used in the calculation of relative bioavailability of natural assemblages (Figure 5B).

Field study location	Fe ligand	Avg k_{up}	Units	Rel. bioavail.	Reference	Notes
SUBARCTIC PACIFIC (>0.2 μm)						
Coastal (station P4)	Fe'	9.2E-01	L ⁻¹ mol ⁻¹ Ch ⁻¹		Maldonado and Price (1999)	Data taken from 1996 study performed along line P which runs from coast (P4, high Fe) to offshore (P26, lower Fe) waters
	DFB	7.3E-03		8.0E-03		
	DFE	7.7E-03		8.3E-03		
Offshore (station P26)	Fe'	5.8E-02				FeL at ratio of 1:5 for DFB and DFE
	DFB	3.2E-03	5.4E-02			
	DFE	3.1E-03	5.2E-02			
SOUTHERN OCEAN >0.8 μm						
South of polar front	Fe'	2.5E-13	L ⁻¹ h ⁻¹		Hassler et al. (2011)	Fe' assumed to be 1 nM Uptake rates from lowest concentration of ligand additions
	DFB	5.0E-14		2.0E-01		
	Monosaccharides	3.8E-13		1.5E+00		
SOUTHERN OCEAN >0.2 μm						
	Fe'	2.6E+02	L ⁻¹ mol ⁻¹ Ch ⁻¹		Maldonado et al. (2005)	Uptake rates from dark experiments
	FeDFB	7.9E+00		3.0E-02		FeL is 1:10 for organic ligands, DFB, and Gallicocatechin
	Gallicocatechin	4.8E+02		1.8E+00		
	<i>in situ</i> ligands	1.1E+02		4.2E-01		
EQUATORIAL PACIFIC >0.2 μm						
Upwelling region	FeDFB	0		0	Wells et al. (1994)	Fe:DFB (1:5); population dominated by <i>Synechococcus</i> spp.
LOCH SCRIDIAN <20 μm						
	Fe'	7.9E-02	L ⁻¹ mol ⁻¹	7.0E-05	Lis and Shaked (in preparation)	Fe:DFB (1:2); population dominated by <i>Pseudo-nitzschia</i> spp. diatoms
	DFB	5.6E-06				
GULF OF AQABA >0.2 μm						
	Fe'	1.6E-01			Lis and Shaked (in preparation)	Fe:DFB (1:2); population dominated by <i>Synechococcus</i> spp.
	FeDFB	2.5E-04		1.6E-03		

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