Iron bioavailability to phytoplankton - an empirical approach

Supplementary material

Hagar Lis, Yeala Shaked, Chana Kranzler, Nir Keren and François M.M. Morel

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Section 1: Calculation of the uptake rate constant k_{in}

The iron uptake rate constant (k_{in}) is used in order to compare the iron uptake abilities of different phytoplankton species and the bioavailability of different Fe substrates. Here we explain in detail how this constant was calculated. The iron uptake rate constant, k_{in} (in units of L cell^{-1} hr^{-1}), is calculated by dividing the cellular uptake rate, ρ (mol Fe cell^{-1} hr^{-1}), by the Fe-substrate concentration in the medium (mol L^{-1}) - see equation 2 in main text. This analysis is valid only when cellular uptake sites are not saturated by the Fe substrate. Figure S1 shows the Michaelis-Menten like relationship between cellular Fe uptake rates and Fe-substrate concentration. Note that the substrate is either free inorganic iron, Fe', or organically bound iron such as FeDFB. Initially, uptake rate increases linearly with substrate concentration until all Fe-uptake sites are saturated with iron and maximal uptake rate is reached (v_{max}). In order to ensure the validity of the k_{in} calculation, care was taken to select data in which iron concentration is linearly correlated to Fe-uptake rates.

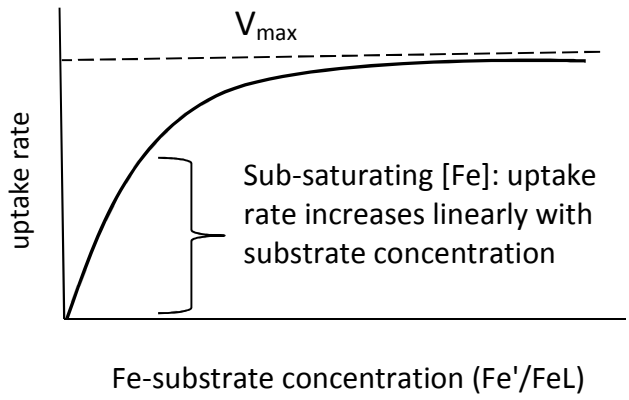


Figure S1 – Iron uptake rate as a function of Fe substrate concentration. All uptake rates included in our data sets fall within the linear region of this graph, well before substrate saturation of the transport systems.

Section 2: Raw data base for figures 1, 2 and 3

The cellular iron uptake rate constants (k_{in}) making up the data base in this contribution originate from original work and an array of previously published studies. The full data set required to calculate k_{in} values and surface areas is summarized in Tables S1-S5. These tables include details regarding organisms, cell size, iron substrates, Fe uptake rates and uptake rate constants. Unless otherwise specified, cell surface area (S.A) was taken from measurements performed in the publication from which the cellular Fe uptake rates of a given organism were extracted. In some studies iron uptake rates are given are given in units of mol Fe gr C^{-1} hr^{-1}. We converted this to mol Fe $cell^{-1}$ hr^{-1} using a carbon per cell conversion factor detailed in the table. For convenience, tables are found in Excel format (also in supplemental material). All references in the notes of these tables are included in the reference list at the end of this file.

Table S1: Fe' uptake by iron-limited eukaryotic phytoplankton

Table S2: FeDFB uptake by iron-limited eukaryotic phytoplankton

Table S3: The uptake of different Fe-substrates by iron-limited eukaryotic phytoplankton. Errors on surface area taken from study in which uptake rates were published if available, if not they were calculated from the range of published cell sizes found in the literature. Errors on k_{in} calculations were either taken from errors published in each study or measurements for the same organism and substrate.

Table S4: Fe' uptake by iron-limited cyanobacteria

Table S5: FeDFB uptake by iron-limited cyanobacteria

Section 3: Experimental methods in generation of original data

This section provides details of the methods involved in generating the original data in this article.

3.1 Trace metal clean techniques

Strict trace metal clean techniques were applied in all culturing and experimental manipulations. Solutions were prepared with double-distilled water (Milli-Q, Millipore, 18.2 mV) and analytical grade chemicals. All work was done under positive pressure HEPA filters. All plastic ware used for culture growth and uptake experiments were soaked in soap for 48 hours, rinsed in deionised water, followed by a 48 hr soak in 10% HCl and a finally rinsed three times with Milli-Q water. After HCl treatment, bottles were filled with Milli-Q water amended with EDTA and stored. All vessels were washed three times with Milli-Q water prior to use. Plastic ware and salt solutions were microwave sterilised while vitamin, trace metal and nutrient additions were filter sterilized through an acid washed 0.2µm filter. All tips were washed three times with microwave sterilized 10% HCl and then three times with microwave sterilized Milli-Q water prior to use.

3.2 Phytoplankton growth and iron limitation

Phytoplankton cultures were grown under constant illumination to avoid the influence of circadian rhythms on iron uptake rates. Growth mediums and conditions, monitoring of growth and indicators of Fe-stress for each species are detailed in the Table S6 below. Eukaryotic culture growth was monitored by counting cells using a coulter counter (Beckman Z3) while prokaryotic cells were monitored via optical density measurements (750nm). Once Fe-limitation was established, cells were harvested in exponential phase, concentrated, washed and suspended in Fe-free uptake medium in preparation for short term Fe-uptake experiments. More details regarding the preparation of cells for uptake experiments can be found in (Shaked et al 2005) for eukaryotic phytoplankton and in (Kranzler et al 2011) for prokaryotic phytoplankton.

Table S6: Phytoplankton media and conditions for culture growth. **References: (1) (Morel et al 1979) (2) (Moore et al 2007) (3) (Guillard 1975) (4) (Medium A which contains nitrate is designated A+ medium. Stevens et al 1973) (5) (Chen et al 1996) (6) (Shcolnick et al 2007)**

Organism	Growth Temperature (°C)	Growth medium [Fe] for limiting and non-limiting conditions	Fe-stress indicators
Emiliania huxleyi	20	Aquil[1] 20nM (lim) 300nM (non-lim)	Decreased growth rate
Nannochloropsis oculata	20	Aquil[1] 20nM (lim) 300nM (non-lim)	Decreased growth rate
Thalassiosira weissflogii	20	Aquil[1] 80nM (lim) 300nM (non-lim)	Decreased growth rate
***Synechococcus* WH7803 and WH8102**	25	AMP[1] 0nM (lim) 300nM (non-lim)	Changes in intracellular photosynthentic pigments (phycocyanin, phycoerythrin and chlorophyll *a* ratios)
***Synechococcus* CCMP1183**	25	f/2[2] 0nM (lim) 300nM (non-lim)	Decreases in intracellular photosynthetic pigments (chlorophyll *a*)
***Prochlorococcus* MED4**	25	AMP1[3] 0nM (lim) 300nM (non-lim)	Decreases in intracellular photosynthetic pigments (chlorophyll *a*)
***Synechococcus* PCC7002**	30	A+[4] 0nM (lim) 1µM (non-lim)	Decreased growth rate and decreases in intracellular photosynthetic pigments (chlorophyll *a*) and a blue shift in the absorption spectrum.
Trichodesmium erythraeum	25	YBCII[5] 0nM (lim) 1µM (non-lim)	Decreased trichome length
***Anabeana* UTEX 2576**	30	YBG11[6] 0.1 µM (lim) 10 µM (non-lim)	Decreased in intracellular photosynthetic pigments (chlorophyll *a*) and a blue shift in the absorption spectrum.

3.3 Short term iron uptake -mediums and experimental conditions

Short term ^{55}Fe uptake experiments were performed in accordance with protocols described in (Shaked et al 2005) and (Kranzler et al 2011). Experiments were performed with ^{55}Fe ($^{55}FeCl_3$, Perkin Elmer). All uptake experiments were performed in growth mediums which contained no trace metals, nutrients or vitamins (i.e. salts only). These were made up of the salt mixes such as Synthetic Ocean Water (SOW) or Turks Island Mix (salt mix in AMP1 medium). With the exception of YBG11 which contains HEPES, uptake mediums contain no organic buffers and 2mM of freshly made trace metal clean $NaHCO_3$ was added as a buffering agent instead. The pH in all experimental media ranged between 7.8 -8.2.

In Fe' uptake experiments, ^{55}Fe was precomplexed to EDTA (Fe:EDTA 1:2) prior to spiking into an EDTA buffered medium. EDTA concentrations in the medium varied with organism (see Table 7 below). In FeL (L= DFB,DFE and Aerobactin) uptake, ^{55}Fe was precomplexed to the ligand of choice (Fe:L 1:1.1) prior to spiking into an `EDTA free medium. Precomplexed iron-ligand solutions were pH adjusted to pH 5-7 and allowed to equilibrate overnight prior to use. After spiking, experimental Fe-uptake media were allowed to equilibrate overnight.

Addition of phytoplankton cells to uptake media marked the start of an uptake experiment. At various times during the 4-8 hr uptake experiments, weighted volumes of the experiment medium were filtered onto polycarbonate filters (eukaryotes and lightly coloured cyanobacteria) or nitrocellulose filters (strongly coloured cyanobacteria such as *Anabaena*) , rinsed with a saline solution, washed with Ti-citrate-EDTA reagent (Tang and Morel 2006) for 2-5 min and then again rinsed with SOW. Duplicate samples were processed at most time points. Nitrocellulose filters from *Anabaena* experiments were processed as described in (Kranzler et al 2011) to prevent chlorophyll quenching of signal. Otherwise, filters were retained for measurement of radioactivity in a Beckman scintillation counter with Quicksafe A scintillation liquid (Zinsser Analytic). Intracellular iron was calculated from the average specific activity (activity of the medium divided by the total iron added).

<u>Table S7:</u> Media and experimental conditions in short term iron uptake experiments. For medium references see Table S6. *SOW - synthetic ocean water.

Organism	Uptake temperature (°C)	Uptake medium	[EDTA] (µM) only present in Fe' uptake experiments
Emiliania huxleyi	Room temp	SOW*	100
Nannochloropsis oculata	Room temp	SOW*	100
Thalassiosira weissflogii	Room temp	SOW*	100
***Synechococcus* WH7803 and WH8102**	Room temp	AMP1 salts (Turk's island salt mix) + 2mM $NaHCO_3$	20
***Synechococcus* CCMP1183**	Room temp	SOW*	20
***Prochlorococcus* MED4**	Room temp	AMP1 salts (Turk's island salt mix) + 2mM $NaHCO_3$	20
***Synechococcus* PCC7002**	30	A+ salts + 2mM $NaHCO_3$	80
Trichodesmium erythraeum	Room temp	SOW*	20
***Anabeana* UTEX 2576**	30	YBG11	16

Section 4: Regression analysis for Fe' and FeDFB data

Iron uptake rate is a function of Fe substrate concentration on the one hand, and cell surface area on the other. We thus plot concentration normalized uptake rates (i.e. the uptake rate constant k_{in}) against cell surface area for all the collected data points in figures 1c and 2b in the main text. Both figures 1c and 2b are shown on log scales. Regression analysis was conducted on a linear scale as shown in Figure S2 and S3 for the Fe' and FeDFB data respectively. Trend line slopes are very close to one and pass near the origin in both cases. This implies direct proportionality between cell surface area and the uptake rate constant. Forcing the trend line through the origin translates to a slope of unity on a log-log scale. The inset in both figures show the trend line passing through the smaller cell size ranges.

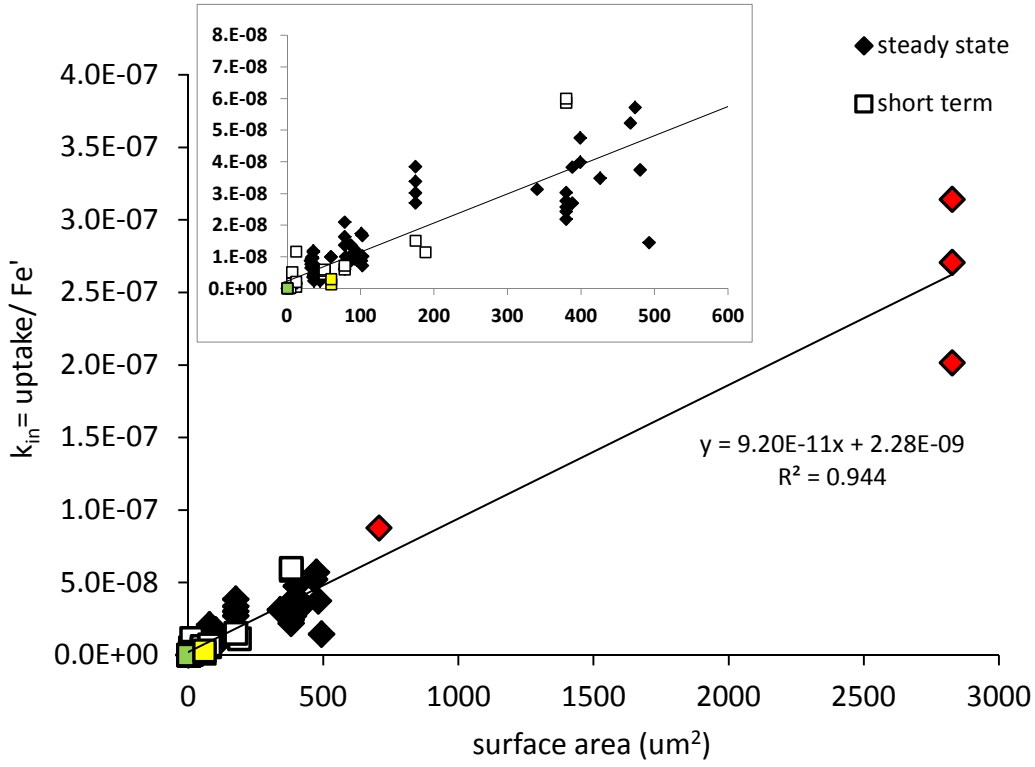
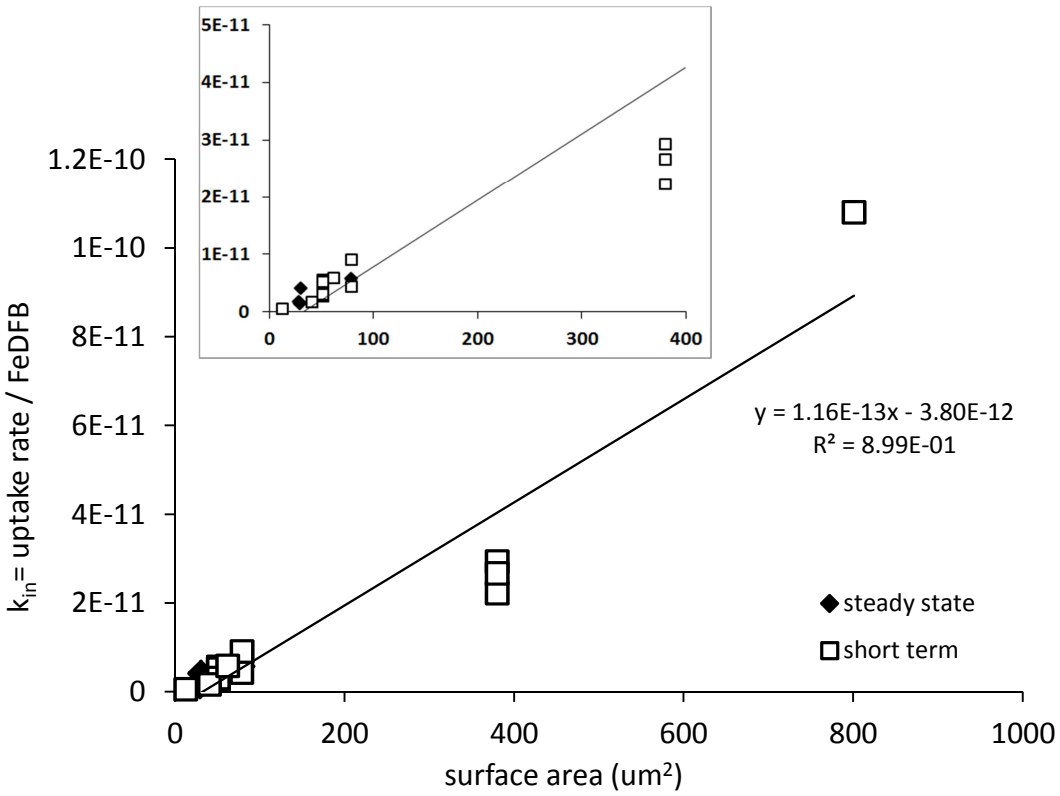


Figure S2 – the Fe' uptake rate constant as a function of cell surface area shown here in a linear scale. The inset shows the data for the smaller size ranges. The linear trend line has not been forced through the origin.



<u>Figure S3-</u> the FeDFB uptake rate constant as a function of cell surface area shown here in a linear scale. The inset shows the data for the smaller size ranges. The linear trend line has not been forced through the origin.

Section 5: Diffusion limitation of iron uptake

The results of our analysis suggest that life in aquatic environments has forced phytoplankton to evolve Fe uptake mechanisms that operate at optimal efficiency and are all limited by the same fundamental physical, chemical or biochemical factors. At very low substrate concentrations, large phytoplankton may be limited by the rate at which molecular diffusion can supply nutrients to the cell surface. Here we assess whether phytoplankton iron uptake rates are diffusion limited. We calculate diffusive flux of iron to a cell which is a perfect sink for iron (i.e. iron concentration at the cell surface is zero) according to (Pasciak and Gavis 1974) :

$$\boldsymbol{J = 4\pi r D S}$$

Where J is the diffusive flux of iron to the cell in units of (mol L^{-1} sec^{-1}), r is cell radius in µm, D is the molecular diffusion coefficient of the iron species in units of (cm^2 sec^{-1}) and S is the concentration of the iron substrate in question in mol L^{-1}. We calculated the diffusive flux of Fe' and FeDFB to the cell surface using the following molecular diffusion coefficients: $D_{Fe'} = 9\ x\ 10^{-6}\ cm^2 sec^{-1}$ and $D_{FeDFB} = 1.1\ x\ 10^{-6}\ cm^2 sec^{-1}$ (Völker and Wolf-Gladrow 1999). We compared the diffusive flux of iron to its uptake rate by phytoplankton for both Fe' (Figure S4) and FeDFB (Figure S5). Diffusive flux (J) and iron uptake rates were normalized to substrate concentration and plotted as a function of cell surface area. Thus, the units of the y-axis are L $cell^{-1}$ hr^{-1}. Hypothetical diffusive flux values were calculated according to the radii of spherical cells.

As can been seen in Figure S4, diffusion may become a limiting factor in the Fe' uptake of spherical cells greater than 70µm in diameter. This is in agreement with findings by (Armstrong 2008). FeDFB uptake on the other hand lies very far from diffusion limitation (Figure S5).

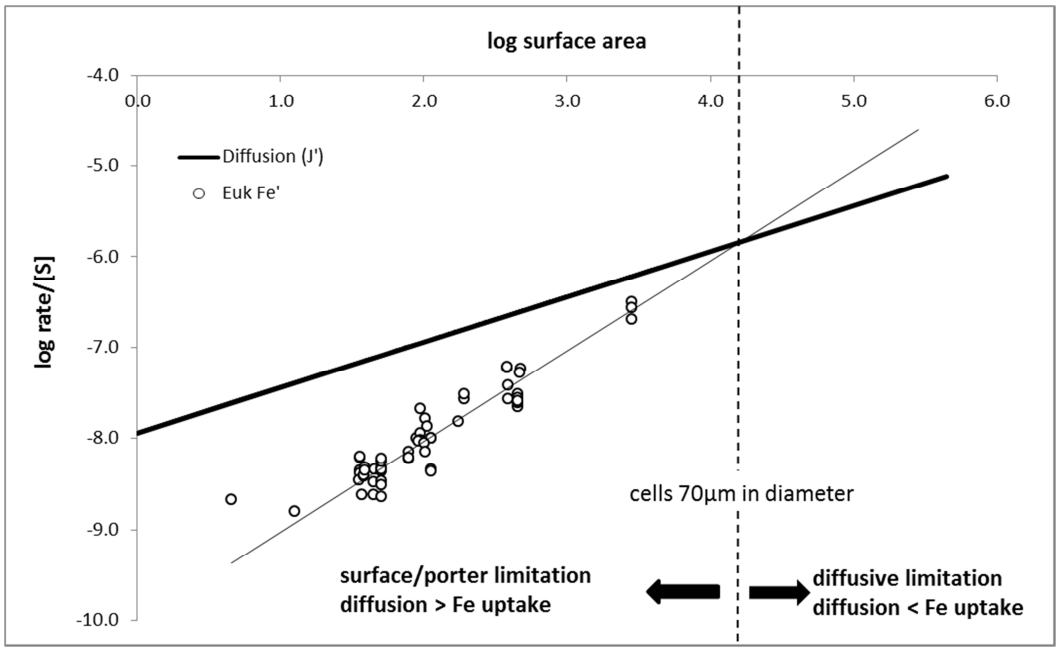


Figure S4 – **Diffusive flux and uptake of Fe' as a function of cell surface area. Diffusive flux of Fe' to the cell exceeds uptake for cells smaller than 70µ in diameter. This means that cells within our data set are not experiencing diffusive limitation of Fe' uptake.**

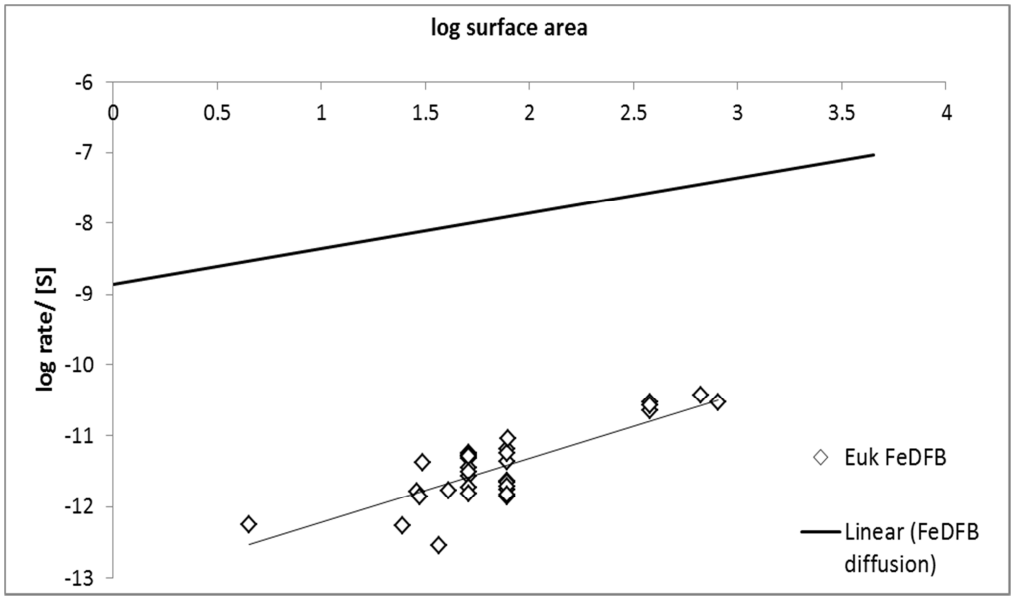


Figure S5 – Diffusive flux versus uptake of FeDFB as a function of cell surface area. Diffusive flux of FeDFB to the cell exceeds uptake for all size ranges. Thus diffusion does not limit FeDFB uptake in phytoplankton.

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