Probing the Bioavailability of Dissolved Iron to Marine Eukaryotic Phytoplankton Using In Situ Single Cell Iron Quotas

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Abstract We present a new approach for quantifying the bioavailability of dissolved iron (dFe) to oceanic phytoplankton. Bioavailability is defined using an uptake rate constant (k_{in-app}) computed by combining data on: (a) Fe content of individual in situ phytoplankton cells; (b) concurrently determined seawater dFe concentrations; and (c) growth rates estimated from the PISCES model. We examined 930 phytoplankton cells, collected between 2002 and 2016 from 45 surface stations during 11 research cruises. This approach is only valid for cells that have upregulated their high-affinity Fe uptake system, so data were screened, yielding 560 single cell k_{in-app} values from 31 low-Fe stations. We normalized k_{in-app} to cell surface area (S.A.) to account for cell-size differences.

The resulting bioavailability proxy (k_{in-app}/S.A.) varies among cells, but all values are within bioavailability limits predicted from defined Fe complexes. In situ dFe bioavailability is higher than model Fe-siderophore complexes and often approaches that of highly available inorganic Fe'. Station averaged k_{in-app}/S.A. are also variable but show no systematic changes across location, temperature, dFe, and phytoplankton taxa. Given the relative consistency of k_{in-app}/S.A. among stations (ca. five-fold variation), we computed a grand-averaged dFe availability, which upon normalization to cell carbon (C) yields k_{in-app}/C of 42,200 ± 11,000 L mol C^{-1} d^{-1}. We utilize k_{in-app}/C to calculate dFe uptake rates and residence times in low Fe oceanic regions. Finally, we demonstrate the applicability of k_{in-app}/C for constraining Fe uptake rates in earth system models, such as those predicting climate mediated changes in net primary production in the Fe-limited Equatorial Pacific.

Plain Language Summary In many oceanic regions, iron exerts strong control on phytoplankton growth, ecosystem structure, and carbon cycling. Yet, iron bioavailability and uptake rates by phytoplankton in the ocean are poorly constrained. Recently, Shaked et al. (2020) established a new approach for quantifying the availability of dissolved Fe (dFe) in natural seawater based on its uptake kinetics by Fe-limited cultured phytoplankton. Here, we extend this approach to in situ phytoplankton, establishing a standardized proxy for dFe bioavailability in low-Fe oceanic regions.

Bioavailability is estimated through single cell Fe uptake constants, calculated by combining measured Fe contents of individual phytoplankton cells collected from multiple regions with concurrently measured dFe concentrations, as well as modeled growth rates. We then utilize this proxy for: (a) comparing dFe bioavailability among organisms and regions; (b) calculating dFe uptake rates and residence times in low-Fe oceanic regions; and (c) constraining Fe uptake parameters of earth system models to better predict ocean productivity in response to climate change.

1. Introduction

Marine phytoplankton generate about half of Earth’s oxygen and play important roles in ocean carbon uptake and cycling (Sigman & Hain, 2012). Phytoplankton photosynthesis is often limited by the supply of macro and micronutrients (Moore et al., 2013; Saito et al., 2008). In particular, the micronutrient iron (Fe), present at sub-nanomolar concentrations in most of the upper waters (Johnson et al., 1997), exerts strong...
control on phytoplankton biomass and production rates, as well as microbial community structure, trophic dynamics and elemental cycling in both the coastal and open ocean (Tagliabue et al., 2017). Significant research efforts to understand, constrain, and accurately model the biogeochemical Fe cycling are underway, given its importance to ocean productivity and the global carbon cycle (Boyd & Ellwood, 2010; Tagliabue et al., 2016).

Over the past four decades, algal physiologists have examined how cultured marine phytoplankton cope with Fe limitation and acquire Fe from their external milieu (reviewed by Marchetti & Maldonado, 2016; Shaked & Lis, 2012; and Sutak et al., 2020). Recent phytoplankton genomic studies have expanded our understanding of Fe homeostasis and uptake, documenting multiple Fe transport pathways (e.g., Allen et al., 2008; Groussman et al., 2015; Kustka et al., 2007; Lommer et al., 2007; McQuaid et al., 2018; Morrissey & Bowler, 2012). Meanwhile, sea-going oceanographers have investigated Fe uptake and cycling in situ plankton communities (e.g., Bowie et al., 2001; Cabanes et al., 2020; Maldonado & Price, 1999). These studies have been complemented by global-scale surveys of Fe concentrations and speciation via the international GEOTRACES program (Anderson, 2020; Mawji et al., 2014; Schlitzer et al., 2018), as well as by efforts to determine the molecular speciation of dFe (e.g., Boiteau et al., 2016, 2018; Macrellis et al., 2001; Mawji et al., 2008). Together, these studies have greatly expanded our understanding of dFe concentration and speciation, as well as sources, and sinks in the upper ocean.

Yet, there remains considerable uncertainty about the connection between dFe speciation and dFe bioavailability to resident phytoplankton. Dissolved Fe speciation in the upper ocean is largely dominated by a heterogeneous pool of organic complexes, mostly molecularly uncharacterized but with distinct chemical reactivities (e.g., Bundy et al., 2014, 2018; Fitzsimmons et al., 2015; Gledhill & van den Berg, 1995; Rue & Bruland, 1995) and undefined bioavailability (e.g., Hassler et al., 2012; Worms et al., 2006; Shaked & Lis, 2012). Furthermore, even if it was possible to link dFe speciation to bioavailability in natural seawater, the speculation of dFe in the surface ocean is highly dynamic, with ligand-exchange, photochemical, and biologically mediated reactions that drive rapid interconversion among dFe species (e.g., Barbeau et al., 2001; Waite, 2001; Shaked, 2008). Additionally, resident phytoplankton actively alter their physiology and transport mechanisms to access and internalize different Fe substrates (e.g., Marchetti et al., 2012), further complicating efforts to constrain the bioavailability of individual chemical Fe species. Considering the dynamics in Fe speciation, it has been suggested that total dFe is the most appropriate indicator of bioavailable Fe in the ocean (Bruland et al., 2001), although others have proposed that labile particulate species might also be important on timescales relevant to biological productivity (Hurst et al., 2010). Most numerical models of ocean biogeochemistry currently assume that all Fe species in the dissolved phase are equally bioavailable, and thus use dFe concentrations in seawater to estimate biological Fe uptake (Tagliabue et al., 2016).

Seeking a simplified and generalized definition and quantification method for Fe bioavailability in the ocean, Shaked et al. (2020) presented a standardized proxy that relies on the uptake kinetics of Fe-limited phytoplankton. The bioavailability of natural dFe was probed through short-term uptake experiments with low-level radiotracer using several cultured Fe-limited phytoplankton species belonging to the major taxa occurring in the ocean. All tested phytoplankton species were found to acquire natural dFe, including organic complexes, at comparable rates when accounting for their surface area (S.A.). Normalizing these uptake rates to cell S.A. and dFe concentration in the water sample, Shaked et al. (2020) established the bioavailability proxy, $k_{in-app}/S.A$. Testing 12 seawater types from different basins and depths, they observed relatively small differences between water types and reported a grand average value for dFe availability in natural seawater.

Here, utilizing in situ phytoplankton and dFe data from the ocean, we extend this approach and evaluate dFe bioavailability across different Fe-limited ocean regions. We first calculated in situ steady-state Fe uptake rates of diverse natural phytoplankton communities by combining single cell Fe content (i.e., quotas) measured with synchrotron X-ray fluorescence with growth rates estimated from a biogeochemical model. Then, utilizing measured, ambient dFe concentrations, we calculated single-cell Fe uptake rate constants ($k_{in-app}$), which upon normalization to cell S.A. ($k_{in-app}/S.A.$) or cell carbon ($k_{in-app}/C$), serve as a standardized proxy of dFe availability. We verify and establish this proxy for Fe-limited phytoplankton and report that dFe availability does not vary systematically with location, temperature, dFe concentra-
tions or phytoplankton groups, enabling calculation of grand mean oceanic dFe bioavailability proxy for low Fe regions. We then utilize this proxy for: (a) comparing dFe bioavailability among organisms and regions; (b) calculating dFe uptake rates and residence times in low Fe oceanic regions; and (c) constraining Fe uptake parameters of earth system models to better predict ocean productivity in response to climate-change.

2. Materials and Methods

2.1. Analytical Approach

Following the framework of Lis, Kranzler, et al. (2015) and Shaked et al. (2020), we infer oceanic dFe availability from uptake rates calculated using single-cell Fe quotas determined with synchrotron X-ray fluorescence and growth rates estimated from a biogeochemical model (details below). We then divide calculated Fe uptake rates by measured ambient dFe concentrations to obtain an uptake rate constant, \( k_{\text{in-app}} \), as shown in Equation 1:

\[
k_{\text{in-app}} \left( \text{L cell}^{-1} \text{d}^{-1} \right) = \frac{\text{Fe quota} \left( \text{mol Fe cell}^{-1} \right) \times \text{growth rate} \left( \text{d}^{-1} \right)}{\text{SW dFe concentration} \left( \text{mol Fe L}^{-1} \right)}
\]

This approach is based on several assumptions, namely that dFe is the substrate for uptake by phytoplankton and that dFe concentrations remain relatively constant during cell growth (i.e., cell Fe quota reflects ambient Fe concentration). We then propose to apply \( k_{\text{in-app}} \) as a standardized proxy of dFe bioavailability in low-Fe regions. The following conditions have to be met for \( k_{\text{in-app}} \) to serve as a bioavailability proxy: (a) cells are Fe-limited or Fe-stressed, such that the high-affinity Fe uptake system is active; (b) \( k_{\text{in-app}} \) remains relatively stable across varying degrees of Fe-stress; (c) \( k_{\text{in-app}} \) is proportional to the surface area (S.A.) of the cell, allowing normalization to S.A.; and (d) \( k_{\text{in-app}} \) remains relatively stable between cells from different taxonomic groups. We test and verify these assumptions and conditions in the results section. See supporting information Section S1 for additional information.

2.2. Datasets and Parameters

Our analysis draws upon published datasets available in data repositories (GEOTRACES intermediate data product, BCO-DMO) and scientific publications (see Figure 1 legend for a full list). We analyzed eight datasets collected during 9 cruises across four ocean basins between 2002 and 2016 (Figure 1). Detailed analytical methods and data collection protocols can be found in the GEOTRACES cookbook (Cutter et al., 2017) and individual publications (Twining et al., 2003, 2004a, 2004b, 2011, 2015a, 2015b, 2019, 2021). Information on cell Fe quota and size measurements, which are key to our analysis, is detailed below. Some published dFe concentrations from SOFeX project (Twining et al., 2004a, 2004b) were found to be contaminated and hence were replaced with values provided by Z. Chase (personal communication), who analyzed the original samples.

2.2.1. Cellular Fe Quota, Cell Dimensions and C Quota

The Fe content of individual phytoplankton cells (i.e., the Fe quota), collected at 20 m depth, was measured by synchrotron X-ray fluorescence (SXRF). Detailed methods are provided elsewhere (Twining et al., 2003, 2004a, 2011, 2015a, 2015b, 2019), and consistent methods and analytical standards have been applied throughout the studies. Carbon quotas for each cell were calculated from biovolume estimates from 2 to 3 cell dimensions from a light micrograph, after applying cell shape, volume, and surface area equations of Hillebrand et al. (1999). Calculated biovolumes were converted to cellular C using the relationships for diatoms and nondiatoms provided by Menden-Deuer and Lessard (2000). The mean number of cells analyzed in each station was 20 (ranged from 10 to 50, Table 1). Cells were broadly classified into three groups: diatoms, flagellates, or picoeukaryotes. Some stations only contained one phytoplankton group, while others had two or three groups (Table 1).
2.2.2. Growth Rate Evaluation

For each station, growth rates were calculated based on sampling month using the PISCES-V2 model (Aumont et al., 2015). Modeled growth rates, affected by a combination of temperature, light, and nutrient limitation, were calculated separately for two phytoplankton functional types: diatoms and nanophytoplankton. For simplicity, all cells other than diatoms (mostly defined as flagellates in the original SXRF analysis) were assigned growth rates of nanophytoplankton. The model-derived growth rates were available at a spatial resolution of 2° in longitude and 2 × cos (latitude), with the resolution enhanced to 0.5 × 0.5° around the equator. The growth rate output of the PISCES-V2 model typically ranged from 0.2 to 0.8 d⁻¹ for stations with varying degrees of Fe-limitation, and as low as 0.03 d⁻¹ for N-limited stations (Table 1). The model growth rates compared well with the few available in situ growth rates reported for these cruises (Landry et al., 2011; Selph et al., 2011). Further details on the estimation of growth rates and its effect on our analysis is provided in the supplementary material (Section S2).

2.3. Dataset Screening and Selection Criteria

We excluded cells collected from artificially Fe-fertilized stations (as during SOFeX; Twining et al., 2004a), and cells collected from the deep-chlorophyll maximum that may have been light limited and thus have relatively high Fe quotas (Maldonado et al., 1999). We calculated $k_{\text{in-app}}$ for the remaining 930 cells collected...
| Project     | Station | Lat (N) | Long (E) | Temp (°C) | [dFe] (nM) | [NO₃] (μM) | log (N/Fe) | Degree of limitation | Model growth rates (d⁻¹) | Average kₘₐₜₜ₁(S.A.) | Included |
|-------------|---------|---------|----------|-----------|------------|------------|------------|---------------------|--------------------------|--------------------------|----------|
| SOFeX       | U5715 & U5721 | −39.3 | −178.6 | | 0.60       | 2.8        | 0.7        | +       | 14                | 0.35  | 3.1 × 10⁻¹⁰ | Yes      |
| South Pacific | U5727 | −39.4 | −178.8 | | 0.59       | 3.5        | 0.8        | +       | 38                | 0.35  | 1.7 × 10⁻¹⁰ | Yes      |
| FeCycle II  | U5731 | −39.4 | −178.7 | | 0.12       | 3.4        | 1.4        | ++      | 23                | 0.23  | 2.2 × 10⁻⁹  | Yes      |
| South Pacific | U5750 | −39.4 | −178.5 | | 0.07       | 2.5        | 1.5        | ++      | 22                | 0.23  | 2.5 × 10⁻⁹  | Yes      |
| EBO4        | U5756 & U5776 | −39.4 | −179.4 | | 0.13       | 3.0        | 1.4        | ++      | 29                | 0.23  | 1.7 × 10⁻¹⁰ | Yes      |
| Equatorial Pacific | 2 | 4.0 | −110.0 | | 0.079      | 0.75       | 1.0        | ++      | 21                | 0.34  | 8.0 × 10⁻⁹  | Yes      |
|              | 4 | 2.0 | −110.0 | | 0.079      | 6.3        | 1.9        | ++      | 21                | 0.43  | 6.6 × 10⁻⁹  | Yes      |
|              | 7 | 0.0 | −110.0 | | 0.079      | 6.2        | 1.9        | ++      | 35                | 0.50  | 6.2 × 10⁻⁹  | Yes      |
|              | 10 | −2.0 | −110.0 | | 0.079      | 7.1        | 2.0        | ++      | 15                | 0.47  | 1.3 × 10⁻⁹  | Yes      |
|              | 12 | −4.0 | −110.0 | | 0.20       | 8.0        | 1.5        | +       | 15                | 0.49  | 2.0 × 10⁻⁹  | Yes      |
|              | 14 | 0.0 | −116.7 | | 0.24       | 7.4        | 1.4        | +       | 19                | 0.49  | 2.1 × 10⁻⁹  | Yes      |
|              | 16 | 0.0 | −120.0 | | 0.15       | 3.5        | 1.4        | +       | 8                 | 0.49  | 3.0 × 10⁻⁹  | Yes      |
|              | 20 | 0.0 | −126.0 | | 0.24       | 7.5        | 1.5        | +       | 18                | 0.47  | 2.9 × 10⁻⁹  | Yes      |
|              | 22 | 0.0 | −128.3 | | 0.20       | 6.6        | 1.5        | +       | 7                 | 0.48  | 2.4 × 10⁻⁹  | Yes      |
|              | 26 | 0.0 | −135.0 | | 0.16       | 6.1        | 1.6        | +       | 40                | 0.49  | 2.5 × 10⁻⁹  | Yes      |
|              | 28 | 0.0 | −138.7 | | 0.32       | 5.8        | 1.3        | +       | 21                | 0.50  | 2.4 × 10⁻⁹  | Yes      |
| EPZT        | 1    | −12.0 | −79.2  | | 0.11       | 7.5        | 1.8        | +       | 26                | 0.44  | 9.3 × 10⁻⁹  | Yes      |
| Equatorial Pacific | 3 | −12.0 | −77.7  | | 0.82       | 19         | 1.4        | --      | 13                | 0.57  | 3.8 × 10⁻¹⁰ | Yes      |
|              | 4 | −12.0 | −77.8  | | 0.41       | 12         | 1.5        | ?       | 23                | 0.57  | 1.4 × 10⁻⁹  | No       |
|              | 5 | −12.0 | −78.2  | | 0.27       | 12         | 1.6        | +       | 12                | 0.57  | 5.5 × 10⁻ⁱ⁰ | Yes      |
|              | 11 | −12.0 | −94.0  | | 0.049      | 0.85       | 1.2        | ++      | 16                | 0.32  | 1.0 × 10⁻⁹  | Yes      |
|              | 15 | −16.0 | −104.0 | | 0.078      | 0.57       | 0.9        | ++      | 17                | 0.12  | 3.5 × 10⁻⁹  | Yes      |
|              | 18 | −15.0 | −112.8 | | 0.087      | 3.8        | 1.6        | +       | 13                | 0.14  | 2.4 × 10⁻⁹  | Yes      |
|              | 26 | −11.7 | −128.0 | | 0.17       | 2.3        | 1.1        | +       | 16                | 0.18  | 1.9 × 10⁻⁹  | Yes      |
|              | 36 | −10.5 | −152.0 | | 0.082      | 0.32       | 0.6        | +       | 29                | 0.082 | 1.5 × 10⁻⁹  | Yes      |
| GeoMICS     | P8    | 48.8  | −128.7 | | 0.27       | 0.35       | 0.1        | +       | 18                | 0.28  | 4.7 × 10⁻¹¹ | Yes      |
| North Pacific | P6  | 48.7  | −127.7 | | 0.46       | 2.0        | 0.6        | ?       | 13                | 0.17  | 4.8 × 10⁻¹¹ | No       |
|              | P4  | 48.7  | −126.7 | | 0.64       | 0.37       | −0.2       | --     | 27                | 0.17  | 8.4 × 10⁻¹¹ | No       |
|              | P1  | 48.6  | −125.5 | | 0.13       | 3.1        | 0.4        | --     | 27                | 0.28  | 6.7 × 10⁻¹¹ | No       |
| IrnBru      | 27.5  | 42.7  | −125.0 | | 0.35       | 17         | 1.7        | +       | 14                | 0.37  | 3.3 × 10⁻¹⁰ | Yes      |
| California Current System | 2 | 38.7 | −123.7 | | 0.57       | 14         | 0.4        | --     | 12                | 0.43  | 1.2 × 10⁻¹⁰ | Yes      |
| IO9N        | 87    | −26.52| 95      | 24.29     | 0.12       | <0.05      | −0.4      | +       | 20                | 0.030 | 1.2 × 10⁻¹¹ | No       |
| Indian Ocean | 127 | −4.53 | 94.87   | 30.18     | 0.06       | <0.05      | −0.1      | +       | 16                | 0.059 | 4.8 × 10⁻¹¹ | No       |
|              | 130  | −3.13 | 94.43   | 30.69     | 0.07       | <0.05      | −0.1      | +       | 18                | 0.067 | 3.3 × 10⁻¹¹ | No       |
|              | 189  | 14.54 | 89.59   | 30.2      | 0.38       | <0.05      | −0.9      | +       | 19                | 0.65  | 9.1 × 10⁻¹¹ | No       |
|              | 194  | 17    | 89.85   | 29.39     | 0.49       | <0.05      | −1.0      | +       | 11                | 2.5   | 2.0 × 10⁻¹⁰ | No       |
### Table 1
Continued

| Project | Station | Lat | Long | Temp | [dFe] (nM) | [NO₃]⁻ (uM) | Degree of limitation | Model growth rates (d⁻¹)ᵃ | Average kₑff/S.A. | Included (Yes/No) |
|---------|---------|-----|------|------|------------|--------------|----------------------|----------------------|----------------|------------------|
| NAZT North Atlantic | 2010-5 | 31.0 | −22.0 | 24 | 0.18 | 0.20 | -0.6 | ? | ++ | 14 | 0.026 | 3.4 × 10⁻¹¹ | No |
| | 2010-9 | 17.4 | −18.3 | 28 | 0.88 | 0.035 | −1.2 | --- | ++ | 19 | 0.16 | 4.3 × 10⁻¹¹ | No |
| | 2010-99 | 17.7 | −31.1 | 27 | 0.42 | 0.026 | −0.9 | --- | ++ | 16 | 0.01 | 0.031 | 1.7 × 10⁻¹¹ | No |
| | 2010-153 | 21.6 | −61.6 | 28 | 1.6 | 0.020 | — | --- | ++ | 10 | 0.032 | 5.4 × 10⁻¹² | No |
| | 2011-1 | 39.7 | −69.8 | 19 | 0.62 | 0.57 | — | --- | ? | 10 | 0.26 | 0.35 | 3.3 × 10⁻¹⁰ | No |

Note. Stations were defined according to the primary limiting nutrient based on N and Fe concentrations and the log₁₀ ([NO₃]/[Fe]) criteria of Browning et al. (2017), aided with published findings on these cruises. The degree of limitation is indicated by a scale of four categories: "−−", "−", "+", "++", ranging from no limitation (−−) to severe limitation (++) for more information see Table S3. Stations not considered Fe-limited were not included in the final assessment of dFe bioavailability (indicated as No in the "Included" column). All samples are from mixed layer. *Model output growth rates were for two phytoplankton groups, diatoms and nanoflagellates (titled here Flag). From 45 unperturbed surface stations from eight research campaigns (including 9 cruises). Then, we defined for each station whether cells are Fe-limited, Fe-replete or N-limited (Table 1, see criteria below).

#### 2.3.1. Determination of Fe Limitation

Only cells from Fe-limited populations were used to probe dFe availability because under these subsaturating dFe conditions the cells upregulate their high affinity Fe-uptake systems (Hudson & Morel, 1990; Maldonado & Price, 2001; Shaked et al., 2005), and their Fe uptake rates are directly proportional to in situ dFe concentrations (for details see Shaked et al., 2020). Each data set was evaluated with regards to hydrography, temperature, nutrient (Fe, NO₃⁻) concentrations, and when available, physiological and molecular markers of Fe-limitation and grow-out experiments conducted in parallel. We evaluated the primary limiting nutrient for phytoplankton growth in each of the stations based on measured concentrations of dFe in nM and NO₃⁻ in μM and the ratio of these according to the criteria of Browning et al. (2017). Stations with [dFe] < 0.6 nM and log₁₀([NO₃]/[Fe]) > 1, were defined as Fe-limited. Stations with [NO₃⁻] < 1 μM and log₁₀([N/Fe]) < 1, were defined as N-limited (regardless of [dFe]). Stations with [dFe] > 0.6 nM were defined as Fe-replete (regardless of log₁₀([N/Fe])). A few stations with [dFe] < 0.6 nM and log₁₀([N/Fe]) ranging between 0 and 1, were defined as co-limited by Fe and NO₃⁻. Only stations defined as Fe-limited or co-limited by Fe and NO₃⁻ were included in the final analysis and used for probing oceanic dFe availability (Table 1). Even under low-Fe conditions, individual cells may accumulate excess Fe (Twining et al., 2021). Thus, the status of Fe-limitation by individual cells was further assessed by their cellular Fe quotas, which typically range between 1 and 50 μmol Fe/mol C (Marchetti & Maldonado, 2016). Cells demonstrating luxury Fe uptake, defined here as Fe: C > 100 μmol Fe/mol C, were excluded from dFe availability analysis (at most five cells per station in only a few of the Fe-limited stations).

#### 2.3.2. Steady State Growth

The calculation of steady-state dFe uptake rates from cell Fe quotas and growth rates is valid only for steady state growth (Sunda & Huntsman, 1997; Sunda et al., 2005), which was assumed to occur a priori in most stations. This steady-state condition was probably not met in some dynamic systems receiving episodic Fe supply, such as upwelling regions or dust impacted areas. Two stations over the Peru shelf were defined as nonsteady state based on a mismatch between [dFe] and mean Fe quota (as compared with other data from the same cruise (Twining et al., 2021)), and were not included in the dFe bioavailability analysis (Table 1).

#### 2.4. Statistical Analysis

Data processing was conducted using R Studio, and statistical testing was conducted with JMP statistical software (v11, SAS). Data were log₁₀-transformed to normalize data, which was confirmed with Shap-
iro-Wilk tests. Comparisons between cell types and between geographic locations were tested with Student t-tests and one-way ANOVAs. An analysis of covariance model (ANCOVA) was used to test for statistically significant effects on $k_{\text{in-app}}$ by simultaneous variations of cell type, geographic location, and dFe (statistical analyses are shown in Sections S3 and S4).

3. Results

3.1. Choice of dFe (Over Fe') as a Probe of Iron Bioavailability in the Ocean

Following Shaked et al. (2020), we consider dFe, the entire pool of dissolved Fe complexes, as the substrate for uptake by naturally occurring Fe-limited phytoplankton when calculating $k_{\text{in-app}}$ (Table S1). The choice of dFe rather than Fe', the sum of inorganic Fe species, may appear to conflict with culture studies that report Fe' as the substrate for uptake (Shaked et al., 2005; Sunda & Huntsman, 1995). However, most culture work has been conducted in media buffered by a large excess of EDTA, in which EDTA complexation intentionally dominates Fe speciation, and the Fe' concentrations are largely in the pM range (Shaked & Lis, 2012; Sunda et al., 2005). While Fe' is undoubtedly an important substrate for phytoplankton uptake in the ocean (Morel et al., 2008), ocean Fe' concentrations calculated from electrochemical Fe speciation measurements fail to account for the rapid in situ cycling of Fe' (Gledhill & Buck, 2012).

Indeed, in situ Fe uptake studies demonstrate that the supply rates of Fe' in surface waters are too slow to account for the rates of Fe uptake, indicating that ocean phytoplankton access organically bound dFe species (Hassler et al., 2011; King et al., 2012; Maldonado & Price, 1999; Maldonado et al., 2005; Mellett et al., 2018).

A comparison of Fe quotas measured in natural phytoplankton populations and laboratory cultures shows 1000-fold divergence when plotted against concurrently evaluated Fe' (Figure 2a). However, when Fe quotas of natural phytoplankton are plotted rather as a function of measured dFe they closely agree with those of the cultures grown in EDTA-buffered media (Figure 2b). This supports our use of dFe as the bioavailable Fe pool in natural systems, following the approach of Shaked et al. (2020).

3.2. Proportionality Between $k_{\text{in-app}}$ and Surface Area (S.A.) of Individual Cells

Previous studies have demonstrated proportionality between Fe uptake rates and S.A. in Fe-limited cells, reflecting physiological maximum density of high-affinity transport proteins at the cell surface (Hudson & Morel, 1990; Lis, Kranzler et al., 2015; Lis, Shaked et al., 2015; Maldonado & Price, 2001; Morel, 2008; Shaked & Lis, 2012; Sunda & Huntsman, 1997; Sunda et al., 2005). Graphically, such proportionality should yield a slope of unity (1) on a log-log plot of $k_{\text{in-app}}$ versus S.A. with a high linear correlation coefficient ($R^2$). Indeed, slopes nearing one and high correlation coefficients ($R^2 > 0.57$) are obtained for all major cruises that sampled Fe-limited phytoplankton in several locations (Figure 3), and for the entire dataset with 560 single-cell data points (Section S3).

The linear relationship between $k_{\text{in-app}}$ and S.A. suggests that, across these broad oceanic regions, Fe-limited cells are subjected to similar constraints on available membrane-space for Fe transport, and can thus provide a common indicator of dFe availability. Subsequently, we normalize $k_{\text{in-app}}$ to cell S.A., and propose the resulting parameter as a measure of dFe bioavailability:
Bioavailability of SW dFe (L μm$^{-2}$ d$^{-1}$) = $k_{\text{in-app}}$ (L cell$^{-1}$ d$^{-1}$) / S.A. (μm$^2$ cell$^{-1}$)

(2)

3.3. Consistency of $k_{\text{in-app}}$/S.A. Among Different Degrees of Fe-Limitation

The degree of Fe-limitation, as deduced from measured dFe concentrations, physiological and molecular Fe-deficiency markers, and grow-out incubations, varies substantially among sites (Table 1). Moreover, at any given station, different species of phytoplankton may experience varying degrees of Fe-limitation, depending on their size, life history, Fe acquisition mechanisms, Fe-requirements and Fe-use efficiencies (Fourquez et al., 2020; Maldonado et al., 2005; Twining et al., 2021). Hence, $k_{\text{in-app}}$/S.A. should remain constant across varying degrees of Fe-limitation for it to provide a reliable measure of dFe availability.

Laboratory studies have shown that, once the activity of the high-affinity Fe uptake system has been maximized, Fe uptake rate constants remain largely unchanged at varying degrees of Fe-limitation (Kustka et al., 2007; Lis, Kranzler, et al., 2015; Maldonado & Price, 2001). High-affinity Fe uptake mechanisms should be expressed even when macronutrients are low, as shown by Caputi et al. (2019) for ISIP gene expression in the sub-tropical Pacific gyres. Thus, $k_{\text{in-app}}$/S.A. should provide a valid estimate of Fe uptake rates across all low-Fe systems. Matching these expectations, average $k_{\text{in-app}}$/S.A. did not differ in populations estimated a priori to be either mildly or strongly Fe-limited (Figure 4). Among Fe-limited populations, $k_{\text{in-app}}$/S.A. shows no statistically significant relationship with dFe. Geometric mean $k_{\text{in-app}}$/S.A. across these Fe-limited populations was $3.16 \pm 0.82 \times 10^{-10}$ L μm$^{-2}$ d$^{-1}$ (±95% confidence interval [CI]; Figure 4). In contrast, $k_{\text{in-app}}$/S.A. was about 10-fold lower in populations...
that were either Fe replete or limited by N (p < 0.0001; Figure 4). Such a drop in \( k_{\text{in-app}}/\text{S.A.} \) matches expectations, since Fe-replete cells downregulate their high affinity Fe transport systems and reduce the density of Fe-transporters on the cell surface (Hudson & Morel, 1993; Maldonado & Price, 2001). This supports our assertion that Fe bioavailability can be assessed at all stations where high-affinity Fe uptake systems are being maximized (dFe < 0.6 nM).

Given the broad use of N/Fe as a Fe-limitation index (as we have done above) and the recognition that large ocean regions may be co-limited by N and Fe (Browning et al., 2017), it is interesting to also consider the applicability of \( k_{\text{in-app}}/\text{S.A.} \) in low-N areas. Our Fe-uptake constant does appear to vary as a function of \( \log_{10} (\text{N}/\text{Fe}) \) (Figure S5); however, this relationship is driven by N-availability controls on phytoplankton growth rates that are independent of Fe availability. There is no relationship between \( k_{\text{in-app}}/\text{S.A.} \) and \( \log_{10} (\text{N}/\text{Fe}) \) when the low-N stations (represented by X's and white symbols in Figure 4 and S5) are removed. Thus, our approach to calculating \( k_{\text{in-app}}/\text{S.A.} \) cannot be applied in regions where macronutrient availability limits growth rates, as Fe uptake and cell growth is decoupled in these systems (Twining et al., 2021). The disconnect can be seen in Figure 4, where calculated \( k_{\text{in-app}}/\text{S.A.} \) are ca. 10-fold below those calculated for cells not limited by N. However, the calculated geometric mean \( k_{\text{in-app}}/\text{S.A.} \) should be applicable to regions with low N and low Fe, as high-affinity Fe uptake appears to occur in low-Fe areas independent of N availability (Caputi et al., 2019).

### 3.4. Consistency of \( k_{\text{in-app}}/\text{S.A.} \) Among Phytoplankton Groups

The dataset contains cells from different groups, which were broadly categorized as diatoms, flagellates, and picoeukaryotes (Table 1). For \( k_{\text{in-app}}/\text{S.A.} \) to provide a reliable measure of dFe availability, the differences among these groups should be small. Shaked et al., (2020) reported a similarity in \( k_{\text{in-app}}/\text{S.A.} \) among several cultured species from different taxa, based on short-term uptake experiments with natural seawater. Here, however, \( k_{\text{in-app}}/\text{S.A.} \) is calculated using cell Fe quotas, which differ between phytoplankton groups (Twining & Baines, 2013; Twining et al., 2021).

Acknowledging the possible variations in \( k_{\text{in-app}}/\text{S.A.} \) among natural communities, we examined the four low-Fe studies for which more than one cell type was analyzed. In FeCycle II and EB04 cruises, diatoms, flagellates, and picoeukaryotes had mostly comparable \( k_{\text{in-app}}/\text{S.A.} \) (Figure 3 and S6). Stronger taxonomic group differences were seen in SOFeX, where least squares geometric mean diatom \( k_{\text{in-app}}/\text{S.A.} \) \((0.51 \times 10^{-10} \text{ L } \mu\text{m}^{-2} \text{ d}^{-1})\) was only 16% of that observed in flagellates \((3.1 \times 10^{-10} \text{ L } \mu\text{m}^{-2} \text{ d}^{-1}); \text{Figures 3d} \) and S6). This difference likely reflects the markedly lower Fe quota-to-volume ratios that can be achieved by Fe-limited Southern Ocean diatoms (Strzepek et al., 2011). In contrast, diatoms in the EPZT cruise had 1.7 times higher \( k_{\text{in-app}}/\text{S.A.} \) than those of flagellates \((5.0 \text{ vs. } 2.9 \times 10^{-10} \text{ L } \mu\text{m}^{-2} \text{ d}^{-1}, \text{Figure S6}) \), possibly reflecting the Fe-storage capacity of some diatoms, especially under differential N and/or Si limitation (De La Rocha et al., 2000; Twining et al., 2021).

Combining these four studies, we found statistically significant but relatively minor (~1.4 fold) differences in \( k_{\text{in-app}}/\text{S.A.} \) between cell types (Section S5). Least-square geometric mean \( k_{\text{in-app}}/\text{S.A.} \) were \( 2.1 \times 10^{-10}, \) \( 3.0 \times 10^{-10}, \) and \( 2.2 \times 10^{-10} \text{ L } \mu\text{m}^{-2} \text{ d}^{-1} \) for diatoms, flagellates, and picoeukaryotes, respectively. The variations in phytoplankton taxa \( k_{\text{in-app}}/\text{S.A.} \) likely reflect unique physiologies related to Fe-sparing and Fe-storage strategies. But, these taxon differences are small relative to the overall variance in station-specific \( k_{\text{in-app}}/\text{S.A.} \) across all stations (~5 fold, Table 1), and hence will exert at most a marginal effect on comparisons of dFe bioavailability.
Having confirmed that $k_{in-app}/S.A.$ is a valid standardized proxy for bioavailability, we further explore the dataset to gain insights on the availability of dFe in the ocean. We compare uptake constants of both individual cells (Figure 5a) and geometric means of discrete stations (Figure 5b) among the various research campaigns. These are further compared to the bioavailability envelope that empirically predicts the upper and lower limits of Fe availability to phytoplankton in seawater (Lis et al., 2015). The upper black line in Figure 5 represents the most bioavailable Fe species, Fe', while the lower green line, the least bioavailable Fe species, FeDFB. The space between them defines the bioavailability envelope, where all Fe-complexes are predicted to reside. We also plot the average uptake rate constant of seawater dFe as probed by Fe-limited phytoplankton (Shaked et al., 2020), under either dim or natural light ($k_{in-app}/S.A = 3.6 \times 10^{-11}$ and $2.1 \times 10^{-10}$ L $\mu$m$^{-2}$ d$^{-1}$, respectively). The grand mean dFe availability calculated here is plotted as a blue line ($k_{in-app}/S.A = 3.2 \pm 0.82 \times 10^{-10}$ L $\mu$m$^{-2}$ d$^{-1}$).

3.5. Comparing $k_{in-app}/S.A.$ Among Cruises and Former Studies and Implications for Oceanic dFe Availability

Significant scatter among individual-cell data is observed on a log-log plot of $k_{in-app}$ versus S.A. yet, none of the cruises stand out compared to the others (Figure 5a). All values plot within the boundaries of the bioavailability envelope, clustering toward the upper range of the envelope, nearer to Fe' than to FeDFB. This
indicates that, as a whole, the availability of the mixed pool of Fe-complexes in seawater is much higher than that of strong Fe-siderophore complexes. In fact, based on our data, we can set a new lower limit of dFe availability in the ocean, which is ~10-fold higher than that defined by the nonphotolabile siderophore FeDFB. Although Fe-binding ligands in the ocean generally have strong complexation constants that are comparable to model siderophores (Gledhill & Buck, 2012), dFe availability in the ocean is significantly higher than for Fe bound to these siderophores. This is likely due to a number of rapid physiological and photochemical Fe cycling processes that occur in natural systems. These processes appear to increase the availability of dFe species by generating the highly bioavailable Fe′ (Barbeau et al., 2001; Maldonado & Price, 2001; Maldonado et al., 2005; Shaked, 2008; Waite, 2001).

Geometric means of k_{in-app}/S.A. at discrete stations spanning a broad geographical range and temperature gradient were rather consistent, generally 2–5 × 10^{-10} L μm^{-2} d^{-1} (Figure 5b). Higher values are found in some of the Equatorial Pacific stations from EB04 and EPZT cruises, which may be due to poorly constrained dFe (for some EB04 stations where measured dFe approached analytical capabilities [Kaupp et al., 2011], or taxon-specific Fe storage at some EPZT stations [Twining et al., 2021]). Nonetheless, focusing on the consistency among most stations, we computed averaged oceanic dFe availability (k_{in-app}/S.A.) of 3.16 ± 0.82 × 10^{-10} L μm^{-2} d^{-1} (mean ± 95% confidence interval). This exceeds the value estimated from cultures under dim laboratory light (Figure 5b, red line), but agrees well with outdoors experiments where photochemical reactions increase dFe availability (Figure 5b orange line; Shaked et al., 2020). The close match between k_{in-app}/S.A. of phytoplankton from culture and the ocean suggests that photochemical reactions in the surface ocean are important to increase dFe bioavailability by temporarily releasing inorganic Fe (Fe′) from Fe complexes. Cells analyzed in this study were collected from the upper 20 m of the water column, where some of the Fe complexes undergo photochemical degradation to form the highly labile but short-lived Fe′.

4. Discussion

The surface area-normalized uptake rate constant (k_{in-app}/S.A.) derived above met all predefined criteria. It is proportional to cell surface area and broadly consistent across diverse phytoplankton communities from low-Fe ocean regions that experience various degrees of Fe limitation. It thus can serve as a proxy of oceanic dFe bioavailability in near-surface waters of low-Fe regions. The grand average k_{in-app}/S.A. of 3.16 ± 0.82 × 10^{-10} L μm^{-2} d^{-1}, which provides a first order estimate of mean dFe bioavailability, agrees with previous, independent estimates and supports the significance of photochemistry in increasing dFe availability in surface waters. Comparison with the published Fe bioavailability envelope indicates that, indeed, most dFe in low-Fe ocean regions appears to be available for uptake by phytoplankton over relevant timescales. Note that our analysis reflects primarily conditions in near-surface waters of Fe-limited regions. Colloids, which were not directly considered in this analysis and may contribute to the dFe pool, could also potentially affect dFe availability; their significance likely increases with depth as they recycle through the photic zone (Boye et al., 2010).

To further apply our proxy, we next normalize it to carbon instead of surface area; this allows us to predict in situ Fe uptake rates and calculate biologically driven residence times of dFe in the surface ocean. Lastly, we apply our uptake rate constants to constrain global model predictions regarding climate change impacts on primary productivity and upper trophic levels in the Fe-limited eastern equatorial Pacific.

4.1. Generating a Carbon-Normalized Bioavailability Proxy (k_{in-app}/C)

A potential drawback in the use of k_{in-app}/S.A. for assessing biological Fe uptake in ocean models is that phytoplankton surface area is rarely measured or incorporated in these models, which instead usually rely on cell C. Most experimental phytoplankton uptake data are obtained by filtration onto specific pore size filters, and converting these data to S.A. introduces large uncertainty. In contrast, C uptake rates are often measured simultaneously with Fe uptake rates, which are then reported normalized to C or chlorophyll (Ellwood et al., 2020; King et al., 2012; Mellett et al., 2018). Hence a C-normalized bioavailability proxy, k_{in-app}/C, with units of L mol C^{-1} d^{-1}, would have wide application in experimental and modeling studies.
Global Biogeochemical Cycles

In the dataset analyzed in this study, cellular C quotas were estimated for each cell from calculated cell volumes using the carbon-to-volume relationships of Menden-Deuer and Lessard (2000). This allows a straightforward conversion of \( \frac{k_{\text{app}}}{\text{S.A.}} \) to \( \frac{k_{\text{app}}}{\text{C}} \), but there are some caveats to consider. For example, while cellular C is proportional to cellular volume, \( \frac{k_{\text{app}}}{\text{S.A.}} \) is proportional to S.A (Figure 3), and cell volume to S.A. ratios are not constant, varying according to cell shape and size. Moreover, carbon-to-volume relationships published by Menden-Deuer and Lessard (2000) differ between diatoms and nondiatoms. Despite these caveats, \( \frac{k_{\text{app}}}{\text{S.A.}} \) is highly correlated with \( \frac{k_{\text{app}}}{\text{C}} \), when comparing both individual cells and station averages (Section S6).

Similar to \( \frac{k_{\text{app}}}{\text{S.A.}} \), the C-normalized Fe bioavailability proxy shows only small variations among most individual stations in a single cruise and among cruises (Figure 6). The calculated grand average (geometric mean) \( \frac{k_{\text{app}}}{\text{C}} \) in our study is 42,200 ± 11,000 L mol C\(^{-1}\) d\(^{-1}\) (mean ± 95% confidence interval) well within the range reported for different regions using independent measurements (Table S4).

### 4.2. Applying \( \frac{k_{\text{app}}}{\text{C}} \) to Predict Fe Uptake Rates in the Ocean

The C-normalized Fe-uptake constant can be used to calculate Fe uptake rates by phytoplankton communities using Equation 3, and calculated rates can be compared to those measured directly in situ.

\[
\text{In situ Fe uptake rate (mol L}^{-1}\text{d}^{-1}) = \frac{k_{\text{app}}}{\text{C}} \times \text{Cellular C or POC (mol L}^{-1}\text{)} \times \text{dFe (mol L}^{-1}\text{)} \quad (3)
\]

We selected studies that conducted on-deck Fe uptake experiments with natural phytoplankton communities using small additions of a radioactive tracer (typically 0.2 nM \(^{55}\)Fe). We compared measured rates with rates calculated using the grand average \( \frac{k_{\text{app}}}{\text{C}} \) (42,200 L mol C\(^{-1}\) d\(^{-1}\)), dFe concentrations in the experiment (i.e., ambient dFe + \(^{55}\)Fe), and POC (particulate organic carbon, Figure 7). For a study lacking POC measurements (Mellett et al., 2018), we used chlorophyll a (Chl) instead of POC and converted the Fe uptake constant to \( \frac{k_{\text{app}}}{\text{Chl}} \) (0.176 L \( \mu \)g Chl\(^{-1}\) d\(^{-1}\), Section S6). Predicted uptake rates are in good match with rates measured in the California upwelling system (Mellett et al., 2018) and the Subarctic Pacific (King et al., 2012), generally within 5%–50% of measured values (Figure 7, mean difference: 18 ± 7%). In the Southern Ocean (Ellwood et al., 2020), predicted rates exceed measured rates, possibly reflecting the somewhat lower dFe availability in this region (perhaps driven by dim irradiance) in accord with lower than average \( \frac{k_{\text{app}}}{\text{C}} \) found in SOFeX (20,400 ± 4000 L mol C\(^{-1}\) d\(^{-1}\); Figure 6, Section S6).
The grand average biomass-normalized uptake rate constant ($k_{\text{in-app}}/C$) provides a simple means to derive Fe uptake rates from field data on a large spatial scale. In this way, our new insight into $k_{\text{in-app}}/C$ and Fe bioavailability can assist experimental design, data interpretation and modeling, taking advantage of the growing number of the high quality dFe measurements generated through the GEOTRACES program. However, care should be taken, as this approach applies only to Fe limited or stressed regions (where phytoplankton uptake systems are saturated). In addition, $k_{\text{in-app}}/C$ is derived from near-surface cells and may differ deeper in the euphotic zone due to variations in Fe speciation and cycling. Because of this, $k_{\text{in-app}}/C$ cannot be a simple replacement for the prognostic Fe uptake scheme in ocean models. These schemes require assumptions to be made around key parameters, like $V_{\text{max}}$ and $K_s$, or feedbacks linked to up- and downregulation of Fe uptake that until now have been impossible to validate. However, $k_{\text{in-app}}/C$ can be easily derived from the model output (Fe uptake rate, Fe quota, and dFe concentration), providing a unique way to assess the skill of modeled iron uptake rates in low Fe regions (see Section 4.4).

The presence of a reasonably constant $k_{\text{in-app}}/C$ for diverse Fe-limited communities across the ocean suggests that there is an emergent limit to the uptake rates that can be realized by these communities. This may follow logically from the underlying concept that Fe uptake is limited by biological surface area available for Fe transporters at the cell surface, which are responsible for accumulating Fe into cell biomass. Iron-limitation also places constraints on cell size (and hence S.A./vol), since Fe uptake by large taxa will be diffusion limited (Timmermans et al., 2001). Comparison of our $k_{\text{in-app}}$ values with estimated maximum diffusive fluxes of Fe' and FeDFB (Section S8), further demonstrates that cell surface/membrane space are restricting dFe uptake in the ocean, while diffusion limitation "kicks in" only for spherical cells greater than 60 μm in diameter (Figure S10). The consistency of this parameter also suggests that these communities have maximized Fe uptake and thus approach the physiological limits measured for monocultures in the laboratory.

Figure 7. Application of the bioavailability proxy $k_{\text{in-app}}/C$ to calculate Fe uptake rates in the ocean. We compare uptake rates measured in several studies (colored bars) with uptake rates calculated according to Equation 3 (hatched bars), using reported dFe and POC (or Chl). Studies are color coded: Blue-Ellwood et al., (2020) Green-Mellett et al., (2018), and Red-King et al., (2012).
4.3. Applying $k_{in-app}/C$ to Evaluate Biologically Derived dFe Residence Times in Low Fe Surface Waters

The C-normalized Fe uptake constant, $k_{in-app}/C$, can help quantify Fe cycling by providing a simple estimate of biological-derived dFe residence time in the upper ocean. Typically, this time is estimated by dividing the Fe concentration (or inventory) by phytoplankton Fe uptake rates. Since Fe uptake rates have been measured only in a handful of studies, biological-derived dFe residence times are rarely estimated. Inserting Equation 3 into the residence time calculation results in the following relationship:

$$\text{Biologically derived dFe residence time (d)} = \frac{1}{k_{in-app}/C \left( \text{L mol C}^{-1} \text{d}^{-1} \right) \times \text{POC (mol L}^{-1} \right)}$$

Equation 4 suggests that the timescales of biological Fe cycling in low Fe systems are a function of phytoplankton biomass (POC) but actually not of dFe. As above, since Fe uptake capabilities are maximized under Fe-limitation, rates of uptake (and hence residence times) are driven by biomass. POC levels in low-Fe regions are generally constrained between 2 and 10 µM (Bowie et al., 2001, 2015; Ellwood et al., 2020; King et al., 2012; Strzepek et al., 2005), resulting in predicted residence rates of 2.4–12 days. These estimates are very similar to residence times determined from direct measurements of Fe uptake and dFe in a range of low-Fe systems (Table S5), suggesting that our approach is robust.

These estimates of biologically derived Fe residence times are notably shorter than published residence times for dFe in the upper ocean. Black et al., (2020) calculated Fe residence times for major ocean basins by combining particulate Fe measurements with flux estimates from sediment traps and $^{234}$Th disequilibrium. They found upper ocean residence times of dissolved Fe, relative to biological Fe export, to range broadly from a month to a decade, suggesting that dFe is cycled 10 or more times, on average, before being exported with sinking biomass. This supports previous assertions that productivity in most systems is supported by recycled Fe (Boyd et al., 2005, 2017) and points to the likely role of biological processes in driving the decoupling between iron and other nutrient cycles (Rafter et al., 2017).

4.4. Applying $k_{in-app}/C$ to Constrain Model Simulations of Fe Uptake Rates

The observationally derived uptake constant $k_{in-app}/C$ can also be used to evaluate Fe uptake rates predicted by numerical ocean models. Iron uptake rates in ocean models are poorly constrained observationally and result from model assumptions regarding a suite of poorly known parameters that represent the cellular affinity and storage capacity for Fe (e.g., Aumont et al., 2015; Stock et al., 2014). Additionally, ocean model performance is usually evaluated and compared via static metrics such as dFe concentrations and distributions, but this approach can belie significant differences in Fe cycling and residence times (Tagliabue et al., 2016) and lead to important uncertainties in the response of Fe limited regions to climate change (Tagliabue et al., 2020). Indeed, a recent effort to evaluate Fe fluxes in the Pacific Ocean concluded that small vertical gradients in dFe obscure easy detection of large, and often opposing, Fe flux terms (Tagliabue et al., 2019). Thus, to improve ocean Fe models requires the ability to evaluate their underlying fluxes, especially those linked to biology. The $k_{in-app}/C$ constant provides a first-order way to do this.

As an example of this application, we use $k_{in-app}/C$ to constrain model simulations assessing the impact of climate change on ocean productivity. Recently, Tagliabue et al. (2020) utilized a comprehensive set of model simulations to predict changes in net primary production (NPP) and upper trophic level biomass in the Eastern Equatorial Pacific under the high emissions RCP8.5 climate change scenario. Their simulations utilized scenarios of high or low Fe uptake, which differ only in the maximum uptake rate applied but lead to differences in the strength of Fe limitation in the upper ocean. Simulations using high or low Fe uptake rates performed similarly in the present day (1986–2005), but the future NPP projections differed markedly (Tagliabue et al., 2020).

This region overlaps the EB04 cruise included in this study, presenting an opportunity to assess the different model scenarios using our field-derived $k_{in-app}/C$. In Figure 8, we plot EB04 cruise track, the observed $k_{in-app}/C$ of each station and the $k_{in-app}/C$ derived from the PISCES model. We first extracted for each station...
the modeled Fe uptake rates (present-day runs). Then, rearranging Equation 3, we derived $k_{\text{in-app}}/C$ from these Fe uptake rates using cell quotas and dFe concentrations (all from the model).

Our field-derived $k_{\text{in-app}}/C$ clearly support the model scenario with lower Fe uptake (Figure 8). The high Fe uptake model scenario shows $k_{\text{in-app}}/C$ that are much higher than EB04 observations, indicating that Fe bioavailability is overestimated in this model scenario. This unique assessment of the modeled biological Fe cycle suggests that the large decline in Equatorial Pacific NPP projected under the high Fe uptake scenario would be less likely to occur (Tagliabue et al., 2020) and highlights the usefulness of our observational based constant for constraining global ocean model predictions. Moreover, this exercise illustrates a means to widely assess the fidelity of modeled phytoplankton Fe uptake for Fe-limited regions across a wide range of available earth system models. In doing so, a novel constraint on the biological Fe cycle component of these models would be provided, which complements the common assessments against bulk properties, such as dFe or chlorophyll.

5. Conclusions

In this study, we quantified dFe bioavailability in the ocean by examining Fe uptake rate constants of in situ eukaryotic phytoplankton. To do so, we combined Fe quotas of 930 natural cells measured with synchrotron X-ray fluorescence, with modeled growth rates, and calculated in situ steady-state Fe uptake rates for single cells from diverse oceanic regions. We then divided these rates by concurrently measured dFe concentrations to estimate in situ Fe uptake rate constants ($k_{\text{in-app}}$). These $k_{\text{in-app}}$ were normalized to surface area or cellular C to derive $k_{\text{in-app}}/S.A.$ or $k_{\text{in-app}}/C$, respectively. The surface area normalized uptake rate constants ($k_{\text{in-app}}/S.A.$) show that cells in low-Fe systems have higher Fe uptake rate constants than cells in Fe-replete or N-limited systems, as expected from physiological studies. In order to use $k_{\text{in-app}}$ as a proxy of dFe bioavailability, only $k_{\text{in-app}}$ for Fe-limited or Fe-stressed cells were included, reducing our dataset to 560 $k_{\text{in-app}}$ values.

\[ \text{Figure 8. Utilization of } k_{\text{in-app}}/C \text{ values to constrain model predictions for the equatorial Pacific Ocean. In the upper panel, red circles show the average } k_{\text{in-app}}/C \text{ of low-Fe stations from the EB04 cruise along the equator. Stations with dFe below the detection limit have been removed. The black and blue symbols show } k_{\text{in-app}}/C \text{ derived from uptake rates used in the model of Tagliabue et al., (2020), corresponding to the stations location. The model was run assuming either high uptake scenarios (black squares) and or low uptake scenarios (blue diamonds). In the lower panel, the cruise track was plotted over satellite-measured chlorophyll (Chl, from Brzezinski et al., 2011), and relevant stations are highlighted in white circles, with cruise station numbers indicated.} \]
Focusing on Fe-limited/Fe-stressed regions, we observed that the surface area normalized uptake rate constants ($k_{\text{up}}$/S.A.) varied by ~five fold, over a broad latitudinal range, indicating consistent behavior across Fe-limited regions and enabling us to estimate a grand average Fe uptake rate constant for low Fe regions. Compared to specific Fe-complexes and species in seawater, the averaged seawater dFe availability is significantly higher than nonphotolabile siderophore-bound Fe, and somewhat lower than inorganic Fe'. The oceanic near-surface $k_{\text{up}}$/S.A. values agree well with culture-based estimates measured under natural sunlight, highlighting the importance of photochemical transformation of dFe in the well-lit surface waters for dFe bioavailability.

Other applications of these in situ dFe uptake rate constants, especially when normalized to cell C, were also illustrated, as we used the $k_{\text{up}}$/C to: (a) validate predictions from an ocean models; and (b) calculate biologically derived Fe residence times in euphotic zones. Furthermore, $k_{\text{up}}$/C can be utilized to estimate Fe uptake rates in remote Fe-limited systems for which POC and growth rates can be inferred from remote sensing (Tanioka et al., 2020). In the future, our Fe bioavailability proxy can be applied, alongside the growing database of phytoplankton cellular Fe quotas (Twining et al., 2021), to provide novel constraints on the biological Fe cycle component of earth system models in Fe-limited systems, which can improve the predictability of the response of upper ocean productivity to climate change.

Conflict of Interest
The authors declare no conflicts of interest relevant to this study.

Data Availability Statement
All data used in our analysis has been previously published and the original studies have been cited in the body of the manuscript. These data are available from the Biological and Chemical Oceanography Data Management Office (BCO-DMO) in the following datasets: https://www.bco-dmo.org/dataset/643270, https://www.bco-dmo.org/dataset/841640, https://www.bco-dmo.org/dataset/841583, https://www.bco-dmo.org/dataset/549122, https://www.bco-dmo.org/dataset/768064.

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