Dear anonymous referee 1

We send responses to referee1's comments for the submitted manuscript "Substantial nutrient consumption in the dark subsurface layer during a diatom bloom: the case study on Funka Bay, Hokkaido, Japan" by Umezawa et al. The title was revised according to the comment. The referee's comments were very helpful, and we have revised the manuscript taking all comments. The modification was highlighted in this response file. We attached an additional figure (spatial distributions of temperature, salinity, and density at surface 1 m) to explain a possibility of subduction.

Best regards

Corresponding author: Atsushi Ooki

Reviewer's comment 1

1) Effects of the physical processes.

The authors denied the vertical mixing and horizontal mixing are the main reasons to decline the nutrient concentration at 30–50 m depth of this area. However, the water density on 15th March is lighter than that of 4th March. The authors did not show the horizontal distributions of density and nutrient concentration. So the subduction processes cannot be denied. When the authors have much data, the nutrient(nitrate)-density plot would help discussion on it. I am concerned about the possibility that the low-nutrient water formed in the euphotic layer in the other area subducted at the observation station. For example, such a phenomenon occurs in an anticyclonic eddy.

Respond to the comment 1

We added a discussion about the subduction with new figures as follows:

Line 300 - 312

4.2.4 Subduction of surface water into the subsurface layer

Fourth, we discuss a possibility if subduction of surface water caused the decrease in nutrient concentrations at the subsurface layer (30 – 50 m) of the observation station 30. At the medium depth (40 m) of the subsurface layer, temperature, salinity, and density were 3.5 – 3.6 °C, 33.64, and 26.7σ, respectively, on these dates. Suppose surface water in certain area of the bay subducted and it reached 40 m-depth at the observation station on 15 March, the subducted surface water should have the same temperature, salinity, and density. The average current speed at 40 m-depth between these dates was 3.3 cm s^{-1} (unpublish data), which was obtained from acoustic doppler current profiler (ADCP) set on the sea floor. The middle layer water at the station could have reached from anywhere of the bay within 11 days. We prepared spatial distributions of temperature, salinity, and density at the sea surface (1 m) on 4 March (Fig. 7a-c). From these spatial distributions, there was not any area that satisfied required temperature, salinity, and density to form subduction water into the subsurface layer (see an enlarged figure 7c). The low-density water (< 26.65σ) was spreading to the back of the bay since 4 March. We considered that the subsurface layer water at the station was not associated with subduction. Thus, we excluded a possibility of subduction as a reason for the nutrient decline.

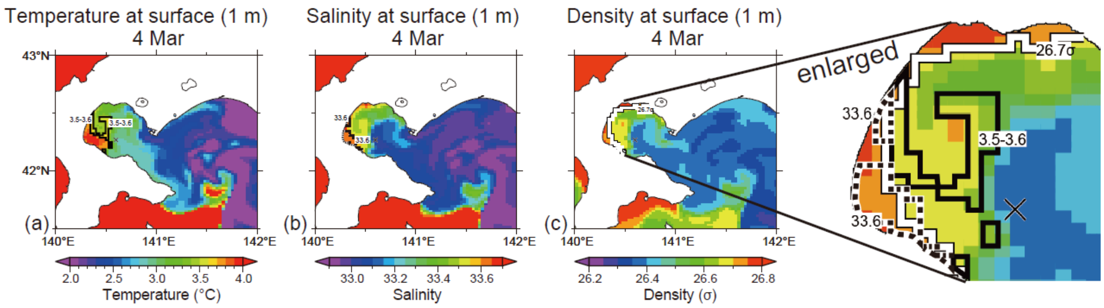

Fig. 7 Horizontal distributions of temperature (a), salinity (b), and density (c) at the surface (1 m) on 4 March 2019. Lines at temperature range 3.5 - 3.6 °C, salinity 33.6, and density 26.7σ were drawn in the figure. The all lines were drawn in an enlarged figure of density. The location of observation station was maked with a cross.

Reviewer's comment 2

The other possible process is the nutrient diffusion process. I don't know the diffusion of this area, > several tens μmol N m−2 d−1 usually occurs in the ocean. The nutrient diffusion occurs with physical disturbance, but diapycnal nutrient flux must be considered. The observations were snapshots, and the authors may not observe the diffusion processes, but the authors must show the nutrient flux at 50 m and 30 m depths are balanced on 4th and 15th March based on the slope of the nitracline and pycnocline, and this process is not the major nutrient decline process.

Respond to the comment 2

According to the referee's comment, we calculated the diffusive transport of nutrients. We added a discussion about the diffusive transport of nitrate as follows:

Line 286 - 299

4.2.3 Diffusive transport between the surface and the subsurface layers

Third, we discuss an effect of diffusive transport of NO_3^- on concentration change at the subsurface layer (30 – 50 m) on 15 March. There are not any diffusive coefficients (Kρ) in Funka Bay. We referred a range of Kρ (= 10^{-6} – 10^{-5} m^2 s^{-1}) measured just below the mixed layer (~ 30 m) at the western subarctic Pacific in summer (Dobashi et al. 2021). Concentration gradients of NO_3^- were –0.000221 μmol m^{-4} (= $\Delta NO_3^-{}_{20m–30m}$ / 10 m), –0.000141 μmol m^{-4} (= $\Delta NO_3^-{}_{30m–40m}$ / 10 m), –0.000115 μmol m^{-4} (= $\Delta NO_3^-{}_{40m–50m}$ / 10 m), and –0.0000135 μmol m^{-4} (= $\Delta NO_3^-{}_{50m–60m}$ / 10 m). The range of diffusive transport of NO_3^- were calculated to be 0.00022 – 0.0022 μmol m s^{-2} between 20 m and 30 m, which could result in concentration change of 0.021 ~ 0.21 μmol L^{-1} at 30 m for 11 days. Concentration changes between 30 m and 40 m and between 40 m and 50 m were calculated to be 0.013 ~ 0.13 μmol L^{-1} and 0.011 ~ 0.11 μmol L^{-1}, respectively. The total concentration change at 30 m, which includes transports from 20 m layer and 40 m layer, ranges from –0.20 μmol L^{-1} (= –0.21 + 0.013) to +0.11 μmol L^{-1} (= –0.021 + 0.13). Ranges of total concentration change at 40 m and 50 m were –0.12 ~ +0.096 μmol L^{-1} and –0.11 ~ –0.024 μmol L^{-1}, respectively. The observed decreases were of 1.6 μmol L^{-1} at 30 m, 2.0 μmol L^{-1} at 40 m, and 2.4 μmol L^{-1} at 50 m between the two dates. Thus, we concluded that diffusive transport of NO_3^- had a minor effect on the concentration decreases at the subsurface layer.

Reviewer's comment 3

2) Impact of the biogeochemistry and primary production

The authors concluded "This consumption could result in reduced new production in the subsurface layer after the bloom, when this layer would once again become part of the euphotic zone, if the diatoms sank to deeper layers." However, I cannot agree with this without the evidence that the diatoms are not increased in this layer. Diatoms have some unique modes of nutrient uptake (Martin-Jezequel et al. 2000). Is the observed nutrient uptake of diatoms in the dark condition not linked to the growth? When the authors have the time-series chlorophyll a concentration data in the laboratory experiments, please show the data and discuss that they did not fix carbon. In the case of cyanobacteria, they grow up in the twilight zone (Sohrin et al. 2011). In addition, the dark condition in the laboratory may be different from the dark condition of the field. Even though the PAR is less than 0.1% at the surface, it was not completely dark in the ocean. Many exciting discussions may be possible: nitrate uptake (new production) may occur in the twilight zone but not contribute to the primary production/ new production is underestimated when the nitrate uptake is not measured in more dark layers.

Respond to the comment 3

We proposed alternative hypotheses (1 and 2) to deduce the influence of nutrient uptake by diatoms in dark subsurface layer in bloom.

1) If the diatom population that had consumed half of the nutrients in the dark subsurface layer sank to the deeper layer during the bloom, then the primary production in the subsurface layer after the bloom, at which time it would be part of the euphotic zone, would be reduced by half compared to the production in the case where there was no nutrient consumption during the dark period.

2) If the diatoms that had consumed nutrients in the dark subsurface layer remained in that layer after the bloom, they would rapidly grow under the returning light conditions when the euphotic zone deepened after the bloom.

As for the first hypothesis, we are sure that nutrient loss from euphotic layer leads decrease in primary production. In the case of Funka Bay, we have noted that the consumption of nutrients in the dark subsurface layer would have an impact outside the bay, because the subsurface water is exchanged with Oyashio water. We need to test if diatoms that have once taken nutrients in darkness can grow rapidly in light afterward and if the light intensity (1% PAR, 0.1% PAR, and complete darkness) has an impact on nutrient uptake. We would like to use Martin-Jezequel et al (2000) and Sohrin et al (2011) as references to plan the future research.

To clarify our viewpoints, we have revised as follows:

Line 315 - 326

We propose alternative hypotheses (1 and 2) to deduce the influence of nutrient uptake by diatoms in dark subsurface layer in bloom.

1) If the diatom population that had consumed half of the nutrients in the dark subsurface layer sank to the deeper layer during the bloom, then the primary production in the subsurface layer after the bloom, at which time it would be part of the euphotic zone, would be reduced by half compared to the production in the case where there was no nutrient consumption during the dark period.

2) If the diatoms that had consumed nutrients in the dark subsurface layer remained in that layer after the bloom, they have a potential to rapidly grow under the returning light conditions when the euphotic zone deepened after the bloom.

In the case of Funka Bay, we note that the consumption of nutrients in the dark subsurface layer would have an impact outside the bay, because the subsurface water is exchanged with Oyashio water.

Reviewer’s comment 4

3) The structure of the manuscript

It was just an opinion, but I am familiar with the manuscript which divides results and discussion. I believe the authors can divide them. However, if the authors considered the present style is better, this is not mandatory. This is the option, too, but the title of the manuscript should be a more appealing one. The present title only attracts local interests. For example, when the authors consider the observed phenomenon possibly occurs everywhere under diatom blooms, the title can be revised as “Significant nutrient consumption in the dark subsurface layer during a diatom bloom: the case study on Funka Bay, Hokkaido, Japan”.

Respond to the comment 4

According to the comment, we divided results and discussions and revised the title.

Reviewer’s comment 5

L14: Times of observations are necessary. Technically, the authors’ observation is not time-series, because the observation was conducted randomly.

Respond to the comment 5

We revised the manuscript accordingly as follows:

Line 349 - 350

We conducted repetitive observations in Funka Bay, Hokkaido, Japan, 15 February, 4 and 15 March, and 14 April 2019.

Reviewer’s comment 6

L21 “We believe that this is the first study to present observational evidence for the consumption of the main nutrients by diatoms in the dark subsurface layer during the spring bloom.” In my opinion, this sentence is not important. Instead of this sentence, the authors should add why they considered the nutrient decline does not occur by physical processes.

Respond to the comment 6

We added a sentence about exclusions of physical processes as an explanation of nutrient reduction in the subsurface layer. We think the sentence “this is the first study to present ---” is important. So, we described it in the abstract.

Line 19 - 20

We excluded possibilities of three physical process, water mixing, diffusive transport, and subduction, as reasons for the decrease in nutrients in the subsurface layer.

Reviewer’s comment 7

L26 “Si: NO3– ratio”. Yes, this is not wrong and described in Harrison et al 2004, but Si(OH)4:NO3 or Si: N ratio is more appropriate. This is just opinion.

Respond to the comment 7

We corrected accordingly.

Reviewer’s comment 8

L31–32: References are required.

Respond to the comment 8

We added a reference (Rosa et al., 2007).

Reviewer's comment 9

L34–35: "From time-series observations in the bay, it is possible to examine the temporal changes of biochemical parameters within the same identified water mass while the water is in the bay." I cannot understand this sentence clearly. What means "while the water is in the bay"?

Respond to the comment 9

We revised the sentence as follows:

Line 35 - 37

From repetitive observations in the bay, it is possible to collect the same identified water mass in different times while the water is in the bay and examine the temporal changes of biochemical parameters within the same identified water mass.

Reviewer's comment 10

L37–39: "A massive spring bloom dominated by diatom species occurs in March every year before the Oyashio water flows into the surface of the bay, and it lasts until late March or early April, when Oyashio water occupies the surface of the bay (Odate 1987; Maita and Odate 1988)." I cannot understand this sentence. Please clarify. Can the author divide it into two sentences?

Respond to the comment 10

We revised the sentence as follows:

Line 39 - 41

A massive spring bloom dominated by diatom species occurs in March every year (Odate 1987; Maita and Odate 1988). Oyashio water flows into the bay from the surface after the begging of the bloom. The bloom lasts until late March or early April. Oyashio water occupies the surface of the bay in April (Kudo and Matsunaga, 1999).

Reviewer's comment 11

L58–61: I could not find any meaning in these two sentences. I cannot see any discussion of VOIs in this manuscript. In addition, the reference is under consideration. So I considered these two sentences should be removed.

Respond to the comment 11

According to the comment, we removed the sentence.

Reviewer's comment 12

Method: L72–73: "Observations in Funka Bay have been reported elsewhere (Shimizu et al. 2017)." What did the authors want to describe? Some information on the observations conducted in 2019 was described in 2017? Describe the details or remove the sentence.

Respond to the comment 12

According to the comment, we removed the sentence.

Reviewer's comment 13

L78: How do the authors calculate the analytical precision? This is very low. Did the authors measure the nutrient concentration of not-frozen samples? If the results are frozen samples, the precisions are too good, in particular, silicate.

Respond to the comment 13

We added information about the determination of precision as follows.

Line 77 - 78

Analytical precision was 0.12% for NO_3^-, 0.21% for NO_2^-, 0.19% for PO_4^{3-}, 0.11% for SiO_2, and 0.34% for NH_4^+ as determined by repeated measurement (n = 7) of reference seawater for nutrient standards (KANSO, standard Lot BZ, Osaka, Japan).

Reviewer’s comment 14

L82: Where Thalassiosira nordenskioeldiicome from? Algae collection?

Respond to the comment 14

We added a sentence accordingly:

Line 83 - 84

A diatom *Thalassiosira nordenskioeldii*, which predominates in the early phase of the spring bloom in Funka Bay (Ban et al 2000), was isolated from natural seawater collected in the western subarctic Pacific Ocean (Oyashio area) in May 2019.

Reviewer’s comment 15

L95: “We set the initial concentrations of nutrients at 23 times those of the first dark incubation.” Why did the authors set so high initial nutrient concentration? The environments are very different from the field observations.

Respond to the comment 15

The added information about the amount of added amount of nutrients as follows:

Line 231-233

The added amount of NO_3^- per chl-a (0.022 = 31.1 μmol L^{-1} / 1426 μg L^{-1}) into the nutrient depleted culture of the first experiment was 9% of the amount of NO_3^- per chl-a (0.24 = 3.4 μmol L^{-1} / 14 μg L^{-1}) in seawater at 30 m on 4 March.

Line 241-244

If the diatoms consumed nutrients much more quickly than we collected the culture sample on day 2, the daily consumption rates are underestimated.

We added enough amount of nutrients in the second incubation experiment. The added amount of NO_3^- per chl-a (10.3 = 743.5 μmol L^{-1} / 72.5 μg L^{-1}) was 43 times of the seawater.

Reviewer’s comment 16

* Did not the authors conduct the microscopic observations? Is Thalassiosira nordenskioeldii the dominant species of the observations? This is very important. Because the other species is dominant, the authors’ incubation experiments are meaningless.

Respond to the comment 16

We added information about microscopic analysis as follows:

Line 228-231

From microscopic image analysis, *Thalassiosira nordenskioeldii* occupied 14.2% of number of phytoplankton cells (n = 1209) collected by plankton net (mesh = 100 µm) on 15 March 2019. Other dominant species were *Chaetoceros* spp. and other *Thalassiosira* sp. We confirmed that *Thalassiosira nordenskioeldii* was one of the dominant species in the spring bloom 2019.

Reviewer’s comment 17

L102–106: Please define the water masses at the materials and methods. When the authors defined in the materials and methods section, the results will be simpler.

Respond to the comment 17

We revised the manuscript accordingly.

Reviewer’s comment 18

L106: “The revised classification result” This is unclear. Did the authors revise in this manuscript or revise in Ooki et al. 2019?

Respond to the comment 18

We used the definition of water mass classification proposed in Ooki e al. (2019). Ooki et al. (2019) made a minor change in the classification by Ohtani and Kido (1980).F

We removed the phrase “The revised classification result”

Reviewer’s comment 19

L115–119: This is not a result. Please define in the materials and methods section.

Respond to the comment 19

We revised the manuscript accordingly.

Reviewer’s comment 20

L123: “the original data in supplementary information of Ooki et al., (submitted).” Is it right? I can see the supplementary information of this manuscript.

Respond to the comment 20

We have used the same chl-a data in the two papers (this paper and unpublished paper). We removed the description “original data in supplementary information of Ooki et al., submitted”.

Reviewer’s comment 21

L124: “The data for chl-a are taken from a related article.” What does it mean? I think it is acceptable to share the data with other manuscripts.

Respond to the comment 21

We removed the description “The data for chl-a are taken from a related article”.

Reviewer’s comment 22

L150–168: These paragraphs were “discussion”. These discussions can be put after the results section because the results after this paragraph are not contained the results of the discussion. For me, this style is hard to follow.

Respond to the comment 22

According to the comment, we moved these paragraphs from “results” to “discussion”.

Reviewer’s comment 23

3.3.2 This section (results of incubation experiments) should be shown before 3.3.1.

Respond to the comment 23

We revised accordingly.

Reviewer’s comment 24

L270: Villareal et al. reported Rhizosolenia, and not Thalassiosira. Do the authors have any evidence on the vertical migration of Thalassiosira? If not, this discussion is speculative. In addition, the authors' names are wrong: Wirtz and Lan Smith (2020) are correct.

Respond to the comment 24

We added some explanations about the vertical migration of Thalassiosira (Richardson and Cullen, 1995). To clarify our viewpoint, we revised as follows (please see the sentence marked with yellow):

Line 328-337

In relation to the second hypothesis, an interesting survival strategy for diatom, *Rhizosolenia*, which forms large aggregations (mats), has been proposed (Villareal et al. 1996; Richardson et al. 1998; Villareal et al., 1999; Villareal et al., 2014). The survival strategy of *Rhizosolenia* is that they consume NO_3^- in the dark subsurface layer, and then migrate to the surface euphotic layer where they have a growth advantage in oligotrophic subtropical open ocean areas. For the coastal marine diatom, *Thalassiosira weissflogii,* was studied to examine changes in buoyancy and ratio of carbohydrate to protein which determine the cell density (Richardson and Cullen, 1995). They revealed that accumulation of carbohydrate as a result of nitrate depletion leads rises in cellular density and sinking speed and that accumulation of protein as a result of nitrate addition after the nitrate depletion leads a positive buoyancy. Several modelling studies reported contributions of primary production by vertically migrating phytoplankton to net primary production. For example, Witz and Lan Smith, (2020) estimated that vertically migrating phytoplankton contributes 7% of net primary production at the subarctic gyre of the western Pacific.