

RESPONSE TO EDITOR'S REVIEW 11/1/24 (RESPONSES IN BLUE)

Dear authors,

Thank you for your responses to the reviewers. All three referees highlighted the interest and high quality of the observations reported in your manuscript. However, they also noted that the paper would benefit from some re-writing, particularly in the Abstract, Introduction and the Conclusions, so that the text is more informative and concise. The figures also need to be revised, because they are quite small and need to be seen at >200% zoom, at which point some become pixelated (e.g. Fig. 4). For consistency, all figures should use the same font type in axis labels and titles. Additional method details should be provided, as indicated by reviewer 3.

I therefore invite you to prepare a revised version of your manuscript, which should address the reviewers' comments as outlined in your letter of response.

Thank you for submitting your work to Biogeosciences.

Best regards,
Emilio Marañón

>>Thank you for your review. We have rewritten the Abstract, Introduction, and Conclusions as noted below in responses to individual reviewers. We have also improved the quality of all images (to a minimum of 300dpi). All figures now use the same font type in axis labels and titles. We have added additional method details as indicated by Reviewer 3. These additional details were prepared by Ichiko Sugiyama, who we therefore now list as a coauthor.

RESPONSE TO REVIEWS 10/31/24 (RESPONSES IN BLUE)

High metabolic zinc demand within native Amundsen and Ross Sea phytoplankton communities determined by stable isotope uptake rate measurements

RC1: ['Comment on egusphere-2024-2085'](#), Yeala Shaked, 24 Aug 2024

The study was conducted in the Amundsen Sea, Ross Sea, and Terra Nova Bay, focusing on phytoplankton in the >3 μm particulate size fraction within these highly productive Antarctic regions. It aimed to investigate the demand for dissolved Zn (dZn) and dissolved cadmium (dCd) in Southern Ocean phytoplankton communities using ^{67}Zn and ^{110}Cd tracer uptake experiments. This is the first study reporting direct measurements of dZn uptake in a natural environment. (due to the risk of contamination during sea-based studies). Some of its main findings include high metal uptake rates (pMetal) for both Zn and Cd, which were consistent with the observed depletion of dZn and dCd in surface waters. Also, high biomass and low seawater pCO₂ were identified as primary factors controlling the increase in particulate Zn (pZn), likely leading to increases in particulate Cd (pCd) due to the upregulation of shared transport systems.

This is a very large dataset. The experiments are well performed, and the many results are presented clearly. The authors are very knowledgeable and try to cover most aspects of the field (surface depletion, link to Si, Zn-Cd replacement, etc.). The product is a rather long, detailed, and informative manuscript, but I wonder if the messages can be streamlined a bit to get the major

points across more clearly. It seems that many writers have contributed their input over time, but then a brave last reader/leader did not integrate the parts and selected and re-wrote the 2-3 key points that should be repeated in the abstract, the end of the intro, and the conclusions. The abstract is too educational and less clear about the major points. The conclusions are interesting and add to the paper, but they are not written as conclusions as instead of a bullet-point short closure, they deal with Irwing-Williams and Cobalt distribution.

Thank you for your appreciation of the study. We agree that our abstract and conclusion should be rewritten, and have done this to better summarize our discussion and capture our take-away findings. We have also made an effort to streamline our text for clarity.

We have updated our abstract to the following text:

“Zinc (Zn) is an essential micronutrient for most eukaryotic phytoplankton. Zn uptake by phytoplankton within the euphotic zone results in nutrient-like dissolved Zn (dZn) profiles with a large dynamic range. The combination of key biochemical uses for Zn and large vertical gradients in dZn implies the potential for rapid rates of Zn removal from the surface ocean. However, due to the ease of contamination at sea, direct measurements of dZn uptake within natural environments have not been previously made. To investigate the demand for dZn and for dissolved cadmium (dCd; a closely related nutrient-like element) within Southern Ocean phytoplankton communities, we conducted ^{67}Zn and ^{110}Cd tracer uptake experiments within the Amundsen Sea, Ross Sea, and Terra Nova Bay of the Southern Ocean. We observed a high magnitude of Zn uptake ($\rho\text{Zn} > 100 \text{ pmol dZn L}^{-1} \text{ d}^{-1}$) into the particulate phase that was consistent with ambient depleted dZn surface concentrations. High biomass and low seawater pCO_2 appeared to contribute to ρZn , which also led to increases in ρCd likely through the upregulation of shared transport systems. These high ρZn measurements further imply that only short timescales are needed to deplete the large winter dZn inventory down to the observed surface levels in this important carbon-capturing region. Overall, the high magnitude of Zn uptake into the particulate fraction suggests that even in the Zn-rich waters of the Southern Ocean, high Zn uptake rates can lead to Zn depletion and potential Zn scarcity.”

We have moved the text in the conclusion detailing the effects on Co to a new paragraph in the Discussion (section 4.3, Effects on dCo cycling).

We have updated our conclusions to the following text: “We have quantified the movement of the trace metals Zn and Cd from the dissolved to the particulate phase within the phytoplankton $>3 \mu\text{m}$ size fraction collected in the Amundsen Sea, Ross Sea, and Terra Nova Bay of the Southern Ocean during austral summer 2017-2018. Our study adapts the stable Cd isotope tracer uptake method (Cox et al., 2014) to the measurement of Zn uptake, and represents the first time series measurements of dZn and Zn uptake in a coastal environment during a bloom event. We have found that these high observed uptake rates were sufficient to draw down the otherwise abundant dZn inventory on seasonal timescales. Our analysis suggests that enhanced total biomass and low pCO_2 act as primary influences on ρZn , and that high ρZn results in dynamic changes in the cycling of both Cd and Co as a consequence of high Zn demand. Low dZn:Si slope values observed in the Ross Sea and Terra Nova Bay further highlighted the intense drawdown of dZn by biota to meet a high metabolic dZn demand. Overall, our study demonstrates that Zn demand is high and rapid enough to depress the inventory of Zn available to phytoplankton, suggesting that Zn has been overlooked as a dynamic limiting micronutrient in primary productivity modeling.”

I like that the discussion has titles and deals with specific topics. I do wonder if all these topics

should be included as, in some cases, the figures are barely mentioned (e.g., Fig. 12). I leave it to the authors, but you may consider removing some topics (like Zn/Si), so that you can develop better other topics. If you decide to leave all these topics, please clarify from the start (intro) that these are your goals and that you can indeed solve/add to the issues raised in the introduction. A table can be an excellent way to present/conclude the different aspects & insights from this study (like the higher Cd uptake rates, the differences in phytoplankton composition between basins and its links to Zn/Si, use of Cd-CA, etc...

Overall, I applaud the authors for a very detailed, analytically challenging, and high-quality study. My suggestions are meant to ease the read by streamlining the paper and increasing its impact.

Thank you—we agree and have edited our introduction so that it clearly sets up the goals of our study. We have moved the Zn/Si information out of the conclusion and into the discussion surrounding the final figure (discussion section 4.4, dZn and dCd relationships with macronutrients).

As Fig.12 makes a relatively minor (but relevant) contribution to the discussion, we have moved it to Supplementary.

RC2: ['Comment on egusphere-2024-2085'](#), Anonymous Referee #2, 14 Sep 2024

These are technically demanding studies that deliver valuable insight into metallobiology as it occurs in a natural environment. The notion that Zn, not the more anticipated Fe, could limit primary productivity due to its depletion via high levels of productivity in the Antarctic Southern Ocean, is a most important and original contribution. The choice of this environment which re-sets its nutrient distribution each year is especially neat.

[Thank you for your appreciation of the study.](#)

The text may benefit from a minor caveat. The estimated uptake rate of ^{67}Zn suggests that (in the extreme case) it would take a mere 25 days to deplete surface Zn from 4.7 nM to an observed level of 1.7 nM. It is noted in the text that this assumes a constant Zn uptake rate, but it may be worth restating the conditions under which the rate was determined for this extreme case (for example): If I understand correctly, the uptake rate was a 24 h measurement using a particulate fraction spiked to 2 nM Zn, but what was the ambient dissolved concentration in the seawater at the time of sampling? Cells contain zinc sensors (like SmtB, ZiaR, Zur in cyanobacteria for example) that modulate Zn uptake, export and storage. Their status at the start of the experiment, then after exposure to the spike, is pertinent to estimating whether actual depletion is liable to be longer or shorter than the 25 day estimate.

Thank you for this suggestion—the observed level of 1.7nM dZn at station 11 is the dissolved Zn concentration in the seawater at station 11, observed at the time of sampling. So to this surface seawater with 1.7nM Zn, we added an additional 2nM Zn spike (as ^{67}Zn) to measure total Zn uptake (our uptake rate equation takes into account both the added spike and the total ambient dZn).

We have added a reference to Fig. 4b (showing dZn concentrations at station 11) to better clarify this, and changed the text to “Taking station 11, for which the highest Zn uptake rate was observed, as an extreme case: with a maximum Zn uptake rate of 158 pmol L⁻¹ d⁻¹, it would take only 25 days to deplete a surface winter concentration of 4.8 nM (that is, the average deepwater (< 200 m) dZn concentration for all stations measured in this study) down to the observed surface concentration of 1.7 nM at station 11 (Fig. 4b), assuming a constant uptake rate and no additional inputs of dissolved Zn (Fig. 10).”

We appreciate that our simple depletion timeframe calculation is meant as a proof-of-concept to

demonstrate that dZn depletion can occur quickly, and important caveats must be included. We have added this text: “An important caveat to this simplistic calculation is that the regulation and production of Zn sensors that modulate Zn uptake, export, and storage will naturally fluctuate in response to changing dZn and therefore cannot be assumed as a constant.”

RC3: '[Comment on egusphere-2024-2085](#)', Anonymous Referee #3, 26 Sep 2024

This study presented a comprehensive biogeochemical dataset from the Southern Ocean and based on that the authors provided knowledge on trace metals uptake in micronutrient-depleted ocean water. The study shows relevance to the Southern Ocean carbon export and climate change. In my opinion, the manuscript was prepared poorly. Starting from the abstract to the conclusion, everything is mixed up. There are a lot of inconsistencies in the methods. There are too many figures with poor quality. Sometimes, the fonts are not readable. I suggest the authors rewrite the manuscript concisely. The current version is too lengthy, but there is little discussion. Here are the comments:

We have made an effort to better clarify our methods, given these comments. We have improved the quality of all figures to a minimum of 300 dpi before adding to the word document and converting to a pdf. In agreement with R1 and this comment, we have rewritten the abstract, introduction and conclusions for clarity.

Line 13-28: This is the general knowledge on the Southern Ocean, can't be the abstract of a research article. Except for the sentence "To investigate.....", the entire first paragraph provides knowledge that can be associated with an introduction but not an abstract. I suggest rewriting the abstract.

In agreement with R1 and this comment, we have rewritten the abstract.

Line 34: On what basis this value is high?

Line 24 was “Overall, we observed a high magnitude of Zn uptake ($> 100 \text{ pmol dZn L}^{-1} \text{ d}^{-1}$) into the particulate phase within these Southern Ocean phytoplankton communities.”

Within the study area, Zn uptake rates ranged from $\sim 10 \text{ pmol/L-d}$ to $>100 \text{ pmol/L/d}$, therefore 100 was on the high end.

Introduction: Overall, shorten the introduction

In agreement with R1 and this comment, we have shortened the introduction.

Line 95-96: Add a supporting reference

Line 95-96 was “The rapid removal of dZn from the surface within the Southern Ocean suggests the possibility for phytoplankton growth to become Zn-limited.”

We have added a reference to our preprint documenting the removal of surface dZn creating Zn stress. As uptake rates are the focus of the present study, we have rewritten the text to: “Substantial removal of dZn from Southern Ocean surface waters appears associated with high biomass blooms and low pCO₂ conditions during austral spring and summer, creating the potential for phytoplankton growth to become Zn and carbon co-limited (Kell et al., 2023; Morel et al., 1994).”

Line 99-102: Antarctic sea releases all trace metals including Zn during melting season. This statement is only valid if the Fe:Zn ratio in the sea ice exceeds than Fe: Zn uptake ratio. I suggest providing supporting information to back this statement.

Lines 99-102 were “While phytoplankton growth in the Southern Ocean is well-known to be primarily limited by Fe availability (Arrigo et al., 2008; Martin, 1990), melting icebergs and ice shelves are known to act as external sources of Fe (Hopwood et al., 2019; Person et al., 2021; St-Laurent et al., 2017) with larger Fe inputs expected from increased ice melt in a warming climate. Increased dFe inputs to surface Antarctic waters may act to relieve Fe stress, but would simultaneously support the development of other nutrient limitations.”

There is a large amount of lithogenic particulate material being entrained in melting of the Antarctic ice shelf. This is due to the ice shelf being anchored on the continent and grinding the seafloor as it moves (including as glaciers) entraining sedimentary iron. Studies have demonstrated large inputs of iron from ice shelf melting both in the form of particulate and subsequently dissolved iron (Planquette et al., 2013). The amount of Fe in crustal material (a standard proxy for lithogenic material) is much higher than for Zn (3.5% as Fe versus 71 ppm (or 0.0071%) as Zn; Taylor and McLennan 1985). Of course, not all of the lithogenic iron (or zinc) is soluble.

We have added two sentences about the lower lithogenic abundance of Zn in entrained material: “The majority of ice-melted Fe input is sourced from particulate lithogenic material (entrained during grounding of ice shelves on the continent and sediments). Fe in crustal material is more abundant than Zn (3.5% as Fe versus 0.0071% as Zn) (Taylor and McLennan, 1985), creating a large inventory of particulate Fe available that can be partially dissolved by biotic and abiotic processes.”

Also note there is a distinction between sea ice and ice shelf and glacier melting, where sea ice is formed from the seawater, whereas the ice shelf is formed on the Antarctic continent and migrates into the ocean through glaciers and ice berg calving. Sea ice also concentrates sedimentary/lithogenic particulate material on its base becoming a capacitor for iron (Noble et al., 2013). The reviewer’s notion of a Fe:Zn ratio seems to focus on the dissolved phase, but as in most coastal environments, the particulate phase plays a major role in iron’s biogeochemical cycling.

Line 107-110: Polynyas receive nutrients from surrounding sea ice. Given the sea ice Fe/Zn ratio is not discussed here, the idea of Zn stress is speculative.

The text was “Coastal polynyas that form within the Amundsen and Ross Seas during austral spring and summer are particularly primed to experience Zn stress as these regions host highly productive seasonal phytoplankton blooms that act as significant carbon sinks (Arrigo et al., 2012).” Please see the discussion above and the added sentence about the crustal Fe:Zn ratio.

DZn content in the Antarctic sea ice is often higher than dFe
(Check <https://www.sciencedirect.com/science/article/pii/S0304420311000107>)

Please see above. Also see review by Lannuzel et al. 2016
(<https://doi.org/10.12952/journal.elementa.000130>) for a more complete discussion.

Line 131: 4) Do you mean living particulate matter?

The text was “The transfer of added isotopes into the particulate phase is the combined result of 1) active transport of metal into cells, 2) nonspecific metal adsorption to cell surfaces, 3) metal

adsorption to non-living particulate organic matter, and 4) metal adsorption to particulate inorganic matter,..."

Adsorption to living particulate matter is described by 2). By 4), we mean adsorption to nonliving particles that do not contain organic carbon. We have rephrased 4) to "metal adsorption to non-living particulate inorganic matter".

Line 176-185: This paragraph should not come under Analyses. It should come under sub-sampling

We have renamed this Methods section "2.3 Sampling for total dissolved metal analyses", and labeled the next paragraph "2.4 Analyses of total dissolved Cd and Zn using isotope dilution".

Line 225-227: Will move to section 2.2.

We agree and have moved this sentence describing plasticware cleaning to section 2.2, 'Preparation of plasticware.'

Line 232: In this study, the reported Cd concentration is high enough (up to ca. 800 pmol/L), so Mo oxide-induced Cd interference is expected to be relatively lower considering seawater Mo concentration. However, in polynyas water, limited knowledge exists on dissolved Mo concentration. If dMo concentrations are enriched in the studied area (Mo-induced Cd concentrations increase linearly with Mo concentration), measured dCd requires re-estimation. I suggest you provide background information on dMo concentration, if available.

Seawater dMo concentrations were not measured in this study, but as Mo is conservative in seawater (Tuit 2003, [The marine biogeochemistry of molybdenum \(mit.edu\)](#)), we do not anticipate dMo being elevated in polynyas. We note that our dCd values were consistent with GSC standard consensus results.

Line 238: Table S3 has been referenced before Table S2. Please check the sequence carefully. What about blank values, detection limits, and sample reproducibility? This information is important before reporting the dissolved trace metal dataset.

The tables are now in correct order.

Regarding reproducibility—The calculation of total metals using the isotope dilution/seawater preconcentration (seaFAST) technique was done in singlicate for 312 samples (12 bottles *26 stations). Time constraints prevented this analysis in technical duplicate (624 samples total), but we note that our dMetal profiles are oceanographically consistent and the GEOTRACES GSC average standard measurements were in agreement with average consensus values (see Table S2). Particulate ⁶⁷Zn and ¹¹⁰Cd measurements were done in technical duplicate. We have prepared a supplemental file showing every measurement, the average of duplicates, and the range of duplicates. For each Zn sample (222 total), the range was less than 10pM, while for each Cd sample (224 total), the range was less than 1.49 pM. We have added this sentence to the Methods, section 2.7 Filter digestion and particulate ICP-MS analysis: "Duplicate values were in good agreement (Supplementary File 1), and the average value was used in further calculations.":

Regarding blanks and the limit of detection—our instrument blanks possessed very low dMetal concentrations, and therefore a low limit of detection. We have added this to a new Methods section, "2.5 Procedural Blanks and limit of detection (LOD)." 2.5 Procedural blanks and limit of

detection (LOD). The text now reads: “Procedural blanks were quantified by preconcentrating 30mL of MiliQ water adjusted to pH 2 with HCl (Optima, Fisher Scientific) to 1mL. Metal concentrations were determined using an external SPEX multi-element standard as described above. The LOD was calculated as 3 x the standard deviation of the blank measurements (13.5 pM Fe, 2.9 pM Ni, 1.6 pM Cu, 38.1 pM Zn, and 0.3 pM Cd).”

Line 281-282: Based on what you made this presumption? In the previous para, you mentioned the published Zn and Cd concentrations of Ross Sea water, which are 2 nM and 300 pM, respectively. Is this concentration lower compared to the HNLC water? Please give a reference value so that you can say the value is depleted.

The text was “As this seawater is naturally depleted in both metals, the spike addition artificially increases the total Zn and Cd present and thus could perturb the response of biology to these additions.”

Natural surface depletion of dZn and dCd is both demonstrated in this manuscript (see depth profiles) and has been documented previously (<https://doi.org/10.1016/j.marchem.2008.07.008>, <https://doi.org/10.1016/j.gca.2019.09.039>).

Line 333-335: The seawater already has dCd and dZn loads (without the adding spikes), which could be taken up by phytoplankton during incubation and form particulate matter. Do you have control data (treatment without adding Cd and Zn)? To understand Pre-existing particulates, the value needs to be subtracted by control.

The text reads “The pre-existing particulate value for ¹¹⁰Cd was obtained from incubation bottles that had Zn added, but no Cd spike. Likewise, the pre-existing particulate value for ⁶⁷Zn was obtained from incubation bottles that had Cd added, but no Zn spike. The ⁶⁷Zn spike solution was confirmed to contain virtually no ¹¹⁰Cd, ¹¹¹Cd, nor ¹¹⁴Cd. The ¹¹⁰Cd spike was likewise confirmed to contain virtually no ⁶⁷Zn, ⁶⁴Zn, nor ⁶⁶Zn.”

We did not include a separate control, completely unspiked bottle due to sampling time/materials constraints (including a third bottle would have increased our sampling load from ~252 bottles (18 stations * ~7 depths * 2 bottles) to ~378 bottles). The text above describes how we got around this issue. Pre-existing particulate ⁶⁷Zn was measured in the +¹¹⁰Cd bottle, and pre-existing particulate ¹¹⁰Cd was measured in the +⁶⁷Zn bottle. The two bottles served as each other’s background. Background (that is, pre-existing particulate) pZn was subtracted from measured pZn, and background pCd was subtracted from measured pCd.

Line 339-341: This is a wild assumption that just is mentioned previously. Why don't you use control to show the magnitude of natural uptake?

Lines 339-341 were “As a result, we assumed that the added ⁶⁷Zn spike did not affect the pre-existing Cd, nor did the ¹¹⁰Cd spike affect the pre-existing Zn. It is assumed that the pre-existing particulate blank was in steady state, i.e. that it represented the Cd or Zn already in the particulate fraction and that any possible natural uptake that could occur during incubation for 24 h was negligible.”

Please see above regarding our sampling constraints. Fig.S1 demonstrates that our technique was sound, with background ⁶⁷Zn:⁶⁶Zn measured in the +¹¹⁰Cd bottle falling on the natural abundance ratio line. The same was true for ¹¹⁰Cd:¹¹⁴Cd measured from the +⁶⁷Zn bottle.

Line 406: What is the detection limit of dFe? It's unusual to see ICPMS and seaFAST can measure as low as 0.01 nM Fe.

Please see above regarding our blanks and LOD. The Fe detection limit was 13.5pM.

Figure 4: I suggest you show the results for control in left panels. I see the control in Figure S1 (left panels) where you showed the ratios. Do the same here as its important.

The same is applicable in the other two study areas.

As mentioned above, we did not include a “control” (ie, completely unspiked) bottle, but rather, the Cd spiked bottles served to quantify the background pZn, and the Zn spiked bottles served to quantify the background pCd. Please see above regarding bottle load. Figure S1 is showing the natural measured abundance (“background”) ratios of particulate $^{67}\text{Zn}:^{68}\text{Zn}$ measured in the ^{110}Cd spiked bottle, and the natural measured abundance (“background”) ratios of particulate $^{110}\text{Cd}:^{111}\text{Cd}$ measured in the ^{67}Zn spiked bottle. Background pZn was subtracted from measured pZn, and background pCd was subtracted from measured pCd. We have removed the word “control” from the figure and replaced it with “background”. We have added this text to the Fig.S1 caption: “The background particulate $^{67}\text{Zn}:^{68}\text{Zn}$ was measured from the ^{110}Cd -spiked bottle. The background particulate $^{110}\text{Cd}:^{114}\text{Cd}$ was measured from the ^{67}Zn spiked bottle.”

Line 524-542: Until this point, it reads like result, and it should go to result.

We have moved this paragraph up into Results, and it is now Results section 3.4.

From here, a few explanations should be mentioned in methods, such as depth-integrated stock. It should also be included that why Chlorophyll normalized uptake rate has been used?

We present chlorophyll-normalized uptake rate data because biomass levels were variable across all stations. Normalization allows for the comparison of uptake rates across all stations by accounting for the density of biomass at each station.

To the Statistics and Plotting section of Methods, we have added this text describing depth-integrated uptake rates: “Depth-integrated uptake rates were calculated using the ‘auc’ function within the Scikit-learn (v0.23.2) Python library.”