

Release of dissolved organic nitrogen by a planktonic community in Akkeshi Bay

Toru Hasegawa^{1,*}, Isao Koike¹, Hiroshi Mukai²

¹Ocean Research Institute, University of Tokyo, 1-15-1, Minamidai, Nakano, Tokyo 164-8639, Japan

²Akkeshi Marine Biological Station, Hokkaido University Akkeshi, Hokkaido 088-1113, Japan

ABSTRACT: We applied conventional incubation experiments combined with a ¹⁵N-tracer technique to evaluate the relative contributions of phytoplankton, micrograzers and copepods to the release of dissolved organic nitrogen (DON) or dissolved nitrogen (DN) in coastal waters from Akkeshi Bay, eastern Hokkaido, Japan. From March to November, water temperature ranged from –1 to 17°C, and chl *a* concentrations showed about 5-fold change (from 2.6 to 12 µg l⁻¹). The calculated rates of DON release by phytoplankton (18 to 28 nmol l⁻¹ d⁻¹) were comparable to those of DN release by copepods (18 to 67 nmol l⁻¹ d⁻¹). However, the DON release rates by micrograzers (140 to 350 nmol l⁻¹ d⁻¹) greatly exceeded those of the other planktonic assemblages. DON release by micrograzers was well coupled to DON uptake by bacteria, indicating that there was a significant nitrogen flux from phytoplankton to bacteria via micrograzers. DON supply in the upper layer would activate microbial food webs and consequently relieve the short supply of dissolved inorganic nitrogen for phytoplankton during the stratified season.

KEY WORDS: DON · ¹⁵N-tracer · Plankton

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INTRODUCTION

In general, primary production in many coastal waters is believed to be nitrogen-limited (Dugdale & Goering 1967, Ryther & Dunstan 1971, Ward et al. 1989, Oviatt et al. 1995). Consequently, the nitrogen cycle has been regarded as one of the central issues for studies on coastal ecosystems. However, one of the components of nitrogen, i.e., dissolved organic nitrogen (DON), has been excluded from the traditional schema of the marine nitrogen cycle (Williams 1995). DON often occupies the largest pool of combined nitrogen in marine environments (Sharp 1983), and previous studies suggest that DON is a potentially important nitrogen source for bacteria and phytoplankton populations (Jackson & Williams 1985, Tupas & Koike 1991, Bronk & Glibert 1993a). Evaluation of DON dynamics in the upper ocean, however, has been ham-

pered by the nature of DON, e.g., the complexity of its composition and high background concentrations.

In planktonic communities, the majority of organic compounds including DON originates from phytoplankton. While bacterial demand for dissolved organic carbon (DOC) was reported to be as much as 10 to 50% of daily primary production (Azam et al. 1983, Cole et al. 1988), DOC release by healthy phytoplankton is generally under 10% of the production (Sharp 1977, Smith et al. 1977, Mague et al. 1980, Larsson & Hagström 1982). This discrepancy implied that heterotrophic grazers played a significant role in DOC release (Jumars et al. 1989). It was also suggested that the grazing process was potentially of importance in DON release (Bronk & Glibert 1993b, Bronk et al. 1998, Bronk & Ward 1999). However, the relative contributions of phytoplankton and heterotrophs (i.e., micro- and macrozooplankton) to DOC and DON release in field conditions have not yet been evaluated.

The aims of this study were (1) to experimentally estimate the relative contributions to DON release caused

*E-mail: thasegaw@ori.u-tokyo.ac.jp

by phytoplankton, micrograzers, and copepods at a subarctic embayment and (2) to discuss the role of DON in coastal marine nitrogen flux. Our results showed that the rates of DON release by micrograzers greatly exceeded those of the other planktonic assemblages, and the DON they supply in the upper layer would be important for relieving the short supply of dissolved inorganic nitrogen (DIN) available to primary producers.

MATERIALS AND METHODS

Akkeshi Bay is located on the eastern Pacific coast of Hokkaido, Japan. All samplings were done at a fixed station (water depth 13 m: 43° 01' N, 144° 52' E) in the bay. Temperature and salinity were measured using a YSI salinometer (Model 33 S-C-T meter) at 1 m intervals from the surface to a depth of 12 m. In this study, we defined organisms that passed through a 94 µm net as microplankton. Because 91 to 100% of chlorophyll *a* (chl *a*) in whole seawater was contained in the <94 µm fraction (Hasegawa unpubl.), our results represented the majority of phytoplankton activity. However, micrograzers' activity on nitrogen flux might be underestimated, since the general definition of micrograzer was <200 µm (Sieburth et al. 1978).

Incubation experiments (<94 µm assemblage). Incubation experiments with size-fractionated seawater (<94 µm) were carried out on 9 March, 25 May, 29 June, 24 August, and 10 November 1998. Conditions of incubation are shown in Table 1. NH₄⁺ regeneration rates were determined by the ¹⁵N-isotope dilution method (Blackburn 1979, Caperon et al. 1979) and NH₄⁺ uptake rates were determined after Glibert et al. (1982). Further, DO¹⁵N release was measured on 25 May, 29 June and 24 August 1998 and expressed as percent extracellular release (PER: percentage of ¹⁵N-labeled DON to ¹⁵N-labeled DON plus ¹⁵N-labeled particulate organic nitrogen [PON], Hasegawa et al. 2000c). Details of the methods used to measure DON release are described in Hasegawa et al. (2000a,c).

The dilution method with ¹⁵N-tracer. DON release rates mediated by micrograzers were estimated by the

dilution method (Landry & Hassett 1982) using pre-incubated seawater with ¹⁵NH₄⁺ to label phytoplankton. After 2 or 3 d pre-incubation, the dilution experiments were performed on 12 March, 28 May, 2 July, 27 August and 12 November 1998 (Hasegawa et al. 2000b). A series of diluted seawater was incubated for 6 (in August) or 12 h (other months) in the dark with the addition of excess cold-NH₄⁺ (ca 8 µM). The relative contributions to DO¹⁵N release by the micrograzers and phytoplankton were tested statistically. To calculate DON release rates by micrograzers, we assumed that the ¹⁵N atom% of DON released is the same as that for PON. Statistical protocols basically followed those described by Andersen et al. (1991) for the estimation of N and P sources contributing to phytoplankton growth and are fully described in Hasegawa et al. (2000b).

Copepod incubation experiments with ¹⁵N-tracer. To estimate dissolved nitrogen (DN) release rates by copepods, incubation experiments were carried out on 12 March, 28 May, 2 July, 27 August and 12 November 1998 using pre-incubated seawater (2 or 3 d) with ¹⁵NH₄⁺ to label phytoplankton. Seawater with copepods (feeding) and without copepods (control) was incubated for 6 (in August) or 12 h (other months) under dark conditions with the addition of excess cold-NH₄⁺ (ca 8 µM). Copepods used in these experiments were at late copepodite stages of the species dominating the macrozooplankton at the sampling time. D¹⁵N release rates by copepods were measured by determining the difference between the ¹⁵N disappearance from PON fraction and the ¹⁵N appearance in the copepods' bodies. To calculate DN release rates by copepods, we assumed that the ¹⁵N atom% of DN released is the same as that for PON. Copepods' NH₄⁺ excretion rates were obtained by measuring the difference in the NH₄⁺ concentrations of the bottles with and without copepods. Details of the incubation procedures are described elsewhere (Hasegawa et al. 2001).

Conversion of obtained ¹⁵N data to DON release rates. We assumed 2 main pathways which are attributed to DO¹⁵N release in a planktonic assemblage. One was direct DO¹⁵N release by phytoplankton (Mykkestad et al. 1989, Biddanda & Benner 1997) and the other DO¹⁵N release which is mediated by grazers (Lampert 1978, Nagata & Kirchman 1991). However, because of the limitations of the methodology we applied, it was difficult to evaluate DON release not associated with ¹⁵N-flux. For example, DON release induced by the lysis of dead cells could not be detected by our methodology. Consequently, our approach did not cover all potential processes of DON release in a planktonic community.

DO¹⁵N is directly released by phytoplankton, the source of which is considered as low molecular weight

Table 1. Dates and conditions of incubation experiments (<94 µm assemblage)

	Incubation temperature (°C)	Incubation time (h)	Light (µE s ⁻¹ m ⁻²)
Mar	3	3	24
May	10	6	70
Jun	13	6	34
Aug	18	1	40
Nov	9	6	43

(LMW) organic compounds in the cell (Bronk & Glibert 1991). These compounds originate from the uptake of DIN and degradation of cellular high molecular weight (HMW) organic compounds (Kanda & Hattori 1988). However, Kanda & Hattori (1988) showed that DIN depletion in culture media resulted in a decrease in the amino acid (component of LMW organic compounds) pool in the cells by more than half. Thus, DIN uptake might be the main source of the LMW pool in those cells. Further, Kanda et al. (1988) showed that the amount of cellular hot ethanol soluble fraction (HESF) was saturated by ^{15}N -labeled compounds after a brief (in most cases ~1 h) time lag from $^{15}\text{NH}_4^+$ addition in the surface waters of the western North Pacific. Although HESF consists of not only LMW but also HMW organic compounds, it is likely that most DO^{15}N is released from the HESF. Thus, in our 1 to 6 h incubation experiments (<94 μm assemblage) with $^{15}\text{NH}_4^+$ addition, we might be able to detect the major portion of direct DON release by phytoplankton. Although DO^{15}N might also be released by micrograzers under our experimental conditions, we assumed that the measured DO^{15}N release was exclusively from phytoplankton. This resulted in some overestimation of phytoplanktonic DON release.

Because we added a high amount of $^{15}\text{NH}_4^+$ (0.7 $\mu\text{mol N l}^{-1}$ for May and June and 1 $\mu\text{mol N l}^{-1}$ for August) compared to ambient concentrations (<0.15 $\mu\text{mol N l}^{-1}$), the addition might have enhanced the rates of NH_4^+ uptake and coupled DON release by phytoplankton. To evaluate DON release rates by phytoplankton assemblages in natural conditions, we assumed that regeneration is the only source of NH_4^+ and the uptake rate of NH_4^+ is equal to the regeneration rate of NH_4^+ within a community. This assumption is reasonable, since ambient NH_4^+ concentrations were undetectable (<0.05 $\mu\text{mol l}^{-1}$, in May and August) or low (0.14 $\mu\text{mol l}^{-1}$, in June) at our study site. Using the measured PER in each month, we calculated the DON release rate by phytoplankton assemblages (A) as follows,

$$A = \text{PER} \times \text{NH}_4^+ \text{ uptake rate} \\ (= \text{NH}_4^+ \text{ regeneration rate}) \quad (1)$$

On the other hand, if grazers release a part of their prey as DON, adequate ^{15}N label in phytoplankton increases the sensitivity of the detection of grazers' DO^{15}N release. We, therefore, used pre-incubated seawater with $^{15}\text{NH}_4^+$ for 2 to 3 d to estimate the effect of grazers on DON release. Further, the addition of cold- NH_4^+ at the start of the grazing experiments would decrease ^{15}N atom% of LMW organic pool in the phytoplankton cells. Thus, the effect of direct DO^{15}N release by phytoplankton might be minimized in our dilution experiments.

Since we measured DN release rates by copepods as the difference between the ^{15}N disappearance from

the PON fraction and the ^{15}N appearance in the copepods' bodies, these rates were a combination of DON and NH_4^+ release. In general, NH_4^+ release by copepods consists of release from the endogenous metabolism and from food-dependent processes (Gardner & Paffenhöfer 1982). During short time grazing experiments with ^{15}N prey, $^{15}\text{NH}_4^+$ release by copepods mainly originated from food-dependent processes. Since we estimated copepods' NH_4^+ excretion from the difference in NH_4^+ concentrations between feeding and control bottles, we could not separate the above 2 processes of NH_4^+ excretion. Consequently, we could not separate DO^{15}N and $^{15}\text{NH}_4^+$ release by copepods and thus compared the obtained DN release rates with the DON release rates of other plankton. In addition, ambient standing stock of copepods in each month was assumed to be 40 $\mu\text{g l}^{-1}$, which is the maximum standing stock value for total macrozooplankton in the bay obtained during 1972 (8 to 40 $\mu\text{g l}^{-1}$).

Chemical, isotopic, chl a and bacterial analyses. NH_4^+ , NO_3^- , NO_2^- and dissolved inorganic phosphorus (DIP) concentrations were determined with a Technicon autoanalyzer (Strickland & Parsons 1972). DON concentrations were measured by the wet oxidation method of Solórzano & Sharp (1980).

To prepare the sample for nitrogen isotopic measurements of NH_4^+ , we applied the conventional steam distillation method of Bremner & Keeney (1965). Samples for the determination of the nitrogen isotopic ratio of DON were prepared by the method described by Slawyk & Raimbault (1995) with some modification (Hasegawa et al. 2000a).

The nitrogen isotopic ratio of DON, PON, and NH_4^+ , and organic carbon and nitrogen contents of particulate organic matter (POM) and copepods were analyzed using a continuous flow mass spectrometer (Tracermass, Europa Scientific) equipped with a CN analyzer (Roboprep-CN, Europa Scientific) (Hasegawa et al. 2000a). The copepods were lyophilized and dry weights were determined on a Cahn microbalance (model 29) prior to analysis. POM on the GF/F filter was acidified with HCl vapor to remove carbonate before analysis. Chl a was determined by the fluorometric method of Strickland & Parsons (1972) as modified by Suzuki & Ishimaru (1990), using a Turner Designs fluorometer. Bacteria were counted by epifluorescence microscopy after DAPI staining (Porter & Feig 1980).

RESULTS

Environmental conditions

Water temperature in Akkeshi Bay showed distinct seasonal change, while salinity was rather constant

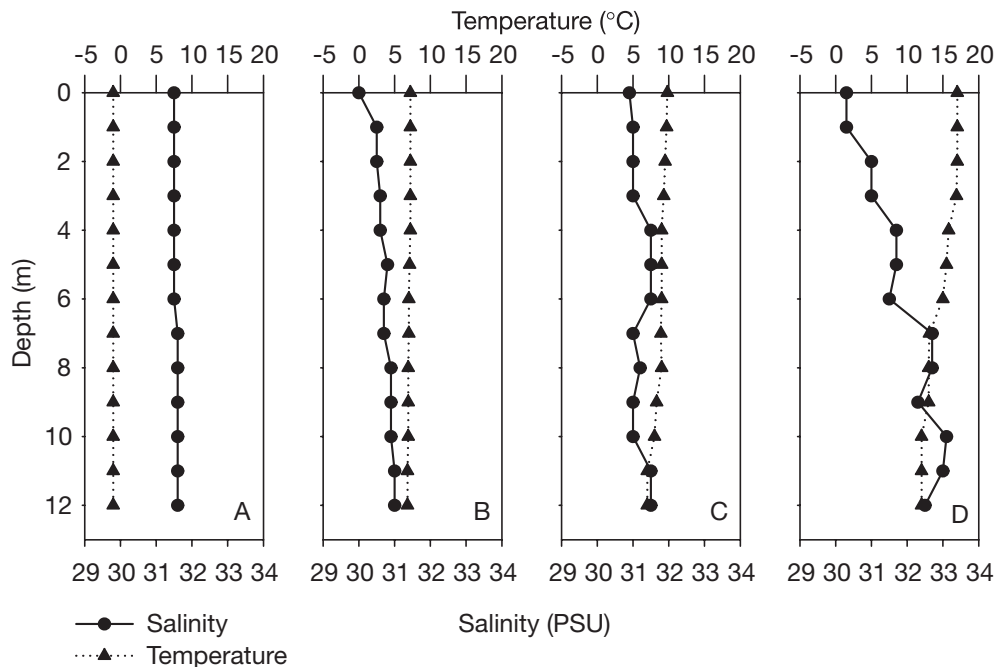


Fig. 1. Vertical distributions of salinity and temperature at a station in Akkeshi Bay. Data are given for (A) 9 March, (B) 28 May, (C) 29 June and (D) 24 August 1998. There are no data for November

except for August (Figs. 1 & 2A). The vertical profiles of temperature and salinity showed stratified condition in August, indicating limitation of nutrient supply to surface water. Concentrations of DIN were low or undetectable except for November (Fig. 2B). Concentrations of DIP were always higher than $0.2 \mu\text{mol l}^{-1}$

and highest in November. Increases in nutrient concentrations in November were caused by the disappearance of the thermocline which had developed in the summer. The DIN/DIP ratios we observed (0 to 11) were lower than the Redfield ratio from March to November (Redfield et al. 1963). DON concentrations

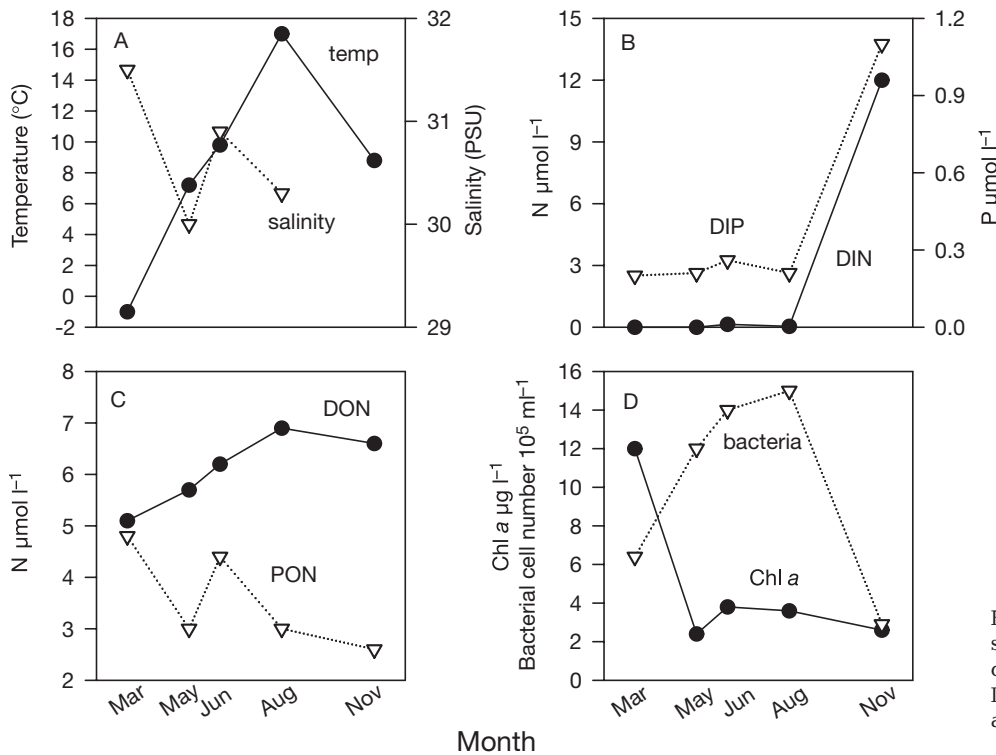


Fig. 2. (A) Temperature and salinity of surface seawater; concentrations of (B) DIN and DIP, and (C) DON and PON; and (D) chl a and bacterial cell number

were always higher than $5 \mu\text{mol l}^{-1}$, and except for November, DON constituted the most abundant nitrogen pool in the bay (Fig. 2C). The high concentration of chl *a* in March indicated a blooming condition, and chl *a* concentrations showed a 5-fold seasonal change (Fig. 2D). The bacterial cell number also changed 5-fold and was highest in August. Low values (0.44 to 1.1) of PON/chl *a* ($\mu\text{mol}/\mu\text{g}$) ratio indicated that PON mainly consisted of phytoplankton (McCarthy & Nevins 1986).

Nitrogen uptake and regeneration

In this study, the incubation times (Table 1) were adjusted depending on the water temperature and chl *a* concentration. Although uptake rates decreased as a hyperbolic function with time of the incubation, the decrease was most remarkable within 30 min (Wheeler et al. 1982) and uptake rates were almost constant from 1 to 24 h of incubation (Harrison 1983). Therefore, estimated rates of NH_4^+ uptake could be comparable among the seasons as the first order of approximation.

In March, the potential NH_4^+ uptake rate by the $<94 \mu\text{m}$ fraction was rather high ($1800 \text{ nmol l}^{-1} \text{ d}^{-1}$), while the chl *a*-specific NH_4^+ uptake rate was low (Table 2). However, the NH_4^+ regeneration rate by the $<94 \mu\text{m}$ fraction was $190 \text{ nmol l}^{-1} \text{ d}^{-1}$ and the NH_4^+ excretion rate by copepods was $17 \text{ nmol l}^{-1} \text{ d}^{-1}$. These values were only 12% of NH_4^+ demand by the planktonic assemblage, suggesting severe NH_4^+ limitation. As the season progressed, NH_4^+ regeneration by the $<94 \mu\text{m}$ assemblage became comparable to the NH_4^+ demand (45% in May and 79% in June) even though the observed NH_4^+ demand was a potential.

In August, both the rates of potential NH_4^+ uptake ($5900 \text{ nmol l}^{-1} \text{ d}^{-1}$) as well as chl *a*-specific NH_4^+ uptake ($1600 \text{ nmol } \mu\text{g}^{-1} \text{ chl } a \text{ d}^{-1}$) of the $<94 \mu\text{m}$ fraction was the highest among the observed seasons. The NH_4^+ regeneration rate by the $<94 \mu\text{m}$ fraction was $830 \text{ nmol l}^{-1} \text{ d}^{-1}$. Copepods excreted $65 \text{ nmol } \text{NH}_4^+ \text{ l}^{-1} \text{ d}^{-1}$, and

the total NH_4^+ regeneration rate throughout the heterotrophic processes was $900 \text{ nmol l}^{-1} \text{ d}^{-1}$, which was 15% of the above-measured NH_4^+ demand.

In November, the estimated rate of NH_4^+ regeneration ($750 \text{ nmol l}^{-1} \text{ d}^{-1}$) exceeded the rate of NH_4^+ uptake ($450 \text{ nmol l}^{-1} \text{ d}^{-1}$), and an accumulation of NH_4^+ ($2.0 \mu\text{mol l}^{-1}$) in ambient water was observed. The chl *a*-specific NH_4^+ uptake rate ($170 \text{ nmol } \mu\text{g}^{-1} \text{ chl } a \text{ d}^{-1}$) was comparable to that of March and June.

Ingestion rates of micrograzers and copepods

Nitrogen-based ingestion rates by micrograzers ranged from 700 to $4800 \text{ nmol l}^{-1} \text{ d}^{-1}$, while those by copepods ranged from 55 to $180 \text{ nmol l}^{-1} \text{ d}^{-1}$ (Table 2; calculated from Hasegawa et al. 2000b, 2001). Although we were unable to estimate the ingestion rates of micrograzers in May and June (Hasegawa et al. 2000b), ingestion rates by micrograzers were 12 to 27 times higher than those by copepods in the other months. This indicated the importance of micrograzers as herbivores in the bay environment. However, copepods might control the food web structure through their grazing pressure on micrograzers.

Release and uptake rates of DON or DN

During the observations from May to August, PER in $<94 \mu\text{m}$ seawater ranged from 2.7 to 4.9% (Hasegawa et al. 2000c). The calculated rates of DON release ranged from 18 to $28 \text{ nmol l}^{-1} \text{ d}^{-1}$ (Table 3).

In the dilution method with ^{15}N -tracer, the release and uptake rates of DON, which were estimated by a forward stepwise multiple regression ($F = 3.6$ to enter the regression), are listed in Table 3 (Hasegawa et al. 2000b). DON release by phytoplankton was not retained in the final regression. DON release rates by micrograzers showed a 2-fold change and ranged from

Table 2. Uptakes rate and regeneration rates of NH_4^+ , and ingestion rates ($\text{nmol l}^{-1} \text{ d}^{-1}$) estimated from incubation experiments in Akkeshi Bay water. Ingestion rates are calculated and/or taken from Hasegawa et al. (2000b, 2001)

	NH_4^+ uptake rate ($<94 \mu\text{m}$)		NH_4^+ regeneration rate		Ingestion rate	
	Total	Chl <i>a</i> specific ^a	$<94 \mu\text{m}$	Copepods	Micrograzers	Copepods
Mar	1800 (± 34) ^b	150 (± 2.9) ^b	190	17 (7.9–22) ^c	1200 (± 220) ^d	74 (64–80) ^c
May	1100 (± 36)	430 (± 15)	500 (± 66) ^b	67 (39–96)	– ^e	55 (41–63)
Jul ^f	720 (± 33)	180 (± 9.3)	570 (± 65)	57 (27–67)	–	72 (66–83)
Aug	5900	1600	830	65 (± 31) ^b	4800 (± 340) ^d	180 (± 0.14) ^b
Nov	450 (± 3.4)	170 (± 0.77)	750 (± 400)	30 (± 2.7)	700 (± 120)	57 (± 3.8)

^a NH_4^+ uptake rate per $1 \mu\text{g}$ chl *a* ($\text{nmol } \mu\text{g}^{-1} \text{ chl } a \text{ d}^{-1}$)
^bRanges of duplicated samples
^cRanges of triplicated samples
^dStandard deviation
^eNo data
^fIncubation experiment for $<94 \mu\text{m}$ was done in June

Table 3. DON and DN flux ($\text{nmol l}^{-1} \text{d}^{-1}$) estimated from the incubation experiment. Release and uptake rates were taken and/or calculated from Hasegawa et al. (2000b,c, 2001)

	DON release		DN release	DON uptake
	Phytoplankton	Micrograzers	Copepods	Bacteria
Mar	– ^a	190 (± 30) ^b	67 (57–73) ^c	170 (± 20) ^b
May	18 (± 0.65)	140 (± 38)	42 (25–52)	100 (± 20)
Jul ^d	28 (± 2.5)	350 (± 29)	46 (39–55)	200 (± 11)
Aug	23 (± 0.97)	280 (± 36)	45 (± 7.2) ^e	170 (± 17)
Nov	–	350 (± 64)	18 (± 7.2)	360 (± 38)

^aNo data
^bStandard error
^cRanges of triplicated samples
^dIncubation experiment for phytoplankton was done in June
^eRanges of duplicated samples

140 to 350 $\text{nmol l}^{-1} \text{d}^{-1}$. Bacterial DON uptake rates ranged from 100 to 360 $\text{nmol l}^{-1} \text{d}^{-1}$ and were comparable to 58–103% of release rates by micrograzers in each month. This result implies bacterial dependence on DON released by micrograzers.

DN release rates by copepods ranged from 18 to 67 $\text{nmol l}^{-1} \text{d}^{-1}$ (Table 3). Percentages of DN release to nitrogen-based ingestion showed an 8-fold change and ranged from 9 to 75%, i.e., copepods release 25 to 91% of their food nitrogen as DN.

Relative importance of DON release by different plankton assemblages in Akkeshi Bay

The calculated rates of DON release by phytoplankton ranged from 18 to 28 $\text{nmol l}^{-1} \text{d}^{-1}$, and in each observed season, these rates were the lowest compared to other planktonic organisms (Table 3). However, the importance of micrograzers as a producer of DON can be clearly indicated in this study (Table 3). DON release rates by micrograzers ranged from 140 to 350 $\text{nmol l}^{-1} \text{d}^{-1}$ and greatly exceeded those by the other planktonic assemblages. DN release rates by copepods, which also included excretion of NH_4^+ , ranged from 18 to 67 $\text{nmol l}^{-1} \text{d}^{-1}$.

DISCUSSION

In this study, we experimentally estimated NH_4^+ uptake and regeneration rates by planktonic assemblages in the <94 μm size range, ingestion rates of copepods and micrograzers, and DON or DN release rates of phytoplankton, copepods and micrograzers at the surface water of Akkeshi Bay from March to November 1998. Below, we discuss estimated nitrogen fluxes which might cause us to reevaluate previous views on nitrogen cycles in coastal environments (Williams 1995).

Seasonal change in nitrogen environment

In Akkeshi Bay, the DIN/DIP ratios were lower than the Redfield ratio (Redfield et al. 1963). Further, DIN concentrations were undetectable or low, whereas DIP concentrations were always higher than 0.2 $\mu\text{mol l}^{-1}$ from March to August. In this period, NH_4^+ regeneration compensated 12 to 79% of potential NH_4^+ uptake. These data strongly suggested that growth of phytoplankton was under the limitation of nitrogenous nutrient supply in the bay.

In August, the observed NH_4^+ regeneration rate was highest in this study. High water temperature (18°C) might be one of the reasons for the highest rate. A high rate of NH_4^+ regeneration would support a relatively high standing stock of phytoplankton (3.6 $\mu\text{g chl a l}^{-1}$), although stratification prevented the supply of inorganic nitrogen from the deep layers (Fig. 1).

In November, DIN and DIP concentrations in the surface water were high, which was a result of the disappearance of thermocline. A high concentration of NH_4^+ (2.0 $\mu\text{mol l}^{-1}$) of ambient water was also found in this month, which was consistent with the estimated rate of NH_4^+ regeneration (750 $\text{N nmol l}^{-1} \text{d}^{-1}$) that exceeded the rate of NH_4^+ uptake (450 $\text{N nmol l}^{-1} \text{d}^{-1}$). Therefore, in this month the supply of nitrogenous nutrients might not have limited the growth of phytoplankton.

Relative importance of DON release by different plankton assemblages

Although, in marine environments, most organic compounds including DON initially originated from photosynthesis of phytoplankton, the contribution of phytoplankton to DON release was the lowest among the examined planktonic assemblages. The estimated rates of direct DON release by phytoplankton ranged from 18 to 28 $\text{nmol l}^{-1} \text{d}^{-1}$ (Table 3). During the observations from May to August, PER showed changes in a narrow range (2.7 to 4.9%), and Hasegawa et al. (2000c) suggested that these low PER values might be common in marine environments. If PER is almost constant seasonally, the contribution of phytoplankton to DON release might increase when a large amount of inorganic nitrogen is taken up, i.e. period during which a bloom develops.

Some field observations showed DOC accumulation after blooming conditions (Ittekkot 1982, Billen & Fon-

tigny 1987). There are 2 representative mechanisms for DOC release by phytoplankton; one is passive (Bjørnsen 1988) and the other is an active process (Fogg 1983). If excess photo-assimilated carbon is accumulated in the cell due to nutrient depletion (Fogg 1983), these 2 mechanisms well explain DOC accumulation into surface water when a bloom dissipates. On the other hand, growth of marine phytoplankton is generally suppressed by nitrogen deficiency (Dugdale & Goering 1967, Ryther & Dunstan 1971, Ward et al. 1989, Oviatt et al. 1995). Therefore, it is unlikely that excess nitrogen compounds accumulate within the cell at the end of bloom. Further, in Akkeshi Bay, spring and fall bloom consisted of large diatoms (Motoda et al. 1977, Taguchi et al. 1994), and a decline in the bloom of diatoms is usually caused by sedimentation rather than cell lysis at the surface (Brussaard et al. 1995), i.e., the dissipation of the bloom might have little effect on surface DON accumulation in the bay.

In laboratory studies, enhanced release rates of DOC by heterotrophs have been reported (Lampert 1978, Copping & Lorenzen 1980, Caron et al. 1985, Strom et al. 1997) and have also been theoretically discussed (Jumars et al. 1989). In field studies, indirect evidence suggested that grazing activities enhanced the rates of DON release (Bronk & Glibert 1993b, Bronk et al. 1998, Bronk & Ward 1999). In these studies, estimated DON release rates were higher at night, when grazing rates tended to increase, than during the day (Durbin et al. 1990). However, due to the lack of suitable methodology, the importance of DON release by herbivorous grazers in the field has not been directly demonstrated.

To obtain direct evaluation of DON release by micrograzers, we applied the ^{15}N -tracer technique with the dilution method (Landry & Hassett 1982). The micrograzer communities might consist of flagellates, ciliates, nauplii of crustaceans and so on in the bay. Because we did not specify community structure in each experiment, knowledge about which species was important for DON release was not available. However, the importance of micrograzers in general as a producer of DON is clearly indicated in this study (Table 3). DON release rates by micrograzers ranged from 140 to 350 $\text{nmol l}^{-1} \text{d}^{-1}$ and greatly exceeded those by other organisms. Further, the DON release rates by micrograzers were well coupled to the DON uptake rates by bacteria (Table 3), indicating that there was a significant nitrogen flux from phytoplankton to bacteria via micrograzers.

Using the ^{15}N -tracer technique with conventional feeding experiments, it was shown that DN release efficiency of copepods compared to their ingestion was high, especially in spring. DN release rates by copepods ranged from 18 to 67 $\text{nmol l}^{-1} \text{d}^{-1}$. Although DN

release rates consisted of DON and NH_4^+ , these rates were 1 order lower than DON release rates by micrograzers (Table 3), suggesting that DON release by copepods is of minor importance.

For grazing by copepods, a 3-fold daily amplitude between high grazing rate at night and low rates during the day is reported (Durbin et al. 1990). Since our grazing experiments (the dilution method and copepod feeding experiment) were carried out only at night under dark conditions (6 or 12 h incubation), our estimation of daily rates might be a 1.5-fold overestimation.

To evaluate DON release rates by phytoplankton, micrograzers and copepods, we designed 3 completely different methodologies in this study. However, because of the complexity of natural planktonic assemblages, it is difficult to identify exactly the activities of the targeted organisms. For example, although the source of measured DO^{15}N release was assumed to be phytoplankton in incubation experiments using the $<94 \mu\text{m}$ assemblage, this was not true in most cases, possibly due to the contribution of micrograzers to DO^{15}N release. In the dilution method with ^{15}N -tracer, although DON release by phytoplankton was rejected statistically, it is difficult to demonstrate that phytoplankton make no contribution to DON release. The method used in the copepod incubation experiments with ^{15}N tracer has the inherent disadvantage that it results in the inclusion of NH_4^+ excretion by copepods. In spite of the difficulties mentioned above, a comparison of the results of these 3 completely different methods showed that micrograzers were the most important contributors to DON release within the observed seasons.

Role of DON in marine ecosystem

Generally, in spring, high concentrations of nutrients and increasing solar radiation stimulate production of diatoms in temperate coastal waters such as Akkeshi Bay (Motoda et al. 1977). An increase in the standing stock of diatoms would stimulate herbivorous grazer activity, and herbivores might continue to release DON within surface marine ecosystems. DON remains in the euphotic layer while most of the diatoms sink to the bottom (Brussaard et al. 1995). Bacteria can grow using the plentiful DON and increase their biomass in this DIN-depleted condition, because bacteria are thought to be the primary consumer of DON in coastal waters (Wheeler & Kirchman 1986, Kirchman et al. 1989, Kroer et al. 1994). A 2-fold increase in bacterial biomass was observed from March to May in the bay (Fig. 2C). Bacteria also regenerate NH_4^+ from DON (Kirchman et al. 1989, Tupas & Koike 1991). Further,

NH_4^+ might be regenerated through the microbial food web (Azam et al. 1983). For the growth of phytoplankton, NH_4^+ regenerated in these ways might be important in DIN-depleted surface waters. DON, which remained in the euphotic zone for a long time compared to PON, stimulates the microbial loop, and finally stimulates primary production.

Indeed, DON release by micrograzers was comparable to NH_4^+ regeneration in $<94 \mu\text{m}$ seawater (Tables 2 & 3), and thus DON constitutes a dynamic pool of nitrogen in coastal environments. Generally, productivity in marine ecosystems is thought to be controlled by the DIN supply available to phytoplankton (Dugdale & Goering 1967, Ryther & Dunstan 1971, Ward et al. 1989, Oviatt et al. 1995). However, 'deposit nitrogen' in the upper layer, i.e. DON, could sustain not only bacterial production but also that of phytoplankton, either directly or through heterotrophic activity. For a clear understanding of planktonic production it is, therefore, necessary to include DON in schema of the marine nitrogen cycle.

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