

Determination of inert and labile copper on GEOTRACES samples using a novel solvent extraction method

Rintaro Moriyasu, James W. Moffett^{*}

Department of Biological Sciences, University of Southern California, 3616 Trousdale Parkway, AHF204, Los Angeles, CA 90089, USA

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ABSTRACT

Copper, in seawater, is predominantly bound by organic ligands of unknown composition. Complexation has been thermodynamically characterized using synthetic ligand competition experiments which assumes equilibrium among all chelators within the system. However, equilibration times are constrained by wall loss issues with the synthetic ligands. Here, a solvent extraction methodology, was utilized to avoid the wall-loss problems. Using an exceptionally high concentration of a strong copper chelator, oxine (8-hydroxyquinoline), at least six hours of equilibration time is required to reach steady state between the competing ligand and the labile copper in seawater. This is much longer than equilibration times used in previously published works. Our method was optimized by using samples from GEOTRACES expeditions in the North Pacific and North Atlantic Oceans. Surprisingly, 60–90% of the copper was not exchangeable with oxine under these conditions. We define this fraction as “inert”, and these data, which include profiles as deep as 1000 m in the North Pacific, suggest that this is a widespread feature. Our results suggest that there are two distinct pools of labile and inert copper, rather than an assemblage of similar complexes with incremental differences in stability constants. The results have important implications for the marine geochemistry of copper and its bioavailability. Complexation has been shown to limit copper bioavailability and influences scavenging and residence time. Moreover, a basic paradigm of copper speciation methodologies, that even strong Cu complexes are relatively labile, is likely incorrect.

1. Introduction

Within aquatic regimes, dissolved copper (dCu) is predominantly bound by organic ligands; indeed, among all of the first-row transition metals, copper (Cu) is known to form the strongest, most stable complexes due to its small ionic radius and its propensity to undergo Jahn-Teller distortion (Irving and Williams, 1953). It has been estimated that $\geq 99\%$ of Cu is bound by ligands (van den Berg et al., 1987; Coale and Bruland, 1988; Moffett and Dupont, 2007; Bundy et al., 2013). This is significant because both Cu bioavailability and toxicity have been found to be functions of free cupric ion concentration ($[Cu^{2+}]$) rather than of total dCu concentration (Coale and Bruland, 1988; Sunda and Lewis, 1978; Sander et al., 2015), and Cu toxicity can occur at $[Cu^{2+}]$ as low as 10^{-12} M for cyanobacteria (Brand et al., 1986).

Marine water column Cu concentrations are generally depleted at the surface and increase linearly with depth; this is thought to be a result of

particulate reversible scavenging as its main sink, mid-depth remineralization, and remobilization via benthic flux (Boyle et al., 1977). The linearity of the depth profile is primarily attributed to reversible scavenging (Little et al., 2013) where the slope of the depth profile is a function of scavenging rate (Richon and Tagliabue, 2019). Copper ligands are also thought to play a major role in controlling the rate of Cu scavenging by particulate matter (Davis and Leckie, 1978; Laglera and van den Berg, 2003). Free Cu^{2+} , rather than Cu species that are strongly bound by organic ligands, will be scavenged more easily; organic-bound Cu, on the other hand, will remain dissolved throughout the water column (van den Berg et al., 1987).

Even among organic ligands that bind Cu, there is a range in binding strength. In many studies, at least two classes of ligands are identified; L_1 ligands have stronger binding strengths and have concentrations comparable to ambient Cu (Moffett et al., 1990; Whitby et al., 2018), while L_2 ligands have weaker binding strengths and are at significantly higher

Abbreviations: CLE-AdCSV, Competitive Ligand Exchange-Adsorptive Cathodic Stripping Voltameter; DTPA, Diethylenetriamine Pentaacetate; FLPE, Fluorinated Low-Density Polyethylene; GFAAS, Graphite Furnace Atomic Absorbance Spectroscopy; ID-ICP-MS, Isotope Dilution-Inductively Coupled Plasma-Mass Spectrometer; PFA, Perfluoroalkoxy Alkanes; SA, Salicylaldehyde.

^{*} Corresponding author.

E-mail address: jmoffett@usc.edu (J.W. Moffett).

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concentrations than L_1 (Bundy et al., 2013). The strength of these ligands have been found to be on a spectrum where ligand types are divided by their conditional stability constants (K^{cond}) of approximately 10^{13} – 10^{16} for L_1 -ligands, and 10^{10} – 10^{13} for L_2 -ligands (Whitby et al., 2018), but these values can vary depending on the window of detection (α) which is a sum of the conditional stability constants (K_1^{cond} and β_2^{cond} for the mono and bis-complex, respectively) of the artificial ligand multiplied by the concentrations of artificially added competing ligand (Eq. 1), used for one particular electrochemical method known as the Competitive Ligand Exchange – Adsorptive Cathodic Stripping Voltammetry (CLE-AdCSV). Marine *Synechococcus* sp. has been found to produce chelators in the presence of high Cu concentrations; these ligands were observed to have similar K^{cond} to L_1 -type ligands in both seawater samples (Moffett et al., 1990) and cultures (Moffett and Brand, 1996). Organic ligands that are not produced by microbial processes, often referred to as humic substances, can also chelate Cu in the open ocean (Whitby and van den Berg, 2015; Whitby et al., 2018).

It has been proposed by Kogut and Voelker (2003) that some strongly binding Cu compounds ($\log K' \geq 13$) are kinetically non-labile. They define “inert Cu” as Cu species that are unexchangeable between natural Cu pools and the synthetic ligand salicylaldoxime on a timescale of up to 48 h. The analytical window, based on of the strength and concentration of the competing ligand used for the electrochemical method, constrains the kinetic binding constant of the strongly binding ligand (K_{CuL}) as being greater than $10^{17.3}$; these workers constrain this value based on the α of salicylaldoxime (SA), at 1 mM concentration ($\log(\alpha_{SA}) = 8.5$). Kogut and Voelker (2003) attribute the formation of this fraction to the complexation of dCu by colloids. They were able to eliminate this fraction and other organic ligands by UV-irradiating their samples prior to measurement.

In Waquoit Bay, MA, Moffett et al. (1997) reported Cu so tightly bound that even 500 μM of benzoylacetone was unable to detect any signal on the CLE-AdCSV; this further illustrates the issues many workers have with this method’s ability to resolve organic-bound Cu. Leeuwen and Town (2005) also demonstrate the kinetic limitations of the CLE-AdCSV method. These workers show that there is a nonequilibrium between the added ligands and strongly bound complexes originating from the slow rates of dissociation which leads to an overestimation in the conditional stability constants of metal-ligand complexes. Leeuwen and Town (2005) claim that no definitive conclusions can be made about this fraction that is undetectable by electrochemical means and raise questions about the validity of conditional stability constants derived by CLE-AdCSV. Ruacho et al. (2020) postulated that inert complexes could explain the persistence of complexed Cu in Pacific deep waters. Further evidence of nonexchangeable Cu was reported by Ma et al. (2006); these workers used Donnan dialysis and a radioisotope, ^{64}Cu ($t_{1/2} = 12$ h), to isotopically label the exchangeable pool of Cu between solid and solution phases in water extracted from soil samples. Ma et al. (2006) also observed an overestimation of labile Cu in their isotope dilution calculation because not all of the organically bound Cu was exchangeable with their radiotracer, and this non-equilibrium, unexchangeable fraction is thought to be colloidal. This Cu pool likely affects local cycling of Cu through prevention of particulate scavenging which could have an effect on global Cu cycling.

In this work, we developed a novel method for measuring the concentration of kinetically inert Cu on the Inductively Coupled Plasma – Mass Spectrometer. This method requires the use of solvent extraction, which was utilized previously as an alternative to CLE-AdCSV (Moffett and Zika, 1987a), while adding a much stronger Cu ligand, 8-hydroxyquinoline, at higher concentrations. This will result in a higher detection window, allowing for maximum extraction of the labile Cu fraction and creating a physical separation from the inert fraction. Moreover, it enables much longer equilibration times (at least two weeks) by eliminating the problem of wall loss that constrains voltammetric methods. The method has been applied to samples from a geographically diverse range of open ocean environments.

2. Sampling

Samples used for the initial development of the method were obtained from GEOTRACES GA03 aboard the R/V *Knorr* (KN204–01), shown on the map in Figure 1. The ship was equipped with the GEOTRACES CTD/rosette (Model 32G, Sea-Bird Electronics). This standard GEOTRACES rosette contains 24 Teflon-coated 12 L GO-FLO bottles (Model 10,812 T, General Oceanics) with hydrographic sensors for dissolved oxygen, algal pigments, conductivity, temperature, pressure, and beam transmittance. These samples were preserved by filtering through 0.2 μm Acropak™ 200 Supor capsule filter (Pall Corporation) and frozen at -20 °C. Locations, where seawater was sampled during KN204–01, are shown in Fig. 1. Samples used for assessing the method through the DTPA (Diethylenetriamine pentaacetate) titration experiment were obtained from Wrigley Institute’s Flow-through seawater system on Santa Catalina Island (33.26°N, 118.29°W), shown on map in Figure 2. These samples were filtered, using 0.2 μm Acropak™ 200 Supor capsule filters, sent back to the lab, and measured within 1 week of collecting.

Samples used for application of the method were taken during the first half of GEOTRACES GP15 aboard the R/V *Roger Revelle* (RR1814) and were sampled at stations 4 (54.7°N, 155.1°W) and 9 (44.5°N, 152.0°W), shown on Fig. 2. Station 9 samples ranged from GEOTRACES Sample # 13048–13,070 (ranging between 25 and 1000 m depths), taken on October 7th, 2018, and consisted of a 12-point depth profile. These samples were also collected using the trace metal clean GEOTRACES CTD/rosette, and total and labile Cu were measured. Samples were filtered through a 0.2 μm Acropak™ 200 Supor capsule filters and frozen, to prevent wall-loss and changes in speciation, from Hilo, HI. Freezing samples at -20 °C have been previously shown to preserve the speciation of Cu in samples (Jacquot and Moffett, 2015).

3. Materials and reagents

3.1. Materials

All seawater samples were frozen and stored within 1 L Fluorinated Low-Density Polyethylene (Nalgene; FLPE) bottles. Sample bottles were acid-washed prior to RR1814 cruise following standard trace element and metal acid washing procedure.

The reaction vessels used for solvent extraction were 15 mL Standard Teflon Vials with rounded interiors (Savillex: Catalog #200–015–20) with 33 mm PFA Closures (Savillex: Catalog #600–033–01) and 7 mL Standard Teflon Vials with flat interiors (Savillex: Catalog #200–007–10) with 24 mm PFA Closures (Savillex: Catalog #600–024–01).

3.2. Reagents

Toluene (Sigma Aldrich; CAS #: 108–88–3), used for solvent extraction, was distilled prior to usage using a Teflon still. A 0.1 molar (M) solution of 8-hydroxyquinoline (Sigma Aldrich; oxine; CAS# 148–24–3; ACS grade) was prepared in Optima™ grade methanol (Sigma Aldrich; CAS# 67–56–1). A 5% solution of Optima™ grade nitric acid (HNO_3 ; Thermo Fisher; CAS# 7697–37–2) in Milli-Q® water (Millipore; 18.2 M Ω /cm) was used for the back-extraction. Concentrated Optima™ grade hydrogen peroxide (Fisher; H_2O_2 ; CAS#: 7722–84–1) was used to treat for organic interference when measuring the concentration of total Cu on GEOTRACES GP15 samples (Bacconnais et al., 2019). For the titration experiment with diethylenetriamine pentaacetate (DTPA) (Sigma Aldrich; CAS# 67–43–6), a 0.1 M solution of the added ligand was made in Milli-Q® water with pH adjusted to 10 using Optima™ grade Ammonium Hydroxide (Fisher; CAS# 1336–21–6).

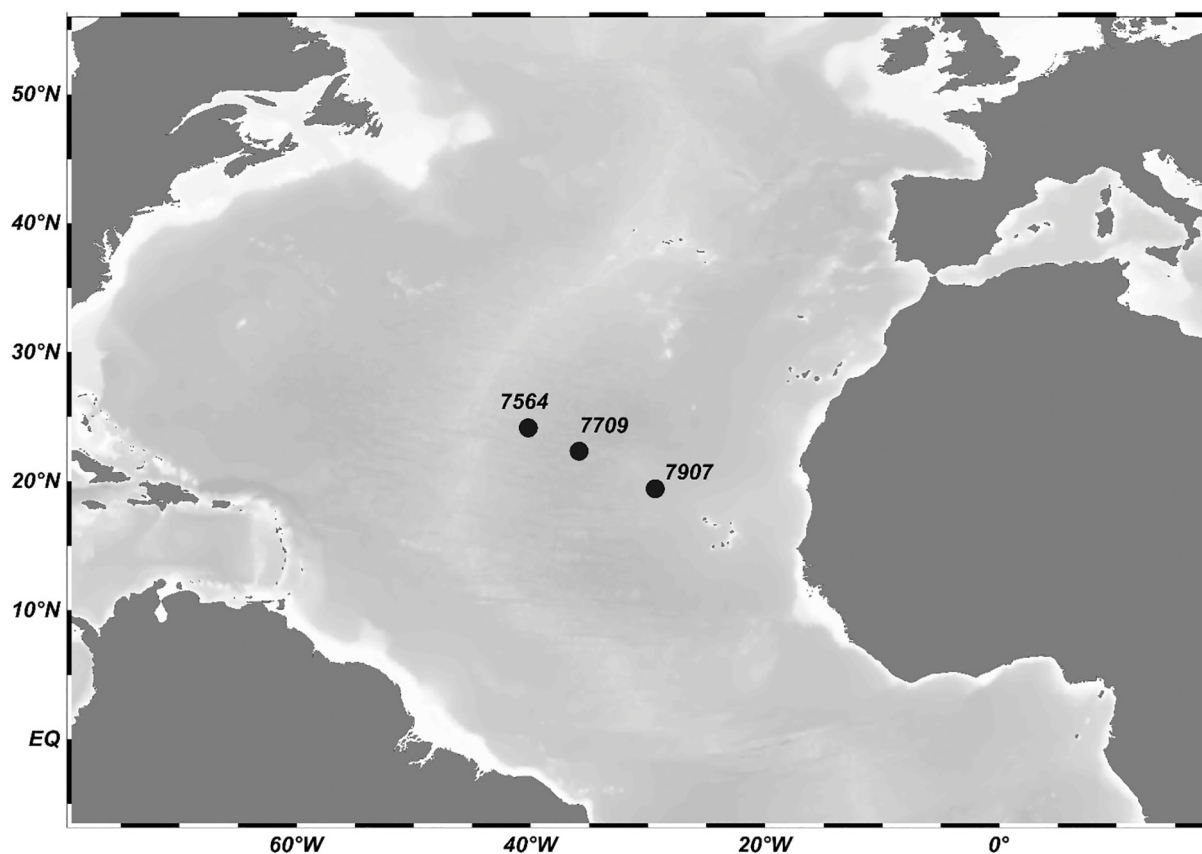


Fig. 1. Map of samples taken from GEOTRACES GA03. Samples taken aboard the R/V *Knorr* on cruise (KN-204-01) between November 6th to December 11th, 2011.

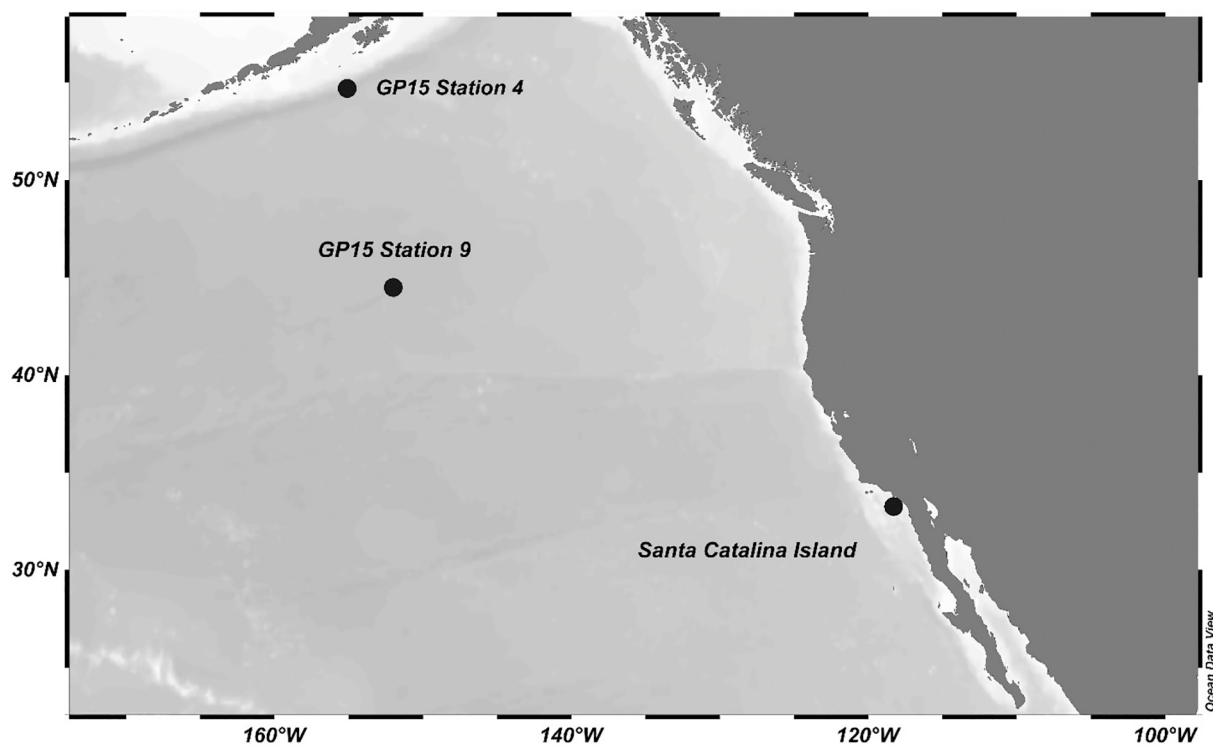


Fig. 2. Map of GP15 Stations 4 (54.7 °N, 155.1°W) and 9 (44.5 °N, 152.0°W), and Santa Catalina Island (33.2°N, 118.0°W).

4. Methods

4.1. Solvent extraction methodology

Frozen samples were defrosted overnight in a water bath. Once defrosted, 15 mL of sample was used to pre-treat the Teflon vials overnight. A 5 mL aliquot of seawater sample was measured into each vial, and 500 μM of oxine was pipetted into each aliquot. A 4 mL aliquot of toluene was added to each vial and allowed to equilibrate on the shaker for 6 h. Once equilibrated, 3 mL of added toluene was pipetted into three separate 7 mL Teflon flasks (1 mL per vial for triplicates) and taken to dryness on a hotplate at 160 $^{\circ}\text{C}$. 1 mL of 5% Optima grade HNO_3 was added to each Teflon vial; the vials were left on a shaker overnight to allow for back-extraction of the labile Cu fraction into aqueous layer. The acid used for back-extraction is then measured using isotope dilution on the Thermo Element 2™ Inductively Coupled Plasma – Mass Spectrometer (ID-ICP-MS) on medium resolution. Due to low concentrations of the labile fraction, ESI Apex Q desolvating nebulizer (Thermo Fisher, Parts #: 1299560) was used during the measurements. Samples were measured within 1 week of defrosting to retain speciation.

Thorough cleaning of Teflon vials, both 7 and 15 mL was essential to avoid significant memory effects. Prior to use, all vials were washed in a 10% HCl solution bath for at least 1-week. This was followed by filling the vials with 3 M Optima grade HNO_3 and leaving them closed on a hotplate at 100 $^{\circ}\text{C}$ overnight. Once removed from the hotplate, the vials were rinsed with high-purity Milli-Q water and filled with 1% Optima grade HNO_3 (Fisher) for storage. Teflon vials and closures were used because they are impermeable to toluene.

Pre-treatment of the Teflon vials used in the equilibration and solvent extraction steps (15 mL vial) is crucial since the acid washing process can leave a pH memory effect on the vials. Un-neutralized acid can cause uneven extraction of labile Cu among replicates, but by leaving 15 mL of the seawater sample in the vials overnight, this was avoided. The sample used to pre-treat is disposed of and replaced with fresh sample for analysis.

The 1 L FLPE bottles used to store and freeze samples were thoroughly cleaned using an acid washing protocol. This consisted of a 10% Citranox (Alconox) solution bath for 1 day, followed by a 10% hydrochloric acid (HCl; Fisher; CAS# 7647-01-0) solution bath for at least 1 week. The bottles were then filled with 1% Optima grade HCl solution and left to soak for 2 days. Between each step of the process, the bottles were thoroughly rinsed out using Milli-Q water. These were then stored and shipped empty inside of 2 re-usable, re-sealable zipper bags.

The toluene used for the solvent extraction method was distilled prior to usage which lowered the Cu blanks to below detection. A simple 375 mL Teflon Sub-boiling Still Assembly (Saville; Catalog# 520-1-1-2) was used with standard heating tape as the heat source on one end. A Styrofoam container filled with ice was placed on the other end of the distillation apparatus to condense the vaporized solvent.

4.2. Total copper measurements

Total Cu measurements for the GEOTRACES GA03 samples were done previously by Jacquot and Moffett (2015) using a single-batch nitrilotriacetic acid (NTA) resin extraction which was followed up by ID-ICP-MS; the procedure is described there. These values were compared with $[\text{Cu}]_{\text{Total}}$ measurements using the solvent extraction method developed in this article which used a mercury lamp to UV-irradiate the seawater sample for 2 h (Jelight Company, Inc.; UVO-Cleaner Model No. 342) in a Teflon vial with a quartz window. Once oxidized, samples were left overnight prior to extraction. This treatment was assumed to destroy organic matter binding both the inert and labile Cu fractions, enabling the yield of our protocol to be determined in a system where 100% of the Cu should be complexed by oxine.

Total Cu measurements for station 9 of GEOTRACES GP15 utilized a method adapted from Rapp et al. (2017). Samples were acidified to a pH

of 1.7 and pre-concentrated using the SC-DX seaFAST (Elemental Scientific; M-SFS2-MG-52) automated pre-concentration solid phase extraction columns and measured on the Element 2 ID-ICP-MS on medium resolution. Measurements were made in triplicates.

5. Competitive ligand exchange

5.1. Theory

The theory behind the competitive ligand exchange/solvent extraction methodology for Cu was developed by Moffett and Zika (1987a) who coupled extraction with analysis on the Graphite Furnace Atomic Absorbance Spectroscopy (GFAAS). That method used acetylacetone, while the current method uses oxine (ox), a much stronger chelator. The theory is analogous to competitive ligand approaches using other analytical methodologies, such as voltammetry. Modifying the equation developed by Campos and van den Berg (1994), we have Eq. (1):

$$\alpha_{\text{Cu(ox)}_x} = \frac{[\text{Cu(ox)}_x]}{[\text{Cu}^{2+}]} = \beta_2^{\text{cond}} [\text{ox}]^2 + K_1^{\text{cond}} [\text{ox}] \quad (1)$$

where $\alpha_{\text{Cu(ox)}_x}$ is the partitioning coefficient between the ligand-reactive “labile Cu” ($[\text{Cu(ox)}_x]$) and cupric ions ($[\text{Cu}^{2+}]$); K_1^{cond} and β_2^{cond} are the conditional stability constants for the mono- and bis- oxine complexes with Cu. The partitioning coefficient is also referred to as the analytical window of the ligand, often used to quantify the binding strength of the added ligand system. Labile Cu is defined as:

$$[\text{Cu}]_{\text{labile}} = [\text{Cu(ox)}_x] = [\text{CuL}]_{\text{weak}} + [\text{Cu}^{2+}] + [\text{Cu}'] \quad (2)$$

where:

$$[\text{Cu(ox)}_x] = [\text{Cu(ox)}_2] + [\text{Cu(ox)}^+] \quad (3)$$

The labile fraction is the sum of weakly-bound organic Cu complexes ($[\text{CuL}]_{\text{weak}}$), free cupric ions, and inorganic Cu species such as CuCO_3 . The latter fraction is referred to as Cu' . This equation can be simplified because, at higher added ligand concentrations, the mono-complex is insignificant (Buck and Bruland, 2005; Bundy et al., 2013). Since we are using 500 μM oxine, Eq. (1) can be simplified down to:

$$\alpha_{\text{Cu(ox)}_2} = \frac{[\text{Cu(ox)}_2]}{[\text{Cu}^{2+}]} = \beta_2^{\text{cond}} [\text{ox}]^2 \quad (4)$$

and

$$[\text{Cu(ox)}_x] = [\text{Cu(ox)}_2] \quad (5)$$

since we are forming mostly the bis-complex with negligible concentrations of the non-extractable, charged mono-complex. Total dissolved Cu ($[\text{Cu}]_T$) in our system is then:

$$[\text{Cu}]_T = [\text{CuL}]_{\text{strong}} + [\text{Cu}]_{\text{labile}} \quad (6)$$

where $[\text{CuL}]_{\text{strong}}$ is the fraction of the Cu pool bound by strongly binding organic ligands. This fraction is so tightly bound that it will not exchange with the added ligands, even with a detection window of 9.52 (log

Table 1

List of Chelators, concentrations, and detection windows ($\text{Log}(\alpha_{\text{AL}})$) used here and previous studies.

	Chelator used	[Chelator] (μM)	Log (α_{AL})
Campos & van den Berg, (1994)	8-hydroxyquinoline (oxine)	1	5.02
Whitby et al., (2018)	Salicylaldehyde (SA)	10	5.1
Bundy et al., (2013)	SA	25	5.8
Kogut & Voelker, (2003)	SA	500; 1000	7.9; 8.5
This Study	oxine	500	9.52

(β_2^{cond}) of 16.12 at 500 μM of oxine) which is a much higher value than those used by previous studies by sometimes orders of magnitudes (Table 1), resulting in:

$$[\text{Cu}]_{\text{inert}} = [\text{CuL}]_{\text{strong}} \quad (7)$$

$$[\text{Cu}]_{\text{labile}} = [\text{Cu}(\text{ox})_2] \quad (8)$$

and

$$[\text{Cu}]_{\text{inert}} = [\text{Cu}]_{\text{T}} - [\text{Cu}]_{\text{labile}} \quad (9)$$

The method developed in this study directly measures $[\text{Cu}]_{\text{labile}}$ while $[\text{Cu}]_{\text{T}}$ is measured using standard seaFAST automated preconcentration columns followed by measurement on the ICP-MS. From the difference, an operationally defined “inert” Cu fraction (bound by organic ligands with $\alpha \geq 9.52$) can be calculated.

5.2. Equilibration time experiment

The equilibration time for the solvent exchange method was varied to see if the amount of time given for ligand-solvent equilibration would change the concentration of extractable, labile Cu. This experiment was also done to ensure that all Cu that is reactive to the added ligand would be extracted. The range of equilibration times used ranged from 1 to 336 h (approximately two weeks). When leaving samples on the shaker during equilibration, all samples were wrapped within a black, opaque bag to prevent photo-oxidation/degradation of Cu-binding ligands.

5.3. DTPA titration experiment

The oxine-toluene system was calibrated against a known Cu chelator, diethylenetriamine pentaacetate (DTPA). This enabled us to calculate the empirical side reaction coefficient ($\alpha_{\text{Cu}(\text{ox})_2}$) (Eq. 1) for the method to compare with other methods and to set lower limits on how strongly bound inert Cu might be. Reliable stability constant data are available for DTPA Cu complexes, compiled in Twiss and Moffett (2002). A seawater sample from Santa Catalina Island was UV-irradiated for 2 h to remove all pre-existing organic chelators of Cu; these samples were then equilibrated for 3 h with varying concentrations of DTPA (0–2.46 mM) before equilibration with oxine. Solvent extraction was performed as above.

5.4. Choice of ligand

Oxine was chosen as the competing ligand primarily for its high affinity with Cu. It was previously used by Campos and van den Berg (1994) as the competing ligand to achieve the highest range of detection windows (6.4–9.0). Comparatively, previous studies that have used solvent extraction have used salicylaldoxime (Dahl, 1968) and acetylacetone (Moffett and Zika, 1987a; Moffett et al., 1990). These competing ligands, however, are more useful for establishing an equilibrium between artificial and natural ligands which enable estimates of natural ligand binding strengths to be calculated; such ligands work best if they have a moderate binding strength that are comparable to natural ligands

(van den Berg and Donat, 1992; Campos and van den Berg, 1994). In comparison, our objective is to overwhelm the naturally occurring ligands so that all forms of Cu that are kinetically labile will be transferred to the oxine-complexed pool. This was achieved by using high concentrations of a strongly binding Cu ligand; this method reaches the highest detection window, α_{AL} (Table 1), allowing for the extraction of most, if not all, chemically labile Cu species, $[\text{Cu}]_{\text{labile}}$.

5.5. Choice of solvent

Toluene (methylbenzene) and carbon tetrachloride were found to be good solvents for the solvent extraction method by Moffett and Zika (1987a). Toluene was chosen over carbon tetrachloride in previous studies, such as Moffett et al. (1990) since it is much less toxic to handle. Both solvents, however, were found to not affect the electrostatic properties of seawater (Moffett and Zika, 1987a).

6. Results

6.1. Equilibration time experiment

The time taken between the addition of ligand-solvent system and extraction was varied for GEOTRACES sample # 12576 ($[\text{Cu}]_{\text{total}} = 1.85 \text{ nM}$; 50 m depth at station 4 of GP15) and can be found in Fig. 3. An equilibration time of $\leq 3 \text{ h}$ resulted in $<10\%$ of $[\text{Cu}]_{\text{total}}$ extracted. When equilibration time is $\geq 6 \text{ h}$, we found $\geq 25\%$ of $[\text{Cu}]_{\text{total}}$ extracted, which we expect to be the maximum extractable/labile Cu. This value does not change for over two weeks which shows that all extractable copper

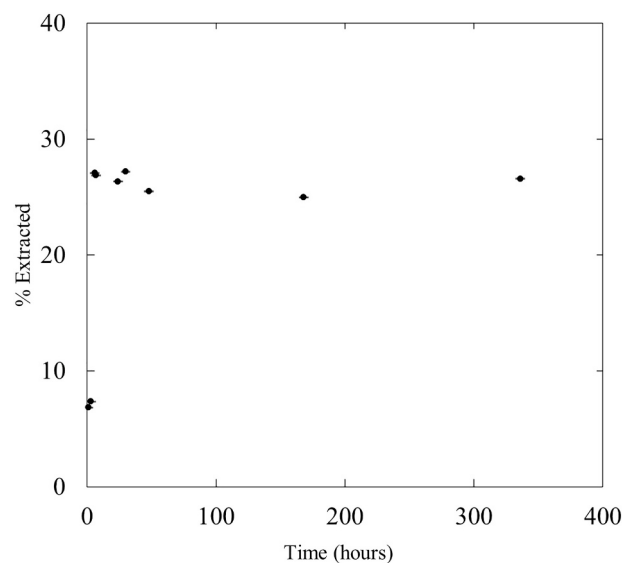


Fig. 3. Sample 12576 (50 m, Station 4 of GP15) had the equilibration time between addition of ligand-solvent system and solvent removal varied. This equilibration time ranged between 1 and 336 h (corresponding to 2 weeks).

Table 2

Frozen samples taken from GEOTRACES GA03 (NAZT) measured using solvent extraction methodology with 500 μM 8-hydroxyquinoline. Samples were analyzed with and without a 2-h UV-irradiation/oxidation step to destroy all naturally existing chelators.

G# ^a	$[\text{Cu}]_{\text{labile}}$ (nM)	SD	Depth (m)	UV-ox. Cu (nM) ^b	SD	$[\text{Cu}]_{\text{Total}}$ (nM) – Jacquot ^c	SD	% Labile	% Inert	% Recovered ^d
7564	0.56	0.06	235.8	1.593	0.002	1.65	0.03	34.9	65.1	97
7709	0.33	0.03	187.1	0.76	0.01	0.84	0.05	45.3	54.7	91
7907	0.36	0.02	95.1	0.85	0.04	0.90	0.06	42.6	57.4	94

^a Refers to GEOTRACES Sample ID number.

^b Samples were UV-irradiated under a mercury lamp for 2 h and extracted with 500 μM oxine.

^c Cu_{T} obtained from BCO-DMO – measured by Jacquot & Moffett., (2014) using nitrilotriacetic acid (NTA) extraction.

^d A % Recovered when comparing totals measured via UV-oxidation solvent-extraction and on the totals previously via NTA extraction.

species with 500 μM oxine were extracted by 6 h. In addition, this experiment shows that there is no significant wall-loss from leaving samples for 2 weeks in the Teflon vials with the solvent.

6.2. Oxine concentration dependence

The oxine concentration for solvent extraction was determined using samples from GEOTRACES GA03 (GEOTRACES # 7564 at 235.8 m and #7709 at 187.1 m) and varying oxine concentration to find the optimal concentration for oxine addition to maximize extraction (Fig. 4). Results of all GA03 samples can be found in Table 2. A concentration of 500 μM of oxine was found to maximize the extraction of $[\text{Cu}]_{\text{labile}}$ while less oxine resulted in $\leq [\text{Cu}]_{\text{labile}}$ and less consistent extractions. There is a general decrease in extractable Cu for additions of oxine $>500 \mu\text{M}$; may be caused by the formation of a charged, copper tris-oxine complex which does not partition into the organic solvent layer.

6.3. DTPA titration experiment

The side reaction coefficient for 500 μM oxine was determined by titrating the oxine with diethylenetriamine pentaacetate (DTPA) added to UV-irradiated seawater from Santa Catalina Island ($[\text{Cu}]_{\text{Total}} = 3.15 \pm 0.06 \text{ nM}$). This conditional stability constant of this Cu ligand in

seawater was previously calculated by Twiss and Moffett (2002); these workers found that $\log(K_{\text{DTPA}}^{\text{cond}})$ is 12.42. By UV-oxidizing the seawater samples, the naturally existing Cu ligands are removed (van den Berg and Donat, 1992; Campos and van den Berg, 1994; Bundy et al., 2013; Whitby et al., 2018). Prior to this experiment, samples from GEOTRACES GA03 were used as standards to show that UV-irradiation of seawater can indeed destroy all pre-existing ligands and can achieve quantitative extraction of total copper (Table 2). $[\text{Cu}]_{\text{T}}$ measured using this method (UV-ox Cu) was compared with those measured by Jacquot and Moffett (2015) who used a nitrilotriacetic acid (NTA) resin extraction with an isotope spike on the ICP-MS. Varying concentrations of DTPA (from 0 to 2.5 mM) were added to seawater solutions free of organic-bound Cu. Copper bound by DTPA forms a charged complex which does not partition into the solvent phase.

The results (Fig. 5) showed that in the absence of any strong, organic Cu ligand, all Cu is extractable, and in the presence of increasing concentrations of DTPA, the concentration of labile Cu (extractable Cu) decreases until below detection. Using the results from the DTPA experiment where $[\text{DTPA}] = 0.98 \text{ mM}$, we calculated the $\log(\beta'_2)^{\text{Cond.}}$ of oxine with the known $\log(K'_1)^{\text{Cond.}}$ of DTPA (Twiss and Moffett, 2002)

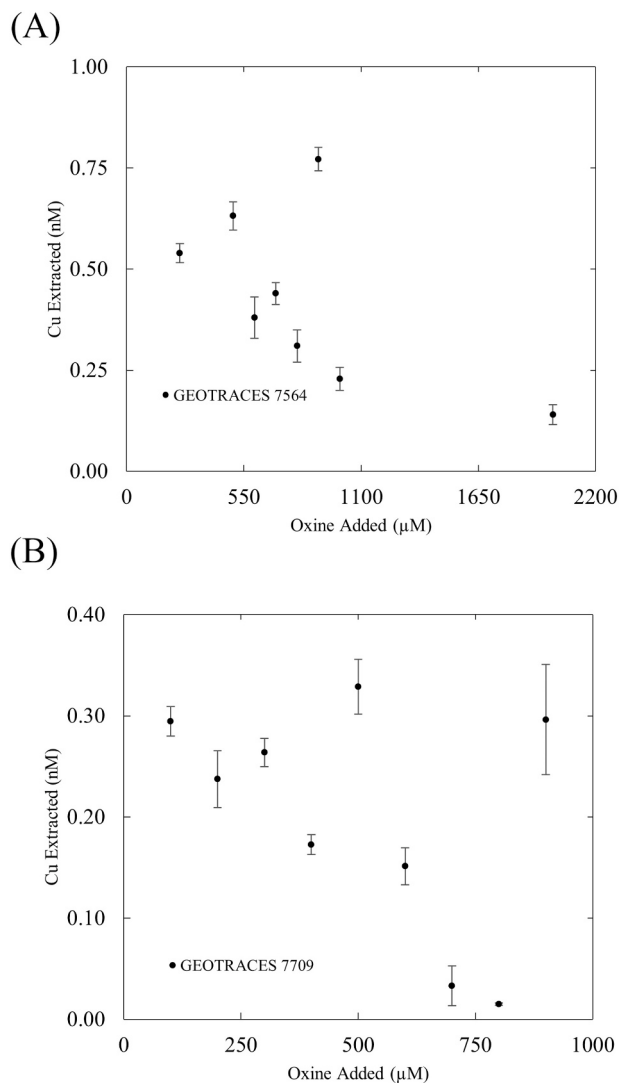


Fig. 4. Copper extracted versus oxine concentration for GEOTRACES Samples 7564 and 7709.

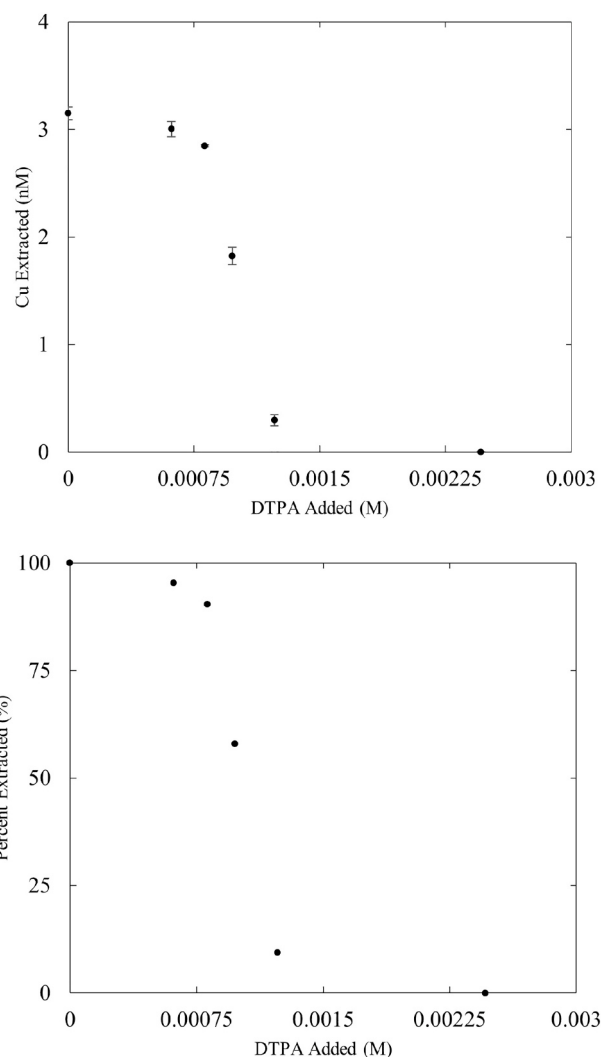


Fig. 5. Results from the Diethylenetriamine Pentaacetate (DTPA) titration experiment. Samples were titrated with 0–2.5 mM DTPA against 500 μM 8-hydroxyquinoline (oxine). Top figure is actual concentration of Cu extracted (nM) from Santa Catalina Island seawater sample against the amount of DTPA added (M). Bottom figure is percent Cu extracted (of total $3.15 \pm 0.06 \text{ nM}$) against added DTPA.

and concentrations of extractable and total Cu. The $\log(\alpha_{\text{Cu(ox)2}})$ was then calculated to be 9.52 (Table 1).

6.4. Extraction of copper without the added ligand

A control experiment, where solvent extraction of Cu was measured without using any added copper ligands, was done previously by Moffett and Zika (1987a). These workers found that using GFAAS, no measurable Cu (less than 1% of the ambient Cu) partitioned into the solvent phase from the aqueous when extraction was done without acetone. This experiment was repeated under the solvent extraction protocol proposed by this study. The sample used was GEOTRACES # 7709 (at 187.1 m depth) from the GA03 cruise. All replicates ($n = 9$) yielded extractable Cu below detection and were unquantifiable (data not shown). Without an added ligand, neither labile nor inert Cu will extract into the solvent phase. This experiment was replicated ($n = 3$) for GEOTRACES #12576 (depth of 50 m and used for the equilibration time variance experiment) for 48 h; similar to equilibrating without oxine for 6 h on #7709, the measured $[\text{Cu}]_{\text{labile}}$ was below detection.

7. Application of method to GEOTRACES samples

7.1. Water column profile

The solvent extraction method reported here was applied to samples from station 9 of GP15 (Fig. 6). Error bars, for inert Cu, were propagated from measurements made for total and labile Cu. Labile Cu was found to be highest at the surface and decreases rapidly below the fluorescence maxima. Below 100 m, $\geq 90\%$ of dCu was found to be consistently inert. This appears to be in stark contrast with samples measured along GA03 ($n = 3$) where depths ≥ 100 m all had between 30 and 50% labile Cu.

A detailed explanation of these trends requires analysis of other samples from this expedition (GP15) and others. However, one explanation is that waters recently exposed to the surface may have more labile Cu through the release of inert Cu by photochemical reactions. This is in line with the findings of Moffett and Zika (1987a, 1987b), who saw an increase in cupric ion activity which they attributed to the photolysis of Cu-binding ligands. These workers saw a 20-fold increase in free cupric ions which are a part of the labile Cu pool. Increase in free cupric ions at the surface was also observed in the oligotrophic Sargasso Sea (Moffett et al., 1990). Moffett and Zika (1987a) also saw a decrease in the organic ligand concentrations below the euphotic zone which they attribute to decomposition. At station 9, however, we observed that most of the dissolved Cu below the euphotic zone to be inert, and our current understanding of Cu-binding ligands would conclude that this inert pool would be strongly bound by organic ligands. Another explanation is that surface waters contain more recent inputs of Cu, and the formation of inert complexes are slow. The differences between the Atlantic and Pacific samples, while preliminary at this stage, could arise because the deep waters in the Atlantic are younger than at comparable depths in the North Pacific. In older waters there is more time for the formation of inert complexes or for the transfer of labile Cu into the inert pool. Moffett et al. (1990) tested whether or not photo-oxidation of seawater removed the strong Cu-binding ligand, the L_1 -class. They left a sample from the Sargasso Sea (140 m depth) in sunlight for 4 h a day for 5 days straight in a quartz flask; the sample was then measured on the CLE-AdCSV where they found that the L_1 -class ligand, which was previously detected, had become undetectable. A control, kept in the dark for the same period of time, resulted in no loss of ligands.

The local labile Cu minima appears to coincide with the chlorophyll maxima. Similar results were reported in Moffett et al. (1990) where the depth of maximum Cu-binding occurred at the chlorophyll maxima as well.

7.2. Implications of inert Cu for the electrochemical determination of free $[\text{Cu}^{2+}]$ and conditional stability constants and concentrations of copper-binding ligands

It is important to note a fundamental difference in the voltammetric ligand competition methodology described above and the solvent extraction method described here. In the former, the objective is to obtain a thermodynamic characterization of naturally occurring Cu complexes by setting up a competition between natural ligands and a synthetic ligand. The experimentalist strives to obtain conditions where there is a significant fraction of Cu complexed in both the naturally occurring labile complex pool and the synthetic complex. This balance minimizes error propagation in the ensuing calculations. By contrast, we seek to add so much oxine to the sample to convert all of the labile Cu into the Cu oxine complex. That essentially makes it impossible to determine any thermodynamic information about the naturally occurring labile ligands. Instead, we can only set lower limits on how strong the “inert complexes” might be. Note that there is no reason why our method could not be used to gain thermodynamic information if, in nature, Cu was dominated by an exceedingly high concentration of a labile ligand. Indeed, we performed that very experiment in our calibration with DTPA.

Consideration of our findings raises the following issue for the electrochemical methods. Those methods assume that all of the dissolved Cu complexes are in equilibrium with the competing ligand in order to perform the calculations. What if equilibrium is obtained with the labile fraction but not the inert fraction? To test this hypothesis, we carried out voltammetric titrations on samples where we had determined labile and inert fractions and performed the speciation calculations both ways.

With electrochemical methods, unknown parameters are solved for using titration data and the following equation:

$$\frac{\text{Cu}[\text{AL}]^2}{[\text{Cu}]_{\text{Total}}} = \frac{\beta_2 [\text{AL}]^2}{1 + \Sigma K_i L_i + \beta_2 [\text{AL}]^2} \quad (10)$$

where $[\text{AL}]$ is the added ligand, $[\text{Cu}]_{\text{Total}}$ is the total dissolved Cu detected by CLE-AdCSV, β_2 is the conditional stability constant of the added ligand, and K_i and L_i are the conditional stability constant and concentration of the naturally occurring ligand, respectively. Modifying Eq. (9) and applying it to (10), we obtain:

$$\frac{\text{Cu}[\text{AL}]^2}{[\text{Cu}]_{\text{Total}} - [\text{Cu}]_{\text{Inert}}} = \frac{\beta_2 [\text{AL}]^2}{1 + \Sigma K_i L_i + \beta_2 [\text{AL}]^2} \quad (11)$$

and therefore,

$$\frac{\text{Cu}[\text{AL}]^2}{[\text{Cu}]_{\text{Labile}}} = \frac{\beta_2 [\text{AL}]^2}{1 + \Sigma K_i L_i + \beta_2 [\text{AL}]^2} \quad (12)$$

This allows for a better approximation of K_i and L_i because the electrochemical titrations are not accurately accounting for inert Cu, causing an overestimation of ligand concentrations and their conditional stability constants.

At station 9, we measured one shallow and one deep sample (25 and 750 m depths) from the profile using CLE-AdCSV, following the protocols outlined in Jacquot and Moffett (2015). Using 2 μM of the Cu chelator salicylaldehyde (SA), a conditional stability constant (K) and ligand concentration ($[L]$) were calculated with a single ligand model on a nonlinear fit, previously described by Gerringa et al. (1995) and Moffett & Dupont, (2007) where:

$$K = \frac{[\text{CuL}]}{[\text{Cu}^{2+}][L_f]} \quad (13)$$

and

$$[L] = [\text{CuL}] + [L_f] \quad (14)$$

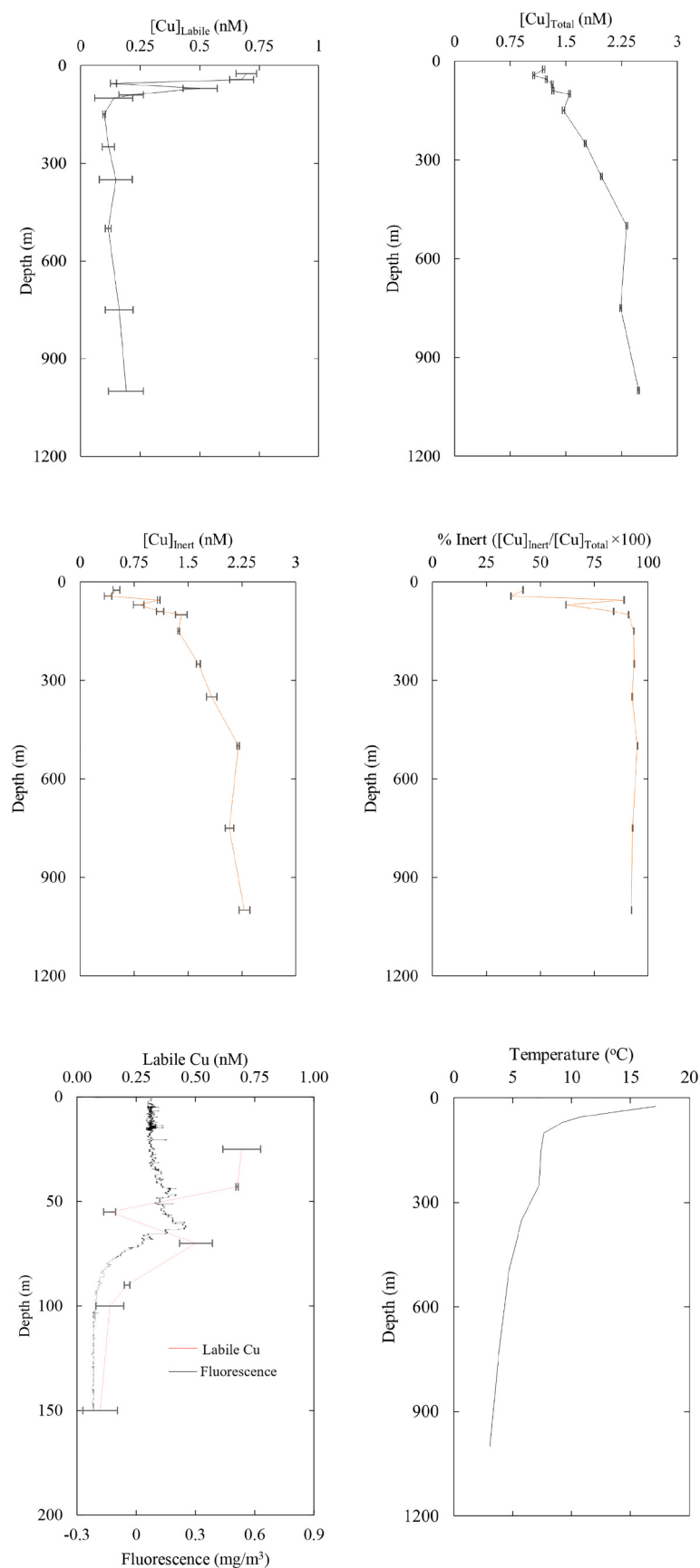


Fig. 6. Depth profiles of labile, total, and inert copper species at station 9 (12-point depth profile), accompanied by profiles of temperature, percent of total copper that is inert at each depth, temperature, and surface labile copper overlaid with surface fluorescence. Concentrations of labile copper is measured on the ICP-MS and indicated by a red profile while concentration of inert copper and percent inert are derived values and indicated in orange. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The α of the natural ligand along with eq. (13) can be rearranged into the reciprocal of the Langmuir isotherm (15).

$$\frac{[CuL]}{[Cu^{2+}]} = \frac{K[L]}{1 + K[Cu^{2+}]} \quad (15)$$

When weak and inorganic ligands are included, the equation becomes:

$$\frac{\Sigma[CuL]}{[Cu^{2+}]} = \Sigma K_i L_i (i>1) + \frac{K[L]}{1 + K[Cu^{2+}]} \quad (16)$$

(Moffett, 1995) which was solved using Sigmaplot version 10.0 for K and [L], assuming $\Sigma K_i L_i (i>1) = 200$. Using the conditional stability constant (K'_1) of the strongest detectable ligand (L_1), $[L_1]$, and total Cu ($[Cu]_T$), the following quadratic can be formed to solve for $[Cu^{2+}]$ (Moffett & Dupont, 2008):

$$0 = -[Cu]_T + (1 + K'_1[L_1] - K'_1[Cu^{2+}])x + K'_1x^2 \quad (17)$$

where,

$$[Cu^{2+}] = \frac{x}{\Sigma_{i=2} K_i L_i} \quad (18)$$

As mentioned previously, $\Sigma K_i L_i (i>1)$ is estimated as 200. Using both $[Cu]_T$ and the labile Cu measured used in this method, the values for K'_1 , $[L_1]$, and $[Cu^{2+}]$ were calculated.

Results for conditional stability constants (K) and ligand concentration ([L]) are reported in Table 3 and Fig. 7A-D. Additional calculations were performed for titration data in the North Atlantic reported in Jacquot and Moffett (2015). These samples were collected at the same stations and similar depths to the data reported in Table 2 (none of the sample depths actually titrated in that study were available for comparison). Based on the data in Table 2, we assumed a representative value of 50% inert Cu for samples on GA03 and applied that assumption to the titration data in Jacquot and Moffett (2015). In each case, subtracting the inert fraction from the total Cu results in a substantially lower conditional stability constant than was obtained by applying a single ligand model using total Cu. This is a more realistic estimate for the binding constant of the complex comprising the labile fraction than the single ligand model, since the existence of an inert fraction implies that there must be at least two ligand pools. The total ligand concentration (Table 3) is defined as the sum of the “labile” ligand calculated from the non-linear regression plus the inert Cu (assuming the inert Cu is a ligand-bound complex). It is larger than the ligand concentration determined without considering inert Cu because the “labile ligand” is similar to the value derived from Eq. (11). The excess ligand, defined as the total ligand concentration minus the total Cu, is much larger when Eq. (11) is used. A higher ligand concentration, combined with a weaker stability constant, means that the free $[Cu^{2+}]$ estimates are basically unchanged with the exception of the 25 m sample at GP15 Sta. 9. These results suggest that future analysis of electrochemical titration data

should include an estimate of the inert fraction.

8. Discussion

The current paradigm underlying all speciation methods for Cu is that it is at equilibrium among all of the competing ligands (natural and artificial) in a titration experiment. Many previous studies using voltammetry, including those that use oxine, limit the equilibration time to less than an hour. Beyond this time period, wall loss within the electrode assembly creates an artifact. Our data suggest that at least 6 h are required to reach a steady state (Figure 3). Beyond that, there is little change between 6 h and two weeks. These findings reveal several problems when using competitive ligand exchange techniques with voltammetry. Firstly, estimates of speciation parameters can change depending on whether or not the inert fraction is excluded from the denominator in the speciation calculations. Secondly, since we showed that 6 h was required for equilibration with the naturally occurring ligands in the labile fraction, even with an exceptionally high competing ligand concentration (Figure 3), the earlier voltammetric methods have likely not reached equilibrium, given equilibration times of an hour or less. This is certainly the case for studies that use oxine, such as Campos and van den Berg (1994). Oxine is likely representative of other ligands, used in voltammetry, which have similar oxygen donor ligands.

Our findings suggest that, when inert Cu is treated separately in calculations, estimates of the stability constants are lower, but the excess ligand concentration increases. Therefore, the estimates of free Cu^{2+} are similar to those determined without considering an inert fraction. This is not surprising because free Cu^{2+} , in a sample, is determined by the equilibrium between Cu and labile ligands in a sample even if this is only a small fraction of the total Cu pool. Recently, there have been important advances in the modeling of titration data derived from competitive ligand methods (Pižeta et al., 2015). However, these models are only useful if the assumptions used to derive the original data are valid.

While “inert” remains operationally defined, the data in Fig. 3 show that the two pools we have designated as inert and labile are completely different in their exchangeabilities. Alternatively, had there been a continuum of ligands with incrementally increasing lability, one might have expected a linear relationship between oxine complex formation and time over the two weeklong period which we did not see. This sharp break between the two pools creates opportunities to characterize each fraction using other tools in the future such as an examination of stable isotope composition. The sharp break also suggests that there may be fundamental differences in the speciation, perhaps with inert Cu consisting of colloids (Jensen et al., 2020), sulfidic nanoparticles (Luther and Rickard, 2005), or proteins; labile Cu is comprised of strong but exchangeable, low molecular weight chelators.

If the valence of inert copper is Cu (I), it is possible that future work with a Cu (I)-selective chelator may extract a larger labile fraction. Such chelators usually contain nitrogen donor ligands, such as 1, 10-

Table 3

Conditional stability constant (K'_1) of strong Copper ligand, strong ligand concentration $[L_1]$, and free cupric ion concentration $[Cu^{2+}]$ measured on two depths from GP15 station 9 and 3 depths from GA03 using Competitive Ligand Exchange – Adsorptive Cathodic Stripping Voltammetry. Data obtained from voltammeter were fitted to a nonlinear regression using Sigmaplot version 8. Total L ($[L]_{Total}$) is defined as the sum of the $[L_1]$ and the concentration of inert Cu while Excess L ($[L]_{Excess}$) is defined as $[L]_{Total}$ subtracted by $[Cu]_{Total}$.

Sample and Cu used	Depth (m)	$[Cu]_{Total}$ (nM)	$\log(K'_1)$	$[L_1]$ (nM)	$[L]_{Total}$ (nM)	$[L]_{Excess}$ (nM)	$\log([Cu^{2+}])$
13050 with labile Cu	750	2.24	14.22	1.43	3.51	1.27	-14.86
13050 with total Cu	750	2.24	14.98	2.54	4.62	2.38	-14.86
13070 with labile Cu	25	1.20	14.99	1.46	3.42	2.22	-14.9
13070 with total Cu	25	1.20	14.79	1.47	3.43	2.23	-14.53
GA03 St. 18 with Labile Cu	110	0.53	14.28	1.26	1.79	1.26	-14.39
GA03 St. 18 with Total Cu	110	1.05	14.63	1.26	1.26	0.21	-14.20
GA03 St. 20 with Labile Cu	135	0.41	13.69	1.29	1.70	1.29	-13.88
GA03 St. 20 with Total Cu	135	0.82	13.94	1.30	1.30	0.48	-13.87
GA03 St. 22 with Labile Cu	51	0.49	13.83	1.22	1.71	1.22	-13.94
GA03 St. 22 with Total Cu	51	0.97	14.24	1.25	1.25	0.28	-13.93

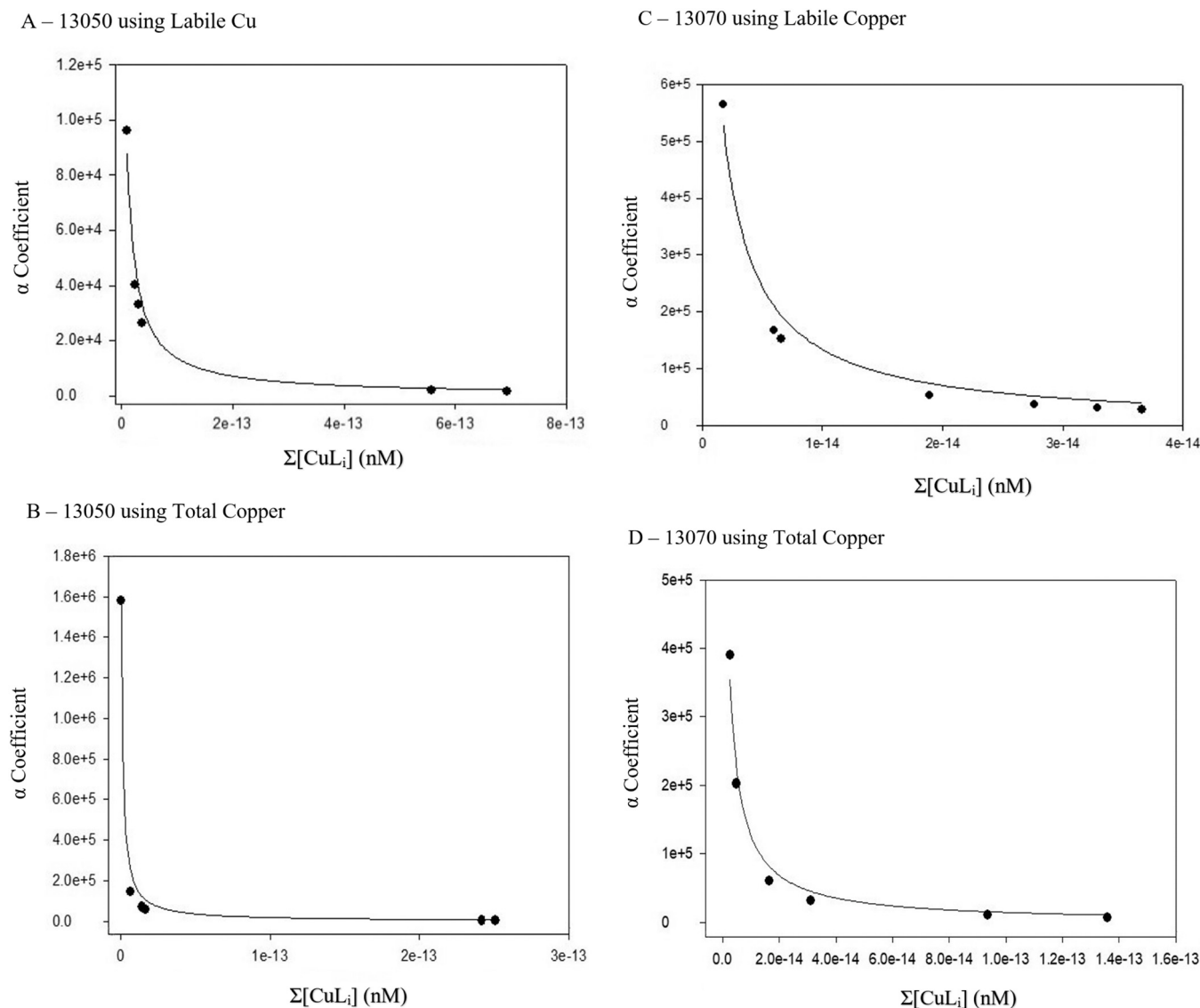


Fig. 7. (A–D). Samples from station 9 (GEOTRACES# 13050 and 13070) of GP15 were analyzed using the traditional Competitive Ligand Exchange – Adsorptive Cathodic Stripping Voltammeter (CLE-AdCSV). Titration data were then run through Sigmaplot vers. 8 to calculate values of K'_1 , $[\text{L}_1]$, and $[\text{Cu}^{2+}]$. Results of this non-linear regression were made using the measured labile Cu concentrations (6A – 13050 and 6C – 13070 using labile Cu) and total Cu concentrations (6B – 13050 and 6D – 13070 using total Cu). These results can be found in Table 3.

phenanthroline, rather than oxygen, such as oxine. Nevertheless, Moffett et al. (1985) and Moffett and Zika (1988) showed that Cu (II) chelators, even those that interact only weakly with Cu (I), can greatly facilitate its oxidation and incorporation into the Cu (II) pool. However, these workers were focusing on simple inorganic complexes of Cu (I). Copper (I) associated with a protein or reduced sulfur may react slowly with an oxygen-donor ligand, such as oxine.

The data presented here are not sufficient to present definitive conclusions about the distributional trends of Cu in the world's oceans. However, it is likely that the inert fraction forms slowly below the surface since there is a higher fraction in the older waters of the North Pacific and lower in the euphotic zone and in the North Atlantic. The presence of an inert fraction of Cu may have important implications for its geochemistry in the water column since it is anticipated that its biological availability and exchange with sinking particles will be strongly inhibited.

Declaration of Competing Interest

None.

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References

- Baconnais, I., Rouxel, O., Dulaquais, G., Boye, M., 2019. Determination of the copper isotope composition of seawater revisited: a case study from the Mediterranean Sea. *Chem. Geol.* 511, 465–480. <https://doi.org/10.1016/j.chemgeo.2018.09.009>.

- van den Berg, C.M.G., Donat, J.R., 1992. Determination and data evaluation of copper complexation by organic ligands in sea water using cathodic stripping voltammetry at varying detection windows. *Anal. Chim. Acta* 257 (2), 281–291. [https://doi.org/10.1016/0003-2670\(92\)85181-5](https://doi.org/10.1016/0003-2670(92)85181-5).
- van den Berg, C.M.G., Merks, A.G.A., Duursma, E.K., 1987. Organic complexation and its control of the dissolved concentrations of copper and zinc in the Scheldt estuary. *Estuar. Coast. Shelf Sci.* 24, 785–797. [https://doi.org/10.1016/0272-7714\(87\)90152-1](https://doi.org/10.1016/0272-7714(87)90152-1).
- Boyle, E.A., Sclater, F.R., Edmond, J.M., 1977. The distribution of dissolved copper in the Pacific. *Earth Planet. Sci. Lett.* 37, 38–54. [https://doi.org/10.1016/0012-821X\(77\)90144-3](https://doi.org/10.1016/0012-821X(77)90144-3).
- Brand, L.E., Sunda, W.G., Guillard, R.R.L., 1986. Reduction of marine phytoplankton reproduction rates by copper and cadmium. *J. Exp. Mar. Biol. Ecol.* 96, 225–250. [https://doi.org/10.1016/0022-0981\(86\)90205-4](https://doi.org/10.1016/0022-0981(86)90205-4).
- Buck, K.N., Bruland, K.W., 2005. Copper speciation in San Francisco Bay: a novel approach using multiple analytical windows. *Mar. Chem.* 96, 185–198. <https://doi.org/10.1016/j.marchem.2005.01.001>.
- Bundy, R.M., Barbeau, K.A., Buck, K.N., 2013. Sources of strong copper-binding ligands in Antarctic peninsula surface waters. *Deep Sea Res. Part II: Top. Stud. Oceanogr.* Southern Ocean Nat. Iron Fertil. 90, 134–146. <https://doi.org/10.1016/j.dsr2.2012.07.023>.
- Campos, M.L., van den Berg, C.M., 1994. Determination of copper complexation in sea water by cathodic stripping voltammetry and ligand competition with salicylaldehyde. *Anal. Chim. Acta* 284, 481–496. [https://doi.org/10.1016/0003-2670\(94\)85055-0](https://doi.org/10.1016/0003-2670(94)85055-0).
- Coale, K.H., Bruland, K.W., 1988. Copper complexation in the Northeast Pacific. *Limnol. Oceanogr.* 33, 1084–1101. <https://doi.org/10.4319/lo.1988.33.5.1084>.
- Dahl, I., 1968. The application of salicylaldehyde in solvent extraction. *Anal. Chim. Acta* 41, 9–14. [https://doi.org/10.1016/S0003-2670\(01\)80354-8](https://doi.org/10.1016/S0003-2670(01)80354-8).
- Davis, J.A., Leckie, J.O., 1978. Effect of adsorbed complexing ligands on trace metal uptake by hydrous oxides. *Environ. Sci. Technol.* 12, 1309–1315. <https://doi.org/10.1021/es60147a006>.
- Gerringa, L.J.A., Herman, P.M.J., Poortvliet, T.C.W., 1995. Comparison of the linear Van den Berg/ Ruzic transformation and a non-linear fit of the Langmuir isotherm applied to Cu speciation data in the estuarine environment. *Mar. Chem.* 48 (2), 131–142. [https://doi.org/10.1016/0304-4203\(94\)00041-B](https://doi.org/10.1016/0304-4203(94)00041-B).
- Irving, H., Williams, R.J.P., 1953. The stability of transition-metal complexes. *J. Chem. Soc.* 3192–3210. <https://doi.org/10.1039/JR9530003192>.
- Jacquot, J.E., Moffett, J.W., 2015. Copper distribution and speciation across the international GEOTRACES section GA03. Deep Sea research part II: topical studies in oceanography, GEOTRACES GA-03 - the U.S. GEOTRACES North Atlantic Transect 116, 187–207. <https://doi.org/10.1016/j.dsr2.2014.11.013>.
- Jensen, L.T., Wyatt, N.J., Landing, W.M., Fitzsimmons, J.N., 2020. Assessment of the stability, sorption, and exchangeability of marine dissolved and colloidal metals. *Mar. Chem.* 220, 103754. <https://doi.org/10.1016/j.marchem.2020.103754>.
- Kogut, M.B., Voelker, B.M., 2003. Kinetically inert Cu in coastal waters. *Environ. Sci. Technol.* 37, 509–518. <https://doi.org/10.1021/es020723d>.
- Laglera, L.M., van den Berg, C.M.G., 2003. Copper complexation by thiol compounds in estuarine waters. *Mar. Chem.* 82, 71–89. [https://doi.org/10.1016/S0304-4203\(03\)00053-7](https://doi.org/10.1016/S0304-4203(03)00053-7).
- Leeuwen, H.P.V., Town, R.M., 2005. Kinetic limitations in measuring stabilities of metal complexes by competitive ligand exchange-adsorptive stripping voltammetry (CLE-AdSV). *Environ. Sci. Technol.* 39 (18), 7217–7225. <https://doi.org/10.1021/es0503672b>.
- Little, S.H., Vance, D., Siddall, M., Gasson, E., 2013. A modeling assessment of the role of reversible scavenging in controlling oceanic dissolved Cu and Zn distributions: modeling oceanic Cu and Zn. *Glob. Biogeochem. Cycles* 27, 780–791. <https://doi.org/10.1002/gbc.20073>.
- Luther, G.W., Rickard, D.T., 2005. Metal sulfide cluster complexes and their biogeochemical importance in the environment. *J. Nanopart. Res.* 7, 389–407. <https://doi.org/10.1007/s11051-005-4272-4>.
- Ma, Y.B., Lombi, E., Nolan, A.L., McLaughlin, M.J., 2006. Determination of labile Cu in soils and isotopic exchangeability of colloidal Cu complexes. *Eur. J. Soil Sci.* 57, 147–153. <https://doi.org/10.1111/j.1365-2389.2005.00723.x>.
- Moffett, J.W., 1995. Temporal and spatial variability of copper complexation by strong chelators in the Sargasso Sea. *Deep-Sea Res. I Oceanogr. Res. Pap.* 42, 1273–1295. [https://doi.org/10.1016/0967-0637\(95\)00060-J](https://doi.org/10.1016/0967-0637(95)00060-J).
- Moffett, J.W., Brand, L.E., 1996. Production of strong, extracellular Cu chelators by marine cyanobacteria in response to Cu stress. *Limnol. Oceanogr.* 41 (3), 388–395. <https://doi.org/10.4319/lo.1996.41.3.0388>.
- Moffett, J.W., Dupont, C., 2007. Cu complexation by organic ligands in the sub-arctic NW Pacific and Bering Sea. *Deep-Sea Res. I Oceanogr. Res. Pap.* 54, 586–595. <https://doi.org/10.1016/j.dsr.2006.12.013>.
- Moffett, J.W., 1987a. Solvent extraction of copper acetylacetonate in studies of copper (II) speciation in seawater. *Mar. Chem.* 21 (4), 301–313. [https://doi.org/10.1016/0304-4203\(87\)90053-3](https://doi.org/10.1016/0304-4203(87)90053-3).
- Moffett, J.W., Zika, R.G., 1987b. Photochemistry of copper complexes in sea water. In: *Photochemistry of Environmental Aquatic Systems*, ACS Symposium Series. American Chemical Society, pp. 116–130. <https://doi.org/10.1021/bk-1987-0327.ch009>.
- Moffett, J.W., Zika, R.G., 1988. Measurement of copper(I) in surface waters of the subtropical Atlantic and Gulf of Mexico. *Geochim. Cosmochim. Acta* 52, 1849–1857. [https://doi.org/10.1016/0016-7037\(88\)90008-7](https://doi.org/10.1016/0016-7037(88)90008-7).
- Moffett, J.W., Zika, R.G., Petasne, R.G., 1985. Evaluation of bathocuproine for the spectro-photometric determination of copper(I) in copper redox studies with applications in studies of natural waters. *Anal. Chim. Acta* 175, 171–179. [https://doi.org/10.1016/S0003-2670\(00\)82729-4](https://doi.org/10.1016/S0003-2670(00)82729-4).
- Moffett, J.W., Zika, R.G., Brand, L.E., 1990. Distribution and potential sources and sinks of copper chelators in the Sargasso Sea. *Deep Sea Res. Part A. Oceanogr. Res. Papers* 37, 27–36. [https://doi.org/10.1016/0198-0149\(90\)90027-S](https://doi.org/10.1016/0198-0149(90)90027-S).
- Moffett, J.W., Brand, L.E., Croot, P.L., Barbeau, K.A., 1997. Cu speciation and cyanobacterial distribution in harbors subject to anthropogenic Cu inputs. *Limnol. Oceanogr.* 42, 789–799. <https://doi.org/10.4319/lo.1997.42.5.0789>.
- Pizeta, I., Sander, S.G., Hudson, R.J.M., Omanović, D., Baars, O., Barbeau, K.A., Buck, K.N., Bundy, R.M., Carrasco, G., Croot, P.L., Garnier, C., Gerringa, L.J.A., Gledhill, M., Hirose, K., Kondo, Y., Laglera, L.M., Nuester, J., Rijkenberg, M.J.A., Takeda, S., Twining, B.S., Wells, M., 2015. Interpretation of complexometric titration data: An intercomparison of methods for estimating models of trace metal complexation by natural organic ligands. *Marine Chemistry* 173, 3–24. <https://doi.org/10.1016/j.marchem.2015.03.006> (SCOR WG 139: Organic Ligands – A Key Control on Trace Metal Biogeochemistry in the Ocean).
- Rapp, I., Schlosser, C., Rusiecka, D., Gledhill, M., Achterberg, E.P., 2017. Automated preconcentration of Fe, Zn, Cu, Ni, Cd, Pb, Co, and Mn in seawater with analysis using high-resolution sector field inductively-coupled plasma mass spectrometry. *Anal. Chim. Acta* 976, 1–13. <https://doi.org/10.1016/j.aca.2017.05.008>.
- Richon, C., Tagliabue, A., 2019. Insights into the major processes driving the global distribution of copper in the ocean from a global model. *Glob. Biogeochem. Cycles* 33, 1594–1610. <https://doi.org/10.1029/2019GB006280>.
- Ruacho, A., Bundy, R.M., Till, C.P., Roshan, S., Wu, J., Barbeau, K.A., 2020. Organic dissolved copper speciation across the U.S. GEOTRACES equatorial Pacific zonal transect GP16. *Mar. Chem.* 225, 103841. <https://doi.org/10.1016/j.marchem.2020.103841>.
- Sander, S.G., Buck, K.N., Wells, M., 2015. The effect of natural organic ligands on trace metal speciation in San Francisco Bay: implications for water quality criteria. *Marine Chemistry* 173, 269–281. <https://doi.org/10.1016/j.marchem.2014.09.015> (SCOR WG 139: Organic Ligands – A Key Control on Trace Metal Biogeochemistry in the Ocean).
- Sunda, W.G., Lewis, J.A.M., 1978. Effect of complexation by natural organic ligands on the toxicity of copper to a unicellular alga, *Monochrysis lutheri*. *Limnol. Oceanogr.* 23, 870–876. <https://doi.org/10.4319/lo.1978.23.5.0870>.
- Twiss, M.R., Moffett, J.W., 2002. Comparison of copper speciation in coastal marine waters measured using analytical voltammetry and diffusion gradient in thin-film techniques. *Environ. Sci. Technol.* 36, 1061–1068. <https://doi.org/10.1021/es0016553>.
- Whitby, H., van den Berg, C.M.G., 2015. Evidence for copper-binding humic substances in seawater. *Mar. Chem.* 173, 282–290. <https://doi.org/10.1016/j.marchem.2014.09.011>.
- Whitby, H., Posacka, A.M., Maldonado, M.T., van den Berg, C.M.G., 2018. Copper-binding ligands in the NE Pacific. *Mar. Chem.* 204, 36–48. <https://doi.org/10.1016/j.marchem.2018.05.008>.