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27th Jun 22

Dear Dr Bundy,

Your manuscript titled "A review of dissolved organic copper-binding ligand sources, sinks, and internal cycling in the ocean" has now been seen by 3 reviewers, and I include their comments at the end of this message. They find your work of interest, but some important points are raised. We are interested in the possibility of publishing your study in Communications Earth & Environment, but would like to consider your responses to these concerns and assess a revised manuscript before we make a final decision on publication.

We therefore invite you to revise and resubmit your manuscript, along with a point-by-point response that takes into account the points raised. Please highlight all changes in the manuscript text file.

In particular, please ensure that your revised manuscript meets the following editorial threshold:

**** Fully justify or adapt your proposed definitions of L1, L2 and L3 ligands and provide a thorough discussion of the difficulties and complexities associated with such definitions ****

We are committed to providing a fair and constructive peer-review process. Please don't hesitate to contact us if you wish to discuss the revision in more detail.

Please use the following link to submit your revised manuscript, point-by-point response to the referees' comments (which should be in a separate document to any cover letter) and the completed checklist:

[link redacted]

**** This url links to your confidential home page and associated information about manuscripts you may have submitted or be reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage first ****

We hope to receive your revised paper within six weeks; please let us know if you aren't able to submit it within this time so that we can discuss how best to proceed. If we don't hear from you, and the revision process takes significantly longer, we may close your file. In this event, we will still be happy to reconsider your paper at a later date, as long as nothing similar has been accepted for publication at Communications Earth & Environment or published elsewhere in the meantime.

We understand that due to the current global situation, the time required for revision may be longer than usual. We would appreciate it if you could keep us informed about an estimated timescale for resubmission, to facilitate our planning. Of course, if you are unable to estimate, we are happy to accommodate necessary extensions nevertheless.

Please do not hesitate to contact me if you have any questions or would like to discuss these revisions further. We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

Best regards,

Joe Aslin

Locum Chief Editor,
Communications Earth & Environment
<https://www.nature.com/commsenv/>
Twitter: @CommsEarth

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Furthermore, please align your manuscript with our format requirements, which are summarized on the following checklist:

[Communications Earth & Environment formatting checklist](https://www.nature.com/documents/commsj-phys-style-formatting-checklist-article.pdf)

and also in our style and formatting guide [Communications Earth & Environment formatting guide](https://www.nature.com/documents/commsj-phys-style-formatting-guide-accept.pdf) .

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- If applicable, a statement regarding data available with restrictions
- If a dataset has a Digital Object Identifier (DOI) as its unique identifier, we strongly encourage including this in the Reference list and citing the dataset in the Data Availability Statement.

DATA SOURCES: All new data associated with the paper should be placed in a persistent repository where they can be freely and enduringly accessed. We recommend submitting the data to discipline-specific, community-recognized repositories, where possible and a list of recommended repositories

is provided at http://www.nature.com/sdata/policies/repositories.

If a community resource is unavailable, data can be submitted to generalist repositories such as figshare or Dryad Digital Repository. Please provide a unique identifier for the data (for example a DOI or a permanent URL) in the data availability statement, if possible. If the repository does not provide identifiers, we encourage authors to supply the search terms that will return the data. For data that have been obtained from publically available sources, please provide a URL and the specific data product name in the data availability statement. Data with a DOI should be further cited in the methods reference section.

Please refer to our data policies at http://www.nature.com/authors/policies/availability.html.

REVIEWER COMMENTS:

Reviewer #1 (Remarks to the Author):

Comments to Ruacho et al.

This manuscript summarizes our current understanding of Cu-binding organic ligands in the ocean, which I agree is very lacking. The authors correctly note that many further studies will be required for us to fully understand how these ligands behave in marine environments. The lack of consensus on the definition of L1, L2, and L3 is also a problem that leads to inconsistencies and difficulties in comparing data between studies. Though I agree that a definition is needed to better characterize the ligands in the ocean, I don't think arbitrarily assigning log K values that separates L1, L2, and L3 is reasonable. If we assume that a variety of ligands exists with a continuous spectrum of binding strengths, the log K values that are used to define L1 and L2 are artefacts of the analytical method (highly dependent of the detection window used, especially in the case where two or more ligands are detected simultaneously using a single titration curve). Hence, the definitions of L1, L2, and L3 should be inherently different and specific to each study (unfortunately) unless we can find a scientifically sound method to distinguish between each pool of ligands. In fact, the use of the complexing capacity ($=[L]*K$) may be more relevant than [L] and K individually since the calculations of [L] and K from the titration curve are not independent (i.e. the value of [L] depends on K, and vice versa). I admit that because our current understanding of the Cu-binding ligands is so lacking, the authors may not agree with my interpretation on this topic. If so, I feel that at least a discussion of this should be included in the review.

I also think that this review is very light on the description of analytical methods for determining Cu-binding organic ligands in seawater. The authors pointed out that the most common method to determine Cu-binding ligands is CLE-ACSV but earlier methods such as anodic stripping voltammetry may be worth mentioning, as well as why cathodic stripping voltammetry is the preferred method. A schematic diagram that shows how the titrations are carried out and an example of titration curve and its linear and non-linear transformations would be greatly appreciated.

This article would also benefit greatly from having a table that compiles all currently available ligand data, including concentrations and log K of L1, L2, L3, and the analytical methods used, in various ocean basins, marginal seas, coastal areas, and even in rivers. In the current manuscript, I often have to comb through a lot of texts to find the information I need. I also think that some ligand data from the North Pacific region were omitted. Below is some data that may be included in this review at the authors' discretion:

1. Li, Li, et al. "Dissolved trace metal distributions and Cu speciation in the southern Bohai Sea, China." *Marine Chemistry* 172 (2015): 34-45.
2. Wong, K. H., Obata, H., Kim, T., Kondo, Y., & Nishioka, J. (2021). New insights into the biogeochemical cycling of copper in the subarctic Pacific: Distributions, size fractionation, and organic complexation. *Limnology and Oceanography*, 66(4), 1424-1439.
3. Wiwit, Wong, K. H., Fukuda, H., Ogawa, H., Mashio, A. S., Kondo, Y., Nishioka, J., & Obata, H. (2021). Wide-range detection of Cu-binding organic ligands in seawater using reverse titration. *Marine Chemistry*, 230, 103927.

Specific comments:

Abstract: Can you state how many papers have you reviewed and how many ligand data points are included in this review?

Line 96: Including a sentence that lists all of the possible Cu-binding ligands before introducing them individually in following paragraphs would make it easier to follow.

Line 179: In this paragraph, it may be more informative to include more detailed discussion about the concept of "analytical window" or "detection window" by listing earlier studies that employ the "multi-detection window" approach and later attempts to unify data from multiple detection windows to improve precision of the speciation calculations.

Thompson, C. M., M. J. Ellwood, and Sylvia G. Sander. "Dissolved copper speciation in the Tasman Sea, SW Pacific ocean." *Marine Chemistry* 164 (2014): 84-94.

Pižeta, I., Sander, S. G., Hudson, R. J. M., Omanović, D., Baars, O., Barbeau, K. A., ... & Wells, M. (2015). Interpretation of complexometric titration data: An intercomparison of methods for estimating models of trace metal complexation by natural organic ligands. *Marine Chemistry*, 173, 3-24.

Line 259: Perhaps indicate here that the different concentrations of SA equate to different detection windows.

Section 4: This section seems a little confusing. The authors started by discussing about the distribution of Cu-binding ligands in the ocean (section 4.1), which could be nicely followed by section 4.8. I think that the inclusion of the various sources and sinks between 4.1 and 4.8 breaks the flow of the discussion. Perhaps sources (4.2-4.6) – sinks (4.7) – distributions (4.1 & 4.8), in that order and each having its own separate section (for eg. Sections 4, 5, 6, respectively), would be easier for readers to follow? Discussing the distributions of Cu-binding ligands at the end also connects well with the following section regarding modelling efforts.

Section 6: This section seems a little redundant. Most of the information repeat what has already been mentioned in section 4. New information regarding the fluxes and removal rates could be easily summarized in a Table following a short explanation, perhaps integrated into the modelling

section (Section 5).

Reviewed by Kuo Hong Wong

Reviewer #2 (Remarks to the Author):

Review Manuscript#: COMMSENV-22-0371

The manuscript entitled 'A review of dissolved organic copper-binding ligand sources, sinks, and internal cycling in the ocean, with Randelle M bundy as the corresponding author is offering a thorough review of the topic. The critical evaluation of the existing literature shows that a common definition of the different ligand classes, based on their stability constants is still missing, and the paper proposes a three-class structure. They further critically discuss the lack of proper inclusion of Cu binding organic ligands in global biogeochemical models, despite their importance for the dissolved Cu concentrations and the bioavailability of Cu to organisms, which can have consequences of primary productivity

The manuscript is presented in good shape and the most recent literature has been included. I recommend the publication of this study with minor changes which I are listed below.

The only major point that surprised me was that the nomenclature of the theory behind metal speciation and CLE-ACSV contained several errors, which must be corrected, or extended. However, since this is well known stuff I still consider the changes minor and able to be done within a two week period.

Comments

Line 77: what about the critical discussion, recommendations, new insights??? Please add sentence here

Line 94pp :The concept of the mixed complexation of CuI and CII needs more evidence: Why would you think so? Is there a reference for that? Are you talking about the ligand pool or individual ligands? For the pool I agree for individual ligands(classes) I disagree.

Line 129: '... molecular formula [C₂₀H₂₁N₄O₈S₂ +M]⁺ (M = metal isotope),...' I think 'isotope must be ion.

Line 139: I don't see any connection with krill grazing? Is there any evidence (REF) for this. If not it is highly speculative

Line 171: Fist subscripted term missing, in KCu₂⁺ or KCu['], i.e. KCuLi,Cu₂⁺

Line 172: Cu['] not explained

Line 181: the term about the analytical window : $AL = K_{AL} \times [AL']$ and $[AL']^-$ This must be $\alpha_{AL} = \dots$ This is only true for 1:1 complexes of (M) here Cu. This must be displayed correctly! Also you should indicate that KAL is for the complex with Cu.

Line 188: '... , while those outside this window will not be detected.' This is not correct. They are

'seen' but the binding parameter may not be determined correctly.

Line 217: reference for IMAC method missing here.

Line 234/235: '... rapidly accelerate our understanding of marine Cu complexation, but very few published studies exist.' This is the place to list them!

Line 299 typo: ...' ligands is unclear.'

Line 329: additionally to the Buck and Bruland paper of 2005 you may add maybe also add the paper of The effect of natural organic ligands on trace metal speciation in San Francisco Bay: Implications for water quality criteria SG Sander, KN Buck, M Wells, Marine Chemistry 2015, 173, 269-281, Where these high L concentrations and the contributions of endmembers were shown even more, using the same data, but a multi window fit.

Line 350pp 'The variability in binding strengths of detected ligands is likely due, in part, to differences in the analytical window used by each study, but could also be due to differences in the type of ligands associated with sediment sources.' Again here the paper SG Sander, KN Buck, M Wells, Marine Chemistry 2015, 173, 269-281, should be used to show that the way the data is analysed also plays a role for the binding parameters.

Line 352: 'Vey few studies...' References must be given here to see what method they used. For example if ASV was used stability constants may be different. I cannot see any ref to Dario Omanovic's work, who has done some studies on Cu and Cu complexation in estuaries and coastal seas. These are usually very systematic.

Line 538: BLM... The following paper should be considered for applying the BLM to Cu toxicity on mussel larvae. Copper toxicity to blue mussel embryos (*Mytilus galloprovincialis*): The effect of natural dissolved organic matter on copper toxicity in estuarine waters R Zitoun, SJ Clearwater, C Hassler, KJ Thompson, A Albert, SG Sander, Science of the Total Environment 2019, 653, 300-314

Line 646: You should add what phytoplankton concentration this number is based on and how you take into consideration huge regional differences. This also neglects the thought that some areas may be Cu limited. Of course the range you estimate is huge, but would that make any difference to the final numbers or uncertainties?

Line 646: What about microbial Cu uptake as a sink?

Line 653: There has also been a work on degradation of Cu-ligands by sunlight in freshwater, but it may still be important as HS are also part of the marine ligand pool Effect of UVB irradiation on Cu²⁺-binding organic ligands and Cu²⁺ speciation in alpine lake waters of New Zealand S Sander, JP Kim, B Anderson, KA Hunter, Environmental Chemistry 2005, 2 (1), 56-62

Would the numbers there change the estimate provided here?

Reviewer #3 (Remarks to the Author):

In this manuscript, the authors review the current state of knowledge of oceanic Cu binding ligands, including their binding strengths, compounds, analytical methods, distributions, sources and sinks, and future modelling. They suggest an updated classification scheme for ligands based on binding strength, and provide some basic estimates of ligand fluxes that could be used in future modelling efforts.

Overall, I appreciate this paper, which I think is a timely contribution summarising a sometimes complex field. I would not call myself an expert in Cu-binding ligands, and I did find two aspects of the manuscript surprising:

1. The paper concludes that rivers and sediments are the most important sources of Cu-binding ligands to the ocean, as opposed to in situ biological sources.

Is this really true/defensible, given the relatively abundant culturing data showing ligand production at concentrations linearly proportional to Cu concentrations in the culture media (as described in section 4.6)? Perhaps what is meant by this statement is that rivers and sediments are the key external sources of Cu-binding ligands to the oceans? Could this additional in situ source be the key difference that results in observed differences between dCu and Cu-binding ligand distributions (e.g., line 496)?

2. I find the new operational definition proposed for L1, L2 and L3 ligands potentially confusing, albeit I recognise that there is no perfect classification scheme.

Historically (and to me), L1 ligands are those with binding strengths $>$ (about) 12, while L2 ligands are weaker (generally about 8-11). These two groupings would approximately correspond to L1 = strong, low molecular weight complexes and L2 = high molecular weight humic acids and the like (i.e. DOM). Because of the widespread use of L1 in the literature for compounds around 12-13, a switch to $>$ 14 must be based on a strong argument. If I understand correctly, the suggestion is that the $>$ 14 ligands are true 'chalkophores'? i.e. Cu-specific ligands? Yet, lots of phytoplankton produce Cu-binding ligands (albeit perhaps not true 'chalkophores') weaker than this.

Ligands with binding strengths $>$ 14 seem to be rather rare (but maybe not?). Plus, as described, there are inherent analytical uncertainties in measuring binding strengths. If L1 Fe ligands are defined as having strengths $>$ 12, why not be consistent for Cu? The term chalkophore could still be reserved for the very strongest ligands (e.g., methanobactin).

Some other suggestions for improvement/more minor questions:

3. It would be helpful to define $\log K_{CuLi,Cu'}$ earlier and more explicitly in the text (appears only at line 171)

4. You are missing some early papers on Cu binding ligands in seawater (analysed by ASV, which is also not described?):

Coale, K. H., Bruland, K.W., 1988. Copper complexation in the Northeast Pacific. *Limnology and Oceanography* 33 (5), 1084–1101

Coale, K. H., Bruland, K. W., 1990. Spatial and temporal variability in copper complexation in the North Pacific. *Deep-Sea Research Part a-Oceanographic Research Papers* 37 (2), 317–336.

Also: Bruland, K. W., Rue, E. L., Donat, J. R., Skrabal, S. A., Moffett, J. W., 2000. Intercomparison of voltammetric techniques to determine the chemical speciation of dissolved copper in a coastal seawater sample. *Analytica Chimica Acta* 405 (1-2), 99–113.

5. The authors might not want to get into this, but there is an interesting Cu isotope story relating to organic ligands, too... e.g., Little et al., 2018, Ryan et al., 2014

Line 56: even greater than 99%, I thought?

Line 66: I'm not sure I agree with the statement 'relatively little attention has been paid ' given there is literature dating back almost 40 years?

Line 71 and 72: Repetition of 'This review'

Line 217: define acronym 'IMAC'

Line 285: Need a noun after 'This'

Line 297: 'studies', yet only one study referenced?

Line 433: A difference with Fe-binding ligands doesn't seem surprising, given Fe is a scarce nutrient, while Cu is often toxic

Line 526-527: Are rivers not the dominant source of dissolved Cu today?

Line 549: Is the acronym GBCM in common usage? I don't find it very intuitive...

Line 561: Need a noun after 'This'

Line 562: and/or that their ultimate sources (biology) are the same

Line 571: Write out 'Iron' at the start of a sentence.

Line 594: 'compiled' might be a better word than 'found'

Line 674: Is this actually possible?

Little, S. H., Archer, C., Milne, A., Schlosser, C., Achterberg, E. P., Lohan, M. C., & Vance, D. (2018). Paired dissolved and particulate phase Cu isotope distributions in the South Atlantic. *Chemical Geology*, 502, 29–43.

Ryan, B. M., Kirby, J. K., Degryse, F., Scheiderich, K., & McLaughlin, M. J. (2014). Copper isotope fractionation during equilibration with natural and synthetic ligands. *Environmental Science & Technology*, 48(15), 8620–8626.

REVIEWER COMMENTS:

Reviewer #1 (Remarks to the Author):

Comments to Ruacho et al.

This manuscript summarizes our current understanding of Cu-binding organic ligands in the ocean, which I agree is very lacking. The authors correctly note that many further studies will be required for us to fully understand how these ligands behave in marine environments. The lack of consensus on the definition of L1, L2, and L3 is also a problem that leads to inconsistencies and difficulties in comparing data between studies. Though I agree that a definition is needed to better characterize the ligands in the ocean, I don't think arbitrarily assigning log K values that separates L1, L2, and L3 is reasonable. If we assume that a variety of ligands exists with a continuous spectrum of binding strengths, the log K values that are used to define L1 and L2 are artefacts of the analytical method (highly dependent of the detection window used, especially in the case where two or more ligands are detected simultaneously using a single titration curve). Hence, the definitions of L1, L2, and L3 should be inherently different and specific to each study (unfortunately) unless we can find a scientifically sound method to distinguish between each pool of ligands. In fact, the use of the complexing capacity ($=[L]*K$) may be more relevant than [L] and K individually since the calculations of [L] and K from the titration curve are not independent (i.e. the value of [L] depends on K, and vice versa). I admit that because our current understanding of the Cu-binding ligands is so lacking, the authors may not agree with my interpretation on this topic. If so, I feel that at least a discussion of this should be included in the review.

***Response:** We agree that it is difficult to operationally define Cu-binding ligands, because we currently do not know very much about the composition of the ligands that comprise the Cu-binding ligand pool in seawater. However, we feel that there is sufficient data from water column observations and from a few model Cu ligands to operationally define some ligand classes. We also strongly feel that this will help to facilitate the comparison between studies using different analytical methods. While complexing capacity is perhaps a better metric to use to compare ligand binding capacity across studies, it unfortunately does not allow you to understand specific sources and sinks of particular ligand classes.*

We have chosen to keep our current operational definitions of ligand classes, but have explained this in much more detail in the current version of the text (section 3.5). Specifically, we use a newly added compilation table (new Table 2) to justify these definitions, along with information about known model ligands that fall nicely within these new ligand categories.

I also think that this review is very light on the description of analytical methods for determining Cu-binding organic ligands in seawater. The authors pointed out that the most common method to determine Cu-binding ligands is CLE-ACSV but earlier methods such as anodic stripping voltammetry may be worth mentioning, as well as why cathodic stripping voltammetry is the preferred method. A schematic diagram that shows how the titrations are carried out and an example of titration curve and its linear and non-linear transformations would be greatly

appreciated.

Response: *We have now added an additional section to the manuscript (section 3.1) that discusses anodic stripping voltammetry methods, and how the analytical window of that method compares to CLE-ACSV. As mentioned above, we also included a new table (Table 2) that lists all studies to date that used ASV or CLE-ACSV to measure Cu-binding organic ligands in the water column.*

This article would also benefit greatly from having a table that compiles all currently available ligand data, including concentrations and log K of L1, L2, L3, and the analytical methods used, in various ocean basins, marginal seas, coastal areas, and even in rivers. In the current manuscript, I often have to comb through a lot of texts to find the information I need. I also think that some ligand data from the North Pacific region were omitted. Below is some data that may be included in this review at the authors' discretion:

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Response: *All of these studies have now been included in the new Table 2.*

Specific comments:

Abstract: Can you state how many papers have you reviewed and how many ligand data points are included in this review?

Response: *We did not include this information in the abstract, but it is now included in Table 2 and in our discussion of how to operationally define Cu-binding ligand classes (section 3.5).*

Line 96: Including a sentence that lists all of the possible Cu-binding ligands before introducing them individually in following paragraphs would make it easier to follow.

Response: *We have now included a sentence that introduces the variety of compounds first, before talking about each compound type individually (now lines 102-103).*

Line 179: In this paragraph, it may be more informative to include more detailed discussion about the concept of "analytical window" or "detection window" by listing earlier studies that employ the "multi-detection window" approach and later attempts to unify data from multiple detection windows to improve precision of the speciation calculations.

Thompson, C. M., M. J. Ellwood, and Sylvia G. Sander. "Dissolved copper speciation in the Tasman Sea, SW Pacific ocean." *Marine Chemistry* 164 (2014): 84-94.

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estimating models of trace metal complexation by natural organic ligands. Marine Chemistry, 173, 3-24.

Response: *We have now reorganized section 3 and added a discussion of ASV, as well as how the analytical window of ASV compares to the range of windows used for CLE-ACSV. We have also included the references you have listed.*

Line 259: Perhaps indicate here that the different concentrations of SA equate to different detection windows.

Response: *We have ended up deleting this sentence after reorganizing section 3.*

Section 4: This section seems a little confusing. The authors started by discussing about the distribution of Cu-binding ligands in the ocean (section 4.1), which could be nicely followed by section 4.8. I think that the inclusion of the various sources and sinks between 4.1 and 4.8 breaks the flow of the discussion. Perhaps sources (4.2-4.6) – sinks (4.7) – distributions (4.1 & 4.8), in that order and each having its own separate section (for eg. Sections 4, 5, 6, respectively), would be easier for readers to follow? Discussing the distributions of Cu-binding ligands at the end also connects well with the following section regarding modelling efforts.

Response: *Thank you for the recommendation on the reordering of this section. We have taken this advice, and now discuss ligand sources, sinks, and then open ocean distributions and internal cycling. We also combined the OMZ section into the microbial ligand source section.*

Section 6: This section seems a little redundant. Most of the information repeat what has already been mentioned in section 4. New information regarding the fluxes and removal rates could be easily summarized in a Table following a short explanation, perhaps integrated into the modelling section (Section 5).

Response: *We decided to keep this section, but have condensed it and tried to keep it from being redundant of section 4.*

Reviewed by Kuo Hong Wong

Reviewer #2 (Remarks to the Author):

Review Manuscript#: COMMSENV-22-0371

The manuscript entitled ‘A review of dissolved organic copper-binding ligand sources, sinks, and internal cycling in the ocean, with Randelle M Bundy as the corresponding author is offering a thorough review of the topic. The critical evaluation of the existing literature shows that a common definition of the different ligand classes, based on their stability constants is still missing, and the paper proposes a three-class structure. They further critically discuss the lack of proper inclusion of Cu binding organic ligands in global biogeochemical models, despite their

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The manuscript is presented in good shape and the most recent literature has been included. I recommend the publication of this study with minor changes which I are listed below.

The only major point that surprised me was that the nomenclature of the theory behind metal speciation and CLE-ACSV contained several errors, which must be corrected, or extended. However, since this is well known stuff I still consider the changes minor and able to be done within a two week period.

Response: *We have edited section 3 to ensure all nomenclature is correct and consistent, and have added a new section discussing ASV methods, including a discussion of the analytical window of this method.*

Comments

Line 77: what about the critical discussion, recommendations, new insights??? Please add sentence here

Response: *We have added a few sentences discussing the major new insights from this review (now lines 78-83).*

Line 94pp :The concept of the mixed complexation of CuI and CII needs more evidence: Why would you think so? Is there a reference for that? Are you talking about the ligand pool or individual ligands? For the pool I agree for individual ligands(classes) I disagree.

Response: *We have clarified here that we mean the ligand pool and not individual ligands.*

Line 129: ‘... molecular formula [C₂₀H₂₁N₄O₈S₂M]⁺ (M = metal isotope),...’ I think ‘isotope must be ion.

Response: *We changed this to [C₂₀H₂₁N₄O₈S₂M]⁺ (M = metal isotope) because we do mean metal isotope (it could be either ⁶³Cu or ⁶⁵Cu) and it was a compound with a +1 charge.*

Line 139: I don’t see any connection with krill grazing? Is there any evidence (REF) for this. If not it is highly speculative

Response: *We removed the reference to krill here and simply left it as “mass feeding events”.*

Line 171: Fist subscripted term missing, in KCu²⁺ or KCu⁺, i.e. KCuLi,Cu²⁺

Response: *We have reorganized the majority of section 3, which now includes a section describing ASV measurements and better defines all of the relevant terms.*

Line 172: Cu’ not explained

Response: *We have now defined Cu'.*

Line 181: the term about the analytical window : $AL = K_{AL} \times [AL']$ and $[AL']$ - This must be $\alpha AL = \dots$. This is only true for 1:1 complexes of (M) here Cu. This must be displayed correctly! Also you should indicate that KAL is for the complex with Cu.

Response: *We have edited this and explained the differences in the analytical window between ASV and CLE-ACSV.*

Line 188: '... , while those outside this window will not be detected.' This is not correct. They are 'seen' but the binding parameter may not be determined correctly.

Response: *We have edited this sentence to reflect this.*

Line 217: reference for IMAC method missing here.

Response: *We have added the reference.*

Line 234/235: '... rapidly accelerate our understanding of marine Cu complexation, but very few published studies exist.' This is the place to list them!

Response: *We have referred to those papers above, but have now repeated the references here as well.*

Line 299 typo: ... 'ligands is unclear.'

Response: *Thank you, this has been corrected.*

Line 329: additionally to the Buck and Bruland paper of 2005 you may add maybe also add the paper of The effect of natural organic ligands on trace metal speciation in San Francisco Bay: Implications for water quality criteria SG Sander, KN Buck, M Wells, Marine Chemistry 2015, 173, 269-281, Where these high L concentrations and the contributions of endmembers were shown even more, using the same data, but a multi window fit.

Response: *We have now added this reference.*

Line 350pp 'The variability in binding strengths of detected ligands is likely due, in part, to differences in the analytical window used by each study, but could also be due to differences in the type of ligands associated with sediment sources.' Again here the paper SG Sander, KN Buck, M Wells, Marine Chemistry 2015, 173, 269-281, should be used to show that the way the data is analysed also plays a role for the binding parameters.

Response: *We have added this reference and the Wells et al. (2013) reference as well.*

Line 352: 'Very few studies...' References must be given here to see what method they used. For example if ASV was used stability constants may be different. I cannot see any ref to Dario

Omanovic's work, who has done some studies on Cu and Cu complexation in estuaries and coastal seas. These are usually very systematic.

Response: *We have added these references.*

Line 538: BLM... The following paper should be considered for applying the BLM to Cu toxicity on mussel larvae. Copper toxicity to blue mussel embryos (*Mytilus galloprovincialis*): The effect of natural dissolved organic matter on copper toxicity in estuarine waters R Zitoun, SJ Clearwater, C Hassler, KJ Thompson, A Albert, SG Sander, *Science of the Total Environment* 2019, 653, 300-314

Response: *This reference has been added.*

Line 646: You should add what phytoplankton concentration this number is based on and how you take into consideration huge regional differences. This also neglects the thought that some areas may be Cu limited. Of course the range you estimate is huge, but would that make any difference to the final numbers or uncertainties?

Response: *We added a sentence about the caveats to this estimate based on regional differences in phytoplankton and bacteria abundances, etc.*

Line 646: What about microbial Cu uptake as a sink?

Response: *We are not sure what the reviewer means by this, but we have discussed that Cu uptake (from ligand bound Cu) is one of the primary sinks for ligands based on our back of the envelope calculations.*

Line 653: There has also been a work on degradation of Cu-ligands by sunlight in freshwater, but it may still be important as HS are also part of the marine ligand pool Effect of UVB irradiation on Cu²⁺-binding organic ligands and Cu²⁺ speciation in alpine lake waters of New Zealand S Sander, JP Kim, B Anderson, KA Hunter, *Environmental Chemistry* 2005, 2 (1), 56-62
Would the numbers there change the estimate provided here?

Response: *The rates calculated in this study were very low compared to the ones we have used in seawater in the current manuscript, so we did not include them here.*

Reviewer #3 (Remarks to the Author):

In this manuscript, the authors review the current state of knowledge of oceanic Cu binding ligands, including their binding strengths, compounds, analytical methods, distributions, sources and sinks, and future modelling. They suggest an updated classification scheme for ligands based on binding strength, and provide some basic estimates of ligand fluxes that could be used in future modelling efforts.

Overall, I appreciate this paper, which I think is a timely contribution summarising a sometimes

complex field. I would not call myself an expert in Cu-binding ligands, and I did find two aspects of the manuscript surprising:

1. The paper concludes that rivers and sediments are the most important sources of Cu-binding ligands to the ocean, as opposed to in situ biological sources.

Is this really true/defensible, given the relatively abundant culturing data showing ligand production at concentrations linearly proportional to Cu concentrations in the culture media (as described in section 4.6)? Perhaps what is meant by this statement is that rivers and sediments are the key external sources of Cu-binding ligands to the oceans? Could this additional in situ source be the key difference that results in observed differences between dCu and Cu-binding ligand distributions (e.g., line 496)?

Response: *Yes, this is an important point and one that we have now clarified in the manuscript. We suggest based on the available data to date, that rivers and sediments are the major external sources of Cu-binding ligands to the ocean. However, we did not even include a biological ligand production flux in our back of the envelope calculations due to lack of data (Table 4). We have now clarified in the manuscript that it is highly likely that there are other important sources (and sinks) of ligands to the ocean that we are still not able to quantify at this time. Also, it is worth noting that biological production of ligands is also likely the ultimate source of the ligands coming from the sediments and/or rivers as well and we have clarified this in the manuscript.*

2. I find the new operational definition proposed for L1, L2 and L3 ligands potentially confusing, albeit I recognise that there is no perfect classification scheme.

Historically (and to me), L1 ligands are those with binding strengths $>$ (about) 12, while L2 ligands are weaker (generally about 8-11). These two groupings would approximately correspond to L1 = strong, low molecular weight complexes and L2 = high molecular weight humic acids and the like (i.e. DOM). Because of the widespread use of L1 in the literature for compounds around 12-13, a switch to $>$ 14 must be based on a strong argument. If I understand correctly, the suggestion is that the $>$ 14 ligands are true 'chalkophores'? i.e. Cu-specific ligands? Yet, lots of phytoplankton produce Cu-binding ligands (albeit perhaps not true 'chalkophores') weaker than this.

Ligands with binding strengths $>$ 14 seem to be rather rare (but maybe not?). Plus, as described, there are inherent analytical uncertainties in measuring binding strengths. If L1 Fe ligands are defined as having strengths $>$ 12, why not be consistent for Cu? The term chalkophore could still be reserved for the very strongest ligands (e.g., methanobactin).

Response: *We think that perhaps the reviewer is thinking about the logK values for Fe-binding ligands here, which do span the ranges mentioned above. We agree that the classification of Cu-binding ligands is tricky though, because much less is known about these ligands compared to Fe and fewer model ligands exist for us to enable a clear operational definition. At the suggestion of another reviewer, we have compiled all known water column Cu-binding ligand studies to date that have quantified ligands and their binding strengths (new Table 2). From this compilation, it is clear that the range of Cu-binding ligand detected in the marine environment spans a huge range covering several orders of magnitude ($\log K = 7.5-16.5$). This is a much larger range than observed for Fe-binding ligands ($\log K \sim 9-13$). If the ASV studies are ignored in this compilation*

due to their low analytical window (see new section 3.1), then the range of reported L_1 values are from $\log K = 11.5-16.5$ ($n = 23$ studies). This is still a big range, and covers studies that use a variety of analytical windows. It also overlaps significantly with the range in reported L_2 values ($\log K = 9.0-15.5$; $n = 24$ studies). Based on this compilation it appears that ligands with $\log K$ values > 14 are prevalent, and because “chalkophores” have a $\log K$ values > 14 , we have stuck with our suggestion to operationally define L_1 Cu ligands as those with $\log K_s > 14$, since methanobactin seems like a good model “chalkophore” and falls within this operational definition. For the L_2 class, terrestrial and marine humics have $\log K_s$ ranging from 10-12. Finally, exopolysaccharides have $\log K_s \sim 8$, which are representative of model L_3 ligands. Since we have model ligands that fall within these classes, we believe it is helpful for the field to operationally define ligand classes based on these stability constants and those that have been observed in the published water column studies to date. We have thus stuck with our original operational definitions, but have explained this much more thoroughly in the text (section 3.5).

Some other suggestions for improvement/more minor questions:

3. It would be helpful to define $\log K_{CuLi,Cu}$ earlier and more explicitly in the text (appears only at line 171)

Response: We have now defined this earlier and more explicitly in the text.

4. You are missing some early papers on Cu binding ligands in seawater (analysed by ASV, which is also not described?):

Coale, K. H., Bruland, K. W., 1988. Copper complexation in the Northeast Pacific. *Limnology and Oceanography* 33 (5), 1084–1101

Coale, K. H., Bruland, K. W., 1990. Spatial and temporal variability in copper complexation in the North Pacific. *Deep-Sea Research Part a-Oceanographic Research Papers* 37 (2), 317–336.

Also: Bruland, K. W., Rue, E. L., Donat, J. R., Skrabal, S. A., Moffett, J. W., 2000.

Intercomparison of voltammetric techniques to determine the chemical speciation of dissolved copper in a coastal seawater sample. *Analytica Chimica Acta* 405 (1-2), 99–113.

Response: We have now added a section describing ASV methods (now section 3.1), which was also recommended by another reviewer.

5. The authors might not want to get into this, but there is an interesting Cu isotope story relating to organic ligands, too... e.g., Little et al., 2018, Ryan et al., 2014

Response: We have not gone into details regarding Cu isotopes in this paper, though this is interesting and definitely relates to Cu-binding ligands and particularly reversible scavenging. We have added these references in section 4.

Line 56: even greater than 99%, I thought?

Response: Yes, we have now changed to this greater.

Line 66: I'm not sure I agree with the statement 'relatively little attention has been paid' given

there is literature dating back almost 40 years?

Response: *We meant compared to Fe-binding ligands, which are now incorporated into Fe biogeochemical models, while Cu ligands are not.*

Line 71 and 72: Repetition of 'This review'

Response: *We have changed this sentence.*

Line 217: define acronym 'IMAC'

Response: *It has now been defined.*

Line 285: Need a noun after 'This'

Response: *This sentence has been changed.*

Line 297: 'studies', yet only one study referenced?

Response: *We have edited this sentence.*

Line 433: A difference with Fe-binding ligands doesn't seem surprising, given Fe is a scarce nutrient, while Cu is often toxic

Response: *Agreed, we have amended this sentence.*

Line 526-527: Are rivers not the dominant source of dissolved Cu today?

Response: *Yes, they also are the dominant source today, but the paper was focused on the Proterozoic.*

Line 549: Is the acronym GBCM in common usage? I don't find it very intuitive...

Response: *It is common, but we had defined it in the header for that section and it is now defined also in the body of the text.*

Line 561: Need a noun after 'This'

Response: *The sentence has been changed to "These modeling efforts..."*

Line 562: and/or that their ultimate sources (biology) are the same

Response: *Yes, we have added that here.*

Line 571: Write out 'Iron' at the start of a sentence.

Response: *We have previously defined iron (Fe) earlier in the manuscript.*

Line 594: 'compiled' might be a better word than 'found'

Response: *This has been changed.*

Line 674: Is this actually possible?

Response: *We changed this sentence to “associated with sinking organic material.”*

Little, S. H., Archer, C., Milne, A., Schlosser, C., Achterberg, E. P., Lohan, M. C., & Vance, D. (2018). Paired dissolved and particulate phase Cu isotope distributions in the South Atlantic. *Chemical Geology*, 502, 29–43.

Ryan, B. M., Kirby, J. K., Degryse, F., Scheiderich, K., & McLaughlin, M. J. (2014). Copper isotope fractionation during equilibration with natural and synthetic ligands. *Environmental Science & Technology*, 48(15), 8620–8626.

15th Sep 22

Dear Dr Bundy,

Your manuscript titled "A review of dissolved organic copper-binding ligand sources, sinks, and internal cycling in the ocean" has now been seen again by our reviewers, whose comments appear below. In light of their advice I am delighted to say that we are happy, in principle, to publish a suitably revised version in Communications Earth & Environment under the open access CC BY license (Creative Commons Attribution v4.0 International License).

We therefore invite you to revise your paper one last time to address the remaining comments of our reviewers. At the same time we ask that you edit your manuscript to comply with our format requirements and to maximise the accessibility and therefore the impact of your work.

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REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

This revised manuscript by Ruacho et al. following the 1st revision is much improved with the addition of Table 2 which lists all the major Cu ligand studies globally, as well as the section on ACSV. The flow of the discussion is also greatly improved.

The authors also added a brief discussion on how the definitions of L1, L2, and L3 were decided, which I find satisfactory with our current knowledge.

Therefore, I recommend this manuscript for publication.

However, there are minor points the authors should address prior to publication:

Line 20: Here, dissolved copper is abbreviated as Cu, but in line 54, it is abbreviated as dCu. Throughout the manuscript Cu is used predominantly while dCu is only used sparingly. In many cases where dCu should be used (such as dCu-binding organic ligands, since those ligands are binding to dissolved Cu), Cu was used instead. The authors should standardize these abbreviations.

Section 3.1: Does ACSV have lower sensitivity (higher detection limit) because it does not use artificial ligand such as SA that help increase the measurement sensitivity? If so, this should be included in the discussion.

Line 442: Here, the authors stated that humic acid bind "strongly" to Cu, whereas in line 393, it was stated that humic acid is a weak ligand. The use of strong and weak to refer to humic acid as a ligand may be a little confusing.

Line 507: How does organic ligand impact the residence time of dCu near hydrothermal vent?

Line 561: the meaning of "free Cu concentration is vital to AOA" is unclear.

Fig. 4: I don't quite understand what the arrow indicating Cu limitation and Cu toxicity is trying to explain. The authors may be indicating that Cu is toxic to phytoplankton in coastal regions because of the higher dCu concentrations. However, studies have shown that both coastal regions and open oceans have similar pCu levels that indicate no Cu toxicity. If there is a justification for the arrow, please include it in the manuscript.

Reviewed by Kuo Hong Wong.

Reviewer #2 (Remarks to the Author):

The authors of the manuscript 'A review of dissolved organic copper-binding ligand sources, sinks, and internal cycling in the ocean' have addressed all comments and recommendations I made during the initial review of the work.

I am satisfied with the revision and recommend the acceptance of this manuscript for publication.

Kind regards
Sylvia Sander

Reviewer #3 (Remarks to the Author):

I am very happy with the revised version of this manuscript, which I think is much improved. Thanks to the authors for their hard work!

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Response: Thank you, we have changed all of the places where it says dCu to say dissolved Cu for clarity.

Section 3.1: Does ACSV have lower sensitivity (higher detection limit) because it does not use artificial ligand such as SA that help increase the measurement sensitivity? If so, this should be included in the discussion.

Response: We did not mention that ASV has a higher sensitivity relative to the more indirect speciation techniques because the methods are measuring different things, and ACSV does not necessarily have a higher sensitivity depending on the instrument being used (including mercury drop size) and the samples being measured (e.g. samples with very low concentrations of labile Cu).

Line 442: Here, the authors stated that humic acid bind "strongly" to Cu, whereas in line 393, it was stated that humic acid is a weak ligand. The use of strong and weak to refer to humic acid as a ligand may be a little confusing.

Response: Thank you, we have fixed this statement.

Line 507: How does organic ligand impact the residence time of dCu near hydrothermal vent?

Response: We changed this sentence to, "If hydrothermal vents are indeed a source of Cu-binding ligands to the overlying water column this would be important to constrain, as it would impact the transport of dissolved Cu in these systems."

Line 561: the meaning of "free Cu concentration is vital to AOA" is unclear.

Response: We edited this sentence for clarity, and it now reads, "These observations indicate a potential link between Cu-complexation in seawater and AOA growth, since AOA appear to primarily take up free Cu."

Fig. 4: I don't quite understand what the arrow indicating Cu limitation and Cu toxicity is trying to explain. The authors may be indicating that Cu is toxic to phytoplankton in coastal regions because of the higher dCu concentrations. However, studies have shown that both coastal regions and open oceans have similar pCu levels that indicate no Cu toxicity. If there is a justification for the arrow, please include it in the manuscript.

Response: We added additional details in the figure caption related to the arrow in Figure 4.

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