

Umezawa et al. present a study investigating the contribution of diatoms to a nutrient decline in the dark subsurface water of Funka Bay (Japan) during and after the surface water bloom occurrence. Their hypothesis is that diatoms from the genera *Thalassiosira* that dominate the primary production in the surface ocean sink and accumulate below the euphotic zone where they continue to consume nutrient in the absence of what they call “photosynthetic growth”. To confirm their hypothesis, authors have designed a laboratory experiment growing *Thalassiosira nordenskiöldii* in the dark and measuring the evolution of nutrient concentration in the medium. The main conclusion of this study reveals that *Thalassiosira nordenskiöldii* grown in culture conditions with replete nutrient concentration is able to consume nitrate in the absence of light. They attempted to demonstrate that this is the only possible explanation for the nutrient drawdown observed in the dark subsurface layer of Funka Bay between March 4<sup>th</sup> and April 14<sup>th</sup>.

Generally speaking, this manuscript is poorly organized, not clearly written and in a poor English wording. I would strongly advise the authors to read carefully their manuscript again and have it read by an English-speaking person. I believe that authors didn't use appropriate methods to evaluate the main objective of the study. Because the incubation experiment has not been replicated the conclusions from this experiment that are supposed to support their main hypothesis regarding the in-situ nutrient drawdown is only speculative. Moreover, the rates estimated from this experiment cannot fully explain the observations. I have detailed my main concerns below and do not recommend publication of this manuscript in its actual state.

#### GENERAL COMMENTS

\*In general, I feel that the introduction is poorly structured and weak. A lot of background about the hydrology but also about the dynamic of the phytoplankton bloom and productivity in Funka Bay is missing. I invite the authors to develop their bibliography and look closer in the literature for studies that have already been conducted in Funka Bay and develop their introduction a little further. For example (and this is not exhaustive), Nakada et al. (2013) for water mass dynamics, Shinada et al. (1999) and Radiarta and Saitoh (2008) for phytoplankton bloom and productivity dynamics in the bay. Most importantly, the objectives of this work are not clearly presented here. Why authors decided to focus on the processes affecting nutrient reduction in the dark subsurface layer during the bloom, what is the purpose of this study?

\*Two main comments for the Material and Method section:

I do not think that 4 sampling days conducted in a random way can be considered as a time series, perhaps a survey...

When investigating biological processes, incubation experiments such as those conducted in this study MUST be replicated (triplicates are a must), otherwise any interpretation coming out from the experiment results is only speculative and conclusion cannot be ensured. This is very important!

\*Section 3.2 of the Discussion is really long compared to other sections and contains a lot of redundancies. I suggest to re-organize this section chronologically instead of per nutrient, for example 3.2.1 pre-bloom, 3.2.2 bloom and 3.2.3 post-bloom situation. This might help reducing the length of this section (especially for chl<sub>a</sub> and nitrate) and remove most of the redundancies.

Moreover, this will allow authors to discuss the evolution of the nutrient ratios which will help confirming or infirming their hypothesis and which has not been explored in the discussion?

\*The conclusion is poorly reflecting the main observations and hypothesis raised by the authors to explain the nutrient drawdown observed in the dark subsurface water of Funka Bay. Indeed, Authors cannot reject the influence of vertical mixing as they did L284-288, they haven't discussed the possibility of nutrient diffusion (active or passive), and the consumption rate of diatoms measured during the incubation experiment are not enough to fully explain the observed nutrient drawdown. The conclusion MUST be revised to truly reflect the main results of this study.

\*Most of the figures of this manuscript either displayed the wrong information or are incomplete.

#### ABSTRACT

The abstract should use non-technical terms. For example, while "mixed-layer" is a common term used in oceanography and it is ok to use it in the abstract, Dark Zone Depth needs to be defined. Another option could be use "below the euphotic zone" instead of "dark zone depth". The main conclusion of the abstract needs to be revised since the nutrient consumption by diatom in the dark layer is likely not the only possible explanation to the observed nutrient drawdown in the subsurface waters of Funka Bay.

L14 and later in the manuscript: February 15<sup>th</sup> to April 14<sup>th</sup> of 2019. Please be careful on the calendar notation along the manuscript.

L16: "On both date"? During the whole time series from Feb 15<sup>th</sup> to Apr 14<sup>th</sup>? or during the bloom from March 4<sup>th</sup> to March 15<sup>th</sup>? By the way, winter water was above the euphotic zone (so above the dark-zone depths) on March 4<sup>th</sup> in fig.2.

L17: Same comment as above, please specify which dates. On fig.4, while  $\text{NO}_3^-$  concentration decreases by approximately half, it is absolutely not the case for  $\text{Si(OH)}_4$  and  $\text{PO}_4^{3-}$  (e.g. at 50m concentrations dropped from 0.7 to 0.6  $\mu\text{mol L}^{-1}$  and from 15 to 13  $\mu\text{mol L}^{-1}$  for  $\text{PO}_4^{3-}$  and  $\text{Si(OH)}_4$  respectively)

L20-23: Authors present a list of several publications that have already studied dark nutrient consumption rates and ratios (see L250-259)

#### INTRODUCTION

L25-26: Surface euphotic zone sounds redundant.

L28: Use either "Si:N ratio" or " $\text{Si(OH)}_4:\text{NO}_3^-$  ratio"

L38: It is actually believed that the intrusion of the Oyashio water in the Bay triggers the diatom bloom by strengthening water column stratification.

L56-58: This is unfortunate since nutrient limitation can change from time to time. This manuscript doesn't have to focus on nutrient limitation in the surface water, however, it would be interesting to have a paragraph (even a short one) about what controls the dynamic of the bloom in the euphotic zone during this study. This is important since nutrient drawdown occurring in the surface layer will likely affect the subsurface biogeochemistry by creating gradient (and potentially diffusive fluxes) of nutrients, or by triggering adaptive response from the biology (such as vertical migration, resting spore formation etc.)

L58-61: VOI are not presented nor discussed in this manuscript. Please remove these sentences or add a figure, present and discuss the results of VOI measurements, and highlight their contribution in answering the objectives of the present study (why VOIs are measured?).

## MATERIEL AND METHODS

L72-73: What does this sentence mean? What kind of observations? Are they relevant for this paper? Please remove this sentence or develop.

L77:  $\text{SiO}_2$  is not a nutrient. This is the formula for particulate silica, silicic acid (the nutrient) is  $\text{Si}(\text{OH})_4$ , please make sure to double-check the notations throughout the manuscript.

L78: Analytical precision: this is almost too good to be true! I am curious to see how this has been calculated?

L83: please use axenic instead of sterile. F/2 medium has approximately  $106 \mu\text{mol.L}^{-1} \text{Si}(\text{OH})_4$ ,  $882 \mu\text{mol.L}^{-1} \text{NO}_3^-$ , and  $36 \mu\text{mol.L}^{-1} \text{PO}_4^{3-}$  (see Guillard and Ryther, 1962; Guillard, 1975 or Andersen et al (2005), Algal Culturing Techniques). It is more likely a modified (not particularly Si-rich) f/2 medium then, although I agree it is still plenty of nutrients for diatoms.

L86: This is really unfortunate since checking for contamination is crucial regarding the objective of the incubation experiment. Indeed, contamination by a population of other micro-algae or bacteria can affect the consumption (or recycling) of  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$  (although it is probably less the case for  $\text{Si}(\text{OH})_4$ ). This have to be mentioned and discussed in the discussion.

L93-97: What was the purpose of this second dark incubation? and why adding much more nutrient compare to the first one? Please explain.

## RESULTS AND DISCUSSION

### 3.1. Hydrographic features

Are the water masses defined based only on salinity? Or did the authors use a T-S diagram to define the distribution (depth range) of the different water masses during the study? If so, it would have been useful to have a figure with a T-S diagram that illustrate the position of water mass end-members (such as in Shimizu et al. 2017) in supplement material at least.

L111: “biogeochemical”

### 3.2 Biogeochemical parameters

My guess is that authors meant “biogeochemical” instead of “biochemical” since biogeochemical refers to processes associated nutrient cycles and nutrient distribution while biochemical is more related to intra-cellular processes.

L115-118: Why authors chose this definition of the mixed layer, please explain AND cite appropriate reference. Same comment for euphotic zone and dark depth zone, appropriate referencing is missing.

L118: in general terms like “much less” should be avoided in papers, please be more specific and give a threshold or a range.

Also, mixed layer, euphotic zone and dark depth zone can be defined in the previous paragraph, since they can be considered as hydrographic features as well.

L120-124: Authors cannot discuss in detail data that are not available either in another ALREADY published work, in a database, in supplement or in the main text of the manuscript. Authors cannot refer to an unpublished work when it concerns data availability, especially

when these data are used to make some of the figures. This is not acceptable! Moreover, I don't understand this sentence since the chl $a$  profile in fig.3 looks pretty low and homogenous on Feb 15 and it seems that the chl $a$  data ARE presented in the supplement...

L125-126: 27 to 30  $\mu\text{g L}^{-1}$  of chl $a$  sounds very high to me. It looks like it's between 1.5 and 2 times higher than what is usually found in the bay (see L126-128). Is it a particularly exceptional year? Why is chl $a$  that high?

L131: Sinking particles and suspended particles are different things. What makes authors conclude that particles can be sinking vs. in suspension? Please develop.

L140: A reference for the annual maximum is missing.

L150-167: Since there is a section specifically discussing the nutrient consumption in the dark subsurface layer, I would suggest to remove those hypotheses here to avoid redundancy with section 3.3. and to add this material to the discussion in section 3.3. This section 3.2 could thus be renamed "nutrient dynamic in the euphotic zone and dark subsurface layer" or something similar.

L168-175: I do not see why the first explanation of vertical mixing suggested for March 4<sup>th</sup> NO $_3^-$  decrease at depth cannot be still valid in March 15<sup>th</sup>. Indeed, authors based their explanation mostly on the AOU profile. However, AOU profile in the 30-50m layer in March 14<sup>th</sup> looks pretty much the same as in March 4<sup>th</sup> (see fig.3).

L180-186: Note that the signal of regeneration is also clearly seen from the strongly positive values of AOU at depth in April 14<sup>th</sup>.

L185: It is important to be more specific here. For example, the sentence can be "The very high PO $_4^{3-}$ , Si(OH) $_4$  and NH $_4^+$  concentrations measured at depth (1.9, 26.1, and 3.2  $\mu\text{mol L}^{-1}$ , respectively) clearly indicate that nutrient regeneration already occurred at the bottom of the water column in April 14<sup>th</sup>".

### 3.3 Nutrient consumption in the dark subsurface layer

I suggest to add a subsection between subsection 3.3.1 and 3.3.2 to discuss a third hypothesis for nutrient drawdown in the dark subsurface layer, the diffusive flux of nutrient, that have been neglected so far in the manuscript but I think could be of significant importance.

L211: It is currently impossible to see the density difference between 5 and 30m in fig. 4 since both density profiles are incomplete. Moreover, it is more about the shape or curvature of the density profile rather than the magnitude of the density difference. Indeed, a smooth and progressive gradient of density will not act as a strong barrier against exchange compared to a sharp change in density (even though this latter is smaller).

L212-214: Please show these data in a figure

L215: Although this might be true, I do not agree with authors since we do not have access to the observations that support this conclusion (there is no figure illustrating the wind speed and the density profiles on fig.4 are incomplete).

L218-228: This could be another subsection focused on discussing horizontal advection

L222-224: A mixing ratio of 8:2 between Funka-Bay and Oyashio waters is a curious way to present the mixing between these two water masses. Won't it be clearer to say that to generate the observed decrease in salinity, a mixing with 25% of Oyashio water (or 1:4 Oyashio water and Funka Bay water) is necessary. By the way, by using salinities of 33.0, 33.47 and

33.58 for Osahio water, Funka Bay water, and the mix between the two of them, respectively; my estimate is 20% (1:5) of Oyashio water needed to produce the observed decrease in salinity.

L227-228: This is not because the vertical mixing cannot explain all the nutrient drawdown that this hypothesis should be excluded, it is more likely not the main driver of the nutrient drawdown. It doesn't mean that it is not happening, it is more likely a combination of different processes. I suggest to rephrase this conclusion.

L231-232: Same comment as above. The nutrient reduction in the subsurface layer is more likely driven by multiple processes, consumption of nutrient by diatom in the dark might be one of them (not the only one).

L237 and after: Please explain the  $\mu\text{g chl}a^{-1} \text{d}^{-1}$  unit used for consumption rates. Chl*a* concentrations during the incubation experiment are not presented in fig.5 nor elsewhere in the manuscript. Also, in the main text  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$  as well as  $\text{Si(OH)}_4$  consumption are discussed but only  $\text{NO}_3^-$  is show in fig.5. The evolution of ALL nutrient concentration MUST be presented somewhere if authors are discussing them in the manuscript.

L238-342: Although calculation of consumption rates cannot be verified since nutrient concentration during the incubation are not presented, it seems that the consumption rate of diatoms estimated from the incubation experiment are very low and cannot fully explain the nutrient drawdown observed in the 30-50m layer between march 4<sup>th</sup> and March 14<sup>th</sup>.

L243-245: Are these consumption rates calculated between day 0 and day 2 such as in the first incubation experiment? I don't think rates from the second incubation experiment can be compared to rates in the first experiment since too many parameters were different between the two experiments (e.g. concentrations were much higher in the second experiment, since diatom uptake usually follows a Michaelis-Menten function, this can change a lot the dynamic of the diatom uptake!). Does this mean that, because the nutrient concentrations in the field were much lower compare to those set up during both experiments, we can assume that the nutrient consumption rates were probably much lower in the water column and thus would probably contribute to a very small amount of the observed nutrient drawdown?

L247-249: I do not understand this sentence. Why would the consumption rate be largely dependent on the chl*a* content in the diatom cells if, as suggested by authors L165, this nutrient consumption occurs "without photosynthetic growth"? Please explain.

L248-249: I totally agree with this statement!

L251: I do not understand this sentence, please rephrase.

L268-273: All diatoms do not migrate. Those vertical migrations have been observed for mats of Rhizosolenia. Do authors have evidence for such a behavior in Thalassiosira? This needs to be discussed here.

L275: What are those assumptions? They need to be presented and discussed here.

## CONCLUSION

L279-280: Once again, I'm not convinced that 4 sampling days can be considered as a time series.

L291-292: I do not agree with this. The nutrient consumption rates estimated from the incubation experiment are too low to explained the nutrient drawdown observed in the 30-50m layer in Funka Bay.

## FIGURES

Figure 1: There is only one station/sampling site in this study. Describe what O (Oyashio water) and T (Tsugaru water) are in the figure caption or legend.

Figure 2: Define the euphotic zone and MLD in the captions instead of in the legend. Be consistent in the formatting of the legend (e.g. WO: Transitional water instead of Transitional Water: WO).

Figure 3: MLD is not at the same depth on Apr 14<sup>th</sup> in fig.2 (40m) and fig.3 (15m), same on March 4<sup>th</sup> fig.2 (~5m) vs. fig.3 (~10m). Please remove the red rectangle that is supposed to show the concentration change in 4-15 March. It actually doesn't help to read the figure. Most importantly this figure involves a lot of interpolations made from a total of four stations. The time coverage is probably not enough and could generate bias in the figure that could lead to misinterpretation. For example, on panel a. it seems that the bloom started soon after the sampling in Feb15, and peaked on March 4<sup>th</sup>, but we know that diatoms can consume nutrients pretty quickly in the mixed layer and the bloom could have started anytime between Feb 15<sup>th</sup> and March 4<sup>th</sup>, same for the termination of the bloom. I would suggest to present these data as profiles which will be more accurate instead of interpolated time-sections.

Figure 4: The scale on the x axis in the temperature, salinity and density panels are not appropriate since the profile corresponding to March 15<sup>th</sup> in all panels is cut at the surface, same for the March 4<sup>th</sup> density profile. It is hard to discuss incomplete profiles. Right now, we cannot conclude that stratification is stronger on March 15<sup>th</sup> as stated L211.

The nutrient concentration difference in panel d, e and f are pretty obvious indeed, no need to add extra arrows. Moreover, the left-pointing arrow on panel c doesn't mean anything since density increases in the mixed layer from March 4<sup>th</sup> to March 15<sup>th</sup>. I would be interesting to add the standard deviations for the nutrient concentration measurements.

Figure 5: Overall, I like this figure. It is clear, although the scale of the x axis on panel b does not make sense (two squares correspond to 4 days (0 to 4) then to two days (4 to 6), then back to 4 days (6 to 10)). I also still don't understand how the authors have defined their set-up conditions (e.g. why adding  $30\mu\text{mol. L}^{-1} \text{NO}_3^-$  in the first experiment and around  $750\mu\text{mol L}^{-1}$  in the second one? Why this threshold of  $1429\mu\text{g L}^{-1} \text{chl}a$  in the first experiment but  $72.5\mu\text{g L}^{-1}$  in the second one? Although I don't remember is the culture experiment has been replicated (Working with biology, I think it is crucial to run that kind of experiments in triplicate, or at least duplicate them) but this figure needs error bars.