



Title	Primary productivity and nitrogen assimilation with identifying the contribution of urea in Funka Bay, Japan
Author(s)	Kudo, Isao; Hisatoku, Takatsugu; Yoshimura, Takeshi; Maita, Yoshiaki
Citation	Estuarine, Coastal and Shelf Science, 158, 12-19 <a href="https://doi.org/10.1016/j.ecss.2015.03.012">https://doi.org/10.1016/j.ecss.2015.03.012</a>
Issue Date	2015-03-14
Doc URL	<a href="http://hdl.handle.net/2115/64692">http://hdl.handle.net/2115/64692</a>
Rights	© 2015, Elsevier. Licensed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International <a href="http://creativecommons.org/licenses/by-nc-nd/4.0/">http://creativecommons.org/licenses/by-nc-nd/4.0/</a>
Rights(URL)	<a href="http://creativecommons.org/licenses/by-nc-nd/4.0/">http://creativecommons.org/licenses/by-nc-nd/4.0/</a>
Type	article (author version)
File Information	Kudo ECSS.pdf



[Instructions for use](#)

Primary productivity and nitrogen assimilation with identifying the contribution of urea in Funka Bay, Japan.

Isao Kudo<sup>1,2\*</sup>, Takatsugu Hisatoku<sup>1</sup>, Takeshi Yoshimura<sup>1\*\*</sup> and Yoshiaki Maita<sup>1</sup>

<sup>1</sup> Graduate School of Fisheries Sciences, Hokkaido University, Hakodate 041-8611, Japan

<sup>2</sup> Graduate School of Environmental Science, Hokkaido University, Sapporo 060-0813, Japan

\*corresponding author: ikudo@fish.hokudai.ac.jp, +81-11-706-2321

\*\*present address: Central Research Institute of Electric Power Industry, Abiko, Chiba, 270-1194, Japan

## Abstract

Primary production is supported by utilization of several forms of nitrogen (N), such as nitrate, ammonium, and urea. Nevertheless, only few studies have measured the concentration and uptake of urea despite its importance as a nitrogenous nutrient for phytoplankton. We measured primary productivity monthly at four depths within the euphotic zone using a clean technique and the  $^{13}\text{C}$  method by a 24 h in situ mooring incubation over a year in Funka Bay, a subarctic coastal area in Japan, to make better updated estimates (re-evaluation) of annual primary production. Nitrogenous (N) nutrient assimilation rates (nitrate, ammonium and urea) were also measured to elucidate the relative contributions of these nutrients to autotrophic production and to distinguish between new and regenerated production. The estimated annual primary production was  $164 \text{ g C m}^{-2}$ , which was 40–60% higher than the previously reported values in the bay. Use of a clean technique and more frequent measurement during the spring bloom may have contributed to higher rates. The production during the spring bloom was  $56.5 \text{ g C m}^{-2}$ , accounting for 35% of the annual production. The maximum daily productivity occurred in the bloom at  $1.4 \text{ g C m}^{-2} \text{ d}^{-1}$ , which is one of the highest values among the world embayments. The annual primary production in the bay was classified as mesotrophic state based on the classification by Cloern et al. (2014). The assimilation rate of nitrate was maximal at  $54 \text{ nmol N L}^{-1} \text{ h}^{-1}$  during the bloom. During the post-bloom periods with nitrate depleted conditions, assimilation rates of ammonium and urea increased and accounted for up to 85% of the total N assimilation. The assimilation rate of urea was almost comparable to that of ammonium throughout the year. Taking urea into account, the  $f$ -ratio ranged from 0.15 under the nitrate-depleted conditions to 0.8 under the spring bloom conditions. These ratios were overestimated by 50% and 10%, respectively, if urea uptake was eliminated. We provide a valuable data for the primary production dataset in the world's ecosystems, and show that urea plays an important role in supporting regenerated production during late spring and summer.

Keywords: new production, spring bloom,  $f$ -ratio, subarctic, clean technique

## 1. Introduction

Primary production is considered to be limited mostly by nitrogen (N) in marine environments (Thomas, 1970; Ryther and Dunstar, 1971). Phytoplankton utilize several forms of N to support their demand, but ammonium ( $\text{NH}_4^+$ ) is preferably utilized (McCarthy et al., 1977; Glibert and McCarthy, 1984). The form of N species utilized by phytoplankton has been used to distinguish the fraction between new and regenerated production (Dugdale and Goering, 1967). New production is defined as the primary production that utilizes N supplied from outside the euphotic zone, mainly by vertical mixing (Dugdale et al., 1992), and the fraction of new production to the total primary production is termed the *f*-ratio (Eppley and Peterson, 1979). In contrast, regenerated production is supported by regenerated N derived from the metabolic products of several biological processes within the euphotic zone. Usually, nitrate ( $\text{NO}_3^-$ ) and  $\text{NH}_4^+$  are considered new and regenerated N, respectively.

Urea is the simplest form of organic N compound, with two N atoms in one molecule. Because urea is hydrolyzed to  $\text{NH}_4^+$  by urease, an enzyme produced by phytoplankton and bacteria, it is utilized by phytoplankton similarly to inorganic N (McCarthy, 1972). Eppley and Peterson (1979) considered urea as regenerated N similar to  $\text{NH}_4^+$ . Wafar et al. (1995) compiled non-inclusion of urea uptake in N uptake, resulting in the overestimation of *f*-ratio differing from 6 to 55%, depending on the type of ecosystem. Following this recognition, measurement of urea uptake has been included recently in N uptake surveys (Joint et al., 2001; Twomey et al., 2005; Torres-Valdes and Purdie, 2006; Buck et al., 2014), but there are only few studies that address the role of urea in marine ecosystems.

Funka Bay (Fig. 1) is a hotspot of Japanese fisheries such as scallop culture, and thus studies have been conducted for a better understanding of the ecosystem in the bay. Funka Bay has an area of  $2.3 \times 10^3 \text{ km}^2$  with mean and maximum depths of 59 and 96 m, respectively. The bay is conical in shape, separated from the Pacific Ocean by a sill at a depth of 60 m at the mouth. Water exchange events mainly occur twice a year, Oyashio water in spring and Tsugaru warm water (TWW) in autumn (Ohtani, 1971). An intense phytoplankton bloom occurs in late February to March, consisting of diatoms. The timing of the bloom is affected by the inflow of Oyashio water, contributing to the earlier occurrence through the development of density stratification (Kudo and Matsunaga, 1999). The bloom is terminated by the exhaustion of  $\text{NO}_3^-$ , and then silicic

acid ( $\text{Si}(\text{OH})_4$ ) is also depleted by the enhanced consumption of  $\text{Si}(\text{OH})_4$  due to thickening diatom frustule (Kudo et al., 2000). After the bloom an increase in settling flux of organic carbon is observed, accounting for 40% of primary production during the bloom (Miyake et al., 1988). Nutrient regeneration takes place actively from April to August at the bottom (Kudo et al., 2007). The bottom water, with an elevated concentration of nutrients, is mixed with the inflowing TWW after September. Some of the regenerated nutrients in the bay return to the surface due to winter vertical mixing, but the rest flows out by the water exchange with TWW. Scallop (*Mizuhopecten yessoensis*) culture is well established in the bay, and annual production is about 0.1 million tons. Scallop feeds on phytoplankton and particulate organic matter (MacDonald et al., 2006; Aya et al., 2013). Therefore the estimation of primary productivity is important to consider a sustainable allowance for scallop culture in the bay.

Maita and Yanada (1978) and Odate and Maita (1988) reported the annual production in Funka Bay as 100 and 118 g C m<sup>-2</sup>, respectively, based on monthly observation and the standard <sup>14</sup>C method (Steeman Nielsen, 1952). The incubation adopted was a tank method using glass bottles, fluorescent bulbs, light attenuation at two light depths of 100 and 60% of surface irradiance ( $I_0$ ) and 3–4 h of incubation. Fitzwater et al. (1982) reported an improved <sup>14</sup>C method (clean sampling and chemicals and polycarbonate incubation bottles) provided up to three times higher primary productivity compared to the conventional standard <sup>14</sup>C method. Therefore primary productivity in Funka Bay should be re-evaluated using the improved method. Objectives of this study are to conduct monthly based primary productivity measurements for a whole year and in situ incubation adopting the clean technique (Fitzwater et al., 1982) to make better estimates of annual production in Funka Bay. Additionally, nitrogenous nutrient uptake rates ( $\text{NO}_3^-$ ,  $\text{NH}_4^+$  and urea) were also measured to elucidate the relative contributions of these nutrients supporting autotrophic production and to distinguish between new and regenerated production because there have been virtually no studies on N utilization by phytoplankton in the bay.

## 2. Materials and methods

## 2.1 Sampling and water analysis

Sampling cruises were conducted monthly aboard the R/V 'Ushio Maru' from March 1999 to March 2001 and the T/V 'Oshoro Maru' in March 2000 and 2001. More frequent sampling (weekly to bi-weekly) was conducted during the spring bloom in March. The sampling station was Stn 30 (42° 16.2'N, 140° 36.0'E, 92 m depth) in Funka Bay (Fig. 1). Salinity and temperature profiles were obtained by a CTD sensor (Sea-Bird 19). Photosynthetically active radiation (PAR) was measured vertically with a Licor Model II-193 (scalar sensor).

Water samples for chlorophyll *a* (Chl *a*) and nutrients were taken vertically with 5 L Niskin samplers. An aliquot of sample was filtered onto a Whatman GF/F filter. The filter was stored frozen in *N,N*-dimethylformamide to extract plant pigments (Suzuki and Ishimaru, 1990). The extracted Chl *a* was measured with a HITACHI F-2000 spectrofluorometer by the method of Parsons et al. (1984). The samples for NO<sub>3</sub><sup>-</sup>, nitrite (NO<sub>2</sub><sup>-</sup>), NH<sub>4</sub><sup>+</sup>, phosphate (PO<sub>4</sub><sup>3-</sup>) and Si(OH)<sub>4</sub> analysis were stored frozen for subsequent analysis with a Technicon Autoanalyzer II (Grasshoff et al., 1999). Urea was analyzed manually following the method of Mulvenna and Savidge (1992). Precision (coefficient of variation, CV) of nutrient analysis was about 1% for all nutrients except urea and 2.5% for urea by replicate analysis of samples at natural concentration level. Detection limits were estimated at around 0.01 μM based on three times the standard deviation of the lowest concentration of samples. In this paper concentration of NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup> and urea was expressed in μmol N L<sup>-1</sup> because one mole of urea produces two moles of NH<sub>4</sub><sup>+</sup> after hydrolysis by urease.

## 2.2 Primary productivity and N uptake

Primary productivity was measured at four different depths (2, 5, 10 and 15 m) from March 1999 to March 2000 by an in situ incubation method and for a depth of 5 m from April 2000 to March 2001. Water samples for primary productivity were collected with a Teflon-coated trace metal-clean lever-action Niskin sampler attached to a Kevlar line. The samples were dispensed into acid-cleaned Nalgene<sup>®</sup> Polycarbonate bottles (250 mL). An aliquot (10% of total inorganic carbon) of <sup>13</sup>C-labelled NaH<sup>13</sup>CO<sub>3</sub> (99 at.%; Shoko Co., Ltd) was added to each bottle (triplicate) immediately after sampling.

Incubation was conducted in situ by mooring each bottle at their corresponding sampling depth for 24 h (1999–2000). After recovery of the incubation bottles on the next day, each sample was filtered onto a pre-combusted GF/F filter, and the filter was stored in a -80°C deep freezer. Incubation in 2000–2001 was conducted for 3 h in on-board flow-through seawater tanks covered with neutral density screen to reduce light intensity to 50% of ambient light. The measurement principles, equipment, and calculations were described by Hama et al. (1983).

The beginning and end of the blooms were defined as  $\text{Chl } a > 2 \mu\text{g L}^{-1}$  and  $\text{Chl } a < 2 \mu\text{g L}^{-1}$ , respectively because  $\text{Chl } a$  never exceeds the level in non-bloom seasons in the bay. Depth-integrated primary productivity was calculated by trapezoidal integration between 2 and 15 m and within the euphotic zone. The base of the euphotic zone was defined as the depth of 1%  $I_0$ . For depth integration to the base of euphotic zone, it was assumed that primary productivity was zero at this depth and decreased linearly from 15 m to this depth.

$\text{NO}_3^-$ ,  $\text{NH}_4^+$  and urea uptake experiments were conducted from March 2000 to March 2001 in duplicate after inoculation with  $^{15}\text{N}$ -potassium nitrate (99.2 at.%; Shoko Co., Ltd),  $^{15}\text{N}$ -ammonium sulfate (99.6 at.%; Shoko Co., Ltd) or  $^{15}\text{N}$ -urea sulfate (99.6 at.%; Shoko Co., Ltd). As the concentrations of these substrates were not known at the time of inoculation, we added  $^{15}\text{N}$  tracer at various amounts to obtain the Michaelis–Menten (MM) equation. The stable  $^{15}\text{N}$  isotope was enriched at 0.1, 1.0 and 5.0  $\mu\text{mol N L}^{-1}$  (additionally 10.0  $\mu\text{mol N L}^{-1}$  during March 2001). Sampling and handling were the same as for the primary productivity experiments, but the sampling layer was fixed at a 5 m depth. The calculation was conducted following the method of Dugdale and Wilkerson (1986). The precision of uptake rate measurements for  $\text{NO}_3^-$ ,  $\text{NH}_4^+$  and urea was about 20% based on triplicate samples. The in situ uptake rate was obtained from putting the in situ concentration of substrate to the best-fitted Michaelis–Menten (MM) equation between substrate concentration and uptake rate. In keeping with the MM equation, it was assumed that the uptake rate was zero when the substrate concentration was 0  $\mu\text{mol N L}^{-1}$ .  $\text{NH}_4^+$  regeneration experiments were not carried out. Thus,  $\text{NH}_4^+$  uptake data are uncorrected for  $^{15}\text{NH}_4^+$  isotope dilution and are likely to have underestimated the true  $\text{NH}_4^+$  uptake rate (Glibert et al., 1982). We also have not estimated potential losses of  $^{15}\text{N}$  to the dissolved organic N (DON) pool (Bronk et al., 1994).

The  $f$ -ratio was calculated by two ways in considering urea uptake or not as follows:

$$f\text{-ratio}_w = \rho_{\text{NO}_3^-} / (\rho_{\text{NO}_3^-} + \rho_{\text{NH}_4^+} + \rho_{\text{urea}}),$$

$$f\text{-ratio}_{w0} = \rho_{\text{NO}_3^-} / (\rho_{\text{NO}_3^-} + \rho_{\text{NH}_4^+}).$$

where  $f\text{-ratio}_w$  and  $f\text{-ratio}_{w0}$  were  $f$ -ratio with urea uptake and  $f$ -ratio without it, and  $\rho_{\text{NO}_3^-}$ ,  $\rho_{\text{NH}_4^+}$  and  $\rho_{\text{urea}}$  were absolute uptake rates of  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ , and urea, respectively.

The isotope compositions of C and N were measured with a combined system of NA1500 (CE Instruments, Italy) CNS elemental analyzer and MAT 252 (Finnigan MAT, Germany) mass spectrometer.

### 3. Results

#### 3.1 Physical environment

The general pattern of seasonal and vertical distribution in temperature and salinity was similar in 1999 and 2000 (Fig. 2A, B). Temperature in February and March was vertically homogenous at less than 5°C. Temperature in the surface layer increased from late March to August and September. Salinity was greater than 33.5 during winter and gradually decreased in the surface layer after April due to the inflow of Oyashio water and freshwater discharge by a spring thaw. Minimum salinity at less than 31.0 was found in the summer surface layer. Salinity increased in the middle and deeper layer after September, indicating the inflow of TWW. Temperature and salinity were vertically homogenous to the bottom in winter due to an intense vertical mixing.

#### 3.2 Seasonal change in nutrients and Chl *a*

$\text{NO}_3^-$  concentration was greater than 10  $\mu\text{mol N L}^{-1}$  in wintertime, decreased in the upper 40 m in March and almost depleted from late May to October (Fig. 2D). This decrease coincided with a spring phytoplankton bloom in March, indicated by a high concentration ( $> 2 \mu\text{g L}^{-1}$ ) of Chl *a* (Fig. 2C). The bloom occurred in March every year, but the depth range where the high concentration of Chl *a* was found differed in each year. A high concentration of Chl *a* ( $> 5 \mu\text{g L}^{-1}$ ) down to 70 m was found in March 2000 and 2001. The duration of the spring bloom was about a month. Fall bloom was

observed in October 2000. In other periods, Chl *a* concentration was low at less than 1  $\mu\text{g L}^{-1}$ .  $\text{NH}_4^+$  concentration was generally low in the surface layer (~40 m) at less than 1  $\mu\text{mol N L}^{-1}$  (Fig. 2E). Accumulation of  $\text{NH}_4^+$  in deeper layer was found after the bloom.  $\text{NH}_4^+$  concentration reached  $> 8 \mu\text{mol N L}^{-1}$  near the bottom in May and July 1999. Then,  $\text{NH}_4^+$  concentration decreased and  $\text{NO}_3^-$  increased due to nitrification at this depth (Kudo et al., 2007).

Urea concentration ranged from under the detection limit to 2.0  $\mu\text{mol N L}^{-1}$  (Fig. 2F). Urea concentration was high in winter and gradually decreased until summer throughout the water column. Low concentration was found in the surface layer in June and July and the layers below 30 m from July to October. A high concentration of urea was found around 10 m in August.

### 3.3 Primary productivity and N uptake rates

Primary productivity ranged from 1.1 to 147.0  $\text{mg C m}^{-3} \text{d}^{-1}$ , where the minimum and the maximum were observed at 15 m in April, 1999 and at 5 m in March, 2000, respectively (Fig. 3). Primary productivity increased during the spring bloom at more than 25  $\text{mg C m}^{-3} \text{d}^{-1}$ . However, productivity in March, 2000 was higher than that in 1999. Productivity after April was consistently low at less than 25  $\text{mg C m}^{-3} \text{d}^{-1}$ .

The depth at 1% of  $I_0$  was 20 m during the spring bloom and deeper than 30 m in other seasons (Fig. 4A). The deepest value was 50 m in late September.

The depth-integrated (0–15 m) primary productivity was maximal during the bloom period, at 720  $\text{mg C m}^{-2} \text{d}^{-1}$  on March 14, 1999 and 1,430  $\text{mg C m}^{-2} \text{d}^{-1}$  on March 13, and declined to 651  $\text{mg C m}^{-2} \text{d}^{-1}$  on March 23, 2000 (Fig. 4B). After the bloom this value ranged from 76  $\text{mg C m}^{-2} \text{d}^{-1}$  in April to 291  $\text{mg C m}^{-2} \text{d}^{-1}$  in August, 1999. The depth-integrated primary productivity within the euphotic zone was 40–60% higher than that between 0 and 15 m except during the bloom period.

The primary production during the spring bloom was 17.6 and 56.5  $\text{g C m}^{-2}$  for 1999 and 2000, respectively. Annual primary production from April 20, 1999 to April 20, 2000 was 164  $\text{g C m}^{-2}$  within the euphotic zone. The production during the spring bloom accounted for 35% of the annual primary productivity.

$\text{NO}_3^-$  uptake rate was high during the spring bloom, at more than 40  $\text{nmol N L}^{-1} \text{h}^{-1}$  and  $\text{NH}_4^+$  and urea uptake rates were low, less than 12  $\text{nmol N L}^{-1} \text{h}^{-1}$  (Fig. 5A).

The  $\text{NO}_3^-$  uptake rate decreased to less than  $10 \text{ nmol N L}^{-1} \text{ h}^{-1}$  afterwards (Fig. 5A).  $\text{NH}_4^+$  and urea uptake rates increased simultaneously in May and August.

The  $f\text{-ratio}_{\text{wo}}$  was always higher than  $f\text{-ratio}_{\text{w}}$  during the study period because of the sizeable contribution of urea uptake to the total N uptake rates (Fig. 5B). During the bloom period,  $f\text{-ratio}_{\text{w}}$  was more than 0.7 in March, 2000 and 0.8 in March, 2001. After the bloom,  $f\text{-ratio}_{\text{w}}$  decreased sharply to less than 0.4 and remained consistently low until November 2000.

### 3.4 N uptake kinetic parameters

The  $\rho_{\text{max}}$  of  $\text{NO}_3^-$  during the spring bloom was high, at more than  $60 \text{ nmol N L}^{-1} \text{ h}^{-1}$ , while in other seasons this was below  $30 \text{ nmol N L}^{-1} \text{ h}^{-1}$  (Fig. 6A). The  $\rho_{\text{max}}$  of  $\text{NH}_4^+$  in August was  $50 \text{ nmol N L}^{-1} \text{ h}^{-1}$ , similar to that during the spring bloom. The  $\rho_{\text{max}}$  of urea was around  $20 \text{ nmol N L}^{-1} \text{ h}^{-1}$  between April and August, while it was not available during the spring bloom in 2000 due to an incomplete fit to the MM equation without data at  $10 \text{ } \mu\text{mol N L}^{-1}$ . The  $K_s$  (half saturation constant: the substrate concentration at  $0.5 \times \rho_{\text{max}}$ ) of  $\text{NO}_3^-$  was generally as low as  $0.5 \text{ } \mu\text{mol N L}^{-1}$  in spring, and a high value was observed in July and October at more than  $1 \text{ } \mu\text{mol N L}^{-1}$  (Fig. 6B). The  $K_s$  of  $\text{NH}_4^+$  during the spring bloom was high at  $5 \text{ } \mu\text{mol N L}^{-1}$  but decreased to less than  $0.6 \text{ } \mu\text{mol N L}^{-1}$  after the bloom. The  $K_s$  of urea was generally low, at less than  $0.6 \text{ } \mu\text{mol N L}^{-1}$ , except in April.

## 4. Discussion

### 4.1 Annual primary productivity

This is the first report on primary production in Funka Bay adopting the improved method (clean sampling and chemicals, and polycarbonate incubation bottles) by Fitzwater et al. (1982) and in situ mooring incubation for 24 h. The annual primary production in Funka Bay resulted in  $164 \text{ g C m}^{-2}$ , 40–60% higher than the previous estimates ( $100$  and  $118 \text{ g C m}^{-2}$ ) by Maita and Yanada (1978), and Odate and Maita (1988) using the standard  $^{14}\text{C}$  method (glass bottles and a Van-Dorn sampler attached to stainless hydrowire) without in situ incubation. The extent of underestimation of

primary productivity by the standard method in the bay was similar to that of Fitzwater et al. (1982). The reason for the underestimation was thought to be deleterious effects of toxic metal contamination, such as copper from hydrowire, sampler and glassware. In the present study no parallel comparison was made for the same sample between the clean method and the standard "non-clean" method. Thus, this may not be the only reason for the higher estimate in this study.

Another reason was a possibility of underestimation during the spring bloom due to the low sampling frequency. The spring bloom occurs between February and March in the bay, and the duration of the bloom is about a month (Kudo and Matsunaga, 1999). The primary production during the spring bloom in 2000 was  $56.5 \text{ g C m}^{-2}$ , equivalent to the difference from the previously reported annual primary production. As the previous estimates are based on monthly measurement, they may miss the precise estimation during the spring bloom.

It is generally recognized that the increase of human activities results in eutrophication in estuaries and coastal zones (Jickells, 1998; de Jonge et al., 2002; Montagna et al., 2002). The progress of eutrophication may lead to the increase in the primary production. However, the catchment area of Funka Bay is less urbanized and  $\text{NO}_3^-$  concentration in the bay before the spring bloom has not changed in the last four decades (Kudo unpubl.). It is unlikely that the higher primary production in this study is caused by an increase in nutrient availability due to eutrophication.

The maximum and minimum values for daily phytoplankton productivity in Funka Bay occur in summer and winter, respectively (Mann, 2000), ranging from ca.  $0.6$  to  $0.2 \text{ g C m}^{-2} \text{ d}^{-1}$ . Incorporating the newly obtained values in the present study (maximum:  $1.4 \text{ g C m}^{-2} \text{ d}^{-1}$ , Fig. 4), Funka Bay has the highest productivity among the embayments compiled (Mann, 2000), and the maximum value occurs in spring, not in summer. Mann (2000), in his book, defines embayments as places where the influence of river runoff and tidal mixing is small and the spring bloom develops according to the classic pattern. Cloern et al. (2014) recently reviewed primary production in the world's estuarine-coastal ecosystems. Funka Bay was classified as mesotrophic state based on the annual primary production in this study and their classification. Productivity during the spring bloom was as high as that in the eutrophic state, but in other seasons it was similar to that in the oligotrophic state because nutrients were depleted in the euphotic zone after the bloom. The overwhelming majority of primary productivity

measurements were reported from European (more than half from the Baltic region) and North American estuarine-coastal waters (Cloern et al., 2014). It is valuable to provide complete dataset for primary production from Asia for better understanding in the world's estuarine-coastal ecosystems.

#### 4.2 N sources fueling primary production

Dissolved inorganic N (DIN:  $\text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+$ ) consisted of more than 80% of  $\text{NO}_3^-$  before the bloom.  $\text{NO}_3^-$  presented at more than  $10 \mu\text{mol N L}^{-1}$ , decreased during the bloom and was depleted at the end of the bloom (Fig. 2C), followed by  $\text{Si(OH)}_4$  depletion (Kudo et al., 2000). After depletion of  $\text{NO}_3^-$ ,  $\text{NH}_4^+$  and urea became predominant for supporting primary production. Seasonal change in the uptake rate of urea showed as similar pattern as that of  $\text{NH}_4^+$  (Fig. 5A). High rates were observed in May and August, when the contribution of urea uptake to the total N uptake was 47 and 34%, respectively. High contributions of urea to the total N uptake were found at 60-80% in the plume region of Chesapeake Bay (Glibert et al., 1991) and up to 50% in the Oslofjord (Kristiansen, 1983). If urea uptake is not considered for the  $f$ -ratio calculation,  $f\text{-ratio}_{\text{wo}}$  was 50 to 93% higher than  $f\text{-ratio}_w$  from April to August (Fig. 5B). During the spring bloom period, this difference was less than 10% because the  $f\text{-ratio}_w$  was more than 0.8, and the major N species utilized was  $\text{NO}_3^-$ . The extent of this overestimation depends on the ecosystem type and was 6, 17, 24, 42 and 55% in upwelling, coastal, polar, oceanic and estuarine waters, respectively (Wafar et al., 1995). Our result during the spring bloom period was close to the value in upwelling waters, where the most utilized N source seems to be  $\text{NO}_3^-$ . In contrast, our non-bloom values fell within the range of oceanic and estuarine waters, where the regenerated nutrients predominate in the system.

Heterotrophic bacteria also take up inorganic N in case they are in insufficient supply of N from the DOM pool. Inorganic N uptake by heterotrophic bacteria accounts for an average of 78 and 32% of the total  $\text{NH}_4^+$  and  $\text{NO}_3^-$  uptake, respectively (Kirchman, 2000). If the GF/F filter (nominal retention size:  $0.7 \mu\text{m}$ ) retains heterotrophic bacteria that also take up  $^{15}\text{N}$ -labelled substrates, thus the estimates of the  $f$ -ratio would be biased. Fouilland et al. (2007) estimated 80% of the total free-living bacteria was retained on Whatman GF/F filters. They also reported that heterotrophic

bacteria accounted for 44-78% of the total  $\text{NH}_4^+$  and  $\text{NO}_3^-$  uptake during a low phytoplankton biomass ( $\text{Chl } a < 2 \mu\text{g L}^{-1}$ ) period. However, phytoplankton accounted for a larger fraction (58–95%) of urea uptake than heterotrophic bacteria during the same period. Although the contribution of heterotrophic bacteria to N uptake was not examined in the present study, when phytoplankton biomass was low after the spring bloom, it seemed that some of the uptake of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  would be attributed to heterotrophic bacteria. Fouilland et al. (2007) reported that when  $\text{Chl } a$  was  $>2 \mu\text{g L}^{-1}$   $\text{NO}_3^-$  uptake accounted for 38–58% of the total N uptake, and phytoplankton uptake was responsible for this. Most of the studies reported on  $f$ -ratio did not consider heterotrophic bacteria, thus the further studies are needed to elucidate for the contribution of heterotrophic bacteria on N uptake.

There are several potential sources of urea in seawater. The concentration of urea was measured in seven rivers flowing into the bay. The concentration was less than  $0.5 \mu\text{mol N L}^{-1}$  (Kudo, unpubl.), which was less than that in the bay. It has been reported that zooplankton excrete urea (Chen and Cheng, 1993; Conover and Gustavson, 1999; Dagg et al., 1980). In the Arctic, copepods excrete urea more than  $\text{NH}_4^+$  (Conover and Gustavson, 1999). Dagg et al. (1980) reported that excretion of urea is less than that of  $\text{NH}_4^+$  in the Peru upwelling regions. In an incubation experiment, we found copepods collected in the bay excreted urea at a similar rate of  $\text{NH}_4^+$  (Kudo, in prep.). Therefore the important source of urea in the bay seemed excretion by zooplankton, not river water. Thus, urea could be considered as regenerated N like  $\text{NH}_4^+$  in the bay.

#### 4.3 N uptake kinetic parameters

The absolute uptake rate of  $\text{NO}_3^-$  ( $\rho_{\text{NO}_3^-}$ ) was the highest at the peak of the spring bloom in 2000 (Fig. 5A) and close to the  $\rho_{\text{max}}$  (Fig. 6A), indicating substrate saturation for uptake. During summer,  $\rho_{\text{NO}_3^-}$  was less than a half of the  $\rho_{\text{max}}$ . To compare  $\rho_{\text{max}}$  with the other areas,  $\rho_{\text{max}}$  was normalized by  $\text{Chl } a$  ( $\rho_{\text{max}}/\text{Chl } a$ ). The  $\rho_{\text{max}}/\text{Chl } a$  for  $\text{NO}_3^-$  ranged from 5 to 12  $\text{nmol N } \mu\text{g Chl } a^{-1} \text{ h}^{-1}$  during the bloom and from 21 to 90  $\text{nmol N } \mu\text{g Chl } a^{-1} \text{ h}^{-1}$  in summer (Fig. 7). Harrison et al. (1996) reported that  $\rho_{\text{max}}/\text{Chl } a$  for  $\text{NO}_3^-$  has a temperature dependency in the surface water of the North Atlantic Ocean, ranging from 2 to 10  $\text{nmol N } \mu\text{g Chl } a^{-1} \text{ h}^{-1}$  at  $< 10^\circ\text{C}$  and from 10 to 50  $\text{nmol N } \mu\text{g Chl } a^{-1} \text{ h}^{-1}$  at  $> 10^\circ\text{C}$ . The value for the bloom in this study fell within the

range at  $< 10^{\circ}\text{C}$ . The summer value was within a similar range to the values reported. Thus, the observed change in  $\rho_{\text{max}}/\text{Chl } a$  for  $\text{NO}_3^-$  was partly explained by the temperature dependency. Small phytoplankton has a higher uptake rate than large phytoplankton (Koike et al., 1986; Probyn, 1990). Shiomoto et al. (1994) also discussed this difference in  $\rho_{\text{max}}/\text{Chl } a$  for  $\text{NO}_3^-$  in conjunction with size composition of phytoplankton assemblages in the summer, i.e. when a smaller fraction is dominant in summer of off-shore region of Oyashio. In Funka Bay, size composition of phytoplankton changed from micro-sized in spring to nano-sized in summer (Odate and Imai, 2003). Thus, the change in size composition may also explain the seasonal change in  $\rho_{\text{max}}/\text{Chl } a$  for  $\text{NO}_3^-$  in the bay.

The  $K_s$  for  $\text{NO}_3^-$  was generally  $< 1 \mu\text{mol N L}^{-1}$ , which agreed well with the values reported in the off-shore region of Oyashio (Shiomoto et al., 1994). The  $K_s$  for  $\text{NH}_4^+$  was as low as  $1 \mu\text{mol N L}^{-1}$  except in the spring bloom, when the high value of  $5 \mu\text{mol N L}^{-1}$  was obtained. High  $K_s$  for  $\text{NH}_4^+$  at  $> 5 \mu\text{mol N L}^{-1}$  was reported for neritic diatoms (Eppley et al., 1969). This explains the higher  $K_s$  for  $\text{NH}_4^+$  during the spring bloom because the bloom consisted of neritic diatoms in Funka Bay (Shimada, 2000).

## 5. Conclusion

Primary productivity was re-evaluated using the clean technique and 24 h in situ incubation in Funka Bay from 1999 to 2001. The new estimate for annual primary production was  $164 \text{ g C m}^{-2}$ , which was 40-60% higher than previously reported estimates by the standard method. This higher estimate may be explained by the clean technique and more frequent measurements during the spring bloom. Local eutrophication is, however, unlikely to play a role in Funka bay. Based on its annual primary production, the bay was classified as mesotrophic (Cloern et al., 2014). Considering N uptake by phytoplankton, urea uptake rate was on a similar order of magnitude than that of  $\text{NH}_4^+$  after the spring bloom. Thus, urea contributed significantly to N uptake during non-bloom periods when the regenerated production was predominant. Urea should be considered as a nutrient when N nutrient utilization or  $f$ -ratio is evaluated.

## Acknowledgements

We thank the captains, officers and crew of the R/V 'Ushio Maru' and T/V 'Oshoro Maru' for their assistance at sampling. We express special thanks to Drs. Mitsuru Yanada, Shuhei Ban, Yutaka Isoda and Choon Weng Lee for their valuable comments and assistance. We also thank Dr. Masao Minagawa for providing the opportunity to use mass spectrometer. This research was partially supported by a Grant-in-Aid for Scientific Research (B) from JSPS (12480145).

## References

- Aya, F. A., Hidaka, Y., Kudo, I. 2013. Clearance rates and ingestion efficiency of the Japanese scallop *Patinopecten yessoensis*. *Plankton Benthos Research*, 8, 134-140.
- Bronk, D. A., Glibert, P. M., Ward, B. B. 1994. Nitrogen uptake, dissolved organic nitrogen release, and new production. *Science*, 265, 183-184.
- Buck, C. M., Wilkerson, F. P., Parker, A. E., Dugdale, R. C. 2014. The influence of coastal nutrients on phytoplankton productivity in a shallow low inflow estuary, Drakes Estero, California (USA). *Estuaries and Coasts*, 37, 847-863.
- Chen, J. C., Cheng, S. Y. 1993. Urea excretion by *Penaeus Japonicus* Bate exposed to different concentrations of ambient ammonia. *Journal of Experimental Marine Biology and Ecology*, 173, 1-9.
- Cloern, J. E., Foster, S. Q., Klecker, A. E. 2014. Phytoplankton primary production in the world's estuarine-coastal ecosystems. *Biogeosciences*, 11, 2477-2501.
- Conover, J. R., Gustavson, K. R. 1999. Sources of urea in arctic seas: zooplankton metabolism. *Marine Ecology Progress Series*, 179, 41-54.
- Dagg, M., Cowles, T., Whitledge, T., Smith, S., Howe, S., Judkins, D. 1980. Grazing and excretion by zooplankton in the Peru upwelling system during April 1997. *Deep-Sea Research*, 27, 43-59.
- de Jonge, V. N., Elliott, M., Orive, E. 2002. Causes, historical development, effects and future challenges of a common environmental problem: eutrophication. *Hydrobiologia*, 475/476, 1-19.
- Dugdale, R. C., Goering, J. J. 1967. Uptake of new and regenerated forms of nitrogen in primary production. *Limnology and Oceanography*, 12, 196-206.
- Dugdale, R. C., Wilkerson, F. P. 1986. The use of <sup>15</sup>N to measure nitrogen uptake in eutrophic ocean; experimental consideration. *Limnology and Oceanography*, 31, 673-689.
- Dugdale, R. C., Wilkerson, F. P., Barber, R. T., Chavez, F. P. 1992. Estimating new production in the equatorial Pacific Ocean at 150°W. *Journal of Geophysical Research*, 29, 681-686.
- Eppley, R. W., Peterson, B. J. 1979. Particulate organic matter flux and planktonic new production in the deep ocean. *Nature*, 282, 677-680.

- Eppley, R. W., Rogers, J. N., McCarthy, J. J. 1969. Half-saturation constants for uptake of nitrate and ammonium by marine phytoplankton. *Limnology and Oceanography*, 14, 912-920.
- Fitzwater, S. E., Knauer, G. A., Martin, J. H. 1982. Metal contamination and its effect on primary production measurements. *Limnology and Oceanography*, 27, 544-551.
- Fouilland, E., Gosselin, M., Rivkin, R.B., Vasseur, C., Msotajir, B. 2007. Nitrogen uptake by heterotrophic bacteria and phytoplankton in Arctic surface waters. *Journal of Plankton Research*, 29, 369-376.
- Glibert, P. M., Garside, C., Fuhrman, J. A., Roman, M. R. 1991. Time-dependent coupling of inorganic and organic nitrogen uptake in the plume of Chesapeake Bay estuary and its regulation by large heterotrophs. *Limnology and Oceanography*, 36, 895-909.
- Glibert, P. M., Lipschultz, F., McCarthy, J. J., Altabet, M. A. 1982. Isotope dilution models of uptake and remineralization of  $\text{NH}_4$  by marine plankton. *Limnology and Oceanography*, 27, 639-650.
- Glibert, P. M., McCarthy, J. J. 1984. Uptake and assimilation of ammonium and nitrate by phytoplankton: indices of nutritional status. *Journal of Plankton Research*, 6, 677-697.
- Grasshoff, K., Kremling, K., Ehrhardt M. 1999. *Methods of seawater analysis*. Wiley-VCH, Weinheim, 600 pp.
- Hama, T., Miyazaki, T., Ogawa, Y., Iwakuma, T., Takahashi, M., Osuki, A., Ichimura, S. 1983. Measurement of photosynthetic production of a marine phytoplankton population using stable  $^{13}\text{C}$ . *Marine Biology*, 73, 31-36.
- Harrison, W. G., Harris, L. R., Irwin, B. D. 1996. The kinetics of nitrogen utilization in the oceanic mixed layer: Nitrate and ammonium interactions at nanomolar concentrations. *Limnology and Oceanography*, 41, 16-32.
- Jickells, T. D. 1998. Nutrient biogeochemistry of the coastal zone. *Science*, 281, 217-222.
- Joint, I., Rees, A. P., Malcolm, E., Woodward, S. 2001. Primary production and nutrient assimilation in the Iberian upwelling in August 1998. *Progress in Oceanography*, 51, 303-320.
- Kirchman, D. L. 2000. Uptake and regeneration of inorganic nutrients by marine

- heterotrophic bacteria. In Kirchman, D. L. (ed.), *Microbial Ecology of the Oceans*. John Wiley and Sons, New York, pp. 261-288.
- Koike, I., Holm-Hansen, O., Biggs, D. C. 1986. Inorganic nitrogen metabolism by Antarctic phytoplankton with special reference to ammonium cycling. *Marine Ecology Progress Series*, 30, 105-116.
- Kristiansen, S. 1983. Urea as a nitrogen source for phytoplankton in the Oslofjord. *Marine Biology*, 74, 17-24.
- Kudo, I., Matsunaga, K. 1999. Environmental factors affecting the occurrence and production of the spring phytoplankton bloom in Funka Bay, Japan. *Journal of Oceanography*, 55, 503-513.
- Kudo, I., Yoshimura, T., Lee, T. W., Yanada, M., Maita, Y. 2007. Nutrient regeneration at bottom after a massive spring bloom in a subarctic coastal environment, Funka Bay, Japan. *Journal of Oceanography*, 63, 791-801.
- Kudo, I., Yoshimura, T., Yanada, M., Matsunaga, K. 2000. Exhaustion of nitrate terminates a phytoplankton bloom in Funka Bay, Japan: change in  $\text{SiO}_4:\text{NO}_3$  consumption rate during the bloom. *Marine Ecology Progress Series*, **193**, 45-51.
- MacDonald, B. A., Bricelj, V. M., Shumway, S. E. 2006. Physiology: Energy acquisition and utilisation. *Scallops: Biology, Ecology and Aquaculture*. Shumway, S.E., Parsons, G.J. Eds. Elsevier. Amsterdam, p417-492.
- Maita, Y., Yanada, M. 1978. Particulate protein in coastal waters, with special reference to seasonal variation. *Marine Biology*, 44, 329-336.
- Mann, K. H. 2000. *Ecology of Coastal Waters*. 2nd ed. Blackwell Science, Malden. p 406.
- McCarthy, J. J. 1972. The uptake of urea by natural populations of marine phytoplankton. *Limnology and Oceanography*, 17: 738-748.
- McCarthy, J. J., Taylor, W. R., Taft, J. L. 1977. Nitrogenous nutrition of the plankton in the Chesapeake Bay. 1. Nutrient availability and phytoplankton preferences. *Limnology and Oceanography*, 22, 996-1011.
- Miyake, H., Yanada, M., Nishi, T., Hoshizawa, K. 1998. Short-time variation in low trophic level productivity and hydrographic conditions in Funka Bay. *Memoir of Faculty of Fisheries, Hokkaido University*, 45, 36-41.
- Montagna, P. A., Alber, M., Doering, P., Connor, M. S. 2002. Freshwater inflow: science, policy, management. *Estuaries*, 25, 1243-1245.

- Mulvenna, F. P. Savidge, G. 1992. A modified manual method for the determination of urea in seawater using diacetylmonoxide reagent. *Coastal and Shelf Science*, 34, 429-438.
- Odate, T., Maita, Y. 1988. Seasonal changes in the biomass of zooplankton and their food requirement in Funka Bay. *Journal of the Oceanographical Society of Japan*, 44, 228-234.
- Odate, T., Imai, K. 2003. Seasonal variation in chlorophyll-specific growth and microzooplankton grazing of phytoplankton in Japanese coastal water. *Journal of Plankton Research*, 25, 1497-1505.
- Ohtani, K. 1971. Studies on the change of the hydrographic conditions in the Funka Bay. II. Characteristics of the water occupying the Funka Bay. *Bulletin of Faculty of Fisheries Hokkaido University*, 22, 58-66. (in Japanese with English abstract)
- Parsons, T. R., Maita, Y., Lalli, C. 1974. *A Manual of Chemical and Biological Methods for Seawater Analysis*. Pergamon Press, New York, 173 pp.
- Probyn, T. 1990. Size-fractionated measurements of nitrogen uptake in aged upwelled waters: Implications for pelagic food webs. *Limnology and Oceanography*, 35, 202-210.
- Ryther, J. H., Dunstar, W. M. 1971. Nitrogen, phosphorus and eutrophication in the coastal marine environment. *Science*, 171, 1008-1013.
- Shimada, H. 2000. Seasonal changes of phytoplankton species composition in Funka Bay. *Bulletin on Coastal Oceanography*, 38, 15-22.
- Shiomoto, A., Sasaki, K., Shimoda, T., Matsumura, S. 1994. Kinetics of nitrate and ammonium uptake by the natural populations of marine phytoplankton in the surface water of the Oyashio region during spring and summer. *Journal of Oceanography*, 50, 515-529.
- Steeman Nielsen, E. 1952. The use of radioactive carbon ( $C^{14}$ ) for measuring organic production in the sea. *Journal de Conseil Permanent International Exploration de la Mer*, 18, 007-140.
- Suzuki, R., Ishimaru, T. 1990. An improved method for the determination of phytoplankton chlorophyll using *N,N*-dimethylfomamide. *Journal of Oceanographical Society of Japan*, 46, 190-194.
- Thomas, W. H. 1970. On nitrogen deficiency in tropical Pacific oceanic phytoplankton: photosynthetic parameters in poor and rich water. *Limnology and*

Oceanography, 15, 380-385.

Torres-Valdes, S., Purdie, D. A. 2006. Nitrogen removal by phytoplankton uptake through a temperate non-turbid estuary. *Estuarine, Coastal and Shelf Science*, 70, 473-486.

Twomey, L. J., Piehler, M. F., Pearl, H. W. 2005. Phytoplankton uptake of ammonium, nitrate and urea in the Neuse River Estuary, NC, USA. *Hydrobiologia*, 533, 123-134.

Wafar, M. V. M., Le Corre, P., L'Helguen, S. 1995. *f*-Ratios calculated with and without urea uptake in nitrogen uptake by phytoplankton. *Deep-Sea Research I*, 42, 1669-1674.

## Figure legends

Fig. 1 Sampling station in Funka Bay.

Fig. 2 Seasonal change in temperature ( $^{\circ}\text{C}$ ) (A), salinity (B), Chl *a* ( $\mu\text{g L}^{-1}$ ) (C),  $\text{NO}_3^-$  ( $\mu\text{mol N L}^{-1}$ )(D),  $\text{NH}_4^+$  ( $\mu\text{mol N L}^{-1}$ ) (E) and urea ( $\mu\text{mol N L}^{-1}$ ) (F).

Fig.3 Seasonal change in primary productivity ( $\text{mg C m}^{-3} \text{d}^{-1}$ ).

Fig.4 Seasonal change in depth of 1% surface irradiance ( $I_0$ ) (A). Seasonal change in integrated primary productivity ( $\text{mg C m}^{-2} \text{d}^{-1}$ )(B). Integration was made between 0 and 15 m and between 0 and 1%  $I_0$ .

Fig. 5 Seasonal change in the absolute uptake rates ( $\rho$ ) of  $\text{NO}_3^-$ ,  $\text{NH}_4^+$  and urea at 5 m (A). Seasonal change in  $f\text{-ratio}_w$  and  $f\text{-ratio}_{w0}$  at 5 m. The  $f\text{-ratio}_w$  and  $f\text{-ratio}_{w0}$  designate  $f\text{-ratio}$  considered urea uptake rate and that not considered it, respectively (B).

Fig. 6 Seasonal change in  $\rho_{\text{max}}$  (A) and  $K_s$  (B) for  $\text{NO}_3^-$ ,  $\text{NH}_4^+$  and urea at 5 m.

Fig. 7 Seasonal change in Chl *a* normalized  $\rho_{\text{max}}$  for  $\text{NO}_3^-$ ,  $\text{NH}_4^+$  and urea at 5 m.



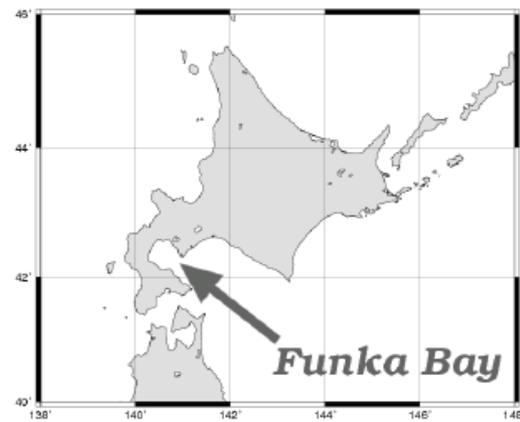
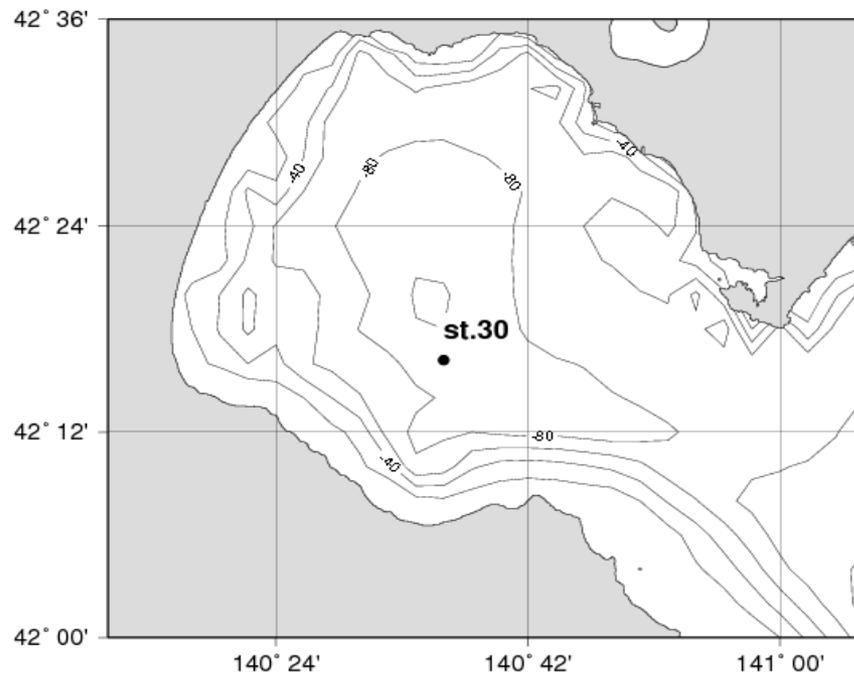


Fig. 1 Kudo et al.

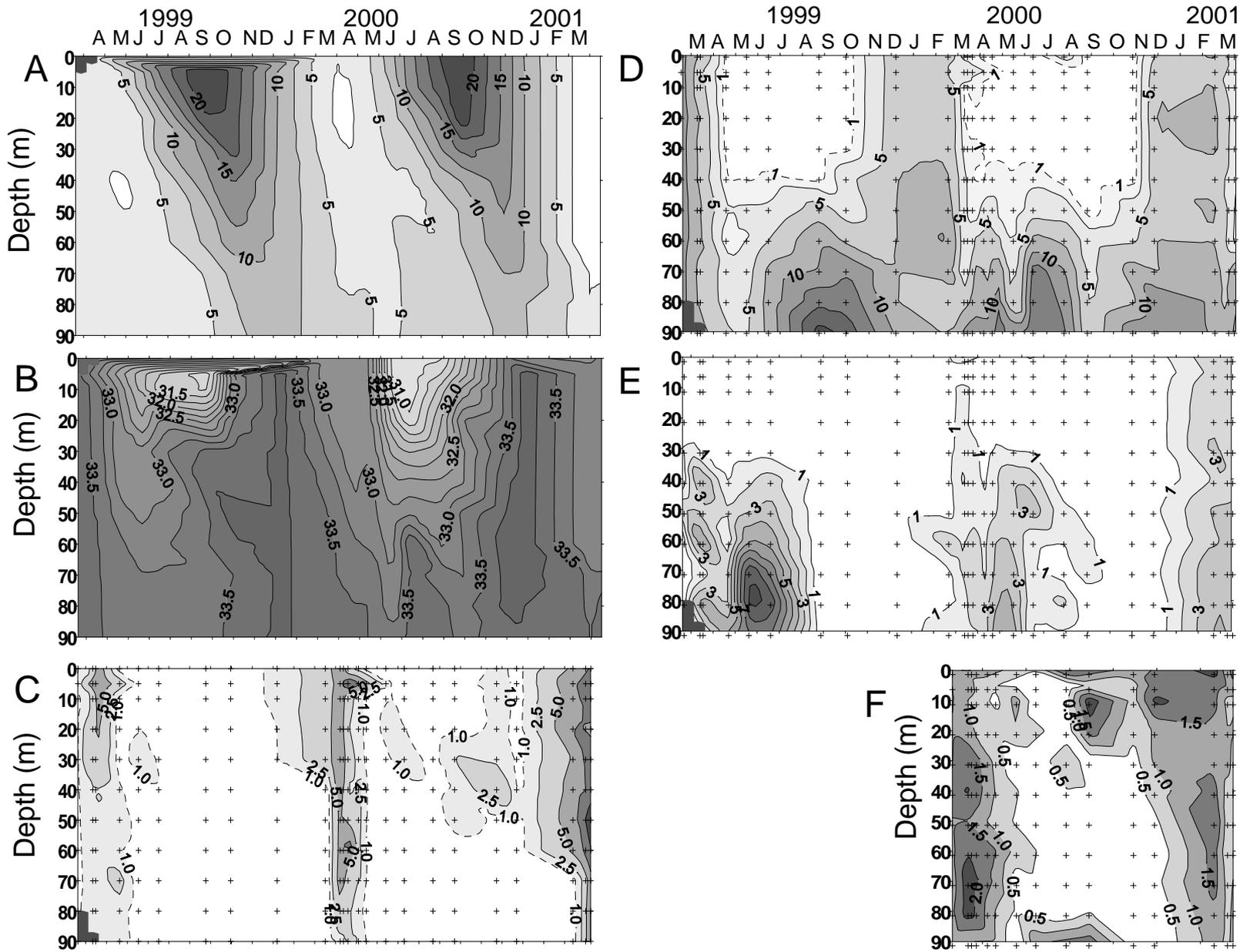


Fig. 2 Kudo et al.

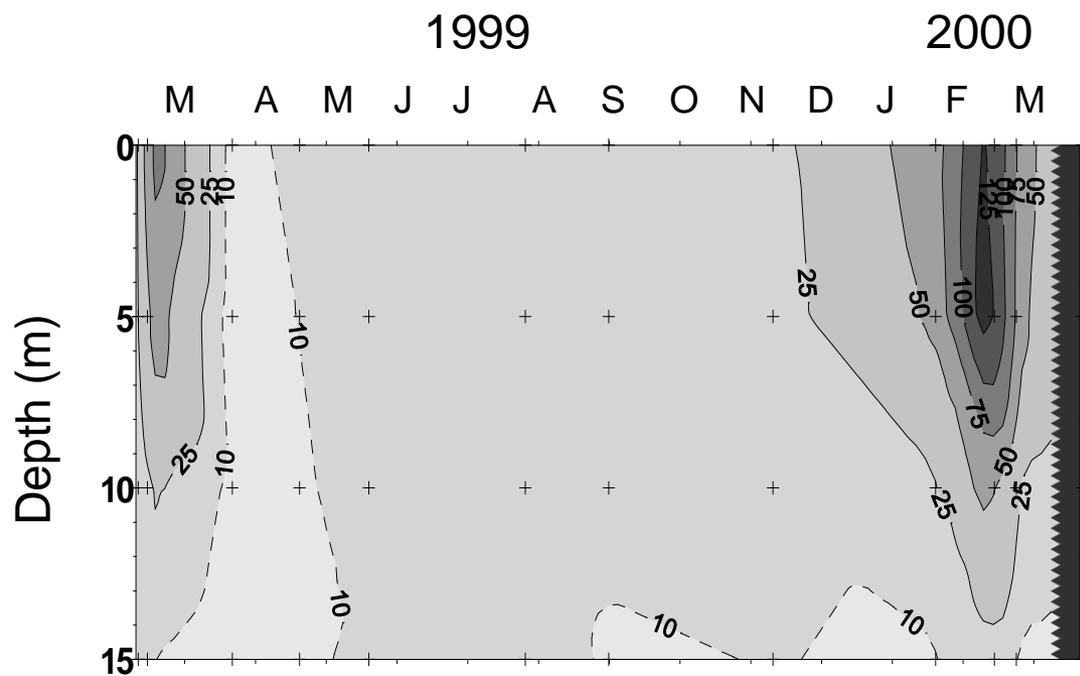


Fig. 3 Kudo et al.

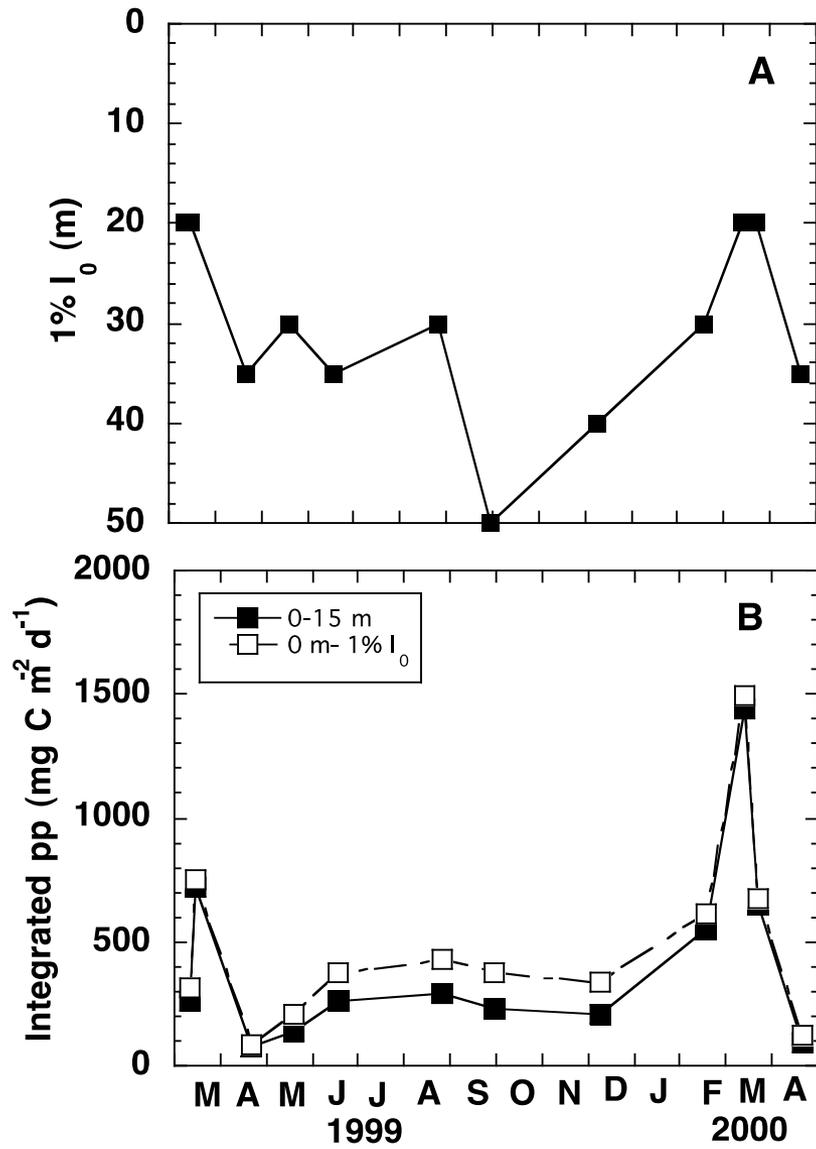


Fig. 4 Kudo et al.

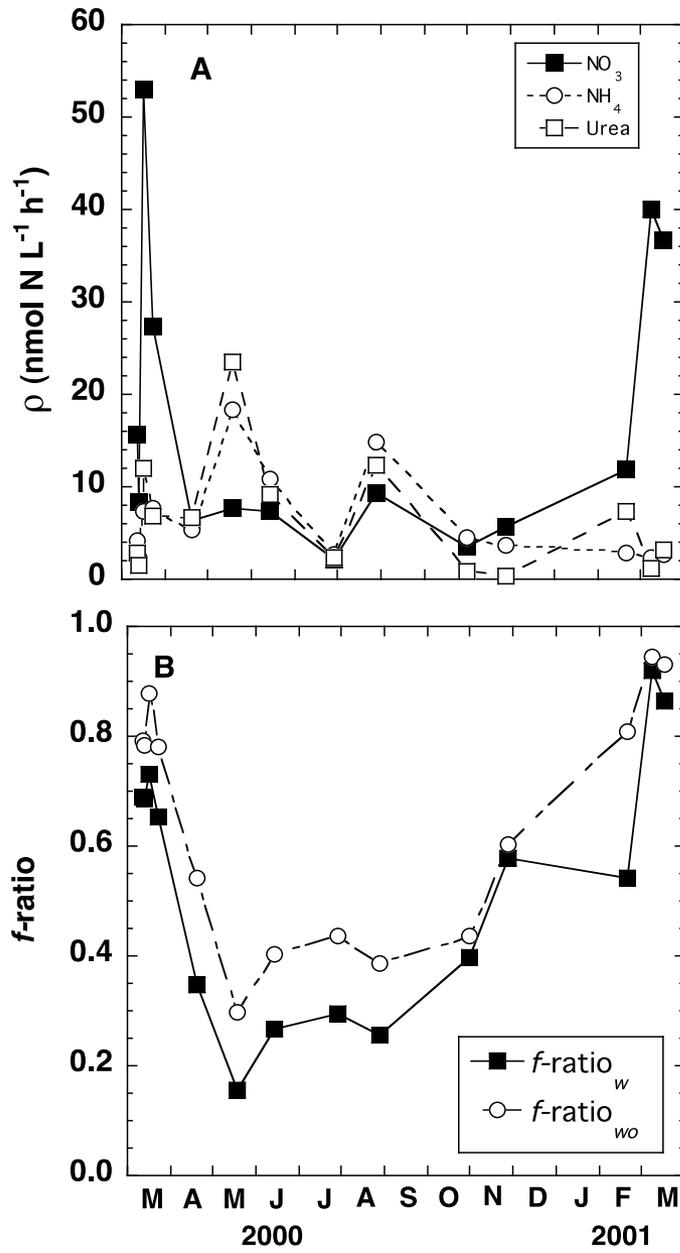


Fig. 5 Kudo et al.

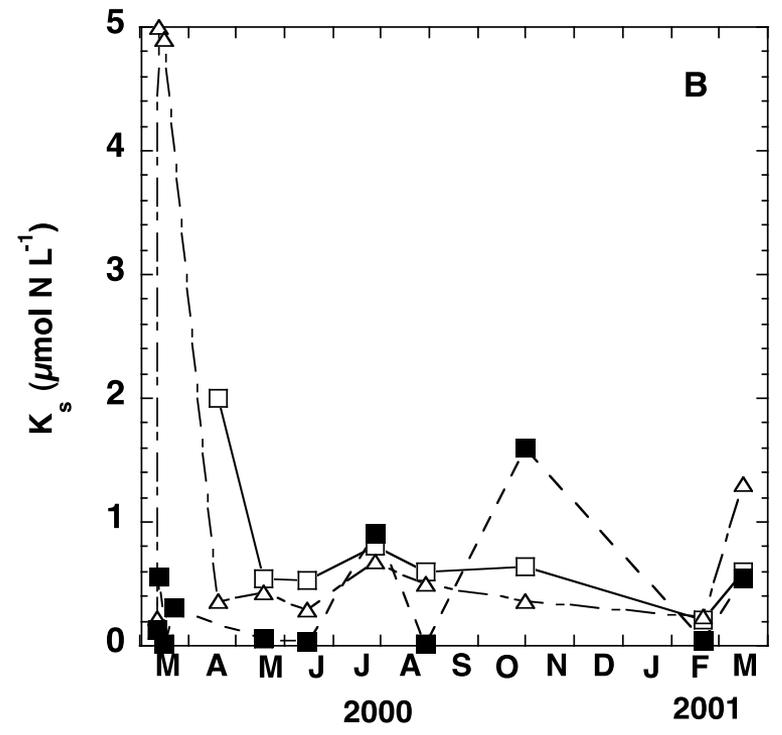
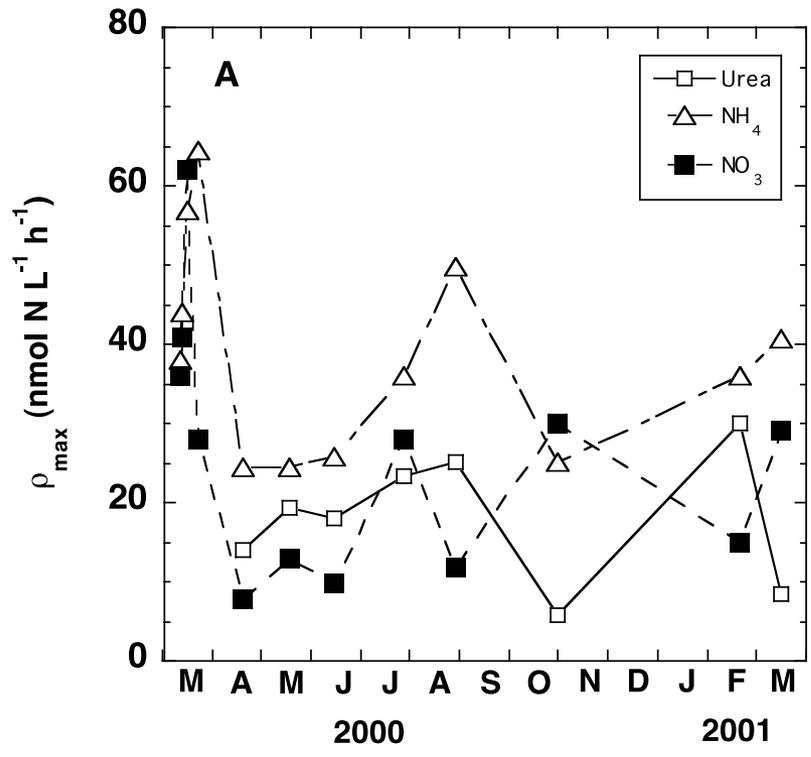


Fig. 6 Kudo et al.

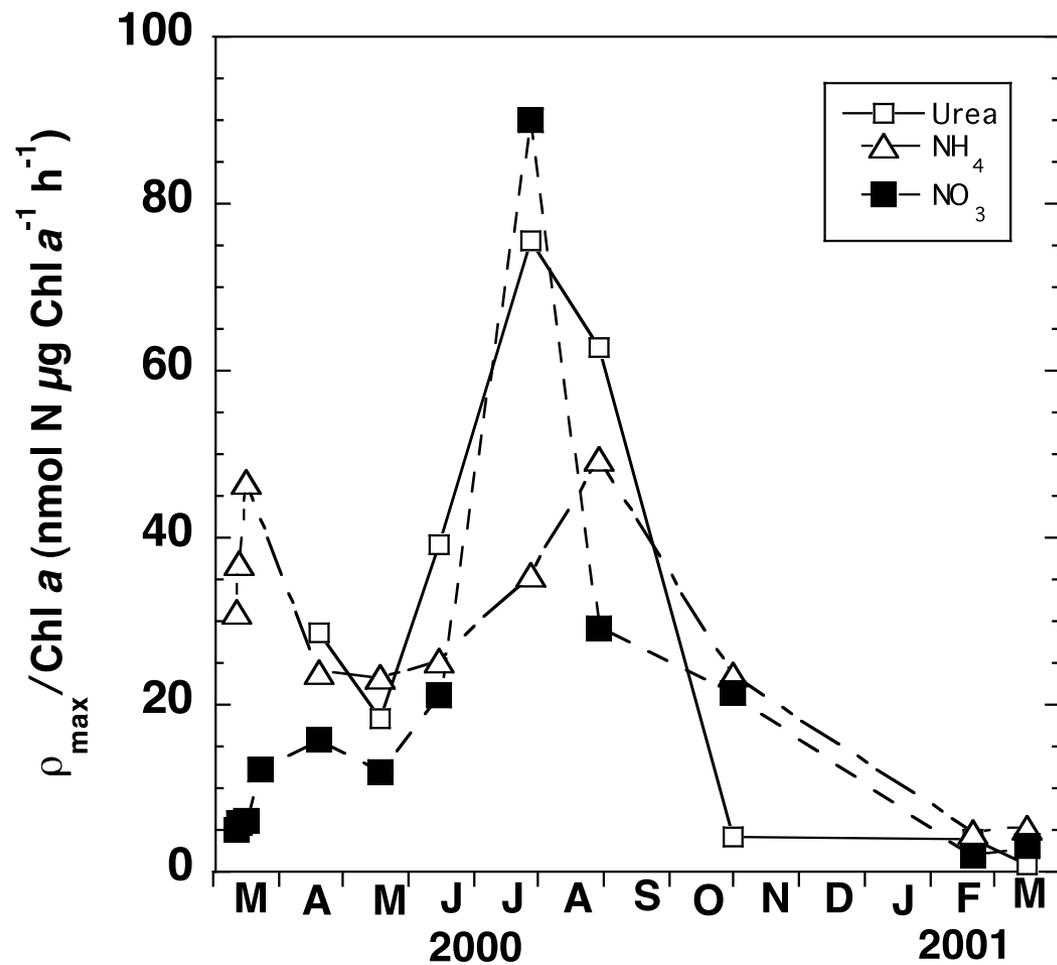


Fig. 7 Kudo et al.