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The effect of acidification on the bioavailability and electrochemical lability of zinc in seawater

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A poorly studied but potentially important consequence of the CO_2-induced acidification of the surface ocean is a possible change in the bioavailability of trace metals, which play a critical role in the productivity and population dynamics of marine ecosystems. We report laboratory and field experiments designed to compare quantitatively the effects of acidification on the bioavailability of Zn, a metal essential to the growth of phytoplankton and on the extent of its complexation by model and natural ligands. We observed a good correspondence between the effects of pH on the rate of Zn uptake by a model diatom and the chemical lability of Zn measured by anodic stripping voltammetry (ASV). In model laboratory systems, the chemical lability and the bioavailability of Zn could either increase or decrease at low pH depending on the mix of complexing ligands. In a sample of coastal surface water, we observed similar increases in the ASV-labile and bioavailable Zn concentrations upon acidification, a result contrary to previous observations. These results, which can likely be generalized to other bioactive trace metals, *mutatis mutandis*, demonstrate the intricacy of the effects of ocean acidification on the chemistry and the ecology of surface seawater.

This article is part of the themed issue 'Biological and climatic impacts of ocean trace element chemistry'.

1. Introduction

As anthropogenic CO_2 dissolves into the ocean it makes surface seawater more acidic and leads to a cascade of chemical changes. Both the increase in CO_2 concentration and the indirect chemical changes that are caused by the decrease in seawater pH have been hypothesized to affect the growth of marine phytoplankton and hence the ecosystems that are supported by their photosynthetic production [1,2].

One potentially important and complex effect of ocean acidification on phytoplankton is that mediated by the changes in the chemical speciation of trace metals, particularly the extent of their binding to chelating compounds, which controls their bioavailability to the microbiota [2–5]. Some metals, like Fe and Zn, are essential for growth [6–8] and others, like Cu, may be toxic [9] such that an acidification-induced change in bioavailability could affect the productivity of marine ecosystems. Most obviously, a change in the bioavailability of Fe could have large consequences for the vast regions of the oceans that are Fe-limited [10]. It is also likely that changes in the bioavailability of trace metals would affect phytoplankton species differentially [4,11] and lead to a shift in the composition of phytoplankton assemblages with consequences for higher trophic levels.

Electrochemical measurements, mostly by anodic stripping voltammetry (ASV) and cathodic stripping voltammetry (CSV), have revealed that several bioactive trace metals, including Fe, Zn and Cu, are bound to strong organic chelating agents in surface seawater [12–15]. It is known from laboratory experiments that chelated metals are typically not available for uptake by phytoplankton. In the presence of a strong chelating agent, metal uptake rates are thus controlled by the free metal concentration, M′ (which includes the inorganic complexes of M with Cl^-, CO_3^{2-}, etc.) [16]. The extent of binding of a metal, M, to any given complexing agent, Y, is generally expected to decrease when the pH decreases because of the competition between M and H^+ for binding to Y. Neglecting the small effect of protonation of inorganic ligands and using α as the average degree of protonation of Y in seawater, the reaction can be written as follows:

$$MY + \alpha H^+ \rightarrow M' + H_\alpha Y. \tag{1.1}$$

As the pH decreases ([H^+] increases), the reaction goes to the right, increasing M′. The first-order expectation is that acidification should thus generally increase the bioavailability of a trace metal. Depending on the acid-based properties of Y, the situation should often be reversed for Fe as a result of the decrease in the concentration of hydroxide complexes, chiefly $Fe(OH)_2^+$ and $Fe(OH)_4^-$, which constitute the bulk of the bioavailable inorganic Fe(III) species [17].

There have been few experiments testing directly the effect of acidification on the bioavailability of trace metals to marine phytoplankton [4,17,18]. Surprisingly, contrary to the expectation of higher M′, and thus higher bioavailability, at lower pH, some field experiments with natural samples from the Gulf of Alaska and the New Jersey coast showed reduced phytoplankton uptake rates for Zn and Cd by the ambient phytoplankton population at lower pH values [18]. Laboratory experiments have shown that the effect of acidification on metal uptake rates in the presence of strong chelators corresponds quantitatively to the calculated change in M′, thus demonstrating that it does not result from a direct effect of pH on the efficiency or regulation of the metal uptake machinery of the cells [17,18]. It is thus the bioavailability of the metals that must have somehow decreased at low pH in the field experiments. This counterintuitive result has been ascribed to a decrease in the concentration of bioavailable complexes of Zn and Cd with weak ligands, L. The bioavailability of a variety of weak Zn complexes—including the cysteine, phytochelatin and histidine complexes, respectively, Zn-Cys, Zn-PC2 and Zn-His—has been previously demonstrated [19]. A decrease in the concentration of the bioavailable metal, M″, which is the sum of the free and bioavailable weak complexes ($M'' = M' + [ML]$), with decreasing pH is possible in the presence of a strong chelator, Y, that has a lower degree of protonation in seawater than the weak ligands L. Under the typical condition $[ML] > M'$, and with α and β

denoting the average degree of protonation of Y and L, respectively, the main reaction is written

$$MY + H_\beta L \rightarrow ML + H_\alpha Y + (\beta - \alpha)H^+. \tag{1.2}$$

If $\beta > \alpha$, the reaction goes to the left when the pH decreases, thus decreasing [ML] and the bioavailable concentration M″. A proof of concept for this *two ligand mechanism* was obtained in Zn-limited laboratory cultures containing EDTA as the strong chelator and cysteine or phytochelatin (the dimer PC2) as the weak ligand [18]: the uptake of Zn and Cd by the diatom *Thalassiosira weissflogii* in the presence of the two ligands indeed decreased at low pH.

In a previous laboratory study with the model ligands EDTA and histidine (His), we demonstrated that the concentration of Zn available for uptake by *T. weissflogii* could be quantified by ASV [20]. While the free Zn concentration, Zn′, was measured by ASV at a plating potential of *ca* −1.1 V (versus Ag/AgCl), the bioavailable concentration Zn″ = Zn′ + [ZnHis] was measured at the slightly more negative plating potential of −1.2 V (for background on the use of electrochemical techniques to study the chemical speciation of trace metals in seawater, see Croot *et al*. [21] and Lewis *et al*. [22]). The good correspondence between the electrochemical and the uptake data confirmed the previous report of the bioavailability of the 1 : 1 Zn-His complex.

The observed agreement between the rate of Zn uptake measured in biological uptake experiments and the labile Zn concentration measured by ASV (at the appropriate plating potential) opens up the possibility of quantifying by electrochemistry the bioavailable Zn in complex chemical systems without direct knowledge of what complexing agents may be present. This would be particularly useful in surface seawater that contains a myriad unidentified organic compounds, many of which may be binding trace metals. Properly applied and interpreted, the same electrochemical techniques that have demonstrated that the bulk of several biologically important trace metals are bound to strong (unknown) chelators in the surface ocean could be useful for quantifying the bioavailability of these metals and how it may change with environmental conditions such as acidification.

Here we report experiments comparing the effects of acidification on the labile concentration of Zn measured by ASV and on the rate of Zn uptake by the model diatom *T. weissflogii*. We observed a quantitative agreement between the two in laboratory media containing a single complexing agent, where the labile and bioavailable Zn concentration increased similarly with decreasing pH. Importantly, a similar correspondence in the changes of the ASV-labile and bioavailable Zn concentration was seen in media containing a strong and a weak complexing agent chosen to evince the opposite pH response as a result of the *two ligand mechanism*. Finally, the effects of acidification on the ASV-labile and bioavailable Zn concentrations in a sample of coastal seawater were also observed to be quantitatively similar. But, instead of resulting in a decreased uptake rate as seen in previous experiments [18], the lower pH resulted in higher ASV lability and bioavailability. Our results both support the usefulness of electrochemical measurements to quantify changes in metal bioavailability and illustrate the complexity of the response of trace metal speciation to ocean acidification.

2. Material and methods

(a) Media preparation and field sample collection

The media were prepared using 0.2 µm filtered and microwave-sterilized Sargasso seawater, collected away from the coast of North Carolina and stored at 4°C, which contains less than 4 nM Zn and approximately 1.2 nM strong Zn ligand, as measured by voltammetry. The media were enriched with chelexed and filter-sterilized macronutrients (100 µM NO_3^-, 10 µM PO_4^{3-}, 100 µM $Si(OH)_4$), filter-sterilized f/2 vitamins and trace metals (20 nM Cu, 120 nM Mn, 10 nM Se, 1 µM Fe) complexed with model ligands in excess (His-only, His + EDTA, and EDTA-only). The concentrations of ligands and Zn in each experiment are given in the figure legends. pH was

adjusted to target experimental values (pH 7.9 and 8.3) by adding ultrapure hydrochloric acid or sodium hydroxide and measured on the total hydrogen ion scale by thymol blue [23].

Surface seawater collected from the New Jersey coast (40.18° N and 73.55° W) in May 2014 using a trace metal clean pumping system containing a background Zn concentration of approximately 10 nM was stored at 4°C until analysis. Aliquots were dispensed into acid-cleaned bottles and enriched with chelexed and filter-sterilized macronutrients (100 µM NO_3^-, 10 µM PO_4^{3-}, 100 µM $Si(OH)_4$) and filter-sterilized f/2 vitamins. The pH was manipulated and measured as described earlier.

(b) Zn speciation calculation and voltammetric measurements

The Zn speciation in the media with model ligands was computed with the thermodynamic equilibrium program MINEQL+ [24], using stability constants taken from the MINEQL database and Morel & Hering [25] and corrected for seawater according to Morel & Hering [25] and Sunda *et al*. [26]. ASV measurements were made by using a static mercury drop electrode (663VA Stand and PGSTAT 128N potentiostat, Metrohm-Autolab), an Ag/AgCl, KCl (3 M) reference electrode with a 3 M KCl salt bridge, and a glassy carbon counter electrode. Pseudopolarograms were obtained in the presence of a 45 mM borate buffer with samples initially purged with high-purity N_2 (5.0, Airgas) for 200 s and before each measurement for 20 s. The pseudopolarography experiments involved the measurement of ASV stripping peak currents at increasingly negative deposition potentials (−0.9 to −1.5 V, $\Delta E = 0.01$ V, deposition time 90 s). After an equilibration time of 10 s, the potential was ramped from −1.2 V to 0 V using a square wave voltammetry (scan rate 0.12 V s^{-1}; amplitude 0.02 V; frequency 60 Hz). ASV titration experiments were performed in 60 ml Teflon bottles spiked with varying Zn concentrations (His-only: 0−60 µM; EDTA + His and EDTA-only: 0−160 µM; NJ coastal seawater: 0−1 µM). To minimize adsorption of analyte onto the wall, the bottles were rinsed with a small aliquot of samples. The samples were allowed to equilibrate overnight in seawater in the presence of a 50 mM borate buffer and various concentrations of Zn adjusted to the target pH values (less than ±0.1 pH units throughout the titrations). The deposition time was varied from 90 s (EDTA and EDTA + His at high reducible Zn concentrations, His-only, NJ coastal seawater) to 1 h (EDTA and EDTA + His at low reducible Zn concentrations). The data were normalized by using the average ratio of the currents measured with both deposition times in the intermediate concentration range. Stripping was performed by square wave voltammetry analogous to the pseudopolarographic analyses. The sensitivity (nA/nM) to convert the measured currents to labile Zn concentrations was determined from the slope of the linear portions of the titration curve at high Zn concentrations when the ligands were completely titrated.

(c) Short-term uptake of Zn

Thalassiosira weissflogii CCMP1336 was obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton in Maine. Cells for short-term uptake were first grown at 20°C with continuous light (80–100 µmol quanta m^{-2} s^{-1}) under Zn-limited conditions. The low-Zn media were prepared using 0.2 µm filtered and microwave-sterilized Sargasso seawater enriched with chelexed and filter-sterilized macronutrients, filter-sterilized f/2 vitamins and trace metals (see above) buffered with 100 µM EDTA. Zn was added as the ZnEDTA complex (1 : 1.1) ($Zn' =$ approx. 6 pM, $Zn_T = 29$ nM at pH 7.9). Acid-cleaned polycarbonate bottles were used for uptake experiments. ^{65}Zn (Oak Ridge National Laboratory) was used as tracer with a specific activity adjusted in each experiment. When EDTA was used as the metal binding ligand, the uptake media were equilibrated overnight before adding Zn. When His was used as the ligand in the two ligand system, Zn was pre-equilibrated with it in a small volume of Milli-Q water for approximately 4 h, and then the complex was added into the seawater with EDTA to equilibrate for another approximately 4 h before adding cells. When His or natural ligands were used as the only metal binding ligand, Zn was equilibrated with the ligand overnight before adding cells.

The concentrations of ligands and Zn in each experiment are given in the figure legends. Zn-limited cells were filtered onto acid-cleaned polycarbonate membrane filters, rinsed five times with 0.2 µm filtered Gulf Stream seawater, resuspended in seawater and dispensed into the uptake bottles. At intervals of 0.5–1 h for a total incubation period of 2–4 h, 20 ml aliquots from each bottle were removed and filtered onto 3 µm polycarbonate membrane filters. Cells were then washed with an oxalate-EDTA solution for 5 min [27], and ^{65}Zn retained on the membrane was measured via liquid scintillation counting. The pH was measured at the beginning and the end of the uptake experiment by thymol blue. To quantify the cell-normalized uptake rate, the cell concentration in each bottle was measured at the end.

3. Results

(a) Effects of pH on the reducible Zn concentration (pseudopolarograms)

Previous experiments at a fixed pH showed a good quantitative correspondence between the labile Zn concentration measured by ASV at a plating potential of −1.2 V (versus Ag/AgCl) and the rate of Zn uptake by *T. weissflogii* in media containing EDTA-only or His + EDTA as complexing agents [20]. ASV measurements under variable pH conditions have rarely if ever been performed, however, so we first compared the stripping currents measured at pH = 8.3 and 7.9 as a function of plating potential (from −0.9 to −1.5 V versus Ag/AgCl) in media containing either His-only or His + EDTA. As seen in figure 1, the shapes of the resulting pseudopolarograms are quite similar and are determined by the reduction of inorganic Zn and Zn-His complexes while Zn-EDTA complexes remain inert. Under all four conditions, the current becomes measurable above −1.05 V and plateaus above −1.4 V. It exhibits two or three inflection points in between as a result of the reduction of Zn(II) from Zn′, ZnHis and Zn(His)$_2$ at increasingly negative potentials, corresponding to the increasing thermodynamic stabilities of the species: Zn′ < ZnHis < Zn(His)$_2$. Importantly, in the presence of His-only, the measured stripping current is systematically higher at pH 7.9 than at pH 8.3, while the reverse is true in the presence of His + EDTA. This is what is expected as a result of the weak acid properties of His in the His-only experiment and the *two ligand mechanism* in the presence of His + EDTA. The inflection point previously observed at −1.2 V [20] is also noticeable in all the pseudopolarograms in this study and is thus again selected for the quantification of Zn″. The current measured at −1.1 V was previously used for quantifying Zn′ [20]. But, in the presence of EDTA, this current is significantly higher at pH = 8.3 than at pH = 7.9, while the value of Zn′ should change very little according to equilibrium calculations (see below). As a result, we chose the slightly lower potential of −1.05 V for the determination of Zn′.

(b) Calculated and measured variations of Zn″ and Zn′ as a function of pH

We compared the ASV-measured values of Zn″ and Zn′ (using plating potentials of −1.2 V and −1.05 V, respectively) with the calculated values over the pH range of 7.6 to 8.4 in media containing His-only and His + EDTA. As shown in figure 2, the qualitative agreement between the experimental data and the calculations is remarkable: the trends in the variations of Zn″ and Zn′ with pH are the same in both, as are the relative values of each throughout the pH range. The quantitative agreement is also quite good, except at high pH where the measured values of Zn″ are somewhat larger than the calculated values. This offset could be due to errors in the calculations of Zn″, which are sensitive to the exact values of the equilibrium constants in the range of interest where both the ZnHis and Zn(His)$_2$ complexes are important. The published values for these constants exhibit some variations and must be extrapolated to the high ionic strength of seawater.

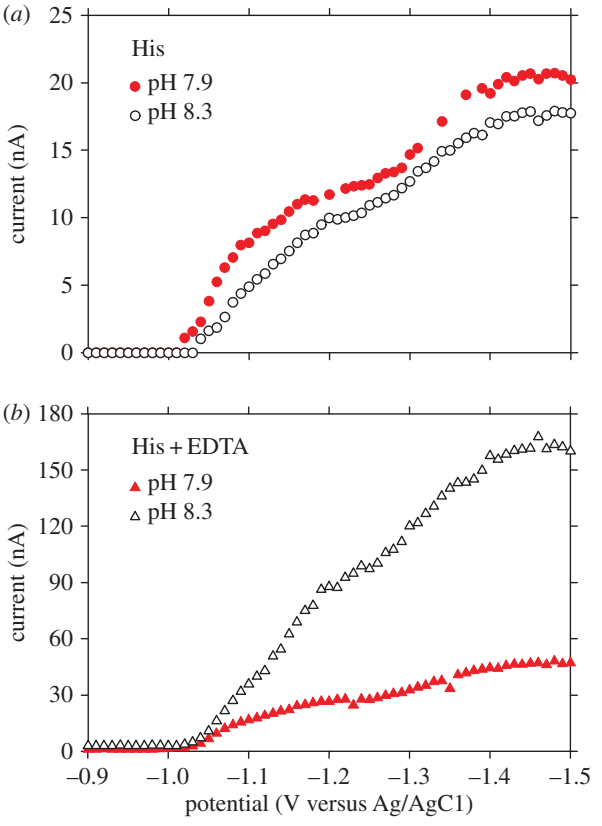

Figure 1. Pseudopolarograms for the reduction of Zn in seawater medium: (*a*) His-only and (*b*) His + EDTA, at pH 7.9 (filled symbols) and pH 8.3 (open symbols) (90 s deposition time). The total Zn and ligand concentration in His-only: 50 nM Zn_T and 40 µM His; His + EDTA: 20 µM Zn_T, 40 µM His and 100 µM EDTA. (Online version in colour.)

(c) Anodic stripping voltammetry-labile Zn concentration versus Zn uptake rate

In the view of the coherence of the ASV data obtained at different pHs, we compared, in the same media, the ASV-labile concentration of Zn and the rate of Zn uptake by *T. weissglogii*, measured in short-term (2–4 h) experiments at pH = 7.9 and 8.3. Three media were used, containing, respectively: (i) 40 µM His-only + 5 nM Zn, (ii) 40 µM His + 100 µM EDTA + 50 nM Zn, and (iii) 100 µM EDTA + 50 nM Zn. As shown in figure 3, the variations in measured Zn″ and Zn uptake rate mirrored each other. As expected, in the presence of EDTA-only, the Zn uptake rate and the ASV-labile Zn concentration were both very low with little influence of pH (figure 3*c*). The addition of His in the EDTA-buffered media increased both Zn″ and the uptake rates considerably, and more so at pH = 8.3 than at pH = 7.9 as expected from the *two ligand mechanism* (figure 3*b*). Under these conditions, the quantitative correspondence in the effects of pH on the measured Zn″ and on Zn uptake rate is excellent.

In the presence of His-only, both Zn″ and the uptake rate increased at lower pH as expected, but the relative increase in uptake rate was somewhat larger than that of Zn″ (figure 3*a*). In this experiment, which required the use of ^{65}Zn as a radiotracer, the total Zn concentration was 5 nM, the minimum concentration that could be dispensed precisely, even under metal clean conditions. Since both the free and the 1 : 1 Zn-His complex are bioavailable, the values of Zn″ (2.3 nM at pH = 8.3 and 3.4 nM at pH = 7.9) were far in excess of the bioavailable Zn in the Zn-limited stock cultures ($Zn' = 6$ pM). As a result, the Zn-limited cells were in the process of downregulating their high-affinity Zn transport system over the course of the two-hour uptake assays [28]. This is evidenced by the large intercept at time zero of the linearly fitted uptake data, which indicates

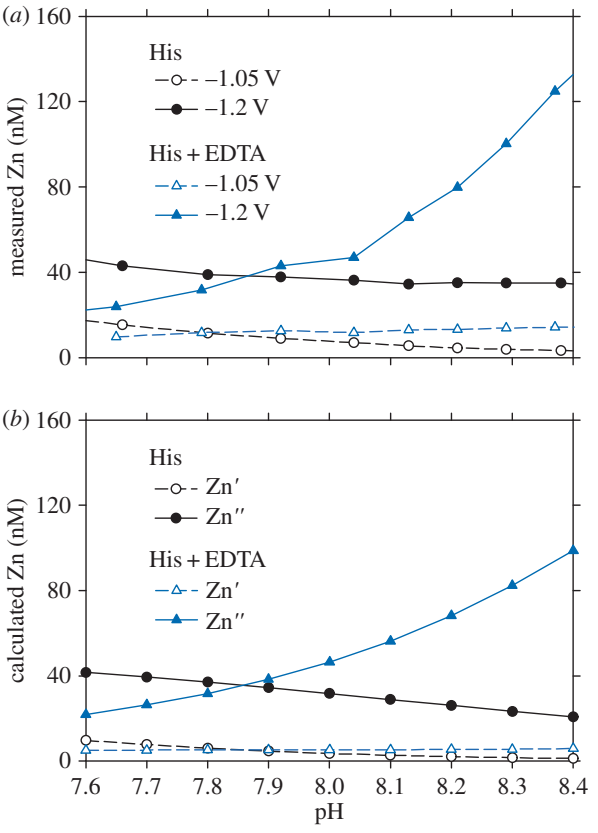

Figure 2. (*a*) ASV-measured values of Zn′ and Zn″ using plating potentials of −1.05 V (open symbols with dashed lines) and −1.2 V (filled symbols with solid lines) (90 s deposition time), respectively, and (*b*) calculated values of Zn′ (open symbols with dashed lines) and Zn″ (filled symbols with solid lines) over the pH range of 7.6–8.4 in media containing His-only (circles) and His + EDTA (triangles). The total Zn and ligand concentration in His-only: 50 nM Zn_T and 40 µM His; His + EDTA: 20 µM Zn_T, 40 µM His and 100 µM EDTA. The conditional equilibrium constants (log *K*) were taken from Morel & Hering [25] and the MINEQL database (ZnEDTA 18.3, ZnHis 7.3 and $Zn(His)_2$ 13.0, at $I = 0$ M). The constants were corrected for an ionic strength (*I*) of 0.5. (Online version in colour.)

a rapid initial decrease in uptake rate (electronic supplementary material, figure S1). Initially, the cells took up both the inorganic and His-bound Zn (as quantified by Zn″) via their high-affinity Zn uptake system, but over time they shifted to take up only the inorganic Zn (as quantified by Zn′) via their low-affinity uptake system. In contrast to the high-affinity one, the low-affinity Zn uptake system of *T. weissflogii* has been shown not to transport the Zn-cysteine complex [19] and the same is likely true of the Zn-His complex. Eventually, the uptake rate is expected to become proportional to Zn′ and be lower by a factor of nearly 3 at pH = 8.3 compared to pH = 7.9 (Zn′ = 0.16 pM versus 0.47 pM).

(d) Anodic stripping voltammetry measurements and uptake rates in field samples

Using a sample of filtered surface seawater from the New Jersey coast, we performed Zn uptake experiments using *T. weissflogii* as the test organism and performed ASV measurements of Zn′ and Zn″ (using plating potentials of −1.05 V and −1.2 V) as well as total Zn concentrations (after UV digestion and acidification). The ASV data show that, in this coastal water sample, the Zn was weakly complexed, with Zn″ values of 4.3 nM and 5.3 nM (at pH = 8.3 and 7.9 respectively; figure 4*a*), compared to a total Zn concentration of approximately 10 nM. As shown in figure 4*a*,*b*,

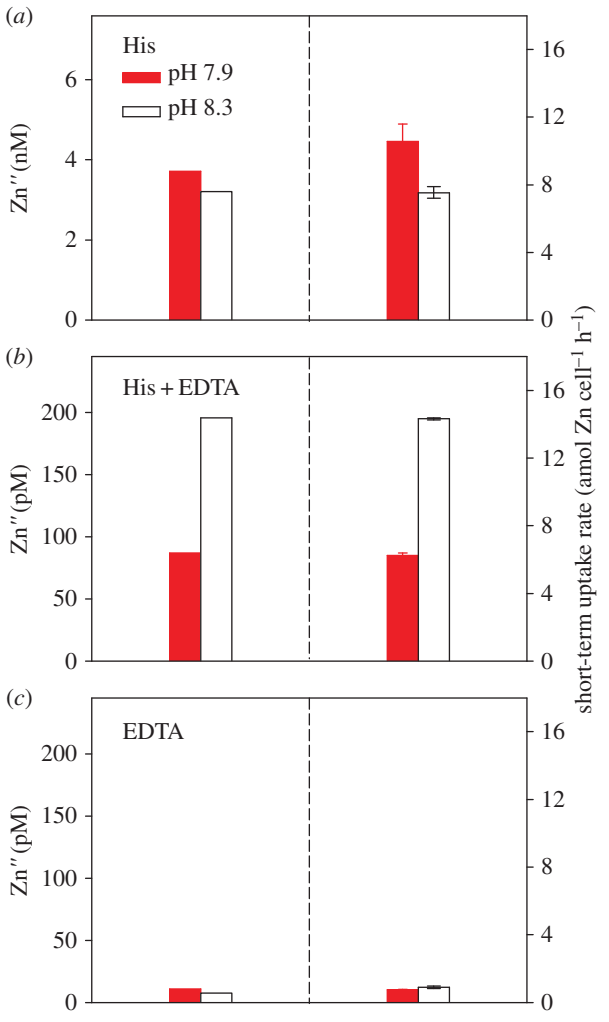

Figure 3. The measured value of Zn″ (i.e. Zn_{labile} at a plating potential of −1.2 V) and the short-term Zn uptake rate by Zn-limited *T. weissflogii* at pH 7.9 and pH 8.3. The total Zn and ligand concentrations in (*a*) His-only: 5 nM Zn_T and 40 µM His; (*b*) His + EDTA: 50 nM Zn_T, 40 µM His and 100 µM EDTA; (*c*) EDTA-only: 50 nM Zn_T and 100 µM EDTA. (Online version in colour.)

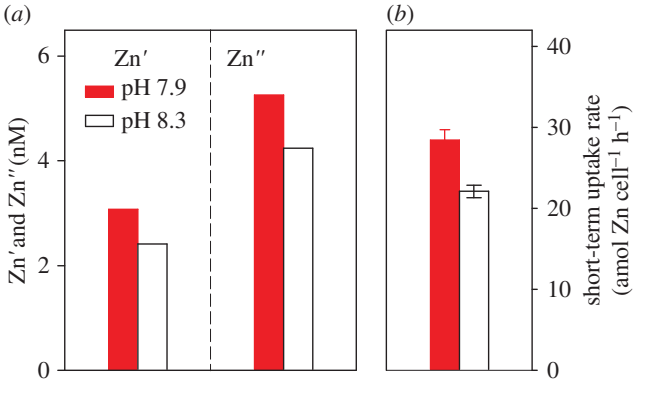

Figure 4. (*a*) The measured values of Zn′ and Zn″ (i.e. Zn_{labile} at plating potentials of −1.05 V and −1.2 V, respectively) and (*b*) the short-term Zn uptake rate by Zn-limited *T. weissflogii* at pH 7.9 and pH 8.3 in New Jersey coastal seawater with the addition of 0.5 nM ^{65}Zn. (Online version in colour.)

we observed a very good quantitative agreement between the effects of pH on Zn″ and on uptake rates: both increased by about 25% when the pH decreased from 8.3 to 7.9.

In this experiment with a field sample, as in the His-only laboratory experiment described above, the previously Zn-limited cells were exposed to high concentrations of bioavailable Zn in the course of the short-term uptake assays. The result was again a downregulation of the high-affinity Zn uptake system of *T. weissflogii* early in the assay as indicated by the high intercept at time zero of the linearly fitted uptake data (electronic supplementary material, figure S1). But in this case the effect of the low pH on the bioavailable Zn concentration, which changes from Zn″ initially to Zn′ eventually, remains nearly constant over time (an increase of 24 versus 28%, figure 4*a*), explaining the good agreement of the Zn speciation and uptake data.

4. Discussion

This study is the first to experimentally investigate simultaneously the effect of acidification on the speciation of Zn and its bioavailability to phytoplankton. It extends previous work that demonstrated a correspondence between the ASV-labile concentration of Zn (measured at an appropriate plating potential) and its bioavailability to phytoplankton at a given pH in media containing a variety of complexing agents [20].

Our laboratory results confirm the bioavailability of weak trace metal complexes, such as the 1 : 1 Zn-His complex, making the quantification of such complexes in surface seawater important for understanding the effect of pH on the interactions of trace elements and the microbiota. Our laboratory data also support the *two ligand mechanism* by which the bioavailability of a trace metal can decrease at low pH in the presence of two ligands, one which makes a bioavailable complex and the other not, when the degree of protonation of the weak ligand in seawater is greater than that of the strong ligand [18]. But our experiments with a New Jersey coastal water sample demonstrated an increased Zn uptake rate at lower pH as is simply expected from the protonation of complexing ligands. This observation, which is contrary to the previous observations of Xu *et al.* [18] in a similar water sample, was supported by a quantitatively similar increase in Zn″, the concentration of bioavailable Zn. The contrast between our new results and those of Xu *et al.* [18] illustrates that the effect of acidification on Zn bioavailability may vary temporally and spatially in both extent and direction. It also indicates that such variability likely reflects changes in the mix of strong and weak ligands that bind Zn in surface seawater over time and space [29].

It seems likely that acidification should have similarly varied effects on the bioavailability of other divalent metals, such as Fe(II), Co(II), Ni(II) and Cu(II), whose complexation by organic ligands in surface seawater is effected through the coordination of the same binding moieties, albeit with different affinities. In the case of a metal such as Cu(II) which forms relatively stable 1:1 and 1 : 2 carbonato complexes, the decrease in $[CO_3^{2-}]$ at low pH can balance out the increased protonation of organic complexing agents and result in little change in the value of Cu′ as observed by Gledhill *et al.* [5] with samples of water from the English south coast. The situation is different for Fe(III) as a result of the formation of hydroxo complexes, chiefly $Fe(OH)_2^+$ and $Fe(OH)_4^-$, that dominate its inorganic speciation in seawater. With the exception of some catecholate siderophores, most chelators consume protons upon binding of $Fe(OH)_2^+$ and $Fe(OH)_4^-$ in seawater such that the value of Fe′ should usually decrease at low pH as observed by Gledhill *et al.* [5]. Accordingly, Fe bioavailability in model systems containing strong chelating agents usually decreases with acidification [17]. The presence of complexing agents that form bioavailable weak Fe(III) complexes should not change this result. A decrease in Fe(III) bioavailability has been observed so far in the few field experiments that have been reported [17]. This result could be different in the presence of excess particulate Fe when acidification increases the dissolved Fe concentration [4].

The correspondence observed so far between ASV measurements and biological uptake rates raises hope that we may be able to quantify experimentally the effects of acidification on the bioavailability of some trace metals without a detailed knowledge of the chemical identity of the many organic ligands that may be complexing those metals at a given time and place. In

fact, as previously discussed, ASV titrations can quantify the bioavailable concentration of a trace metal M″, including the free and weakly complexed species, without even revealing the presence of the weak complexes [20]. One issue is the correspondence between the bioavailability and the electrochemical lability of metals in the presence of dissolved organic matter that exhibits a continuous range—often a bimodal distribution—of affinities for trace metals. Additional field experiments, comparing ASV-labile concentrations of Zn, Cd and Cu to uptake rates by model organisms and ambient populations, will be necessary to generalize the validity of the approach. This should be relatively straightforward in the case of Cd, which like Zn has a convenient radiotracer (^{109}Cd) for biological experiments at very low concentrations; it will more difficult for Cu which will require performing biological uptake experiments either without the help of a radiotracer or with the short-lived ^{67}Cu.

Measurements by competitive ligand exchange, CSV, have been very useful in demonstrating that some metals like Fe, which cannot be measured by ASV, are complexed to strong ligands in surface seawater [12]. But the use of CSV for studying the effects of pH on the chemical speciation and bioavailability of these metals is not straightforward as changes in pH affect the metal affinity of the added plating ligand and its adsorption on the electrode surface, thus requiring a re-calibration of the system at each experimental pH value to be tested. This is unfortunate for Fe is known to limit the growth of phytoplankton in large regions of the ocean such that the potential effect of acidification on Fe bioavailability is of particular interest.

5. Conclusion

The question of how marine ecosystems will be affected by ocean acidification is one that stretches the limits of our scientific knowledge. Our understanding of the global biogeochemical cycles of elements, and of the physics, chemistry and biology of the oceans is still too incomplete to provide us with robust predictive capabilities regarding the consequences of as pervasive a change as that of the CO_2 concentration in the atmosphere. As discussed above, even the seemingly simple question of how acidification of surface seawater should affect the bioavailability of trace metals to the biota is complicated. Depending on the mix of ligands to which it is bound, the bioavailability of a trace metal may increase or decrease as the pH decreases. With very few exceptions, the chemical identities of the organic compounds that complex metals in seawater are unknown. Even if the application of new analytical techniques offers the hope of identifying the major strong chelators in seawater [12], it is doubtful that we will obtain a detailed knowledge of all the strong and weak ligands that control the bioavailability of the metals. The nature and concentration of such ligands likely change over space and time. In this situation, our ability to quantify the effects of acidification on trace metal bioavailability will be enhanced by the further development and validation of techniques such as ASV, that provide an overall quantification of the 'reactivity' of the metals of interest.

Data accessibility. Supporting data are provided in the electronic supplementary material.

Authors' contribution. J.-M.K. carried out the laboratory work and analysed data. O.B. participated in the design of the study and interpreted data. F.M.M.M. designed the research, interpreted the data and wrote the paper. All authors gave final approval for publication.

Competing interests. The authors declare no competing interests.

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