Sato et al. conducted a field study to investigate the distribution of Gamma A, a non-cyanobacterial diazotroph and the effect of grazing by microzooplankton on Gamma A (and cyanobacterial diazotrophs). As someone with knowledge of the N cycle (including diazotrophy) but not necessarily in microzooplankton and/or grazing, this was a very interesting read and I think it will attract different audiences (i.e., those interested in the N cycle, those interested in NCD and those interested in grazing). The manuscript is well-written, well-prepared and I enjoyed reading it. I only have minor comments.

We deeply acknowledge Referee#5's constructive comments and appreciate the opportunity to respond to them. In the revised manuscript, we have added the result of generalized linear mixed model (GLMM) analyses to assess the potential controlling factors on Gamma A abundance, taking into account the sampling non-independence. Referee#5's comments on our analysis helped us improve our manuscript significantly. We will address the comments one by one as shown below.

General comments:

Although it is shortly mentioned upon in the introduction, there are two top-down controls on the distribution of Gamma A: viral infections and zooplankton grazing. In the dilution experiment, the seawater was prefiltered with a 200 µm mesh whereas the particle-free seawater was prepared by filtration through a 0.2 µm filter. Thus, both filtration set-ups would allow for the presence of viral particles. How does the dilution method distinguish between viral lysis or grazing. Does the grazing mortality rate (m) calculated via the dilution method not represent the combined effect of the two top-down processes? Besides that, there also might be an interaction between the grazers and viruses. See e.g.: doi:10.1093/plankt/fbv011. I would like the authors to elaborate a bit more on the potential effect of viruses in their experimental set-up in the discussion.

Generally, the mortality rate calculated from dilution experiments is considered to represent only grazing mortality and does not include viral lysis (Staniewski and Short, 2018; Landry et al., 1998). As Referee #5 noted, both filtration set-ups allow for the presence of viral particles. Therefore, the serial dilution with 0.2 µm-filtered water does not create a proportional gradient of viral particle density (but does create a proportional gradient of grazers, phytoplankton, and Gamma A). This means that the proportional difference in apparent growth rate with serial dilution (slope of regression) should reflect grazing mortality, but not viral lysis. To specifically quantify the viral effect, an additional set-up using 30–100 kDa filtration would be required (known as "modified" dilution experiments), as found in the reference provided by Referee #5 (Pasulka et al., 2015). Potential interactions between grazers and viruses, as discussed in Pasulka

et al. (2015), also become apparent and a problem only through the modified dilution experiments. Therefore, we did not add an explanation on the potential effects of viruses in our set-up. However, since readers may have similar concerns regarding viral impacts in our dilution method, we added the following note to our experimental set-up at L170–172.

"It should be noted that, as viral particles are generally smaller than 0.2 µm and there is no serial gradient of viral density in the diluted bottles, the current calculated mortality rate should theoretically be attributed to microzooplankton grazing mortality (Staniewski and Short, 2018). "

Statistical analysis

I do not think a Pearson's correlation analysis is the right statistical method to explain the distribution of Gamma A. For instance, it is mentioned that gamma A has significant correlations with both nitrate and phosphate concentrations. However, there is a very clear correlation between [NO3-] and [PO43-] (see figure S6 and table 2), which could confound the statistical analysis. These measurements are not independent and a mixed-effect model is required for the statistical analysis.

We appreciate referee#5's constructive suggestion. As suggested, we applied generalized linear mixed model (GLMM) analysis to examine the abundance and grazing mortality rate (m) of Gamma A incorporating cruises as a random effect. The results of GLMMs were consistent with those of the simple correlation analysis. Temperature had a significant positive effect on Gamma A abundance (coefficient = 1.75, $p = 2.3\times10^{-3}$), while nitrate had a significant negative effect (coefficient = –2.17, $p = 5.3\times10^{-5}$). Grazing mortality rate (m) also had a significant negative relationship with Gamma A abundance (coefficient = –0.39, $p = 6.0\times10^{-4}$) at 25 % light depth where dilution experiments were conducted. We revised the method section (L173–187) and result section (L269–278) and updated Table 2 to include the GLMM results.

NifH is a biomarker for potential nitrogen fixation but since the experimental set-up did not include any transcriptomic or proteomic analysis, it remains a potential. I would like the authors to elaborate a bit more about this in the discussion (for instance in chapter 4.2). Does Gamma A have other means to utilize nitrogen-compounds (e.g., ammonium, nitrate, urea) or is nitrogen-fixation the only method to get cellular nitrogen?

We agree. The presence of *nifH* indicates only the potential of nitrogen fixation, and thus transcriptomic or proteomic analysis should be done to confirm the importance of Gamma A as an active diazotroph in the Kuroshio region. We added sentences like below at L305–308.

" Still, it should also be noted that current study only analyzed *nifH* DNA, not RNA or protein, and the dominance in the *nifH* DNA pool does not necessarily mean the most active diazotrophs (Shiozaki et al., 2017). To confirm the importance of Gamma A as an active diazotroph in the study region, future studies should apply transcriptomic or proteomic approaches."

As for other means to utilize nitrogen-compounds, there is no comprehensive information on nitrogen metabolic potential of Gamma A, so its genomic content should be investigated by an omics or isolation approach in the future as mentioned at L332–333.

Specific comments:

Introduction

l60: in situ in italic

Thank you for the comment, but according to author guidelines 'in situ' should not be italic in the manuscript.

l75: I do not understand this sentence. What stable isotope ratio is a proxy for diazotrophy? Everything containing N has a δ15N value, but when is this a proxy for nitrogen fixation? Elaborate.

Thank you for the comment. The δ15N of particulate organic matter (POM) is considered a proxy for nitrogen fixation because it reflects the nitrogen source utilized by organisms. Nitrogen derived from diazotrophs generally shows lower δ15N than that derived from other autotrophs using nitrate supplied from below the euphotic zone (Minagawa and Wada, 1986; Carpenter et al., 1997). Accordingly, δ15N and nitrogen fixation have significant correlations in oligotrophic regions like the subtropical waters and Kuroshio (Horii et al., 2018). In the Kuroshio region, Kodama et al. (2021) observed that nitrate depletion and decreased $\delta^{15}N$ values during summer and indicated a contribution of nitrogen fixation to the nitrogen pool in this season. We revised the sentence accordingly (L75–77).

l78-l79: These results suggest the importance of NCDs, including Gamma A, and a distinct….

Corrected.

l83: plays is to strong, change to "might play"

Corrected.

Results

Figure 4c: In l229, the significant positive correlation between μmax and m is mentioned ($r = 0.83$, $p < 0.01$). Put these statistics in the graph.

We appreciate the comment. We add the statical result in Figure 4c.

Figure S6: Temperature in the second column (not tempereture)

Corrected.

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