Response to Reviews 7/2/25 (Responses in blue)

Zinc stimulation of phytoplankton in a low carbon dioxide, coastal Antarctic environment: evidence for the Zn hypothesis

RC1: 'Comment on egusphere-2025-1609', Anonymous Referee #1, 25 Apr 2025

Kell et al., report an incubation based study to test whether primary producers in an Antarctic coastal environment respond to increased Zn availability. Whereas light, Fe and to a lesser extent Mn, are well established as drivers of productivity in Antarctic coastal ecosystems, any effects of Zn have not been well explored. The authors use multiple lines of argument to show that a state of co-limitation by Fe and Zn is possible. I am a trace metal chemist so cannot comment in depth on the metaproteomic or metatranscriptomic analyses. Overall I think the subject is topical and the text provides some interesting insights into Zn dynamics.

Minor comments (by line number):

I have not read much about dZn concentrations around Antarctica, I assume because it has not been measured much, if some values are reported in the literature I would find it interesting to refer to them in a few sentences just to understand what sort of range and normal profile should be expected in these coastal environments.

> Thank you for this comment, which allowed us to realize that additional context regarding dZn around Antarctica is needed in the introduction. There are actually several studies that have documented the distribution of dZn around Antarctica, and these measurements typically show nutrient-like vertical profiles. An example from Sieber et al 2020 showing typical nutrient-like profiles is linked in the paper below (see their Figure 2), in addition to our prior study showing nutrient-like profiles of dZn in the study region (Kell et al., 2024)

Sieber, M., Conway, T.M., de Souza, G.F., Hassler, C.S., Ellwood, M.J. and Vance, D., 2020. Cycling of zinc and its isotopes across multiple zones of the Southern Ocean: Insights from the Antarctic Circumnavigation Expedition. *Geochimica et Cosmochimica Acta*, *268*, pp.310-324.

Kell, R.M., Chmiel, R.J., Rao, D., Moran, D.M., McIlvin, M.R., Horner, T.J., Schanke, N.L., Sugiyama, I., Dunbar, R.B., DiTullio, G.R. and Saito, M.A., 2024. High metabolic zinc demand within native Amundsen and Ross sea phytoplankton communities determined by stable isotope uptake rate measurements. *Biogeosciences*, *21*(24), pp.5685-5706.

> We propose to add the following text to the introduction to introduce the current knowledge of dZn measurements in the Southern Ocean:

"Vertical profiles of dZn in the Southern Ocean have been measured previously. Zn has not historically been considered as a limiting micronutrient in the Southern Ocean due to the upwelling of nutrient-rich waters that bring dZn to nanomolar concentrations only a couple hundred meters below the surface. Yet nutrient-like profiles of dZn are evident throughout this region, with surface depletion due to biological uptake decreasing this large inventory in the upper water column (Fitzwater et al. 2000; Coale et al. 2005; Baars and Croot 2011; Sieber et al. 2020; Kell et al. 2024).

Additionally, both model-based estimates (Roshan et al. 2018) and direct field measurements (Kell et al. 2024) of Zn uptake in this region have demonstrated a substantial biological demand for Zn in surface waters, leading to significant dZn drawdown. This is consistent with and genomic and laboratory studies indicating an elevated Zn demand in polar phytoplankton (Twining and Baines 2013; Ye et al. 2022).

42-43 The concept of Zn limitation mainly applies to low pCO2 environments which arise in various coastal areas for different reasons, it is not clear to me how pCO2 in these "low" CO2 zones will respond to future climate change as this likely depends on shifts in productivity, upwelling and freshwater discharge in addition to a slow increase in atmospheric pCO2, so I didn't find this framing of changes in global CO2 to be particularly relevant to the main story. I would have been more interested to know why these low pCO2 zones exist, but maybe even this is getting a little away from the main focus of the text and I think the text would be fine without it.

> Line 42-43 was: “This study definitively establishes that Zn limitation can occur in the modern oceans, opening up new possibility space in our understanding of nutrient regulation of NPP through geologic time, and we consider the future of oceanic Zn limitation in the face of climate change.”

>In this study, biology was the driver of the observed decrease in pCO2, rather than freshwater input from glacial and sea ice melt. Please see our comment below. Due to the connection between Zn limitation and C acquisition, we feel maintaining this description is valuable, and as described below, we will modify the text to clarify this.

50 I would refer instead to the later Browning and Moore work (2023) if referring mainly to secondary limitation

> Line 50 was “Yet there is increasing evidence that other micronutrients such as zinc (Zn), cobalt (Co), and vitamin B12 can also influence phytoplankton productivity, often as secondary limiting nutrients after N, P, or Fe are added (Moore et al. 2013).”
> Thank you—we will add the 2023 Browning and Moore reference to this sentence.

68 I would suggest avoiding the term 'prejudice' as this implies unreasonable deductions. Consider that incubations to assess trace metal (co)/serial limitation are generally limited by the number of bottles that can be incubated simultaneously, so inevitably experiments lean towards designs which focus on the most deficient element, which is usually Fe, and perhaps include some combination of Mn, Co and Zn. This isn't unreasonable, but yes I agree with the notion that it means that co- or serial limitation by trace metals other than Fe has probably been under-appreciated to date. Perhaps the authors could rephrase.

> Line 68 was “Whether due to the early negative results, the few positive findings, or a general prejudice against considering additional factors in controlling marine productivity, it is our experience that there is currently no broad community recognition that zinc limitation is a process that could affect primary productivity in any region of the oceans, leaving the original ‘zinc hypothesis’ unresolved (Morel et al. 1994).”

> We appreciate this sentiment. The choice of language was based on our experience in prior submissions at other journals where reviewers vociferously argued that zinc could not be limiting in nature, despite our multiple lines of evidence and additional blank analyses. We propose to change the text to “Whether due to the early negative results, the few positive findings, or the practical

constraints of co-limitation studies in the field that limit the number of micronutrients tested, it is our experience that there is currently no broad community recognition that zinc limitation is a process that could affect primary productivity in any region of the oceans, leaving the original 'zinc hypothesis' unresolved (Morel et al. 1994)."

105 Not sure what 'total dissolved Fe' is, would just 'dissolved Fe' (and 'dissolved Zn') throughout not be clearer?

> We refer to our metal data as "total dissolved" metals following GEOTRACES terminology (see the GEOTRACES cookbook, Section 3.2 Total dissolved (filtered) samples: https://geotracesold.sedoo.fr/images/Cookbook.pdf)

128 I assume N+N means nitrate plus nitrite? Maybe define at first use (apologies if I missed this)

> Thank you for catching this, as N+N was not explicitly defined previously.

>We propose to change the text to "Consistent with high macronutrient abundance in this region, surface macronutrient concentrations were partially depleted at the experimental site with 64%, 46%, and 29% decreases in nitrate+nitrite (N+N), phosphate (P), and silicate (Si), respectively, comparing 10 m and average deep water (200 – 1000 m) values (Fig. 1o)."

142 Apologies if my terminology is wrong - is there a possibility of independent co-limitation i.e. both Zn and Fe produce positive, independent responses in the same species/groups?

> Line 142 was "However, addition of Zn alone (+Zn) also resulted in significantly higher chl a content compared to the controls ($p = 0.011$), implying that a subset of the incubated phytoplankton population benefitted from the addition of Zn alone, without additional Fe, and may thus have been experiencing primary Zn limitation (Fig. 2a)."

> Your interpretation is correct: that we saw a significant response in chl a with +Zn alone, this suggests Type I (Independent) Co-limitation, which we referred to in this sentence as "primary" Zn limitation.

>For clarity, we will change the text to "However, addition of Zn alone (+Zn) also resulted in significantly higher chl a content compared to the controls ($p = 0.011$), implying that a subset of the incubated phytoplankton population benefited from the addition of Zn alone, without additional Fe. This observation is consistent with independent co-limitation (Saito et al. 2008) (Fig. 2a), where two nutrients (such as Fe and Zn) each independently limit different subpopulations or processes, and adding either nutrient alone yields a response."

280-286 Do lab culture metal:P ratios diverge from field ratios? If so a comparison to whatever natural Zn:P ratios are available would be more convincing.

> Lines 280-286 were "Particulate Zn:C ratios reported previously in Zn-limiting culture studies of the diatom *Thalassiosira pseudonana* (Sunda and Huntsman 2005) were converted to Zn:P ratios using the Redfield ratio (Redfield 1958) (Supplementary Table 5). We then compared these ratios and associated growth rates with particulate Zn:P measured within biomass collected at 10, 25, 50 and 100 m at the experimental site. At each of these surface depths, Zn:P measured at the experimental site was ~ 2E-4 mol:mol, which, in comparison to cultured diatom Zn:P ratios, fell within the range of

severely Zn-limited growth rates (Supplementary Figure 6), again demonstrating the propensity for Zn-limited growth in this region and corroborating the incubation results.”

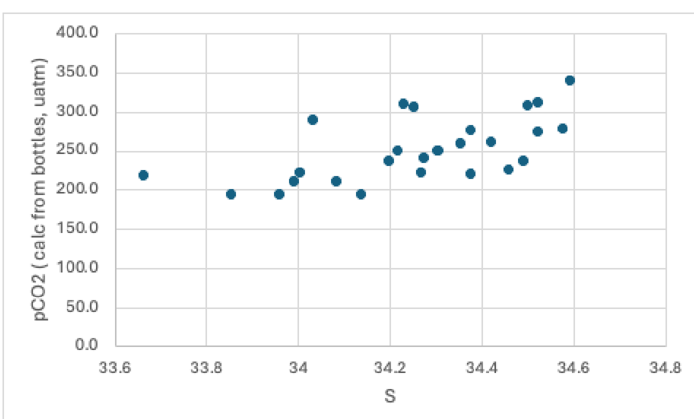
> Metal:P ratios reported by Sunda (Sunda 2012;https://doi.org/10.3389/fmicb.2012.00204REF) do align with field expectations in many cases on the low/limiting side. The reason we are using the Zn:P ratios from culture studies is because those studies were conducted under conditions of Zn limitation, allowing us to define a Zn-limiting threshold. Prior Zn:P ratios from the field have not yet been connected to Zn-limiting conditions in the field, so unfortunately aren’t useful in this comparison.

327-330 Not sure I agree with the logic of the connection here. Yes atmospheric pCO2 is rising, but what are the drivers of low pCO2 in these coastal areas where CO2 is low? If productivity or freshwater discharge in these regions increases (which is quite plausible in some of the low pCO2 areas highlighted), this may well maintain these regions in a state of low CO2 in the future even with increasing atmospheric pCO2.

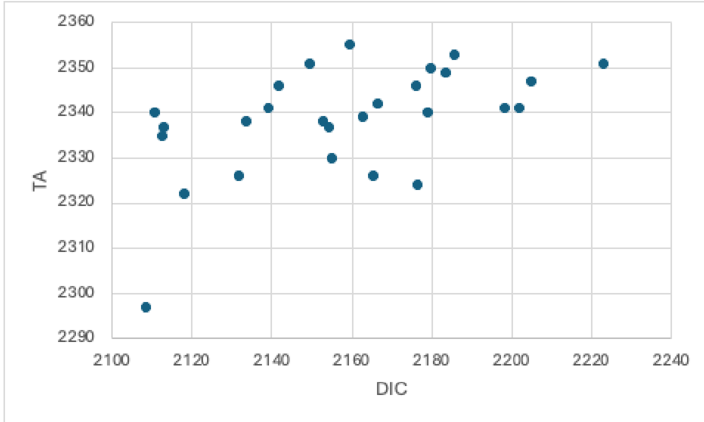
>Lines 327-330 were “We then compared this laboratory-determined Zn/C limitation threshold estimate to both the in situ 221 µatm pCO2 measured at our field study site, and to the historical, global trend in surface ocean pCO2 (Fig. 4a,b). Global surface ocean pCO2 levels are rapidly rising above both the laboratory-estimated 259 µatm pCO2 Zn/C limitation threshold and our field observation value of 221 µatm (Jiang et al. 2023) (Fig. 4a,b). Though only a fraction of the modern-day surface ocean is currently at ≤ 250 ppm pCO2 (predominantly comprised of polar regions; Fig. 4c), this represents a large decrease in oceanic extent compared to only 100 years ago (Fig. 4d).”

> To address this comment, we have calculated the impact of freshwater dilution on pCO2 and found it to be small relative to biological uptake, as shown in the figures below.

> In this study, we documented a ~45% decrease in pCO2 within Terra Nova Bay (~221 µatm) compared to values outside of TNB (~400µatm). Biology was the driver of this decrease in pCO2, rather than freshwater input from glacial and sea ice melt. This is evident in the physicochemical data, where over the measured salinity range (S=33.6-34.8), the effect of simple dilution by fresh water input (DIC=Total Alkalinity=0) would result in a reduction of pCO2 by only ~8-9 ppm (Plot A below). The signals we observe are much larger than that, consistent with a large phytoplankton uptake driver. The total alkalinity (TA) also does not change proportionally with DIC in this region (Plot B below), which is also not consistent with dilution driving a conservative mixing of TA and DIC.



(A)

(B) 

Plots of pCO_2 and Salinity (A) and TA and DIC (B) showing the range of variability observed during the CICLOPS expedition with Terra Nova Bay and sampling sites located outside of the bay showing properties consistent with biological CO_2 drawdown rather than freshwater dilution.

>We will add this text to the manuscript draft to emphasize that the reduction of pCO_2 within Terra Nova Bay was driven by biology rather than freshwater input: "In this study, we documented a ~45% decrease in pCO_2 within Terra Nova Bay (~221 µatm) compared to values outside of TNB (~400µatm). Biology was the driver of this decrease in pCO_2, rather than freshwater input from glacial and sea ice melt. This is evident in the physicochemical data, where over the measured salinity range (S=33.6-34.8), the effect of simple dilution by fresh water input (DIC=Total Alkalinity=0) would result in a reduction of pCO_2 by only ~8-9 ppm. The signals we observe are much larger than that, consistent with a large phytoplankton uptake driver. The total alkalinity (TA) also does not change proportionally with DIC in this region, which is also not consistent with dilution driving a conservative mixing of TA and DIC."

> We agree that the future trajectory of pCO_2 in coastal regions will be influenced by a complex interplay of factors, including biological productivity and freshwater discharge. Our intention was not to pinpoint future pCO_2 trends in these regions, but rather to highlight that Zn status may be an important and underexplored factor influencing phytoplankton physiology and carbon cycling under low pCO_2 conditions. But it is true that our original text only discussed what would happen if pCO_2 only increased globally (ie, less Zn limitation, maybe). The reviewer is making the good point that localized regions of low pCO_2 regions could still persist.

> To caveat and clarify that our interpretation is not definitive, but rather intended to motivate further investigation into the role of Zn in coastal biogeochemistry, we will add this text to the discussion:

> "On the other hand, it is likely that despite rising pCO_2 levels, some coastal regions will continue to experience episodic or persistent low pCO_2 due to high productivity (as observed in this study), freshwater inputs, or other regional processes. Though we do not attempt to model future pCO_2 dynamics in these areas, our results suggest that Zn status may continue to be an important physiological constraint under low pCO_2 conditions, particularly in productive coastal systems. As such, Zn limitation should be considered as part of the broader framework for understanding carbon cycling in these regions, especially as they play a disproportionate role in global carbon export."

400 (and elsewhere in the methods), reference format is duplicated

> Thank you for catching this, we have corrected these duplicated references.

463-469 I assume the authors know this is not ideal, leaving samples unacidified for months usually lowers recovery, although having said that the effects of this on dZn appear to be not too bad, maybe add a comment (see Jensen et al., 2020, Assessment of the stability, sorption, and exchangeability of marine dissolved and colloidal metals)

>Lines 463-469 were “The analysis of total dissolved metals for this expedition has been described previously (Kell et al. 2024). Briefly, seawater collected shipboard by pressure-filtering X-Niskin bottles through an acid-washed 142 mm, 0.2 µM polyethersulfone Supor membrane filter (Pall) within 3 hours of rosette recovery using high purity (99.999%) N2 gas and stored at 4ºC. All sample collection occurred shipboard within an on-deck trace metal clean van. Samples were acidified to pH 1.7 with high purity HCl (Optima) within 7 months of collection and were stored acidified at room temperature for over 1 year prior to analysis.”

> We appreciate that 7 months is a long time to wait prior to acidification, but this is short compared to the Jensen 2020 study (they stored samples unacidified for 22 months). We used a much longer acidification time (>1 year) compared to Jensen (5 months) to allow ample time for desorption from the polyethylene bottle walls. In addition, the Jansen study decanted their seawater samples to a new bottle prior to acidification, which loses all the wall-bound metals in the original bottle. Importantly, in this study we acidified our seawater in the original collection bottle to redissolve metals that had adsorbed to the walls.
>We will add the following text to this Methods paragraph: “This extended acidification time was used to counteract any loss of metal due to adsorption to the bottle walls (Jensen et al. 2020).”