Dear anonymous referee2

We will send responses to referee's comments of "Substantial nutrient consumption in the dark subsurface layer during a diatom bloom: the case study on Funka Bay, Hokkaido, Japan" (revised title) by Umezawa et al. The referee's comments were very helpful, and we have revised the manuscript taking all comments. Corrections in the revised manuscript (and the responses to referee's comments) are highlighted.

Best regards,

Corresponding author: Atsushi Ooki

Contents of response letter

Revised chapter structure

Response to reviewer 2

GENERAL COMMENTS

Reviewer's comment 1

*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 productivityin 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 arenot 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?

Response to the comment 1

According to the comment, we added a reference (Nakada et al 2013) and clarified the purpose of this study. We revised as follows:

Line 31-58

Dissolved iron and nitrate (NO_3^-) supplied from below the surface to the surface euphotic zone through winter vertical water mixing sustain spring phytoplankton bloom in the Oyashio region (Nishioka et al. 2011). Most previous studies about marine primary production have concerned with the nutrient consumption by phytoplankton in the euphotic zone because most phytoplankton species, except for dinoflagellates (e.g. Cullen and Horrigan 1981), are commonly assumed to be incapable of moving actively between the surface mixed layer and below the surface (subsurface layer). A few studies have focused on the vertical migration of a diatom, *Rhizosolenia*, to uptake nutrients in the subsurface layer and grow in the euphotic zone in the oligotrophic subtropical Pacific (Villareal et al. 1996; Richardson et al. 1998; Villareal et al., 1999; Villareal et al., 2014). As for the subarctic area, a modelling study that simulated a lot of chl-a profiles, taking into phytoplankton's migration behaviour, demonstrated that vertically migrating phytoplankton can pump up considerable amount of nutrient to the surface layer from the dark subsurface layer and contributes 7% of net primary production at the subarctic gyre of the western Pacific, Oyashio region (Witz and Lan Smith, 2020). These previous studies have not yet shown observational evidence of nutrient reduction associated with consumption by phytoplankton in the dark subsurface layer, however, nutrient reduction in the dark subsurface layer have been found in the Funka Bay, Hokkaido, Japan (Kudo and Matsunaga, 1999), which faces to the Oyashio-Kuroshio transitional area in the western North Pacific.

Oyashio water reaches the area off the coast of Hokkaido, Japan, where the subtropical water derived from Kuroshio or the Tsugaru warm current waters are also found (Rosa et al., 2007). A small portion of Oyashio water enters Funka Bay in early spring. The bay water exchanges twice a year, with cold Oyashio water in early spring and Tsugaru warm water in early fall (Ohtani 1971). The Oyashio water, a cold and low salinity water, flows into the bay along the northern coast of the bay, forming an anticlockwise flow from late of March to middle of April (Nakada et al. 2013). From repetitive observations in the bay, it is possible to collect seawater samples originated from the same water mass in different times when the water remains in the bay during the observation period and then examine the temporal changes of biogeochemical parameters within the same water mass. For example, temporal changes in nutrients (Kudo and Matsunaga 1999; Kudo et al. 2000), dissolved iron (Hioki et al. 2015), volatile organic iodine (Shimizu et al. 2017) and isoprene (Ooki et al. 2019) have been examined in relation to primary production in the bay. In the Funka Bay, diatom bloom initiates in late winter, February, before Oyashio water flows into the bay (Kudo and Matsunaga, 1999). A massive spring bloom dominated by diatom species occurs in March every year (Odate 1987; Maita and Odate 1988) when Oyashio water flows into the bay. The bloom lasts until late March or early April when Oyashio water occupies the surface of the bay (Kudo and

Matsunaga, 1999).

Line 73-76
In this paper, we examine the temporal variation of nutrient concentrations in Funka Bay from the early phase of the diatom bloom (February) to post-bloom (April) through repetitive observations in 2019. And we focused on the processes affecting nutrient reduction in the dark subsurface layer during the bloom to show evidence of nutrient consumption by diatoms in darkness.

Reviewer's comment 2
*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…
Response to the comment 2
According to the comment, we used "repetitive" instead of "time-series".

Reviewer's comment 3
When investigating biological processes, incubation experiments such as those conducted in this study MUST be replicated (triplicates are a must), otherwise any interpretation coming outfrom the experiment results is only speculative and conclusion cannot be ensured. This is very important!
Response to the comment 2
According to the comment, we carried out the third and fourth incubation experiments additionally (each quadruplicated, n=4). The results of the third and fourth experiments were close to the nutrient reduction found at 30–50-m depths in Funka Bay. However, the dark consumption rates of our experiments and previous studies had wide range values. Thus, we concluded that "dark consumption by diatoms had a potential to reduce nutrients by half in the dark subsurface layer of Funka bay".

We added sentences as follows:
Line 116-125
In the third and fourth experiments, diatom culture *Thalassiosira nordenskioeldii* had grown in modified f/2 medium (NO_3^-, 175 µmol L^{-1}; PO_4^{3-}, 6.5 µmol L^{-1}; $Si(OH)_4$, 18.8 µmol L^{-1}) for 10 and 11 days, respectively. The chl-*a* concentrations in the pre-culture mediums for the third and fourth experiments reached 145 and 198 µg L^{-1}, respectively. The concentrations of NO_3^-, PO_4^{3-}, and $Si(OH)_4$ in the pre-cultured mediums dropped as follows: NO_3^-, 9.27 µmol L^{-1}; PO_4^{3-}, 0.42 µmol L^{-1}; and $Si(OH)_4$, < 1 µmol L^{-1} for the third experiment; NO_3^-, 0.66 µmol L^{-1}; PO_4^{3-}, 0.42 µmol L^{-1}; and $Si(OH)_4$, < 1 µmol L^{-1} for the fourth experiment. We added nutrients (stock f/2 medium) into the pre-culture mediums, after which concentrations (day 0) were as follows: NO_3^-, 186 µmol L^{-1}; PO_4^{3-}, 7.7 µmol L^{-1}; and $Si(OH)_4$, 16.1 µmol L^{-1} for the third experiment; NO_3^-, 213 µmol L^{-1}; PO_4^{3-}, 9.1 µmol L^{-1}; and $Si(OH)_4$, 21.2 µmol L^{-1} for the fourth experiment. Each pre-culture medium (160 mL) was divided into 4 cell-cultivation flasks and put in darkness at 6 °C. On days 1, 2, and 3, 8 mL and 1 mL of incubation medium (n = 4) were filtered to measure nutrient and chl-*a* concentrations, respectively.
Line 316-322
In the third and fourth experiment, the added amount of NO_3^- per chl-a (0.77 – 0.96) was 1.9 – 2.4 times of the seawater at 40 m on 4 March. The results of the third and fourth experiments (Fig. 7a-h) demonstrated that the consumption rates, which were calculated from the concentration difference of nutrient between day 0 and day 3 and the initial chl-a concentrations (day 0), were 0.034 – 0.043 µmol (µg chl-*a*)$^{-1}$ d^{-1} for NO_3^-, 0.0059 – 0.0086 µmol (µg chl-*a*)$^{-1}$ d^{-1} for PO_4^{3-}, and 0.034 – 0.035 µmol (µg chl-*a*)$^{-1}$ d^{-1} for $Si(OH)_4$. The estimated ΔNO_3^- (–4.3 ~ –5.4 µmol L^{-1}), ΔPO_4^{3-} (–0.75 ~ –1.1 µmol L^{-1}), and $\Delta Si(OH)_4$ (–4.3 ~ –4.3 µmol L^{-1}) were close to the actual decreases between the two

dates: ΔNO_3^-, –2.0 μmol L^{-1}; ΔPO_4^{3-}, –0.12 μmol L^{-1}; $\Delta Si(OH)_4$, 3.7 μmol L^{-1}.
Line 335-336
Although the dark consumption rates had wide ranges, we concluded that dark consumption by diatoms had a potential to reduce nutrients by half in the dark subsurface layer of Funka Bay.

Reviewer's comment 4
*Section 3.2 of the Discussion is really long compared to other sections and contain 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 reducingthe length of this section (especially for chl*a* and nitrate) and remove most of the redundancies.
Response to the comment 4
According to the reviewers', we have largely reconstructed the manuscript . We divided the chapter "results and discussion" into separate chapters.

Reviewer's comment 5
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?
Response to the comment 5
According to the comment, we added a chapter as follows:
Line 247-257
3.3 Limiting factor of primary production during the bloom
On 15 February before the occurrence of massive diatom bloom, the average concentrations of NO_3^-, PO_4^{3-} and $Si(OH)_4$ at the surface 0 – 10 m were 9.1, 0.86 and 19.8 μmol L^{-1}, respectively. On 4 March at the peak of the bloom, the average concentrations of NO_3^-, PO_4^{3-} and $Si(OH)_4$ at the surface 0 – 10 m were 0.34, 0.43 and 5.6 μmol L^{-1}, respectively. Uptake ratios of N:P and Si:N at the surface between 15 February and 4 March were 20.5 {= (9.1 – 0.34) / (0.86 – 0.43)} and 1.62 {= (19.9 – 5.6) / (9.1 – 0.34)}, respectively. Similar uptake ratios during diatom bloom in Funka Bay have been reported to be N:P = 15.6 – 23.6 and Si:N = 1.9 – 2.7 (Kudo and Matsunaga 1999). From the uptake ratio of N:P, NO_3^- in the surface water could have been depleted since 4 March. On 15 March at the decline phase of the bloom, the average concentrations of NO_3^-, PO_4^{3-} and $Si(OH)_4$ at the surface 0 – 10 m were 0.54, 0.37 and 4.5 μmol L^{-1}, respectively. Since sufficient amount of $Si(OH)_4$ remained in the surface water on 15 March, we considered that the N-depletion in the surface water limited primary production after the peak of the bloom.
Line 334-335
In the dark subsurface layer of Funka Bay, N-depleted diatoms sunk from the surface after the peak of bloom could have enhanced NO_3^- consumption in darkness.

Reviewer's comment 6
*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.
Response to the comment 6
We added discussions about possibilities of diffusive transport of nitrate and entrainment of

subducted water from the surface. And we revised the explanation about the results of incubation experiments.

Line 360 – 387

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 decrease at the subsurface layer (30 – 50 m) on 15 March. There is not any previous study to have measured 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 sum of concentration changes at 30 m, which include 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 the sum of concentration changes 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 these dates. Thus, we concluded that diffusive transport of NO_3^- had a minor effect on the concentration decreases at the subsurface layer.

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 4 and 15 March (Fig. 2). 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 water should have the same temperature, salinity, and density as it had been at the surface. 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 at the station. The middle layer water at the station could have reached from anywhere of the bay within 11 days. We obtained the spatial distributions of temperature, salinity, and density at the sea surface (1 m) on 4 March using the ocean reanalysis product provided by Meteorological Research Institute in Japan (Fig. 8a-c).
From these spatial distributions, there was not any area that satisfied required temperature (3.5 – 3.6 °C), salinity (33.64), and density (26.7σ) to form subduction water at 40-m depth of the observation station, see an enlarged map of Fig. 8c. 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.

Line 144-152

2.5 Spatial distributions of temperature, salinity, and density at the sea surface

Spatial distributions of temperature, salinity, and density at the sea surface (1 m) were obtained from an ocean reanalysis product provided by Meteorological Research Institute in Japan. This is produced with an operational system for monitoring and forecasting the status of coastal and open-ocean waters around Japan (the JPN system; Hirose et al., 2020). The JPN system includes a double-nested ocean model, the core of which is a Japanese coastal model with a horizontal resolution of 2

km. Three sub-models are interconnected using a nesting technique: a global model (horizontal resolution ~100km), a North Pacific model (horizontal resolution ~10km), and Japanese coastal model (horizontal resolution ~2km). A four-dimensional variational method is applied to the North Pacific model as the assimilation scheme. The process of tides and river runoff are taken into consideration in this JPN system. See the technical report for more detail (Hirose et al. 2020).

Line 428-454

Conclusion

We conducted repetitive observations in Funka Bay, Hokkaido, Japan, from 15 February to 14 April 2019 during and after the spring bloom. We found reductions in nutrient concentrations in the dark subsurface layer both before and after the peak of the bloom and concluded that the latter reduction was caused by dark consumption by diatoms that had grown in the euphotic zone and then sank to the dark subsurface layer. We reached this conclusion using the following rationale.

(1) From the dark incubation experiments, we confirmed that the diatom *Thalassiosira nordenskioeldii*, which is one of the dominant diatom species in the bloom of Funka Bay, could consume nutrients in darkness at substantial rates. Although the consumption rates varied over a wide range, we concluded that dark consumption of nutrient by diatom that had been growing at the surface and then sank into the subsurface layer had a potential to reduce nutrient by half in the dark subsurface layer (30–50 m).

(2) We excluded water mixing, diffusive transport, and subduction as possible reasons for nutrient reduction in the subsurface layer between 4 March and 15 March. First, the stratification between the surface and subsurface layers was strengthened after 4 March, and therefore we considered vertical mixing of water between the layers to be limited. The small decline in salinity at 30 m and no change in salinity at 40–50 m means that mixing with low-salinity Oyashio water could not explain the nutrient reduction, even if the Oyashio water had no nutrients. Second, we estimated the diffusive transport of NO_3^- to have a minor effect on concentration decrease at the subsurface layer. Third, we showed that there was not any area that satisfied required surface temperature, salinity, and density to form subduction water at the subsurface layer (medium depth of 40 m) at the observation station on 15 March. Thus, we excluded the possibilities of subduction as a reason for the nutrient reduction.

The consumption of nutrients in darkness has been studied in many simulated in-situ incubation experiments, with the goal of understanding dark consumption during a daily cycle within the euphotic zone. We believe that this is the first study to demonstrate observational evidence of consumption of the three main nutrients (NO_3^-, PO_4^{3-}, and $Si(OH)_4$) by diatoms in the dark subsurface layer during a bloom. 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. Further research is needed examining the survival strategies of diatoms consuming nutrients in the dark subsurface layer.

Reviewer's comment 7

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.

Response to the comment 7

According to the comment, we added a definition “dark-zone depths (0.1% of surface PAR depth) “ in the abstract. We defined the euphotic-zone depth and the dark-zone depth as 1% and 0.1%, respectively, of surface PAR depth. Thus, we used the term, “dark-zone depth”.

We added explanations that the physical processes were excluded from reasons of the nutrient reduction.

Line 21-22

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 8

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

Response to the comment 8

Professional scientific editors (ELSS, Inc., Tsukuba, Japan) recommended to use “15 February to 14 April”. If the BG editor requires proof reading by professional editors for the revised manuscript, we will offer proof reading to ELSS again.

Reviewer’s comment 9

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

Response to the comment 9

We have revised as “on 4 and 15 March”.

Yes, winter water was found above the euphotic zone depth on 4 March. Deeper winter water remained below the dark-zone depths from 4 March to 15 March.

Reviewer’s comment 10

L17: Same comment as above, please specify which dates. On fig.4, while NO_3^- concentration decreases by approximately half, it is absolutely not the case for $Si(OH)_4$ and PO_4^{3-} (e.g. at 50m concentrations dropped from 0.7 to 0.6 µmol L^{-1} and from 15 to 13 µmol L^{-1} for PO_4^{3-} and $Si(OH)_4$ respectively)

Response to the comment 10

According to the comment 9, we specified the two dates (4 and 15 March) at the prior sentence, thus “these dates” is specified as “4 and 15 March”.

Reviewer’s comment 11

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

Response to the comment 11

According to the comment, we removed the sentence from abstract.

Reviewer's comment 12
L25-26: Surface euphotic zone sounds redundant.
Response to the comment 12
According to the comment, we removed "surface" form "surface euphotic" throughout the manuscript.

Reviewer's comment 13
L28: Use either "Si:N ratio" or "$Si(OH)_4$:NO_3^- ratio"
Response to the comment 13
According to the comment, we used "Si:N ratio".

Reviewer's comment 14
L38: It is actually believed that the intrusion of the Oyashio water in the Bay triggers the diatombloom by strengthening water column stratification.
Response to the comment 14
We recognize that diatom growth begins in late winter before the Oyashio water flows into the bay and massive diatom bloom in the bay is triggered by intrusion of the Oyashio water.
According to the comment, we revised as follows:
Line 55-57
In the Funka Bay, diatom bloom initiates in late winter, February, before Oyashio water flows into the bay (Kudo and Matsunaga, 1999). A massive spring bloom dominated by diatom species occurs in March every year (Odate 1987; Maita and Odate 1988) when Oyashio water flows into the bay.

Reviewer's comment 15
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.)
Response to the comment 15
According to the comment, we added a chapter about nutrient ratio and nutrient limitation as follows:

Line 247-257

3.3 Limiting factor of primary production during the bloom

On 15 February before the occurrence of massive diatom bloom, the average concentrations of NO_3^-, PO_4^{3-} and $Si(OH)_4$ at the surface 0 – 10 m were 9.1, 0.86 and 19.8 μmol L^{-1}, respectively. On 4 March at the peak of the bloom, the average concentrations of NO_3^-, PO_4^{3-} and $Si(OH)_4$ at the surface 0 – 10 m were 0.34, 0.43 and 5.6 μmol L^{-1}, respectively. Uptake ratios of N:P and Si:N at the surface between 15 February and 4 March were 20.5 {= (9.1 – 0.34) / (0.86 – 0.43)} and 1.62 {= (19.9 – 5.6) / (9.1 – 0.34)}, respectively. Similar uptake ratios during diatom bloom in Funka Bay have been reported to be N:P = 15.6 – 23.6 and Si:N = 1.9 – 2.7 (Kudo and Matsunaga 1999). From the uptake ratio of N:P, NO_3^- in the surface water could have been depleted since 4 March. On 15 March at the decline phase of the bloom, the average concentrations of NO_3^-, PO_4^{3-} and $Si(OH)_4$ at the surface 0 – 10 m were 0.54, 0.37 and 4.5 μmol L^{-1}, respectively. Since sufficient amount of $Si(OH)_4$ remained in the surface

water on 15 March, we considered that the N-depletion in the surface water limited primary production after the peak of the bloom.

Reviewer's comment 16

L58-61: VOI are not presented nor discussed in this manuscript. Please remove these sentencesor 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?).

Response to the comment 16

According to the comment, the reference about VOI was removed from there.

Reviewer's comment 17

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

Response to the comment 17

According to the comment, we removed the sentence.

Reviewer's comment 18

L77: SiO_2 is not a nutrient. This is the formula for particulate silica, silicic acid (the nutrient) is $Si(OH)_4$, please make sure to double-check the notations throughout the manuscript.

Response to the comment 18

According to the comment, we changed "SiO_2" to "$Si(OH)_4$" throughout the manuscript.

Reviewer's comment 19

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

Response to the comment 19

We measured reference seawater for nutrient standards (KANSO). The precisions of repetitive analysis are pretty good.

According to the comment, we added an explanation about the precision as follows:

Line 91-93

Analytical precision was 0.12% for NO_3^-, 0.21% for NO_2^-, 0.19% for PO_4^{3-}, 0.11% for $Si(OH)_4$, and 0.34% for NH_4^+ as determined by repetitive measurement (n = 7) of reference seawater for nutrient standards (KANSO, standard Lot BZ, Osaka, Japan).

Reviewer's comment 20

L83: please use axenic instead of sterile. F/2 medium has approximately 106 $\mu mol.L^{-1}$ $Si(OH)_4$ 882 $\mu mol.L^{-1}$ NO_3^-, and 36 $\mu mol.L^{-1}$ PO^{3-} (see Guillard and Ryther, 1962; Guillard, 1975 or Andersen et al (2005), Algual Culturing Techniques). It is more likely a modified (not particularlySi-rich) f/2 medium then, although I agree it is still plenty of nutrients for diatoms.

Response to the comment 20

According to the comment, we used "axenic" instead of "sterile" and added a word "modified".

Reviewer's comment 21

L86: This is really unfortunate since checking for contamination is crutial regarding the objectiveof the incubation experiment. Indeed, contamination by a population of other micro-algae or bacteria can affect the consumption (or recycling) of NO_3^- and PO^{3-} (although it is probably less the case for $Si(OH)_4$). This have to be mentioned and discussed in the discussion.

Response to the comment 21

According to the comment, we added an explanation about the bacterial contamination as follows:

Line 297-299

Since we did not check the bacterial contamination after the experiment, bacterial consumption and/or recycling of nutrients in the culture might have an effect on the results. We assumed that the bacterial activity had a less effect on nutrient changes in the high-density diatom culture.

Reviewer's comment 22

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

Response to the comment 22

Silicic acid was almost depleted on day 2 of the first experiment. If the diatoms consumed $Si(OH)_4$ much more quickly than we collected the culture sample on day 2, the daily consumption rates are underestimated. So, we added much more nutrients in the second experiment.

According to the comment, we added explanations as follows:

Line 306-309

Silicic acid was almost depleted on day 2. If the diatoms exhausted $Si(OH)_4$ more quickly than we collected the culture sample on day 2, the daily consumption rates are underestimated.

In the second experiment, we added excess amount of nutrients into the nutrient-depleted medium, in which cultured diatoms were in a decline phase of growth.

Reviewer's comment 23

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 watermass end-members (such as in Shimizu et al. 2017) in supplement material at least.

Response to the comment 23

According to the comment, we added an illustration of water mass definition as supplement material.

Reviewer's comment 24

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 whilebiochemical is more related to intra-cellular processes.

Response to the comment 24

According to the comment, we used "biogeochemical" instead of "biochemical".

Reviewer's comment 25

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, appropriatereferencing is missing.

Response to the comment 25

According to the comment, we added references and explanations why we adapted the threshold 0.125 kg m^{-3} as follows:

Line 135-143

The surface mixed layer was defined as the layer in which density differences (Δσ) were within 0.125 kg m^{-3} relative to the density at 5-m depth. The threshold Δσ = 0.125 kg m^{-3} is often used for monthly mean of mixed layer in oceanic climate studies (Spall 1991), while the threshold Δσ = 0.01 kg m^{-3} is used for snap-shot observations (Thomson and Fine 2003). We used the maximum threshold Δσ = 0.125 kg m^{-3} to ensure that the subsurface layer water had not mixed with the surface layer during intervals (11 days to a month) between our observations. The euphotic-zone depth was defined as the depth at which photosynthetically active radiation (PAR) was 1% of the surface PAR, where amount of photosynthesis is equal to respiration (Marra 2004). We defined the dark-zone depth at which PAR was 0.1% of the surface PAR, where amount of photosynthesis is approximately tenth part of the 1% PAR depth taking into account the light intensity only as a limiting factor of photosynthesis.

Reviewer's comment 26

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.

Response to the comment 26

According to the comment, we revised the sentence. Please see the response to the comment 25.

Reviewer's comment 27

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…

Response to the comment 27

According to the comment, we added chl-a data in supplementary information of this paper.

Reviewer's comment 28

L125-126: 27 to 30 μg 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?

Response to the comment 28

Yes, chl-a concentrations of 27 to 30 μg L^{-1} are very high. We will examine the mechanism of such a high chl-a event as future research.

Reviewer's comment 29
L131: Sinking particles and suspended particles are different things. What makes authors conclude that particles can be sinking vs. in suspension? Please develop.
Response to the comment 29
We can not make a distinction between sinking particle and suspended particle. We used "sinking" in the manuscript.

Reviewer's comment 30
L140: A reference for the annual maximum is missing.
Response to the comment 30
The annual maximum level (~20 μmol L^{-1}) of nitrate was found in the bottom water in summer (e.g. Kudo et al. 2007).
According to the comment, we removed a phrase "the annual maximum".

Reviewer's comment 31
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 thusbe renamed "nutrient dynamic in the euphotic zone and dark subsurface layer" or something similar.
Response to the comment 31
We have largely reconstructed the manuscript. We divided the chapter "results and discussion" into separate chapters.

Reviewer's comment 32
L168-175: I do not see why the first explanation of vertical mixing suggested for March 4th NO_3^- decrease at depth cannot be still valid in March 15th. Indeed, authors based their explanation mostly on the AOU profile. However, AOU profile in the 30-50m layer in March 14th looks prettymuch the same as in March 4th (see fig.3).
Response to the comment 32
The reason for the same AOU values in the subsurface layer between 4 and 15 March is that net O_2 production had not occurred there.
According to the comment, we added AOU profiles (Fig. 5f) to show that the values stayed unchanged between the two dates. And we changed a phrase "are slightly decrease" to "remained almost the same values".

Reviewer's comment 33
L180-186: Note that the signal of regeneration is also clearly seen from the strongly positivevalues of AOU at depth in April 14th.
Response to the comment 33
According to the comment, we added a sentence about AOU increase in the bottom water as follows:
Line 234-235
Obvious increase of AOU in the deep water (80 – 95-m depth) was found from 15 March (average 20.9 μmol L^{-1}) to 14 April (average 56.0 μmol L^{-1}), see Fig. 4b and Fig. 5f.

Reviewer's comment 34

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

Response to the comment 34

According to the comment, we added explanations about rises in bottom water concentrations in detail as follows:

Line 215-217

In the deep water (80–95 m), the NO_3^- concentrations slightly increased with time since 15 March: 5.38 µmol L^{-1} on 4 March, 5.26 µmol L^{-1} on 15 March, and 6.60 µmol L^{-1} on 14 April.

Line 231-238

In contrast to the subsurface layer, the average concentrations of PO_4^{3-} and $Si(OH)_4$ in the deep layer (80 – 95-m depth) increased with time; 0.78 µmol L^{-1} and 15.3 µmol L^{-1} on 4 March, 0.89 µmol L^{-1} and 22.3 µmol L^{-1} on 15 March, and 1.57 µmol L^{-1} and 25.1 µmol L^{-1} on 14 April, respectively. Obvious increase of AOU in the deep water (80 – 95-m depth) was found from 15 March (average 20.9 µmol L^{-1}) to 14 April (average 56.0 µmol L^{-1}), see Fig. 4b and Fig. 5f. Because the obvious increase of PO_4^{3-} coincided with the rise in AOU, it likely resulted from remineralization following the decomposition of organic matter suspended in the bottom water or settled on the seafloor. The increase of $Si(OH)_4$ in the bottom water is likely resulted from dissolution of biogenic silica settled on the seafloor.

Line 242-246

Because the NH_4^+ concentrations were at their lowest during winter with total column average of 0.25 µmol L^{-1} on 15 February, the signal from remineralization could be clearly detected on 15 March with average of 0.54 µmol L^{-1} at the subsurface water (30 – 50 m). The deep water NH_4^+ concentrations obviously increased with time since 4 March: 0.31 µmol L^{-1} on 4 March, 0.95 µmol L^{-1} on 15 March, and 3.05 µmol L^{-1} on 14 April.

Reviewer's comment 35

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 hypothesisfor 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.

Response to the comment 35

We added a discussion about the diffusive transport of nitrate. Please see the response to the comment 6.

Reviewer's comment 36

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 asharp change in density (even though this latter is smaller).

Response to the comment 36

According to the comment, we added CTD profile (Fig.2) to show all plot and used the density gradient instead of the magnitude of density difference.

Line 341-342

Because the density (σ) gradient between 20-m and 30-m depths, ($\sigma_{30m} – \sigma_{20m}$) / (30 m – 20 m), 0.0033 (kg m^{-3} m^{-1}) on 4 March substantially increased to 0.021 (kg m^{-3} m^{-1}) on 15 March,

Reviewer's comment 37
L212-214: Please show these data in a figure
Response to the comment 37
The data can obtain from the JMA website. We added a URL of download site as follows:
Line 345
https://www.data.jma.go.jp/risk/obsdl/index.php

Reviewer's comment 38
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 andthe density profiles on fig.4 are incomplete).
Response to the comment 38
According to the comment, we revised the figure (CTD profiles in Fig. 2) to show all plots.

Reviewer's comment 39
L218-228: This could be another subsection focused on discussing horizontal advection
Response to the comment 39
We added a discussion about a possibility of subduction water (subducted water horizontally moved to the observation station). Please see the response to the comment 6.

Reviewer's comment 40
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 Oyashiowater 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.

Response to the comment 40
According to the comment, we revised the sentence as follows:
Line 353-355
A mixing between 20% of Oyashio water and 80% of Funka Bay water at 30 m would change the salinity at 30-m depth from 33.58 (on 4 March) to 33.47 (on 15 March).

Reviewer's comment 41
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 oneof them (not the only one).
Response to the comment 41
We agree with the comment 41 that the nutrient reduction in the subsurface layer is driven by multiple processes. We estimated that the diffusive transport occupied approximately 10% of the reduction and concluded that the consumption by diatom had the most important effect on the reduction. Please see the response to the comment 6.

Reviewer's comment 42

L237 and after: Please explain the μg chl*a*$^{-1}$ 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 NO_3^-, PO_4^{3-} as well as $Si(OH)_4$ consumption are discussedbut only 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.

Response to the comment 42

According to the comment, we added an explanation of the consumption rate and the values of chl-a concentrations in the figure. We used the chl-a concentrations on day 0 for the calculations of nutrient consumption rate per unit chl-a.

And, we added figures of the dark incubation results for PO_4 and $Si(OH)_4$ and added a sentence explaining chl-a changes during the dark incubations.

Line 299-301

The daily consumption rates per unit chl-a amount calculated from the concentration difference of nutrients between day 0 and day 2 and the initial concentration of chl-a (1426 μg L^{-1}) of the dark incubation

Line 322-323

The chl-a concentrations were increased in darkness from 145 μg L^{-1} (day 0) to 250 μg L^{-1} (day 3) for the third experiment and from 198 μg L^{-1} (day 0) to 294 μg L^{-1} (day 3) for the fourth experiment.

Revised figures: Fig. 6 and Fig. 7 (attached below)

Reviewer's comment 43

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 4th and March 14th.

Response to the comment 43

According to the comments, we additionally conducted the third and fourth experiments. We explained that "although the dark consumption rates had wide ranges, we concluded that dark consumption by diatoms had a potential to reduce nutrients by half in the dark subsurface layer of Funka Bay" as follows:

Line 317-322

The results of the third and fourth experiments (Fig. 7a-h) demonstrated that the consumption rates, which were calculated from the concentration difference of nutrient between day 0 and day 3 and the initial chl-a concentrations (day 0), were 0.034 – 0.043 μmol (μg chl-*a*)$^{-1}$ d^{-1} for NO_3^-, 0.0059 – 0.0086 μmol (μg chl-*a*)$^{-1}$ d^{-1} for PO_4^{3-}, and 0.034 – 0.035 μmol (μg chl-*a*)$^{-1}$ d^{-1} for $Si(OH)_4$. The estimated ΔNO_3^- (–4.3 ~ –5.4 μmol L^{-1}), ΔPO_4^{3-} (–0.75 ~ –1.1 μmol L^{-1}), and $\Delta Si(OH)_4$ (–4.3 ~ –4.3 μmol L^{-1}) were close to the actual decreases between the two dates: ΔNO_3^-, –2.0 μmol L^{-1}; ΔPO_4^{3-}, –0.12 μmol L^{-1}; $\Delta Si(OH)_4$, 3.7 μmol L^{-1}.

Line 335-336

Although the dark consumption rates had wide ranges, we concluded that dark consumption by diatoms had a potential to reduce nutrients by half in the dark subsurface layer of Funka Bay.

Reviewer's comment 44

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?

Response to the comment 44

We revised the time interval for the consumption rate calculation of the second experiment to between day 0 and day 2. We added explanations about the amount of added nitrate per unit chlorophyll. The observed nitrate amount per unit chlorophyll in the bay were within the range of added nitrate amount per unit chlorophyll in cultures of dark incubations.

Line 293-295

The added amount of NO_3^- per chl-a (0.022 = 31.1 μmol L^{-1} / 1426 μg L^{-1}) was 6% of the ratio of NO_3^-/chl-a (0.40 = 4.8 μmol L^{-1} / 12 μg L^{-1}) in seawater at 40 m on 4 March.

Line 309-310

The added amount of NO_3^- per chl-a (10.3 = 743.5 μmol L^{-1} / 72.5 μg L^{-1}) was 26 times of the seawater at 40 m on 4 March

Line 316-317

In the third and fourth experiment, the added amount of NO_3^- per chl-a (0.77 – 0.96) was 1.9 – 2.4 times of the seawater at 40 m on 4 March.

Reviewer's comment 45

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 nutrientconsumption occurs "without photosynthetic growth"? Please explain.

Response to the comment 45

Although we did not count cell number in the seawater sample collected in the bay and the sample of incubation experiment, capacity of nutrient consumption in darkness might depend on the cell density and nutrition conditions for each cell. We will examine property of nutrient consumption in darkness associated with chl-a, cell number, nutrition condition of diatom cell as future research.

Reviewer's comment 46

L248-249: I totally agree with this statement!

Response to the comment 46

The main purpose of this study is to show the observational results of nutrient reduction in the dark subsurface layer associated with consumption by diatom, excluding possibilities of physical processes. Investigations of dark consumption properties associated with nutrition condition of diatom cell are remained for future research.

Reviewer's comment 47

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

Response to the comment 47

We revised the sentences as follows:

Line 324-326

Cochlan et al. (1991) carried out onboard incubations with a diatom dominating natural seawater setting dark periods of 2–4 hours after light periods. They have reported dark consumption rates for NO_3^- of 0.09–0.14 μmol (μg chl-*a*)$^{-1}$ d^{-1}, which are close to the results from our second incubation.

Reviewer's comment 48

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.

Response to the comment 48

According to the comment, we added a reference about the changes in buoyancy of Thalassiosira (Richardson and Cullen, 1995).

Line 411-415

For the coastal marine diatom, *Thalassiosira weissflogii,* was studied to examine changes in buoyancy in relation to ratios 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.

Reviewer's comment 49

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

Response to the comment 49

According to the comment, we revised the sentence as follows:

Line 420-422

These previous studies have not yet found any evidence of decrease in NO_3^- in the dark subsurface layer from observation. If the hypothesis of diatoms' migration strategy proposed by previous studies is true, the results of our study will provide evidence for the decrease in NO_3^- in the dark subsurface layer associated with the diatoms' strategy.

Reviewer's comment 50

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

Response to the comment 50

We used "repetitive" instead of "time-series" in the revised manuscript.

Reviewer's comment 51

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

Response to the comment 51

We carried out additional incubation experiments. The results were close to the observed nutrient reduction rate per chlorophyll. Please see the response to the comment 43.

Reviewer's comment 52

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.

Response to the comment 52

According to the comment, we described “T: Subtropical Tsugaru current” and “O: Subpolar Oyashio current” in the figure legend.

Reviewer’s comment 53

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 TransitionalWater: WO).

Response to the comment 52

According to the comment, we described the definition of the euphotic-zone and mixed-layer depths in the caption and corrected the legends, “WO: Transitional Water”.

Reviewer’s comment 54

Figure 3: MLD is not at the same depth on Apr 14th in fig.2 (40m) and fig.3 (15m), same on March 4th 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 4th, but we know that diatoms can consume nutrients pretty quickly in the mixed layer and the bloom could have started anytime between Feb 15th and March 4th, 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.

Response to the comment 54

We think that the rectangle showing the depth-temporal range (30-50m, 4-15 March) helps to read the figure. According to the comment, we added figures of vertical profile of chl-a, nutrients and AOU on (Fig. 5) to avoid the misinterpretation. We corrected the MLD on Apr 14th in the figure and revised a sentence as follows:

Line 184-185

15 March Chl-*a* concentrations had decreased at all depths by 15 March, however, there were still high levels (0–10 m, 11.0–16.2 μg L^{-1}) within the euphotic surface mixed layer (0 – 18 m) and in the deeper dark layer (20–95 m, 2.3–7.8 μg L^{-1}).

Fig.5

Please see an attached figure below.

Reviewer’s comment 55

Figure 4: The scale on the x axis in the temperature, salinity and density panels are not appropriate since the profile corresponding to March 15th in all panels is cut at the surface, same for the March 4th density profile. It is hard to discuss incomplete profiles. Right now, wecannot conclude that stratification is stronger on March 15th 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 4th to March 15th. I would be interesting to add the standard deviations for the nutrient concentration measurements.

Response to the comment 55

According to the comment, we revised the CTD profiles (Fig.2) to show all and remove the arrows.

Although we determined the analytical precision of nutrient measurements by repetitive analysis of standard seawater (KANSO), we did not measure the seawater sample more than three times a sample. Thus, we could not show the standard deviation on the figure.

Fig.2
Please see an attached figure below.

Reviewer's comment 56
Figure 5: Overall, I like this figure. It is clear, although the scale of the x axis on panel b does notmake sense (two squares correspond to 4 days (0 to 4) then to two days (4 to 6), then back to 4days (6 to 10). I also still don't understand how the authors have defined their set-up conditions(e.g. why adding 30µmol. L^{-1} NO_3^- in the first experiment and around 750µmol L^{-1} in the second one? Why this threshold of 1429µg L^{-1} chl*a* in the first experiment but 72.5µg 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.

Response to the comment 56
According to the comment, we revised the figure. The initial concentrations of chl-a were the results of pre-cultivation for growing diatom till nutrients were once exhausted in the light condition. According to the comment, we carried out additional experiments (n = 4). We revised the figures of the first and second experiments and added figures of the additional third and fourth experiments.

Fig. 6 and Fig.7
Please see attached figures below.

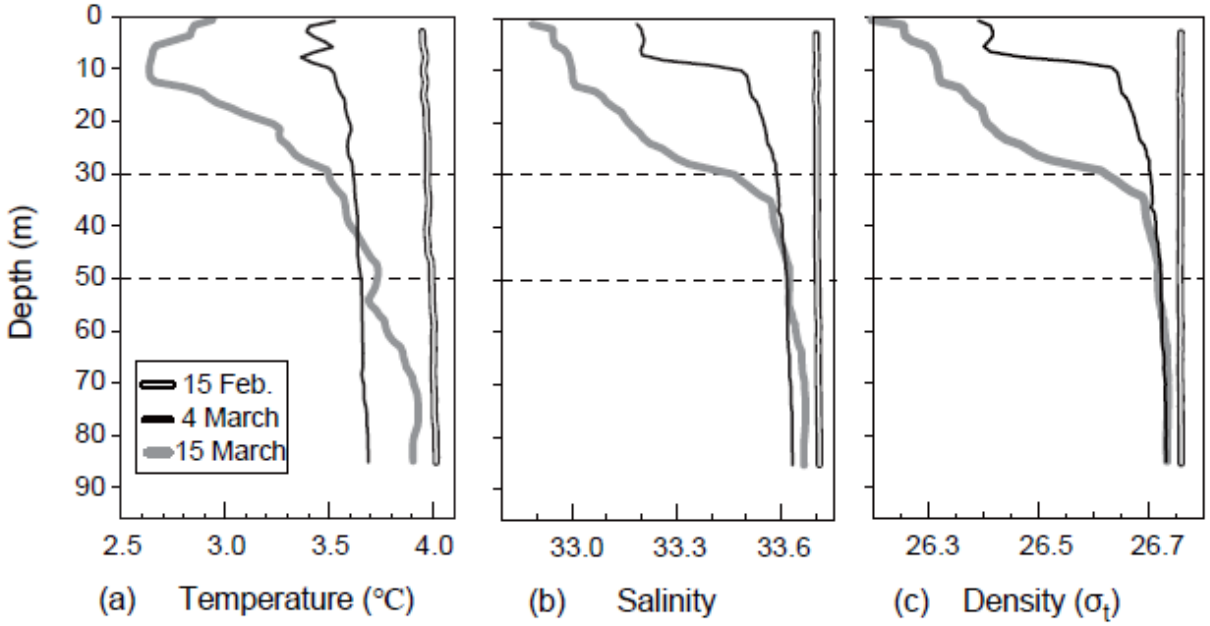

Fig. 2 Vertical profiles of temperature (a), salinity (b), and density (c) at station 30 in Funka Bay, Japan, on 15 February, 4 March, and 15 March.

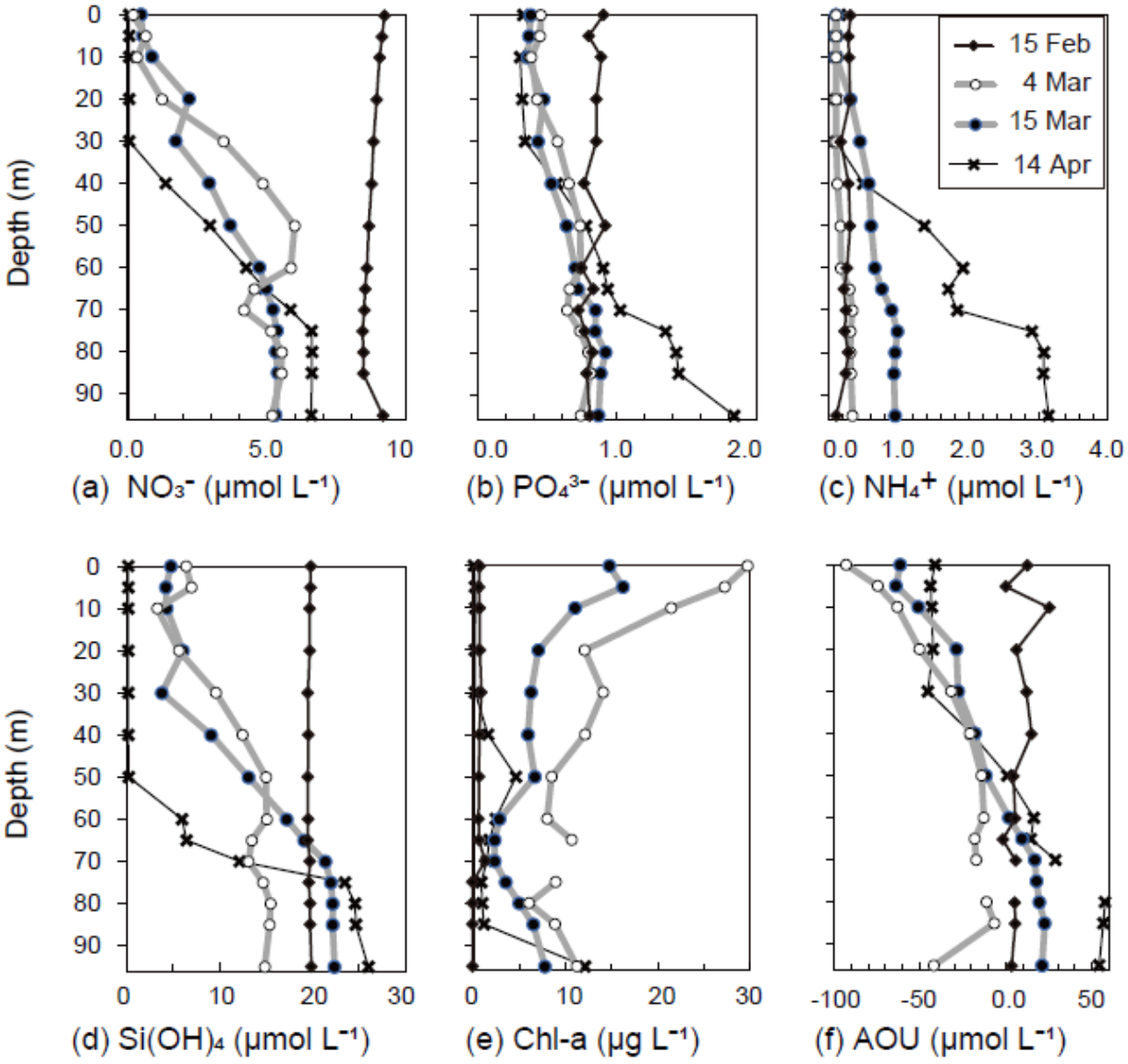

Fig. 5 Vertical profiles of NO_3^- (a), PO_4^{3-} (b), NH_4^+ (c), $Si(OH)_4$ (d), Chl-a (e), and AOU (f) at station 30 in Funka Bay, Hokkaido, Japan, on 15 February, 4 and 15 March, and 14 April 2019.

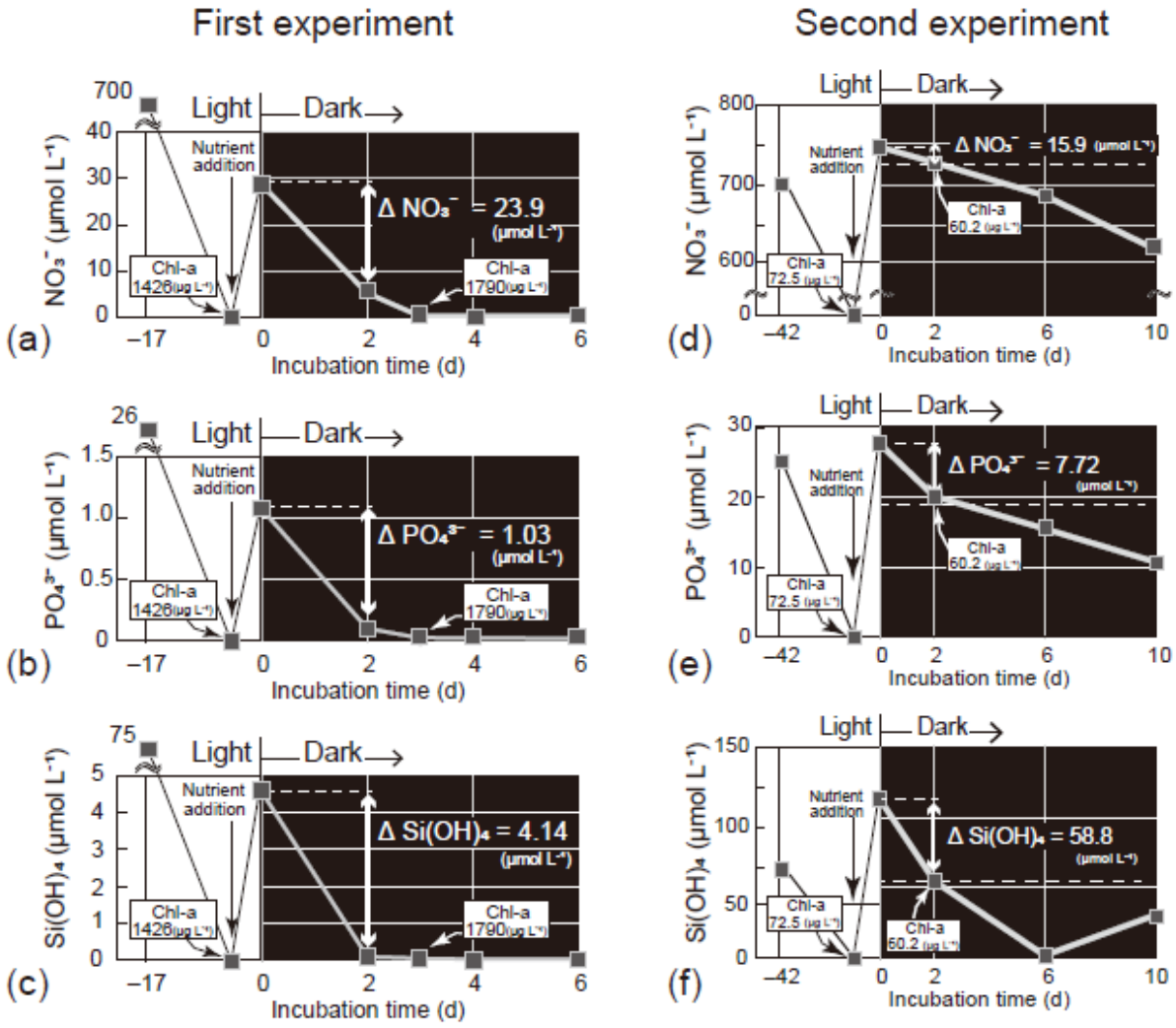

Fig. 6 Temporal change of the nutrient concentrations in the dark incubation experiment using the diatom Thalassiosira nordenskioeldii for NO_3^- (a), PO_4^{3-} (b), and $Si(OH)_4$ (c) of the first experiment and NO_3^- (d), PO_4^{3-} (e), and $Si(OH)_4$ (f) of the second experiment. The diatom was pre-cultured for 17 or 42 days under light conditions before nutrients were added. Each incubation bottle (n=1) with nutrient addition was put in darkness on day 0.

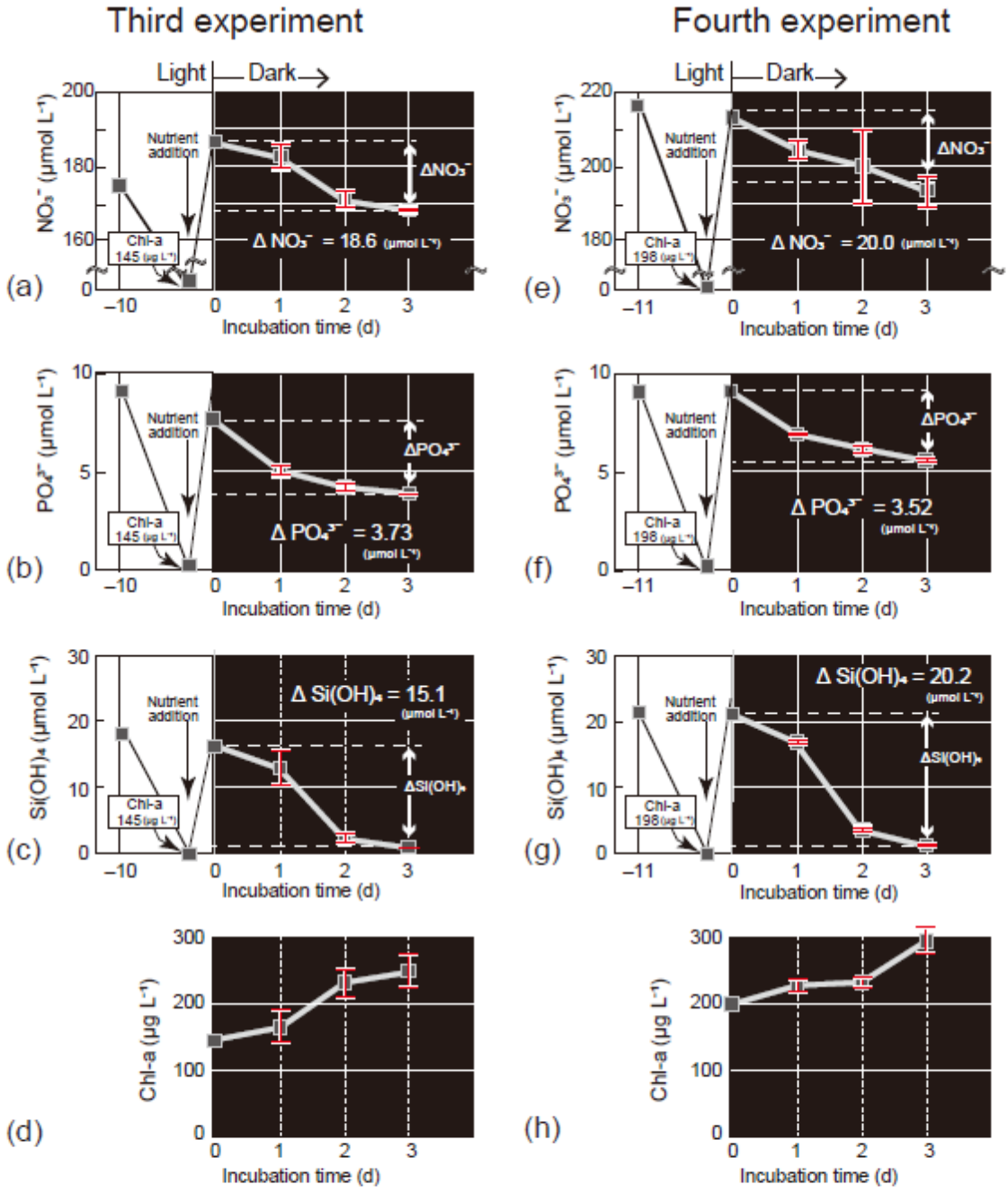

Fig. 7 Temporal change of the nutrient and chl-a concentrations (mean ± 1stdev, n = 4) in the dark incubation experiment using the diatom Thalassiosira nordenskioeldii for NO_3^- (a), PO_4^{3-} (b), $Si(OH)_4$ (c), and chl-a (d) of the third experimet and for NO_3^- (e), PO_4^{3-} (f), $Si(OH)_4$ (g), and chl-a (h) of the fourth experiments. The diatom was cultured for 10 or 11 days under light conditions before nutrients were added. Incubation bottles (n=4) with nutrient addition was put in darkness on day 0.

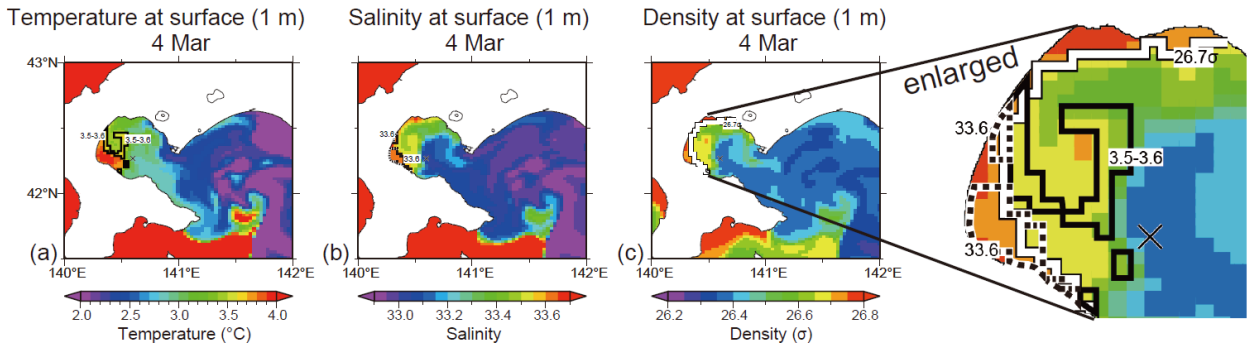

Fig. 8 Horizontal distributions of temperature (a), salinity (b), and density (c) at the surface (1 m) of the Funka-Bay on 4 March 2019. Lines at temperature range 3.5 - 3.6 °C, salinity 33.6, and density 26.7σ were drawn in the figure. The all boudary lines were drawn in an enlarged figure of density. The location of observation station was maked with a cross. The ocean reanalysis product using an operational system for monitoring and forecasting the status of coastal and open-ocean waters around Japan (the JPN system) was provided by Meteorological Research Institute in Japan.