Estuarine, Coastal and Shelf Science 158 (2015) 12–19

Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

journal homepage: www.elsevier.com/locate/ecss

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

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ARTICLE INFO

*Article history:*
Received 2 September 2014
Accepted 8 March 2015
Available online 14 March 2015

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

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 $^{13}C$ 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 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 the higher rates. The production during the spring bloom was 56.5 g C $m^{-2}$, accounting for 35% of the annual production. The maximum daily productivity occurred in the bloom at 1.4 g C $m^{-2}$ $d^{-1}$, 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 nmol N $L^{-1}$ $h^{-1}$ 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.



## 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 ($NH_4^+$) 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 ($NO_3^-$) and $NH_4^+$ 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 $NH_4^+$ by urease, an enzyme produced by phytoplankton and bacteria, it is

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http://dx.doi.org/10.1016/j.ecss.2015.03.012
0272-7714/

utilized by phytoplankton similarly to inorganic N (McCarthy, 1972). Eppley and Peterson (1979) considered urea as regenerated N similar to $NH_4^+$. 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 $2.3 \times 10^3$ 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 $NO_3^-$, and then silicic acid ($Si(OH)_4$) is also depleted by the enhanced consumption of $Si(OH)_4$ 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., 1998). 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$^{-2}$, respectively, based on monthly observation and the standard $^{14}$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 $^{14}$C method (clean sampling and chemicals and polycarbonate incubation bottles) provided up to three times higher primary productivity compared to the conventional standard $^{14}$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 ($NO_3^-$, $NH_4^+$ 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.

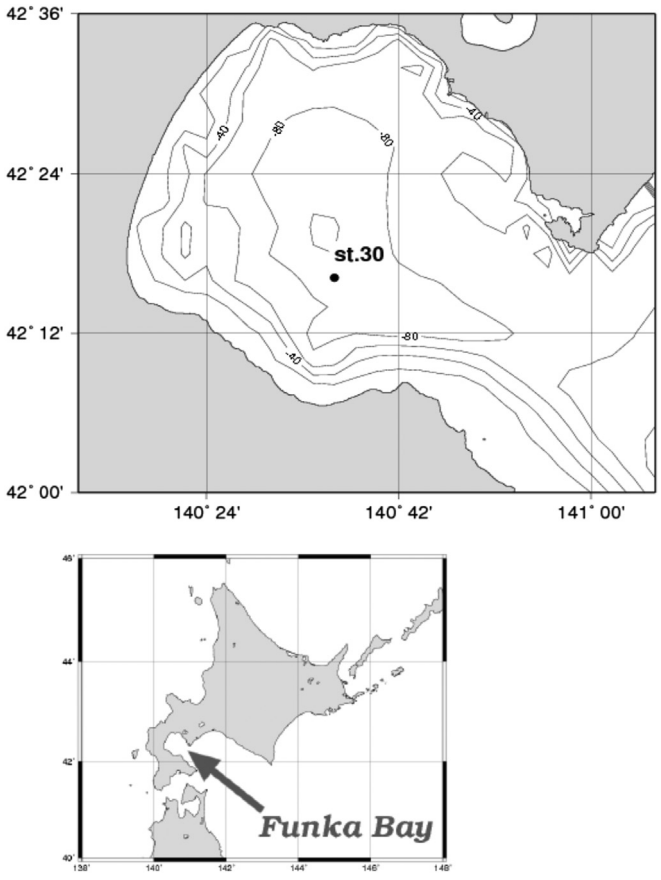


**Fig. 1.** Sampling station in Funka Bay.

## 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 biweekly) 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 $NO_3^-$, nitrite ($NO_2^-$), $NH_4^+$, phosphate ($PO_4^{3-}$) and $Si(OH)_4$ 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 μmol L$^{-1}$ based on three times the standard deviation of the lowest concentration of samples. In this paper concentration of $NO_3^-$, $NO_2^-$, $NH_4^+$ and urea was expressed in μmol N L$^{-1}$ because one mole of urea produces two moles of $NH_4^+$ after hydrolysis by urease.