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Evidence for the production of copper-complexing ligands by marine phytoplankton in the Canadian Arctic and subarctic NE Pacific

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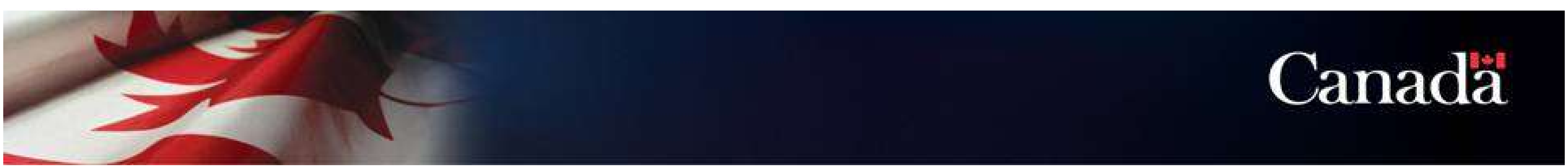


Marine Organic Ligands

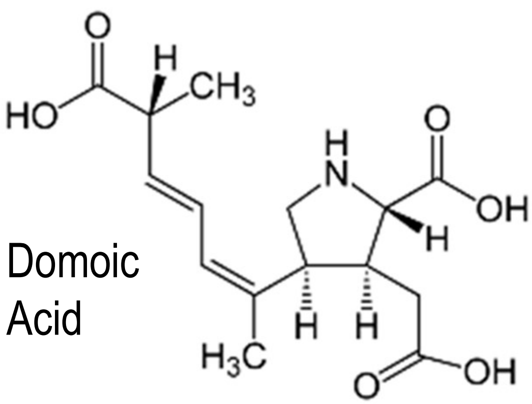
- certain metals (e.g. Cu, Fe and other first-row transition elements) are biologically important as micronutrients but present at very low levels (< 0.05 nmol/kg) in seawater.

- these and other metals (Hg, Pb) can be toxic to marine organisms depending on concentration and speciation.

- some of the organic molecules (DOM) present in seawater can bind and form complexes with metals.

- these marine organic ligands have a profound effect on the distribution, bioavailability and toxicity of trace metals.

Speciation of Metals in Seawater



Domoic Acid

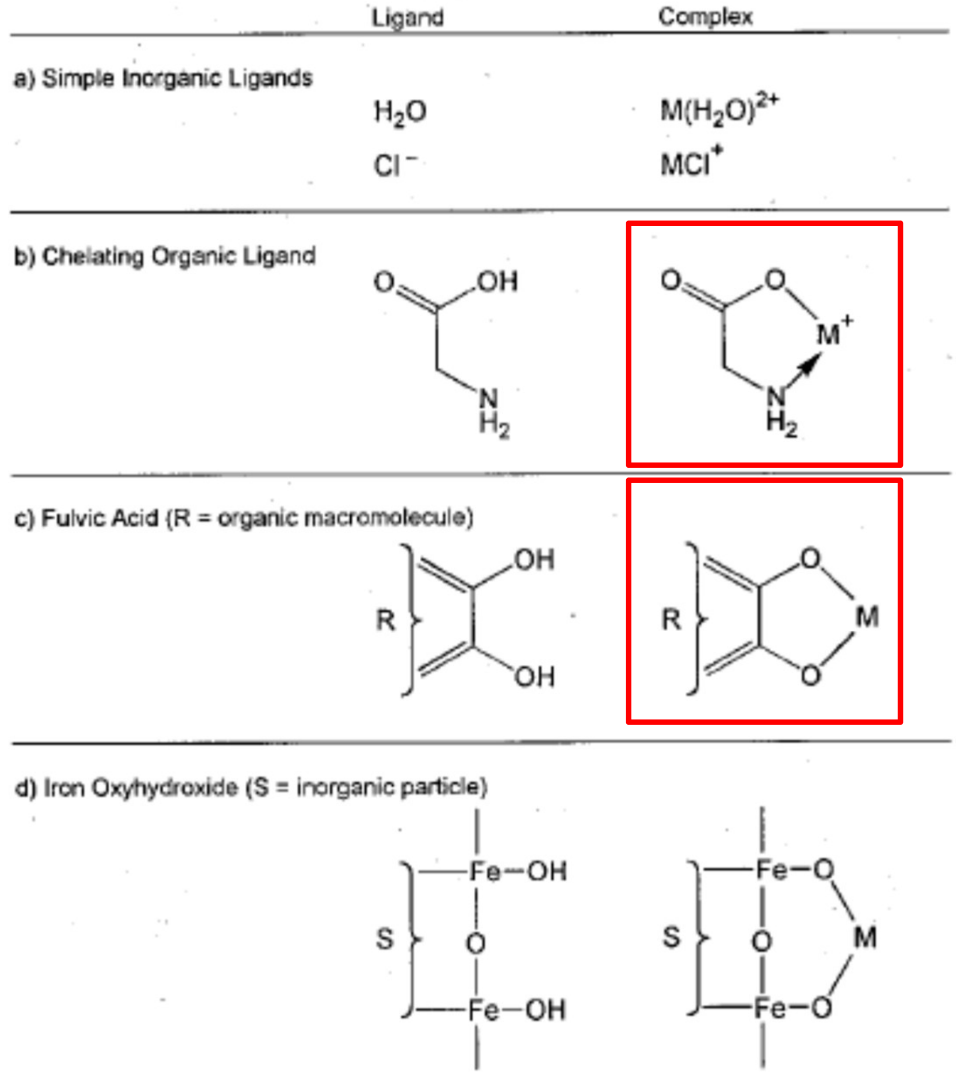


Figure 1.1 Complexation of a divalent metal ion (M^{2+}) by some of the ligands found in natural waters [2]. Certain uncharged ligand atoms (e.g. N, O, S) can form bonds with the metal ion by donating lone pair electrons (→), as shown in (b).

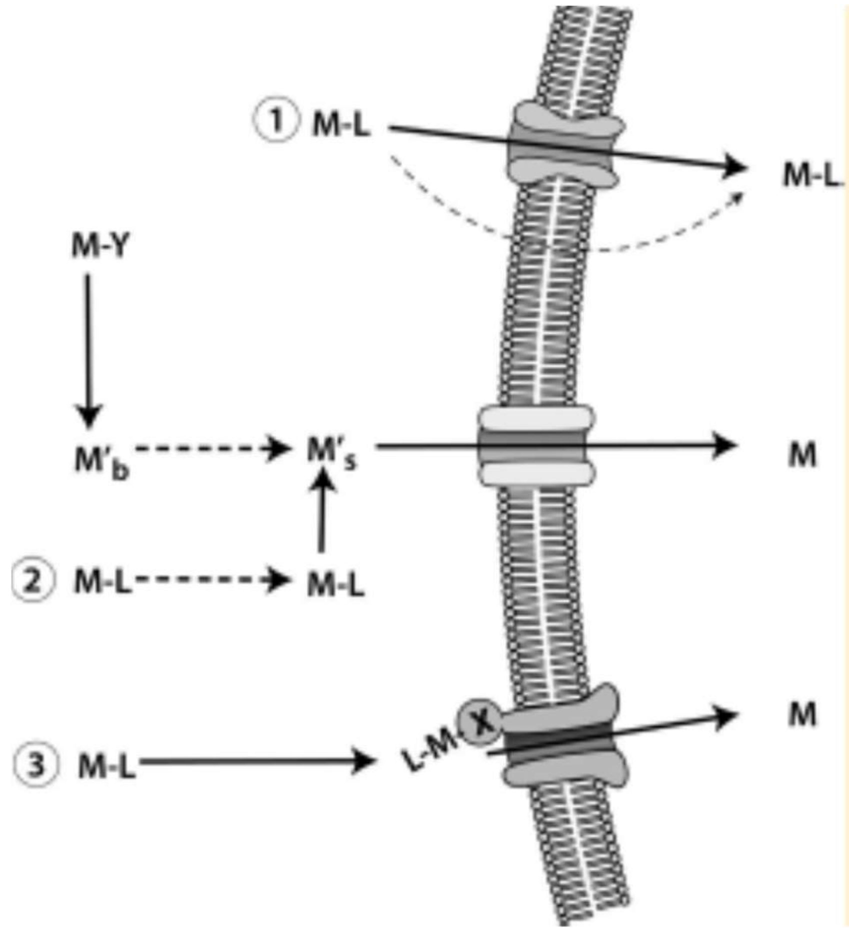
Metal Uptake by Marine Phytoplankton

M = metal ion

L = weak ligand

Y = strong ligand

X = uptake molecule



Aristilda L. *et al.* (2012) Weak organic ligands enhance zinc uptake in marine phytoplankton. *Environmental Science and Technology* **46**: 5438-5445

DFO Organic Ligands Research

- little is known about the structure and ecological role of marine organic ligands.

- identification and structural analysis would help us to better understand their involvement in the uptake and utilization of trace elements by marine phytoplankton.

[Sally Bensusen, NASA EOS]

- carbon fixation, oxygen production, biomass/p.p., HABs *vs.* ocean warming, hypoxia, acidification and fertilization.

Canadian Arctic GEOTRACES

- collaboration between universities and DFO scientists to study trace elements and isotopes in the Canadian Arctic.

- funded by the NSERC Climate Change and Adaptation Research (CCAR) program (2013-2018) to help build research capacity to better understand and predict:

    - future evolution of key physical and biochemical processes.

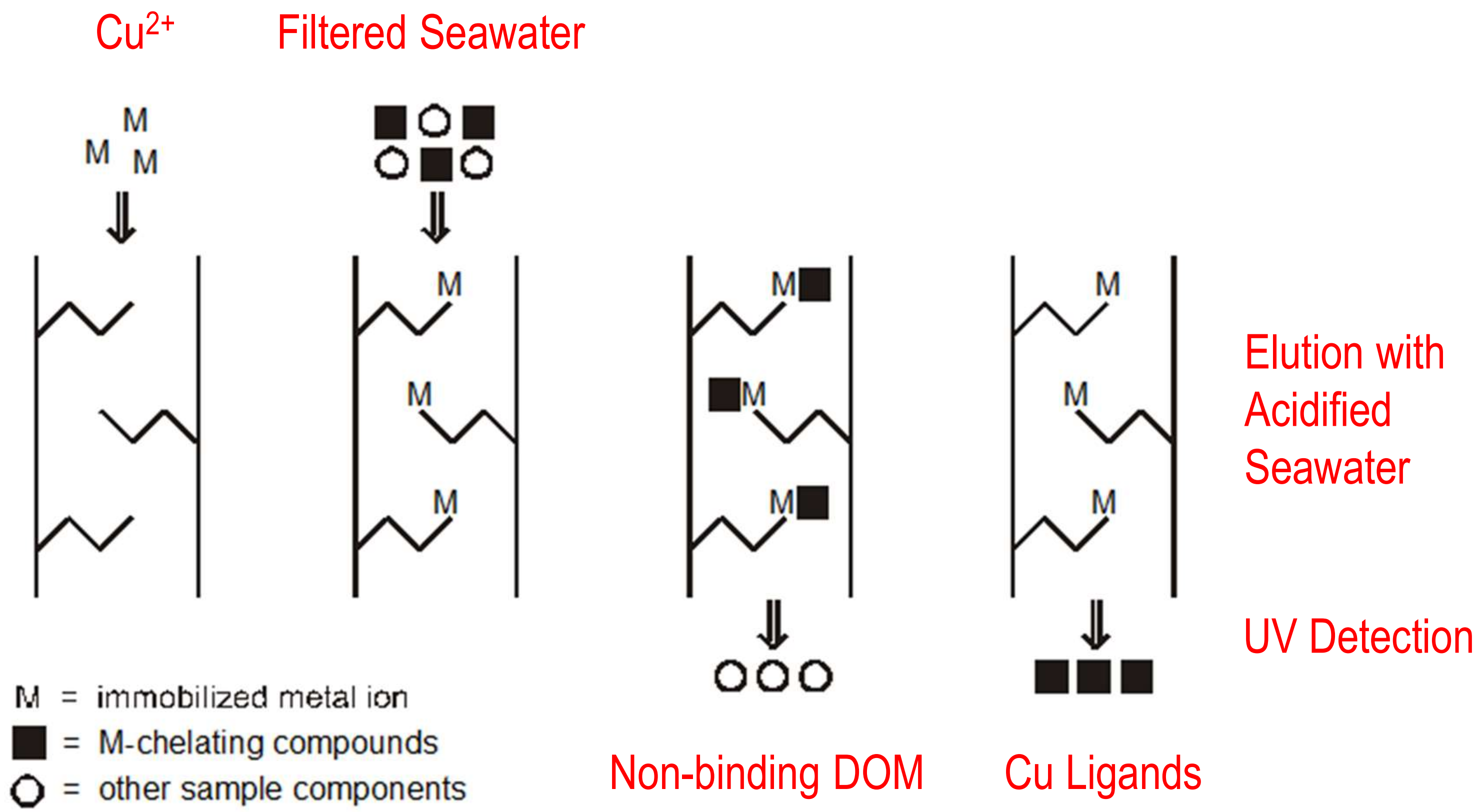
    - climate feedback mechanisms mediated by changing circulation, biological productivity, and cycling of climate-active gases.

    - Arctic contaminant dispersal and cycling, and their potential climate sensitivity over the coming decades.

Organic Ligands Project

- develop and refine IMAC and MS/MS procedures for isolating and identifying organic ligands in seawater using model Cu-binding ligands (e.g. 8-hydroxyquinoline).
- validate procedures by analyzing coastal and oceanic seawater samples collected in the NE Pacific Ocean.
- apply procedures to samples collected during the Canadian Arctic GEOTRACES cruise (Fall 2015).
- compare experimental results with other/published data and develop hypotheses regarding the role of organic ligands in mediating the biological uptake and utilization of trace metals in the NE Pacific and Arctic Ocean.

Cu(II)-IMAC of Marine Ligands



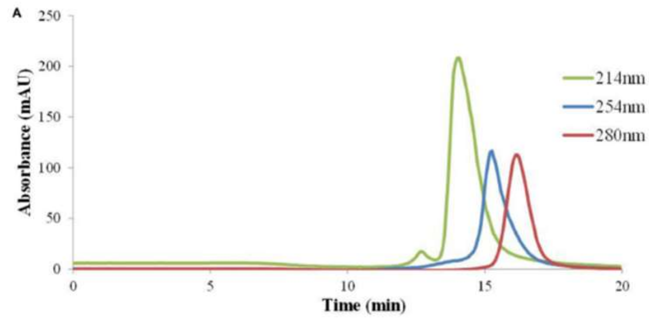
Gordon (1992) Isolation of compounds with affinity for copper from seawater using immobilized copper ion affinity chromatography. *Marine Chemistry* **38**: 1-12

IMAC and MS of Marine Cu Ligands

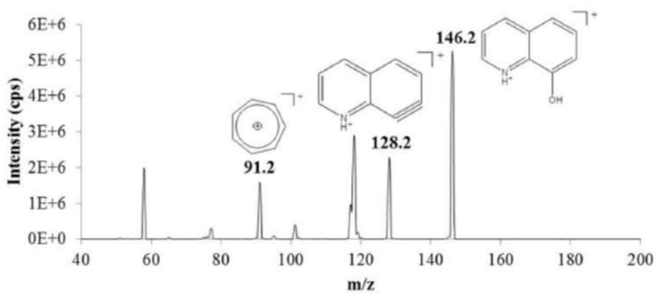
Sampling

0.2-µm Filtration

Cu(II)-IMAC

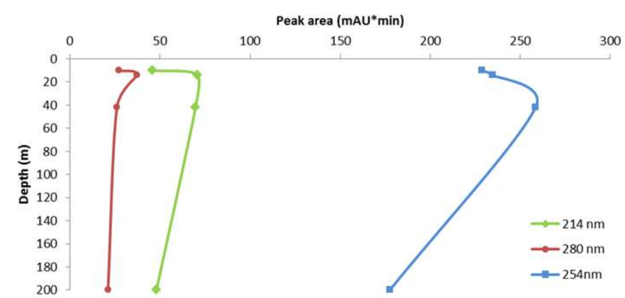


UV Detection



Electrospray MS/MS

SPE



Depth Profiling

Nixon and Ross (2016) *Frontiers in Marine Science*: doi 10.3389/fmars.2016.00246

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IMAC of Model Ligand 8-HQ

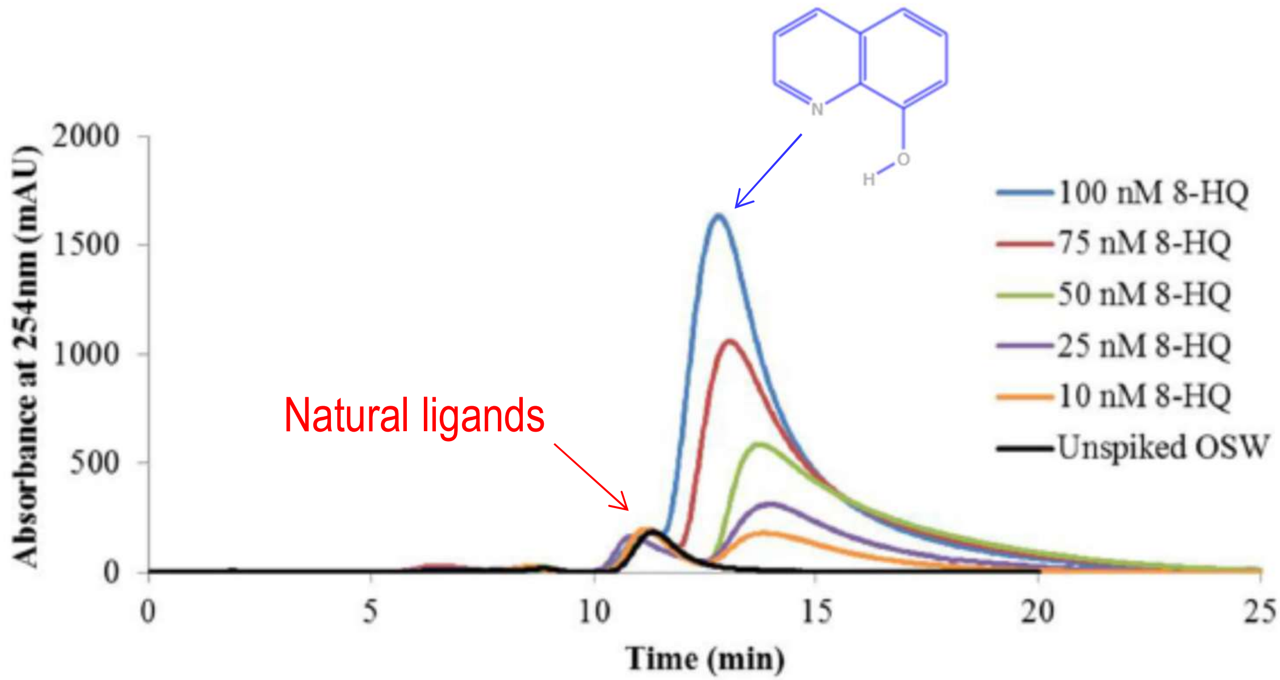


FIGURE 1 | Immobilized copper(II)-ion affinity chromatography (Cu(II)-IMAC) of oceanic surface seawater (OSW) spiked with the model ligand 8-hydroxyquinoline (8-HQ). One-liter OSW samples containing up to 100 nmoles of added 8-HQ were fractionated by Cu(II)-IMAC while monitoring UV absorbance of the eluent at 254 nm using the LKB system (see text). All samples gave rise to a peak at 11 min, attributed to natural copper ligands present in OSW, whereas samples spiked with 8-HQ gave rise to a second elution peak at 12–14 min with area proportional to the amount of ligand added to the OSW sample ($r^2 = 0.9927$).

Nixon and Ross (2016) *Frontiers in Marine Science*: doi 10.3389/fmars.2016.00246

Tandem Mass Spectrometry (MS/MS) of Model Ligand (8-HQ)

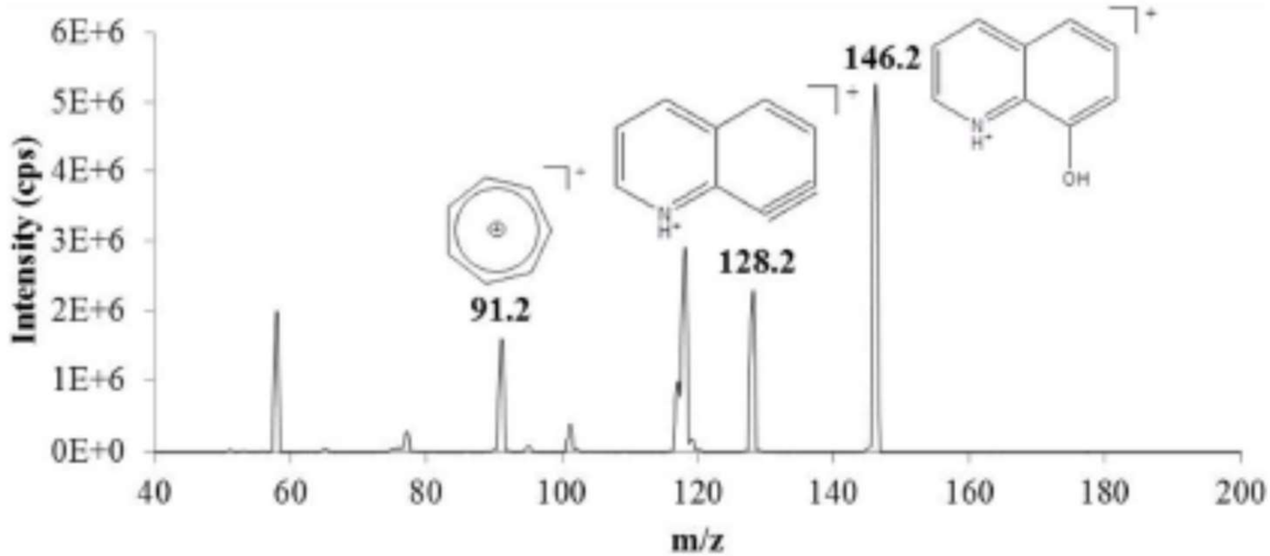
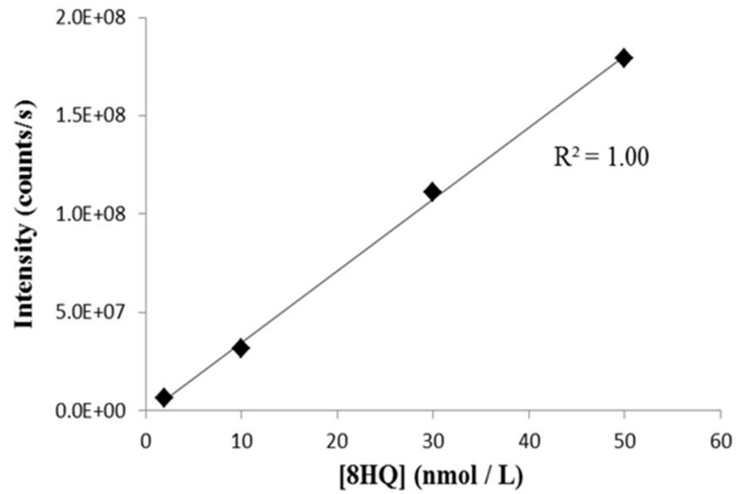


FIGURE 2 | Product-ion mass spectrum of the protonated molecular/precursor ion (*m/z* 146.2) of 8-hydroxyquinoline (8-HQ) obtained by electrospray ionization-tandem mass spectrometry (ESI-MS/MS). Precursor-to-product ion transitions corresponding to loss of water (*m/z* 146.2 > 128.2) and formation of the stable tropylium ion (*m/z* 146.2 > 91.2) were selected for multiple reaction monitoring (MRM) to provide accurate quantification and unambiguous identification of 8-HQ in methanol extracts obtained by solid phase extraction (SPE) (see text).

Nixon and Ross (2016) *Frontiers in Marine Science*: doi 10.3389/fmars.2016.00246

Identification and Quantification by Multiple Reaction Monitoring (MRM)



- linear calibration obtained using methanol standards containing 2 to 50 nmol/L of model ligand (8-HQ).

Identification and Quantification by Multiple Reaction Monitoring (MRM)

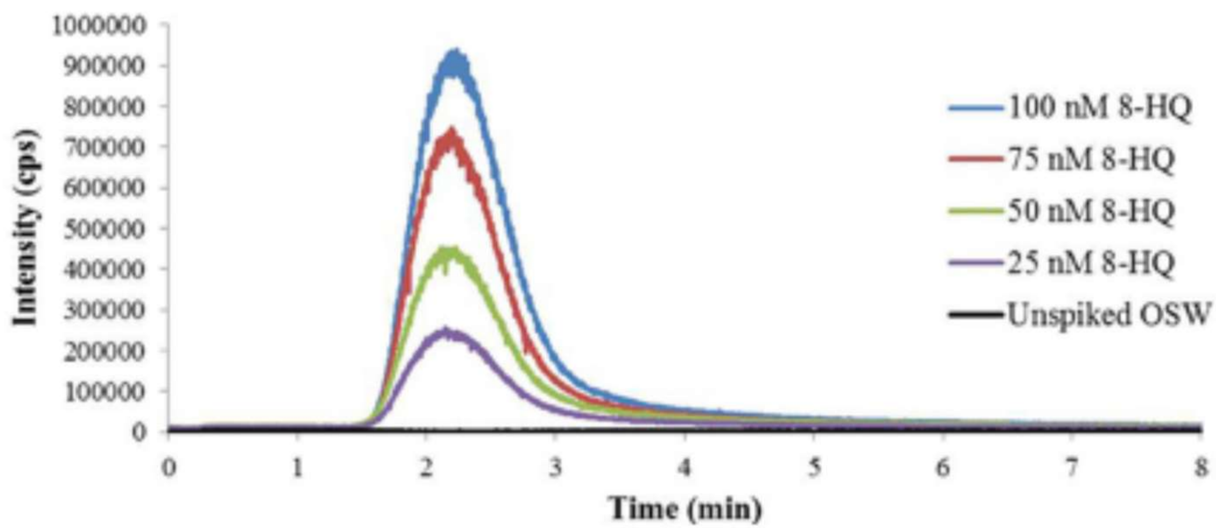


FIGURE 3 | Multiple reaction monitoring (MRM) chromatograms for the *m/z* 146.2 > 91.2 transition used to quantify the model ligand 8-hydroxyquinoline (8-HQ) in Cu(II)-IMAC fractions desalted by solid-phase extraction (SPE). The fractions correspond to the model ligand peaks observed in the Cu(II)-IMAC chromatogram obtained by fractionating 1-L surface seawater samples spiked with 0–100 nmoles 8-HQ. Ten microliter of each SPE extract were analyzed by flow-injection electrospray ionization tandem mass spectrometry (FI-ESI-MS/MS) using 1 mM ammonium acetate in 70% acetonitrile at 100 µL/min as the carrier solvent.

Nixon and Ross (2016) *Frontiers in Marine Science*: doi 10.3389/fmars.2016.00246

Evaluating Ligand Recovery during IMAC and Solid Phase Extraction

TABLE 1 | Recovery of the model ligand 8-hydroxyquinoline (8-HQ) from seawater by immobilized copper(II)-ion affinity chromatography (IMAC) and solid-phase extraction (SPE).

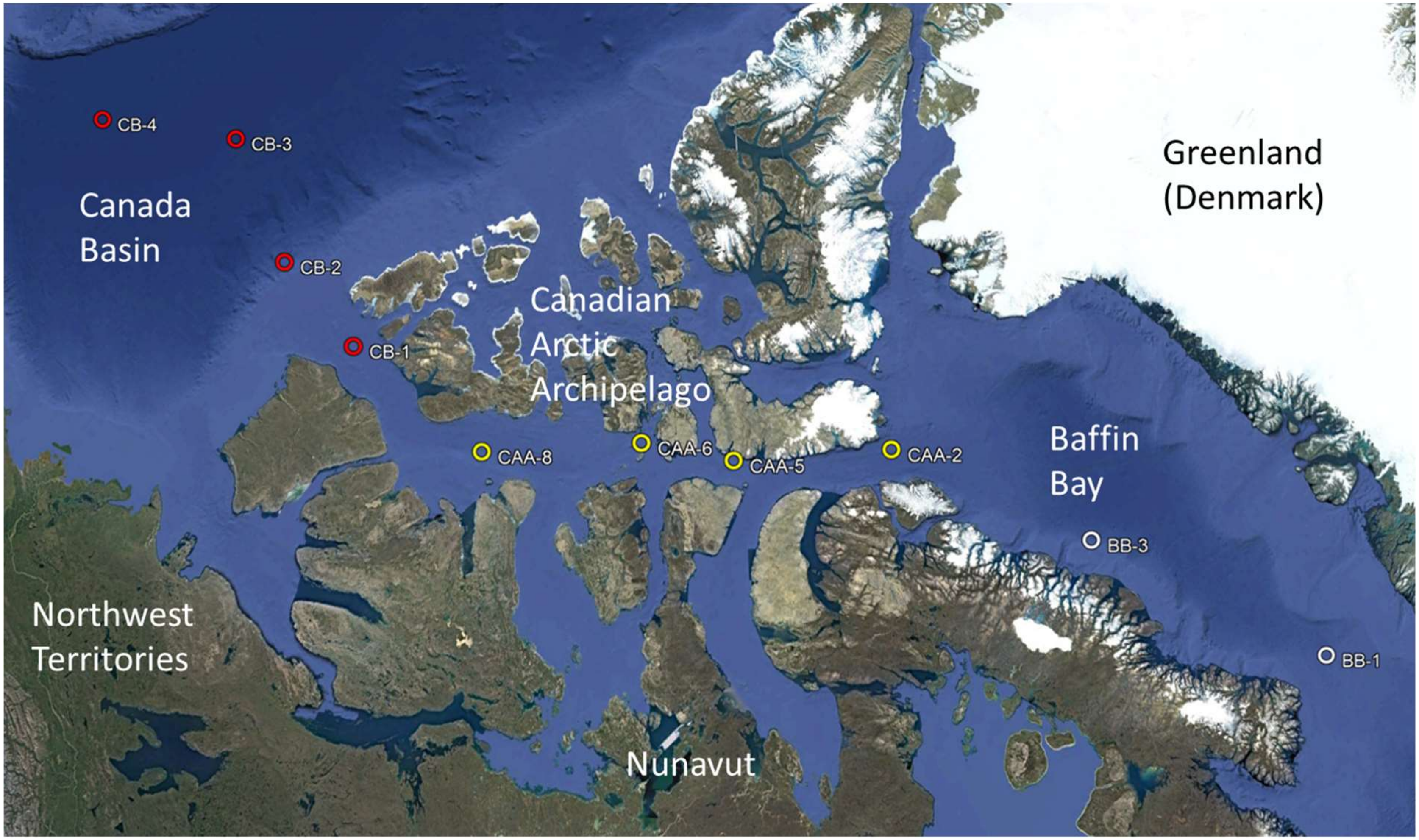
8-HQ added to SW sample (nmoles)	8-HQ measured in SPE extract (nmoles)	8-HQ corrected for SPE recovery (nmoles)	% of IMAC fraction used for SPE	8-HQ in IMAC fraction (nmoles)	% added 8-HQ in IMAC fraction	% total IMAC peak in IMAC fraction	% added 8-HQ recovered in IMAC Peak
25	8.0	10.8	46	23.5	94.0	95	98.0
25	8.0	10.8	53	20.4	81.8	97	84.3
50	12.5	16.8	46	36.5	73.0	99	73.8
100	56.2	75.5	100	75.5	75.5	90	83.9
				Mean	81.1		85.2

Nixon and Ross (2016) *Frontiers in Marine Science*: doi 10.3389/fmars.2016.00246

NGCC • CCGS

AMUNDSEN

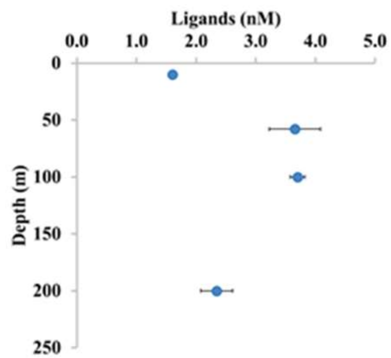
Sampling Locations (Sep/Oct 2015)



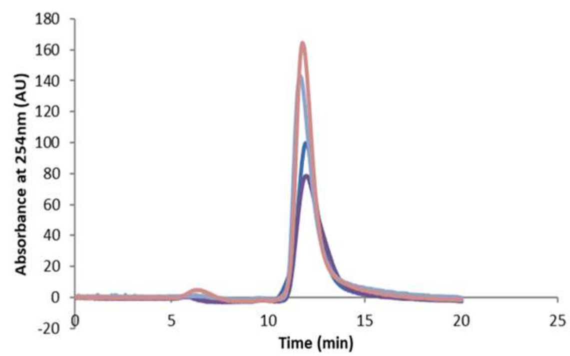
Challenges of Arctic Fieldwork...

IMAC of Arctic Cu Ligands

Sampling **Filtration** **Cu(II)-IMAC**



Depth Profile



UV Detection

Nixon et al. (2019) *Marine Chemistry*: doi 10.1016/j.marchem.2019.103673

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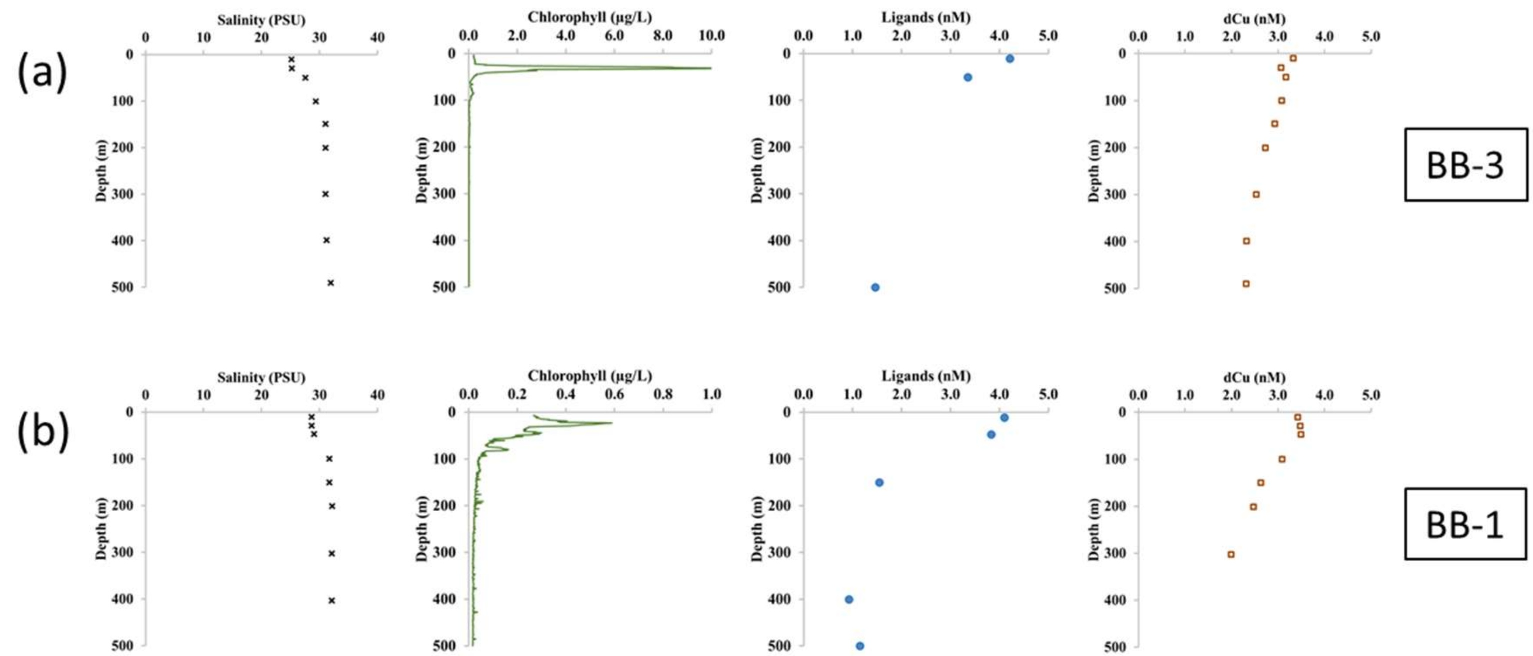
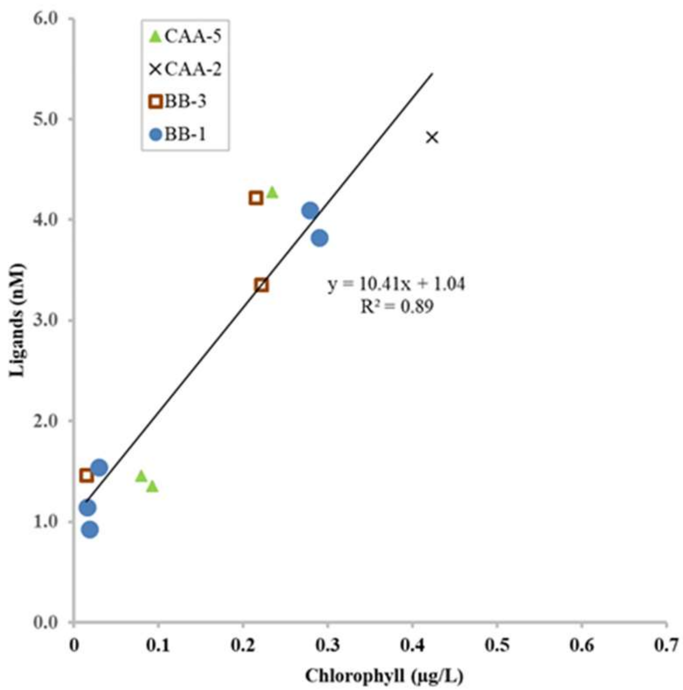
Profiles of Salinity, Chlorophyll, Cu Ligands and dCu in Baffin Bay

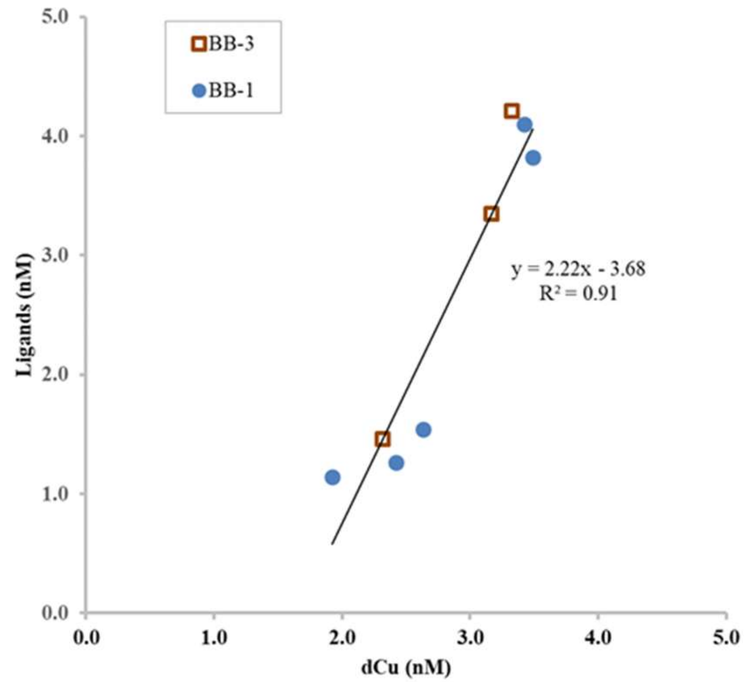


- Cu ligands most abundant near the chlorophyll maximum.

Nixon et al. (2019) *Marine Chemistry*: doi 10.1016/j.marchem.2019.103673

Cu Ligands vs. Chlorophyll and dissolved Cu in Baffin Bay

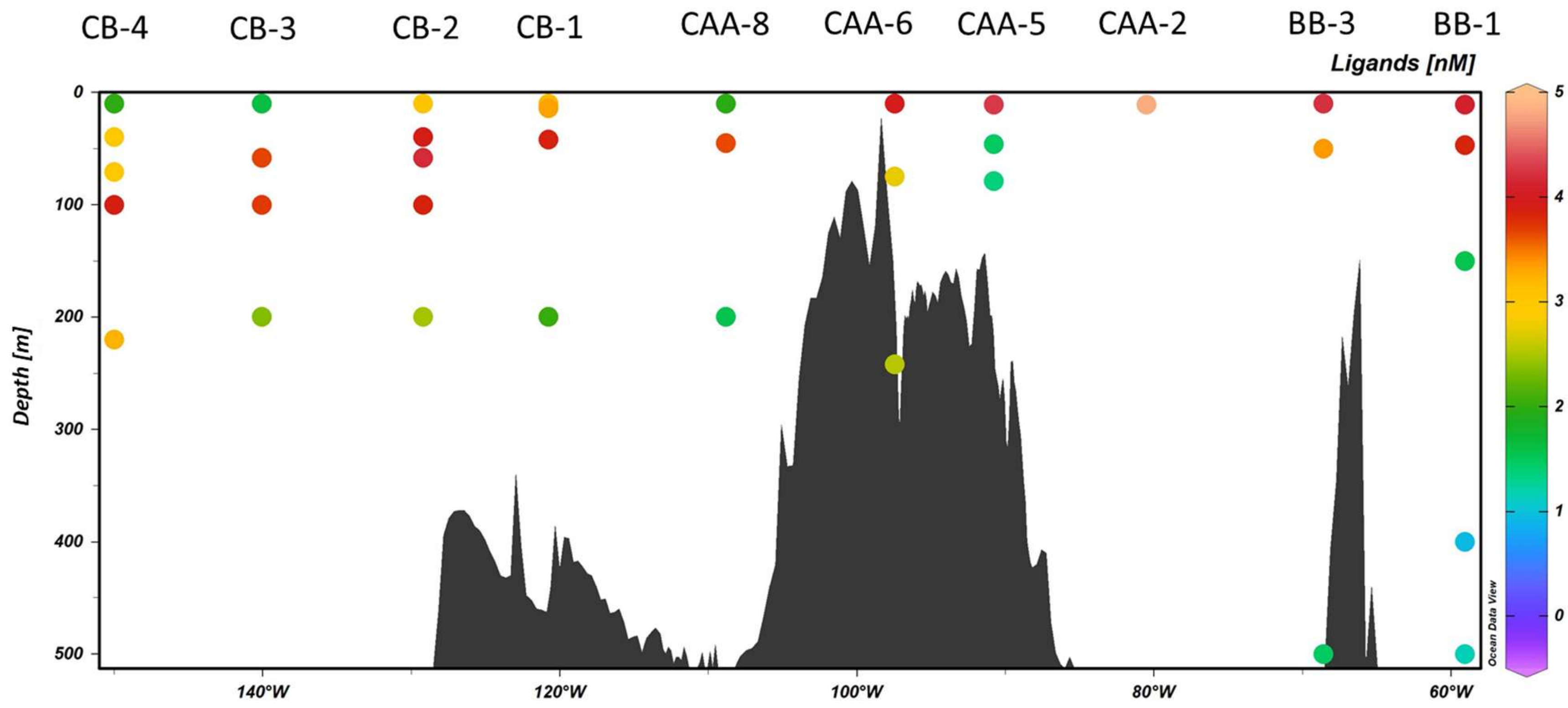




- Cu ligand concentration is correlated with chlorophyll and dCu, suggesting that phytoplankton are a major source.

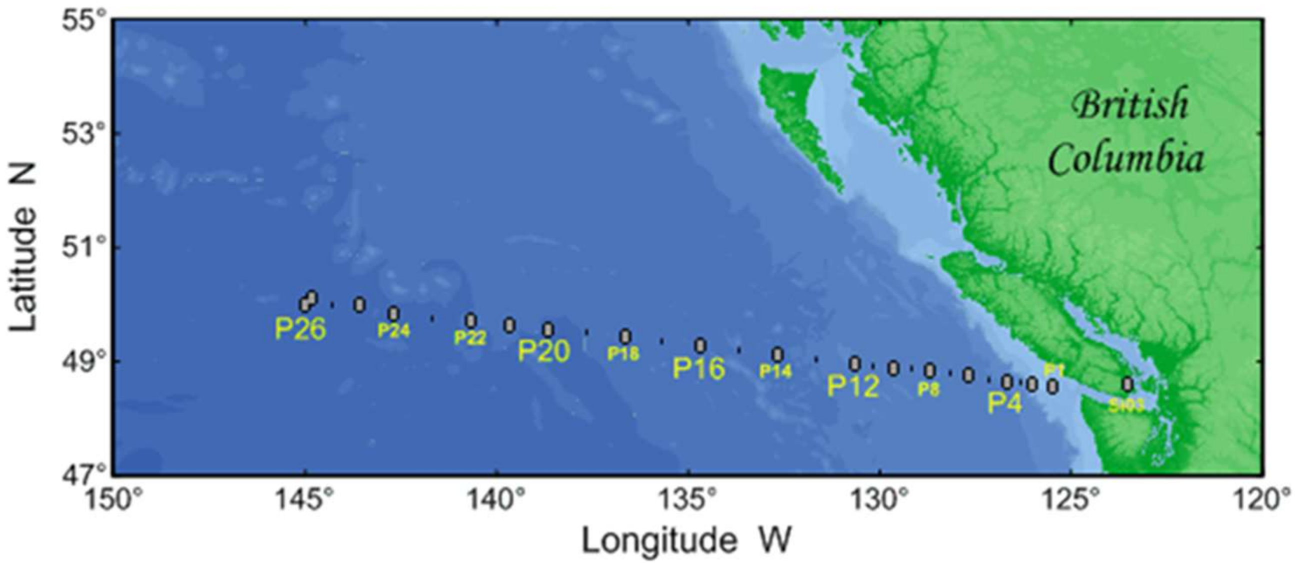
Nixon et al. (2019) *Marine Chemistry*: doi 10.1016/j.marchem.2019.103673

Distribution of Cu Ligands across the Canadian Arctic



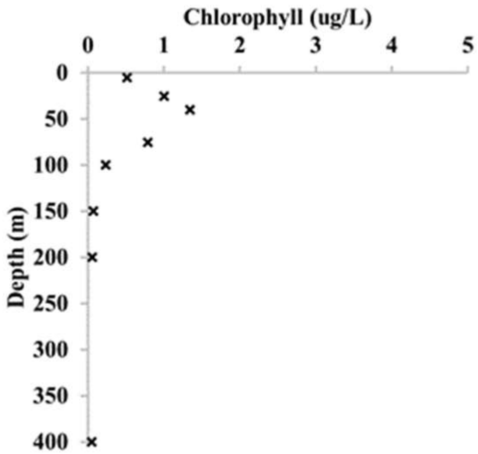
Nixon et al. (2019) *Marine Chemistry*: doi 10.1016/j.marchem.2019.103673

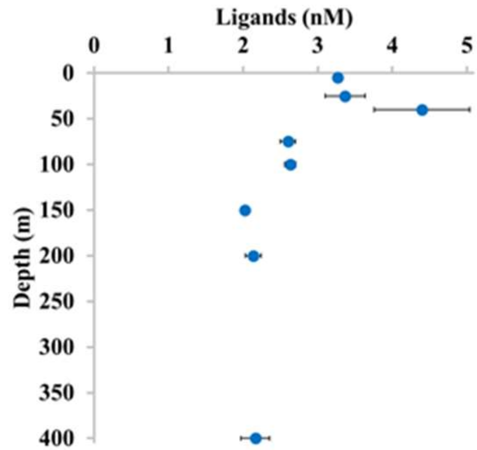
Copper Ligands in the NE Pacific

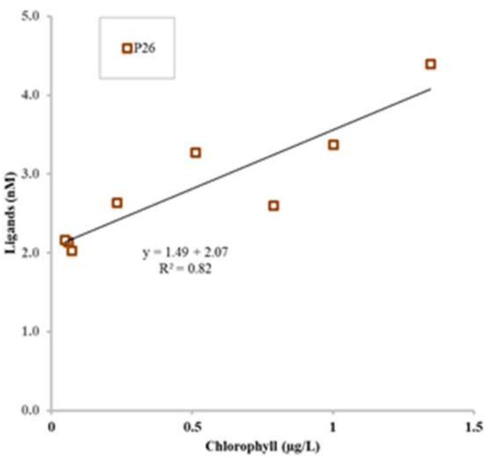


- copper-complexing ligands have also been isolated by Cu(II)-IMAC from filtered seawater samples collected along Line P.
- this includes a depth profile of Cu ligands obtained at station P26 (a.k.a. Station P) in June 2017.

Cu Ligands vs. Chlorophyll at Station P







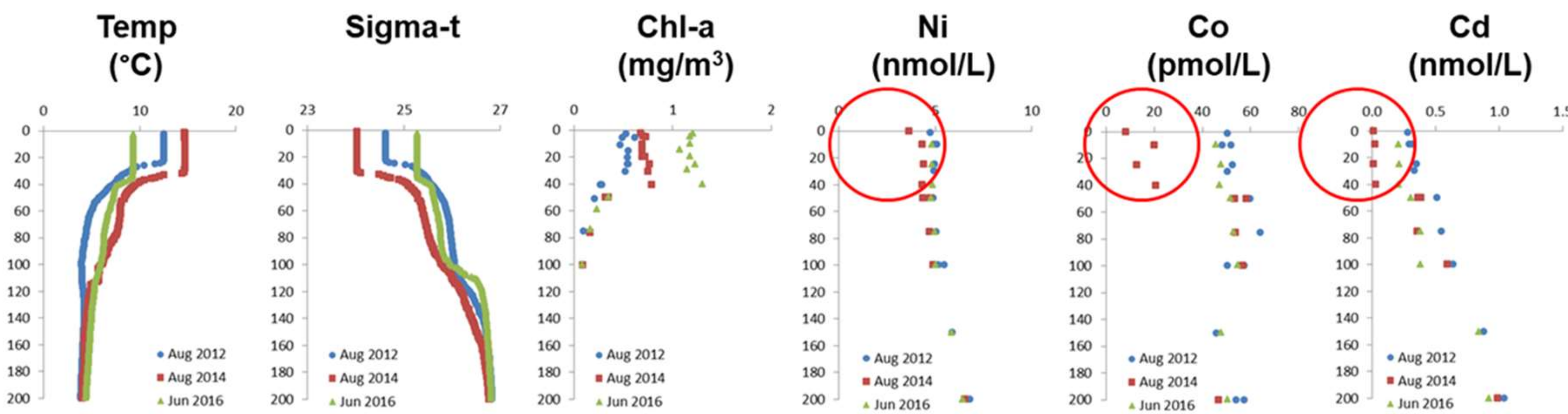
- as in the Canadian Arctic, Cu ligand concentration appears to be correlated with chlorophyll.
- however, the intercept suggests that not all ligands are associated with phytoplankton, and that other sources (e.g. humic substances) may also be significant.

Implications for Climate Change

- the ability of ligand functional groups to bind micronutrient trace metals is affected by water properties.
- lower pH (acidification) inhibits the binding of trace metals leading to higher (potentially toxic) free-ion concentrations.
- equilibria between different chemical forms (speciation) also affected by temperature (warming) and oxygen (hypoxia).
- climate change may affect the ability of phytoplankton and other organisms to regulate metal uptake and/or exposure.

Implications for Climate Change

- increased stratification prevents renewal of micronutrients drawn down by phytoplankton, limiting primary production and altering species distributions (e.g. to smaller cells).
- micronutrients at P26 during the 2014 warming anomaly:



- modeling based on known ligand structures will help to predict climate change impacts on primary productivity and sequestration of atmospheric CO_2 by marine phytoplankton.

Summary

- Cu(II)-IMAC can be used to profile the distribution of copper-binding organic ligands in the marine environment.
- results obtained during the Canadian Arctic GEOTRACES program are the first profiles of copper ligands in the Arctic.
- highest relative abundance of Cu ligands coincides with the chlorophyll maximum (phytoplankton) in these waters.
- similar results were obtained from station P26 in the NE Pacific.
- IMAC produces fractions suitable for MS and MS/MS analysis.
- future work is aimed at optimizing the recovery, identification and structural analysis of ligands in seawater and algal cultures.

Thanks!

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