Exhaustion of nitrate terminates a phytoplankton bloom in Funka Bay, Japan: change in SiO_4:NO_3 consumption rate during the bloom

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ABSTRACT: Time series observations over 36 h were carried out in Funka Bay, Japan, to complement a 6 yr nutrient-dynamics study during a diatom spring bloom, especially focusing on nitrate and silicate as limiting nutrients. At the beginning of the study, nutrient concentrations were high (NO_3 = 10 μM, SiO_4 = 20 μM and PO_4 = 0.8 μM). Nitrate was depleted first during the night while silicate and phosphate remained at 10 and 0.3 μM, respectively. High nitrate reductase activity was observed the following morning, indicating the assimilation of nitrate in situ by the dominant phytoplankter, *Chaetoceros socialis*. The consumption ratio of SiO_4:NO_3 increased by 3 times from 0.83 before the peak to 2.5 after the peak of the bloom. This change was also obvious in the elemental composition of phytoplankton, showing an increase in Si:C and Si:N ratios by a factor of 2 to 4 after the peak of the bloom, while C:N ratio remained constant at around 7. As a consequence of this change in the consumption rate of SiO_4 and NO_3, both silicate and nitrate were finally depleted in the photic zone.

KEY WORDS: Diatom bloom · Nitrate · Silicate · Limiting factor · Consumption rate · Phytoplankton

INTRODUCTION

A spring bloom is generally observed in neritic subarctic and temperate areas and during this period much of the annual 'new production' occurs because nitrate is a main N source. The spring bloom is important to the pelagic ecosystem, with respect to biogeochemical cycles of nutrient as well as trophodynamics and fisheries. This production accounts for one-third of the annual production in some areas (Smetachek 1980, Peinert et al. 1982, Kudo & Matsunaga 1999). The critical event triggering the start of the spring bloom is when the mixed-layer depth is shallower than the critical depth (Sverdrup 1953). The factors affecting this event were reported for several cases as light (Tett 1990) or vertical stability (Townsend & Spinrad 1986).

The magnitude of the amount of primary production is usually dependent on the initial level of 1 limiting nutrient. Usually, phytoplankton biomass was found to be limited by nitrate (Harrison et al. 1981, Hunts-

man et al. 1981) or silicate (Dugdale 1972, Brink et al. 1981). It has been suggested that the initial ratio of these 2 nutrients was important in determining which nutrient becomes exhausted first (Kamykowski 1974, Levasseur & Therriault 1987). The ratio of SiO_4:NO_3 varies over a wide range (from 0.5 to 3.3) in the upwelling zone (Levasseur & Therriault 1987). They also reported that at the time of phytoplankton bloom development nitrate was exhausted first in a water mass with a silicate to nitrate ratio >1 and silicate was exhausted where the ratio was <1. However, there is some controversy about which nutrient is the first to limit the phytoplankton bloom in Funka Bay, Japan. Tsunogai & Watanabe (1983) reported that silicate exhaustion derived the senescence of the bloom and silicate was the key nutrient determining phytoplankton species composition in Funka Bay. The initial ratio of SiO_4:NO_3 was 2 in Funka Bay, suggesting nitrate limitation by the definition by Levasseur & Therriault (1987). However, exhaustion of both nitrate and silicate was observed in the photic zone at the end of the bloom.

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In this study, we examined which nutrient limits the phytoplankton bloom in Funka Bay. Weekly to monthly observations and an intensive 36 h observation were conducted to demonstrate the change in nutrients and elemental compositions in phytoplankton. These results provide information for not only local interest, but also general aspects in terms of limiting nutrient and phytoplankton ecology.

MATERIALS AND METHODS

Weekly to monthly sampling was carried out aboard the RV 'Ushio Maru' during 1987 to 1992. The sampling stations were Stn 17 (42° 9.5' N, 140° 48.0' E) from 1987 to 1990 and Stn 30 (42° 16.2' N, 140° 36.0' E) in 1991 and 1992 in Funka Bay, Japan (Fig. 1). A time series was conducted over 36 h at Stn 30 using the TS 'Oshoro Maru' from March 10 to 12, 1996. Samples were taken from 10 and 40 m depth over an interval of 3 to 4 h using a Go-flo sampler attached to a Kevlar wire. Complete vertical profiles were conducted 3 times during the observation period. Nitrate reductase activity (NRA) was assayed by the method of Berges & Harrison (1995). One liter of subsample was filtered through a Whatman GF/F filter and the filter was ground in a buffer solution with a teflon homogenizer

which was cooled in an ice-water bath. The activity was finally measured as the amount of reduced nitrate which was colorimetrically measured during the reaction time and expressed as $\mu mol\ NO_2\ l^{-1}\ min^{-1}$. Chlorophyll a (chl a) was measured fluorometrically after extraction in N,N-dimethylformamide (Suzuki & Ishimaru 1990). The filtered sample was stored frozen for later analysis of nutrients with a Technicon Autoanalyzer II. Salinity and temperature were monitored with a Neil Brown CTDO sensor.

Phytoplankton samples were collected by vertically towing a plankton net (30 μm mesh) between 0 and 30 m followed by passage through a 300 μm net to remove large zooplankton. The elemental composition of phytoplankton was measured with a Yanaco CHN analyzer MT-2 for C and N. The silicon content of phytoplankton was measured by digestion with 1% Na_2CO_3 at 85°C for 3 h and measured as reactive silicate by the method of DeMaster (1981). As the interference from lithogenic silica to the results was corrected, the measured Si content represents biogenic Si or cellular Si content in phytoplankton.

RESULTS

Change in nutrient concentration during bloom

During our study a spring bloom in Funka Bay generally occurred in early March to April, while the onset of the bloom occurrence was different in each year. This onset was dependent on the timing of the formation of a pycnocline due to the inflow of less saline water (Kudo & Matsunaga 1999). A typical change in nutrients and chl a during the bloom was shown in 1991 (Fig. 2). The spring bloom in 1991 occurred at the end of March, with chl a reaching a maximum of more than 10 $\mu g\ l^{-1}$. At this time, both nitrate and silicate in the photic zone were 5 and 19 μM, respectively. How-
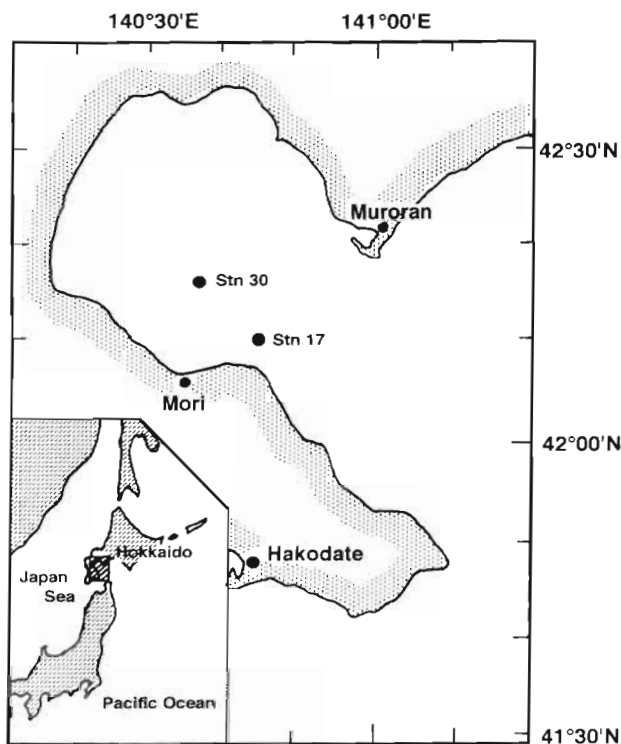

Fig. 1. Sampling stations in Funka Bay, Japan. Stn 17 was occupied during 1987–1990 and Stn 30 during 1991 and 1996
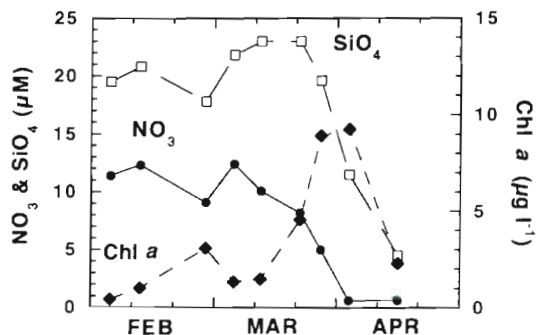

Fig. 2. Change in concentrations of nitrate, silicate and chlorophyll a during the spring bloom in 1991. Concentrations were expressed as an average over 0 to 20 m

ever, nitrate and silicate were almost depleted by the middle of April, which was 2 wk after the peak of the bloom. Average concentrations of nitrate and silicate over 0 to 30 m in April were 0.32 and 2.5 µM, respectively. However, these weekly or monthly sampling intervals do not provide a clear answer as to which nutrient terminates the phytoplankton bloom in Funka Bay: nitrate or silicate.

Time-series observation

Vertical profiles of salinity and temperature at the moored station did not change significantly over a 36 h period with a distinct thermocline and halocline around 40 m (Fig. 3). Judging from temperature and salinity profiles and following the definition of a water mass by Ohtani (1971), the upper layer was occupied by Oyashio Water and the deeper layer was Funka Bay Winter Water. Oyashio Water usually flows into the bay in February or March covering over the existing Funka Bay Winter Water which has a higher density. This inflow had already occurred in the upper layer. This indicated that the water column remained stable during the observation period but the possibility of some horizontal movement occurring could not be ruled out.

Nitrate and silicate at the beginning of the observation were almost homogeneously vertically distributed at about 10 and 20 µM, respectively (Fig. 3). At 10:00 h on March 11 a rapid drawdown of nitrate and silicate was observed in the photic zone. The concentrations of nitrate and silicate decreased to 2 and 10–14 µM, respectively. These concentrations and distributions
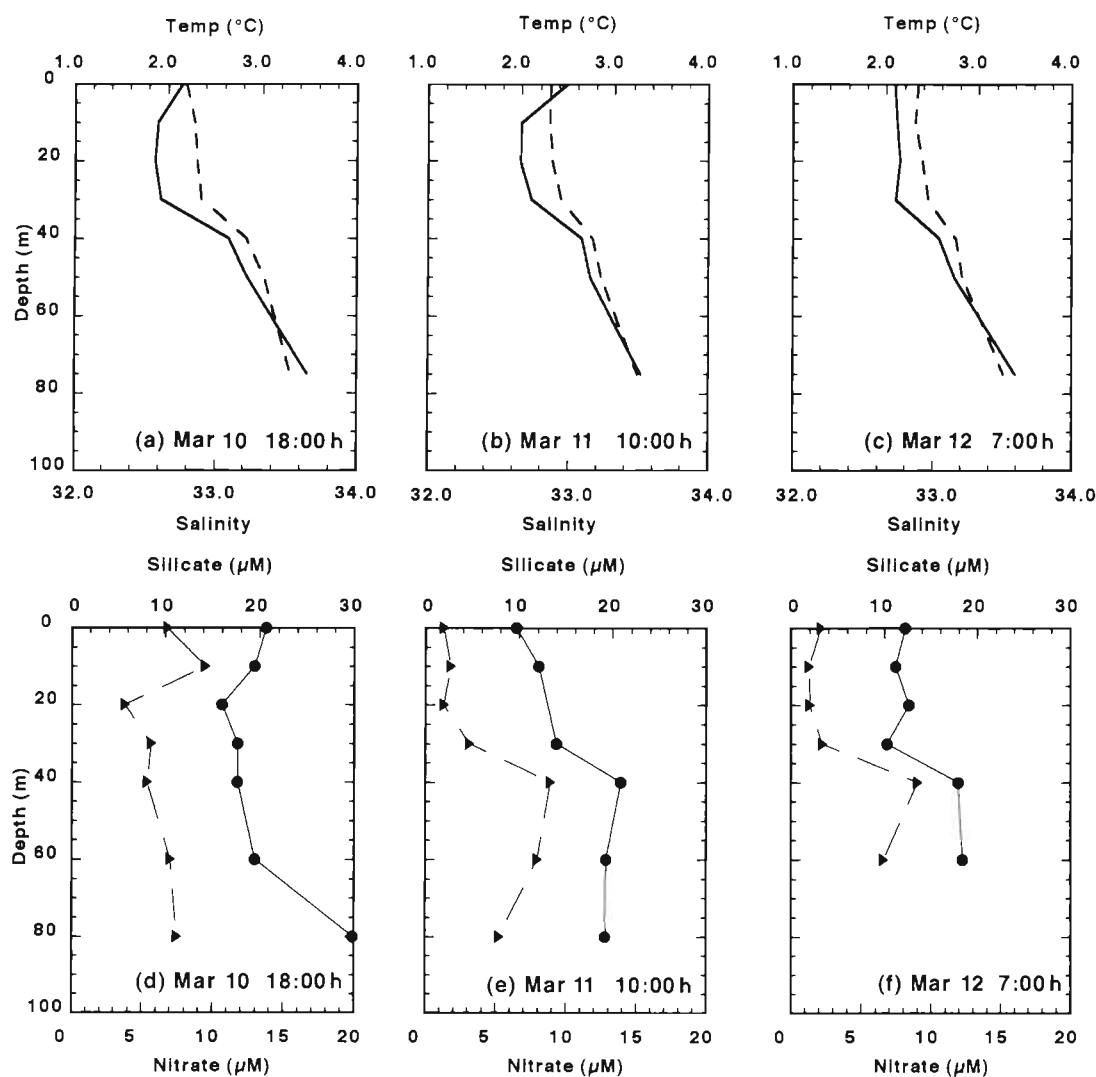

Fig. 3. Vertical distribution of temperature, salinity, nitrate and silicate at Stn 30 during a time series observation in March 1996: (a to c) temperature (——) and salinity (– – –). (d to e) Vertical distribution of nitrate (– –▶– –) and silicate (——●——)
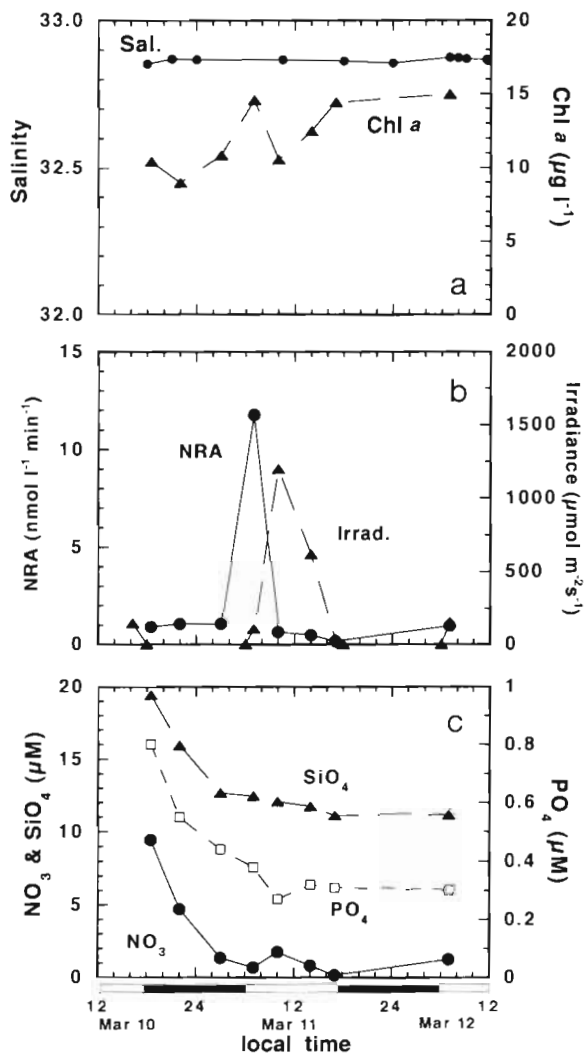
Fig. 4. (a) Change in salinity and chlorophyll *a*, (b) nitrate reductase activity (NRA) and irradiance, and (c) nitrate, phosphate and silicate at Stn 30 during a time series observation from 10 to 12 March 1996. All data except irradiance were measured at 10 m. Irradiance was measured on deck

did not change until the end of the observation period at 07:00 h on March 12. Phosphate was about 0.8 µM at the beginning and also decreased, to 0.3 µM, at the end of the observation period (not shown). More detailed data were obtained from 10 m depth (Fig. 4). Salinity was constant at 32.85, indicating that the water column was stable. Chl *a* increased from 10 to 15 µg l⁻¹ during the observation period, although some scatter was observed during the daytime on March 11. The change in chl *a* indicated that this observation was conducted during the middle to the end of the spring bloom. All nutrients decreased in the first 12 h of the dark period and nitrate was almost exhausted (<1 µM) by the morning of March 11. Silicate and phosphate were not depleted, but decreased from 20 to 12 µM

and from 0.8 to 0.3 µM over the same period, respectively. The consumption ratio of SiO_4:NO_3 at 10 m was 0.9 and that of NO_3:PO_4 was 18, which is similar to the Redfield ratio. High activity of NRA was observed in the morning, which could account for the amount of the decrease in nitrate, 9 µM when integrated over time in Fig. 4. The activity of NRA indicated that the decrease in nutrient was not derived from a water mass exchange, but from the *in situ* assimilation by phytoplankton. These data clearly demonstrate that the spring bloom in Funka Bay was terminated by the depletion of nitrate in the photic zone.

Change in nutrient consumption rate with time

The initial concentrations of nitrate and silicate were almost the same in every year in Funka Bay (Kudo & Matsunaga 1999). The standing stocks of nitrate and silicate from February to April in 1987 to 1992 were plotted in Fig. 5 to elucidate the relative consumption rate of nitrate and silicate during the bloom. The higher standing stock of nitrate (>100 mmol m⁻²) represents the earlier stage of the bloom, and the lower standing stock (<100 mmol m⁻²) represents the later stage of the bloom. The regression equations are given in Fig. 5, where the linearity is highly significant (p < 0.01).

The consumed silicate to nitrate ratio at the earlier stage of the bloom was 0.83, while at the later stage it was 2.5, which is 3 times higher than the ratio in the earlier stage. A change in the intercept from 261 µM at the initial stage to 64.8 µM at the later stage was also observed. The initial consumption ratio of silicate to
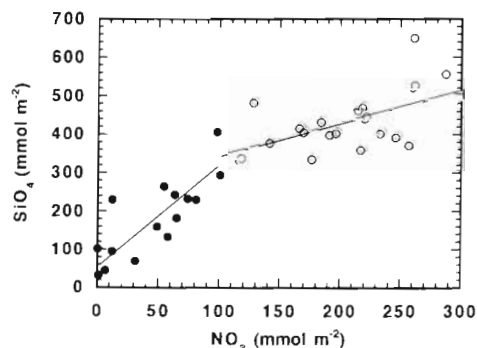

Fig. 5. Standing stock of nitrate versus silicate from 1987 and 1992. Standing stock was calculated by integrating over 0 to 30 m. A standing stock >100 mmol m⁻² represents an earlier stage of the bloom (O) and a standing stock <100 mmol m⁻² represents a later stage (●). Solid lines represent the regression lines. Equations were $SiO_4 = 261 + 0.83 NO_3$ (r = 0.57, n = 21) for the standing stock >100 mmol m⁻² and $SiO_4 = 64.8 + 2.50 NO_3$ (r = 0.79, n = 15) for the standing stock <100 mmol m⁻²

nitrate was similar to the ratio of 0.9 during the time-series observation. An increase in the consumption ratio will result in the depletion of silicate at the end of the bloom.

Elemental composition change in plankton net sample

The elemental composition of phytoplankton revealed that the C:N ratio did not change to any great extent from February to April. An average ratio for C:N was 6.8 ± 0.6 (± 1 SE, n = 16) (Fig. 6). In contrast to the constant value of C:N, Si:C and Si:N ratios increased during the spring bloom just after the peak of the bloom, indicating that these increases were derived from the increase in the biogenic Si content. The time of the increase was different for each year due to the different timing of the bloom occurrence, which was
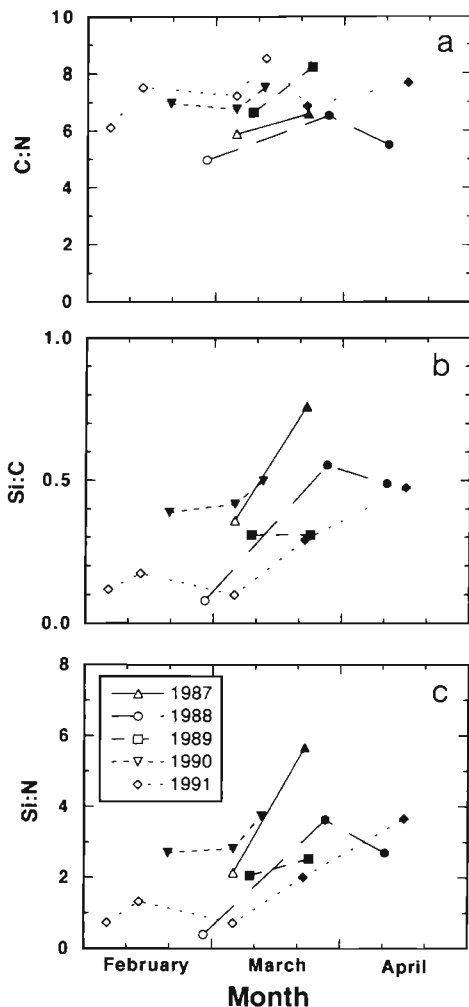

Fig. 6. Changes in cellular ratios in phytoplankton during 1987 and 1991. (a) C:N, (b) Si:C, (c) Si:N. Filled symbols designate the plots after the peak of the bloom in each year

mainly dependent on the stability of the water column (Kudo & Matsunaga 1999). The bloom occurred in early March of 1989 and 1990. The biogenic Si in these years was already high in early March, although in other years it increased from the middle of March, which coincided with the late occurrence of the bloom.

DISCUSSION

Tsunogai & Watanabe (1983) observed the depletion of silicate at the end of a spring bloom in Funka Bay and concluded that this depletion was the factor determining the termination of the bloom. However, they only focused on the change in silicate and phosphate, not on that of nitrate. We compiled the available data on nutrients in Funka Bay and found that both nitrate and silicate were exhausted at the end of the bloom if no external supply of nutrients occurred (Kudo & Matsunaga 1999). Previous observations during the spring bloom in Funka Bay were only conducted weekly and therefore it was not evident which nutrient terminated the phytoplankton bloom in Funka Bay, Si or N. In the present study, daily and hourly time-series observations enabled us to document the final stage of the spring bloom and we observed nitrate depletion in the photic zone while silicate remained at 10 µM.

After reaching the peak, which was identified by the maximal chl *a* concentration, cell division ceased due to the depletion of N. Silicate assimilation continued after the depletion of nitrate until the 10 µM of silicate was used up. In reflection, these changes in the ambient concentrations of nitrate and silicate during the bloom, the cellular content of Si or relative content of Si to N should increase after the depletion of nitrate and until the remaining silicate is taken up. The change of cellular Si was well demonstrated in the change in Si:C and Si:N ratio during and after the bloom (Fig. 6). We define 'termination of growth' as a decline in the bloom as a result of unfavorable growth conditions, such as depletion of nutrients or low irradiance. After the depletion of nitrate, internal nitrate may allow cells to divide at least 1 more time. Under natural conditions, it is difficult to observe this phenomenon because of small-scale perturbation in the water column or horizontal advection.

Primary production at the surface, as measured by the ^{14}C method, at the peak of the bloom was 120 µg C l^{-1} h^{-1} while 1 wk after the peak it dropped 75% to 30 µg C l^{-1} h^{-1} and it was 1 µg C l^{-1} h^{-1} in April. The ammonium concentration in Funka Bay was about 1 µM in the photic zone and did not change during the bloom. The elevated ammonium concentrations exceeding 3 µM occurred below 60 m after May due to the decomposition of organic material produced by the

bloom, which sunk to the bottom. Judging from the change in primary production and ammonium, and low temperatures of 3°C, we consider the influence of regeneration of nutrients in the photic zone to have been of limited magnitude in our study period.

The usage of a 30 µm mesh plankton net has the disadvantage of missing smaller diatoms, and the removal of zooplankton using a 300 µm mesh net from the collected sample would likely include microzooplankton in the measured elemental composition of phytoplankton. Our collection method may not completely retrieve diatoms quantitatively, but our results represent the elemental composition of diatoms typically appearing in the bay because the dominant diatom species during the bloom in Funka Bay are chain-forming diatoms such as *Thalassiosira* and *Chaetoceros* (Nakata 1982, Odate 1987), whose chain length is usually more than 50 µm (Hasle & Syvertsen 1997). The abundance of microzooplankton during the bloom in Funka Bay was generally low compared to that during the summer season (Odate & Maita 1988). If any of these zooplankton were included in the results of the elemental composition in this study they contributed only to C and N, not to Si, because of the absence of Si in their bodies (unless they contained diatoms in their guts).

Nitrate uptake rates and NRA values correlate well in natural plankton (Blasco et al. 1984) and laboratory culture work (Berges & Harrison 1995). The observed mismatch between the nitrate disappearance at night and the high NRA the following morning would account for the diel variation in nitrate assimilation and reduction. The intracellular storage of nitrate showed pronounced high values at midnight (Pettersson & Sahlsten 1990) and NRA exhibited a rapid increase with the onset of daylight (Martinez et al. 1987).

It has been reported that N limitation in the presence of silicate can induce the resting spore formation for many *Chaetoceros* species (Hargraves & French 1980, Garrison 1981, Oku & Kamatani 1997). Oku & Kamatani (1995) showed that the formation of resting spores required 3 times more Si than vegetative cells. In other words, the increase in cellular Si content in a diatom might be an indication of resting spore formation. Vegetative cells of *Chaetoceros* sp. were commonly observed in Funka Bay during the spring bloom (Nakata 1982, Odate & Maita 1990). Odate & Maita (1990) observed a high density of resting spores of *Chaetoceros* sp. in the 50 to 80 m depth layer of Funka Bay in late April at $>10^5$ spores l^{-1}, while the density of resting spores in other season was $<10^3$ spores l^{-1}. They also reported a maximum flux of spores measured by a sediment trap at the same place occurred in late April (2.0×10^8 spores m^{-2} d^{-1}). *Chaetoceros socialis* was the dominant diatom species during our time-series obser-

vations in 1996 (N. Shiga pers. comm.). Therefore, the observed depletion of nitrate may accelerate the increase in Si content in the diatom which is necessary to induce the formation of resting spores and this will lead to silicate depletion following nitrate depletion at the end of the spring bloom in Funka Bay. Rey & Skjoldal (1987) also observed a large decrease in the concentration of silicate below the nitracline and a formation of resting spores of the dominant species *Chaetoceros socialis* after the bloom in the Barents Sea. The formation of resting spores is considered as an effective strategy for preserving a seed population of the species for the following years and escaping from degradation and grazing by zooplankton.

In the world's oceans, the silicate concentration does not always exceed nitrate concentration. Silicate usually becomes a limiting nutrient in the southern hemisphere, while nitrate becomes limiting in the northern hemisphere in the Pacific Ocean (Kamykowski & Zentara 1985). In a Norwegian fjord, the average concentration of silicate and nitrate was 7 and 12 µM respectively, and silicate depletion was observed at the end of the spring bloom (Skjoldal & Wassmann 1986). It is generally thought that the silicate to nitrate ratio of 1 is the crucial ratio to determine which nutrient becomes limiting, silicate or nitrate (Levasseur & Therriault 1987). Our results, however, suggested that the assimilation ratio of silicate to nitrate changed during the growth phase. This may allow a range of both silicate and nitrate exhaustion at different silicate to nitrate ratios in an original seawater.

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