REVIEW ARTICLE

Sources, sinks, and cycling of dissolved organic copper binding ligands in the ocean

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Organic Cu-binding ligands have a fundamental influence on Cu distributions in the global ocean and they complex >99% of the dissolved Cu in seawater. Cu-binding ligands however, represent a large diversity of compounds with distinct sources, sinks and chemical properties. This heterogeneity makes the organic Cu-binding ligand pool difficult to study at the global scale. In this review, we provide an overview of the diversity of compounds that compose the marine Cu-ligand pool, and their dominant sources and sinks. We also summarize the most common analytical methods to measure ligands in marine water column samples. Generally, ligands are classified according to their conditional binding strength to Cu. However, the lack of a common definition for Cu ligand categories has previously complicated data inter-comparison. To address this, we provide a general classification for Cu-binding ligands according to their binding strength and discuss emerging patterns in organic Cu-binding ligand distributions in the ocean according to this classification. To date, there is no global biogeochemical model that explicitly represents Cu ligands. We provide estimates of organic Cu-binding ligand fluxes at key interfaces as first order estimates and a first step for future modeling efforts focused on Cu and Cu-binding ligands.

opper (Cu) is a widely studied trace metal due to its importance for various biological functions[1]. It also plays a role as a toxicant when present in high concentrations of the unbound ion, Cu^{2+} [2–4]. The cycling of dissolved Cu (Cu) in the oceans, estuaries and rivers has been relatively well-studied over recent years[5–13]. Recent knowledge acquired largely thanks to the international GEOTRACES program (https://www.geotraces.org) and new bio-geochemical models has allowed us to gain fresh insights into natural and anthropogenic Cu sources as well as removal processes[14,15]. However, despite growing datasets on dissolved Cu in seawater and the development of some of the first biogeochemical models for Cu[9,12,16,17], many of the internal cycling processes impacting global Cu distributions such as regeneration and scavenging are not well understood. Organic Cu-binding ligands are one aspect of the biogeo-chemical cycling of Cu that remains to be further explored and likely plays an important role in governing the reactivity of Cu in the ocean. In seawater greater than 99% of dissolved Cu is complexed to organic ligands[18], maintaining Cu in the dissolved phase as well as controlling the concentration of Cu^{2+} and thus playing an important role in Cu bioavailability and toxicity[19]. The pool of organic ligands that bind dissolved Cu in seawater consists of a heterogeneous mix of compounds with varying Cu-binding strengths and reactivities[5,7,11,20–22]. These organic ligands are operationally grouped according to their conditional binding strength ($\log K_{CuL_i,Cu^{2+}}$) with the stronger ligands termed "L_1", followed by L_2, L_3, …, L_n. While much work is yet to be done, advances in laboratory experiments and a dramatic increase in Cu-binding ligand mea-surements in the ocean are beginning to shed light on the sources and sinks of these ligands[5,7,16,23].

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Despite the recognition of the importance of ligands in dissolved iron (Fe) cycling, relatively little attention has been paid in the literature to Cu-binding ligands and their impact on the oceanic inventory of Cu. For example, it has been established that including a dynamic ligand cycle for dissolved Fe-binding ligands is essential for reproducing the measured in situ datasets of dissolved Fe profiles from GEOTRACES basin-scale sections[24,25]. However, a similar exercise has not yet been performed for Cu, despite the knowledge that the majority of dissolved Cu in seawater is associated with ligands. This review discusses our current understanding of the biogeochemical cycling of Cu-binding ligands in seawater. We summarize what is known so far about the chemical identity of Cu-binding ligands, including how we measure these ligands using the most commonly employed analytical techniques (voltammetry). Then we provide a general overview of the nature and importance of Cu complexation in marine waters with a focus on how observational work can complement biogeochemical modeling efforts to better understand the role that Cu-binding ligands may play in impacting the reactivity and bioavailability of Cu in the marine environment. Based on a compilation of all water column Cu-binding ligand studies to date ($n = 54$ studies), we find that rivers and sediments are the most important external sources of Cu-binding ligands to the open ocean, while photodegradation and microbial uptake are the dominant sinks in surface waters. Future efforts that characterize fluxes of Cu-binding ligands and their internal cycling are critical for better understanding Cu cycling in the global ocean.

Components of the marine Cu-binding ligand pool

Characterization of Cu-binding ligands is complicated by the broad assortment of chemical compounds contributing to the organic complexation of dissolved Cu in the marine environment. In fact, the ligand pool is most likely a continuum of compounds of varying Cu-binding capacities, with some ligand types themselves exhibiting multiple complexation sites (e.g., humic substances[26]). As such, in some cases it may not be appropriate to characterize ligands into groups (L_1, L_2 etc.) defined by their conditional stability constants at all[27]. However, identifying key ligand groups or key functional groups dominating marine Cu complexation can teach us about the processes that control the distribution and bioavailability of Cu throughout the water column. In oxic seawater, the +II oxidation state of inorganic Cu is thermodynamically favored[28], although Cu can be reduced to the +I oxidation state by photochemical and biological processes[29,30]. Around 10% of the dissolved Cu in ocean surface waters has been shown to be Cu(I)[31], with potentially as much as 80-90% as Cu(I) in estuarine waters[11,32]. Ligands containing nitrogen and oxygen-based-binding groups tend to stabilize the +II oxidation state, while ligands with sulfur-binding groups can stabilize the +I state, which acts to reduce Cu^{2+} and relieve Cu toxicity[33,34]. It is likely that the Cu-binding ligand pool present in seawater is complexing a combination of either Cu(I) or Cu(II), depending on the identity of the ligand.

Several different classes of organic molecules have been identified in seawater that have the capacity to bind Cu, including humic-like substances, exopolysaccharides, thiols and other low molecular weight microbially produced compounds. All of these compounds vary in terms of their overall binding strengths and their impact on Cu cycling. Humic-like substances and exopolysaccharides are components of the dissolved organic matter (DOM) pool that can bind Cu and other metals[35–37], likely through carboxylic and nitrogen-containing functional groups[38]. In the marine environment, these substances are largely derived from marine algae and its decomposition[39,40], while terrestrially derived humics contribute to Cu complexation in estuarine and coastal waters[11,26,41–43]. Humic-like substances have been found to bind to dissolved Cu with $\log K_{CuL_i,Cu^{2+}}$ ranging from 10 to 12[26,43] while exopolysaccharides bind Cu with $\log K_{CuL_i,Cu^{2+}} < 8$[44].

In addition to marine and terrestrial humic-like substances and exopolysaccharides, low molecular weight microbially produced ligands also contribute to the Cu-binding ligand pool in seawater. For example, methanobactins are multidentate ligands that are defined as chalkophores, which have a very high binding affinity for Cu. Chalkophores are thought to be similar to Fe-binding siderophores, in that they have a high specificity for Cu and may be used either to facilitate the uptake of Cu or perhaps to detoxify Cu[45]. Methanobactins have a $\log K_{CuL_i,Cu^{2+}} > 14$[46,47]. They are secreted by methanotrophs, which require Cu for various enzymes[48]. Previously thought to be limited to a few key organisms, methanotrophs have now been found in estuaries, open-ocean waters, deep sea sediments, methane seeps and hydrothermal vents[49–52]. Different species of methanotrophs are also now hypothesized to be ubiquitous within the biosphere, with methanobactin biosynthesis potentially extending beyond methane oxidizers[53], suggesting their role in marine Cu complexation may so far be underestimated.

Other microbially produced compounds that are not considered chalkophores, but can also bind Cu with high affinity are likely important Cu-binding ligands in seawater (Table 1). Reduced sulfur substances such as thiols like glutathione, cysteine and thiourea are important for metal detoxification in cell metabolism and have been found to bind Cu in seawater[54]. Phytochelatin, an oligomer of glutathione, is ubiquitously produced by marine algae for metal detoxification, particularly for Cu[55–57]. Similarly, metallothionein is a family of cysteine-rich, low molecular weight proteins synthesized by marine algae, which also play a role in protection against metal toxicity and oxidative stress[58]. Various thiols and thiol groups have been detected in pore waters, estuaries, coastal and open-ocean waters globally[11,54,59–65] with $\log K_{CuL_i,Cu^{2+}}$ ranging from 11 to 16 depending on the oxidation state of the Cu and the stoichiometry of the ligand complex[11,34,60,66].

Other uncharacterized Cu ligands have also been found in marine waters. Recent work on identifying the chemical structures of microbially produced Cu-binding compounds in the southern Pacific Ocean found one major ligand type was a compound of molecular formula $[C_{20}H_{21}N_4O_8S_2M]^+$ ($M = $ metal isotope), containing several azole-like metal-binding groups as well as a sulfur group[67]. Additional ligands were also identified in this study that bound both nickel and Cu. Although the ultimate source of these ligands is unknown, it is likely they are biologically produced[67].

Hemocyanins are Cu-containing metalloproteins responsible for transporting oxygen in the blood of many marine invertebrates such as cephalopods, mollusks and crustaceans, including copepods and krill. Similar to the role of hemes in the marine Fe cycle[68,69], hemocyanins could be potentially important contributors to the marine Cu cycle and Cu-binding ligand pool, but have received little attention in marine Cu studies to date. Various other Cu-complexing compounds have also been identified in marine waters, such as domoic acid[70]. Such specific compounds may be important to Cu bioavailability either on very localized scales or periodically, such as during mass feeding events or during large harmful algal bloom events (e.g., domoic acid). Overall, we have only just begun to unravel the identities of Cu-binding ligands in seawater.

Measuring copper-binding ligands

In this section, we present the most commonly used methods for characterizing Cu-ligand concentrations and binding strengths in

Table 1 Organic copper-binding ligands produced by marine microbes.

Region or microbe	Species	Ligand class	Ligand concentration (nmol L^{-1})	log$K_{CuL_i, Cu^{2+}}$	m/z	Molecular formula, functional groups, ID	Method	References
Coccolithophore	Hymenomonas carterae	-	-	-	-	-	ASV	Croot et al. (2000)[140]
	Emiliania huxleyi	L$_1$	54	15.6	-	-	CLE-CSV	Leal et al. (1999)[142]
	Emiliania huxleyi	L$_2$	145–160	11.6	-	Arginine-cysteine (Arg-Cyc) and glutamine-cysteine (Gln-Cys)	CLE-CSV	Dupont et al. (2004)[141]
Cyanobacteria	Synechococcus WH 7803	-	40	-	-	-	ASV	Croot et al. (1999)[138]
	Synechococcus WH 7803	L$_2$	15–110	12.3–13.3	-	-	CLE-CSV	Moffett and Brand (1996)[19]
	Synechococcus PCC 7002	L$_2$	139	12.2	-	-	CLE-CSV	Lawrence (1998)[139]
Dinoflagellate	Amphidinium carterae	-	75	-	-	-	ASV	Croot et al. (1999)[138]
	Prorocentrum micans	-	-	-	-	-	ASV	Croot et al. (2000)[140]
Diatoms	Skeletonema costatum	-	47	-	-	-	ASV	Croot et al. (1999)[138]
	Thalasiosira weissflogii	-	1–30	-	-	Glutathione; thiol	HPLC	Tang et al. (2005)[218]
Heterotrophic bacteria	Vibrio alginolyticus	L$_2$	31	11.0	-	-	ASV	Gordon et al. (2000)[96]
Methane-oxidizing bacteria	-	L$_1$	-	-	-	Methanobactins; thioamide groups	ion exchange and hydrophobic interaction chromatography	Zahn and DiSpirito (1996)[219]
Brown algae	Fucus vesiculosus	L$_2$	20–800	11.2	-	-	CLE-CSV	Gledhill et al. (1999)[220]
Algae and Fungi	-	-	-	-	-	Phytochelatins; thiol	HPLC	Ahner et al. (1995)[145], Zenk (1996); Kawakami et al. (2006)
Eukaryotes	-	-	-	-	-	Glutathione; thiol	HPLC, CSV	Dupont et al. (2004); Dupont and Ahner (2005); Kawakami et al. (2006)[221]
Southern Equatorial Pacific surface waters	-	-	0.05–0.15	-	572.009	[C$_{20}$H$_{21}$N$_4$O$_8$S$_2$Cu]$^+$; azole	LC-ICP/ESI-MS	Boiteau et al. (2016)[67]

Organic copper-binding ligands that have been observed to be produced by microbes in culture or in natural seawater. The "–" denotes that parameter was not determined.

seawater. Then, we propose new operational definitions of Cu-ligand classes based on measured stability constants and known model ligands, and call for a consensus on defining these ligand pools to facilitate comparisons across datasets and analyses in future studies.

Anodic stripping voltammetry (ASV). The earliest methods to measure the organic complexation of dissolved Cu in seawater used anodic stripping voltammetry (ASV)[71,72]. This method involves titrating a natural sample with dissolved Cu, and measuring the "ASV-labile" Cu pool over the course of the titration. The ASV-labile Cu pool is predominantly a mixture of inorganic Cu complexes (Cu′), which includes any free or hydrated Cu^{2+} and $Cu(CO_3)^0$. However, ASV-labile Cu could also include organically complexed Cu (CuL) if the complexes are not kinetically stable. This method is a direct speciation technique, in that the sample is not perturbed beyond the addition of dissolved Cu during the titration, and the labile Cu is measured directly. In this method, the labile Cu′ is reduced to Cu^0 and forms a Cu-mercury amalgam over a certain deposition time. The Cu^0 is then oxidized ("stripped") out of the mercury and the current is measured by the instrument. The peak heights are plotted against the total Cu in the sample (Cu_T = dissolved Cu + added Cu) producing a titration curve. Kinetically stable Cu is not electrochemically active, and thus only labile Cu′ will adsorb to the mercury drop at the deposition potential (voltage) used in ASV measurements. When the total dissolved Cu in a sample is calculated, the difference between the total dissolved Cu and ASV-labile Cu is said to equal the strongly organically complexed Cu. The ligand concentration (L) and binding strength ($\log K_{CuL_i,Cu^{2+}}$) can be calculated based on the analytically measured current, and different linearization techniques[73–75]. However, any species that dissociate at the applied plating potential will contribute Cu to the labile Cu peak, including known Cu-binding ligands such as thiols, therefore ASV measurements can overestimate labile Cu and thus underestimate ligand concentrations.

An important aspect of ASV is the analytical window, or the detection window of the method. The analytical window is defined by α, or a side reaction coefficient. Since ASV detects only labile Cu, which only includes inorganic Cu, then the α for ASV is approximately equal to the inorganic side reaction coefficient for Cu, or $\alpha_{Cu} = \sum[CuX_i]/[Cu^{2+}]$, where $\sum[CuX_i]$ is the sum of all inorganic Cu complexes. Thus, the α for ASV ~ α_{Cu}, which is equal to 10–20 depending on the salinity and pH of the seawater[76]. The analytical window is often expressed as a log value, so for ASV the log α for pH 8.2 seawater is 1.3[76,77]. Techniques with higher analytical windows will be able to measure stronger organic Cu complexes while lower analytical windows will target weaker Cu complexes. The side reaction coefficient of the natural ligands in a sample is defined by $\alpha_L = K_L \times [L']$ where K_L is the conditional stability constant of the natural ligands and $[L']$ is the concentration of ligands not already bound to Cu. When α_L is within an order of magnitude on either side of the α of the chosen analytical method, those ligands will be detected, while those outside this window will not be detected accurately[78]. The ASV method has a very low analytical window since it only measures labile Cu complexes (inorganic Cu), and thus studies using ASV can generally only measure weak Cu-binding ligands[77].

Competitive ligand exchange-adsorptive cathodic stripping voltammetry (CLE-ACSV). More recently, the most common methods for understanding the Cu-binding ligand pool in seawater use a variation on the ASV technique and were first employed in the 1980s[79–81]. These methods are called competitive ligand exchange-adsorptive cathodic stripping voltammetry (CLE-ACSV) as described for Cu in Campos and van den Berg[18]. With this method, aliquots of a filtered seawater sample are titrated with increasing concentrations of dissolved Cu, in the presence of a buffer to maintain a constant pH (usually near 8). A well-characterized artificial ligand is then added to compete with the natural ligands for dissolved Cu complexation. It is recommended that the dissolved Cu additions range from +0 to at least 10x the ambient dissolved Cu concentration with at least two initial +0 nmol L^{-1} Cu amendments, across 10 or more titration points (ideally 15 points)[82,83]. After a period of equilibration (15 min to overnight[5,18,20,84]), each sample aliquot of the titration is analyzed sequentially using ACSV, whereby a hanging mercury drop electrode is used to apply a potential during the deposition step, in which the Cu and added ligand (AL) complex adsorb onto the mercury drop while the solution is stirred. The potential is then scanned in a negative direction and the reduction current of the Cu–AL complex is measured. This is repeated for each aliquot and the height of this peak is recorded and plotted against the concentration of dissolved Cu, ultimately producing the titration curve. This titration data is then interpreted using either linear[81,85,86] or non-linear[87] transformations to determine the ambient ligand concentration $[L_i]$ (where i denotes ligand class) and binding strengths ($K_{CuL_i,Cu^{2+}}$ or $K_{CuL_i,Cu'}$) of the natural Cu-binding ligand pool. Conditional stability constants for these ligand classes are expressed as either $K_{CuL_i,Cu^{2+}}$ or $K_{CuL_i,Cu'}$, where $K_{CuL_i,Cu^{2+}} = K_{CuL_i,Cu'} \times \alpha_{Cu}$. The technique described above is known as a "forward" titration, but Nuester and van den Berg[88] describe a "reverse" titration in which the AL is spiked in increasing concentrations while the dissolved Cu remains constant. The "reverse" titration technique is employed in circumstances when high dissolved Cu concentrations could result in the [dissolved Cu] >[L] such as around hydrothermal sites or in some coastal environments[89]. Both techniques have their advantages; the "forward" titrations are more commonly employed for determining Cu-binding ligand parameters.

Similar to ASV, the identity of the AL and the AL concentration together determine the analytical window, which in turn controls the range of binding strengths of the ligands that may be detected. The analytical window of the chosen AL is defined by $\alpha_{Cu(AL)_x} = K_{AL} \times [AL']^x$ and $[AL']^x$ is the concentration of the AL not bound to Cu, raised to the power of the stoichiometry of the Cu–AL complex[78,90]. In CLE-ACSV the [AL] ≫ dissolved Cu, so effectively the [AL] = [AL']. The resulting ligand concentrations and conditional stability constants measured with this technique thus represent an average of the different ligands that are detected within the analytical window applied. Depending on the AL, the $\alpha_{Cu(AL)_x}$ can vary several orders of magnitude[77], generally from $\log \alpha_{Cu(AL)_x}$ 2.1–6.5[77]. These methods are useful in that they allow calculation of the ligand parameters and resulting Cu^{2+} concentrations with minimal alteration of the natural sample, similar to ASV. However, aside from the $\log K_{CuL_i,Cu^{2+}}$, they do not provide information on the identity of the compounds that might make up the organic Cu-ligand pool in seawater, and are restricted by the detection window. An additional important consideration for these methods is the amount of dissolved Cu that is exchangeable with the added ligand. Findings from Moriyasu and Moffett demonstrate that dissolved Cu in older deep waters is primarily found in an inert form, with implications for speciation calculations[91]. When dealing with samples with a high percentage of inert Cu, an overestimation of a ligand's conditional stability constant will occur unless the inert fraction of dissolved Cu is omitted from the total dissolved Cu used in speciation calculations.

Direct quantification of ligand groups using voltammetry. Complementary voltammetric analyses can provide additional information on the identity of Cu-binding ligands by quantifying the electrochemical response of specific Cu-binding ligand groups within a sample, such as electroactive humic substances and thiols[92]. Here, the voltammetric peaks corresponding to the organic compounds being measured are quantified by additions of commercial standards (e.g., thiols such as glutathione and thioacetamide, and humic or fulvic acid isolates, typically from the Suwannee River (SRHA or SRFA)). These compound concentrations are then converted to a corresponding Cu-binding concentration using a ligand-binding ratio with Cu that has been measured independently for the standard being used. One caveat for this technique is that it is assumed the Cu-binding capacities of the natural ligands are the same as the standards and the method cannot discriminate the target ligand from other similar compounds present in the natural sample. For example, exopolymeric substances can be confused with humic substances, or multiple thiol peaks can coalesce and include sulfide, due to the compounds having similar electrochemical behaviors. These methods are continually being developed and improved[93] and they are fast and have low detection limits, allowing for a deeper insight into the key classes of Cu-binding ligands present in seawater than CLE-ACSV alone can provide.

Additional methods for characterizing the Cu-ligand pool. In addition to voltammetric techniques, several additional methods for characterizing the Cu-binding ligand pool in seawater and pore waters have also been used. These methods include liquid chromatography coupled to inductively coupled plasma mass spectrometry and electrospray ionization mass spectrometry (LC-ICP/ESI-MS) or Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), and Cu(II)-immobilized metal affinity chromatography (IMAC). Mass spectrometry techniques like LC-ICP/ESI-MS or FT-ICR-MS are used on samples that have first been extracted from seawater onto a column using solid phase extraction, and then these compounds are eluted in methanol or another solvent and the compounds are then separated using reverse phase chromatography coupled to a mass spectrometer[67,94]. Standards are generally not commercially available for the natural microbially produced Cu-binding ligands that might be found in seawater, so quantifying these compounds via mass spectrometry techniques is a challenge. However, some putative compounds have been quantified using LC-ICP-MS, where Cu-containing compounds are first identified by peaks in ^{63}Cu on the ICP-MS, and then these peaks are quantified and matched to their mass to charge ratio (m/z) found using LC-ESI-MS[67].

In the Cu(II)-IMAC method, the IMAC column is first loaded with Cu^{2+} ions and then the sample is passed over the column to capture organic compounds with an affinity for Cu^{95}. These compounds are then eluted and measured either via UV-adsorption or mass spectrometry[96,97]. This method is promising in that it is able to isolate only Cu-binding ligands while other solid phase extraction techniques capture a myriad of organic compounds. Both the Cu(II)-IMAC method and the mass spectrometry methods that have been employed thus far to identify and characterize the organic Cu-binding ligand pool in seawater have the potential to rapidly accelerate our understanding of marine Cu complexation, but very few published studies exist[95–99]. Importantly, new work also suggests that a large fraction of dissolved Cu (up to 90%) in deep waters may be inert, which has implications for these analytical methods as well as for dissolved Cu reactivity and bioavailability[91]. Future work that aims to characterize Cu-binding ligands in the marine environment will be particularly insightful to our understanding of the marine Cu cycle.

A call for an operational consensus on copper ligands. Although many methods have been used to characterize organic Cu speciation, we largely focused our discussion here based on lessons learned from voltammetry Cu speciation studies, which are the methods that have been employed for the bulk of the GEOTRACES studies and many other basin-scale datasets. All of the voltammetric studies that have been completed in the water column to our knowledge are summarized in Table 2 ($n = 54$ studies). These studies are those that only focused on measuring Cu-binding ligands in the water column of estuarine, coastal and open-ocean areas, excluding studies from specific sources such as hydrothermal vent fluids, pore waters, freshwater, or culture studies. Ligands characterized through voltammetric methods are usually categorized as either "strong" (L_1) or "weak" (L_2 or L_3) based on the measured $\log K_{CuL_i,Cu^{2+}}$, but the measured stability constants span several orders of magnitude ($\log K_{CuL_i,Cu^{2+}} = 7.5–16.5$; Table 2) and there is no consensus in the literature for Cu-binding ligands on what constitutes a L_1, L_2, or L_3 ligand for Cu. This is complicated by the fact that different analysts use different analytical windows, which influences the range of ligands that can be detected accurately by a given method. For organic Fe-binding ligands, Gledhill and Buck[100] proposed that ligand classes should be consistently operationally defined between studies based on their stability constants, where the strongest L_1 ligands are defined as ligands with $\log K_{FeL_1,Fe'} > 12$. There are known model ligands in seawater that fall within this operational class such as siderophores, which are compounds that bind Fe with a similar or greater binding strength than this operational definition. These operational definitions allow identified compounds to be connected with a particular ligand class, and enable comparisons across studies or distinct analytical windows.

However, much less is known about Cu-binding ligands in seawater and what compounds might comprise both the strong and weak Cu-binding ligand pool, and many studies only define a single ligand class (Table 2). Based on the few model Cu-binding ligands that we know of, we can start to operationally define some ligand classes. For example, the model chalkophore methanobactin binds to Cu with conditional binding strengths of 14–16[46,101]. Some of the strongest Cu-binding ligands observed in seawater have $\log K_{CuL_i,Cu^{2+}} > 14.0$ Kleint et al.[102] report $\log K_{CuL_i,Cu^{2+}}$ of 14.1 in shallow hydrothermal vents[102] and $\log K_{CuL_i,Cu^{2+}}$ of 14–16 have been reported in coastal waters around Scotland and in the NE Pacific[5,42]. Several additional studies in the Pacific[5–7,89,103,104], North Atlantic[16,78,80,105,106], and Southern Ocean[21,107] also all report stability constants exceeding 14.0 (Table 2). With this available observational data and the knowledge that model chalkophores fit within the range of binding strengths observed in seawater, we propose that L_1 ligands should be classified as ligands with stability constants ≥ 14.0. The majority of the Cu speciation studies compiled in Table 2 report ligands with $\log K_{CuL_i,Cu^{2+}}$ ranging from 9.0 to 15.5 ($n = 24$ studies), which likely represent an average of both strong and weak ligands. Model weaker ligands such as humic substances have $\log K_{CuL_i,Cu^{2+}} = 10.0–12.0$[26], while thiols have a range of $\log K_{CuL_i,Cu^{2+}}$ values from 11.0 to 16.0[34,66]. Finally, another model weaker ligand domoic acid has a $\log K_{CuL_i,Cu^{2+}} = 10.3$[70]. Based on the range of observed stability constants of these known model ligands, and the prevalence of weaker ligands detected in voltammetric studies (Table 2) we recommend ligands with $\log K_{CuL_i,Cu^{2+}}$ 10.0–14.0 should be operationally defined as L_2

Table 2 All voltammetry studies on organic copper-binding ligands.

Region	Depth (m)	Filter (μm)	[Cu] (nmol L⁻¹)	Method	$[L_1]$, $[L_2]$, $[L_3]$ (nmol L⁻¹)	$\log K_{Cu_{L_1},Cu^{2+}}$, $\log K_{Cu_{L_2},Cu^{2+}}$, $\log K_{Cu_{L_3},Cu^{2+}}$	References
North Pacific $n = 13$	Surface	0.45	<31	ASV	<30	-	Srna et al., 1980[222]
	Surface	0.45	4.25–23.2	ASV	1.25–22.20	-	Nilsen and Lund, 1982[223,c]
	Surface, deep	0.3	0.62	ASV	4.1–5.13		Donat et al., 1986[224]
	0–1400	0.3	0.57–2.19	ASV	0.62–2.4, L_1; 0.63–10.20, L_2	10.42–12.24; 8.24–9.24	Coale and Bruland, 1988[71]
	0–500	0.3	0.5–1.5	ASV	0.74–2.64, L_1; 0.20–10.29, L_2	10.21–12.24; 8.07–9.95	Coale and Bruland, 1990[72]
	0–3000	0.2	1.5–4.5	CSV	2.5–6.5	12.7–14.1	Moffett and Dupont, 2007[103]
	0–3000	0.45	0.62–3.7	CSV	1.54–6.10	12.84–15.11; 12.7–13.6	Buck et al., 2012[104]
	Surface and near bottom	0.2	24.3–43.1	CSV	22.7–53.2, L_1; 67.6–321, L_2	9.8–10.5	Li et al., 2015[225]
	0–1400	0.2	1.2–3.3	CSV	1.2–4.0, L_1; 1.1–20.1, L_2	15.0–16.5; 11.6–13.6	Whitby et al., 2018[5]
	0–58	0.2	0.6–4.7	CSV	0.7–6.9, L_1; 2.9–31, L_2	14.0–15.7	Wong et al., 2019[6]
	0–2000	0.2	0.6–2.5	CSV	0.9–4.5, L_1; 2.8–27, L_2	12.0–13.3; 13.2–14.5	Wong et al., 2021[226]
	Surface	0.2	2.9–124.8	Reverse CSV	3–121	13.0–16.2	Wiwit et al., 2021[227]
	100	0.2	1.14	CSV	1.4–2.0, L_1; 7.7–11.3, L_2	15.6–16.5; 13.8–14.0	
South Pacific $n = 2$	0–1000	0.2	0.26–1.75	CSV	1.55–6.46	12.5–14	Jacquot et al., 2013[8]
	0–5000	0.2	0.22–5.33	CSV	0.75–8.88	12.29–14.53	Ruacho et al., 2020[7]
North Atlantic $n = 14$	Surface	0.45	3.14–9.44	ASV	7–50	-	Duinker and Kramer, 1977[228]
	0–140	0.22	8.18–25.97	ASV	0.3–9.59	-	Hasle and Abdullah, 1981[229]
	Surface	-	3	ASV	0.615	12.2	Piotrowicz et al., 1982[230]
	Surface	-	2.3	CSV	11, L_1; 33, L_2	10.2	van den berg, 1984[79]
	0–3000	0.45	1.2–5.9	ASV	23–488	7.56–10.3; 7.80–8.21	Buckley and van den berg, 1986[231]
	Surface, deep	0.45	13–3.5	ASV	50–82		Kramer, 1986[232]
	Surface	0.4	0.92–3.2	CSV	1.2–73	10.5–15.9	Donat and van den berg, 1992[233]
	Surface	0.2	0.9–2	CSV	1.4–4.5	11.9–13.2	Moffett, 1995[234]
	Surface	0.2	12.7–27.9	ASV	16–38, L_1; 15–40, L_2; 54–100, L_3	>12; Mean 8.8; Mean 7.7	Kozelka and Bruland, 1998[235]
	Surface	0.2	40.8	ASV	11.5, L_1; 48.6–104, L_2	14.3	Croot et al., 1999[38,b]
	Surface	0.2	31.1–92.0	CSV	13–16, L_1; 13–15, L_2; 46–57, L_3	10.8–11.7; 12.3–12.5; 10.1–10.5	Bruland et al., 2000[77]
	Surface	0.2	12.6–13.6	ASV		8.8–9	Bruland et al., 2000[77]
	Surface	0.2	1.4–19.2	CSV	5.7–23.2, L_1; 9–17.6, L_2	11.5–15.6; 10.6–11.5	Oldham et al., 2014[105]
	0–5000	0.4	0.43–3.07	CSV	1.6–13.1, L_1; 2.1–31.9, L_2	14.0–15.1; 12.6–14.1	Jacquot et al., 2015[16]
	Surface	0.2	0.07–4.03	CSV	0.82–5.26	12.5–14.8; 13.4–14.4	Arnone et al., 2022[106]
South Atlantic $n = 1$	0–5000	0.2	0.5–3.5	CSV	0.5–2.6	11.6–14.6	Zitoun et al., 2021[236]
Gulf of Mexico $n = 1$	Surface	0.2	12–6.0	CSV	1.7–73, L_1; 8.7–281.2, L_2; 0.9–22.8	10.7–12.1; 12.6–14.5	Mellett and Buck, 2020[181]
Indian Ocean $n = 1$	Surface	0.45	1.96–3.24	CSV	4.13–17.8	12.1–15.7	Donat and van den berg, 1992[233]
Estuary	Surface	0.45	10.7–152	CSV	26–206, L_T (single fit)	11.7–12.78	van den Berg et al., 1987[80]

Table 2 (continued)

Region	Depth (m)	Filter (µm)	[Cu] (nmol L⁻¹)	Method	[L₁], [L₂], [L₃] (nmol L⁻¹)	$\log K_{CuL_1,Cu^{2+}}$, $\log K_{CuL_2,Cu^{2+}}$, $\log K_{CuL_3,Cu^{2+}}$	References
n = 17							
	Surface	0.45	45–48	CSV	23–152, L₁ (2ligand fit)	13.2–14.9	Donat et al., 1994[237]
				CSV	44–131, L₂ (2ligand fit)	11.5–12.8	
				CSV	13, L₁	13.5	
	Surface	0.2	3.8–117	CSV	60–80, L₂	9.0–9.6	Moffett et al., 1997[119]
				CSV	10.5–33.3, L₁	12.57–14.3	
	Surface	0.2	7.4–27.9	CSV	39.6–135.7, L₂	9.94–11.72	Wells et al. 1998[238]
				ASV	16–38, L₁	Exceeded window	
				ASV	16–107, L₂	8.2–10.5	
				ASV	40–100, L₃	8.8–9.0	
	Surface	0.2	7–23.6	CSV	9.6–32.5, L₁	14.51–16.14	Laglera and van den berg, 2003[153]
				CSV	13.7–299, L₂	12.7–13.46	
	Surface	0.22	81.9–190.1	CSV	66.6–160.7	12.5–12.9	Dryden et al., 2004[239],a
	Surface	0.2	2.9–22.7	CSV	7–229	13.5	Shank et al., 2004[126]
	Surface	0.45	17.9–49.9	CSV	22–265, L₁	13.1–14.3	Buck and Bruland 2005[20]
				CSV	55–135, L₂	12–12.9	
	Surface	0.2	5.6–6.4	CSV	116–132	13.5	Shank et al., 2006[173],a
	Surface	0.22	13.1–24.7	CSV	26–56	11.8–12.3	Dryden et al., 2007[60]
	0–115	0.2	4.08–6.12	CSV	2.98–7.23	12.9–15.6	Jacquot et al., 2014[240]
	Surface	0.2	11.6–54.5	CSV	21.9–57.5	12.2–13.8	Whitby and van den Berg, 2015[43]
	Surface	0.22	2.9–65	CSV	16–51, L₁	14.0–15.2	Whitby et al., 2017[11]
				CSV	30–112, L₂	12.1–13.4	
	0–40	0.2	2.03–2.65	CSV	1.95–4.21, L₁	13.7–16.0	Wong et al., 2018[241]
				CSV	6.3–33, L₂	11.1–12.8	
	Surface	0.02	3–77	ASV	6–120, L₁	9.4–11.4	Dulaquais et al., 2020[41]
		0.45	5–82	ASV	8–147, L₂	11.4–13.4	
	Surface	0.22	5–50	ASV	3.5–63, L₁	9.6–10.8	Padjan et al., 2021[115]
				ASV	17–104, L₂	8.2–9.0	
	Surface	0.015	0.7–28.3	CSV	1.3–66.3, L₁	13.2–15.3	Hollister et al., 2021[10]
		0.2	0.7–42.8	CSV	2.8–83.2, L₂	12.6–14.2	
Southern n = 5	Surface	0.45	3.93–8.81	ASV	1.57	–	Batley and Florence, 1976[242]
	180	0.4	2.16–2.4	CSV	2.5–4.13, L₁	15.7–16.4	Buck et al., 2010[107],a
				CSV	0.3–1.8, L₂	14.6–15.5	
	Surface	0.4	1.33–2.84	CSV	2.26–8.04, L₁	13.57–16.0	Bundy et al., 2013[21]
				CSV	2.54–8.32, L₂	12.73–13.33	
	0–3500	0.2	0.32–3.41	CSV	1.08–5.22	12.00–13.64	Thompson and Ellwood, 2014[12]
	Surface	0.2	0.47–0.55	CSV	1.5–2.5, L₁	12.64–13.57	
				CSV	7.4–7.76, L₂	11.2–11.72	
	0–3000	0.2	0.85–2.66	CSV	–	>13	Heller and Croot, 2015[84]
				CSV	23.6–73.3, L₂	11.1–12.14	

All published voltammetry (ASV, CLE-ACSV) studies to date that characterized organic Cu-binding ligands in the water column.
aDenotes a study that measured water column samples from photochemical or biological incubation experiments.
bIncludes studies that measured model Cu-binding ligands.
cStudies that performed an acidification and a digestion.
"–" denotes samples where a particular ligand class was not detected or reported.

ligands. There are no known model ligands with binding strengths less than 10.0, though several studies have identified ligands in seawater with weaker binding constants (Table 2) and freshwater algae have been shown to exude exopolysaccharides with $\log K_{CuL_i,Cu^{2+}} < 10.0$[44]. We therefore recommend that any ligands that are characterized with a $\log K_{CuL_i,Cu^{2+}} < 10.0$ should be classified as L_3 ligands (Table 3). By operationally defining these ligand classes based on the available observational data and known model Cu-binding ligands, we hope future studies will be able to discern emerging patterns in strong and weak Cu-binding ligand cycling.

Sources, sinks, and internal cycling
Sources of Cu-binding ligands
Rivers and margins. Rivers and estuaries are important margin sources for organic Cu-binding ligands. In estuaries, dissolved Cu concentrations typically show conservative behavior, governed by the mixing of high dissolved Cu river water with lower dissolved Cu seawater (Fig. 1)[108–115]. Deviations from the conservative trend have also been observed, as a result of sorption to solids, colloidal flocculation and/or biological uptake[41,108,110,116,117], or Cu inputs along the estuary[20]. Anthropogenic activity can enhance the supply of dissolved Cu through the contamination of aerosols, rivers and groundwater, particularly in coastal areas[15,118–120].

Table 3 Operational definitions of organic copper-binding ligands in seawater.

Operationally defined ligand class	$\log K_{CuL_i,Cu^{2+}}$
L_1	>14.0
L_2	10.0-14.0
L_3	<10.0

Proposed operational definitions of Cu-binding ligand classes in seawater using CLE-ACSV methods.
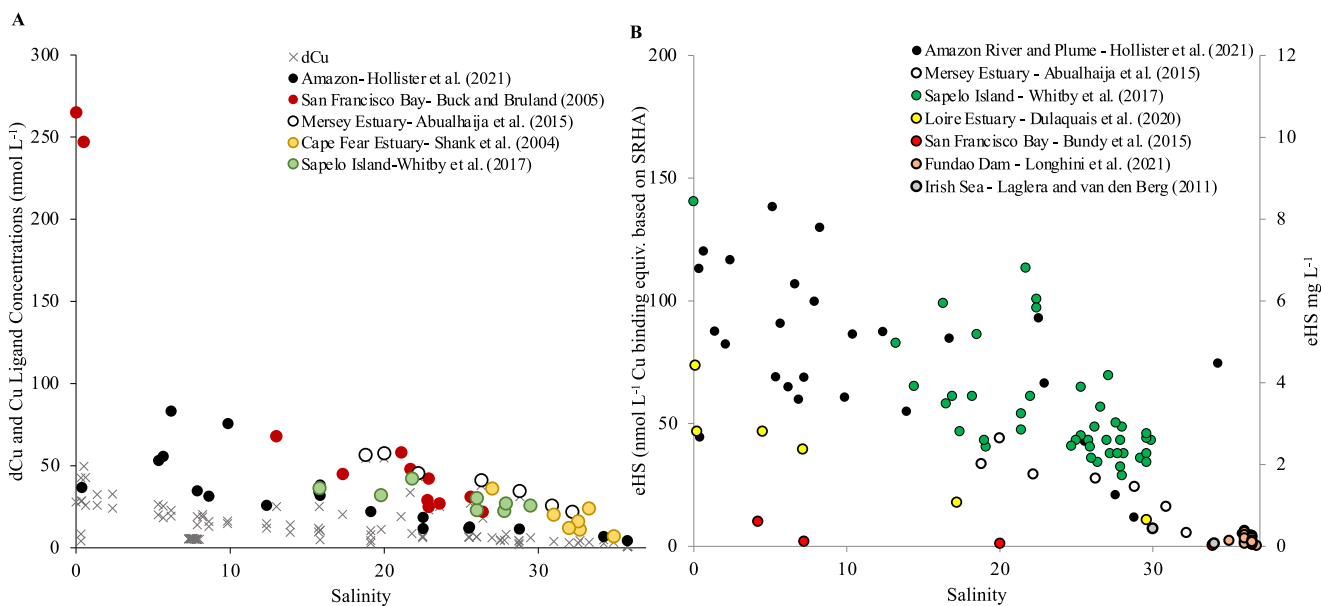
The dissolved Cu in rivers and estuaries is largely organically complexed (>99%), and therefore organic Cu-binding ligands play an important role in the cycling of dissolved Cu in estuarine systems (Fig. 1). For example, humic substances bind strongly to dissolved Cu, and the flocculation of dissolved Cu can occur when humic substances mix with seawater[121,122], although the stabilization of dissolved Cu-humic complexes may also mitigate this removal process[123]. Flocculation during estuarine mixing removes 20−40% of the dissolved Cu[59,124], and up to 97% of the ligands[10], reducing dissolved Cu and Cu-ligand concentrations to the levels typically found in coastal waters[10,60,125,126]. The Cu-binding ligand concentrations are also generally conservative along the salinity gradient in most studies (Fig. 1). However, few studies have examined the true riverine endmember, so it is possible that non-conservative mixing occurs in the low salinity end of estuaries (Fig. 1), possibly due to flocculation of a colloidal fraction of ligands and dissolved Cu[10]. Data from Buck and Bruland (2005)[20,127,128] for example, found much higher Cu-ligand concentrations at the low salinity end of the estuary compared to dissolved Cu, and the excess Cu-binding ligands (excess ligand = [ligand] – [dissolved Cu]) decreased as salinity increased (Fig. 1). Whether there are more sources of Cu-binding ligands in freshwater, or Cu ligands are preferentially scavenged relative to dissolved Cu, is unknown.

Anthropogenic activities that lead to elevated dissolved Cu in rivers and the margins may also trigger ligand production in the estuary. Cyanobacteria such as *Synechococcus* are particularly sensitive to Cu toxicity even at very low levels of free Cu[19]. To mitigate the effects of Cu toxicity, *Synechococcus* have been shown to produce strong Cu-binding ligands and release them into solution to mediate Cu toxicity and alter the bioavailability to the in situ dissolved Cu[19]. This process might be an important source of Cu-binding ligands in coastal environments, particularly where total dissolved Cu is high and cyanobacteria are abundant. Anti-fouling paint itself has also been found to be a source of Cu-binding ligands in addition to dissolved Cu[129].

Fig. 1 Dissolved copper and organic ligands in estuarine systems. Data from select studies showing typical **A** dissolved Cu concentrations (*x*) and organic Cu-binding ligand concentrations (circles), and along the river to seawater continuum. **B** Concentrations of electroactive humic substances expressed in terms of their potential Cu-binding equivalent concentration, based on values calculated for SRHA in Whitby et al.[43]. Amazon, Mersey, and Sapelo data use the CuHS technique[43]; San Francisco Bay, Fundao Dam, and Irish Sea use the FeHS technique[243-245], while Loire Estuary uses the MoHS technique[92]; different methods have been shown to give comparable eHS concentrations[35,246].

Sediments. Shelf sediments are another important source of Cu-binding ligands to the ocean[7,21,84] and can be a source of L_1 ligands[7,21]. In the Equatorial Pacific, strong L_1 ligands ($\log K_{CuL_i,Cu^{2+}}$ >14.0) were detected off the coast of Peru, which are some of the strongest ligands that have been detected from shelf sources. These ligands corresponded with a plume of ^{228}Radium (^{228}Ra) from the continental shelf[7,130]. Bundy et al.[21] detected intermediate L_2 ligands ($\log K_{CuL_i,Cu^{2+}}$ 10.0–14.0), from the Antarctica Peninsula shelf and Heller and Croot[84] similarly detected intermediate ligands in this region. The variability in binding strengths of detected ligands is likely due, in part, to differences in the analytical window and data processing method used by each study[127,128], but could also be due to differences in the type of ligands associated with sediment sources. Studies in estuarine and coastal marine sediments have reported multiple ligand classes, with one study in a shallow lagoon finding two types of ligands, with $\log K_{CuL_i,Cu^{2+}}$ 14.2 and 12.5, which were associated with thiol compounds[59]. Another coastal study also found one relatively stronger and one relatively weak ligand, but both with $\log K_{CuL_i,Cu^{2+}}$ <10.0, which, with our new operational definition would thus be characterized as L_3 ligands[131]. Additional observations are needed to gain a better understanding of the class of ligands coming from sediments. It is important to understand whether dissolved Cu emanating from sediments is likely to be associated with either weaker or stronger Cu-binding ligands, because this would impact the longevity and residence time of sediment-derived dissolved Cu and its export offshore. Weaker ligands have thus far been found in higher concentrations than stronger ligands in continental shelf regions, however even low concentrations of strong ligands will be primarily responsible for binding dissolved Cu from margin sources if they are present in excess of dissolved Cu. Determining the sources of strong ligands in near shore environments is important to constrain in future studies, to uncover the impact of ligands in stabilizing external margin sources of dissolved Cu to the ocean.

Hydrothermal vents. Hydrothermal vents play an important role for trace metal biogeochemistry as vents can provide an influx of trace metals to the overlying waters[132,133]. Hydrothermal vents may also be a source of Cu-binding ligands[22,134–136]. Samples originating from within the vent or its immediate surroundings have been observed to have very high concentrations of Cu-binding ligands, ranging from 29 to 4460 nmol L^{-1}, especially when compared to ligand concentrations found within a typical open-ocean profile (typically ranging from less than 1 nmol L^{-1} to 4–6 nmol L^{-1}). Within waters impacted by hydrothermal venting, thiols and small proteins are thought to be responsible for at least some fraction of the Cu complexation[22]. A possible source of Cu-binding ligands within vent fluids may come from vent microbes who have been shown to produce ligands in order to buffer against high labile Cu concentrations immediately surrounding the vent field[135]. Though samples within and immediately around a hydrothermal vent have been shown to have Cu-binding ligand concentrations up to 1000-fold of those found within the water column, it is not known what fraction of these ligands make it into the neutrally buoyant plume and potentially impact the global cycling of ligands and dissolved Cu[136]. Current available water column data from GEOTRACES expeditions GA03[16] and GP16[7] do not show a surplus of Cu-binding ligands near hydrothermal vents in the neutrally buoyant plumes, though the sampling on these expeditions happened tens to hundreds of meters above the ridge. If hydrothermal vents are indeed a source of Cu-binding ligands to the overlying water column this would be important to constrain, as it would impact the transport of dissolved Cu in these systems.

Microbial production of Cu-binding ligands. Similar to Fe-binding ligands, a variety of marine microbes have been shown to produce Cu-binding ligands[137]. Cu-binding ligand production, usually in response to Cu stress, has been observed in cultures of heterotrophic bacteria[96], cyanobacteria[19,96,138,139], dinoflagellates[138,140], coccolithophores[141,142], and diatoms[138]. Voltammetric measurements of these ligands in culture showed a range in $\log K_{CuL_i,Cu^{2+}}$ from 11.0 to 15.6[137] (Table 1). Cu-binding ligands such as glutathione[141], phytochelatins and metallothioneins[55,57,143–146] are used to detoxify Cu, while compounds such as methanobactins and staphylopines have been shown to facilitate Cu uptake[47,101].

Although many Cu-binding ligands produced by microbes in culture have been characterized, much less is known about the impact of microbial Cu-ligand production in seawater. The similar binding strengths of ligands observed in culture compared to those measured in seawater suggest that at least a portion of the Cu-ligand pool in seawater likely has a microbial source. Despite known ligand production from phytoplankton and bacteria, studies have only rarely identified a correlation with productivity or biomass and Cu-binding ligands[99]. Thiols, for example, are produced by many phytoplankton and bacteria and yet a strong connection between productivity and thiols has not been observed in the marine environment. Unlike siderophores for Fe, thiols can be produced for a variety of reasons, including dealing with free radicals[147], or to detoxify other metals[143,148–152]. Thus, the lack of a significant relationship of thiols with productivity is perhaps not surprising. It is clear however, that Cu-binding ligand production in the marine environment appears to be distinct from that of Fe ligands, likely related to differences in the role of these metals as micronutrients and toxicants. Further research is necessary to determine what might trigger Cu-binding ligand production by microbes in seawater. It is important to note however, that many of the other sources of Cu-binding ligands that have been observed (e.g., sediments, hydrothermal vents) are likely ultimately from a microbial source. Thus, understanding microbial production of ligands is critical.

A few studies have connected voltammetric measurements of the Cu-binding ligand pool with ligand characterization techniques via mass spectrometry[23,97] or specialized voltammetric techniques aimed at characterizing specific Cu compounds such as thiols[61,93,103,153]. Initial work that characterized Cu-binding ligands in seawater used Cu(II)-IMAC columns to capture Cu-containing organic compounds and then elute and measure them via UV-adsorption or mass spectrometry[96,97]. This work found that the extracted Cu compounds contained nitrogen and thiol-like functional groups, and were similar to some ligands that had been found in algal cultures. Cu-binding ligands identified using Cu(II)-IMAC in the northeast Pacific along Line P also correlated with chlorophyll *a* and phaeopigments across both near shore and offshore stations and in different seasons, providing additional evidence for a biological ligand source[98]. Voltammetric studies have also routinely noted high ligand concentrations and low dissolved Cu concentrations in surface waters and in the chlorophyll maximum[8,16,103]. In the eastern South Pacific, Boiteau et al.[67] identified several distinct Cu-binding compounds via LC-ICP/ESI-MS techniques in surface waters with azole-binding groups, some of which also bound nickel (section "Components of the marine Cu-binding ligand pool"). The Cu-binding ligands identified in this study were higher near shore and decreased offshore, along with the dissolved Cu. At present, the sources of these ligands are unknown, but are presumed to be biological in origin. Further studies able to identify Cu-binding ligands in seawater will provide essential next steps in characterizing microbial sources of Cu ligands in seawater.

Microbial production of Cu ligands is likely particularly important in oxygen minimum zones (OMZs), as Cu may be a limiting factor for microbial production in these regions. Microbial production in OMZs is dominated by ammonia oxidizing archaea (AOA) and bacteria (AOB). Recent studies using genome sequencing techniques revealed that AOA and AOB produce a wide range of Cu containing enzymes[154–156]. Moreover, experiments from Amin et al.[157] showed that AOA ammonia oxidation rates and cell density were strongly decreased in Cu-limiting conditions, and totally inhibited in the absence of Cu. They also observed a strong dependency of AOA-specific growth to Cu^{2+} concentrations, with limiting effects below 10^{-13} mol L^{-1} and potentially toxic effects above 10^{-11} mol L^{-1}. These observations indicate a potential link between Cu complexation in seawater and AOA growth, since AOA appear to primarily take up free Cu. Experiments by Amin et al.[157] indicated that the AOA *N. maritimus* may be a source of strong Cu-binding ligands when grown under Cu-replete conditions. In these conditions, AOA may produce Cu-binding ligands to regulate Cu^{2+} concentrations at optimal levels for growth. However, Jacquot et al.[8] argued that the low Cu^{2+} concentrations observed in the Pacific OMZ was the result of high consumption rates of Cu, leading to low Cu^{2+}, rather than high ligand production rates leading to low Cu^{2+}. They suggested that the elevated ligand concentrations found in these regions may have originated from remineralization of organic particles or advected dissolved organic material from the shelf. Direct microbial ligand production cannot be determined from these studies, because the identities of the ligands are unknown and thus any potential biosynthesis genes for ligand production cannot be explored. Whether organisms in OMZs are producing organic Cu-binding ligands to regulate growth is an important future area of dissolved Cu biogeochemistry to constrain.

Sinks of Cu-binding ligands
Microbial Cu-ligand uptake. In addition to Cu-binding ligand production, microbes may also be a sink for Cu-binding ligands. Initial work in culture studies showed that Cu uptake rates were driven primarily by Cu^{2+} concentrations and not the total dissolved Cu concentration in the culture media[4,158,159]. These observations led to the understanding that uncomplexed Cu (Cu^{2+}) might be the only bioavailable form of Cu. However, additional studies demonstrated that eukaryotic Cu uptake rates exceeded those that would be expected from simply a diffusive supply of Cu^{2+} by 2–1000-fold, suggesting that at least some marine phytoplankton are capable of accessing organically bound Cu[2,101,160–166]. It was unknown at the time however, whether in situ marine phytoplankton and bacteria were able to take up Cu from the natural Cu ligands present in seawater. Semeniuk et al.[167] provided the first such evidence that this was indeed the case, and showed that dissolved Cu in the northeast Pacific was taken up 5 times faster than would be expected based on simply Cu′. This was then substantiated with a more detailed study that showed definitively that phytoplankton and bacteria were able to take up naturally present strong and weak Cu-ligand complexes[168]. Thus, it appears that Cu may act similarly to dissolved Fe in that Cu likely has an "envelope" of bioavailability[169,170], where Cu′ is the most bioavailable, but some microbes can also utilize Cu from a range of ligand complexes. Bioavailability is likely related to the reducibility of Cu(II) to Cu(I) from ligand complexes, as Cu uptake transporters (known as CTR) target Cu(I) and appear to be relatively common uptake systems in some diatoms[166,171]. Semeniuk et al.[168] also suggested that some phytoplankton may use weaker Cu-binding ligands as a "weak ligand shuttle" to move dissolved Cu from strong complexes to weaker, more reducible Cu complexes
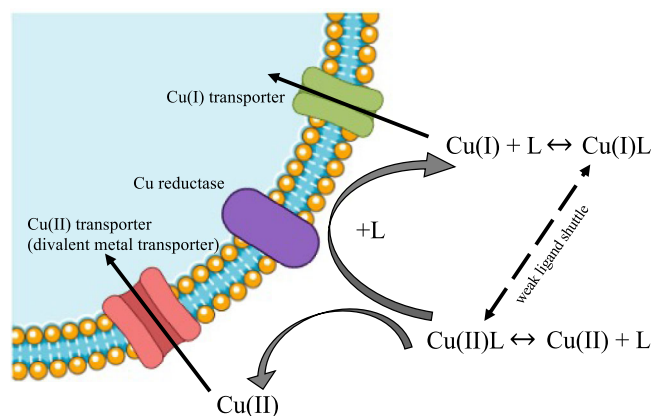

Fig. 2 Copper uptake mechanisms in the marine environment. Known mechanisms of copper uptake associated with organic copper-binding ligands in the marine environment by microbes.

(Fig. 2). For example, cysteine has been shown to enhance Cu bioavailability[172]. Regardless, there is still much to explore with respect to how Cu speciation impacts dissolved Cu bioavailability to marine phytoplankton and bacteria, and how microbial uptake impacts Cu-binding ligand concentrations.

Photochemical degradation. Cu-binding ligands are well known to be photochemically degradable by photosynthetically active radiation and UV-light[31,173–177]. The exact mechanism of the photodegradation is not as well known as it is for some Fe-binding ligands such as siderophores[178]. However, both humic substances and thiols are known to be photochemically degradable[62,177,179]. The photochemical effects on Cu-binding ligand capacity have also been observed in incubation experiments[107,180] and in seasonal differences in Cu-binding capacities in the Gulf of Mexico[181]. Although the photochemical degradation of Cu-binding ligands is well known, the bulk of the studies focused on this topic have been in estuaries and less explored in the open ocean. It is very likely that photochemical degradation of Cu-ligand complexes impacts the bioavailability of Cu[180], so further work in this important area will shed important insights on ligand cycling.

Global open-ocean distributions of Cu-binding ligands. While the sources and sinks of dissolved Cu have been explored recently in basin-scale studies and biogeochemical modeling efforts[7–9,16,182], the relevant sources and sinks and internal cycling of Cu-binding ligands are not as well understood. Advances in metal speciation data processing have facilitated high sample throughput leading to large-scale datasets of dissolved Cu speciation. Open-ocean data from the Pacific and Atlantic oceans shows patterns in Cu-binding ligand distributions that are similar to distributions of dissolved Cu, with low nanomolar concentrations near the surface and quasi-linear increasing concentrations with depth[5,7,8,12,16] (Fig. 3). The highest concentrations of Cu-binding ligands have been detected in the deep waters (>3000 m) from full depth ocean profiles, and near bottom sediments (concentrations up to 4–6 nmol L^{-1}). In a comparison of Cu-binding ligand distributions between the Pacific and North Atlantic basins, higher concentrations of both dissolved Cu and Cu-binding ligands were observed on average in the Pacific basin. This observation was interpreted as an accumulation of Cu-binding organic ligands in older Pacific waters (Fig. 3B[7]). The difference in ligand concentrations in deep waters of the Pacific compared to the Atlantic are not as great as the differences in dissolved Cu concentrations however, leading to
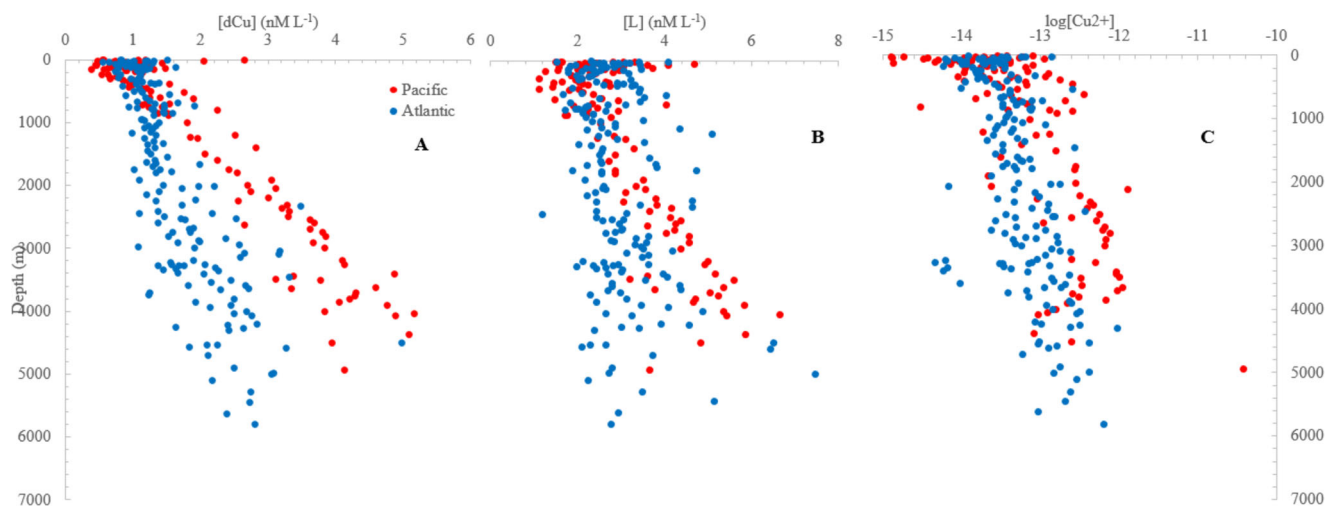
Fig. 3 Dissolved copper and ligand data for the North Atlantic and South Pacific Oceans. A Averaged dissolved Cu data for the Pacific and North Atlantic oceans. **B** Averaged Cu-binding ligand data for the Pacific and North Atlantic oceans collected from available datasets. **C** Averaged free Cu, log[Cu^{2+}], from available datasets for the Pacific and North Atlantic oceans[5,7,8,12,16,247].

some higher Cu^{2+} concentrations in the deep Pacific (Fig. 3C)[7], though not approaching toxicity thresholds for cyanobacteria (~10^{-11} mol L^{-1}). Organic ligands appear to "buffer" free Cu concentrations to within a relatively narrow range in both the Atlantic and Pacific Ocean basins (Fig. 3), perhaps approaching levels that are even limiting to growth for some organisms. Recent work that suggests that a large fraction of the dissolved Cu in seawater is likely inert to exchange[91], and the observation that a large portion of dissolved Cu is associated with very strong ligand complexes[7,16] is important for considering open ocean dissolved Cu reactivity. The impact that organic ligands have on the cycling of dissolved Cu on the basin scale also has important implications for understanding whether dissolved Cu is reversibly scavenged throughout the water column, as has been proposed by a modeling study[182] and some work on Cu isotopes[183,184].

While some strides have been made in identifying ligands in surface waters, we still do not have a thorough understanding of the identities of Cu ligands. For example, algal cultures release Cu-binding ligands such as thiols[55,142], and Boiteau et al.[67] identified Cu-binding ligands in the surface waters of GP16 where biological utilization of dissolved Cu is occurring, but the ultimate origin of those ligands is unknown. Though Cu-binding ligands are starting to be identified in open-ocean waters[67], these ligands have not been connected to micro-organisms or have been shown to be produced under specific circumstances. Available data from the NE Pacific suggested up to 32% of Cu-binding ligand pool was made up of humic substances and thiol type ligands[5]. While the study of humic substances and thiols in the open ocean is important for understanding the role of some key contributors to marine Cu speciation, this demonstrates that there is still a large portion of the Cu-binding ligand pool that remains unidentified, particularly in the deep ocean.

Internal cycling. Despite recent ocean-basin-scale datasets on the distributions of Cu-binding ligands, we know very little about their internal cycling. A comparison of data from the North Atlantic[16] and the South Pacific[7] suggests that both dissolved Cu and Cu-binding ligands accumulate in the oldest waters of the deep Pacific, implying that ligands are likely produced along with dissolved Cu during regeneration of sinking particles[7,16]. Indeed, Fe and Cu-binding humic-like compounds were recently found to be directly produced during particle degradation[185]. Deep profiles of Cu ligands from both the Atlantic and Pacific also suggest that

Cu-binding ligands are scavenged onto sinking particles. Although Cu displays a nearly linear profile with depth indicative of an influence of reversible scavenging[9], Cu-binding ligands do not have the same profile (Fig. 3). Cu-binding ligands tend to remain in excess of dissolved Cu even near the ocean bottom, but the amount of excess ligand decreases with depth[7,16]. It is unclear whether this is due to a lack of deep ligand sources, degradation of Cu-binding ligands with depth, or a scavenging of Cu-binding ligands onto particles. However, it is clear that the cycling of organic Cu-binding ligands is not always entirely coupled to that of dissolved Cu. Owing to our lack of knowledge on the sources, sinks and internal cycling of Cu-binding ligands we cannot yet estimate a residence time for these complexes. The only estimate of a residence time for deep ocean Fe-binding ligands was 779–1035 years[186], but no estimates for residence times for ligands in surface waters have been proposed. Many processes acting on Fe-binding ligands also likely overlap for Cu-binding ligands, so it is possible they have similar residence times. However, even the residence time of Cu is up for debate[9,187] and a lot of work remains to be able to better understand the residence time of both dissolved Cu and Cu-binding ligands.

Modeling the copper-binding ligand pool
The rarity of in situ measurements and lab experiments concerning Cu-binding ligands limits our current understanding of their global cycling and their potential importance for ocean biogeochemistry. In this context, models represent a valuable tool since they enable the testing of different hypotheses regarding the influence of different processes on ligand cycling. In this section, we present some of the modeling work that focus on Cu-binding ligands. These models differ in scope, scale and complexity, but they allow for studying specific aspects of Cu-ligand cycling. We then briefly discuss how information from these different models could be integrated into global ocean models.

Geochemical models. Geochemical models can be used to constrain some external sources of ligands. For instance, Sander and Koschinsky[22] and Stüeken[188] studied the impacts of hydrothermal sources on oceanic budgets of Cu-binding ligands. These models represent the thermodynamic mixing effects between hydrothermal fluids and the overlying water column. Both fluids have fixed Cu and ligand concentrations with fixed binding strength (log$K_{CuL_i, Cu^{2+}}$). One fundamental hypothesis of these

models is that some ligands originate from hydrothermal vents, but that the majority are already present in the water column and that both ligand sources have similar complexing capacities. Using box models to integrate the results over the ocean, these studies revealed that 2–20% of total dissolved Cu in the modern ocean is supplied by hydrothermal vents and stabilized by organic ligands in the deep water column. Stüeken[188] also used this geochemical thermodynamic modeling method to represent past hydrothermal vents and showed that rivers became the dominant ocean dissolved Cu source in the Proterozoic.

Models of Cu chemical speciation. Metal ions that bind to natural organic matter such as humics vary according to metal-substrate affinity constants, reaction stoichiometries, competition between different ions, and may be impacted by water pH and salinity. Many thermodynamic chemical models exist to represent metal ion binding with humic substances and oxides in fresh-water, seawater and soils[189–194]. These models provide affinity constants for the binding of different metals (including Cu) with humics as well as descriptions of competition effects between different metals for humics. These models are frequently used in ecotoxicological assessments to identify metal contamination in freshwater, estuarine and soil systems. Furthermore, chemical speciation models are used to predict Cu toxicity to plankton and fish through the use of Biotic Ligand Models (BLM)[195]. These models are frequently used by scientists and governments to evaluate Cu contamination and toxic impacts on soils, freshwater and estuarine systems[196–200].

The work of Hirose[201] specifically addressed Cu speciation and binding with organic ligands in marine systems. This author used similar principles of thermodynamic chemistry modeling to study different roles of Cu ligands, including the protecting role of weak ligands for microbes against Cu toxicity in high Cu regions[201], Cu-Fe competition for strong ligands, which may reduce Fe concentrations and lead to Fe deficiency[202], or the buffering effects of the excess ligands, which may shield Cu speciation from the effects of ocean acidification[203].

Towards integrated modeling of Cu ligands in global biogeo-chemical models (GBCM). Because of the importance of Cu in regulating microbial production and due to competing hypotheses about the shape of its vertical profile, Cu has recently been included in two global models[9,187]. However, both these models lack a representation of Cu-ligand cycling. Only in Richon and Tagliabue[9] are Cu ligands explicitly represented with a uniform concentration and complexing capacity. Even though we are far from representing the 3-dimensional complexity of Cu-ligand cycling in global biogeochemical models (GBCMs), results from small scale modeling and increased availability of Cu-binding ligand distributions may provide some future directions for GBCM development.

The geochemical and other types of box models may provide information on the external sources of Cu ligands. Even though they do not provide constraints on the ligand fluxes from hydrothermal or riverine sources, models of thermodynamic equilibrium seem to confirm that the ligands from external sources have similar complexing capacity to those already present in the water column. These modeling efforts may indicate that there is no change in the complexing capacity of ligands during their cycling in seawater or that their ultimate sources (e.g., microbes) are the same.

Chemical speciation models provide useful information on the complexing capacities and the kinetics of Cu-ligand reactions. However, these models showed that there is a range of complexing capacities depending on the chemical nature of the

ligand. Therefore, more studies on the nature of Cu ligands are necessary in order to accurately represent Cu complexing in GBCMs. Results from BLM and other toxicity models can inform GBCMs with Cu concentration thresholds, which can be implemented to represent the toxic impacts of Cu on oceanic species. This type of information has already been used by Prosnier et al.[204] to model Cu toxicity on freshwater *Daphnia*. Fe cycling is well represented in GBCMs and results such as Hirose (2007)[202] may be used to represent the competition between Cu and Fe for ligands and the potential impacts on global ocean productivity. Finally, future studies that define Cu-ligand distributions in the operationally defined classes stated in this manuscript, will facilitate cross comparisons between regions, analysts and analytical methods.

## Synthesis: towards an integrated view of organic Cu-ligand cycling

Based on our review of current knowledge and understanding of Cu-ligand cycling, we summarize Cu-ligand sources, sinks and internal cycling in the global ocean and in the following section attempt to calculate some of the first global fluxes of Cu-binding ligands (Fig. 4).

Constraining the external sources of Cu-binding ligands to the oceans. Several studies indicate that hydrothermal vents may be sources of Cu ligands (section "Hydrothermal vents"). However, the nature of the ligands found near vent sites is up for debate[22,135] and recent measurements found no surplus of ligands near vent sites[7,8]. Therefore, based on the current understanding, it is difficult to assess the global contribution of hydrothermal vents to the global Cu-ligands budget and we have not attempted to calculate it here.

Many studies on riverine ligands highlighted a statistically significant relationship between L_1 concentrations and dissolved organic carbon (DOC)[42,126,205,206]. This relationship is summarized in Supplementary Table 1. Based on the data we compiled, we derive a generic relationship between DOC fluxes and L_1 concentrations: $[L_1] = 0.0002[DOC] - 0.0367$ ($R^2 = 0.8129$). This equation allows the calculation of a global estimate of riverine sources of strong ligands based on global estimates of DOC riverine fluxes[207–209], yielding a global riverine source of ligands between 30 and 1000 Gmol L^{-1} yr^{-1}.

A few studies have estimated the sediment-water exchange flux of Cu ligands. Shank et al.[126] measured ligand fluxes between 850 and 870 nmol m^{-2} day^{-1} (with standard deviation about ±600). Santos-Echeandía et al.[210] calculated Cu and ligand fluxes from tidal pore waters in a salt marsh estuary in Portugal between 2 and 6 mol m^{-2} day^{-1}. However, ligand concentrations in estuaries are usually high, therefore the local inputs from sediments may have limited impacts on overall ligand budgets. Murray et al.[211] estimated the global tidal flats area to be about 128×10^9 m^2, so using Santos-Echeandia's estimate of ligand exchange from tidal flooding gives about 0.2 Gmol L yr^{-1} of strong ligand sourced from tidal exchange. Using a global estimate of oceanic shelf areas (using the ORCA2 grid), we calculate a sediment source of Cu ligands of about 5.7 Gmol L yr^{-1}.

Several studies have measured aerosol ligand to organic carbon ratios[212–214]. Overall, a higher ligand to carbon ratio is observed in samples taken in a forest throughfall[212,213] than in coastal plains[214], indicating that vegetation may be a source of Cu ligands in aerosols. Based on these studies, we found an average ligand/organic carbon ratio around 450 nmol L^{-1} mg^{-1} C in rainwater. Multiplying this ratio by Kanakidou et al.[215] estimate of the global wet deposition of organic carbon to the oceans
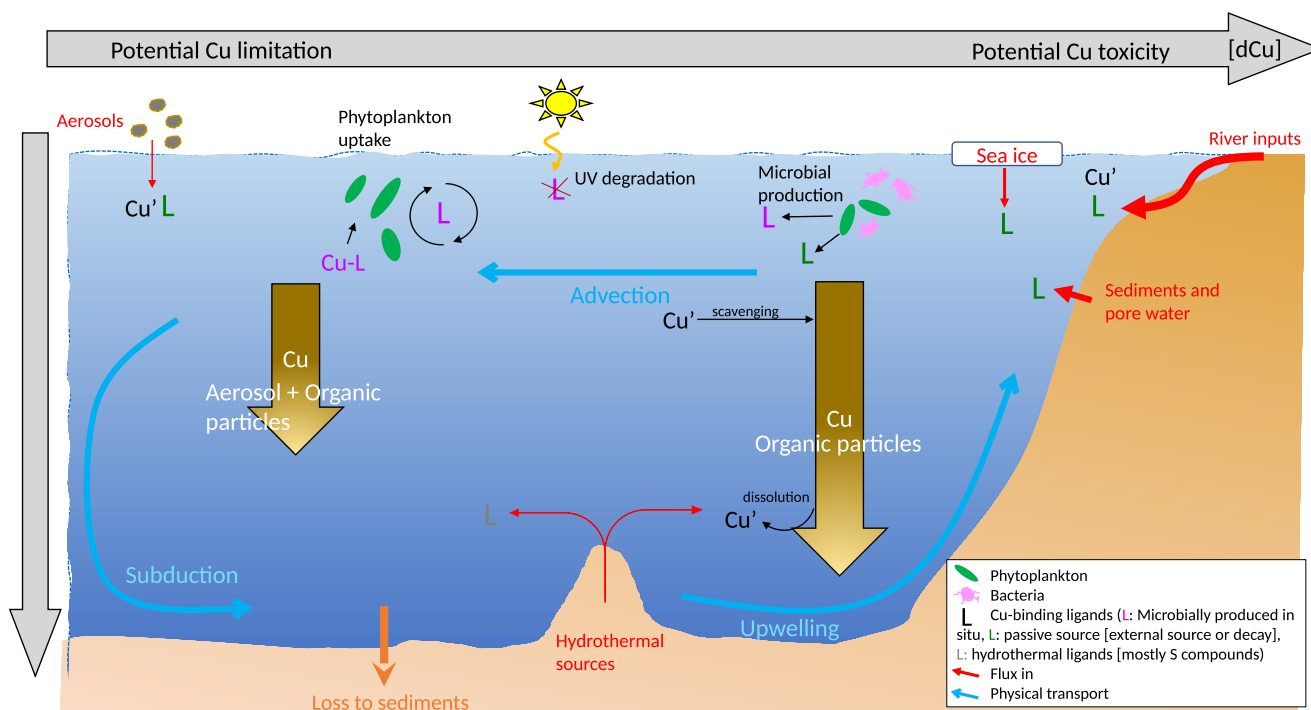
Fig. 4 Current understanding of the cycling of organic copper-binding ligands in the ocean. Schematic of copper-binding ligand cycling in the global ocean. Source fluxes that have been included in Table 4 are shown as red arrows, while sink fluxes that are shown in Table 4 are orange arrows. The flux of ligands to sediments is assumed based on assumptions of steady state. Additional ligand sources and sinks where flux calculations have not been completed are represented by black arrows. The potential for copper limitation or copper toxicity is shown to generally follow total copper concentrations, with the potential for copper toxicity greatest in coastal regions and the potential for copper limitation being greatest in open-ocean regions.

(230 Tg C yr^{-1}) gives an estimate of 0.1 Gmol L yr^{-1} of Cu ligands from aerosol wet deposition. This estimate comes with a wide uncertainty, both because of the large differences in the ligand/organic carbon ratios in the literature, and the large uncertainties in global aerosol deposition fluxes.

To our knowledge, the only estimates of Cu-ligand concentrations in ice are from Bundy et al.[21] who reported ligand concentrations measured in sea ice, glacier ice, and algal-influenced sea ice from Admiralty Bay (Antarctica) of 12.5, 2.7, and 26.15 nmol L^{-1}, respectively.

Abernathey et al.[216] estimated the Southern Ocean water flux from sea ice and glacier water to be, respectively, 15,750 and 1575 Gt yr^{-1}. When multiplied by Bundy et al.[21] estimates of Cu-ligand concentrations, we obtain a potential Cu-ligand source from sea ice between 0.2 and 0.5 × 10^{-3} Gmol L^{-1} yr^{-1} and a potential source from glacier water of around 4 × 10^{-6} Gmol L^{-1} yr^{-1}.

Several observations have shown that different phytoplankton species produce Cu ligands in conditions of Cu limitation or Cu toxicity (section "Microbial production of Cu-binding ligands"). However, quantitative estimates of Cu-ligand production rates are still missing. To our knowledge, Echeveste et al.[217] provided the only ligand production flux by the coccolithophore *E. huxleyi* (about 12.5 fM cell^{-1} in high Cu conditions). Unfortunately, this estimate is difficult to generalize to the global ocean and more work on microbial ligand production would be necessary to quantify the magnitude of this source at the ocean scale. While it is likely that microbial ligand production is one of the dominant sources of ligands to the ocean, the current lack of rate measurements makes it impossible to estimate the value of this source given the current datasets.

Constraining the sinks of copper-binding ligands in the ocean. Based on Semeniuk et al.[168] estimates, the uptake of ligand-bound Cu by phytoplankton is about 50–250 pmol Cu L^{-1} d^{-1}, which makes 18–6750 mmol Cu m^{-3} yr^{-1}. Generalizing this consumption rate to the global surface ocean (0–100 m) gives a rough estimate of the magnitude of the Cu-ligand sink from phytoplankton uptake of 0.6–270 Gmol L yr^{-1}. This estimate has a large range and uncertainties, as regional differences in microbial abundances and community composition will have large impacts on this flux.

Using samples from Cape Fear, Shank et al.[126] estimated a degradation rate of Cu-binding ligands by sunlight of about 0.28 d^{-1}, which is much higher than the DOC photooxidation rates estimated at about 0.01 d^{-1}. This degradation rate constant can be used in the following equation to estimate ligand concentrations:

$$L_i = L_{i0}e^{-kt} \qquad (1)$$

where L_{i0} is the initial ligand concentrations and at the time of exposure in days, and k is the degradation rate (estimated around 0.28 d^{-1}). Using a global ligand source of about 500 Gmol L yr^{-1} supplied to the surface ocean, calculated from the previous section's estimates, we obtain an estimate for global ligand photodegradation of about 123 Gmol L yr^{-1}.

Missing sinks and uncertainties. The fluxes discussed in the previous two sections are summarized in Table 4 and should be considered as first order estimates giving the magnitude of the sources and sinks and their large uncertainties. From this table, we can conclude that rivers are likely the major source of

Table 4 Global estimated fluxes of copper-binding ligands.

Source or sink	Global estimate (Gmol yr^{-1})	References
Rivers	30–1000	Muller and Batchelli (2003)[42], Hoffman et al. (2007)[205], Shank et al. (2004)[126]
Sediments	5.7 (continental shelf) 0.2 (tidal exchange)	Shank et al. (2004)[126], Santos-Echeandia et al. (2013)[210]
Aerosols (wet deposition)	0.1	Kanakidou et al. (2012)[215], Hou et al. (2005)[213], Karavoltos et al. (2017)[248], Witt et al. (2007)[214]
Sea ice	0.2–0.5 × 10^{-3}	Abernathey et al. (2016)[216], Bundy et al. (2013)[21]
Glacier (estimates for the Southern Ocean)	4 × 10^{-6}	
Phytoplankton uptake	0.6–270	Semeniuk et al. (2015)[168]
Photodegradation	123	Shank et al. (2006)[173]

Estimates of global oceanic fluxes of organic Cu-binding ligand sources and sinks. We have not included hydrothermal or microbial sources despite both likely being an important source, as the appropriate data was not readily available.

Cu-binding ligands to ocean waters, with sediments and aerosols being second-order sources. Sea ice and glacier melt probably have a minor impact on Cu-ligand budgets, however, sea-ice algae may be a strong source of Cu ligands and subsequent release after sea-ice melt may locally influence Cu cycling. The estimates for Cu-ligand sinks show that both phytoplankton uptake and photodegradation may have comparable magnitude, however, these estimates have large uncertainties that may span several orders of magnitude. If we assume steady state for the global Cu-ligand budget, the global ligand sink to sediments can be estimated on the order of hundreds of Gmol L yr^{-1}, making sedimentation likely the greatest sink for Cu ligands. However, we found no in situ estimate for Cu-ligand loss to sediments. Future research efforts should focus on constraining this sink by measuring ligands associated with sinking organic material.

Conclusions

Organic Cu-binding ligands bind the majority of the dissolved Cu in the ocean and are recognized as being important for dissolved Cu cycling, and yet much remains to be discovered about their sources, sinks and internal cycling, and none of the existing Cu biogeochemical models include a dynamic cycle of Cu-binding ligands. Here, we summarized the current knowledge of Cu-ligand cycling in the global ocean with a focus on large-scale open-ocean processes based largely on basin-scale GEOTRACES datasets and found that margin sediments and rivers are the major sources of Cu-binding ligands in seawater, and sedimentation, microbial uptake and photochemical degradation are the major sinks. Future studies that focus on understanding Cu-binding ligand fluxes and identifying Cu-binding ligands in seawater will be particularly insightful for future modeling efforts, and for understanding the impact of Cu-binding ligands on Cu bioavailability to marine organisms.

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A.R. led the writing of the manuscript and generated figures, C.R., H.W., and R.B. helped write and edit the manuscript and contributed to making the figures and tables.

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