Dear anonymous referee1

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 referee 1**

**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 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 374 - 387**

**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.

**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 360 - 373**

**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.

**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 at maximum 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 or migrated to the upper layer, they have a potential to rapidly grow under the returning light conditions when the euphotic zone deepened after the bloom.

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 as future research. 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 393 - 422**

Nutrient uptake by diatoms in dark subsurface layer in bloom would have impacts on primary production and distribution of phytoplankton in bloom and post-bloom. We propose alternative hypotheses (1 and 2) to deduce the influence of nutrient uptake in the dark subsurface layer.

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 at maximum 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 or migrated to the upper layer, 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 was exchanged with Oyashio water.

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 euphotic zone 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 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. Several modelling studies have suggested 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.

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 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 removed the sentence "this is the first study to present ---" from abstract.

**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 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 50 - 53**

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.

**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 55 - 58**

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).

**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 revised as follows:

Line 50 - 56

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.

**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 91 - 93**

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 97 - 99**

We conducted dark incubation experiments four times using a diatom *Thalassiosira nordenskioeldii*, which predominates in the early phase of the spring bloom in Funka Bay (Ban et al 2000). *Thalassiosira nordenskioeldii* was isolated from natural seawater collected in the western subarctic Pacific Ocean 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**

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

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 308-310

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. 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.

**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 289-292

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”. And, we added chl-a data in supplement material.

**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:

Line 408-418

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 euphotic zone 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 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. Several modelling studies have suggested 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.

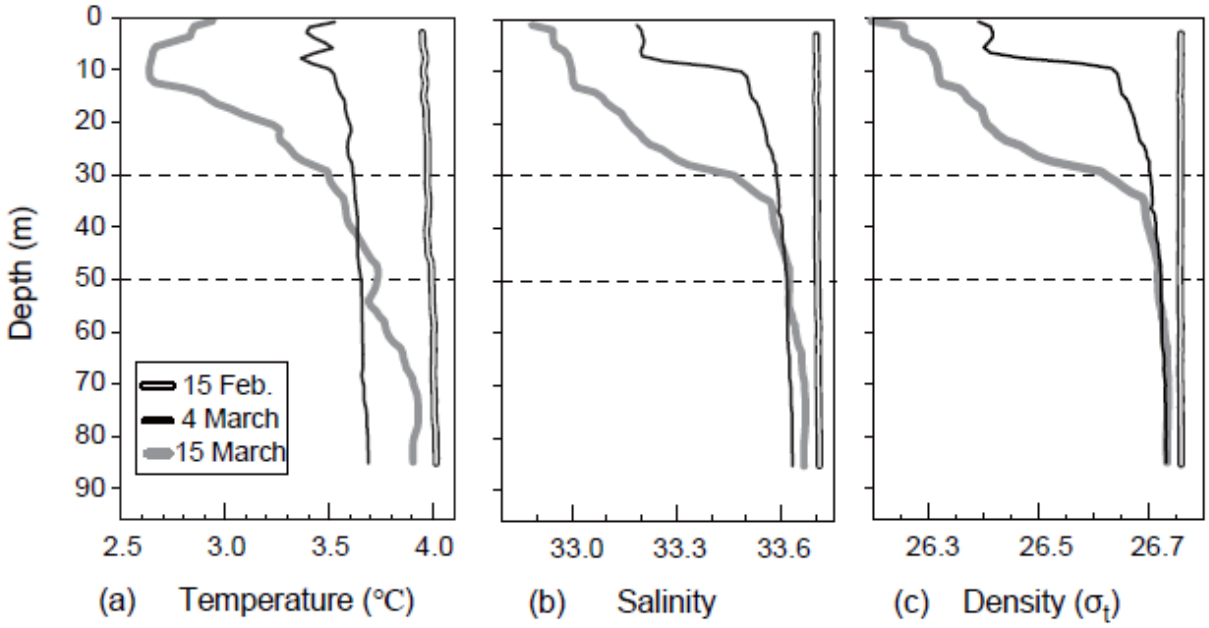


Fig. 2 Vertical profiles of temperature (a), salinity (b), and density (c) at station 30 in Funka Bay, Japan, on 15 February, 4 March, and 15 March.

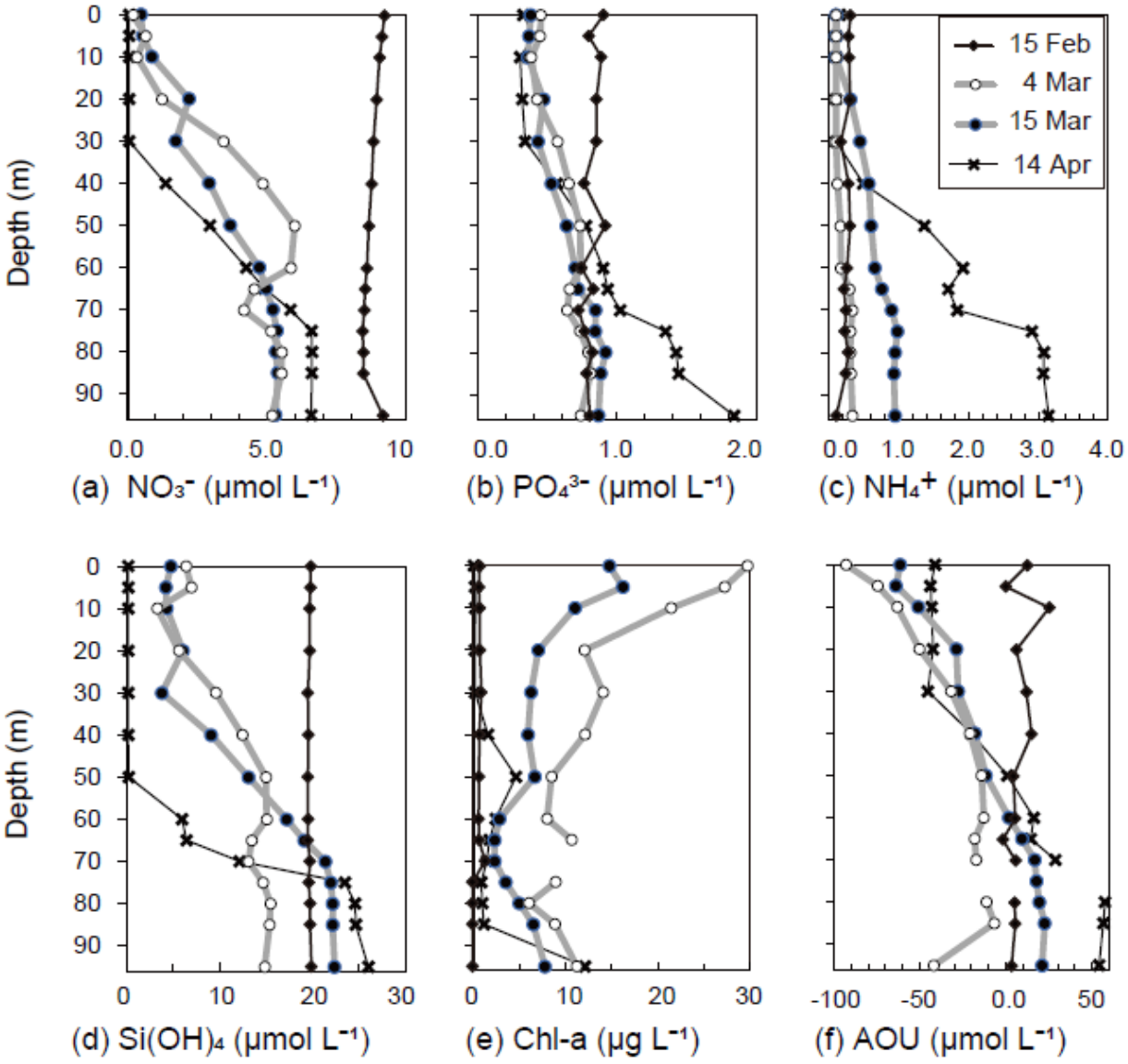


Fig. 5 Vertical profiles of $NO_3^-$ (a), $PO_4^{3-}$ (b), $NH_4^+$ (c), $Si(OH)_4$ (d), Chl-a (e), and AOU (f) at station 30 in Funka Bay, Hokkaido, Japan, on 15 February, 4 and 15 March, and 14 April 2019.

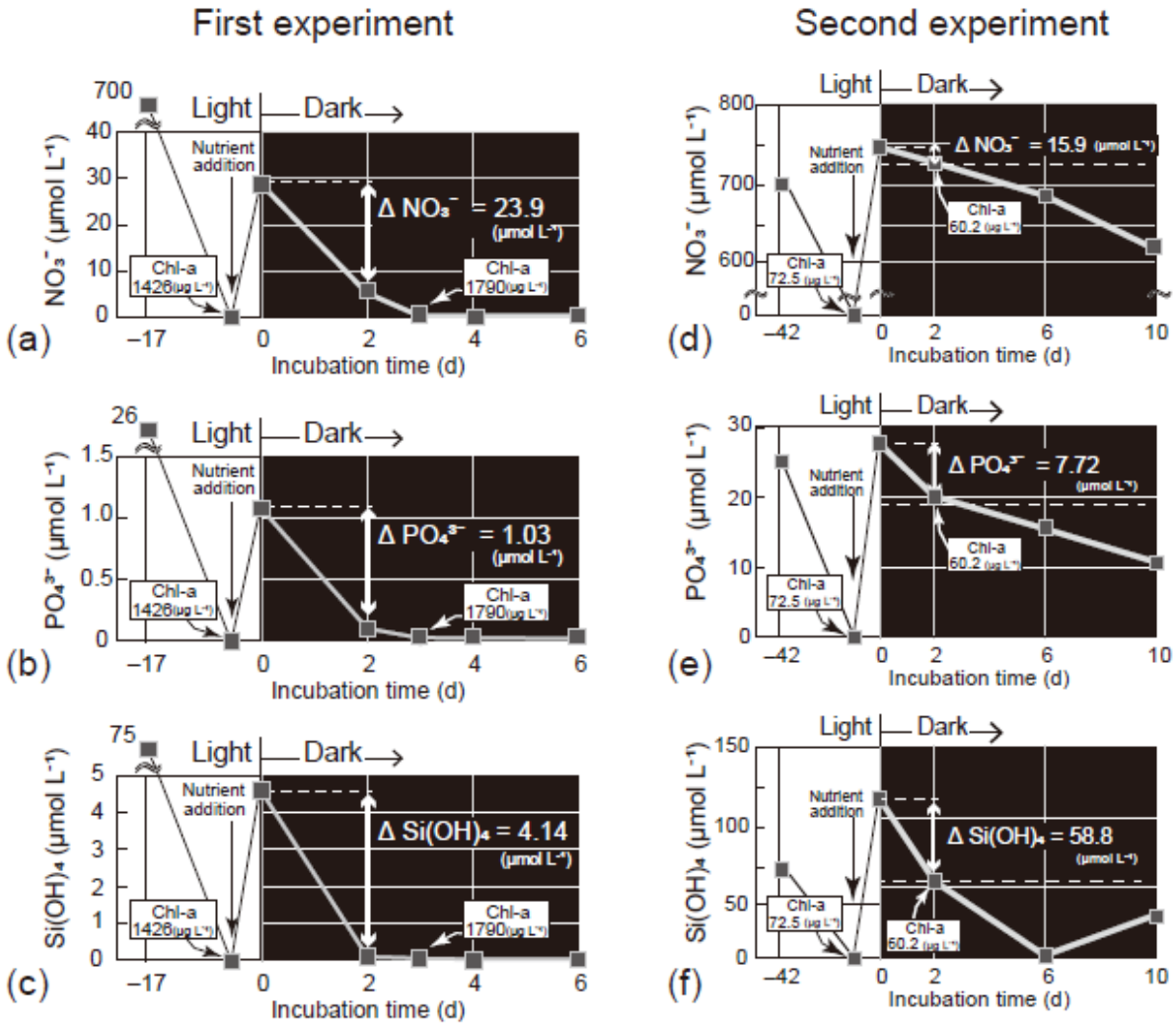


Fig. 6 Temporal change of the nutrient concentrations in the dark incubation experiment using the diatom Thalassiosira nordenskioeldii for $NO_3^-$ (a), $PO_4^{3-}$ (b), and $Si(OH)_4$ (c) of the first experiment and $NO_3^-$ (d), $PO_4^{3-}$ (e), and $Si(OH)_4$ (f) of the second experiment. The diatom 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.

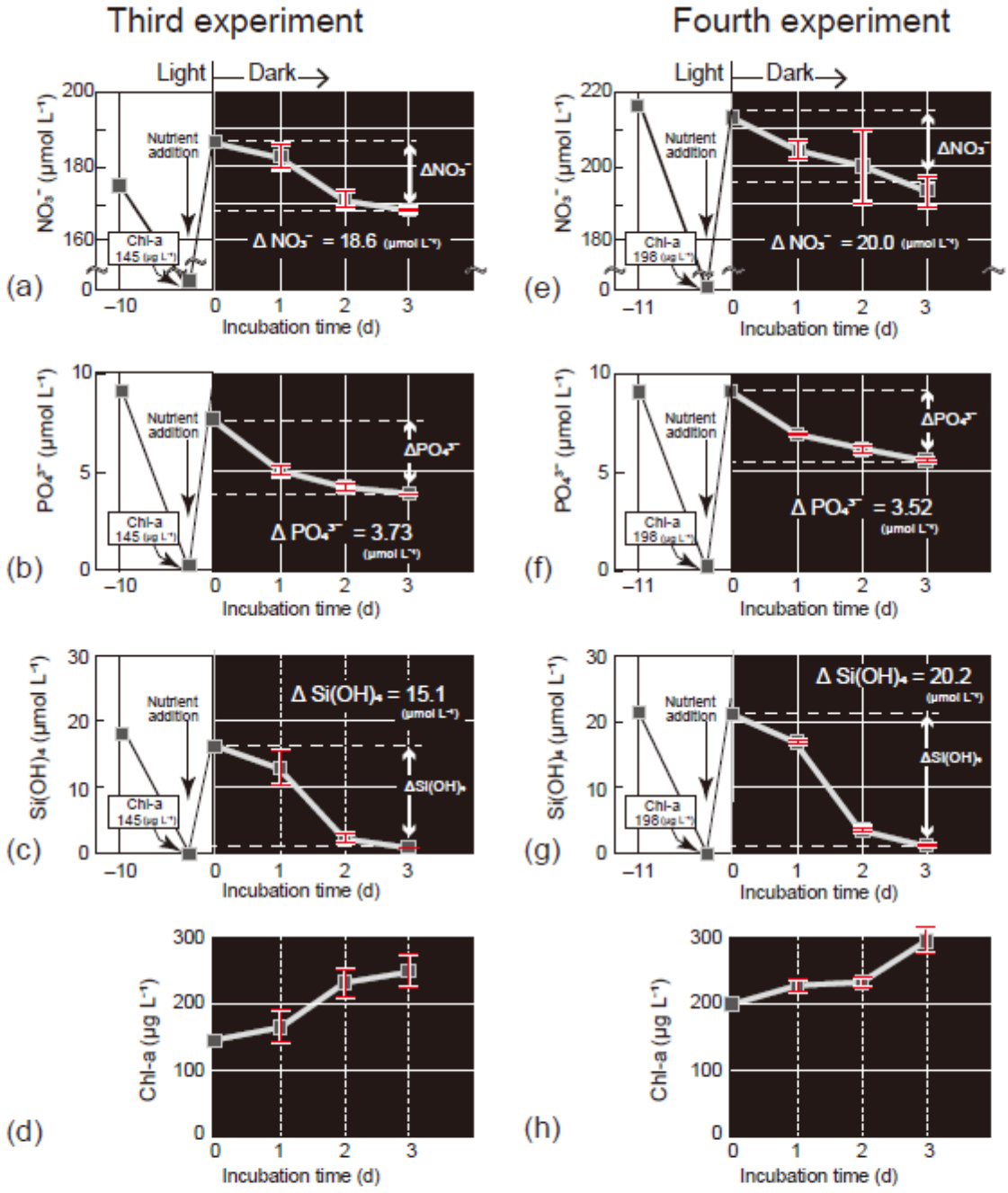


Fig. 7 Temporal change of the nutrient and chl-a concentrations (mean ± 1stdev, n = 4) in the dark incubation experiment using the diatom Thalassiosira nordenskioeldii for $NO_3^-$ (a), $PO_4^{3-}$ (b), $Si(OH)_4$ (c), and chl-a (d) of the third experimet and for $NO_3^-$ (e), $PO_4^{3-}$ (f), $Si(OH)_4$ (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.

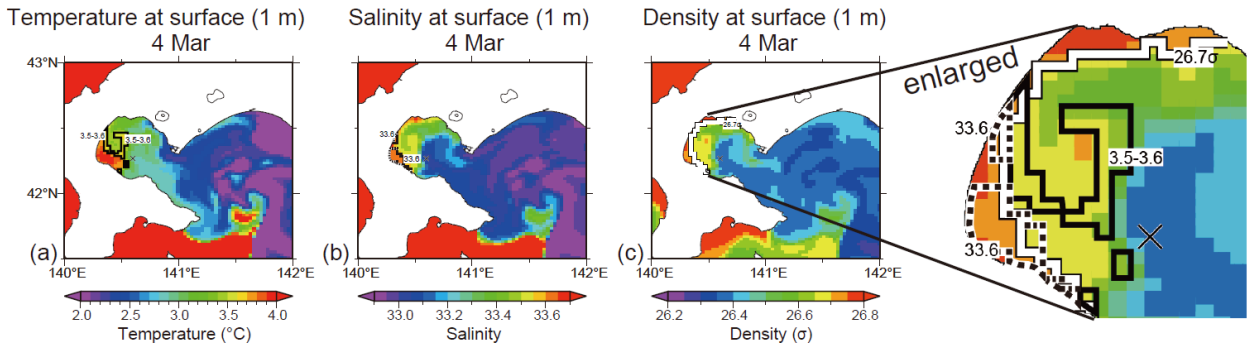


Fig. 8 Horizontal distributions of temperature (a), salinity (b), and density (c) at the surface (1 m) of the Funka-Bay on 4 March 2019. Lines at temperature range 3.5 - 3.6 °C, salinity 33.6, and density 26.7σ were drawn in the figure. The all boudary lines were drawn in an enlarged figure of density. 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.