The complex provenance of Cu-binding ligands in the South-East Atlantic

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\textbf{ABSTRACT}

Organic ligands play a key role in the marine biogeochemical cycle of copper (Cu), a bio-essential element, regulating its solubility and bioavailability. However, the sources, abundance, and distribution of these ligands are still poorly understood. In this study, we examined vertical Cu speciation profiles from the South-East Atlantic (GEOTRACES section GA08). Profiles were collected from a range of ocean conditions, including the Benguela upwelling region, the oligotrophic South Atlantic Gyre, and the Congo River outflow. In general, the lack of a significant correlation between most of the parameters assessed here with Cu speciation data obscures the provenance of Cu-binding ligands, suggesting that Cu speciation in the South-East Atlantic is influenced by a complex interplay between biotic and abiotic processes. Nevertheless, the total dissolved Cu (Cu\textsuperscript{2+}) illustrated an allochthonous origin in the working area, while Cu-binding ligands showed both an allochthonous and a biogenic, autochthonous origin. Pigment concentrations showed that the phylogeography of different microorganisms influenced the spatial features of the Cu-binding ligand pool in the South-East Atlantic. Allochthonous Cu-binding ligand sources in the upper water column are likely associated with dissolved organic matter which originated from the Congo River and the Benguela upwelling system. Deep water ligand sources could include refractory dissolved organic carbon (DOC), resuspended benthic inputs, and lateral advected inputs from the shelf margin. The degradation of L\textsubscript{1}-type ligands and/or siderophores in low oxygen conditions may also be a source of L\textsubscript{2}-type ligands in the deep. Free Cu ion levels (1.7 to 156 fM), the biologically available form of Cu, were below the putative biolimiting threshold of many marine organisms. Two classes of ligands were found in this study with total ligand concentrations ([L]) ranging from 2.5 to 283.0 nM and conditional stability constants ($\log K_{\text{CuL}}$) ranging from 10.7 to 14.6. The Cu speciation values were spatially variable across the three subregions, suggesting that biogeochemical processes and sources strongly influence Cu speciation.

\textbf{1. Introduction}

Greater understanding of the distribution and speciation of trace elements in the ocean is critical for understanding their geochemical cycling and impact on biological processes (Flemming and Trevors, 1989; Heller and Croot, 2015; Sunda, 2012; Sunda, 1994). Copper (Cu) is an essential component of various enzymatic pathways, respiratory proteins, and oxidases for many marine organisms, including phytoplankton (Barón et al., 1995; Lopez et al., 2019). However, high Cu concentrations can retard vital processes or even be lethal at levels above the essential range (Klaune and Singhasemanon, 2011). For Cu, and other bioactive metals, the impact on marine biota is not determined by the total dissolved metal concentration ([Cu\textsuperscript{2+}]; Anderson and Morel, 1978; Brand et al., 1986; Sunda and Lewis, 1978) but rather by the concentration of the bioavailable form, which is generally the free metal-ion concentration ([Cu\textsuperscript{2+}]). Whilst some forms of dissolved organically complexed Cu are also bioavailable (Kong and Price, 2020; Lorenzo et al., 2005; Semeniuk et al., 2015), it is generally accepted that

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Cu$^{2+}$ is the most bioavailable form of Cu to marine organisms. [Cu$^{2+}$] > 1.0 pM have been shown to adversely affect some microorganisms (Amin et al., 2013; Annett et al., 2008; Brand et al., 1986; Glass and Orphan, 2012; Guo et al., 2010; Jacquot et al., 2014; Mann et al., 2002), while [Cu$^{2+}$] ≤ 0.01 pM (≤ 0.2 pM for ammonia-oxidizing Archaea; Amin et al., 2013; Jacquot et al., 2014) have been found to be bio-limiting (Maldonado et al., 2006; Peers et al., 2005; Sunda and Huntsman, 1995). Whilst these values are commonly cited as 'critical Cu$^{2+}$ thresholds', they are species-dependent and can vary between different marine organisms (Lopez et al., 2019). For example, eukaryotic phytoplankton such as diatoms, coccolithophorids, and dinoflagellates are in general more tolerant to [Cu$^{2+}$] than small cyanobacteria (Synchococcus and Prochlorococcus) (Lopez et al., 2019).

Approximately 89 to 99% of the Cu$_T$ in seawater exists as organic complexes (Buck et al., 2007; Coale and Bruland, 1990; Moffett and Dupont, 2007; van den Berg et al., 1987), resulting in [Cu$^{2+}$] within the ‘Goldilocks’ range, that is neither too low to sustain biological uptake, nor too high to inhibit essential metabolic processes of marine organisms (Brand et al., 1986; Bruland et al., 2003; Kåune and Singh, 2009; Mann et al., 2002; Maldonado et al., 2006; Tang et al., 2001). However, [Cu$^{2+}$] can increase to potentially toxic levels when the Cu complexation capacities of organic ligands are saturated in the water column (Mann et al., 2002; Moffett et al., 1997), for instance due to high Cu$_T$ inputs from rivers, groundwater discharge, atmospheric deposition, resuspended sediments, benthic inputs, upwelling and mixing from deep water, and hydrothermal vent inputs (Bruland et al., 2003; Heller and Croot, 2015; Little et al., 2018; Pohl et al., 2011; Sander et al., 2007). Furthermore, Cu$^{2+}$ can decrease to deficient levels under conditions of high biomass production, low Cu$_T$ input, extensive scavenging, or high levels of strong Cu-binding complexes such as organic matter (Sadiq, 1992; Jacquot and Moffett, 2015).

Despite progress being made in assigning elemental formulas to Cu-binding ligands (Boiteau et al., 2016), their direct structural characterization in seawater remains challenging owing to analytical and methodological restraints (Boiteau et al., 2016; Donat et al., 1997; Gordon et al., 1996; Vraspir and Butler, 2009). To date, the primary tool employed for the study of Cu-binding ligands (L) is via electrochemical analysis, employing voltammetric techniques, which enable the partial characterization of the Cu-binding ligand pool via estimation of [L] and conditional stability constants (logK$_{CuL}$, Cu$_2L^{-}$; cond) of prevalent CuL-complexes (Campos and van den Berg, 1994; Thompson et al., 2014). With these two parameters, together with [Cu$_T$], the [Cu$^{2+}$] can be calculated for a specific sample. Commonly, stability constants of Cu-binding ligands in seawater are divided into two ligand classes, i.e. L$_1$ (logK$_{CuL}$, Cu$_2L^{-}$; cond > 12 and up to 16; (Croot et al., 2000; Vraspir and Butler, 2009, Bundy et al., 2013; Muller and Batchelli, 2013, Whitby et al., 2018)) and L$_2$ (logK$_{CuL}$, Cu$_2L^{-}$; cond < 12), following the definition of Vraspir and Butler (2009), even though it is likely that there is a continuum of organic ligands present (Heller and Croot, 2015).

Voltammetry does not inform ligand composition, beyond characterising the stability of the CuL-complexes, but field and laboratory studies have found a strong link between L$_1$ and thiols, and L$_2$ and humic substances (Donat et al., 1994; Laglera and van den Berg, 2003; Whitby et al., 2017; Whitby and van den Berg, 2015). Voltammetric analyses of microbial exudates of laboratory-based cultures showed that thiols can be actively produced by various marine microorganisms with logK$_{CuL}$, Cu$_2L^{-}$; cond in the range of 10–13 in response to high [Cu$^{2+}$] in the growth medium (Croot et al., 2000; Dupont et al., 2004; Keleven et al., 2012; Leal et al., 1999; Moffett et al., 1990; Moffett and Brand, 1996; Vasconcelos et al., 2002). These logK$_{CuL}$, Cu$_2L^{-}$; cond values have also been measured for natural marine systems in the photic zone where primary production occurs, suggesting that at least a fraction of the Cu-binding marine ligand pool is of biogenic nature (passively or actively produced) rather than derived from refractory compounds (Coale and Bruland, 1988; Croot et al., 2000; Leal et al., 1999; Mann et al., 2002; Thompson et al., 2014). Passive generation pathways of marine biogenic Cu-binding ligands are thought to arise from grazing, bacterial remineralization, and viral lysis (Gledhill and Buck, 2012; Laglera et al., 2020a; Poovrin et al., 2011; Sato et al., 2007). Marine sediments, refractory DOC, and particles from shelf margins have been suggested as non-biological sources of L$_1$- and L$_2$-class Cu-binding ligands in the deeper ocean and coastal regions (Chapman et al., 2009; Thompson et al., 2014; Jacquot and Moffett, 2015; Ruacho et al., 2020; Hassler et al., 2020). In coastal and estuarine systems, terrestrial-derived ligands, such as humics, have also been recorded to comprise a large fraction of the prevalent Cu-binding ligand pool (Abaullahjia et al., 2015; Bundy et al., 2015; Laglera and van den Berg, 2009; Shank et al., 2004). Additionally, some of the L$_2$-type ligands may be photochemical degradation products of L$_1$-type Cu-binding ligands, such as refractory DOC, in the euphotic zone (Laglera and van den Berg, 2006; Hassler et al., 2020).

So far, the sources, sinks, and the fate of Cu-binding ligands in the natural marine environment are not well-defined and Cu speciation data is disproportionately focussed on the North Atlantic, the Pacific, and the Southern Ocean (Jacquot et al., 2013; Jacquot and Moffett, 2015; Heller and Croot, 2015; Mellett and Buck, 2020; Ruacho et al., 2020; Thompson et al., 2014; Whitby et al., 2018). Here we present Cu speciation and associated CuL complexation data from thirteen stations along the GEOTRACES section GA08 in the South-East Atlantic, a region of major trace metal inputs such as from the Namib Desert (Frank et al., 2016). Samples were collected across a diverse range of environments, ranging from the biologically productive Benguela upwelling region to the oligotrophic South Atlantic Gyre, and the trace metal-rich Congo River outflow. These diverse biogeochemical settings can be expected to have an impact on the spatial pattern of Cu speciation. The overall aim of this study was to enhance insights into the factors that influence Cu speciation and to identify the sources of Cu-binding ligands in the South-East Atlantic. Characterising the provenance of Cu-binding ligands will help to clarify the bioavailability and fate of Cu$_T$ in the ocean and will thus enhance the ability of biogeochemical models to capture global Cu$_T$ distributions in the current and future ocean.

2. Materials and methods

2.1. General

Thirteen stations were sampled for Cu$_T$ and Cu speciation parameters in the Angola and northernmost Cape Basin on the GEOTRACES section GA08 (RV Meteor expedition M121; November 22 and December 27, 2015; Fig. 1 and Table 1; Supplementary Table S1; Frank et al., 2016).

Stations were sampled in the shelf waters off west Africa and along two zonal transects perpendicular to the coast, at 3°S and 29°S. The sample sites were located in three unique oceanographic regions including an area dominated by the Angola Current (AnC), the Benguela Current (BC), and the South Atlantic Current (SAC) (Berger et al., 1998; Stramma and England, 1999), and in areas of major trace metal input from upwelling, sedimentary, riverine, and aeolian sources (Table 1; Frank et al., 2016). Both regular stainless-steel conductivity-temperature-depth-profiler-rosette (CTD) and non-contaminating, trace-metal-clean CTD-rosette (TM-CTD) deployments were conducted at all sites.

2.2. Hydrographic setting

The oceanographic setting during the collection of samples for the expedition is reported in Rahlf et al. (2020). In brief, the BC, the dominant and nutrient-rich surface current of the Cape Basin, is fed by the eastwards flowing SAC (Fig. 1; Berger et al., 1998; Stramma and England, 1999). The BC has a well-defined equatorward flow parallel to the coast of southwest Africa until it meets the warm tropical, poleward-flowing waters of the AnC at the Angola-Benguela Front (ABF) between 16°S and 17°S (Berger et al., 1998; Hutchings et al., 2009; Rahlf et al., 2020; Stramma and England, 1999). The region in the Cape Basin,
between ~34°S and ~15°S, is generally referred to as the Benguela Upwelling System, which is characterized by several upwelling cells with the most prominent ones located at 20°S (Northern Namibian Cell), 23°S (Central Namibian Cell), and 27°S (Lüderitz Cell) (Hutchings et al., 2009; Shannon, 2006; Siegfried et al., 2019). The cyclonic Angola Gyre (AG), an offshore upwelling zone, is together with the AnC the major oceanographic feature of the Angola Basin (Berger et al., 1998; Rahlf et al., 2020). The southward flowing AnC is influenced to the north by the Congo River plume providing offshore estuarine circulation (Berger et al., 1998).

The surface waters in the northern part of the Angola Basin are dominated by the presence of Tropical Surface Water (TSW), while the mixed layer in the southern part of the Angola Basin and Cape Basin is occupied in large by Subtropical Surface Water (STSW; Fig. 2; Rahlf et al., 2020). Both the TSW and the STSW progress equatorward and overlie the South Atlantic Central Water (SACW) (Rahlf et al., 2020; Stramma and England, 1999). Nutrient-rich, northward flowing Antarctic Intermediate Water (AAIW) forms the intermediate water mass in the sampling region between 500 and 1300 m water depth (Rahlf et al., 2020; Shannon, 2006; Talley, 1996; Whitworth III and Nowlin Jr, 1987), which overlies the northwards propagating oxygen-poor and nutrient-rich Upper Circumpolar Deep Water (UCDW) (Stramma and Schott, 1999). The North Atlantic Deep Water (NADW) is the deepest near bottom water mass in the Angola Basin, which moves southwards between ~1500 m and ~4500 m (Rahlf et al., 2020). In the Cape Basin, near the bottom, the Lower Circumpolar Deepwater (LCDW) and

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**Table 1**

| Station | Location       | Key Features                          | Region     | Distance to the coast |
|---------|----------------|---------------------------------------|------------|-----------------------|
| 4       | Shelf Waters   | Upwelling, Desert Input               | BC         | Nearshore station     |
| 13      | Shelf Waters   | Congo River                           | AnC        | Nearshore station     |
| 14      | Shelf Waters   | Congo River                           | AnC        | Shelf station         |
| 15      | Shelf Waters   | Congo River                           | AnC        | Shelf station         |
| 17      | E-W Transect 3°S| Sediment Resuspension, Shelf Input    | AnC        | Nearshore station     |
| 18      | E-W Transect 3°S| Sediment Resuspension, Shelf Input    | AnC        | Shelf station         |
| 19      | E-W Transect 3°S| Sediment Resuspension, Shelf Input    | AnC        | Shelf station         |
| 20      | E-W Transect 3°S| Sediment Resuspension, Shelf Input    | AnC        | Shelf station         |
| 37      | W-E Transect 29°S| Oligothrophic waters                  | SAC        | Open ocean station    |
| 38      | W-E Transect 29°S| Oligothrophic waters                  | SAC        | Open ocean station    |
| 43      | W-E Transect 29°S| Sediment Resuspension, Shelf Input, Upwelling | BC          | Shelf station         |
| 44      | W-E Transect 29°S| Sediment Resuspension, Shelf Input, Upwelling | BC          | Nearshore station     |
| 51      | Shelf Waters   | Upwelling, Urban Discharge, Desert Input | BC         | Nearshore station     |

Oceanographic regions as in Fig. 1.

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Fig. 1. Sampling locations along the GEOTRACES section GA08. Stations sampled for Cu speciation analysis are indicated by diamonds, while black dots represent all the stations sampled during the expedition. General oceanographic surface features of the sampling area are indicated by white dashed lines and include the South Equatorial Current (SEC), the Angola Benguela Front (ABF), the Angola Front (AF), the South Equatorial Counter Current (SECC), and the Angola Gyre (AG). Coloured diamonds indicate the grouping of Cu speciation data into oceanographic regions with red, yellow, and green diamonds reflecting sites influenced by the Angola Current (AnC), the Benguela Current (BC), and the South Atlantic Current (SAC), respectively (Louropoulou et al., 2020). The map was adapted from Rahlf et al. (2020). The figure was produced using Ocean Data View v.5.2.1 (Schlitzer, R. Ocean Data View. 2020, https://odv.awi.de/ ). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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Antarctic Intermediate Water (AAIW), Upper Circumpolar Deep Water (UCDW), and temperature were retrieved from the calibrated CTD (Seabird SBE911 plus) equipped with oxygen sensors. Water-masses: Tropical Surface Water (TSW), Subtropical Surface Water (STSW), South Atlantic Central Water (SACW), Antarctic Intermediate Water (AAIW), Upper Circumpolar Deep Water (UCDW), North Atlantic Deep Water (NADW), and Antarctic Bottom Water (AABW). Numbers above the graph indicate the station numbers.

Antarctic Bottom Water (AABW) carry denser water northwards (Stramma and Schott, 1999) until they reach the Walvis Ridge, which acts as a topographic barrier (Rickli et al., 2009). See Supplementary Fig. S1 for a 3D view of the working area.

2.3. Sample collection and analysis for Cu\textsuperscript{II} and Cu speciation

Sample collection for Cu\textsuperscript{II} and Cu speciation analysis followed established trace-metal-clean sampling protocols (Cutler et al., 2010; Sander et al., 2009). Full depth seawater samples were obtained using a TM-CTD rosette loaded with 24 × 12 L trace-metal-clean Go-Flo bottles (General Oceanics Inc.) and equipped with a Seabird SBE911 plus calibrated CTD including two independently calibrated optical oxygen sensors. Upon recovery, Go-Flo bottles were transferred to the onboard trace-metal-clean sampling container over-pressured with HEPA filtered air (class 100). Subsamples for Cu speciation and Cu\textsuperscript{II} analysis were filtered through 0.2 μm AcroPak-500 cartridge filters (Pall) under N\textsubscript{2} pressure and collected, following three 10% volume rinses with sample seawater, into acid-cleaned low-density polyethylene bottles.

Filtered Cu\textsuperscript{II} samples were acidified on board in a laminar flow bench to pH 1.9 using ultra-pure grade HCl (UpA grade, Romil). The Cu\textsuperscript{II} samples were stored in the dark and shipped to GEOMAR (Kiel; Germany) for analysis via isotope dilution ICP-MS using an Element XR (Thermo Scientific) following Rapp et al. (2017). For Cu\textsuperscript{II}, the limit of detection, defined as 3 times the standard deviation of the procedural blank, was 62 pM. Results for certified reference samples (GSP, GSC, and SAFe S) agreed well with the GEOTRACES consensus values (Supplementary Material (Supplementary Table S3). Peak height was chosen as the characteristic signal value after background correction. Measurements were carried out at room temperature. Voltammetric cell cups were rinsed with Milli-Q-water between titrations to avoid carry-over between samples. To ensure the reliability of the data, [Cu\textsubscript{II}] of a certified reference seawater sample (NASS-7, National Research Council, Canada) was analysed multiple times by a standard addition method using CLE-AdCSV. Electrochemical parameters were adopted from the Cu speciation analysis. The result (n = 12; 3.33 ± 0.43 nM) of the certified reference sample was consistent with the published consensus value (3.07 ± 0.22 nM).

Final titration curves were interpreted in the speciation program ProMCC (https://sites.google.com/site/daromosoft/home/promcc; Omanovic et al., 2015). logK\text{CuL} and logK\text{CuSA} values and [L] were derived for each titration using the one-ligand and two-ligand complete complication-fitting model (Omanovic et al., 2015). The model with the best-fit and the least fitting error was used for data acquisition. Side reaction coefficients and stability constants of SA of each sample were obtained with van den Berg’s ion-pairing model for seawater (EXCEL worksheet - ion-pairing model for seawater, written by C.M.G. van den Berg in 2014) using sample specific salinity values, a SA concentration of 5 μM, an ambient temperature of 21 °C, and a pH of 8.1. The conditional stability constants for SA were set to logK\text{CuSA}~9.54 and logK\text{CuSA}~14.96, and the inorganic side reaction coefficient (\text{Cu(II)}) was set to 25. More details of the parameters are provided in the Supplementary Material (Supplementary Table S4). The resulting detection window of the method (D\text{SA}%) was 4.23 logD\text{SA}, calculated as the sum of the product of [SA] and K\text{CuSA}~cond, for Cu\text{II} (Apte et al., 1988; Laglera and Filrella, 2015). Complexation capacities (logK\text{CuL} for Cu\text{II} with [L] and [CuSA]~cond, calculated as the product of [L] and K\text{CuSA}~cond, for Cu\text{II}) of non-bound ligands by Cu; Gledhill and Gerringa, 2017) of the estimated Cu-binding ligands fell within one order of magnitude of D\text{SA}, indicating that Cu speciation parameters were reliably determined, except for Station 20 at 800 m depth (D\text{SA}~4.23 ± 1.0; logK\text{CuSA}~3.8–5.8; Supplementary Table S4) (Apte et al., 1988; Laglera and Filrella, 2015).
2015). It has to be noted, however, that the here reported titration range from 0 to 80 nM Cu might not have been enough to saturate prevalent ligands, and thus an underestimation of the estimated [L] cannot be excluded, especially for [L] > 80 nM. Nevertheless, the chosen titration maximum followed the study of Heller and Croot (2015) and was up to 20 x higher than the highest measured [Cu_L] in the working area and also higher than the titration range of other Cu speciation studies using CSV (up to 25 nM; Jacquot and Moffett, 2015; Thompson et al., 2014; Ruacho et al., 2020), and thus the here reported titration range was deemed appropriate to saturate most natural ligands.

Of the 79 samples analysed, the titration curves of 15 samples could not be adequately fitted in the ProMCC software, potentially due to [Cu_L] already exceeding [L] in the initial samples (Buck and Bruland, 2005a, 2005b). These samples were thus not used in the dataset (Supplementary Table S5). Due to problems with the voltammetric measurements, a further 8 samples had to be re-thawed and re-measured (Supplementary Table S5). While one freezing/thawing cycle was demonstrated in our laboratory to maintain the Cu-binding ligand speciation sufficiently (Sander et al., 2005), multiple freezing and thawing has not been studied systematically and artefacts may occur. However, the complexation parameters of the 9 samples that underwent two freezing/thawing cycles before being measured were comparable to the parameters of adjacent sites and thus the data was deemed acceptable.

2.4. Sample collection and analysis for DOC and macronutrients

Water samples for DOC were collected at every station using the stainless-steel CTD-rosette equipped with Niskin bottles. Water samples were collected in pre-combusted glass ampoules and acidified to pH < 2 to remove inorganic carbon. Samples were stored in the dark at 4 °C and shipped to GEOMAR for analysis by high temperature combustion using a Shimadzu TOC analyser following Badr et al. (2003). The DOC results for a certified reference sample provided by Hansell Laboratory (n = 10; 44.2 ± 5.6 μM; Rosenstiel School of Marine and Atmospheric Science (RSMAS): Batch 18-2018, Lot # 10-18, DSR (700 m)) agreed with the published consensus value (43–45 μM).

Niskin and Go-Flo bottles were also sampled for PO₄, NO₃, Si, and NH₄ and analysed on board using an auto-analyser (Quattro Seal Analytical) following Hydes et al. (2010). Macro-nutrient analysis was validated with a certified reference sample (BW KANSO). The precision of the reference samples was around 0.7% and there was no significant difference between the measured values of KANSO and the consensus values.

2.5. Sample collection and analysis for phytoplankton biomass and composition

Seawater samples collected from the stainless steel CTD-rosette through the upper ~200 m of the water column were also used to characterize phytoplankton biomass and community structure. Water was collected in 10 L opaque carboys and processed immediately after collection. Samples (0.62–4.00 L depending on biomass) were filtered onto glass fibre filter papers (GFF; Machery-Nagel, 0.7 μm pore size), stored frozen at ~80 °C, and shipped to GEOMAR for analysis. Samples were extracted in 90% acetone in plastic vials by homogenization of the filters using glass beads in a cell mill, centrifuged (10 min, 5200 rpm, 4 °C), and the supernatant was filtered through 0.2 μm PTFE filters (VWR International). Pigment concentrations in the supernatant were determined by reverse-phase high performance liquid chromatography (HPLC; Dionex UltiMate 3000 LC system, Thermo Scientific) following Van Heukelen and Thomas (2001). Pigments were identified via examination of diagnostic retention times and absorption spectra. Pigment standards were from Sigma-Aldrich (USA) and the International Agency for 14C Determination (Denmark). Estimates of the contributions of individual phytoplankton types to chlorophyll-a (chl-a) were calculated using CHEMTAX (version 1; Mackey et al., 1996) applying pigment ratios from Higgins et al. (2011).

2.6. Statistical analysis

Pearson correlation coefficients were used to examine the effect of abiotic and biotic parameters on [Cu₄] and Cu speciation parameters. The chosen level of statistical significance was p ≤ 0.05. Statistical analysis was performed using the computing environment R (corplot library; version 3.1.2; R Development Core Team, 2016).

3. Results and discussion

Stations were clustered into three different oceanographic regions (AnC, BC, SAC) and also grouped together regarding their distance to the shore, i.e. nearshore, shelf (stations situated on the continental margin), and open ocean stations (Fig. 1, Table 1). Data were considered as being from the same depth interval when the depth difference was less than ±5 m.

3.1. Total dissolved Cu in the South-East Atlantic

Elevated surface [Cu₄] (< 100 m) were measured in the Angola Basin, matching elevated concentrations of a suite of other trace elements (median of 0.70 nM and up to 1.71 nM Cu; Fig. 3; Barraqueta et al., 2017 and Barraqueta et al., 2019; Bianchi et al., 2013; Jourdin et al., 2006; Vieira et al., 2020; Van Der Loeff et al., 1997) and likely associated with freshwater inputs from the Congo River (Vieira et al., 2020; Liu et al., 2021). Elevated [Cu₄] in the nearshore Cape Basin were likely associated with Benguela upwelling of trace metal rich deep-water rather than atmospheric inputs, which are expected to be low in this region (BC station: median of 0.93 nM and up to 2.12 nM Cu₄; Mahowald et al., 2005; Barraqueta et al., 2019; Noble et al., 2012). The stations situated further offshore in the Cape Basin and influenced by the SAC had a median [Cu₄] of 0.66 nM in the upper water column (up to 1.27 nM). This lower concentration agrees with levels reported by Boyé et al. (2012), Little et al. (2018), and Pohl et al. (2011), and is presumably a result of greater distance from coastal sources and weaker upwelling in offshore subtropical and oligotrophic gyre waters. Generally, higher median [Cu₄] were found in the upper water column of nearshore stations relative to the upper water column of shelf stations and open ocean stations, suggesting a dominance of allochthonous Cu₄ sources in the South-East Atlantic (0.93 nM, 0.72 nM, and 0.66 nM, respectively).

The vertical distribution of [Cu₄] in the South-East Atlantic generally showed the familiar near linear increases with depth (Supplementary Fig. S2) (Boye et al., 2012; Little et al., 2018), suggesting that [Cu₄] in the three subregions are mainly controlled by biological uptake in surface waters and remineralization at depth. Some stations also showed various Cu₄ maxima in the subsurface and near the continental shelf (Fig. 3), which has been observed previously in the South-East Atlantic (Boyé et al., 2012) and other marginal regions in the world (Bruland, 1980; Pohl et al., 2011). These subsurface maxima may partly reflect benthic inputs and entrainment of shelf sediments as suggested by several concurrent Mn maxima (Liu et al. (2021), with Mn acting as a proxy for lithogenic input, and previously published isotope data (Little et al., 2018; Rahlf et al., 2020), sediment studies (Robinson et al., 2002; Orani et al., 2018; Orani et al., 2019), and trace metal data (Noble et al., 2012) off the coast of Namibia.

[Cu₄] correlated well with macronutrient concentrations at stations influenced by the SAC, but not with stations further north, influenced by the BC and AnC, conveying a more complex combination of factors acting on element concentrations and distributions in these two regions (SAC: PO₄ with R² > 0.76 and p < 0.01; NO₃ with R² > 0.76 and p < 0.01; Si with R² > 0.95 and p < 0.01; Supplementary Table S6). Oxygen did not show a correlation with Cu₄ but waters with dysoxic conditions
<60 μM kg⁻¹ oxygen, Supplementary Table S10; Gray et al., 2002) had higher median \([Cu_1]\) than waters with oxic redox conditions (dysoxic: 0.89 nM Cu₂⁺; oxic 0.83 nM Cu₂⁺), which has been observed previously in the eastern tropical South Pacific (Jacquot et al., 2013). Elevated \([Cu_1]\) in conditions where \(H_2S\) exists, are surprising, because \([Cu_1]\) in these regimes should be low as a result of the formation of insoluble CuS precipitates (Guinoiseau et al., 2019; Roshan and Wu, 2015; Saito et al., 2003). However, sulphides have also been shown to act as ligands, enhancing rather than diminishing the solubility of trace metals during dysoxic conditions (Al-Farawati and van den Berg, 1999; Jacquot et al., 2013). Brüchert et al. (2006) observed high concentrations of \(H_2S\) in surface sediments and bottom waters of the Namibian coast and thus the elevated \([Cu_1]\) observed in the dysoxic waters of the BC and AnC regions may partly be driven by sulphide complexation. More studies are, however, needed to identify the role of sulphides on Cu speciation in the South-East Atlantic. A more detailed description on Cu₇ circulation in the working area, including sources and sinks, will be provided in Liu et al. (2021).

### 3.2. Cu speciation in the South-East Atlantic

Total Cu-binding ligand concentrations, \([L_1]\) (\([L_1] = [L_4] + [L_2]\)), ranged from 2.5–283.0 nM with \([L_1]\) ranging from 0.3–73.0 nM and \([L_2]\) from 0.7–281.2 (Fig. 3; Supplementary Table S5 and Fig. S2). Previous measurements of \([L]\) in open ocean areas in the Atlantic by Jacquot and Moffett (2015) and Heller and Croot (2015) using CLE-AdCSV and SA ranged from 0.8–73 nM, a range lower than observed here. This difference is likely due to one or a combination of (i) difference in sampling locations, (ii) lower analytical detection windows (Jacquot and Moffett, 2015: [SA] of 1, 2, and 2.5 μM; Heller and Croot, 2015: [SA] of 1 and 2 μM (Bruland et al., 2000), (iii) lower equilibration times after SA addition (Jacquot and Moffett, 2015: 30 min; Heller and Croot, 2015: 6–12 h), (iv) different fitting models and numbers of ligand classes included in the speciation model (Jacquot and Moffett, 2015: non-linear Gerringa fitting model using one-ligand model; Heller and Croot, 2015: non-linear Gerringa fitting model using one- and two-ligand models) (Pizeta et al., 2015), and/or (v) incomplete titration of natural ligands or a non-linear response of the sensitivity of the titration curve as a function of the \([Cu_1]\), which would result in lower \([L_1]\) estimates. The Scatchard plot within the ProMCC software (Omanovic et al., 2015) indicated the presence of a second ligand class for 12 out of the 64 fit-able stations in the working area (Supplementary Table S5). Four of these stations had \([L_2]\) estimates outside of the \(Cu_1\) titration range leading to large associated errors and thus a possible overestimation of \([L_1]\), which might also explain the different \([L_1]\) in the Atlantic by different studies. Sensitivities of the \(L_2\)-titration curves within the titration range were, however, similar to the sensitivities of those outside the titration range, and estimated \([L_2]\) were thus deemed reliable. Since the fitted sensitivity is based on the entire titration it is possible to fit titration curves that may not have been out-titrated. Further, all reported Cu speciation parameters fall within D₉₅ and the here reported results were thus considered credible.

The binding strengths (log\(K_{CuL, Cu_{2+}}\)) of Cu-binding ligands ranged from 10.7 to 14.6 with log\(K_{CuL, Cu_{2+}}\) values averaging 12.7 ± 0.7 and 11.4 ± 0.5 (mean ± s.d.), respectively (Fig. 3; Supplementary Table S3). These log\(K_{CuL, Cu_{2+}}\) values are lower than previously reported values measured in the Atlantic (Jacquot and Moffett, 2015; Heller and Croot, 2015). However, these studies used shorter equilibration times during Cu titrations which can result in lower \([L_1]\) and accordingly higher estimates for log\(K_{CuL, Cu_{2+}}\) (Heller and Croot, 2015). As indicated by the wide range of log\(K_{CuL, Cu_{2+}}\) and log\(K_{CuL, Cu_{2+}}\) in the current study, the prevalent Cu-binding ligands seem to be structurally diverse, implying a complex assemblage of Cu-binding ligands of various sources in the South-East Atlantic (log\(K_{CuL, Cu_{2+}}\): 3.78–5.78; Supplementary Table S3). Irrespective of the subregion, \([L_1]\) were in excess of \([Cu_1]\), sometimes exceeding \([Cu_1]\) by 3 orders of magnitude, with >99.7% of Cu bound to organic ligands (Supplementary Table S3). This >99% organic
complexation of Cu(I) is a common feature of oceanic Cu speciation and generally maintains [Cu(II)] in the ‘Goldilocks’ range (Buck et al., 2007; Coale and Bruland, 1990; Donat and Van den Berg, 1992; Jacquot and Moffett, 2015; Moffett et al., 1990; Tang et al., 2001; van den Berg et al., 1987). The here estimated [Cu(II)] were extremely low, ranging from 1.7 to 156.0 nM, but compare well to [Cu(I)] that have been found elsewhere in the Atlantic (Jacquot and Moffett, 2015). This output provides further evidence that, despite various local Cu(I) sources, [Cu(II)] are generally strongly complexed throughout the Atlantic by prevalent Cu-binding ligands, which restrict [Cu(II)] to a narrow concentration range in most of the ocean (Jacquot and Moffett, 2015).

No statistically significant correlations (\(R^2 \geq 0.70; p \leq 0.05\)) between Cu speciation parameters and physical and chemical parameters were found across the dataset as a whole, except for [Si] and [Cu(II)]. This positive correlation together with the positive correlation between [Si] and [Cu(I)], is in agreement with previous studies in the Atlantic (Heller and Croot, 2015) and is suggestive of opaline particles being a major source of Cu(I) and Cu(II) in the South-East Atlantic ([Si] and [Cu(II)]; \(R^2 = 0.78\) and p-value < 0.01; [Si] and [Cu(I)]; \(R^2 = 0.95\) and p-value < 0.01; Supplementary Table S6 and S7; Heller and Croot, 2015). These correlations, however, only hold for stations influenced by the SAC and disappear for stations influenced by the BC and AnC. This output suggests a more complex combination of factors acting on [Cu(II)] in these two dynamic regions such as mixing, upwelling, scavenging, river input, and especially biological uptake by diatoms, which are the dominant phytoplankton species in the coastal Atlantic (Fig. 5). As with other physical parameters, oxygen showed no significant correlation with [L1] or [L2] across the whole dataset, but waters with low oxygen concentrations (< 60 \(\mu\)M kg\(^{-1}\) oxygen) did display lower median values compared to higher oxygen waters, pointing toward an L1 degradation in dysic waters (low oxygen: 4.0 nM L1 and 11.5 nM L2; high oxygen: 14.0 nM L1 and 18.1 nM L2). Median concentrations of the L2-type ligands were much higher in low oxygen waters in relation to high oxygen waters, supporting the former statement of L1 degradation and indicating that dysic environments are a source of weak Cu-binding ligands in the South-East Atlantic (oxic: 65.5 nM; dysic: 162.1 nM). The Median log\(K_{\text{CuL}}\) and log\(K_{\text{CuL}}\) values were higher in dysic waters with values of 13.2 and 4.68 compared to values of 12.5 and 4.64 in oxic waters. These differences in the chemical properties of the Cu-binding ligand pool suggest that ligands likely derive from different sources and/or experience different fates in different redox regimes. The existence of ligands in low oxygen waters, even though at lower concentrations than in oxic waters, can also explain the above mentioned high [Cu(I)] in the dysic regimes, despite the fact that these environments usually act as Cu sinks owing to CuS precipitation (Roshan and Wu, 2015; Saito et al., 2003). There was little difference in median [Cu(II)] within the different redox regimes despite the drastic difference in log\(K_{\text{CuL}}\), log\(K_{\text{CuL}}\), log\(K_{\text{CuL}}\), and log\(K_{\text{CuL}}\) (oxic: 20.8 M Cu(II); dysic: 21.5 M Cu(II)). L2-type Cu-binding ligands thus appear to keep the [Cu(II)] under low oxygen conditions at similar levels to that of oxic conditions, despite higher [Cu(I)]. This data raises the question about the importance of L2-type Cu-binding ligands to act as a buffer to maintain the “Goldilocks-principle” in the ocean and which processes are at play to keep [Cu(II)] to a low narrow range throughout most of the water column of the South-East Atlantic. The general lack of correlation between Cu speciation parameters and physical and chemical values across the dataset suggests that Cu speciation is likely influenced by an interaction of several local processes, sources, and sinks.

Statistically significant correlations between the Cu speciation parameters and physical and chemical parameters were only found for some parameters across the three subregions (Supplementary Table S7). In the Angola Basin, [NH₄] showed a positive correlation with [L1], suggesting that both weak and strong Cu-binding ligands derive from terrestrial sources, akin to NH₄ (\(R^2 = 0.74\) and p-value < 0.01; Supplementary Table S7; Andzi-Barhe and Bouak, 2013). The oligotrophic waters of the offshore Cape Basin illustrated a negative correlation between [Cu(II)] and temperature as well as [L1] and [DOC] and [L1] and [DOC] (\(R^2 = -0.73\) and p-value < 0.01, \(R^2 = -0.86\) and p-value < 0.01, and \(R^2 = 0.86\) and p-value < 0.01, respectively; Supplementary Table S7). The two latter trends indicate that DOC quality rather than quantity is an important factor for the Cu-binding ligand pool at the stations influenced by the SAC. The importance of DOC quality over quantity for Cu speciation is also highlighted in the literature (Cooper et al., 2014; Deryutten et al., 2015; Zitoun et al., 2019). In the coastal Cape Basin oxygen concentrations had a negative correlation with [L1] (\(R^2 = 0.77\) and p-value < 0.01). This trend aligns with a study by Thompson et al. (2014) in the Tasman Sea, suggesting a degradation of strong Cu-binding ligands in low oxygen environments, perhaps through bacterial utilization. More detail about Cu-binding ligand sources in the South-East Atlantic can be found below.

### Table 2

| Parameter | ANC | BC | SAC | Nearshore | Shelf | Open Ocean |
|-----------|-----|----|-----|-----------|-------|------------|
| Cu(I) (nM) | 0.70 | 0.93 | 0.66 | 0.93 | 0.72 | 0.66 |
| DOC (mM) | 68.3 | 49.8 | 42.6 | 55.1 | 54.7 | 42.6 |
| L1 (nM) | 14.9 | 25.3 | 19.6 | 14.7 | 21.2 | 19.6 |
| log\(K_{\text{CuL}}\), Cu(II) | 12.6 | 12.5 | 12.2 | 12.6 | 12.4 | 12.2 |
| log\(K_{\text{CuL}}\), Cu(I) | 4.73 | 4.75 | 4.38 | 4.75 | 4.74 | 4.38 |
| L2 (nM) | 93.1 | 43.0 | 144.1 | 64.6 | – | – |
| log\(K_{\text{CuL}}\), Cu(II) | 11.1 | 11.9 | 11.3 | 6.3 | 11.1 | – |
| log\(K_{\text{CuL}}\), Cu(I) | 4.14 | 4.50 | 4.31 | 4.15 | – | – |
| L1 (nM) | 23.1 | 37.7 | 19.6 | 15.6 | 33.8 | 19.6 |
| Cu(II) (nM) | 15.2 | 15.4 | 26.2 | 14.3 | 15.8 | 26.2 |
| Si (mg m\(^{-3}\)) | 0.094 | 0.310 | 0.073 | 0.310 | 0.143 | 0.073 |
| Chl-a (\(\mu\)g m\(^{-3}\)) | 0.53 | 0.58 | – | 0.66 | 0.48 | – |
| Synechococcus (\% total chl-a) | 0.04 | 0.02 | 0.06 | 0.02 | 0.06 | 0.03 |
| Prochlorococcus (\% total chl-a) | 0.06 | 0.03 | 0.13 | 0.02 | 0.13 | 0.06 |
| Dinoflagellates (\% total chl-a) | 0.03 | 0.06 | 0.07 | 0.05 | 0.07 | 0.05 |
| Haptophytes (\% total chl-a) | 0.28 | 0.48 | 0.56 | 0.29 | 0.56 | 0.30 |
| Pelagophytes (\% total chl-a) | 0.04 | 0.05 | 0.11 | 0.04 | 0.13 | 0.06 |
| Chlorophytes (\% total chl-a) | 0.02 | 0.01 | – | 0.02 | 0.01 | – |
| Cryptophytes (\% total chl-a) | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Prasinophytes (\% total chl-a) | 0.08 | 0.05 | 0.10 | 0.06 | 0.10 | 0.08 |

Results were grouped by oceanographic region (ANC, BC, SAC) and distance to the shore (nearshore, shelf, and open ocean) (Fig. 1; Table 1). Individual sample results are provided in the accompanying supplement (Tables SS and S8).
m) were higher in the Cape Basin influenced by the BC compared to those in the upper water column influenced by the SAC and the AnC (Table 2; Fig. 3). This trend indicates that the nutrient-rich Benguela upwelling system may play a role as a Cu-binding ligand source. Further, \([L_1]\) and \([L_2]\) were generally lower at nearshore stations relative to shelf and open ocean stations, suggesting a dominance of autochthonous sources of Cu-binding ligands in the surface ocean of the South-East Atlantic. This spatial trend contrasts with Cu speciation studies of Boiteau et al. (2016) and Ruacho et al. (2020) in the eastern and tropical Pacific, respectively, but accords with the study of Jacquot et al. (2013) in the eastern tropical South Pacific, highlighting that the spatial distribution of Cu-binding ligands throughout the ocean is rather complex. A second Cu-binding ligand class could only be resolved in the upper water column (<100 m) of five stations influenced by the BC and AnC (station 13, 15, 18, 20, and 51; Supplementary Table S5 and Fig. S2). In particular, \(L_2\)-type ligands were only found at seven depths at nearshore stations close to the Congo River outflow, at one station within the Benguela Upwelling System, and at two shelf stations along the 3°S transect. Thus, the presence of \(L_2\)-type ligands is rather localised, suggesting a local source and/or a biogeochemical process that is driving \(L_2\)-characteristics in the South-East Atlantic. However, the presence of weaker ligands might have been difficult to detect analytically due to the challenge of resolving ligands with similar log\(K_{\text{CuL, Cu2-}}\) cond values within a single analytical detection window (Sander et al., 2011; Wells et al., 2013). Further, median \([L_2]\) were much higher at nearshore stations in relation to shelf stations, indicating that \(L_2\)-type ligands are important in controlling \([Cu^{2+}]\) in coastal areas of the South-East Atlantic, namely areas that commonly show higher and more variable Cu\(\text{II}\) inputs from terrestrial sources (Table 2; Bundy et al., 2013).

The median values of log\(K_{\text{CuL, Cu2-}}\) cond and log\(K_{\text{CuL, Cu2-}}\) were lowest at stations influenced by the SAC and highest at nearshore stations followed by shelf and open ocean stations (Table 2; Fig. 3; Supplementary Table S3). log\(K_{\text{CuL, Cu2-}}\) indicates the capacity of natural ligands to bind Cu (Gledhill and Gerrings, 2017). Despite higher \([L_1]\) and \([L_2]\) at offshore stations in relation to nearshore stations, lower log\(K_{\text{CuL, Cu2-}}\) cond and log\(K_{\text{CuL, Cu2-}}\), with associated higher \([Cu^{2+}]\) at offshore stations may reflect a photodegradation of Cu-binding ligands into ligands with much weaker log\(K_{\text{CuL, Cu2-}}\) cond, log\(K_{\text{CuL, Cu2-}}\) cond in oligotrophic waters of the South-East Atlantic, which aligns with findings of Mellett and Buck (2020) from the eastern Gulf of Mexico. Taken together, Cu-binding ligand concentrations were highest in the BC and over the shelf but with median log\(K_{\text{CuL, Cu2-}}\) cond and log\(K_{\text{CuL, Cu2-}}\) cond values, while near-shore stations had stronger ligands with stronger binding capacities but with lower Cu-binding ligand concentrations. These differences in \([L_1]\), \([L_2]\), log\(K_{\text{CuL, Cu2-}}\) cond, and log\(K_{\text{CuL, Cu2-}}\) cond, in the upper water column of the three subregions and with distance to the coast likely points towards various sources of Cu-binding ligands and various driving factors of Cu speciation in the South-East Atlantic. That each subregion may be influenced by different Cu-binding ligands with different complexion capacities is also elucidated by Fig. 4. However, Cu-binding ligands closer to the coast, at stations influenced by the AnC and BC, seemed to be more similar than those at offshore stations influenced by the SAC.

\([Cu^{2+}]\) in the upper water column ranged from 6.0 to 55.4 fM, comparing well with previously reported \([Cu^{2+}]\) in the Atlantic (Jacquot and Moffett, 2015), with lowest concentrations at stations influenced by the AnC and BC, as well as at nearshore- and shelf stations (Table 2; Fig. 3). The here reported \([Cu^{2+}]\) estimates are well below the level found to be biolimiting for many marine microbial cultures (Amin et al., 2013; Maldonado et al., 2006; Peers et al., 2005; Sunda and Huntsman, 1995) and thus may lead to ecological impacts on residing microorganisms. It is, however, currently unknown if the \([Cu^{2+}]\)-biolimiting threshold determined in culture studies transfers to natural seawaters, with typically much more dilute microbial biomass (Lopez et al., 2019). Thus, it is likely that the biological demands of natural phytoplankton assemblages might be sustained by low \([Cu^{2+}]\). Further, some phytoplankton species are known to produce specific ligands to acquire trace metals even during deficient conditions to fulfill their growth requirements (Zhang et al., 2019). For instance, siderophores and exopolymERIC substances (EPS) that specifically bind to Fe are produced under Fe-limiting conditions by various phytoplankton (Maldonado et al., 2005; Hassler et al., 2015). A similar response by microorganisms may also be possible during Cu\(^{2+}\)-limiting conditions in the South-East Atlantic. In addition, some microorganisms can access organically complexed Cu (Lorenzo et al., 2005; Quigg et al., 2006; Semeniuk et al., 2015) and thus biolimitation might not be solely dependent on \([Cu^{2+}]\). Further research is needed to improve current understanding of the “Goldilocks’ principle” and to assess how much of the organically complexed Cu is indeed biologically available in the natural marine environment.

### 3.4. Cu speciation in the deep-water column of the South-East Atlantic

Similar to previous studies (Jacquot and Moffett, 2015; Heller and Croot, 2015), the vertical distribution of \(L_2\) and \(L_1\) showed generally high concentrations in the upper water column and decreased gradually with depth with some profiles showing subsurface maxima (Fig. 3, Supplementary Table S5 and Fig. S2). Strong conditional stability constants remained relatively homogenous with depth often showing slightly higher log\(K_{\text{CuL, Cu2-}}\) cond values in the upper water column compared to deep waters. Strong binding capacities (log\(K_{\text{CuL, Cu2-}}\) cond) generally decreased with depth. Weaker ligands were only detected in subsurface waters of Station 15, 18, and 20, and in the subsurface and close to the seafloor of Stations 4, 13, 15, 18, 19, 20, and 51 (Supplementary Table S5 and Fig. S2). The spatial trend of \(L_2\)-type ligands together with log\(K_{\text{CuL, Cu2-}}\) cond and log\(K_{\text{CuL, Cu2-}}\) cond suggests a benthic input of weaker Cu-binding ligands or a \(L_1\)-type ligand degradation as the most likely source of \(L_2\) to the deep South-East Atlantic. As expected, \([Cu^{2+}]\) generally increased with depth as \([L_1]\) and \([L_2]\) decreased and \([Cu^{2+}]\) increased, due to remineralization (Heller and Croot, 2015; Jacquot and Moffett, 2015; Thompson et al., 2014; Whitby et al., 2018). However, \([Cu^{2+}]\) also showed some local minima in the mid water column and close to the seafloor for most stations (Supplementary Table S3). These subsurface minima generally correlated with observed \([L]\) maxima, suggesting that \([Cu^{2+}]\) is possibly buffered in the deep water column of the South-East Atlantic by a combined effect of scavenging and organic complexion (Jacquot and Moffett, 2015). \([Cu^{2+}]\) never exceeded 1.5 pM, with 0.01–0.05 pM at 3000 m in oligotrophic waters, indicating that \([Cu^{2+}]\) is strongly buffered in deep oceanic waters, which is also

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**Fig. 4.** Calculated \(-\log ([Cu^{2+}])\) versus calculated \(-\log (K_{\text{CuL, Cu2-}})\) of the whole water column in the South-East Atlantic. Data is shown for each of the three subregions.
highlighted in other studies that found $[\text{Cu}^{2+}]$ of 0.08–2.7 pM at ~3000 m in the North Pacific and Mid-Atlantic (Buck et al., 2012; Jacquot and Moffett, 2015).

3.5. Phytoplankton biomass and community structure in the South-East Atlantic

The chl-$a$ concentrations at the sites sampled for Cu-binding ligands ranged from 0.005 to 2.418 mg m$^{-3}$ (Figs. 5, 6; Supplementary Tables S8). These ranges compare well to previously published values of the Congo River Basin, the southern Benguela, and the oligotrophic waters of the South Atlantic (Weeks et al., 2006; Hopkins et al., 2013; Browning et al., 2014) The median [chl-$a$] in the upper water column (<100 m) of the coastal Cape Basin were much higher relative to the levels in the upper water column of the Angola Basin and the offshore Cape Basin (Table 2; Fig. 5), thereby following the macronutrient distribution.

As expected, akin to the macronutrient distribution, median [chl-$a$] in the upper water column decreased with distance to the shore. Diagnostic pigment analyses indicated that diatoms and haptophytes contributed most of the chl-$a$ in the upper water column of regions influenced by the AnC and BC, whereas haptophytes, Prochlorococcus, and pelagophytes contributed most to the upper water column influenced by the SAC (Table 2). Generally, diatoms and haptophytes dominated the microbial community at nearshore and shelf stations, while open ocean stations were dominated by haptophytes (Table 2). Taken together, akin to the macronutrients, $\text{Cu}_T$, and Cu speciation parameters, both the phytoplankton biomass and composition showed a strong spatial variability, suggesting that the phylogeography of different phytoplankton groups may indeed influence the spatial features of the Cu-binding ligand pool in the working area.

An opposing trend between median values of $[\text{Cu}^{2+}]$ and [chl-$a$] was observed in the upper water column, with the highest $[\text{Cu}^{2+}]$ values found in oligotrophic waters, where there were the lowest [chl-$a$] (Table 2, Figs. 5, 6). At stations influenced by the SAC, $[\text{Cu}^{2+}]$ were within the Goldilocks’ threshold and thus a limitation of other micronutrients, especially Fe that is commonly limited in oligotrophic waters, could be responsible for the prevalent low phytoplankton productivity (Annett et al., 2008; Brand et al., 1986; Irving and Williams, 1953; Maldonado et al., 2006; Sunda et al., 1981; Browning et al., 2017). This hypothesis is supported by the work of Browning et al. (2017), which showed a co-limitation of Fe and N in bioassay experiments with oligotrophic waters sampled adjacent to the South Atlantic Gyre. That said, this does not imply that $[\text{Cu}^{2+}]$ is independent from the chl-$a$ biomass in the working area, but rather points out that other nutrients likely superimpose any effect of this parameter on phytoplankton productivity. Lowest median $[\text{Cu}^{2+}]$ were found at nearshore stations which also showed highest median phytoplankton biomass (Fig. 6), thereby

Fig. 5. Phytoplankton biomass (a) and community composition (b) in the surface waters of the working area (3 m). The relative contribution of the different phytoplankton classes to total chl-$a$ was determined by CHEMTAX.

Fig. 6. Surface cross section of Cu speciation parameters, chlorophyll-a concentration (chl-$a$), and phytoplankton community composition along the GEOTRACES section GA08.
being suggestive of elevated Cu\(^{2+}\) demand of prevalent phytoplankton communities drawing down [Cu\(^{2+}\)] to nearly depleted levels below the Goldlocks’ threshold (Litchman et al., 2006; Semeniuk et al., 2016). This conclusion agrees well with the fact that rapidly growing diatoms with very fast Cu uptake rates (Guo et al., 2012) dominate the phytoplankton community in the upper water column of nearshore stations, contributing up to 98% to the total chl-a. Overall, the results indicate that [Cu\(^{2+}\)] may at least play some role in driving microorganism biomass in the South-East Atlantic, even though no significant correlation was evident between [Cu\(^{2+}\)] and [chl-a] (R\(^2\) = -0.01; p-value = 0.97; Supplementary Table S9). This lack of correlation between [chl-a] and [Cu\(^{2+}\)] suggests either that Cu\(^{2+}\) is not the only bioavailable Cu form in the South-East Atlantic and/or that different phytoplankton species have different Cu\(^{2+}\) uptake rates which obscures any relationship. To develop a fuller picture of the biological importance of Cu\(^{2+}\) in the South-East Atlantic, bioassays targeting the understanding of Cu bioavailability, and co-limitation and competition with other macronutrients and micronutrients are needed.

The spatial distribution of dinoflagellates, haptophytes, and pелагофиты, was in accordance with the distribution of median [Cu\(^{2+}\)] in the upper water column with the relative contribution of these phytoplankton species to chl-a being higher at stations influenced by the SAC than at stations influenced by the BC and AnC (Figs 5, 6). This trend may be suggestive of these nano- and micro-plankton species requiring less Cu\(^{2+}\) (i.e. slower Cu uptake rates) than other phytoplankton groups, such as diatoms, that dominate waters with lower [Cu\(^{2+}\)] in the upper water column of the coastal Cape Basin and the Angola Basin (Table 2). However, the higher [Cu\(^{2+}\)] in oligotrophic waters could also result from less Cu\(^{2+}\) drawdown by scavenging, or higher photodegradation of Cu-binding ligands rather than biological driving factors (Sadig, 1992; Laglera and van den Berg, 2006; Jacquot and Moffett, 2015; Hassler et al., 2020). Across the whole dataset, [Cu\(^{2+}\)] generally showed no significant correlation with the contribution of different phytoplankton groups to total chl-a levels (Supplementary Table S9). Cu is a bioessential element for marine microorganisms, and the lack of a correlation between [Cu\(^{2+}\)] and the contribution of different phytoplankton groups to total chl-a levels points towards a complex interaction between micronutrients, phytoplankton, and other physiochemical processes.

### 3.6. Origin of organic Cu-binding ligands in the South-East Atlantic

#### 3.6.1. Ligand sources in the upper water column

The spatial distribution of Cu-binding ligands in the upper water column (< 100 m) suggests an autochthonous, biogenic Cu-binding ligand source in the surface ocean of the South-East Atlantic even though the correlation with [chl-a] was statistically insignificant (Supplementary Table S9). This interpretation matches Cu speciation studies elsewhere (Boiteau et al., 2016; Heller and Croft, 2015; Jacquot and Moffett, 2015; Ruacho et al., 2020; Thompson et al., 2014). [L\(_1\)] generally increased with distance from the coast in the upper water column with an opposite order of logK\(_{CuL, Cu22}\)\(_{cond}\) values (Table 2), suggesting that a terrestrial, allochthonous input is likely not the dominant source of strong Cu-binding ligands to the surface waters of the working area. This conclusion is contrary to the one made for Ca\(_7\) which showed a clear continental origin. The spatial distribution of [L\(_1\)], with [L\(_1\)] generally being lower at nearshore stations relative to shelf and open ocean stations, contrasts with DOC and chl-a levels, but does coincide with an increase in the contribution of haptophytes, phytoplankton, and prasinophytes to the total chl-a concentration. While this data does not indicate that L\(_1\)-type ligands were necessarily autochthonously produced by residing phytoplankton in the upper water column, it provides an intriguing possibility. Studies with phytoplankton showed that Fe-binding ligands can be produced under Fe-limiting conditions to facilitate Fe uptake (Maldonado et al., 2005; Hassler et al., 2015) and thus similar processes cannot be ruled out to exist for Cu\(^{2+}\). Thus, the greater production of Cu-binding ligands by oceanic phytoplankton during supposedly Cu\(^{2+}\)-limiting conditions in oligotrophic waters may explain the higher [L\(_1\)] in offshore compared to coastal waters (Guo et al., 2012; Semeniuk et al., 2016), especially since oceanic phytoplankton are known to have higher metabolic Cu requirements compared to coastal strains (Annett et al., 2008; Guo et al., 2012; Maldonado et al., 2006; Peers et al., 2005; Semeniuk et al., 2016). Further, microorganisms including cyanobacteria, dinoflagellates, diatoms, and coccolithophores are known to exude ligands during high Cu\(^{2+}\) stress conditions with logK\(_{CuL, Cu22}\)\(_{cond}\) in the order of 10 to 13.3 (Moffett and Brand, 1996; Croft et al., 2000; Leal et al., 1999; Pistocchi et al., 2000; Vasconcelos et al., 2002; Vraspir and Butler, 2009; Thompson et al., 2014). These organisms are also present in the South-East Atlantic and estimated logK\(_{CuL, Cu22}\)\(_{cond}\) were similar to those estimated for Cu\(^{2+}\)-stressed phytoplankton cultures. Thus, it is conceivable, albeit not tested, that, akin to siderophores, strong Cu-binding ligands can be actively produced by affected microorganisms during limiting conditions to facilitate the uptake of the bioessential Cu\(^{2+}\).

Cu\(^{2+}\)-limitation can also lead to a passive ligand release pathway via, for example, cell lysis of dead phytoplankton and/or virus-mediated lysis of unfit phytoplankton cells, two ligand sources which have been increasingly recognized as important components of the marine metal-binding ligand pool, especially for Fe (Bonnain et al., 2016; Poovrin et al., 2011; Sato et al., 2007). The oligotrophic offshore stations illustrated the lowest median logK\(_{CuL, Cu22}\)\(_{cond}\) and since low logK\(_{CuL, Cu22}\)\(_{cond}\) are commonly associated with passive production pathways (Poovrin et al., 2011; Sato et al., 2007; Whitty et al., 2018) it can be suggested that some of the Cu-binding ligands detected in the upper water column of the South-East Atlantic do originate from passive ligand release processes (Table 2).

A fraction of the organic Cu-binding ligand pool in the upper water column might also originate from biogenic pathways not directly related to phytoplankton, bacteria, or viruses. For instance, Cu-binding ligands have been recognized to be linked to humic substances, zooplankton grazing, and organic matter breakdown and degradation (Gledhill and Buck, 2012; Whitty et al., 2017; Whitty et al., 2018). While the correlations between [DOC] and [L] were insignificant across the dataset as a whole, DOC and Cu-binding ligands did show a decrease in concentrations with distance to the shore along the Congo River transect and the 29°S transect (Fig. 7). This trend suggests that the Congo River and the Benguela upwelling system act as sources of DOC and Cu-binding ligands to the South-East Atlantic. Prevalent ligands might be linked to DOC, since bulk DOC has been shown to complex metals (Abualhajia et al., 2015; Nogueira et al., 2017), which may explain the simultaneous decrease of [DOC] and [L] along the two transects, while [Cu\(^{2+}\)] increases. Thus, the data suggests that some of the Cu-binding ligands found in the working area have a terrestrial source in the upper water column, which is in line with previous evidence found in other ocean basins (Bundy et al., 2013). Generally, the metal complexation capacities of DOC are only related to a small fraction of the bulk DOC pool, a fraction commonly related to humic-like substances and exopolysaccharides (Buck et al., 2017). Hence the lack of a correlation between [L] and DOC is not surprising. Taken together, the Cu-binding ligand pool in the upper water column of the South-East Atlantic seems to be influenced by various processes of active, passive, and indirect Cu-binding ligand production. These processes need to be disentangled in the future to enhance current understanding of the cycling of Cu\(_7\) and other metals, in the current and future ocean.

Correlation analysis between Cu-binding ligand concentrations and [chl-a] or phytoplankton group contributions, both across the dataset as a whole and for the defined subregions, showed no significant correlation (Supplementary Table S9). Nevertheless, a visual inspection of Fig. 6 suggests that pелагофиты and haptophytes contribute to the L\(_1\)-pool in oligotrophic offshore waters, while diatoms contribute to the L\(_2\)-pool at station 43 and 44. The strong Cu-binding ligands in the Benguela Upwelling System seem to accord well with the presence of
Synechococcus and prasinophytes, whereas diatoms and haptophytes seem to be the driving forces for the presence of \(L_1\) in the Angola Basin. This spatial correlation suggests that marine microbes produce a least some of the prevalent Cu-binding ligands, despite the lack of correlation between Cu-speciation parameters and residing phytoplankton. It is plausible that different phytoplankton species produce (actively or passively) varying amounts of Cu-binding ligands with different chemical properties (different \(\log K_{\text{CuL}}\), \(\log K_{\text{CuL}^2}\), multidentate binding capacity, non-specific metal binding) (Coale and Bruland, 1988; Heller and Croot, 2015; Mann et al., 2002). Consequently, \([L]\) would therefore not correlate with [Cu\(^{2+}\)], [chl-\(a\)] concentration, and/or specific phytoplankton groups (Coale and Bruland, 1990; Heller and Croot, 2015). Further, previous studies (Browning et al., 2017; Louropoulou et al., 2020) observed Fe and N-Fe co-limitation of phytoplankton in the South-East Atlantic, thereby indicating that the prevalent ligands might also derive from active siderophore production owing to Fe-limitation (Maldonado et al., 2005; Hassler et al., 2015). Biologically produced siderophores bind Fe with remarkable affinity, but siderophores with softer donor atoms can also interact with Cu\(^{2+}\) (Johnstone and Nolan, 2015). Thus, the ligands found in the working area may have binding sites with non-specific metal affinities and hence can bind to multiple metals, which may explain the lack of correlation between Cu-speciation parameters and phytoplankton. These assumptions, however, warrant further in-situ studies focusing on Cu speciation in Cu\(^{2+}\) limited phytoplankton cultures and cultures co-limited by various micronutrients.

Overall, the complexity of processes, sources, and sinks in the upper water column of the South-East Atlantic makes it challenging to adequately pinpoint the source or sources of Cu-binding ligands. This finding is supported by the work of Boiteau et al. (2016) who showed that organic Cu-binding ligands in surface waters near the coast of the eastern South Pacific were comprised of a diverse and complex mixture of chromatographically unresolved polar compounds. Cu-binding ligands in the upper water column of the South-East Atlantic likely derive from a complex interaction of various autochthonous and allochthonous processes and pathways. As for autochthonous processes, it currently remains unresolved, whether the prevalent Cu-binding ligands originate from biological active production to cope with deficient [Cu\(^{2+}\)], from passive release processes such as cell lysis, or whether both sources play a role in the South-East Atlantic. What can be said though is, that the phylogeography of prevalent phytoplankton species does influence the spatial features of Cu-binding ligands in the working area.

### 3.6.2. Ligand sources in the deep ocean

As shown in Fig. 3 and Supplementary Fig. S2, the vertical distribution of \(L_T\) decreased gradually with depth. This decrease suggests a degradation of ligands, especially \(L_1\)-type ligands, in deep waters, thereby matching earlier observations in the Atlantic (Jacquot and Moffett, 2015; Heller and Croot, 2015). The UCDW and the NADW showed sharp increases in \([L]\), possibly pointing towards different biogeochemical processes (degradation and bioremineralization), dynamics, and/or sources of ligands in different water masses with different age structures (Fig. 3; Supplementary Fig. S2; Moffett and Dupont, 2007; Ruacho et al., 2020). Ruacho et al. (2020) previously observed higher \([L_1]\) and detected \(L_2\)-type ligands in older water masses and suggested this was due to the degradation of stronger ligands into slightly weaker ligands along the ocean circulation as water masses age. We also found \(L_2\)-type ligands in deeper waters (Supplementary Table S5), potentially a result of the same mechanism. It thus appears that the age-structure of water masses might indeed have an influence on \([L_T]\) and Cu speciation in the deep ocean. However, further work is required to establish the viability of this assumption.

\([L_T]\) also showed some local maxima in deep waters close to the shelf margin and the sea floor (Fig. 3; Supplementary Table S5 and Fig. S2). These maxima were potentially related to remineralized sinking organic matter, lateral advected inputs from the coast and shelf, and/or resuspended benthic sediments including refractory DOC (Coale and Bruland, 1990; Jacquot and Moffett, 2015; Semeniuk et al., 2016; Hassler et al., 2020). Especially in the BC region Cu-binding ligands are likely derived from resuspended sediments since this region is influenced by a very strong coastal upwelling system (Hutchings et al., 2009; Shannon, 2006). This assumption is supported by the estimated \(\log K_{\text{CuL}}\) values for these waters, which were in line with those commonly reported for marine sediment inputs and pore waters (\(\log K_{\text{CuL}}\) values: 11 to >14; Chapman et al., 2009; Jacquot and Moffett, 2015; Ruacho et al., 2020; Skrabal et al., 1997; Thompson et al., 2014). However, unambiguously decoupling between each source is difficult.

Experiments of Boyd et al. (2010) showed a release of \(L_2\)-type Fe-binding ligands during particulate organic carbon (POC) remineralization by heterotrophic bacteria. It is thus conceivable that bacterial remineralization of POC may also play a key role for \(L_2\)-type Cu-binding ligands in the South-East Atlantic. However, this trend has so far not been studied systematically for Cu and POC data is not available for the GO8 section. Nevertheless, when \([L_2]\) are compared to [DOC], high \([L_T]\) were associated with high [DOC] near the ocean bottom and along the shelf ridge, thereby supporting the assumption that biogenic particle decomposition, as proposed for Fe-binding organic ligands (Fukushima et al., 1996; Ghantous et al., 1998; Ibisanmi et al., 2011), can act as a source of Cu-binding ligands to the subsurface of the South-East Atlantic. The correlation between DOC and \(L_T\) was, however, insignificant.
the marine environment is key to understanding the biogeochemical studies might help to disentangle the origin of Cu-binding ligands to ligand sources in the ocean. Some of the major issues and uncertainties restricting confident evaluation of Cu-binding ligands in the natural marine environment are not well-defined and statements on the ligand provenance rely on indirect assumptions based on correlation analysis of Cu speciation parameters with other data collected in the water column. While this analysis remains a useful starting point for evaluating Cu-binding ligand sources in natural marine environments, this method suffers from several challenges and uncertainties restricting confident evaluation of Cu-binding ligand sources in the ocean. Some of the major issues and uncertainties of the here used correlation approach are listed below:

- **Small sample size** - no significant correlation was found between most Cu speciation parameters and chl-a or prevalent phytoplankton groups. This lack of correlation was potentially due to the restricted number of samples that were taken at the same depth for comparison purposes, which added a level of uncertainty to the data interpretation.

- **Data separation** - to facilitate data interpretation, the data were separated into three subregions with each region having their own unique oceanographic setting. However, the South-East Atlantic is dynamic and complex and therefore correlations are likely sensitive to the definition of the oceanographic regions.

- **Dynamic and complex environment** - caution must be applied in using one data snapshot from one point in time to draw conclusions about the sources, sinks, and processes of Cu speciation parameters in dynamic environments such as the South-East Atlantic. Conclusions can be consolidated if fluxes, rates, residence times, and interactions of various processes, i.e. Cu input, biological uptake, remineralization, scavenging, photochemical ligand degradation, and vertical mixing processes, are quantified and understood.

- **Complex interaction between ligands and phytoplankton** - one of the biggest remaining challenges in marine trace metal biogeochemistry is to understand the impact of phytoplankton communities on trace metal bioavailability via ligand production and how in turn this affects marine primary production. Rapid changes in phytoplankton activity and species composition owing to changes in abiotic (temperature, light, macronutrient concentrations, trace metal limitation or toxicity), or biotic factors (grazing) can rapidly alter phytoplankton communities and potentially also their ligand production. Furthermore, it has been shown that different species have different metal affinities, with some species being able to access organically bound metals, while others are not (Kong and Price, 2020; Lorenzo et al., 2005; Semeniuk et al., 2015). This complexity impedes the correlation analysis between Cu speciation parameters and any other parameters analysed here.

- **Different chemical characteristics** - the distribution and concentration of organic ligands generally reflect the distribution and concentration of different sources and different source species, and it is quite possible that different phytoplankton species produce varying amounts of Cu-binding ligands with different chemical properties. The biogenic production of ligands potentially varies owing to different release processes, Cu requirements, uptake rates, and sensitivities which are regulated by multiple and potentially interacting abiotic and biotic factors (Coale and Bruland, 1990; Heller and Croot, 2015; Pistocchi et al., 2000). [L] would, therefore, not show a trend with DOC, [chl-a], and/or specific phytoplankton groups (Coale and Bruland, 1990; Heller and Croot, 2015). In addition, organic ligands can have various residence times, i.e. Fe-binding ligands are known to have a longevity ranging from a couple of hours to >1000 years (Hassler et al., 2017), which makes any correlation between ligands with dynamic phytoplankton communities, which change on timescales of days to seasons, challenging.
while Cu-binding ligands originated from both autochthonous and allochthonous production and release pathways. Ligand concentrations were generally high in the working area and thus Cu\(^{2+}\) uptake, or a passive production owing to, for example, cell lysis during limiting conditions. Despite the lack of a significant correlation between Cu speciation parameters with any of the other parameters analysed here, a visual inspection of the data suggests that diatoms and haptophytes in the Angola Basin, Synecchococcus, diatoms, and prasinophytes in the coastal areas of the Cape Basin, and pelagophytes in the oligotrophic waters of the Cape Basin, may contribute at least a small fraction to the Cu-binding ligand pool in the surface waters of the South-East Atlantic. Allochthonous Cu-binding ligand sources in the surface ocean can be attributed to refractory DOC and bulk DOC that was transported into the working area via the Congo River and the Benguela Upwelling System. L(T) was commonly found in the upper water column of stations in the Angola Basin and in the subsurface above the seafloor of shelf and open ocean stations. The presence of L(T)-type ligands in surface waters suggested a passive source such as photodegradation of L(T), decaying phytoplankton, cell lysis, and microbial organic matter breakdown in the surface ocean, while subsurface Cu-binding ligands likely reflect a ligand sources from remineralized sinking organic matter, sulphides and L(T) degradation in low oxygen waters, resuspended benthic inputs, or lateral advected inputs from the shelf margin.

The poor correlation between Cu speciation parameters with measured abiotic and biotic parameters suggest that there is no simple relationship between Cu speciation parameters and environmental factors in the working area. While the current work provides a first insight into the environmental fate of Cu in the Angola and northernmost Cape Basin and expands our knowledge of Cu speciation in the South-East Atlantic, we need a better understanding of Cu-binding ligands and their dynamics to progress Cu speciation research. We, therefore, suggest a greater focus of future studies on the characterization of the chemical structure of organic ligands, the identification of their sources, and the quantification of factors and processes controlling Cu speciation using appropriate complementary analytical techniques including voltammetry, isotope geochemistry, NMR, HPLC-MS, and FT-ICR-MS. As scientists learn more about the Cu-binding ligand concentration, distribution, kinetics, residence times, and the individual compounds that make up the bulk of the ligand pool, using a targeted and integrative multi-method approach, new insights into the provenance, diversity, variability, production/release pathways, and fate of marine Cu-binding organic ligands will be gained. This information will pave the way for modellers to develop more accurate biogeochemical models for Cu in marine systems and enhance the current understanding of the “Goldilocks’ principle”.

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**Declaration of competing interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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**Appendix A. Supplementary data**

Supplementary data to this article can be found online at [https://doi.org/10.1016/j.marchem.2021.104047](https://doi.org/10.1016/j.marchem.2021.104047).

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