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Nutrient consumption by diatoms in the dark subsurface layer of Funka Bay, Hokkaido, Japan

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Abstract. We conducted time-series observations in Funka Bay, Hokkaido, Japan, from 15 February to 14 April 2019. The diatom spring bloom peaked on 4 March and started declining on 15 March. Funka Bay winter water remained below 30-m depth, which was below the surface mixed-layer and dark-zone depths on both dates. At depths of 30–50 m, concentrations of NO_3^-, PO_4^{3-}, and $Si(OH)_4$ decreased by half between these dates even in darkness. Incubation experiments using the diatom *Thalassiosira nordenskioeldii* showed that this diatom could consume nutrients in darkness at substantial rates. We conclude that the nutrient reduction in the subsurface layer (30–50 m) could be explained by dark consumption by diatoms that had been growing in the surface waters and then sank to the subsurface layer. 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. Nutrient consumption in this layer might have a substantial influence on the primary production during and after the spring bloom.

1 Introduction

The supply of nutrients to the surface euphotic zone has a potent influence on regulating marine primary production. Numerous studies have examined nutrient utilization by marine biota in relation to the nutrient cycles among the surface euphotic zone and conterminous zones (e.g., below the euphotic zone, atmosphere, rivers). In the subarctic North Pacific Ocean, the higher $Si:NO_3^-$ ratio in the surface of the western gyre (Oyashio region) leads to a diatom-dominant population, and the lower ratio

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in the eastern gyre leads to a reduced diatom population; both subarctic gyres are known to be high-nitrate, low-chlorophyll (HNLC) regions, where depletion of dissolved iron (D-Fe) limits the primary production (Harrison et al. 2004).

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. A small portion of Oyashio water enters Funka Bay, Hokkaido, Japan, 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). 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. Temporal changes in nutrients (Kudo and Matsunaga 1999; Kudo et al. 2000) and isoprene (Ooki et al. 2019) have been examined in relation to primary production in the bay. 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). The spring diatom bloom ends because of nitrate depletion, but silicate is further consumed after the nitrate depletion (Kudo et al. 2000). After the bloom, phosphorus depletion in the bay occasionally limits the primary production (Yoshimura and Kudo 2011). D-Fe is not depleted (>3 nmol L^{-1}) in the surface waters of the bay in April (post-bloom) (Hioki et al. 2015), so D-Fe would not limit primary production. One-third of annual primary production occurs during the spring bloom (Kudo and Matsunaga 1999). Thus, depletion of macronutrients (N, P, Si) is the dominant limiting factor for production in the bay. After the bloom, there is extensive settling and sedimentation of particulate organic matter on the seafloor (Miyake et al. 1998), and nutrient regeneration rapidly occurs in the bottom water just after the sedimentation (Kudo et al. 2007).

Although most previous studies in Funka Bay have focused on nutrient consumption in the surface euphotic zone and nutrient regeneration in the bottom water, Kudo and Matsunaga (1999) have pointed out that NO_3^- concentrations in the dark subsurface layer decreased during the spring blooms in 1991 during their observations from 1988 to 1992, and they mentioned that the decrease was due to dilution of water by vertical mixing. In figures of time-depth section of NO_3^- concentration in Kudo and Matsunaga, it seems that decreases in NO_3^- below the surface have occurred during the bloom of all years. This raises the question as to why the nutrient reduction in the dark subsurface layer occurs so frequently, and if it can be attributed to vertical mixing every year, as the surface layer in the bay usually rapidly becomes stratified in spring.

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 time-series observations in 2019, focusing on the processes affecting nutrient reduction in the dark subsurface layer during the bloom. We do not discuss which nutrients limit the primary production in the surface layer during the bloom and post-bloom, because nutrient limitation during the spring bloom in the bay has been reported elsewhere (e.g. Kudo and Matsunaga 1999; Kudo et al. 2000; Yoshimura and Kudo 2011). In our time-series observations in Funka Bay, we measured volatile organic iodine compounds (VOIs) in seawater and sediment as well as nutrients. An overview of chlorophyll-*a* (chl-*a*) concentrations in 2018–2019 in the bay in association with the VOI changes has already been reported (Ooki et al., submitted).

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2 Materials and methods

2.1 Shipboard observations

Shipboard observations were conducted in Funka Bay, Hokkaido, Japan, on 15 February, 4 and 15 March, and 14 April 2019. We used the training ship (T/S) *Ushio-maru*, operated by the Faculty of Fisheries Sciences, Hokkaido University. Water samples were collected at station 30 (St. 30; 40°16.2′N, 140°36.0′E; bottom depth, 96 m) located in the center of Funka Bay (Figure 1, right panel). Seawater samples were collected in 2.5-L Niskin bottles attached to a rosette multi-sampler along with a conductivity-temperature-depth (CTD) probe (SBE 19 plus, Sea-Bird Electronics, Inc.). Surface water was collected with a plastic bucket, and bottom water was collected approximately 1 m above the seafloor using a Van Dorn sampling bottle. The sampling depths were 0, 5, 10, 20, 30, 40, 50, 60, 65, 70, 75, 80, 85, and 95 m (1 m above the seafloor). Observations in Funka Bay have been reported elsewhere (Shimizu et al. 2017).

2.2 Analytical procedures

Chl-*a* concentrations in discrete seawater samples (100 mL) were measured using the fluorometric Welschmeyer method (Welschmeyer 1994) and a Turner Designs fluorometer (model 10-AU-005). Concentrations of nutrients (NO_3^-, NO_2^-, NH_4^+, SiO_2, and PO_4^{3-}) in discrete seawater samples were measured by colorimetric methods using a QuAAtro system (BL-tec). 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^+. Dissolved oxygen was determined by Winkler titration using a 798 MPT Titrino analyzer (Metrohm, Herisau, Switzerland). Apparent oxygen utilization (AOU) was calculated by subtracting the measured oxygen concentration from the dissolved oxygen concentration at saturation under in situ temperature and salinity (Hansen 1999).

2.3 Incubation experiments to test for nutrient consumption by diatoms in darkness

A sterile culture of the diatom *Thalassiosira nordenskioeldii*, which predominates in the early phase of the spring bloom in Funka Bay (Ban et al 2000), was grown in Si-rich f/2 medium (NO_3^-, 700 µmol L^{-1}; PO_4^{3-}, 26 µmol L^{-1}; $Si(OH)_4$, 75 µmol L^{-1}) at 6 °C. We used a 250-mL cell-cultivation flask with a vent cap (VTC-F75V, Violamo). Incubation procedures were carried out under sterile conditions; however, we did not check for contamination after the incubation. When the chl-*a* concentration in the medium reached 1426 µg L^{-1} on day 17 of culture, the concentrations of NO_3^-, PO_4^{3-}, and $Si(OH)_4$ in the medium had dropped below 0.1, 0.05 and 0.1 µmol L^{-1}, respectively. We regarded the diatoms in the medium on day 17 as being nutrient-depleted. We added nutrients (stock f/2 medium) into the nutrient-depleted diatom culture, after which concentrations were as follows: NO_3^-, 31.1 µmol L^{-1}; PO_4^{3-}, 1.15 µmol L^{-1}; and $Si(OH)_4$, 4.75 µmol L^{-1}. The incubation bottle was put in a dark incubator at 6 °C for 6 days. On days 0, 2, 3, 4, and 6, 10 mL and 100 µL of incubation medium were filtered to measure nutrient and chl-*a* concentrations, respectively, using the same methods as for the measurements in seawater samples.

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We also conducted a second dark incubation using another diatom culture *Thalassiosira nordenskioeldii* that had grown for 42 days. The chl-*a* concentration in this culture was only 72.5 μg L^{-1}, which was one twentieth of that in the first dark incubation, implying that it was in a decline phase. We set the initial concentrations of nutrients at 23 times those of the first dark incubation. On days 0, 1, 2, and 10, 10 mL and 1 mL of incubation medium were filtered to measure nutrient and chl-*a* concentrations, respectively.

3 Results and Discussion

3.1 Hydrographic features

There are two main water masses in Funka Bay throughout the year. Tsugaru water that originates from the subtropical North Pacific and Oyashio water that originates from the subarctic North Pacific. The subtropical Tsugaru water has higher salinity (33.6–34.2), and is modified into winter water by winter cooling. The subarctic Oyashio water has lower salinity (32.6–33.0), and is modified into low density summer water (S) by solar radiative heating and freshwater input. These four water masses were first described by Ohtani and Kido (1980). Ooki et al. (2019) added the transitional waters to the water-mass classification: changing from winter water to Oyashio water (W-O), from Oyashio water to summer water (O-S), from summer water to Tsugaru water (S-T), and from Tsugaru water to winter water (T-W). The revised classification results in the temporal variation of water-mass structure shown in Figure 2.

On 15 February 2019, Funka Bay was occupied by winter water at all depths. The influence of Oyashio water (W-O or Oyashio) was evident at the surface (0–7 m) on 4 March and had extended to the deeper layer (0–24 m) by 15 March, and to 0–52 m on 14 April. Winter water remained in the deeper layer below the W-O or Oyashio waters. Here we focus on the temporal changes in biochemical parameters at the depth range of 30–50 m where winter water remained in Funka Bay between 4 and 15 March. Water mixing at that depth range will be discussed in section 3.3.1.

3.2 Biochemical parameters

We prepared time-depth sections of biochemical parameters, showing the surface mixed-layer, euphotic-zone, and dark-zone depths (Figure 3a–e). The surface mixed layer was defined as the layer in which density differences were within 0.125 kg m^{-3} relative to the density at 5-m depth. 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. We defined the dark-zone depth at which PAR was 0.1% of the surface PAR, where amount of photosynthesis is much less than respiration.

3.2.1 Chl-*a*

15 February The surface mixed layer reached at least 85-m depth on 15 February, but the chl-*a* profile was not vertically uniform. Chl-*a* concentrations between 0 and 70 m were relatively high, ranging from 0.65 to 1.4 μg L^{-1} (average, 0.81 μg L^{-1}), compared with concentrations in the deeper layer (75–95 m), where they were below the detection limit (<0.05 μg L^{-1}); see

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the original data in supplementary information of Ooki et al., (submitted). We suggest that on this date, diatom growth had just started as an early phase of the spring bloom. The data for chl-*a* are taken from a related article.

4 March By 4 March the chl-*a* concentrations had substantially increased at all depths. Notably high concentrations (27–30 μg L^{-1}) were found in the surface mixed layer (0–9 m). The depth range of the mixed layer was almost the same as that of the euphotic zone (0–13 m). In Funka Bay, a chl-*a* maximum of 10–20 μg L^{-1} has been found at the peak of the diatom bloom in March of every year (Odate et al. 1993; Kudo and Matsunaga 1999; Kudo et al. 2007). We believe that we observed the peak of the diatom spring bloom on 4 March. Below the surface mixed layer, there were high chl-*a* concentrations (6.0–14.1 μg L^{-1}) even in darkness. The high chl-*a* levels in the dark subsurface layer suggested that large amounts of diatom aggregates, which had been produced in the surface layer, were sinking from the surface to the deeper layer and becoming suspended there.

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

14 April On 14 April the chl-*a* concentrations were very low in the surface euphotic zone (0–40 m, 0.05–1.6 μg L^{-1}) and in the deeper dark layer (50–85 m, 0.85–4.6 μg L^{-1}) except for the bottom water just above the sea floor (95 m, 12.2 μg L^{-1}). We believe that the spring diatom bloom had terminated by 14 April. A local chl-*a* maximum concentration of 4.6 μg L^{-1} was found at 50-m depth, just below the euphotic zone.

3.2.2 Nitrate

15 February On 15 February, nitrate (NO_3^-) concentrations were vertically uniform (8.4—9.2 μmol L^{-1}) and were at the annual maximum.

4 March By 4 March, the NO_3^- concentrations had substantially decreased to 0.15–0.60 μmol L^{-1} in the depth range of 0–10 m within the surface euphotic zone (0–13 m). The decrease in the surface was due to consumption by diatoms, which had rapidly grown during the spring bloom. Below the surface (20–95 m), the NO_3^- concentrations had also decreased, to 1.9–6.0 μmol L^{-1}, which was approximately half of the concentrations in February. Because the dark-zone depth was 60 m on 15 February and 18 m on 4 March, the depth range of 60–95 m was dark on both dates. Thus, there could have been no photosynthesis-related NO_3^- consumption in the dark layer (60–95 m). In the dark layer, NO_3^- concentrations had decreased, to 4.1 – 5.8 μmol L^{-1}, which was 60% of the concentrations in February. We propose two explanations for the decrease in NO_3^- concentrations in the dark layer between 15 February and 4 March.

The first possible explanation is that NO_3^- was consumed by diatoms during their growth in the upper euphotic layer after 15 February, and then this water, in which NO_3^- had been consumed during photosynthesis, mixed vertically with the deeper water (60–95 m) through winter cooling before the water became stratified by 4 March. The possibility of vertical mixing between these two dates is supported by the temporal variation of AOU, which is defined as the difference between the equilibrium saturation concentration and the measured concentration of oxygen in the water; i.e., positive and negative AOUs suggest net consumption (respiration) or net production (photosynthesis) of oxygen in water, respectively. On 15 February,

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the absolute value of AOU over most of the water column was the lowest during the observation period (average, 6.2 μmol L^{-1}; range, –3 to 23 μmol L^{-1}), suggesting that there was no significant net O_2 production throughout the total water column. By 4 March, AOU values had dropped to –14 to –94 μmol L^{-1} at 0–50 m, and to –7 to –42 μmol L^{-1} at 60–95 m, even in darkness. Note that the decrease of water temperature between the two dates (Δtemp = –0.45 °C) could have caused an increase in AOU (ΔAOU = +3.6 μmol L^{-1}) due to the increase in the solubility of oxygen. The large negative AOU in the euphotic layer on 4 March was apparently due to photosynthetic O_2 production. The negative AOU in the deeper dark layer (60–95 m) on 4 March was thought to be due to mixing with surface water, in which AOU had been lowered by photosynthesis, before the water became stratified by 4 March.

The second possible explanation is that the diatoms, which had grown at the surface and then settled to the deeper layer, consumed NO_3^- in darkness without photosynthetic growth. The possibility of nutrient consumption in darkness is discussed in section 3.3. We believe that both explanations apply to the decrease in NO_3^- concentrations in the deeper layer between these two dates; however, we could not separate their effects.

15 March On 15 March, the NO_3^- levels at 0–10-m depth (0.39–0.79 μmol L^{-1}) within the surface mixed layer (0–18 m) had not changed since 4 March. The euphotic zone depth (1% PAR) and dark zone depth (0.1% PAR) had deepened to 22 m and 30 m, respectively. The NO_3^- concentrations in the dark subsurface layer (30–50 m) below the mixed layer had decreased substantially, to 1.6–3.6 μmol L^{-1}, approximately half those on 4 March at the same depth range. In this subsurface layer, AOU values increased slightly between 4 and 15 March, suggesting that there was not any influence from photosynthetic O_2 production and therefore no photosynthetic NO_3^- consumption. We hypothesized that the diatoms that had settled from the surface to the subsurface layer consumed NO_3^- in darkness. This possibility will be discussed in section 3.3. The NO_3^- concentrations in the deeper layer (60–95 m) had not changed since 4 March.

14 April By 14 April, the euphotic zone depth had deepened to 47 m. The NO_3^- concentrations had decreased to below the detection limit (<0.05 μmol L^{-1}) in the upper euphotic layer (0–30 m) and decreased to 1.4 μmol L^{-1} in the lower euphotic layer (40 m). Because the influence of Oyashio water extended from the surface to the subsurface layer, these decreases occurred simultaneously with the water exchange. It is possible that the NO_3^- concentration in the original Oyashio water had already been diminished by diatom consumption before the water entered the bay. In the deeper water (60–95 m), the NO_3^- concentrations slightly increased within winter water. There is a time lag for regeneration of NO_3^- in bottom water after organic matter decomposition because the regeneration of NO_3^- follows the remineralization of NH_4^+ from organic matter and its oxidation (nitrification). A time lag of 1–2 months for NO_3^- regeneration after NH_4^+ regeneration has been observed every year in Funka Bay (Kudo et al. 2007). Thus, the signal from NO_3^- regeneration could not be seen during the spring bloom, and a slight signal was detected on 14 April. In contrast, signals from the regeneration of PO_4^{3-}, $Si(OH)_4$, and NH_4^+ in bottom water were obvious on 15 March, in the decline phase of the bloom, as discussed in the next two sections.

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3.2.3 Phosphate and silicate

Overall, temporal variations in PO_4^{3-} and $Si(OH)_4$ concentrations were very similar to those of NO_3^-. We found decreases in these nutrients in the dark subsurface layer of 60–95 m on 4 March, and 30–50 m on 15 March. On 4 March, the concentrations of PO_4^{3-} and $Si(OH)^-$ in the dark subsurface layer (30–50 m) were 0.66 μmol L^{-1} and 12.4 μmol L^{-1}, respectively, decreasing to 0.53 μmol L^{-1} and 8.7 μmol L^{-1} on 15 March. We concluded that the reasons for these decreases were the same as for NO_3^-. In contrast, PO_4^{3-} and $Si(OH)_4$ concentrations in the deeper layer (70–95 m) increased after 15 March after the peak of the bloom. Because these increases coincided with the rise in AOU, they likely resulted from remineralization following the decomposition of organic matter suspended in the bottom water or settled on the seafloor.

3.2.4 Ammonium

Temporal variations in NH_4^+ concentrations were similar to those of the other nutrients, except for substantial increases of NH_4^+ in the subsurface and bottom layers on 15 March. We considered these increases to be due to remineralization of organic matter suspended in the water column or settled on the seafloor. Because the NH_4^+ concentrations were at their lowest during winter, the signal from remineralization could be clearly detected on 15 March.

3.3 Nutrient consumption in the dark subsurface layer

We found decreases in the nutrient concentrations in the dark subsurface layer between 15 February and 4 March, and between 4 March and 15 March. The latter reduction could not have been affected by photosynthetic consumption by diatoms because there was no light available for photosynthesis. Here we discuss the possible reasons for the nutrient reductions between 4 and 15 March.

3.3.1 Water mixing as a possible explanation for nutrient reduction

First, we discuss the possibility that vertical mixing between the surface mixed layer, which already had reduced nutrient levels, and the subsurface layer (30–50 m) resulted in the observed decrease in nutrient concentrations in the subsurface layer. Because the density difference (Δσ) between the surface mixed layer at 5 m and the subsurface layer at 30 m had increased between 4 March (Δσ = 0.29 kg m^{-3}) and 15 March (Δσ = 0.34 kg m^{-3}), the stratification between these layers had strengthened (Figure 4c). Additionally, there was no bad weather during this period; the wind speeds were relatively low, with daily averages of 3.0–5.9 m s^{-1}, although low air temperature lasted, with daily averages of 1.0 – 5.3 °C (data from Muroran Observatory, Meteorological Agency of Japan; https://www.jma.go.jp/jma/index.html). The effect of low salinity water inflow on the density decrease at the surface layer overcame the effect of cooling on density increase (Fig. 4). From these observations, we

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excluded the possibility of vertical mixing between the two layers as an explanation for the decrease in nutrients in the subsurface layer.

We also considered the possibility of horizontal mixing of subsurface water with Oyashio water entering from the surface of the bay. Oyashio water is characterized by its low salinity. Because the salinity at 30 m declined from 33.58 on 4 March to 33.47 on 15 March, it is possible that the influence of low-salinity Oyashio water extended to 30 m. Since the salinity of Oyashio water at 10-m depth on 15 March, where the minimum temperature (2.6 °C) was found suggesting an appearance of the main body of Oyashio water, was 33.0, we assumed that the salinity of original Oyashio water was 33.0. A mixing ratio of 8:2 between Funka-Bay water at 30 m on 4 March and Oyashio water would change the salinity at 30-m depth from 33.58 (on 4 March) to 33.47 (on 15 March). Even if the concentrations of nutrients in the original Oyashio water were 0 μmol L^{-1}, the mixing ratio of 8:2 (Funka-Bay water:Oyashio water) would reduce the nutrient concentrations at 30-m depth by only 20%. In reality, the NO_3^- concentration at 30 m was decreased by half between these two dates (Figure 4d), and the salinities at 40- and 50-m depths did not change (Figure 4b). Thus, we excluded the possibility of mixing with Oyashio water as a reason for nutrient reduction in the subsurface layer.

3.3.2 Nutrient consumption by diatoms in darkness

We concluded that the only possible explanation for nutrient reduction in the subsurface layer was consumption by diatoms that were sinking from the surface and suspended in the dark subsurface layer during the bloom. To examine this possibility, we conducted dark incubation experiments using the diatom *Thalassiosira nordenskioeldii*, which predominates during the spring bloom in Funka Bay (Ban et al. 2000). The first incubation experiment results demonstrated that the diatom culture, which had been depleted in nutrients before the start of the experiment, rapidly consumed nutrients in darkness within seven days after the nutrient addition (Figure 5a). The daily consumption rates calculated from the concentration difference between day 0 and day 2 of the dark incubation were 0.0090 μmol (μg chl-*a*)$^{-1}$ d^{-1} for NO_3^-, 0.00037 μmol (μg chl-*a*)$^{-1}$ d^{-1} for PO_4^{3-}, and 0.0015 μmol (μg chl-*a*)$^{-1}$ d^{-1} for $Si(OH)_4$. From these consumption rates, we estimated the concentration decreases (ΔNutrients) in the subsurface layer for the 11 days between observation dates using an observed chl-*a* concentration of 11.54 μg L^{-1} (average on 4 March). The estimated ΔNO_3^- (–1.1 μmol L^{-1}), ΔPO_4^{3-} (–0.05 μmol L^{-1}), and $\Delta Si(OH)_4$ (–0.18 μmol L^{-1}) were 1/2–1/20 of the actual decreases in the subsurface layer 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}.

The results of the second incubation experiment (Figure 5b), in which cultured diatoms were in a decline phase of growth, demonstrated that the consumption rates were 0.11 μmol (μg chl-*a*)$^{-1}$ d^{-1} for NO_3^-, 0.053 μmol (μg chl-*a*)$^{-1}$ d^{-1} for PO_4^{3-}, and 0.41 μmol (μg chl-*a*)$^{-1}$ d^{-1} for $Si(OH)_4$, 12–173 times those of the first dark incubation. We assumed that the high consumption rates per unit chl-*a* by second experiment were due to high consumption rates per unit cell, of which chl-a content was considerably small, although we did not count the cell number density. We considered that the rates are largely dependent on

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the chl-*a* content in the diatom cells and the initial concentrations of nutrients. It will be necessary to set better experimental conditions in future research to more accurately estimate the nutrient consumption in the dark subsurface layer.

Cochlan et al. (1991) 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. They used onboard incubations with a diatom dominating natural seawater for dark periods of 2–4 hours after being under light conditions. Many previous studies have focused on the dark consumption within the day–night cycle in the euphotic layer. Onboard simulated in-situ incubations yielded dark:light ratios of NO_3^- consumption rates of 0–0.67 (Nelson and Conway 1979), 0–1.0 (Conway and Whitledge 1979), and 0–0.51 (Cochlan et al. 1991). These previous works have reported wide ranges of dark consumption rates and ratios. Cochlan et al. (1991) reported that the dark:light uptake ratio was greater in N-impoverished waters than in N-replete waters, suggesting that dark uptake is enhanced by nutrient stress. They also mention the importance of N uptake by heterotrophic bacteria, citing studies where uptake by heterotrophic bacteria ranges from half the uptake by phytoplankton to half of the total N uptake.

3.4 The influence of nutrient consumption by diatoms in the dark subsurface layer

If the diatom population that had consumed half of the nutrients in the dark subsurface water sank to the deeper layers 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. Alternatively, if the diatoms that had consumed nutrients in the dark subsurface layer remained suspended in that layer after the bloom, they could rapidly grow under the returning light conditions when the euphotic zone deepened after the bloom. 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.

An interesting survival strategy for diatoms has been hypothesized where the diatoms that consume NO_3^- in the dark subsurface layer migrate to the surface euphotic layer where they have a growth advantage in subtropical open ocean areas (Villareal et al. 1996; Richardson et al. 1998; Villareal et al., 1999; Villareal et al., 2014) and subtropical and subarctic open ocean areas (Witz and LanSmith, 2020). These previous studies have not yet found any evidence of decrease in NO_3^- in the subsurface layer from time-series observation, however, we firstly provided the evidence of decrease in NO_3^- in the dark subsurface layer during the diatom bloom. As for the reduction in $Si(OH)_4$ concentrations found in the dark subsurface layer of the Barents Sea, it has been suggested that diatoms settling from the surface consume $Si(OH)_4$ in the dark subsurface layer to form spores (Rey and Skjoldal 1987). We can make some assumptions about the influences and strategies of diatoms in relation to nutrient consumption in dark subsurface layers, however, there is not yet sufficient observational data for a complete explanation. Further research is needed to examine these possible strategies and their impacts on biogeochemical cycles.

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4 Conclusions

We conducted time-series observations in Funka Bay, 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 surface euphotic layer and then sank to the dark subsurface layer. We reached this conclusion using the following rationale.

(1) We excluded water mixing as a possible reason 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. Second, 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.

(2) From the dark incubation experiments, we confirmed that the diatom *Thalassiosira nordenskioeldii*, which dominates the bloom in Funka Bay, could consume nutrients in darkness at substantial rates. Although the consumption rates varied over a wide range, we concluded that the nutrient reduction in the dark subsurface layer (30–50 m) could be explained by dark consumption by diatoms that had been growing at the surface and then sank into the subsurface layer.

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 surface 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.

Competing interests.

The authors declare that they have no conflict of interest.

Author contributions.

A.O. designed the research and conducted the observations. S.U. analysed the data. A.O. and S.U. conducted the diatom incubations in darkness. Y.N. conducted the sterile diatom incubations. M.T., H.A. and D.N. supported the data analysis. H.O. and T.T. designed the *Ushio-maru* observations. A.O. and S.U. wrote the manuscript with contributions from all co-authors.

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https://doi.org/10.5194/bg-2021-357
Preprint. Discussion started: 6 January 2022

References

Ban, S., Lee, H-W., Shinada, A.,and Toda, T.: In situ egg production and hatching success of the marine copepod Pseudocalanus newmani in Funka Bay and adjacent waters off southwestern Hokkaido, Japan: associated to diatom bloom. J. Plankton Res., 22, 907–922, 2000.

Cochlan, W. P., Price, N. M., and Harrison, P. J.: EFFECTS OF IRRADIANCE ON NITROGEN UPTAKE BY PHYTOPLANKTON - COMPARISON OF FRONTAL AND STRATIFIED COMMUNITIES, Marine Ecology Progress Series, 69, 103-116, 10.3354/meps069103, 1991.

Conway, H. L. and Whitledge, T. E.: DISTRIBUTION, FLUXES AND BIOLOGICAL UTILIZATION OF INORGANIC NITROGEN DURING A SPRING BLOOM IN THE NEW-YORK BIGHT, Journal of Marine Research, 37, 657-668, 1979.

Harrison, P. J., Whitney, F. A., Tsuda, A., Saito, H., and Tadokoro, K.: Nutrient and plankton dynamics in the NE and NW gyres of the subarctic Pacific Ocean, Journal of Oceanography, 60, 93-117, 10.1023/B:JOCE.0000038321.57391.2a, 2004.

Hioki, N., Kuma, K., Morita, Y., Miura, D., Ooki, A., Tanaka, S., Onishi, H., Takatsu, T., Kobayashi, N., and Kamei, Y.: Regeneration dynamics of iron and nutrients from bay sediment into bottom water of Funka Bay, Japan, Journal of Oceanography, 71, 703-714, 10.1007/s10872-015-0312-6, 2015.

Kudo, I., and Matsunaga, K.: Environmental factors affecting the occurrence and production of the spring phytoplankton bloom in Funka Bay, Japan. J. Oceanogr., 55, 505–513, 1999.

Kudo, I., Yoshimura, T., Yanada, M., and Matsunaga, K.: Exhaustion of nitrate terminates a phytoplankton bloom in Funka Bay, Japan: change in SiO4,: NO3 consumption rate during the bloom, Marine Ecology Progress Series, 193, 45-51, 10.3354/meps193045, 2000.

Kudo, I., Yoshimura, T., Lee, C. W., Yanada, M., and Maita, Y.: Nutrient regeneration at bottom after a massive spring bloom in a subarctic coastal environment, Funka Bay, Japan, Journal of Oceanography, 63, 791-801, 10.1007/s10872-007-0067-9, 2007.

Maita, Y., and Odate, T.: Seasonal changes in size fractionated primary production and nutrient concentrations in the temperate neritic water of Funka Bay, Japan. J. Oceanogr. Soc. Japan, 44, 268–279, 1988.

Miyake, H., Yanada, M., Nishi, T., and Hoshizawa, K.: Short-time variation in low trophic level productivity and hydrographic conditions in Funka Bay. Mem. Fac. Fish. Hokkaido Univ., 45, 36–41, 1998.

Nelson, D. M. and Conway, H. L.: EFFECTS OF THE LIGHT REGIME ON NUTRIENT ASSIMILATION BY PHYTOPLANKTON IN THE BAJA CALIFORNIA AND NORTHWEST AFRICA UPWELLING SYSTEMS, Journal of Marine Research, 37, 301-318, 1979.

Odate, T.: Temporal and horizontal distribution of the diatom community during the spring bloom in Funka Bay, southern Hokkaido. Bull. Plank. Soc. Japan, 34, 33–42, 1987.

https://doi.org/10.5194/bg-2021-357
Preprint. Discussion started: 6 January 2022

Odate, T, Yanada, M., Mizuta, H., and Maita, Y.: Phytoplankton carbon biomass estimated from the size-fractionated chlorophyll a concentration and cell density in the northern coastal waters from spring bloom to summer. Bull Plankton Soc Japan 39, 127–144, 1993.

Ohtani, K.: Studies on the change of the hydrographic conditions in the Funka Bay. II. Characteristics of the water occupying the Funka Bay. Bull. Fac. Fish., Hokkaido Univ., 22, 58–66 (in Japanese), 1971.

Ohtani, K., and Kido, K.: Oceanographic structure in Funka Bay. Bull Fac Fish Hokkaido Univ., 31, 84–114, 1980 (in Japanese with English abstract).

Ooki, A., Shida, R., Otsu, M., Onishi, H., Kobayashi, N., Iida, T., Nomura, D., Suzuki, K., Yamaoka, H., and Takatsu, T.: Isoprene production in seawater of Funka Bay, Hokkaido, Japan, Journal of Oceanography, 75, 485-501, 10.1007/s10872-019-00517-6, 2019.

Ooki, A., Minamikawa, K., Meng, F., Hirawake, T., Ueno, H., Nosaka, Y., Takatsu, T.: Marine sediment as a source of methyl and ethyl iodides in coastal oceans, Communications Earth & Environment, under review.

Rey, F. and Skjoldal, H. R.: CONSUMPTION OF SILICIC-ACID BELOW THE EUPHOTIC ZONE BY SEDIMENTING DIATOM BLOOMS IN THE BARENTS SEA, Marine Ecology Progress Series, 36, 307-312, 10.3354/meps036307, 1987.

Richardson, T. L., Cullen, J. J., Kelley, D. E., and Lewis, M. R.: Potential contributions of vertically migrating Rhizosolenia to nutrient cycling and new production in the open ocean, Journal of Plankton Research, 20, 219-241, 10.1093/plankt/20.2.219, 1998.

Shimizu, Y., Ooki, A., Onishi, H., Takatsu, T., Tanaka, S., Inagaki, Y., Suzuki, K., Kobayashi, N., Kamei, Y., and Kuma, K.: Seasonal variation of volatile organic iodine compounds in the water column of Funka Bay, Hokkaido, Japan, Journal of Atmospheric Chemistry, 74, 205-225, 10.1007/s10874-016-9352-6, 2017.

Villareal, T. A., Pilskaln, C. H., Montoya, J. P., and Dennett, M.: Upward nitrate transport by phytoplankton in oceanic waters: balancing nutrient budgets in oligotrophic seas, Peerj, 2, 10.7717/peerj.302, 2014.

Villareal, T. A., Woods, S., Moore, J. K., and CulverRymsza, K.: Vertical migration of rhizosolenia mats and their significance to NO3- fluxes in the central north Pacific gyre, Journal of Plankton Research, 18, 1103-1121, 10.1093/plankt/18.7.1103, 1996.

Welschmeyer, N.A.: Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and pheopigments. Limnol. Oceanogr., 39, 1985–1992, 10.4319/lo.1994.39.8.1985, 1994.

Wirtz, K. and Smith, S. L.: Vertical migration by bulk phytoplankton sustains biodiversity and nutrient input to the surface ocean, Scientific Reports, 10, 10.1038/s41598-020-57890-2, 2020.

Yoshimura, T. and Kudo, I.: Seasonal phosphorus depletion and microbial responses to the change in phosphorus availability in a subarctic coastal environment, Marine Chemistry, 126, 182-192, 10.1016/j.marchem.2011.06.003, 2011.

https://doi.org/10.5194/bg-2021-357
Preprint. Discussion started: 6 January 2022

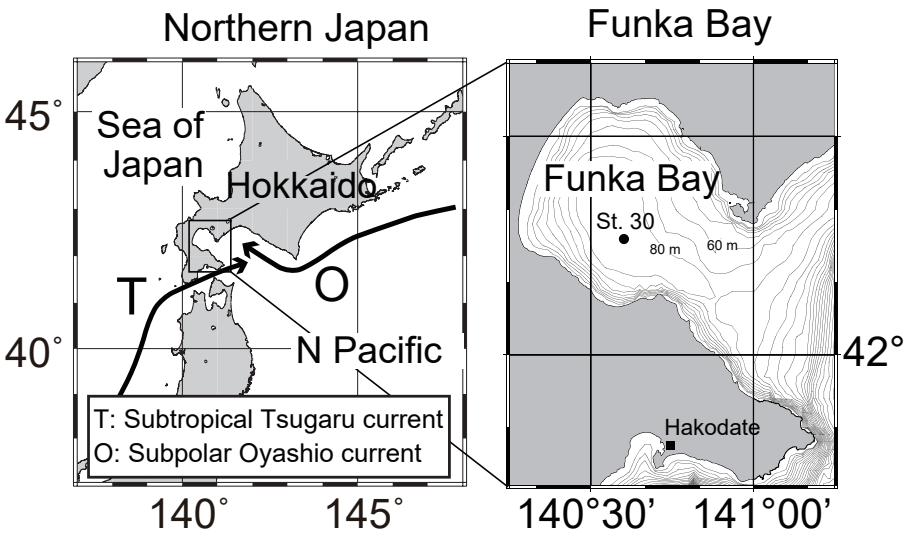

Fig. 1 Sampling sites in Funka Bay, Hokkaido, Japan

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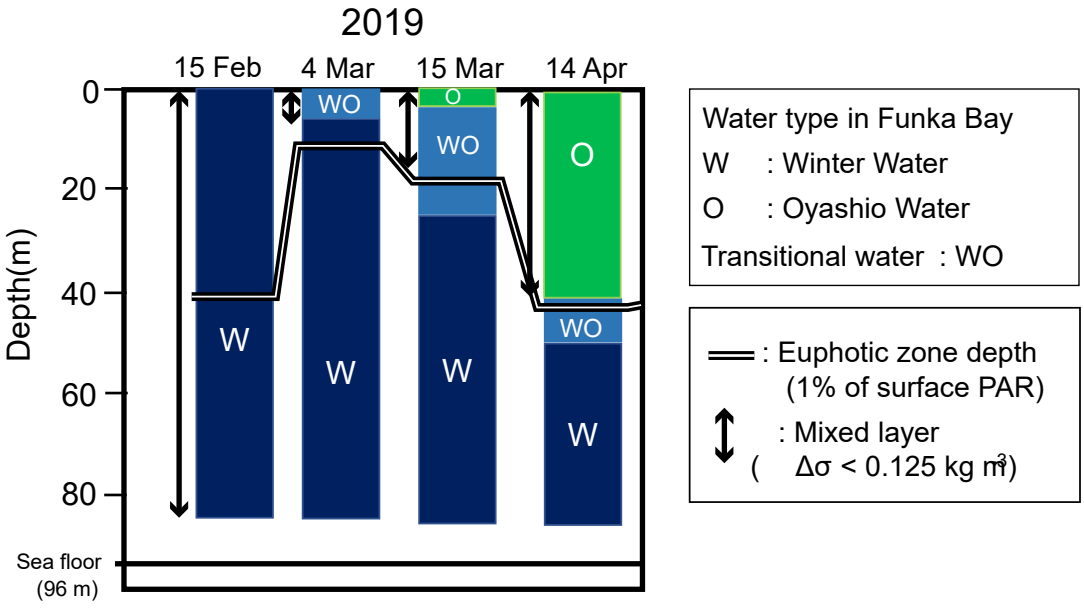

Fig. 2 Time series of water-mass structure at station 30 in Funka Bay, Japan. The two main water masses are winter water (W) and Oyashio water (O). Transitional water (W-O) is a water changing from winter water to Oyashio water by mixing. Euphotic-zone depth and surface mixed-layer depth (MLD) are also shown. PAR, photosynthetically active radiation

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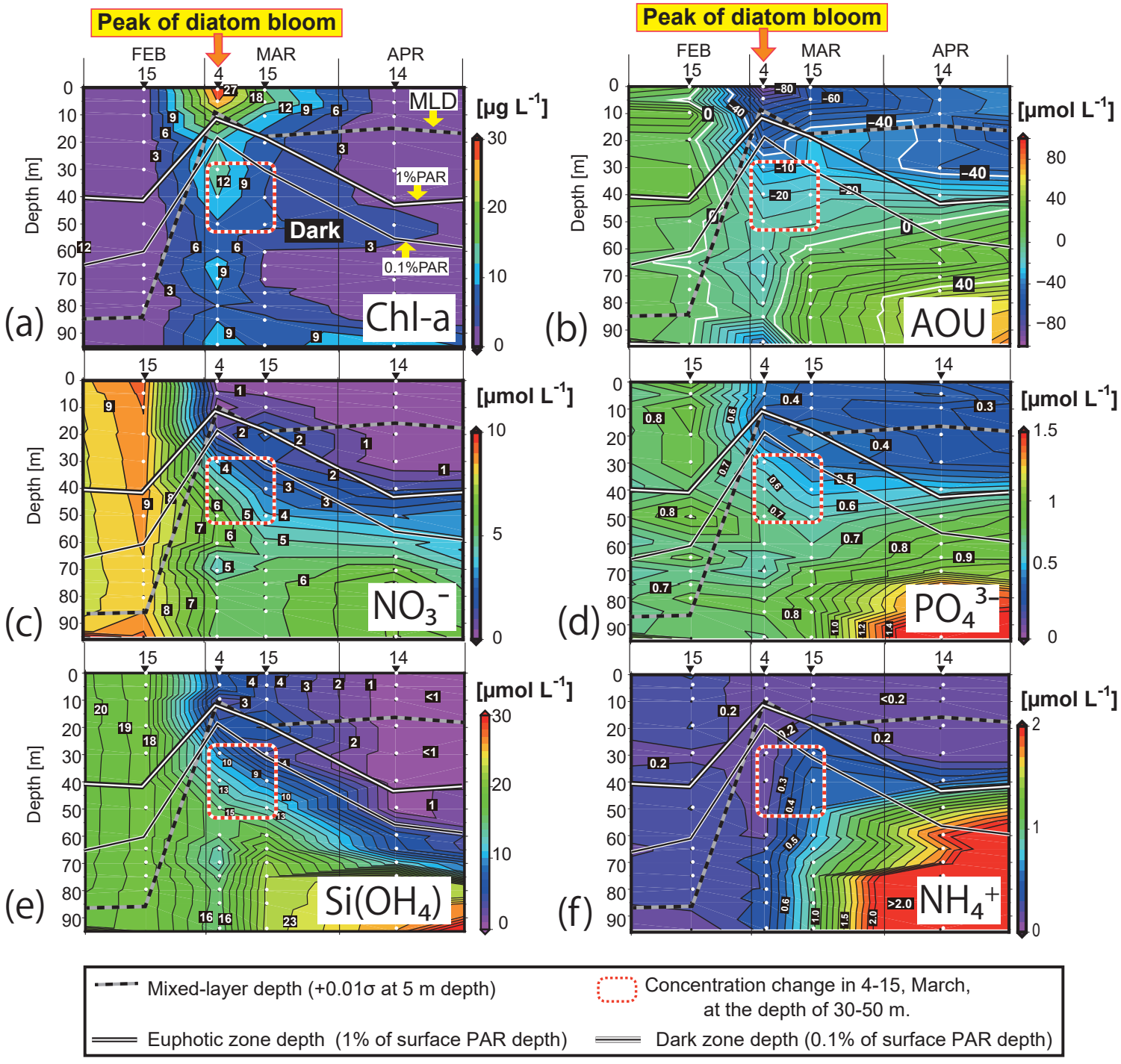

Fig. 3 Time–depth sections of chl-a concentration (a), apparent oxygen utilization (AOU) (b), and concentrations of NO_3^- (c), PO_4^{3-} (d), $Si(OH)_4$ (e), and NH_4^+ (f) in the water column in Funka Bay, Japan. Water was collected on 15 February, 4 and 15 March, and 14 April 2019; white circles indicate sampling depths. Solid white lines indicate the euphotic-zone depth (1% PAR). Solid black lines indicate the dark-zone depth (0.1% PAR). Black-and-white dotted lines indicate surface mixed-layer depth. Squares outlined with red-and-white dotted lines indicate the subsurface layer (30–50 m) on 4 and 15 March, where nutrient reductions were observed. PAR, photosynthetically active radiation

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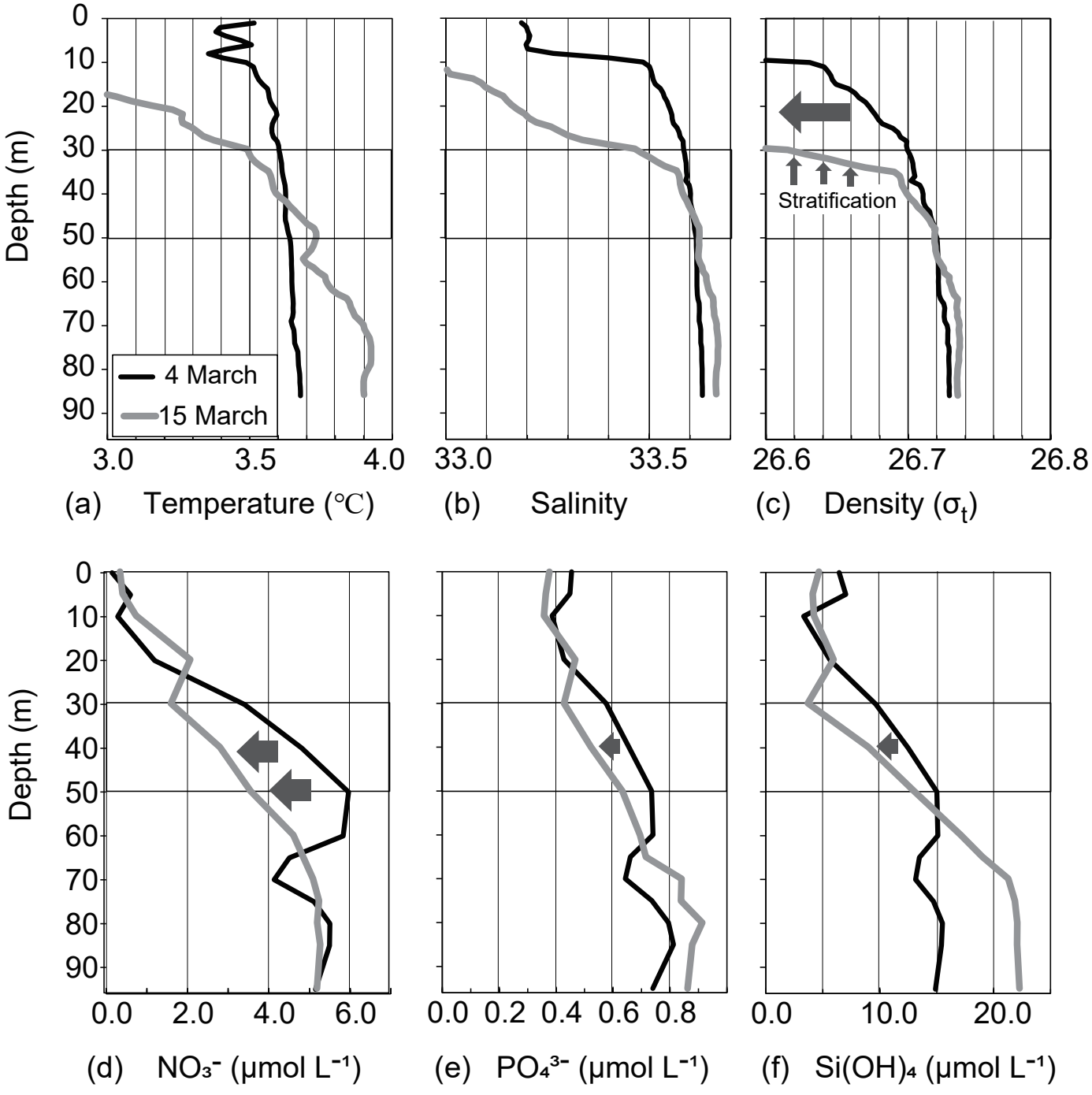

Fig. 4 Vertical profiles of temperature (a), salinity (b), density (c), and concentrations of NO_3^- (d), PO_4^{3-} (e), and $Si(OH)_4$ (f) at station 30 in Funka Bay, Japan, on 4 and 15 March. Left-pointing arrows in (C–F) highlight the obvious decreases between 4 and 15 March.

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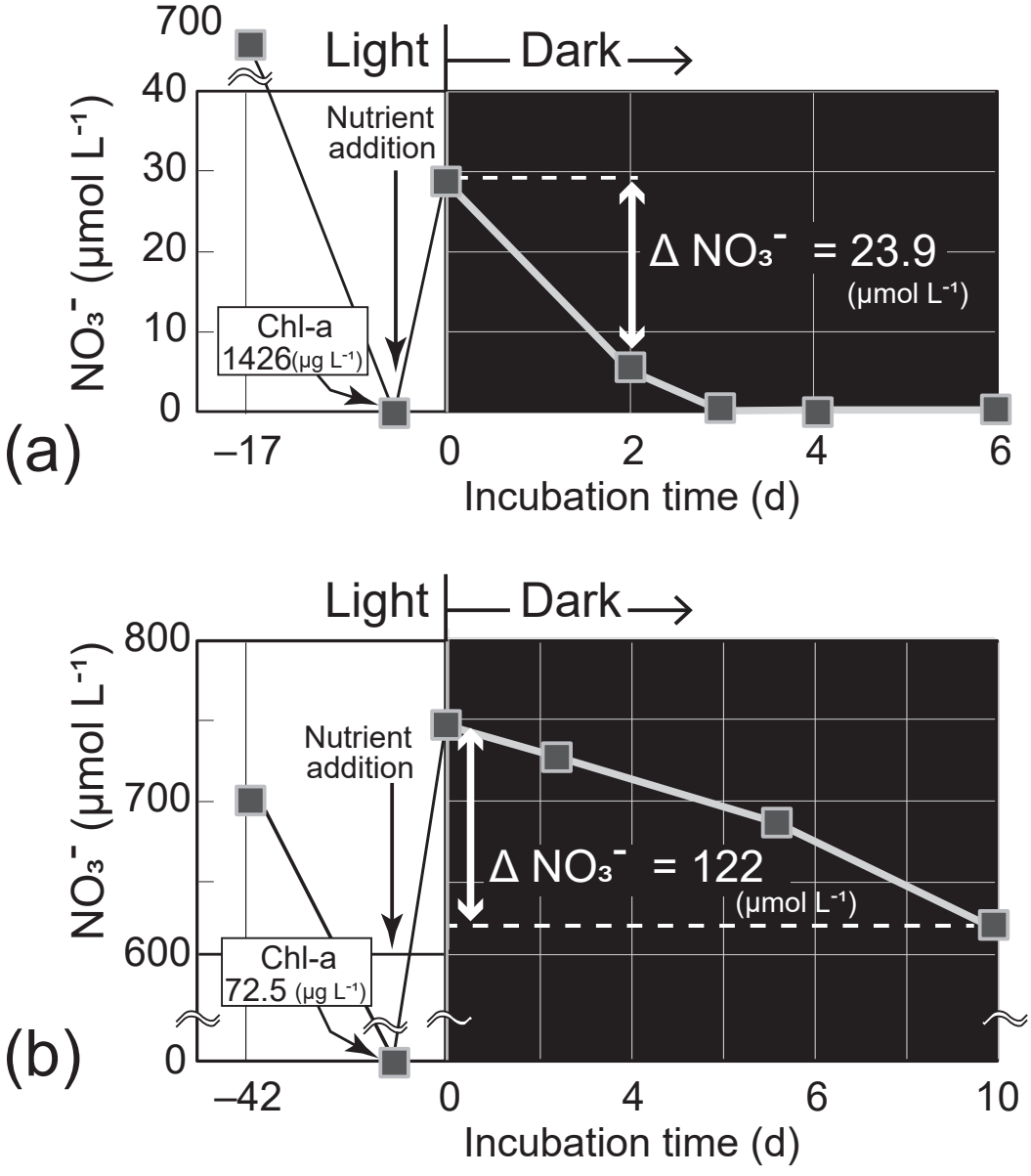

Fig. 5 Time series of the NO_3^- concentration in the dark incubation experiment using the diatom Thalassiosira nordenskioeldii. The diatom was cultured for 17 days at the first experiment (a) and 42 days at the second experiment (b) under light conditions before nutrients were added and it was moved to dark conditions.