ANNUAL REVIEWS

Annual Review of Marine Science

Metal Organic Complexation in Seawater: Historical Background and Future Directions

James W. Moffett[1] and Rene M. Boiteau[2,3]

[1]Department of Biological Sciences, University of Southern California, Los Angeles, California, USA; email: jmoffett@usc.edu

[2]College of Earth, Ocean, and Atmospheric Sciences, Oregon State University, Corvallis, Oregon, USA

[3]Department of Chemistry, University of Minnesota, Minneapolis, Minnesota, USA; email: rboiteau@umn.edu

Annu. Rev. Mar. Sci. 2024. 16:577–99

First published as a Review in Advance on September 18, 2023

The *Annual Review of Marine Science* is online at marine.annualreviews.org

https://doi.org/10.1146/annurev-marine-033023-083652

Keywords

organic ligands, chelators, transition metals, scavenging, bioavailability, coordination, siderophore

Abstract

The speciation of most biologically active trace metals in seawater is dominated by complexation by organic ligands. This review traces the history of work in this area, from the early observations that showed surprisingly poor recoveries using metal preconcentration protocols to the present day, where advances in mass spectroscopy and stable isotope geochemistry are providing new insights into the structure, origin, fate, and biogeochemical impact of organic ligands. Many long-standing hypotheses about the specific biological origin of ligands such as siderophores in seawater are finally being validated. This work has revealed the complexity of organic complexation, with multiple ligands and, in some cases, timescales of ligand exchange that are much slower than originally thought. The influence of organic complexation on scavenging is now a key parameter in biogeochemical models of biologically essential metals, especially iron. New insights about the sources and sinks of ligands are required to enhance the usefulness of these models.

HISTORICAL BACKGROUND

The inorganic composition and physical chemistry of seawater are very well characterized (Millero 2001). There is also great deal of information about the concentration and distribution of trace elements in seawater (Schlitzer et al. 2018). With all of this information, it is fairly straightforward to predict the speciation and redox chemistry of virtually every element in seawater based on thermodynamic data for their respective interactions with the major inorganic components of seawater. Yet, with the huge advances in the analytical chemistry of trace elements, particularly transition metals, in the 1970s, it became clear that there were fundamental differences between the predicted chemistry of individual elements and their actual behavior.

For most elements, preconcentration was required for the analysis of transition metals by graphite-furnace atomic absorption spectrophotometry—the principal means of analysis at the time. Preconcentration methods were developed utilizing ion exchange resins, solvent extraction, or coprecipitation of metal chelates and evaluated with artificial seawater. However, recoveries in natural samples were exceptionally low unless preconcentration was preceded by acidification or ultraviolet irradiation (Batley & Florence 1976, Bruland et al. 1979). Furthermore, measured concentrations of some dissolved metals, such as iron (Fe), often exceeded their inorganic solubilities in seawater (Byrne & Kester 1976). These observations led many investigators to hypothesize that metals were complexed by organic ligands. (While the terms ligand and chelator are often used interchangeably in this research, chelator denotes a polydentate ligand with at least two bonds to the central metal ion; when the structure is unknown, the more generic term, ligand, is preferable.) Organic complexation could explain low yields, since ambient ligands would compete with the synthetic chelators used in analytical protocols. Moreover, UV irradiation would destroy organic ligands. Acidification would protonate or hydrolyze natural ligands, while synthetic ligands used in preconcentration such as dithiocarbamates would be unaffected because of their exceptionally low acid dissociation equilibrium constants.

The hypothesis of organic complexation also supported the comparative properties of each element. For example, the discrepancy between the actual and predicted recovery was greatest for copper (Cu) (Bruland et al. 1979). This is in accord with the well-known trend (Irving–Williams order) of increasing stability of complexes formed by first-row transition metals with increasing atomic number to a maximum with Cu(II) (Bruce 1987). Divalent manganese (Mn), by contrast, showed comparable recoveries in artificial seawater and seawater, and there is still no evidence for the organic complexation of Mn(II). One notable exception was Fe, for which evidence for organic complexation remained inconclusive much longer. In part, that was because Fe(III) is strongly hydrolyzed at seawater pH, and the effect of acidification on recovery could be rationalized by converting hydrolyzed forms into more reactive forms. Fe is also a highly contamination-prone element, and that issue had to be resolved first.

By contrast, the analytical chemistry of trace elements that are not expected to form complexes did not require UV irradiation, including oxyanions (such as molybdate and vanadate) and cations expected to form weak complexes (such as aluminum, gallium, barium, and the rare earths). The formation of weak complexes for these cations could not be ruled out, but they do not interfere with preconcentration methodologies.

By the late 1970s, oceanographers had begun to look beyond organic complexation as merely an analytical challenge and started thinking about its implications for geochemistry and biology. This period saw a rapid increase in our understanding of the role of metals in biology, particularly the impact of metals on phytoplankton and bacteria at the base of marine food chains. Trace metals are micronutrients, and metal uptake by bacteria and phytoplankton can also be viewed as an exchange reaction between cellular uptake sites and ligands in solution. Biologists working with marine microbes in culture recognized analogies between commonly used metal ion buffers in media, such as ethylenediaminetetraacetic acid (EDTA) and citrate, and naturally occurring ligands (Brand et al. 1986). Metal ion buffers control bioavailability by regulating the steady-state concentration of free metal ions in the media, which is what the organism perceives.

Early researchers recognized that organic ligands in seawater have the same vital role as metal ion buffers in media—maintaining metal ions at ranges that are neither too toxic nor growth limiting (Sunda 1989, Sunda & Huntsman 1998). For example, Cu toxicity is especially important at the base of marine food chains. Sunda and coworkers showed that Cu toxicity was associated with free cupric ion (Cu^{2+}) concentrations and that at the ambient Cu concentrations found in seawater, the ambient cupric ion concentration would be lethal to most phytoplankton taxa if Cu was not organically complexed (Sunda 1989, Sunda & Huntsman 1998). Indeed, organic complexation lowers free cupric ion concentrations by three to five orders of magnitude compared with the concentrations in inorganic seawater.

Anthropogenic Cu contamination can lead to specific effects on Cu-sensitive taxa when strong ligands become saturated. Moffett et al. (1997) showed that when water was advected into two small boat harbors, a fivefold increase in total dissolved Cu derived from antifouling paint increased the free cupric ion concentrations by three orders of magnitude when strong ligands were saturated. This increase contributes to the demise of the Cu-sensitive cyanobacterium *Synechococcus* sp. as well as the induction of phytochelatin production by eukaryotes as a metal stress response (Ahner et al. 1997). Subsequently, Mann et al. (2002) argued that photochemical decomposition of Cu ligands in the Sargasso Sea mixed layer—and the resultant increase in biologically available Cu—is associated with the emergence of Cu-tolerant, high-light-adapted ecotypes of *Prochlorococcus* sp.

Many workers also looked at terrestrial systems for insight into marine processes. In the case of Fe, the free ion concentration is insufficient to sustain growth in many regimes because of competition from natural ligands and particle surfaces. Many microbes produce their own Fe-binding organic ligands, called siderophores, to form complexes that can be taken up without a free ferric ion intermediate (Neilands 1995). However, it would take nearly four decades for conclusive evidence of siderophores to be found in seawater.

One important consideration was the impact of organic complexation on the distribution and residence time of metals in the water column. Initially, it was thought that organic complexation would enhance the removal of metals from the water column, because attempts to characterize marine organic matter were (and often still are) based on extracting materials onto hydrophobic resins. This process selects for a fraction of marine dissolved organic matter with a nonpolar component, and such material has strong metal-binding properties, in agreement with other work using terrestrial organic materials. Such compounds might be expected to adhere to sinking particles (Balistrieri et al. 1981, Davis 1984), providing a pathway for metal removal. Subsequent work has shown that most organically bound metals are not particularly particle reactive and has favored the alternative hypothesis that on balance, organic complexation increases the residence time of metals in the water column (Gledhill & Buck 2012). In that respect, it is similar to synthetic complexation with chelators such as EDTA (Hunter et al. 1988), although, as we discuss further below, a strict analogy with EDTA is not consistent with some key properties of marine ligands (see point 4 in the next section).

Systematic Investigation of Metal Complexation Using Titrations

The first systematic studies of organic complexation in seawater were aimed at determining the conditional stability constants and concentrations of the organic ligands. Many such studies have been based on a well-established methodology from chemistry utilizing competitive ligands (Helfferich 1962).

Consider a solution containing a ligand with a known concentration but unknown binding strength. A competing ligand of known concentration and binding strength is added that forms a complex with the metal. Partitioning of the metal between each complex can be quantified and used to determine the unknown stability constant. The complex with the known ligand must have some analytically useful property that can be measured without perturbing the equilibrium between added and ambient ligands. While competing ligand experiments with colorimetric or fluorometric metal-binding probes are typically employed by chemists in simple solutions, these approaches are not sensitive enough for seawater applications. In oceanography, much work has instead utilized adsorptive cathodic stripping voltammetry (CSV) to determine the concentration of competing ligand complexes that adsorb onto a dropping mercury electrode and undergo a redox reaction as the potential on the electrode is changed. The binding strengths of a broad variety of metal complexes can be determined by this technique. These electrochemically active competing ligands span a wide range of conditional stability constants, enabling useful methods to be developed in seawater with a wide range in the binding strength of naturally occurring complexes. Moreover, since only a small fraction of the metal actually adsorbs onto the electrode, from a mass balance perspective the equilibrium between the components in seawater is preserved. This is an important consideration because error propagation in the calculations arising from ligand competition becomes significant if the fraction of metal bound with the added ligand is either too small or too large.

Competitive ligand exchange (CLE) methods provide a direct measurement of the side-reaction (α) coefficient, which is the product of the ligand concentration and the conditional stability constant for the metal complex with that ligand. The α coefficient provides a useful parameter to quantify how strongly a metal is bound to a ligand and assess what the biologically available free ion concentration is (assuming that we also know the total metal concentration). The relative value of α coefficients for two or more competing ligands will determine what fraction of the metal forms a complex with each ligand. For the competing ligand, it can be adjusted by changing the ligand concentration or changing the ligand to yield a range comparable to that of the natural ligands in a given sample. This versatility is a powerful feature of ligand competition methods.

Although measurements of α are analytically simple, it is far more useful to know the conditional stability constant and ligand concentrations independently. Without knowing the ligand concentration, we cannot assess how the free ion concentration will change as the total metal increases or decreases, especially if the ligand concentration is at or near the total metal concentration. As mentioned above, a fivefold increase in Cu concentration in a polluted harbor resulted in a thousandfold increase in free Cu concentrations due to saturation of strong Cu-binding ligands (Moffett et al. 1997).

To determine ligand concentrations, investigators have coupled competitive ligand experiments with titrations of added metals and measured how the α coefficient changes. These data can be analyzed to determine the conditional stability constant (K) and ligand concentration (L) using simple linear transformations such as Scatchard plots or nonlinear regression fits to Langmuir isotherms and by making simplifying assumptions about the number of distinct ligands present and the metal–ligand stoichiometry. But more recently, numerical approaches have enabled the characterization of multiple ligands, which is closer to the actual condition. Considerable progress in this area has been associated with a Scientific Committee on Ocean Research working group on metal organic complexation (Pižeta et al. 2015). Indeed, the initial focus on two ligand classes (strong and weak) has given way to the idea of a continuum of ligands, with some of the least abundant having a large importance because of their high stability constants.

Another useful approach is to use very strong competing ligands to remove as much of the ambient Cu from natural ligands as possible, which has been referred to as a reverse titration

(Nuester & van den Berg 2005). For elements such as Cu, this technique has revealed the existence of progressively stronger ligands at lower and lower concentrations. In principle, a reverse titration could remove all naturally complexed Cu, but there are kinetic and analytical obstacles to this approach, as discussed below.

CLE approaches have been applied to a wide range of biologically active transition metals, and a few important generalizations have emerged:

1. Transition-metal speciation is typically dominated by very strong ligands in a concentration range similar to that of the elements themselves. Thus, small increases in metal concentrations (or decreases in ligand concentrations) lead to large changes in free metal ion concentrations (Moffett 1995, Buck & Bruland 2005). Fe speciation data from the tropical Pacific GEOTRACES GP16 section show this clearly, with excess ligand concentrations seldom exceeding the dissolved Fe concentrations by more than a factor of two (**Figure 1**).
2. CLE measurements in cultures or incubations of bacteria and phytoplankton reveal production of ligands with estimates of *K* similar to those found in the water using comparable methodologies (Moffett & Brand 1996, Mellett et al. 2018).
3. Coastal waters typically have higher ligand concentrations than offshore waters. Higher ligand concentrations are typically associated with higher metal concentrations. Indeed, in many systems, metal and ligand concentrations are tightly coupled over significant gradients in the concentration of both. Moreover, while there are many examples of metals exceeding strong binding ligands, particularly in anthropogenically influenced regimes, strong ligands significantly exceed the total metal concentration only in short-term events like blooms (see below). This suggests the presence of feedbacks that might regulate the relationship between the two parameters.
4. The ligands are highly selective for specific metals (Morel et al. 2003). Surprisingly, adding high concentrations of a second metal to a titration does not change the outcome of a titration, even if the second metal is higher in the Irving–Williams order. While this might be a kinetic artifact, it also has been thought to imply that they are chelators of specific biological origin rather than a molecule like EDTA, which forms complexes with a variety of metals.
5. The strongest ligands for the elements that form strong complexes, such as Fe and Cu, have high concentrations throughout the water column (Gledhill & Buck 2012, Jacquot & Moffett 2015) and throughout the ocean basins, as seen in **Figures 1** and **2**. For elements that form weaker complexes, such as zinc (Zn) and cadmium (Cd), the strongest complexation occurs in the upper water column (Bruland 1992, Ellwood 2004), and the speciation at depth appears to be dominated primarily by inorganic or weak organic complexes.

CLE methods have been widely used, but there have always been concerns about the assumption of equilibrium that is implicit in CLE. Most workers have assumed that an approach to steady state provides reasonable confidence that the distribution between competing ligands is close to equilibrium. But this assumption is constrained by the principal problem with ligand exchange methods that rely on CSV: wall-loss effects. Metal complexes that adsorb onto mercury-drop electrodes are also likely to adsorb onto container walls. This issue is particularly acute for Fe complexes with ligands used in speciation studies: Steady state is often reported after equilibration times as short as 15 min, and it can be impossible to prove that this is a sufficient time to reach equilibration because of signal loss that occurs beyond 15 min owing to wall loss.

To investigate whether wall-loss effects might be an issue for CLE methods with Cu, Moriyasu & Moffett (2022) modified a CLE method developed for CSV using the chelator 8-hydroxyquinoline (oxine). However, they determined the partitioning of the Cu oxine complex into an organic solvent rather than onto a mercury electrode. Wall loss was negligible because

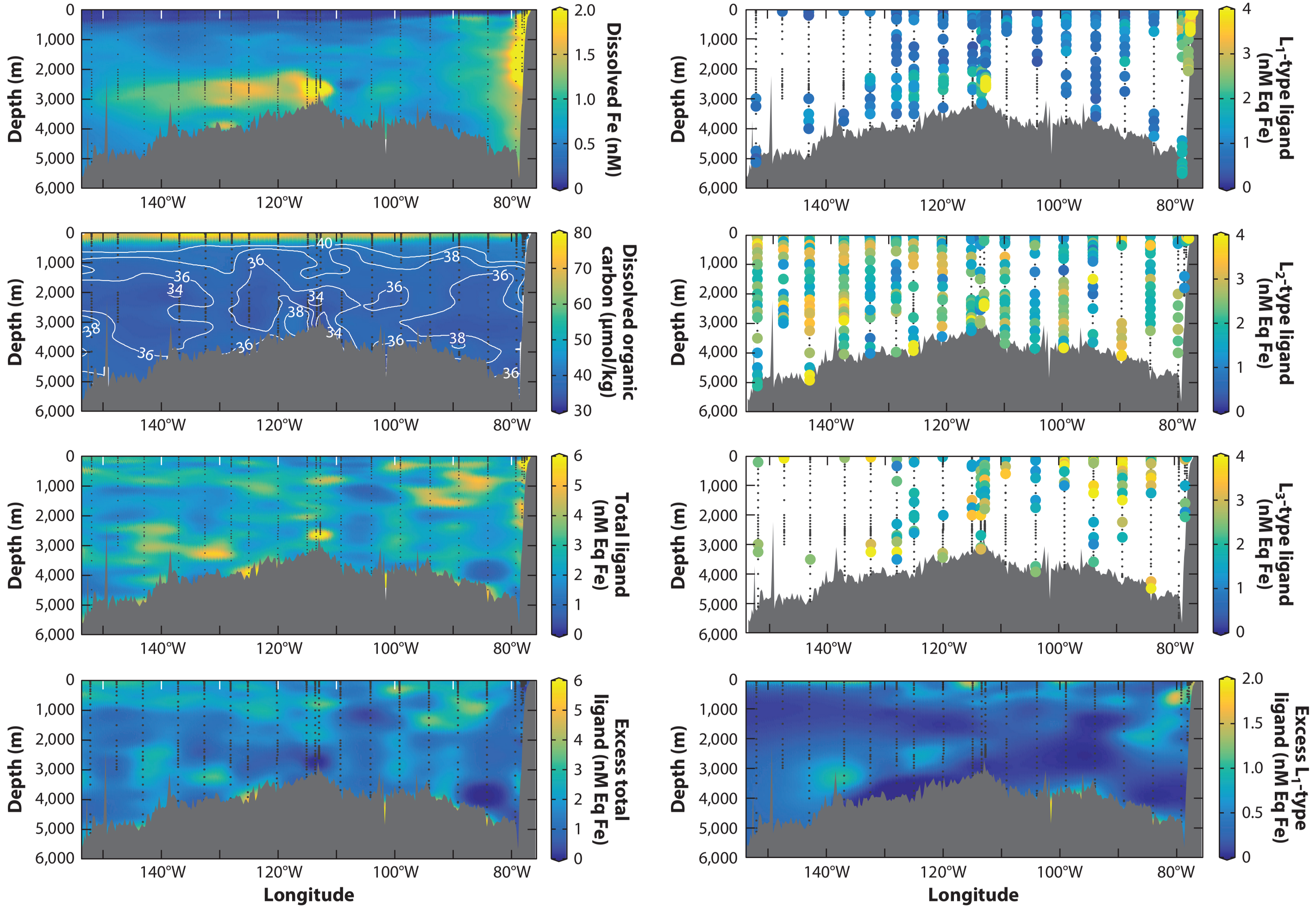

Figure 1

Fe concentration and Fe speciation data determined by adsorptive competitive ligand exchange–cathodic stripping voltammetry on the zonal GEOTRACES GP16 section from the Peruvian margin to 150°W. Note the hydrothermal source of Fe in the center of the section. Abbreviation: nM Eq Fe, nanomolar equivalent of Fe. Figure adapted with permission from Buck et al. (2018).

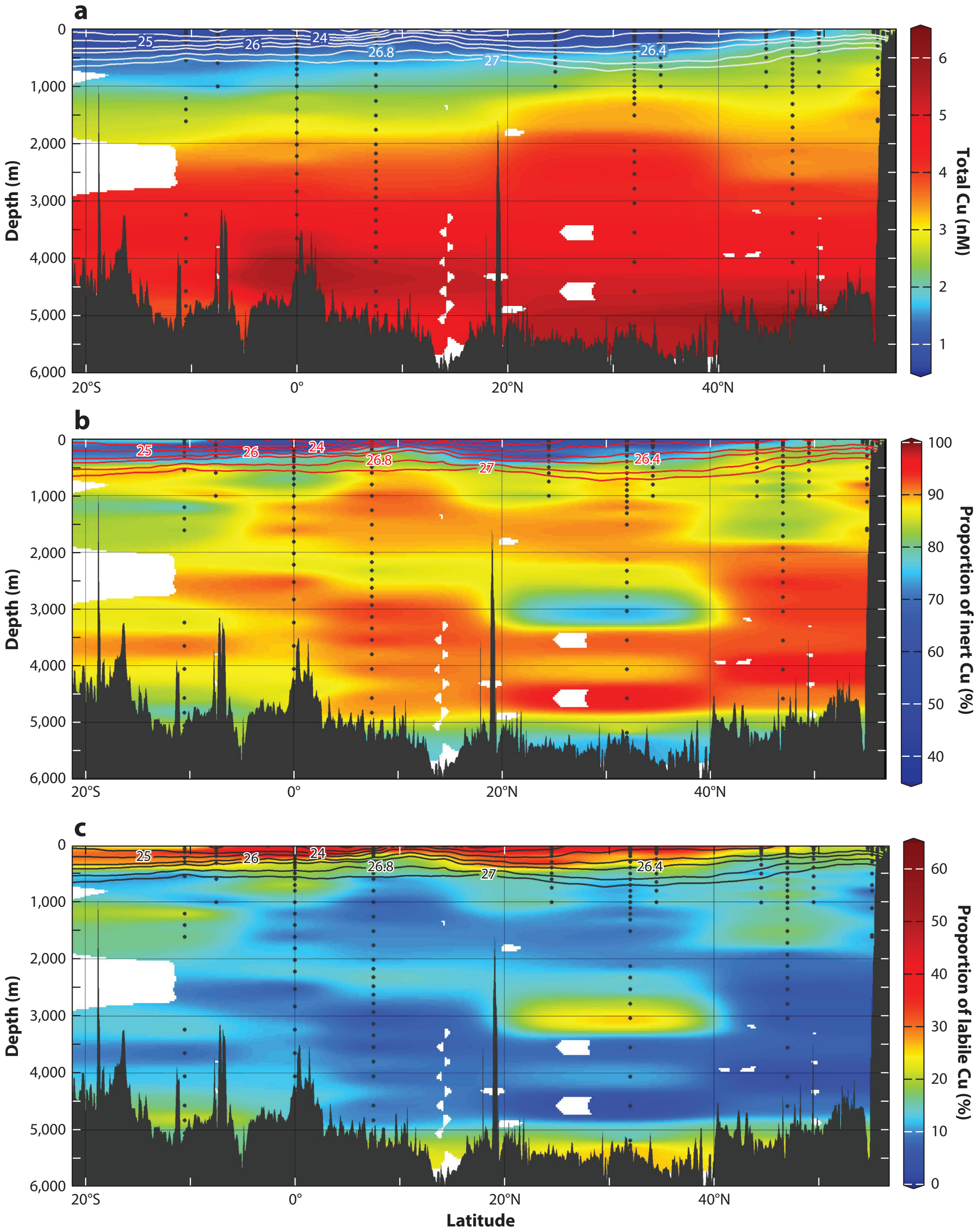

Figure 2

Sections of (*a*) total dissolved Cu, (*b*) percentage of inert Cu, and (*c*) percentage of labile Cu from the GEOTRACES GP15 section. Black dots represent sampling locations. Figure adapted from Moriyasu et al. (2023).

the solvent continuously washed the complex off the wall surfaces, extending the equilibration time from minutes to weeks. They showed that a minimum of 6 h was essential to achieve a steady state that persisted for at least 6 weeks. This suggests that CLE data for Cu speciation may overestimate ligand binding strength if it does not reach equilibrium.

Another advantage of a solvent extraction-based approach is that high concentrations of a competing ligand can be used—levels that would cause unacceptable baseline problems at the mercury electrode interface. In principle, this approach enables us to determine what concentration of competing ligand is required to complex all of the added Cu. The results of Moriyasu & Moffett (2022) were surprising. With 500-μM oxine—which led to a side-reaction coefficient much higher than those of previous methods (and with an equilibration time of 6 h to 3 weeks) in most of the water column—less than 10% of the Cu formed a complex with the oxine. The remaining 90–97% was inert to exchange with the added ligand despite long equilibration times and high added ligand concentrations. This means that the prevailing model of a continuum of labile complexes at some level of equilibrium needs to be replaced by a model with two distinct pools of Cu: labile complexes that reach equilibrium with inorganic Cu and refractory complexes that are kinetically inert to exchange.

The concept of an inert Cu fraction was originally developed by Kogut & Voelker (2003) using CSV. They made the case that when additional increments of competing ligand or equilibration time no longer increase the fraction of metal complexed by a ligand, it is more realistic to consider the remaining fraction to be inert rather than assign it a stability constant, which would merely be a lower bound. However, they had a much lower α coefficient and shorter equilibration time than was allowed by solvent extraction.

Moriyasu et al. (2023) characterized inert Cu on the GEOTRACES GP15 section from Alaska to Tahiti in 2018. **Figure 2** shows that the fraction of Cu that is inert is much lower in the euphotic zone than in deeper waters. Moriyasu et al. (2023) argued that this low inert fraction is probably due to a photochemically driven transformation of inert Cu into more labile forms. This transformation has important implications for Cu uptake by organisms because the inert Cu fraction these workers identified is probably not directly biologically available.

Another element that has an inert complex is cobalt (Co). Co is dominated by a nonexchangeable fraction in oceanic waters, but increasing Co in coastal and reducing regimes is primarily labile, probably Co^{2+} (Saito & Moffett 2001, Saito et al. 2005, Hawco et al. 2016).

Recent work has highlighted the importance of nanoparticles in the speciation of Fe, Cu, and Cd in hydrothermal vents, where they are probably associated with sulfur (Yücel et al. 2011, Gartman et al. 2014, Findlay et al. 2019). Nanoparticles may also be inert to CLE methods, and those findings suggest that other elements besides Cu have an inert fraction as well.

The Relationship Between Metal Complexation and Metal Residence Times, and the Emergence of Feedback Relationships

Natural ligands measured by electrochemical titration methods are often operationally divided into classes based on their binding strengths to Fe (L_1, L_2, etc.) (Rue & Bruland 1995, Gledhill & Buck 2012, Whitby et al. 2018). In most regions of the ocean, the concentration of total ligands exceeds the concentration of dissolved metal. High excess ligand concentrations are found in surface waters and at the deep chlorophyll maximum. Studies have shown that excess Fe ligands with particularly strong binding constants ($\log K'_{\mathrm{cond}} = 11.1$–$13.7$) increase in concentration in highly productive, Fe-depleted waters (Boye et al. 2003, Gerringa et al. 2006, Buck & Bruland 2007); in incubation experiments with microbes forced to Fe limitation (Gledhill et al. 2004; Mawji et al. 2011, 2008); and in mesoscale Fe addition experiments (Rue & Bruland 1997, Kondo et al. 2008).

These studies indicate that ligands are actively produced by microbes in response to metal deficiency. Based on the strong binding constant measured by CSV, it was speculated early on that at least a portion of the Fe ligands in surface waters may include microbial siderophores—high-affinity Fe-binding compounds that are secreted by microbes in response to Fe deficiency. Since siderophores are inherently linked to biological dissolution and uptake of metals, this observation led to hypotheses that marine microbes play an active role in keeping dissolved micronutrient metals in solution and regulating their bioavailability through organic ligand production.

Inorganic or weakly complexed dissolved metals are largely removed from seawater by scavenging to particles, and thus transition-metal chemistry is ultimately controlled by ligands that are at or near the ambient concentrations of the metals. Metals subsequently added during titration experiments are much less tightly complexed. Perhaps the best example of that is the near-1:1 relationship between dissolved Cu and strong ligands (L_1) in the Scheldt estuary. Van den Berg et al. (1987) hypothesized that strongly complexed Cu was stabilized in the dissolved phase, whereas weakly complexed Cu was more readily scavenged, particularly in the high-particle regime of the Scheldt. Any Cu added to the system in excess of the ambient strong ligand concentration would be rapidly scavenged. Thus, in contrast to the system described for Fe above, in the Scheldt, production of strong Cu ligands never leads to an excess. This led to the intriguing hypothesis that the distribution of metals under conditions where scavenging rates are high would be controlled by the concentration of strong ligands. Johnson et al. (1997) proposed that ligand complexation accounts for the accumulation of dissolved Fe in the deep ocean. Surprisingly, Fe has nutrient-like depth profiles in the water column, in contrast to scavenged-type elements such as aluminum, which has decreasing concentrations with depth (Bruland & Lohan 2006, Boyd & Ellwood 2010). However, in contrast to nutrient elements and metals such as Zn and Cd, the concentrations of Fe do not increase from the deep Atlantic to the deep North Pacific. It has been proposed that there is an upper limit on how much dissolved Fe can accumulate that is constrained by the ligand concentration.

This discussion raises an important question: If the concentration of ligands controls the concentration of metals, what controls the concentration of the ligands? This is a question that cannot be addressed without more information about ligands' structure and origin. Are they compounds of recent biological origin, with specific biological functions associated with metal binding? Or are they older material that has undergone much more diagenetic alteration?

Moffett & Brand (1996) showed that cultures of the ubiquitous marine cyanobacterium *Synechococcus* sp. make a ligand that has similar binding properties as naturally occurring ligands. Moreover, they showed that cultures that were stressed with added Cu produced enough ligands to complex the added Cu but no excess. This suggested that *Synechococcus* sp. and perhaps other microbes synthesize chelators via some type of regulated process that optimizes conditions for growth. For Cu, this would lead to what we observe in the ocean, where free Cu^{2+} concentrations are below toxic thresholds for most marine organisms but not low enough to limit growth. Perhaps such a system arises for other metals as well.

This hypothesis has never been tested, and there are several alternative explanations as well. There is no evidence from the molecular biology of *Synechococcus* for such regulation. The 1:1 relationship reported by Moffett & Brand (1996) could have arisen from other causes. A significant fraction of the Cu added by those workers was incorporated into the bacterial biomass or lost to the container walls. Thus, while the production of ligands under Cu stress definitely increased, the 1:1 relationship could simply be the result of preferential removal of any excess Cu from solution (analogous to the Scheldt estuary) rather than a tightly regulated system. **Figure 3** presents a generalized scheme that summarizes how feedbacks among metals, ligands,

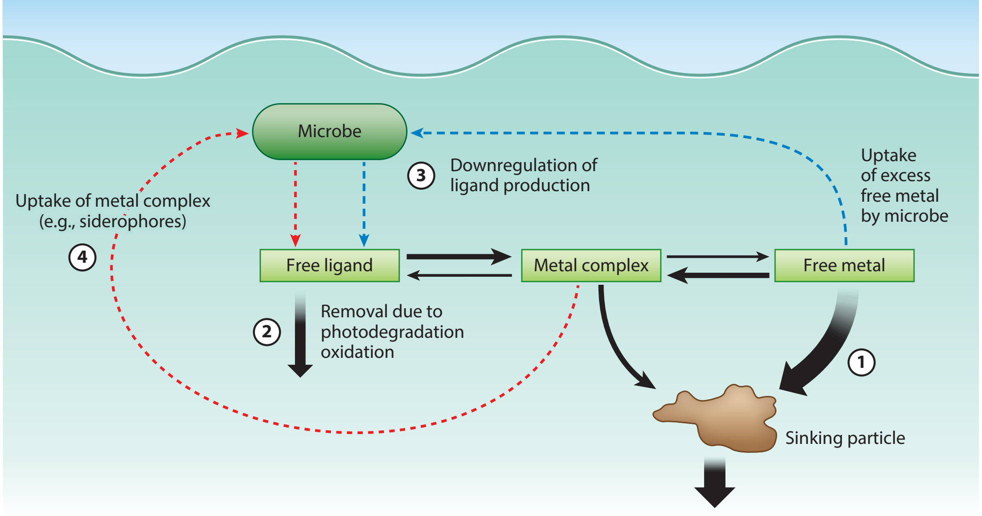

Figure 3

Summary of processes thought to contribute to the close relationship between strong ligands and total metal concentrations for many biologically active metals in seawater. (①) Uptake of metals onto sinking particles (i.e., scavenging) is much stronger for free metals than for metal complexes. (②) Free ligands may be more readily degraded than very stable metal complexes. (③) Production of chelators in response to metal stress may be downregulated when excess free metal is low. (④) Siderophore production (and perhaps production of analogous chelators of other elements) is downregulated when the element is no longer limiting, to avoid unnecessary production of excess ligand.

and particles (living and nonliving) might lead to feedbacks contributing to the close similarities between dissolved metal and strong ligand concentrations.

Johnson et al. (1997) proposed that organic complexation extends Fe residence time in deep waters and that there is a threshold strong ligand concentration of approximately 0.6 nM that is fairly uniform throughout the oceans. Remarkably, that assumption is still a key feature of current models of global Fe distribution, such as Pelagic Interactions Scheme for Carbon and Ecosystem Studies (PISCES) (Aumont et al. 2015, Tagliabue et al. 2016). In PISCES, rates of Fe removal above and below this threshold are very different, and this has been a useful component of models that successfully model Fe distribution. The data in **Figure 1** show that the assumption of a uniform ligand concentration is an oversimplification. In coastal regions, there is probably even more variability.

An important finding for virtually all of the transition metals is that the ambient metals are always more tightly complexed than the added metals in the titration. This observation accounts for the close relationship between metal and ligand concentrations. But could it arise from an experimental artifact? What if the equilibration times are too short in the current experimental setups? Wall loss, bacterial growth, and other artifacts constrain how much time can be allowed between addition of a metal (as a simple inorganic ion) and analysis. To determine what timescales are relevant for complexation, we should consider uptake of a metal by a suspended particle as a competing process with complexation by a dissolved ligand. Consider the fate of a Cu^{2+} ion introduced into the ocean water column. It will quickly form the $CuCO_3$ complex, the predominant inorganic form, before forming stronger complexes with organic ligands. But it may also react with binding sites on microbes or suspended or sinking detrital material. The rate of the latter process will depend on location in the water column. Consider the deep water column, where particles are scarce. The half-life associated with incorporation of dissolved Cu onto suspended particles may be days to weeks. Shouldn't investigators be considering similar timescales for strong complex formation?

It is also important to consider the role of weaker ligands, which have concentrations higher than those of the ambient metal and are therefore the ones detected in titrations. While they do not dominate speciation in most cases, they may play an important role as shuttles between strong complexes and uptake sites on cells and sinking particles, and may thus have a large impact on rates (Hassler et al. 2011). Moreover, they play an important role in the complexation of metals such as Cu in contaminated regimes (Moffett et al. 1997).

In recent years, the importance of humic substances as metal chelators, particularly for Fe and Cu, has also been studied. It is important to digress briefly to describe what a humic substance is. Humic acids and fulvic acids are operationally defined organic materials isolated from natural waters. They have specific definitions associated with the processes used to extract them, and there is a large literature about their chemical properties. In contrast to these extraction-based operational definitions, Whitby & van den Berg (2015) have used the term humic substances to describe organic material in seawater that has similar electrochemical properties to humic and fulvic acids that are consensus reference materials (Abualhaija et al. 2015, Whitby et al. 2020). Their work has suggested that humic substances may play an important role in Fe chelation, particularly as a weaker but more abundant ligand class. The origin of these humic-like ligands remains unclear, but these authors have noted the electrochemical similarities between humic-like ligands detected in deep ocean waters and ligands detected from humic and fulvic acid reference material of terrestrial origin.

THE IDENTITY OF NATURALLY OCCURRING LIGANDS

While electrochemical studies have provided indirect evidence that organic metal-binding ligands are actively produced by microbes, supplied by sediments, and possibly present in excess in deep waters, these approaches are unable to provide information on the chemical identity, biological source, or bioavailability of metal–ligand complexes. Over the past decade, new approaches have been developed to study the molecular composition of marine ligands that complement information from electrochemical methods. To study these individual metal–organic ligand complexes, they must first be concentrated from seawater using solid-phase extraction, which captures a fraction of the total dissolved organic matter and metals based on hydrophobic interactions (Waska et al. 2014). The metal ligands within these mixtures must then be separated and detected. Some of the first approaches to detect individual Fe-binding ligands relied on size exclusion and thin-layer chromatography paired with colorimetric detection (Macrellis et al. 2001). Molecular-level quantification of ligand-bound metal was later obtained using high-pressure liquid chromatography (HPLC) combined with inductively coupled plasma mass spectrometry (ICP MS). Early studies that employed HPLC–ICP MS to measure siderophore concentrations in natural seawater used additions of gallium rather than Fe to saturate and quantify siderophores due to its lower background contamination and interferences (Mawji et al. 2008). Further developments enabled the direct quantification of Fe complexes from seawater by employing trace-metal clean HPLC and ICP MS instruments equipped with a collision cell to remove contamination and isobaric interferences (Boiteau et al. 2013) and eventually entire suites of trace elements, including Fe, Co, Mn, Cu, Zn, and nickel (Ni) (Li et al. 2021, Dewey et al. 2023).

To identify these ligands, the same chromatography is combined with electrospray ionization mass spectrometry (ESI MS) (Mawji et al. 2008, 2011; Velasquez et al. 2011, 2016; Boiteau et al. 2016a,b). The use of Fourier transform mass analyzers (Orbitrap or ion cyclotron resonance) enables measurements of the mass of the intact metal–organic ligand complexes with ultrahigh mass resolution and accuracy (<1 ppm mass error). Computational algorithms can then be used to determine the molecular formula of the metal–ligand complexes by searching for mass peaks that match the natural abundance isotopic patterns and mass defects associated with trace elements and then correlating the relative intensities of these molecular ions with the intensities of the peaks observed by HPLC–ICP MS (Baars et al. 2014, Boiteau & Repeta 2015). In some cases, these formulae have been validated by exchanging the ligand with another rare metal that has diagnostic isotopic patterns (Mawji et al. 2008, Deicke et al. 2014). From these formula-annotated chromatographic features, a molecular structure can be determined by obtaining tandem mass spectrometry fragmentation spectra for those molecular ions and matching them to spectra of authentic standards.

Although the development and applications of these molecular approaches are still nascent, an emerging picture of at least three distinct compositional categories of organic ligands largely mirrors the perceptions of ligand composition that have been inferred from electrochemical measurements. First, a large fraction of metal–organic ligand complexes appear in HPLC–ICP MS chromatograms as a series of sharp chromatographic peaks that represent specific metal-complexing biomolecules. In the case of Fe ligands, many of these species have been identified as known microbial siderophores. Dozens of structurally distinct siderophores have now been observed and quantified in seawater as well as seawater enrichments, indicating a diverse array of specific microbial strategies that compete to maintain a bioavailable pool of dissolved Fe (**Figure 4**).

Beyond these structurally identifiable siderophores, a second group of discrete chromatographically resolved trace element ligands have been identified at the mass or molecular formula level,

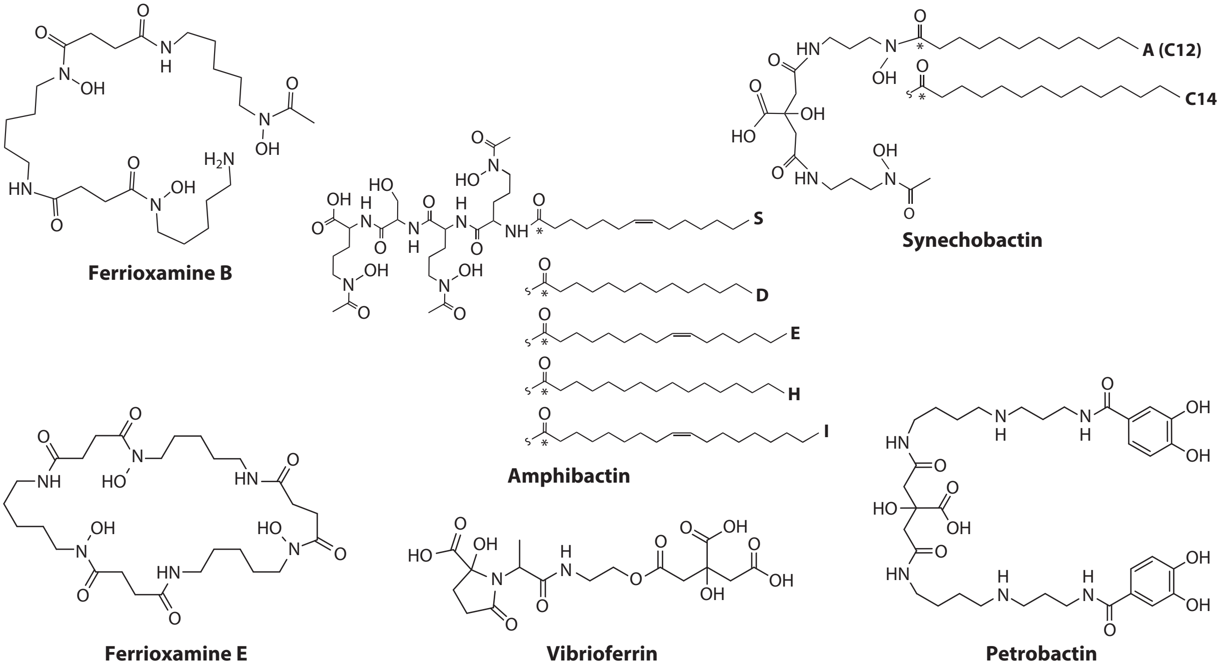

Figure 4

Molecular structures of siderophores that have been observed in the ocean.

but they do not match the mass or fragmentation spectra of any known molecules present in major chemical databases such as PubChem. These orphan molecules represent novel chemical structures produced by unknown organisms and biosynthesis pathways, and we currently lack databases of identified molecules that could be used to determine what they are and what functions they carry out. Determination of their full chemical structure, biological source, and ecological function relies on the identification of specific isolated organisms that can produce them. Laboratory studies of these isolates may then investigate the biosynthesis pathways as well as the conditions under which the molecules are produced. Many Fe ligands and essentially all Cu, Ni, and Zn ligands that have been detected in seawater thus far are orphan molecules. This reflects a major knowledge gap regarding how these other metals are regulated by microbial pathways in the ocean.

In addition to discrete peaks, HPLC–ICP MS data are rich in information on metals bound to a third and typically abundant component: a chromatographically unresolved complex mixture (UCM). These materials appear as broad humps of fluctuating intensities in the HPLC–ICP MS chromatogram, indicating the superposition of many metal-binding small molecules that are not fully resolved by the chromatographic separation. These UCMs account for a significant portion of the total Fe, Cu, Ni, and Zn ligands that are measured by HPLC–ICP MS.

MICROBIAL ORGANIC LIGANDS

There has long been evidence that strong organic ligands originate from microbial activity in the surface ocean. Dust deposition events often result in rapid changes in microbial communities and the dissolved ligand pool. Bioassay experiments across the North Atlantic have shown strong positive biological responses of marine bacteria, phytoplankton, and diazotrophs to dust amendments in waters with dissolved Fe concentrations of less than 2 nM (Blain et al. 2007, Marañón et al. 2010, Langlois et al. 2012). Fe fertilization is often accompanied by the production of strong organic ligands in surface waters. For example, mesoscale Fe fertilization experiments in the equatorial Pacific, the subarctic North Pacific, and the Southern Ocean resulted in a rapid increase in strong ligand concentration (up to fourfold) that accompanied large phytoplankton blooms (Rue & Bruland 1997, Boye et al. 2005, Kondo et al. 2008). Similar increases in dissolved Fe ligand concentrations have been observed in response to natural increases in surface-water dissolved Fe (Fitzsimmons et al. 2015). These observations have been explained as a microbial adaptation to environments where Fe is supplied in transient pulses. The production of ligands when Fe becomes available may result in the Fe staying in the surface ocean as Fe(III) chelates instead of sinking as mineral particles or precipitates. The studies cited above suggest that microbes play a major, although poorly understood, role in solubilizing and regulating the bioavailability of Fe in the surface ocean.

Studies of ligand composition by HPLC–ICP MS–ESI MS have observed recurring trends in the composition and concentration of siderophores across different nutrient regimes that reveal new linkages between metal distributions and biological activity. One key finding has been that the organic speciation of trace metals is quite dynamic and varies across major oceanographic biomes. **Figure 5** shows chromatograms from samples collected in Fe-rich coastal waters and Fe-starved high-nitrate, low-chlorophyll waters, in which more than 23 different discrete compounds were chromatographically resolved. Application of HPLC–ICP MS–ESI MS to the characterization of ligands in coastal upwelling regions along the Peruvian margin (Boiteau et al. 2016a) and California Current system (Boiteau et al. 2019) revealed similar trends. Siderophore concentrations were up to fivefold higher in the high-nitrate, low-chlorophyll samples and were dominated by amphibactins and amphiphilic siderophores with cell membrane affinity. In coastal and oligotrophic regions, amphibactins were replaced with lower concentrations of hydrophilic

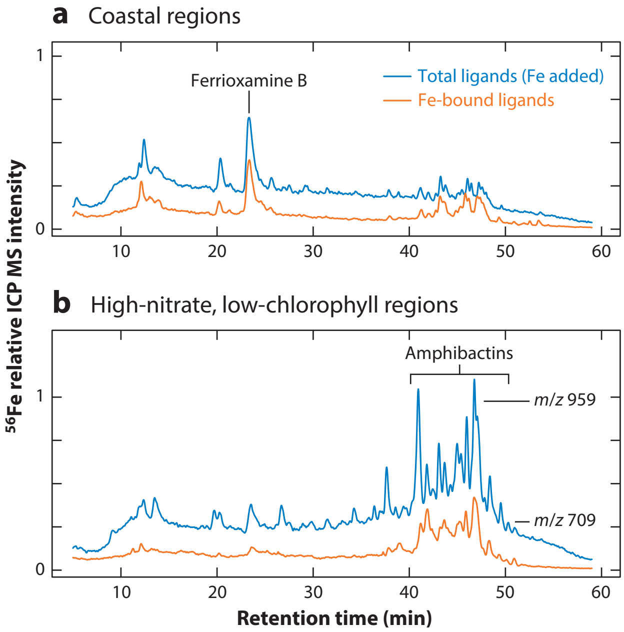

Figure 5

High-pressure liquid chromatography (HPLC)–inductively coupled plasma mass spectrometry (ICP MS) chromatograms of samples collected from the (*a*) coastal and (*b*) Fe-limited high-nitrate, low-chlorophyll regions of the eastern tropical Pacific. The orange line indicates ligands bound to naturally occurring Fe, and the blue line indicates total ligands after the addition of excess Fe to saturate uncomplexed ligands, showing the diversity of organic ligands that can be detected in different regions of the surface ocean. Peak labels indicate identified siderophores as well as the masses of unidentified complexes. Figure adapted from Boiteau et al. (2016a).

ferrioxamine siderophores. Siderophores can also vary over short spatial scales and with depth. For example, high-resolution analyses of Fe ligands through a coastal eddy showed changes in ligand composition and concentration on 10-km spatial scales (Boiteau et al. 2019). A subsurface maximum in siderophore concentrations has also been observed between 200 and 500 m at Station ALOHA, corresponding to depths where dissolved Fe concentrations remain low but dissolved nitrate concentrations are elevated below a seasonal thermocline (Bundy et al. 2018).

Siderophores have also been observed within ocean waters with higher dissolved Fe concentrations, such as nepheloid layers near coastal benthic boundaries (Boiteau et al. 2019) and oxygen minimum zones (Moore et al. 2021). Since siderophore production is active only when microbes are Fe deficient, these results imply that there may be a biological source of strong organic ligands in the mesopelagic and that Fe bioavailability can be low even when concentrations of dissolved Fe are relatively high. These studies highlight the variability in ligand composition across the ocean and the ability of marine microorganisms to quickly respond to changes in Fe availability by producing high concentrations of organic ligands, including siderophores.

Identifying siderophores in the ocean provides insight into the microbes responsible for producing them as well as their bioavailability. Surprisingly, the major siderophores that have been observed in the open ocean to date are only known to be produced by heterotrophic bacterial taxa (especially gammaproteobacteria) that represent a ubiquitous but minor fraction of the entire population. Using similar techniques, Gledhill and colleagues also identified ferrioxamines and amphibactins in seawater incubations carried out in the Atlantic Ocean with various carbon substrates to drive Fe limitation of heterotrophic microbes (Gledhill et al. 2004; Mawji et al. 2008, 2011). Since siderophores can only be utilized by microbes that possess dedicated uptake pathways that recognize specific molecules, it remains unclear whether these bacterial siderophores can supply Fe that is available to the major marine phytoplankton that sustain primary production. However, recent work has uncovered new pathways by which major phytoplankton groups, including cyanobacteria (Basu et al. 2019, Gledhill et al. 2019, Hogle et al. 2022) and diatoms (Kazamia et al. 2018, Coale et al. 2019), can likely take up siderophores that they cannot produce. It may be that symbiotic relationships between specific phycosphere bacteria that can solubilize Fe and these abundant primary producers govern Fe uptake throughout much of the ocean, but the specific interactions remain unclear.

Decades of previous work to characterize the structure and role of siderophores from cultures of marine bacterial isolates has enabled the identification of marine Fe-binding ligands found in the ocean as specific siderophores that facilitate dissolution and biological uptake (Sandy & Butler 2009). The focus on siderophore discovery has been motivated by the long-recognized importance of dissolved Fe as an essential micronutrient that limits microbial growth. In comparison with Fe-binding siderophores, few metallophores for other metals have been identified from cultures, and thus most of the marine metal–organic ligand complexes for other metals remain orphan molecules without a known physiological function. However, their isolation from seawater and characterization by HPLC–ICP MS has raised new questions regarding how trace metals may compete to bind with the same ligand sites. For example, ligands that bind to both Cu and Ni have been found in the subtropical North Pacific (Boiteau et al. 2016b). The extent to which marine metallophores are selective for specific metals and mediate competition for scarce micronutrient resources remains largely unknown, and further investigations of metal-binding competition are needed.

UNRESOLVED COMPLEX MIXTURES OF ORGANIC LIGANDS

The complexity and spatial distribution of metal–UCM complexes suggest that these unresolved organic ligands represent a polydisperse mixture of highly degraded organic molecules. A major fraction of dissolved organic matter in the ocean is composed of carboxylic acid–rich small molecules that have been speculated to be capable of acting as organic ligands for metals within the ocean (Hertkorn et al. 2006). Although molecular formulae for many purely organic components of dissolved organic matter have been determined by ultrahigh-resolution mass spectrometry, the assignment of molecular formulae corresponding to these unresolved metal–organic ligand complexes is not yet as developed as it is for discrete biomolecules. The major analytical challenge to overcome is the limited dynamic range of high-resolution mass spectrometers, where the more abundant non-metal-containing ions suppress the detection of less abundant metal-containing species. Improved analytical approaches that better resolve dissolved organic matter mixtures and increase detection of minor components are needed to characterize the molecular composition of metal–UCM complexes.

The concentrations of Fe–UCM complexes typically increase with depth, suggesting a benthic source and surface-water sink for these ligands (Boiteau et al. 2019). In a study along the California coast, metal amendment experiments showed that Fe-binding sites associated with UCM ligands from the low-oxygen benthic boundary were nearly saturated with Fe. These results suggested that UCM ligands may be important for stabilizing dissolved Fe released from reducing sediments for transport within the oxic water column. Results from this study also suggested that UCM ligands are removed or transformed as they reach the surface (**Figure 6**).

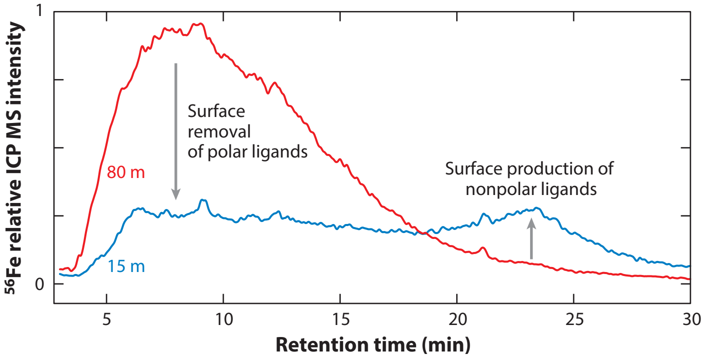

Figure 6

High-pressure liquid chromatography (HPLC)–inductively coupled plasma mass spectrometry (ICP MS) chromatogram of total Fe ligands from an upwelling site along the California coast. Deeper waters (80 m; *red line*) near the benthic boundary (bottom depth 100 m) have elevated concentrations of polar ligands eluting between 5 and 15 min and a lower concentration of nonpolar ligands eluting between 20 and 25 min.

Degradation in the surface ocean may be photochemical or microbial and could transform ligands produced in benthic environments into weaker, more nonpolar molecules. This hypothesis aligns with a recent laboratory study by Hassler et al. (2020), who demonstrated that solar irradiation of deep-sea ligands reduced Fe binding. Riverine sources of metal–UCM complexes have also been observed, although these complexes are largely removed upon mixing with seawater in a manner that relates to the chemical hardness of the metal (Gledhill et al. 2022a). Thus, the extent to which UCM ligands may be derived from terrestrial versus marine organic matter remains unclear. The proposed sources of UCM ligands are similar to those of electroactive humic-like ligands, which have been reported to have maximum concentrations in shallow coastal settings and in the open ocean at intermediate depths (500–1,000 m) (Laglera & van den Berg 2009, Abualhaija et al. 2015, Whitby & van den Berg 2015, Whitby et al. 2020). However, direct comparisons of their distributions are needed to assess the extent to which they represent the same ligands.

It is also important to determine the relationship between the Cu–UCM complexes quantified by HPLC–ICP MS and the inert Cu reported by Moriyasu et al. (2023). It seems plausible that there is overlap between these operationally defined fractions. Finally, it is important to consider the contribution of colloids to these various fractions. Colloids comprise from 20% up to >80% dissolved Fe and Cu in seawater (Fitzsimmons et al. 2015, Jensen et al. 2021), so they could contribute to some of the inert fraction. Cu nanoparticles have also been identified as an important species for Cu derived from hydrothermal vents (Gartman et al. 2014), but there is no evidence for their persistence outside of hydrothermal plumes, making it hard to tell whether they could account for a significant portion of inert Cu at basin scales.

Linking the compositional information obtained by HPLC–ICP MS to ligand information obtained by CSV or other bulk approaches requires new ways of determining the physical parameters of the species detected by HPLC–ICP MS that can be compared with those measured with other analyses. Isotopic exchange of ligand-bound metals in seawater monitored by HPLC–ICP MS is a promising new approach to determine metal–ligand dissociation rate constants of suites of naturally occurring ligands (Boiteau & Repeta 2022). These analyses highlight the sluggish Fe exchange kinetics of microbial siderophores (**Figure 7**) and demonstrate that the UCM fraction represents weaker ligands with faster Fe dissociation constants, consistent with the observation of weaker ligands by CSV that dominate the marine ligand pool.

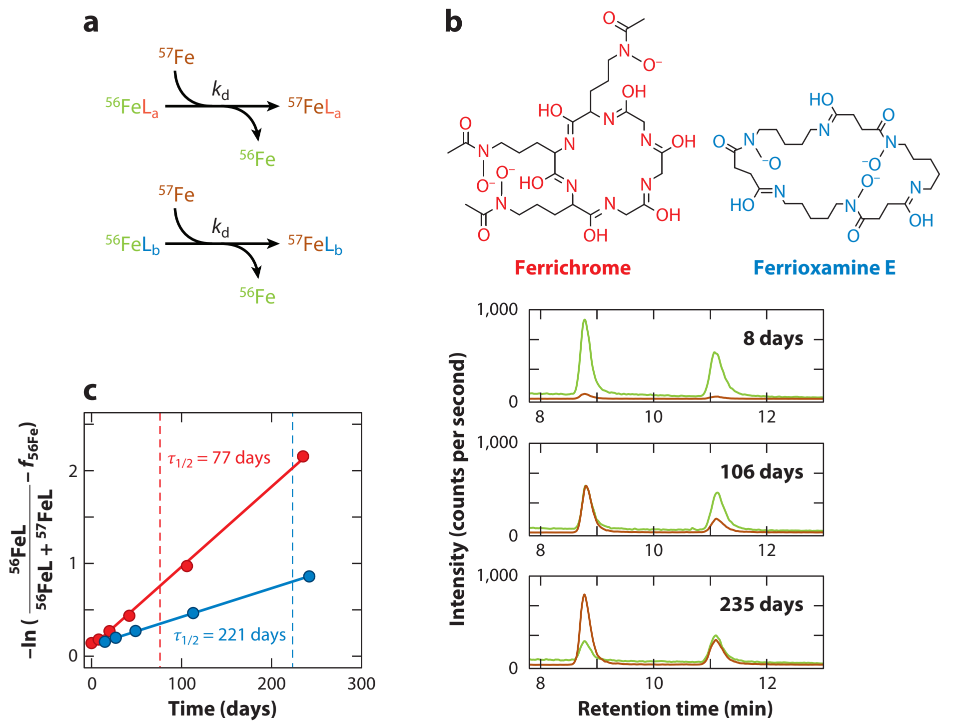

Figure 7

Determination of organic ligand dissociation rates through isotope exchange monitored by high-pressure liquid chromatography (HPLC)–inductively coupled plasma mass spectrometry (ICP MS). Excess ^{57}Fe is added to seawater and gradually exchanges with naturally occurring ^{56}Fe bound to organic ligands present in the sample with some dissociation rate constant (k_d) (panel *a*). Chromatograms collected at three time points are shown for the HPLC–ICP MS separation and detection of two siderophore standards dissolved in seawater, ferrichrome (*red*) and ferrioxamine E (*blue*), as they exchange ^{56}Fe for added ^{57}Fe (panel *b*). Over time, ^{56}Fe (*green trace*) is lost from each compound at different rates and replaced with ^{57}Fe (*orange trace*). To determine the dissociation rate constants (k_d), log-transformed Fe isotope ratios measured by HPLC–ICP MS (^{56}Fe/^{56}Fe + ^{57}Fe) relative to the total mole fraction of ^{56}Fe in solution ($f_{56\text{Fe}}$) were plotted over time (panel *c*). Solid lines show the linear fits to the data, with a slope equal to k_d. Dashed lines indicate the half-life ($\tau_{1/2} = \ln 2/k_d$) of the Fe complex with respect to dissociation. Figure adapted with permission from Boiteau & Repeta (2022); copyright 2022 American Chemical Society.

LIGAND-MEDIATED EFFECTS ON METAL ISOTOPE FRACTIONATION

The marine geochemistry of metal isotopes is a rapidly developing field, particularly for transition metals. Organic complexation is likely to have a significant impact on isotopic fractionation of metals, and this has been most thoroughly investigated for Fe. The stable isotope ratio of dissolved Fe (δ^{56}Fe) has shown promise for determining the relative importance of different Fe sources and how changes in their fluxes might affect biogeochemical cycling (Lacan et al. 2008, Radic et al. 2011, Conway et al. 2013). However, using δ^{56}Fe in such a way relies on knowledge of the fractionation effects of organic ligands that result in offsets between the δ^{56}Fe signatures of Fe from different sources. While several mechanisms are capable of mediating Fe mineral dissolution from dust or sediments in seawater, such as proton-promoted or reductive dissolution by reactive oxygen species, it is ultimately stabilization by organic ligands that keeps that dissolved Fe in solution (Baker & Croot 2010).

Simple laboratory experiments have revealed that organic ligands also have a strong but complex effect on dissolved δ^{56}Fe. Experiments with the weak ligand oxalate and goethite demonstrated a strong initial kinetic isotope fractionation (−2.6‰), which evolved toward heavier δ^{56}Fe compositions (+0.5‰) as a function of the fraction dissolved, a change attributed to equilibrium processes (Wiederhold et al. 2006). Such a finding is consistent with both the observation of isotopically heavy Fe (+0.6‰) bound to the strong bacterial siderophore desferrioxamine B in equilibrium with inorganic Fe and the observation that equilibrium ligand-bound δ^{56}Fe scales linearly with binding strength (Morgan et al. 2010). Similarly, Fe dissolved from hornblende, but not from goethite, was isotopically light in the presence of siderophores, with a kinetic isotope effect that increased with the affinity of the ligand (Brantley et al. 2004). Such studies suggest that isotopic fractionation is not only ligand specific but also dependent on the identity of the solid phase. Thus, Fe mineral composition, reaction timescales, and organic ligand composition all likely play an influential role in the end-member signature of δ^{56}Fe in the oceans, although these dynamics remain poorly constrained.

SUMMARY

The past decade has seen considerable progress in the identification of a variety of biologically produced chelators, new insights into metal complexation kinetics and the role of nanoparticles, and other advances. But challenges remain. While there are now data for the distribution of many siderophores in the water column, this was only possible because we already knew the structures of these compounds from prior studies on laboratory cultures. We still do not have the ability to move beyond molecular formulae for most of the metal–organic ligand complexes observed in seawater thus far. More importantly, the compounds that form discrete peaks are only a small fraction of the total metals isolated by their reverse phase extraction. The remainder are spread among a UCM of ligands that cannot be fully separated into individual compounds by chromatography, and improved analytical approaches are needed to characterize the molecular composition of these molecules. Moreover, a significant fraction (in many cases a majority) of the chelators are not retained by reverse-phase columns. Important advances here will probably focus on new isolation approaches using multiple extraction approaches and more powerful mass spectrometry tools to glean structural information in the absence of sufficient material for tools such as nuclear magnetic resonance spectroscopy.

The inert Cu findings suggest that kinetic artifacts associated with CLE must be evaluated more critically, certainly for Cu but probably for other metals as well. They also suggest that current models of titration data are in error because they assume all of the metal they are fitting is in equilibrium with the ligands, which is not the case if there is a sizable inert fraction.

Recent advances in transition-metal isotope geochemistry offer continuing opportunities for new insight. Beyond Fe, other elements with useful isotope ratios include Cu, Zn, and Ni. Isotopic fractionation of Cu in labile versus inert fractions may provide insight into the timescales of exchange between them, as well as transformations and sources at boundaries. There is already considerable information on fractionation effects associated with organic Cu complexation that will be useful in interpreting these data.

Microbiologists have identified analogues for siderophores for other metals, including chalkophores for Cu (Kenney & Rosenzweig 2018). It seems likely that such compounds will be identified soon in the ocean—if not in the open water column, then in specialized environments where there is a high Cu demand associated with methane oxidation and nitrous oxide reduction (Kenney & Rosenzweig 2018). Interestingly, a lanthanophore for lanthanum has also been produced in cultures of a bacterium, where lanthanum is required for methanol oxidation

(Roszczenko-Jasińska et al. 2020). Further research is needed not only to identify metallophores for these metals in the ocean but also to identify them in marine monocultures in order to link them to producing organisms.

Metal organic complexation is important in other natural waters and in engineered systems such as bioreactors, and there are likely synergies between fields that have yet to be realized. Metal limitation in aquatic systems with high concentrations of organic matter can influence enzymes that mediate important transformations of radiatively important trace gases, such as methane and nitrous oxide. Furthermore, organic complexation is a major challenge in remediation because metal complexes can be exceedingly difficult to remove from waste streams. Recent ocean studies have leveraged metal speciation models developed initially to describe metal binding to terrestrial humic substances to investigate how various metal cations compete to bind with marine ligands (Gledhill et al. 2015, 2022b), and there are opportunities to further develop speciation models that consider multiple metals and ligands simultaneously. It is important that there be more cross-talk among workers in these areas in the future to develop common approaches and methodologies.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

This work was funded by grants to R.M.B. from the Simons Foundation (award 621513), the National Science Foundation (awards 1829761 and 2149091), and the Department of Energy's Genomic Sciences Program in the Office of Biological and Environmental Research (Lawrence Livermore National Laboratory Biofuels Science Focus Area grant SCW1039). J.W.M. was supported by the National Science Foundation (awards 1756415 and 0927387).

LITERATURE CITED

Abualhaija MM, Whitby H, van den Berg CMG. 2015. Competition between copper and iron for humic ligands in estuarine waters. *Mar. Chem.* 172:46–56

Ahner BA, Morel FMM, Moffett JW. 1997. Trace metal control of phytochelatin production in coastal waters. *Limnol. Oceanogr.* 42(3):601–8

Aumont O, Éthé C, Tagliabue A, Bopp L, Gehlen M. 2015. PISCES-v2: an ocean biogeochemical model for carbon and ecosystem studies. *Geosci. Model Dev.* 8(8):2465–513

Baars O, Morel FMM, Perlman DH. 2014. ChelomEx: isotope-assisted discovery of metal chelates in complex media using high-resolution LC-MS. *Anal. Chem.* 86(22):11298–305

Baker AR, Croot PL. 2010. Atmospheric and marine controls on aerosol iron solubility in seawater. *Mar. Chem.* 120(1–4):4–13

Balistrieri L, Brewer PG, Murray JW. 1981. Scavenging residence times of trace metals and surface chemistry of sinking particles in the deep ocean. *Deep-Sea Res. A* 28(2):101–21

Basu S, Gledhill M, de Beer D, Prabhu Matondkar SG, Shaked Y. 2019. Colonies of marine cyanobacteria *Trichodesmium* interact with associated bacteria to acquire iron from dust. *Commun. Biol.* 2(1):284

Batley GE, Florence TM. 1976. Determination of the chemical forms of dissolved cadmium, lead and copper in seawater. *Mar. Chem.* 4(4):347–63

Blain S, Quéguiner B, Armand L, Belviso S, Bombled B, et al. 2007. Effect of natural iron fertilization on carbon sequestration in the Southern Ocean. *Nature* 446(7139):1070–74

Boiteau RM, Fitzsimmons JN, Repeta DJ, Boyle EA. 2013. Detection of iron ligands in seawater and marine cyanobacteria cultures by high-performance liquid chromatography–inductively coupled plasma-mass spectrometry. *Anal. Chem.* 85(9):4357–62

Boiteau RM, Mende DR, Hawco NJ, McIlvin MR, Fitzsimmons JN, et al. 2016a. Siderophore-based microbial adaptations to iron scarcity across the eastern Pacific Ocean. *PNAS* 113(50):14237–42

Boiteau RM, Repeta DJ. 2015. An extended siderophore suite from *Synechococcus* sp. PCC 7002 revealed by LC-ICPMS-ESIMS. *Metallomics* 7(5):877–84

Boiteau RM, Repeta DJ. 2022. Slow kinetics of iron binding to marine ligands in seawater measured by isotope exchange liquid chromatography-inductively coupled plasma mass spectrometry. *Environ. Sci. Technol.* 56:3770–79

Boiteau RM, Till CP, Coale TH, Fitzsimmons JN, Bruland KW, Repeta DJ. 2019. Patterns of iron and siderophore distributions across the California Current System. *Limnol. Oceanogr.* 64(1):376–89

Boiteau RM, Till CP, Ruacho A, Bundy RM, Hawco NJ, et al. 2016b. Structural characterization of natural nickel and copper binding ligands along the US GEOTRACES Eastern Pacific Zonal Transect. *Front. Mar. Sci.* 3:243

Boyd PW, Ellwood MJ. 2010. The biogeochemical cycle of iron in the ocean. *Nat. Geosci.* 3(10):675–82

Boye M, Aldrich AP, van den Berg CM, de Jong JT, Veldhuis M, de Baar HJ. 2003. Horizontal gradient of the chemical speciation of iron in surface waters of the northeast Atlantic Ocean. *Mar. Chem.* 80(2–3):129–43

Boye M, Nishioka J, Croot PL, Laan P, Timmermans KR, de Baar HJW. 2005. Major deviations of iron complexation during 22 days of a mesoscale iron enrichment in the open Southern Ocean. *Mar. Chem.* 96(3–4):257–71

Brand LE, Sunda WG, Guillard RR. 1986. Reduction of marine phytoplankton reproduction rates by copper and cadmium. *J. Exp. Mar. Biol. Ecol.* 96(3):225–50

Brantley SL, Liermann LJ, Guynn RL, Anbar A, Icopini GA, Barling J. 2004. Fe isotopic fractionation during mineral dissolution with and without bacteria. *Geochim. Cosmochim. Acta* 68(15):3189–204

Bruce MR. 1987. A stability ruler for metal ion complexes. *J. Chem. Educ.* 64(5):402

Bruland KW. 1992. Complexation of cadmium by natural organic ligands in the central North Pacific. *Limnol. Oceanogr.* 37(5):1008–17

Bruland KW, Franks RP, Knauer GA, Martin JH. 1979. Sampling and analytical methods for the determination of copper, cadmium, zinc, and nickel at the nanogram per liter level in sea water. *Anal. Chim. Acta* 105:233–45

Bruland KW, Lohan MC. 2006. Controls of trace metals in seawater. *Oceans Mar. Geochem.* 6:23–47

Buck KN, Bruland KW. 2005. Copper speciation in San Francisco Bay: a novel approach using multiple analytical windows. *Mar. Chem.* 96(1–2):185–98

Buck KN, Bruland KW. 2007. The physicochemical speciation of dissolved iron in the Bering Sea, Alaska. *Limnol. Oceanogr.* 52(5):1800–8

Buck KN, Sedwick PN, Sohst B, Carlson CA. 2018. Organic complexation of iron in the eastern tropical South Pacific: results from US GEOTRACES Eastern Pacific Zonal Transect (GEOTRACES cruise GP16). *Mar. Chem.* 201:229–41

Bundy RM, Boiteau RM, McLean C, Turk-Kubo KA, McIlvin MR, et al. 2018. Distinct siderophores contribute to iron cycling in the mesopelagic at Station ALOHA. *Front. Mar. Sci.* 5:61

Byrne RH, Kester DR. 1976. Solubility of hydrous ferric oxide and iron speciation in seawater. *Mar. Chem.* 4(3):255–74

Coale TH, Moosburner M, Allen AE. 2019. Reduction-dependent siderophore assimilation in a model pennate diatom. *PNAS* 116(47):23609–17

Conway TM, Rosenberg AD, Adkins JF, John SG. 2013. A new method for precise determination of iron, zinc and cadmium stable isotope ratios in seawater by double-spike mass spectrometry. *Anal. Chim. Acta* 793:44–52

Davis JA. 1984. Complexation of trace metals by adsorbed natural organic matter. *Geochim. Cosmochim. Acta* 48(4):679–91

Deicke M, Mohr JF, Bellenger J-PP, Wichard T. 2014. Metallophore mapping in complex matrices by metal isotope coded profiling of organic ligands. *Analyst* 139(23):6096–99

Dewey C, Kaplan DI, Fendorf S, Boiteau RM. 2023. Quantitative separation of unknown organic–metal complexes by liquid chromatography–inductively coupled plasma-mass spectrometry. *Anal. Chem.* 95(20):7960–67

Ellwood MJ. 2004. Zinc and cadmium speciation in subantarctic waters east of New Zealand. *Mar. Chem.* 87(1–2):37–58

Findlay AJ, Estes ER, Gartman A, Yücel M, Kamyshny A Jr., Luther GW III. 2019. Iron and sulfide nanoparticle formation and transport in nascent hydrothermal vent plumes. *Nat. Commun.* 10(1):1597

Fitzsimmons JN, Hayes CT, Al-Subiai S, Zhang R, Morton P, et al. 2015. Daily to decadal variability of size-fractionated iron and iron-binding ligands at the Hawaii Ocean Time-series Station ALOHA. *Geochim. Cosmochim. Acta* 171:303–24

Gartman A, Findlay AJ, Luther GW III. 2014. Nanoparticulate pyrite and other nanoparticles are a widespread component of hydrothermal vent black smoker emissions. *Chem. Geol.* 366:32–41

Gerringa LJ, Veldhuis MJW, Timmermans KR, Sarthou G, De Baar HJW. 2006. Co-variance of dissolved Fe-binding ligands with phytoplankton characteristics in the Canary Basin. *Mar. Chem.* 102(3–4):276–90

Gledhill M, Achterberg EP, Li K, Mohamed KN, Rijkenberg MJAA. 2015. Influence of ocean acidification on the complexation of iron and copper by organic ligands in estuarine waters. *Mar. Chem.* 177(3):421–33

Gledhill M, Basu S, Shaked Y. 2019. Metallophores associated with *Trichodesmium erythraeum* colonies from the Gulf of Aqaba. *Metallomics* 11(9):1547–57

Gledhill M, Buck KN. 2012. The organic complexation of iron in the marine environment: a review. *Front. Microbiol.* 3:69

Gledhill M, Hollister A, Seidel M, Zhu K, Achterberg EP, et al. 2022a. Trace metal stoichiometry of dissolved organic matter in the Amazon plume. *Sci. Adv.* 8(31):2249

Gledhill M, McCormack P, Ussher S, Achterberg EP, Mantoura RFC, Worsfold PJ. 2004. Production of siderophore type chelates by mixed bacterioplankton populations in nutrient enriched seawater incubations. *Mar. Chem.* 88(1–2):75–83

Gledhill M, Zhu K, Rusiecka D, Achterberg EP. 2022b. Competitive interactions between microbial siderophores and humic-like binding sites in European shelf sea waters. *Front. Mar. Sci.* 9:855009

Hassler CS, Cabanes D, Blanco-Ameijeiras S, Sander SG, Benner R. 2020. Importance of refractory ligands and their photodegradation for iron oceanic inventories and cycling. *Mar. Freshw. Res.* 71(3):311–20

Hassler CS, Schoemann V, Nichols CM, Butler EC, Boyd PW. 2011. Saccharides enhance iron bioavailability to Southern Ocean phytoplankton. *PNAS* 108(3):1076–81

Hawco NJ, Ohnemus DC, Resing JA, Twining BS, Saito MA. 2016. A dissolved cobalt plume in the oxygen minimum zone of the eastern tropical South Pacific. *Biogeosciences* 13(20):5697–17

Helfferich F. 1962. Ligand exchange. I. Equilibria. *J. Am. Chem. Soc.* 84(17):3237–42

Hertkorn N, Benner R, Frommberger M, Schmittkopplin P, Witt M, et al. 2006. Characterization of a major refractory component of marine dissolved organic matter. *Geochim. Cosmochim. Acta* 70(12):2990–3010

Hogle SL, Hackl T, Bundy RM, Park J, Satinsky B, et al. 2022. Siderophores as an iron source for picocyanobacteria in deep chlorophyll maximum layers of the oligotrophic ocean. *ISME J.* 16(6):1636–46

Hunter KA, Hawke DJ, Choo LK. 1988. Equilibrium adsorption of thorium by metal oxides in marine electrolytes. *Geochim. Cosmochim. Acta* 52(3):627–36

Jacquot JE, Moffett JW. 2015. Copper distribution and speciation across the International GEOTRACES Section GA03. *Deep-Sea Res. II* 116:187–207

Jensen LT, Lanning NT, Marsay CM, Buck CS, Aguilar-Islas AM, et al. 2021. Biogeochemical cycling of colloidal trace metals in the arctic cryosphere. *J. Geophys. Res. Ocean*s 126(8):e2021JC017394

Johnson KS, Gordon RM, Coale KH. 1997. What controls dissolved iron concentrations in the world ocean? *Mar. Chem.* 57(3–4):137–61

Kazamia E, Sutak R, Paz-Yepes J, Dorrell RG, Vieira FRJ, et al. 2018. Endocytosis-mediated siderophore uptake as a strategy for Fe acquisition in diatoms. *Sci. Adv.* 4(5):eaar4536

Kenney GE, Rosenzweig AC. 2018. Chalkophores. *Annu. Rev. Biochem.* 87:645–76

Kogut MB, Voelker BM. 2003. Kinetically inert Cu in coastal waters. *Environ. Sci. Technol.* 37(3):509–18

Kondo Y, Takeda S, Nishioka J, Obata H, Furuya K, et al. 2008. Organic iron (III) complexing ligands during an iron enrichment experiment in the western subarctic North Pacific. *Geophys. Res. Lett.* 35(12):L12601

Lacan F, Radic A, Jeandel C, Poitrasson F, Sarthou G, et al. 2008. Measurement of the isotopic composition of dissolved iron in the open ocean. *Geophys. Res. Lett.* 35(24):L24610

Laglera LM, van den Berg CMG. 2009. Evidence for geochemical control of iron by humic substances in seawater. *Limnol. Oceanogr.* 54(2):610–19

Langlois RJ, Mills MM, Ridame C, Croot P, LaRoche J. 2012. Diazotrophic bacteria respond to Saharan dust additions. *Mar. Ecol. Prog. Ser.* 470:1–14

Li J, Boiteau RM, Babcock-Adams L, Acker M, Song Z, et al. 2021. Element-selective targeting of nutrient metabolites in environmental samples by inductively coupled plasma mass spectrometry and electrospray ionization mass spectrometry. *Front. Mar. Sci.* 8:630494

Macrellis HM, Trick CG, Rue EL, Smith G, Bruland KW. 2001. Collection and detection of natural iron-binding ligands from seawater. *Mar. Chem.* 76(3):175–87

Mann EL, Ahlgren N, Moffett JW, Chisholm SW. 2002. Copper toxicity and cyanobacteria ecology in the Sargasso Sea. *Limnol. Oceanogr.* 47(4):976–88

Marañón E, Fernández A, Mouriño-Carballido B, Martínez-García S, Teira E, et al. 2010. Degree of oligotrophy controls the response of microbial plankton to Saharan dust. *Limnol. Oceanogr.* 55(6):2339–52

Mawji E, Gledhill M, Milton JA, Tarran GA, Ussher S, et al. 2008. Hydroxamate siderophores: occurrence and importance in the Atlantic Ocean. *Environ. Sci. Technol.* 42(23):8675–80

Mawji E, Gledhill M, Milton JA, Zubkov MV, Thompson A, et al. 2011. Production of siderophore type chelates in Atlantic Ocean waters enriched with different carbon and nitrogen sources. *Mar. Chem.* 124(1–4):90–99

Mellett T, Brown MT, Chappell PD, Duckham C, Fitzsimmons JN, et al. 2018. The biogeochemical cycling of iron, copper, nickel, cadmium, manganese, cobalt, lead, and scandium in a California Current experimental study. *Limnol. Oceanogr.* 63(S1):S425–47

Millero FJ. 2001. *The Physical Chemistry of Natural Waters*. New York: Wiley-Intersci.

Moffett JW. 1995. Temporal and spatial variability of copper complexation by strong chelators in the Sargasso Sea. *Deep-Sea Res. I* 42(8):1273–95

Moffett JW, Brand LE. 1996. Production of strong, extracellular Cu chelators by marine cyanobacteria in response to Cu stress. *Limnol. Oceanogr.* 41(3):388–95

Moffett JW, Brand LE, Croot PL, Barbeau KA. 1997. Cu speciation and cyanobacterial distribution in harbors subject to anthropogenic Cu inputs. *Limnol. Oceanogr.* 42(5):789–99

Moore LE, Heller MI, Barbeau KA, Moffett JW, Bundy RM. 2021. Organic complexation of iron by strong ligands and siderophores in the eastern tropical North Pacific oxygen deficient zone. *Mar. Chem.* 236:104021

Morel FMM, Milligan AJ, Saito MA. 2003. Marine bioinorganic chemistry: the role of trace metals in the oceanic cycles of major nutrients. In *Treatise on Geochemistry*, Vol. 6: *The Oceans and Marine Geochemistry*, ed. HD Holland, KK Turekian, pp. 113–43. Amsterdam: Elsevier

Morgan JLL, Wasylenki LE, Nuester J, Anbar AD. 2010. Fe isotope fractionation during equilibration of Fe-organic complexes. *Environ. Sci. Technol.* 44(16):6095–101

Moriyasu R, Moffett JW. 2022. Determination of inert and labile copper on GEOTRACES samples using a novel solvent extraction method. *Mar. Chem.* 239:104073

Moriyasu R, Seth G, John SG, Bian X, Yang SC, Moffett JW. 2023. Cu exists predominantly as kinetically inert complexes throughout the interior of the equatorial and North Pacific Ocean. *Glob. Biogeochem. Cycles* 37(7):e2022GB007521

Neilands JB. 1995. Siderophores: structure and function of microbial iron transport compounds. *J. Biol. Chem.* 270(45):26723–26

Nuester J, van den Berg CM. 2005. Determination of metal speciation by reverse titrations. *Anal. Chem.* 77(1):11–19

Pižeta I, Sander SG, Hudson RJM, Omanović D, Baars O, et al. 2015. Interpretation of complexometric titration data: an intercomparison of methods for estimating models of trace metal complexation by natural organic ligands. *Mar. Chem.* 173:3–24

Radic A, Lacan F, Murray JW. 2011. Iron isotopes in the seawater of the equatorial Pacific Ocean: new constraints for the oceanic iron cycle. *Earth Planet. Sci. Lett.* 306(1–2):1–10

Roszczenko-Jasińska P, Vu HN, Subuyuj GA, Crisostomo RV, Cai J, et al. 2020. Gene products and processes contributing to lanthanide homeostasis and methanol metabolism in *Methylorubrum extorquens* AM1. *Sci. Rep.* 10(1):12663

Rue EL, Bruland KW. 1995. Complexation of iron(III) by natural organic ligands in the Central North Pacific as determined by a new competitive ligand equilibration/adsorptive cathodic stripping voltammetric method. *Mar. Chem.* 50(1–4):117–38

Rue EL, Bruland KW. 1997. The role of organic complexation on ambient iron chemistry in the equatorial Pacific Ocean and the response of a mesoscale iron addition experiment. *Limnol. Oceanogr.* 42(5):901–10

Saito MA, Moffett JW. 2001. Complexation of cobalt by natural organic ligands in the Sargasso Sea as determined by a new high-sensitivity electrochemical cobalt speciation method suitable for open ocean work. *Mar. Chem.* 75(1–2):49–68

Saito MA, Rocap G, Moffett JW. 2005. Production of cobalt binding ligands in a *Synechococcus* feature at the Costa Rica upwelling dome. *Limnol. Oceanogr.* 50(1):279–90

Sandy M, Butler A. 2009. Microbial iron acquisition: marine and terrestrial siderophores. *Chem. Rev.* 109(10):4580–95

Schlitzer R, Anderson RF, Dodas EM, Lohan M, Geibert W, et al. 2018. The GEOTRACES intermediate data product 2017. *Chem. Geol.* 493:210–23

Sunda WG. 1989. Trace metal interactions with marine phytoplankton. *Biol. Oceanogr.* 6(5–6):411–42

Sunda WG, Huntsman SA. 1998. Processes regulating cellular metal accumulation and physiological effects: phytoplankton as model systems. *Sci. Total Environ.* 219(2–3):165–81

Tagliabue A, Aumont O, DeAth R, Dunne JP, Dutkiewicz S, et al. 2016. How well do global ocean biogeochemistry models simulate dissolved iron distributions? *Glob. Biogeochem. Cycles* 30(2):149–74

van den Berg CM, Merks AG, Duursma EK. 1987. Organic complexation and its control of the dissolved concentrations of copper and zinc in the Scheldt estuary. *Estuar. Coast. Shelf Sci.* 24(6):785–97

Velasquez IB, Ibisanmi E, Maas EW, Boyd PW, Nodder S, Sander SG. 2016. Ferrioxamine siderophores detected amongst iron binding ligands produced during the remineralization of marine particles. *Front. Mar. Sci.* 3:172

Velasquez IB, Nunn BL, Ibisanmi E, Goodlett DR, Hunter KA, Sander SG. 2011. Detection of hydroxamate siderophores in coastal and Sub-Antarctic waters off the South Eastern Coast of New Zealand. *Mar. Chem.* 126(1–4):97–107

Waska H, Koschinsky A, Ruiz Chancho MJ, Dittmar T. 2014. Investigating the potential of solid-phase extraction and Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) for the isolation and identification of dissolved metal-organic complexes from natural waters. *Mar. Chem.* 173:78–92

Whitby H, Planquette H, Cassar N, Bucciarelli E, Osburn CL, et al. 2020. A call for refining the role of humic-like substances in the oceanic iron cycle. *Sci. Rep.* 10(1):6144

Whitby H, Posacka AM, Maldonado MT, van den Berg CM. 2018. Copper-binding ligands in the NE Pacific. *Mar. Chem.* 204:36–48

Whitby H, van den Berg CM. 2015. Evidence for copper-binding humic substances in seawater. *Mar. Chem.* 173:282–90

Wiederhold JG, Kraemer SM, Teutsch N, Borer PM, Halliday AN, Kretzschmar R. 2006. Iron isotope fractionation during proton-promoted, ligand-controlled, and reductive dissolution of goethite. *Environ. Sci. Technol.* 40(12):3787–93

Yücel M, Gartman A, Chan CS, Luther GW III. 2011. Hydrothermal vents as a kinetically stable source of iron-sulphide-bearing nanoparticles to the ocean. *Nat. Geosci.* 4(6):367–71