Distribution of copper-complexing ligands in Canadian Arctic waters as determined by immobilized copper(II)-ion affinity chromatography

Richard L. Nixon, Sarah L. Jackson, Jay T. Cullen, Andrew R.S. Ross

1. Introduction

Dissolved organic ligands that form stable complexes with trace metals in natural waters play an important role in the biological utilization and geochemical cycling of these metals (Sunda, 1991; Donat and Bruland, 1995; Kraemer et al., 2015). In seawater, dissolved copper (dCu) exists almost exclusively as copper(II) complexes formed by organic ligands whose origin, identity, and function remain poorly defined (Vraspir and Butler, 2009). Previous studies have shown that copper(II)-complexing ligands are often concentrated near the chlorophyll maximum (Donat et al., 1986; Coale and Bruland, 1988; Moffett et al., 1990), implying that growing phytoplankton assemblages are a significant source of these ligands. Culturing experiments (McKnight and Morel, 1979, 1980; Moffett et al., 1990; Moffett and Brand, 1996; Leal et al., 1999; Gledhill et al., 1999; Croot et al., 2000; Gordon et al., 2000; Dupont et al., 2004; Wiramanaden, 2006; Wiramanaden et al., 2008; Buck et al., 2010; Semeniuk et al., 2015) support the hypothesis that certain marine algae produce ligands to regulate copper uptake.

Electrochemical methods have been used to estimate the concentrations and binding affinities of dissolved copper(II) ligands in the Atlantic (van den Berg, 1984; Huizenga and Kester, 1983; Buckley and van den Berg, 1986; Kramer, 1986; Hering et al., 1987; Sunda and Hanson, 1987; Moffett et al., 1990; Donat and van den Berg, 1992; Waska et al., 2015), Pacific (Donat et al., 1986; Coale and Bruland, 1990; Midorikawa and Tanoue, 1996; Buck and Bruland, 2005; Thompson et al., 2014), Indian (Donat and van den Berg, 1992) and Antarctic Ocean (Bundy et al., 2013) and in the sub-Arctic waters of the North Pacific and Bering Sea (Coale and Bruland, 1988; Moffett and Dupont, 2007; Whitby et al., 2018) as well as in coastal and estuarine waters (Hering et al., 1987; Apte et al., 1990; Donat et al., 1994; Gordon et al., 1996; Moffett et al., 1997; Skrabal et al., 2000; Laglera and van den Berg, 2003; Dryden et al., 2004; Whitby and van den Berg, 2015). We are not aware of any published studies of copper ligands in the high Arctic.

Copper ligands can be isolated from other components of marine dissolved organic matter (DOM) using immobilized copper(II)-ion affinity chromatography (IMAC) (Gordon, 1992; Donat et al., 1997). We have shown that IMAC can be used to determine the relative abundance

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of copper ligands in coastal and oceanic waters of the northeast Pacific based upon their UV absorbance (Nixon and Ross, 2016). In addition to measuring the distribution of copper ligands this relatively simple and robust method provides fractions suitable for ligand characterization by mass spectrometry (Ross et al., 2003; Vachet and Callaway, 2003; Nixon and Ross, 2016) and other analytical techniques (Midorikawa and Tanoue, 1996; Cottrell et al., 2014).

As part of the Canadian Arctic GEOTRACES program we used IMAC to isolate and quantify dissolved copper(II)-binding ligands in samples collected at different depths from 10 locations in the Canada Basin, Canadian Arctic Archipelago (CAA), and Baffin Bay. Results were used to create depth profiles for this operationally-defined fraction of copper ligands. Two types of profiles were observed; one with mid-depth maxima below 30 m, seen in the Canada Basin and western CAA, and one with near-surface maxima above 30 m, observed in the eastern CAA and Baffin Bay. The depth of highest ligand concentration was generally found to coincide with the chlorophyll maximum depth. Results constitute one of only a few studies of copper speciation in Arctic waters and the first comprehensive survey of copper ligands across the Canadian Arctic.

2. Materials and methods

2.1. Reagents

Reagents and solvents were analytical and high performance liquid chromatography (HPLC) grade, respectively, unless otherwise noted. Hydrochloric acid (HCl) was obtained from Anachemia (Vancouver, BC), ethylenediamine tetraacetic acid (EDTA) from Fisher (Pittsburgh, PA), and copper (II) sulfate (99.995% pure) and 8-hydroxyquinoline from Sigma-Aldrich (St. Louis, MO). Ultrapure deionized water (18 MΩ cm) was prepared using an EMD Super-Q system from Millipore (Billerica, MA). Artificial seawater was prepared by dissolving 32 g of Instant Ocean ( Blacksburg, VA) in one litre of ultrapure water and acidified by adding HCl to a concentration of 10 mM (pH 2.1).

2.2. Collection of seawater samples

Samples were collected from the CCGS Amundsen as part of GEOTRACES sections GN02 (10/07/2015–20/08/2015) and GN03 (4/09/2015–1/10/2015) covering an area from 56°N to 77°N and 53°W to 150°W in the Canadian Arctic. Sampling was carried out at 10 stations along a transect between the Canada Basin and Baffin Bay (Fig. 1, Table 1). Samples were collected at depths ranging from 10 m to a maximum of 240 m in the CAA and Canada Basin and 500 m in Baffin Bay. A trace-metal clean sampling system consisting of a powder-coated aluminium frame holding twelve 12-L Teflon-coated Go-Flo bottles and tethered by a 4000-m 4-member conducting Vectran cable encased in polyurethane (Cortland Cable Company, Cortland, NY) (Measures et al., 2008) was used to collect seawater samples. These were gravity-filtered through 0.2-μm Acropak filters (Pall Corporation, Port Washington, NY) on board the ship in a HEPA filtered environment. The filtrate was collected in 1-L high density polyethylene (HDPE) bottles (Nalgene®) and stored at ~20 °C for copper ligand measurements. Where possible, two or more replicate samples were collected for ligand analysis at each depth. Filtrate was also collected in 500-mL low density polyethylene (LDPE) bottles (Nalgene®) and acidified to pH 1.7 using SeaStar Baseline HCl (SeaStar Chemicals, Sidney, BC) for storage and dCu analysis. Sampling bottles were pre-cleaned according to GEOTRACES protocols (Cutler et al., 2010).

2.3. Analysis of seawater samples

IMAC was carried out as previously described (Nixon and Ross, 2016) using a flow rate of 1 mL/min and a manually operated dual-column system incorporating a single wavelength UV detector (LKB Bromma Uvicord S, Pharmacia Biotech, Uppsala, Sweden). Briefly, two 5-mL Hi-Trap Chelating Sepharose HP columns (part no. 17-0409-03, GE Healthcare, Mississauga, ON) were rinsed with 30 mL of deionized water and charged with 50 μmoles of Cu²⁺ ions by passing 5 mL of a 10 mM copper(II) sulfate solution through each column. The columns were rinsed with 5 mL of ultrapure water and equilibrated with 20 to 30 mL of acidified artificial seawater before passing 1 L of filtered seawater sample through each column. Retained compounds were then eluted with acidified artificial seawater for 20 to 30 min, during which the UV absorbance of the eluent was monitored at 254 nm. Absorbance was plotted digitally using custom-built software and peak areas determined using the Riemann method. The columns were regenerated with 15 mL of a 50 mM EDTA solution before rinsing with deionized water.

Chromatograms recorded during IMAC of Arctic seawater samples contained a peak (Fig. 2) corresponding to the elution of UV-absorbing DOM with an affinity for immobilized Cu²⁺ ions (Nixon and Ross, 2016). This peak represents an operationally-defined fraction of the dissolved copper(II)-binding ligands present in seawater (see Discussion). Peak area was converted to ligand concentration using a linear calibration (y = 44.16x + 88.17, r² = 0.998) generated by analyzing, in triplicate, seawater samples spiked with 0 to 100 nmol/L of 8-hydroxyquinoline, a model ligand used to develop and validate the IMAC method (Nixon and Ross, 2016).

Dissolved copper was measured by triple-quadrupole inductively coupled plasma-tandem mass spectrometry (ICP-MS/MS) following offline pre-concentration by solid-phase extraction, as previously described (Jackson et al., 2018).

Salinity was measured at each station using a sensor (Sea-Bird, Belleville, WA) attached to a conductivity/temperature/depth (CTD) rosette. In vivo chlorophyll-a fluorescence was measured in the Canada Basin and at station CAA-8 using a fluorometer (Seapoint, Brentwood, NH) attached to the CTD rosette, and with a separate fluorometer (Turner Designs, Sunnyvale, CA) at other stations. Fluorescence measurements were converted to chlorophyll-a concentrations using calibration factors based upon chlorophyll analysis of discrete water samples from selected stations (Michel Gosselin; personal communication). These and other oceanographic data, including hydrographic measurements (AI Mucci; personal communication), were made available by Amundsen Science Data Collection (2015) and by participating GEOTRACES scientists via a shared database hosted by the University of British Columbia (Kristina Brown; personal communication). Contemporaneous measurements of thiol, humic substances, and fluorescent dissolved organic matter in the Canada Basin and CAA, published by Gao and Guéguen (2018), were also used to interpret the copper ligand profiles determined by IMAC.

3. Results

Hydrographic data collected during the 2015 Canadian Arctic GEOTRACES expedition identified three main water masses in the Canada Basin and CAA (Gao and Guéguen, 2018). Surface waters (SW; top 30 m, salinity < 32 PSU) were dominated by runoff and ice melt in the Canada Basin and western CAA and influenced by warmer, more saline Atlantic waters in the eastern CAA. Arctic outflow waters (OW; 30 to 300 m, salinity < 33.7 PSU) incorporated Pacific summer and winter waters in the Canada Basin and river input and ice melt in the CAA. Deep waters (DW; below 300 m, salinity > 34.5 PSU) were dominated by warm, saline Atlantic water. Salinity profiles help to relate these water masses to the distributions of chlorophyll, copper ligands, and dCu across the Canadian Arctic (Figs. 3-5).

3.1. Canada Basin

Ligand depth profiles in the Canada Basin were similar in shape, featuring a mid-depth maximum of between 3.7 and 4.2 nM that
shoaled eastwards from 100 m at CB-4 (Fig. 3a) to 42 m at CB-1 (Fig. 3d). Analysis of duplicate samples (Fig. 2) showed that ligand concentrations were reproducible with an average relative standard deviation of 10.9%. Error bars indicating plus or minus one standard deviation from the mean are shown wherever replicate samples were analyzed (Figs. 3-5). Stations CB-3 and CB-4 had surface ligand concentrations of 1.6 and 2.0 nM, respectively, compared with 3.2 nM at CB-1 and 3.0 nM at CB-2, where ice cover was greatest (Table 1). Chlorophyll maxima in the Canada Basin were close to the depth of highest ligand concentration (Fig. 3). The ligand maximum extended below the chlorophyll maximum at stations CB-2, CB-3 and CB-4. The ligand concentration at 220 m was 3.2 nM at CB-4 whereas ligand concentrations at 200 m ranged from 2.0 to 2.5 nM at other CB stations. Concentrations of dCu in the Canada Basin were > 4 nM at the surface and remained at around 4 nM until a point below the chlorophyll maximum at which dCu started decreasing with depth to < 3 nM at 250 m.

3.2. Baffin Bay

Ligand profiles in Baffin Bay were distinct from those observed in the Canada Basin, the highest concentrations (> 4 nM) being found near the surface (Fig. 4). Chlorophyll maxima in Baffin Bay were again close to the depth of highest ligand concentration. Baffin Bay had relatively low ligand concentrations below 200 m (between 0.9 and 1.5 nM). Surface concentrations of dCu were lower in Baffin Bay than in the Canada Basin with maximum values approaching 3.5 nM. Dissolved copper concentrations gradually decreased with depth to between 2.0 and 2.3 nM at 500 m.

![Map showing sampling locations in the Canada Basin, Canadian Arctic Archipelago, and Baffin Bay](map.png)

**Fig. 1.** Selected locations at which samples were collected for copper ligand and dissolved copper analysis in the Canada Basin (CB), Canadian Arctic Archipelago (CAA), and Baffin Bay (BB) during the 2015 Canadian Arctic GEOTRACES expedition (map: Google Earth).

**Table 1**

<table>
<thead>
<tr>
<th>Station</th>
<th>Latitude (N)</th>
<th>Longitude (W)</th>
<th>Sampling date (2015)</th>
<th>Ice cover (/10)</th>
</tr>
</thead>
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<tr>
<td>CB-4</td>
<td>75° 00.0 ′</td>
<td>150° 00.0 ′</td>
<td>14-Sep</td>
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</tr>
<tr>
<td>CB-3</td>
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<td>140° 03.0 ′</td>
<td>11-Sep</td>
<td>1</td>
</tr>
<tr>
<td>CB-2</td>
<td>75° 48.6 ′</td>
<td>129° 13.8 ′</td>
<td>9-Sep</td>
<td>4</td>
</tr>
<tr>
<td>CB-1</td>
<td>75° 07.8 ′</td>
<td>120° 34.2 ′</td>
<td>7-Sep</td>
<td>1</td>
</tr>
<tr>
<td>CAA-8</td>
<td>74° 08.4 ′</td>
<td>108° 49.8 ′</td>
<td>23-Sep</td>
<td>0</td>
</tr>
<tr>
<td>CAA-6</td>
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<td>97° 27.6 ′</td>
<td>15-Aug</td>
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</tr>
<tr>
<td>CAA-5</td>
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<td>90° 48.6 ′</td>
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<td>0</td>
</tr>
<tr>
<td>CAA-2</td>
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<td>79° 30.0 ′</td>
<td>10-Aug</td>
<td>0</td>
</tr>
<tr>
<td>BB-3</td>
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<td>68° 36.0 ′</td>
<td>5-Aug</td>
<td>0</td>
</tr>
<tr>
<td>BB-1</td>
<td>66° 51.0 ′</td>
<td>59° 03.6 ′</td>
<td>3-Aug</td>
<td>2</td>
</tr>
</tbody>
</table>

![Immobilized copper(II)-ion affinity chromatography](chromatography.png)

**Fig. 2.** Immobilized copper(II)-ion affinity chromatography of duplicate samples collected at station CB-3 in the Canada Basin.
3.3. Canadian Arctic Archipelago

Ligand profiles featuring mid-depth and near-surface maxima were both observed in the CAA. The profile at CAA-8 (Fig. 5a) resembled those seen in the neighboring Canada Basin with a maximum ligand concentration of 3.6 nM at 45 m, the chlorophyll maximum depth. The dCu profile at CAA-8 was also similar to those observed in the Canada Basin, decreasing with depth from a surface maximum of 4.0 nM to 2.5 nM at 200 m.

Ligand profiles at stations CAA-5 and CAA-6 (Fig. 5b,c) resembled those seen in Baffin Bay with highest ligand concentrations (~ 4 nM) near the surface. Ligand concentrations below 50 m showed little variation but were significantly lower at CAA-5 (< 2 nM) than at CAA-6 (2 to 3 nM), approaching those observed at depth in Baffin Bay. Again, chlorophyll maxima at these stations were close to the depth of highest ligand concentration. Water collected from 11 m at station CAA-2 (Fig. 5d) had the highest recorded ligand concentration of 4.8 nM (no other samples were collected at this station). Profiles of dCu at CAA-5 and CAA-6 showed relatively little variation with depth, averaging 3.2 nM between the surface and 250 m. At CAA-2, dCu decreased sharply from 3.2 nM at 43 m to 2.8 nM at 60 m before falling steadily to 2.4 nM at 200 m.

4. Discussion

4.1. Operational definition of copper ligands

Electrochemical studies have confirmed that marine copper-binding ligands can be isolated from seawater using IMAC; for example, Donat et al. (1997) demonstrated removal of both stronger (L1) and weaker (L2) classes of copper ligands from seawater and detection of L1 in pooled IMAC eluents. Nevertheless, it is possible that certain ligands may not be retained or detected during IMAC, including multi-dentate ligands unable to form stable ternary complexes with IDA–Cu²⁺ (Paunovic et al., 2005; Nixon and Ross, 2016) or those that show weak UV absorbance. However, relative retention times for natural and...
model ligands observed using the same IMAC method (Ross et al., 2003; Nixon and Ross, 2016) suggest that we are monitoring ligands with an affinity for Cu²⁺ comparable to the L₁ class. We have also shown, by spiking samples with excess Cu²⁺, that most ligands entering the IMAC column in complexed form are retained (Nixon and Ross, 2016), presumably via dissociation of the complex or interaction of the complexed copper with the column resin. Hence, the predominant peak observed in the IMAC chromatograms of all Arctic samples represents an operationally defined fraction of UV-absorbing ligands with similar and relatively strong affinities for Cu²⁺. The contributions to IMAC peak area made by different types and sources of ligands will depend upon their relative abundance and UV absorbance. However, the consistency of our ligand measurements in terms of their relationship with chlorophyll (Figs. 3-6) and with L₁ ligand concentrations determined by voltammetry in the sub-Arctic northeast Pacific and Bering Sea (Moffett and Dupont, 2007; Whitby et al., 2018) suggest that they provide a reliable indication of the distribution and concentrations of copper(II)-complexing ligands in Arctic waters.

4.2. Profiles with maxima below 30 m

Mid-depth ligand maxima were observed in the Canada Basin and at station CAA-8, coinciding with the chlorophyll maximum in the OW layer (Gao and Guéguen, 2018). The shallowing of these maxima between station CB-4 (Fig. 3a) and station CAA-8 (Fig. 5a) is consistent with an eastward shoaling of the OW layer, as indicated by the corresponding salinity profiles. Ligand concentrations were relatively low (< 2.0 nM) near the surface at stations CB-4, CB-3 and CAA-8 where lack of ice cover (Table 1) may promote photo-oxidation of certain ligands (Barbeau, 2006). However, stations CB-1 and CB-2 (Fig. 3) had similar ligand concentrations (around 3 nM) near the surface despite low ice cover at CB-1, suggesting that other factors are important in determining ligand abundance.

Complementary studies (Gao and Guéguen, 2018) indicate that thiols, which include biologically-derived ligands like glutathione, are relatively abundant at the chlorophyll maximum in the Canada Basin and eastern CAA. Naturally occurring thiols have been shown to complex copper in estuarine waters (Laglera and van den Berg, 2003) and are known to be produced by some cyanobacteria under conditions of metal stress (Singh et al., 1999). We have shown that glutathione can be recovered from seawater by IMAC (Nixon and Ross, 2016) although absorbance at 254 nm is relatively weak. Analysis of fluorescent dissolved organic matter using UV–vis spectrophotometry and parallel factor analysis (Gao and Guéguen, 2018) found that protein-like component C4 (associated with in situ biological production) and the absorption coefficient at 355 nm (a₃₅⁵, a proxy for terrestrial DOM) were also relatively high in the OW layer of the Canada Basin and western CAA. Taken together, these results suggest that (i) marine phytoplankton or cyanobacteria associated with the chlorophyll maximum could be a significant source of the copper ligands captured by IMAC, and (ii) humic substances and other terrestrial DOM contribute to the pool of copper ligands recovered from the OW layer.

4.3. Profiles with maxima above 30 m

Ligand profiles with near-surface maxima were observed in Baffin Bay and at CAA-5 and CAA-6 where the chlorophyll maximum was relatively shallow. Ligand concentrations measured at 10 m in Baffin Bay were among the highest recorded (> 4 nM) whereas those below 150 m were among the lowest (1.5 nM or less). The difference in ice cover at stations BB-1 and BB-3 (Table 1) appeared to have little effect on ligand concentrations near the surface, implying that other factors are more important in determining ligand abundance. Indeed, relatively high a₃₅⁵ values in surface waters at CAA-2 (Gao and Guéguen, 2018) suggest that contributions from terrestrial DOM to copper ligands in the eastern Canadian Arctic are likely to be greater near the surface. This is consistent with the high ligand concentration (4.8 nM) measured at CAA-2 (Fig. 5d).

4.4. Sources of copper ligands

The highest ligand concentration at each station was generally observed in the vicinity of the chlorophyll maximum. This is consistent with ligand profiles generated using electrochemical methods (Donat et al., 1986; Coale and Bruland, 1988; Moffett et al., 1990) supporting the hypothesis that marine phytoplankton and cyanobacteria are significant sources of copper-complexing ligands in seawater. To further investigate the relationship between ligand abundance and phytoplankton biomass we plotted ligand concentration against chlorophyll concentration at each station. The results fall into three geographically distinct groups.

The first group consists of the four stations in the Canada Basin (Fig. 6a). A positive linear correlation (y = 1.62x + 3.01, r² = 0.76) between ligand and chlorophyll concentrations was observed for nine of
the eighteen samples in this group. These include samples collected from within the chlorophyll maxima, and from near the surface at CB-1 and CB-2. The intercept, which represents the extrapolated ligand concentration in the absence of chlorophyll, may serve as a proxy for humic substances (HS) and other copper-binding DOM not associated with phytoplankton. To test this hypothesis we converted the intercept (3 nM) to IMAC peak area (221 AU.min) using the original calibration curve (see Methods and Materials). We then generated a second IMAC calibration by analyzing seawater spiked with 0 to 200 μg C/L of an equal mixture of three HS standards (3S101H, 2S101F and 3S101F; International Humic Substance Society, St. Paul, MN) based upon the carbon content of each standard (International Humic Substance Society, 2019). We used this calibration \((y = 1.63x + 16.20, r^2 = 0.995)\) to convert the intercept peak area to an equivalent average HS concentration of 126 μg C/L. This value lies within the range of HS-like concentrations (55 to 145 μg C/L) measured by voltammetry in the OW layer of the Canada Basin (Gao and Guéguen, 2018), implying that a significant proportion of HS binds copper.

The remaining Canada Basin samples fall into two sub-groups, each within a narrow range of low chlorophyll concentrations (Fig. 6a). The sub-group with ligand concentrations > 3.7 nM included samples from below the chlorophyll maximum at stations CB-2, CB-3 and CB-4. The highest ligand concentrations at CB-3 and CB-4 were actually measured below the chlorophyll maximum depth (Fig. 3a,b). Zooplankton grazing of phytoplankton is known to occur in the Canada Basin (Yang et al., 2015) and may release metal-binding DOM into the water column (Laglera et al., 2019). Capture of this material by IMAC below the chlorophyll maximum would result in ligand concentrations higher than those expected on the basis of chlorophyll measurements, as seen for this sub-group. Samples with ligand concentrations < 2.5 nM include those collected near the surface at stations CB-3 and CB-4 and from 200 m at stations CB-1, CB-2 and CB-3. Photo-oxidation of copper ligands in open surface waters and bacterial degradation of copper-binding DOM at depth may have contributed to lower than expected ligand concentrations in these samples.

The second group of stations consists of CAA-6 and CAA-8 (Fig. 6b)
which lie to the east of the Canada Basin. These showed a strong linear
correlation between ligand and chlorophyll concentrations
\( y = 4.25x + 1.40, r^2 = 0.99 \). Applying our HS calibration to the in-
tercept gave an equivalent value of 82 \( \mu g \) C/L, which lies within range
of HS-like concentrations (19 to 190 \( \mu g \) C/L) measured in the Canada
Basin and CAA (Gao and Guéguen, 2018). The third group consists of
CAA-2, CAA-5 and the two stations in Baffin Bay (Fig. 6c). These also
showed a strong linear correlation between ligand and chlorophyll
concentrations (\( y = 10.41x + 1.04, r^2 = 0.89 \)) with a steeper slope
and a smaller intercept than for stations to the west. Applying the HS
calibration to this intercept gave an equivalent value of 72 \( \mu g \) C/L.
The apparent decrease in copper-binding HS between the Canada Basin and
Baffin Bay is consistent with complementary studies (Gao and Guéguen,
2018) that found terrestrial DOM to be highest in the western part of
the study region. Although the source of mid-depth humic-rich waters
remains unknown, previous studies (Wheeler et al., 1997) have im-
plicated terrestrial runoff as a significant contributor to bulk DOM in
the Canadian Arctic. On the other hand, an increase in the slope of
ligand vs. chlorophyll plots between the Canada Basin and Baffin Bay
(Fig. 6a-c) may indicate greater production of copper ligands by phy-
toplankton in the eastern part of the study region.

The distribution of copper ligands across the Canadian Arctic can be
seen more clearly by combining ligand profiles from station CB-4 in the
Canada Basin to station BB-1 in Baffin Bay (Fig. 7). The resulting section
shows that the depth of highest ligand concentration shoals steadily
from west to east across the Canada Basin and through the CAA to
Baffin Bay, tracking both the chlorophyll maximum and apparent in-
puts of terrestrial DOM to the OW layer in the west and SW layer in the
east (Gao and Guéguen, 2018).

4.5. Comparison with dissolved copper

Given that exposure to copper can influence the production of
copper-complexing ligands by phytoplankton (see Introduction) we also
examined the relationship between chlorophyll, ligand and dCu con-
centrations. Dissolved copper concentrations were generally found to
be highest at the surface, decreasing steadily to a point below the
chlorophyll maximum at which dCu begins to decline more rapidly. The
depth at which this occurs in the Canada Basin decreases from about
200 m at CB-4 (Fig. 3a) to 75 m at CB-1 (Fig. 3d) which is consistent
with a shoaling of the OW layer, and the associated chlorophyll and
ligand maxima. The highest dCu concentrations measured at these
stations (~ 4.5 nM) are unlikely to induce toxicity or limitation among
phytoplankton, whether in response to the presence of copper or not. This is also true of stations in the CAA and, in particular, Baffin
Bay where higher dCu concentrations coincide with ligand maxima.
The possibility that some of these ligands may be produced in response to copper was further investigated by plotting ligand concentration against dCu (Fig. 6d). A strong correlation ($y = 2.22 \times -3.68$, $r^2 = 0.91$) between ligand and dCu concentrations was observed in Baffin Bay, where phytoplankton appear to be a significant source of copper ligands (Fig. 6c). The plot shows that ligand concentration increases with dCu in a ratio of approximately 2:1, which is consistent with complexation of Cu$^{2+}$ by smaller ligands of the kind known to be recovered by IMAC (Nixon and Ross, 2016). An extrapolated dCu concentration of 1.7 nM in the absence of any copper ligands captured by IMAC is consistent with the recovery of a sub-set of copper-binding substances and other marine dissolved organic matter (Nixon and Ross, 2016). The omission of certain ligands by IMAC may also have contributed to the apparent lack of a consistent relationship between ligand concentration and dCu in the Canada Basin and CAA (not shown).

4.6. Comparison with phytoplankton taxonomy

A wide variety of organisms have been shown to produce copper-complexing ligands in seawater including eukaryotic phytoplankton (Robinson and Brown, 1991; Anderson, 1984; Croot et al., 2000), diatoms (Zhou and Wangersky, 1985; Morelli et al., 1989; Gerrings et al., 1995; Croot et al., 2000), dinoflagellates (Croot et al., 2000) and coccolithophores (Leal et al., 1999; Croot et al., 2000; Echeveste et al., 2018). Culturing experiments have also shown that high-affinity copper ligands can be produced by copper-sensitive marine phytoplankton and cyanobacteria (Leao et al., 2007) in response to metal stress (Singh et al., 1999) and to regulate copper uptake (Semeniuk et al., 2015).

Phytoplankton are the most significant contributors to bulk DOM in the Canadian Arctic (Wheeler et al., 1997) although comprehensive taxonomic data are not readily available. Heterotrophic dinoflagellates and other flagellates represent most of the biomass in the water column whereas diatoms are abundant near sea-ice interfaces (Gosselin et al., 1997), which were largely absent at our sampling locations (Table 1). Large phytoplankton are highly productive near ice-vegetated archipelagic stations while small phytoplankton dominate in the low productivity open waters of the Canada Basin and Baffin Bay (Varela et al., 2013). Decreasing C:N ratios further suggest an ongoing increase in the relative abundance of small phytoplankton and heterotrophs in the Canada Basin, due to freshening of surface waters (Crawford et al., 2015).

Available data from the 2015 GEOTRACES cruise in the eastern Canadian Arctic (Michel Gosselin: personal communication) show that most of the chlorophyll detected in Baffin Bay is associated with cells between 0.7 and 5 μm in diameter concentrated at a depth of about 30 m, whereas a significant proportion of the chlorophyll signal at stations in the central and eastern CAA is associated with cells larger than 20 μm residing at greater depths. Similarities between the distribution of this chlorophyll signal and of the copper ligands measured by IMAC in the eastern Arctic (Fig. 7) suggests that picoplankton, which consist mainly of eukaryotes in these waters (Tremblay et al., 2009; Ardyne et al., 2011), may be a significant source of copper ligands (Moftett et al., 1990; Moffett and Brand, 1996; Croot et al., 2000; Gordon et al., 2000; Wiramanaden, 2000). Techniques to identify and hence determine the origin of ligands captured by IMAC are being developed (Nixon and Ross, 2016).

5. Conclusion

Immobilized copper(II)-ion affinity chromatography (IMAC) was used to profile the distribution of an operationally-defined fraction of dissolved copper ligands across the Canadian Arctic. Significant correlations between ligand and chlorophyll concentrations suggest that marine phytoplankton and/or cyanobacteria could be an important source of copper ligands, particularly in the eastern Canadian Arctic. The correlation between ligand and dissolved copper concentrations in Baffin Bay is consistent with biological production and IMAC recovery of small organic ligands. Comparisons with published data for humic substances and other marine disolve organic matter suggest that both in situ biological production and terrestrial input contribute to the pool of copper ligands captured by IMAC.

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