Response to Reviews 7/3/25 (Responses in blue)

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

RC2: 'Comment on egusphere-2025-1609', Anonymous Referee #2, 06 Jun 2025

This is an excellent study focused on teasing apart the complexities of phytoplankton nutrition, with a specific emphasis on overlooked and traditionally difficult-to-study trace metal nutrients in the environment, i.e., zinc. This study provides several lines of evidence that, in addition to iron (Fe), zinc (Zn) can also limit phytoplankton growth. Most of my comments are minor and largely relate to the accessibility and readability of the text.

>Thank you for your appreciation of the study.

To make the manuscript accessible to researchers outside marine environmental studies, it would be useful to define terms, such as “polynya”.

> We will update the text using “polynya” to read: “A large phytoplankton bloom was present as indicated by high (> 3000 ng L-1) chlorophyll fluorescence concentrations in January that waned into February (Fig. 1d). This observation of high productivity is characteristic of Antarctic polynya environments, which are recurring regions of open water surrounded by sea ice (Arrigo et al. 2012).”

It would also help the reader when describing taxonomic classifications to give a little more information. An example, is line 89. For readers who are not experts in algal phylogeny to add “and the haptophyte Phaeocystis” or something similar, when first mentioning this alga. Is "Phaeocystis" used to refer to this alga (or algae) because the species is unknown or there are likely multiple species?

> Line 89 was “This phytoplankton community initially consisted of a mixed assemblage of both diatoms as indicated by fucoxanthin (fuco, Fig. 1e) and Phaeocystis as verified by shipboard microscopy and as indicated by 19’-hexanoyloxyfucoxanthin (19’-hex, Fig. 1f).”

> Phaeocystis antarctica (Phaeocystis hereon) is the dominant Phaeocystis species in this region (Arrigo et al., 1999 https://doi.org/10.1126/science.283.5400.365; DiTullio et al., 2003 https://doi.org/10.1029/078ARS03). It is true that all ZCRP hits for Phaeocystis (shown in Fig. 3) do indeed have P. antarctica as the best hit species.

> We will update the text to read: “This phytoplankton community initially consisted of a mixed assemblage of both diatoms as indicated by fucoxanthin (fuco, Fig. 1e) and the haptophyte *Phaeocystis* as verified…”

Sentences, such as “Pronounced and progressively deepening total dissolved Zn (dZnT) depletion over time was observed, with dZn depleted down to an average of 0.82 ± 0.47 nM at 10 m over all TNB stations (Fig. 1g)”, are difficult to read and understand without re-reading. For instance, I had initial confusion about the use of “deepening” and “down”. For instance, it appears “deepening” refers to depth in the water column, but the use of “down” refers to the dissolved Zn concentration decreasing? As this is a complex study, the authors need to be careful with word choice.

> For clarity we will reword this text to: “Additionally, we observed pronounced depletion of total dissolved Zn in surface waters across all TNB stations, with an average concentration of 0.82 ± 0.47 nM at 10 m (Fig. 1g). Notably, as the bloom progressed, this depletion extended progressively deeper into the water column (Fig. 1g), indicative of strong Zn uptake and export from the euphotic zone.”

Since the first section of the results was largely previously published, this section could be shortened and/or moved to the methods section as a description of the sampled sites.

>We request to keep this brief section to provide environmental context without the reader having to read an additional manuscript.

Some clarity is needed with respect to whether the same station was measured temporally, or whether each date represents a different station.

>Each station (Table S2) was sampled only once, therefore each date represents a different station, but since all stations were spatially within TNB, we refer to this as temporal sampling of the region. >The text will be updated to: “Twenty-six stations within Terra Nova Bay (TNB) were temporally sampled over the course of one month (January 9 – February 18, 2018) during the 2017-2018 CICLOPS expedition (Fig. 1a; Supplementary Table 2) to concurrently characterize the natural progression of the phytoplankton bloom and biogeochemical changes in the water column (Kell et al. 2024). These stations were spatially distinct (each unique station was sampled once), but given that all were in relatively close proximity to each other within TNB (within a 52 km radius), we have combined the stations to create a temporal analysis of the region.

Do the authors have data to estimate the Zn content of the phytoplankton (or at least the plankton community) in sampled waters compared to how this value changes when the cells are fed Fe, Zn, and Fe+Zn? This would perhaps get at a better understanding of the community’s Zn quota in relation to the amount of Zn that would be considered to be limiting, sufficient, or a luxury.

> This is a good suggestion, however, we did not save material from the experiments to conduct these measurements. Often material in these experiments is somewhat limited, time is short during experimental breakdown, and Zn in particular is notoriously difficult to collect particulate metal data from in the field due to the ease of contamination. We were not sure at the time that the Zn stable isotope uptake studies we conducted concurrently on this cruise would work, as our previous attempts had been contaminated, and prior to the accompanying study there were no field Zn stable isotope uptake data in the oceans. Hence, the experimental studies on laboratory-grown representative strains serves as a useful, and available, comparison.

ZCRP-A belongs to a very large and phylogenetically complex family. Algae, in particular, have an unusual number of paralogs from this family and many of these paralogs have distinct evolutionary origins. Are the authors confident that the “ZCRP-A” proteins identified are orthologs vs. potentially distinct paralogs of the characterized ZCRP-A? Similarly, the ZIPs are another complicated family, with some members expressed during Zn deficiency and others during Fe deficiency.

> We appreciate the reviewer’s thoughtful comment regarding the complexity of the COG0523 and ZIP protein families and potential functional divergence among algal paralogs. Our identifications of ZCRP-A and ZIP protein hits (contigs) within the water column and incubation metaproteomic data

are based on high confidence BLAST hits to diatom reference ZCRP-A sequences (which we have characterized as related to Zn functionality; Kellogg et al., 2022 https://doi.org/10.1038/s41467-022-29603-y) and diatom reference ZIP1 protein sequences (Supp Table 4). This does not definitively distinguish orthologs from paralogs, so we have referred to them simply as “homologs”. It is true that we are inferring Zn-related functionality to all metaproteomic contigs identified as ZCRPA or ZIP homologs, given the response to +Zn in the incubations and the scarce Zn concentrations in the surface water column in this study. To further increase our confidence that the metaproteomic contigs identified here have functionality with Zn (rather than other divalent metal cations), we entered each contig sequence into SHOOT (https://genomebiology.biomedcentral.com/articles/10.1186/s13059-022-02652-8) (https://shoot.bio/), which constructs a phylogenetic tree using the input sequence and identifies orthologs using the SHOOT database of organisms. Of the 21 unique contigs assigned as ZCRPA homologs, 19 were confirmed to be T.pseudonana orthologs, while 2 were assigned as orthologs to the Zn-related COG0523 E.coli proteins YjiA and YeiR. All 5 unique ZIP-assigned contigs were confirmed to be orthologs of T.pseudonana ZIP (Pfam PF02535). We propose to add this SHOOT analysis data of our identified ZCRPA and ZIP contigs as a new supplementary table.