

Interactive comment on “Nutrient limitation of phytoplankton in anticyclonic eddies of the northern South China Sea” by X. Ning et al.

Anonymous Referee #2

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This ms is about the limitation of phytoplankton in 2 anticyclonic eddies in the China Sea. Authors wanted to demonstrate limitation using in vitro nutrients enrichment experiments. But I have important comments dealing with "phytoplankton analysis" and "Nutrient enrichment experiments"

Remarks in relation with phytoplankton: The main problem with this ms is the high difference between "physical/chemical" and "biological/diversity" analysis and discussion. Physical parameters are well presented and discussed, unlike biological parameters. Authors have to integrate more informations in relation to phytoplankton analysis, have to be more precise in results and have to discuss more the evolution of communities. A good example of my comment is given by: 1) the sentence at the end of page 4602: "Comparing the results on shift of size fractionated Chl-a with that of dominant group

and species after the nutrient enrichments, it was found that the most dominant fraction of Chl-a was always nanoplankton, which did not fit the results obtained from identification and cell counting in the phytoplankton dominated by diatoms, which belongs mostly to netplankton"

and

2) the wrong analysis written page 4604: talking about different diatoms, they say : "These could very easily pass through the 20 μm mesh into nanoplankton fraction, when size fractionation was performed, although without any vacuum, resulting in the overestimation of nanoplankton and underestimation of the net plankton biomasses" This is a wrong analysis, mainly for 2 reasons: A) The authors compared a proxy of the biomass (size-fractionated chl a) with abundance (numbers of cells), which is very difficult (=impossible) without measuring the biovolume of each counted species and B) The authors only use the Utermöhl method (inverted microscope) to count phytoplankton. With this method, only microphytoplankton can reasonably be counted. Nano- and pico-plankton are therefore underestimate, only because they can't be observed.

In the same way, authors could give more details in MM concerning the use of the inverted microscope. Did all the 500 ml of sea water was analyze for each sample? How many cells were count? What was the lower size of cell counted? Did mixo- and hetero-trophic dinoflagellates were also count? Authors also have to give all the bibliographic references used to confirm species determination. In "Results and discussion" part, often, when authors talked about all the phytoplankton community, it's wrong because they referred only to "microphytoplankton groups". They can talk about all the community only when they referred to the size fractionated Chl-a. Page 4599 : Authors talk about Synechococcus sp. How did they count this species? It's not written in the ms. In the 4.2.4 part of "Results and Discussion", about "phytoplankton communities", there are no references! Authors must discuss the results, in relation with other published works. In Table 2, authors must give authorities for each dominant species. Do the authors are sure about the determination of *Scrippsiella trochoidea*?

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(publication have already demonstrated the presence of cryptic species in this genus).
Fig. 8: In this graph, do you present the means \pm SE, or the means \pm SD? So, it's the average of 2 values (because you did 2 experiments)? It may be better to give only the 2 values associated to each incubation time.

Remarks concerning Nutrient enrichment experiments: I don't understand something concerning those experiments. If each bottle contains 4 L, you said that "subsamples were taken for measuring size-fractionated Chl-a, phytoplankton species identification and cell counts for each test and control bottles at time 0h, 12h, 24h, 48h, 72h, 96h and 108h for samples taken at Station". You take 0.5 L for the count of phytoplankton species. You never wrote the volume you need for size-fractionated Chl-a, but even without this volume, if you take a minimum of 0.5 L at each time, there was only 0.5 L in each bottles at the end of each experiment. So a maximum of 0.5 L/7 times could be used for Chl-a (= less than 0.1 L) 2 remarks: A) In my lab., using the same technique for Chl-a (fluorescence method), we need 1 L to have a good estimation of Chl-a with concentrations closed to your initial concentrations. How can you obtain results of "size-fractionated Chl-a" with less than 0.1 L? B) You have a great difference between the initial and final volume in your incubation bottle. Don't you think that could be a problem, especially the "bottle effect"?

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