

Significance of bacteria in urea dynamics in coastal surface waters

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ABSTRACT: Bacterial urea production and decomposition were studied in samples from coastal waters in the Southern California Bight (the Bight), USA, and an estuarine system of the Mankyung and Dongjin rivers (MD estuary) in Korea. Bacterial urea production ranged from undetectable to 139 nM d⁻¹, and the mean value of bacterial urea production (58 nM d⁻¹, n = 6) was equivalent to 35–91 % of the estimated phytoplankton N demand in the Bight. The rates of bacterial production of urea were 2 orders of magnitude higher than the bacterial urea decomposition rates. Consequently, bacteria were consistently net producers of urea in the euphotic zone. The concentration-dependence of urea decomposition showed the presence of a high affinity but low capacity system (K_1+S_n : 26 to 33 nM, V_{max} : 3 to 11 nM d⁻¹). The low K_m values indicate that in typical seawater samples, which have >100 nM urea, the bacterial ureolysis system is always near-saturated. The significance of bacteria as urea producers should be incorporated into models of nitrogen regeneration in surface waters.

KEY WORDS: Bacteria · Urea production · Urea decomposition · Coastal waters

INTRODUCTION

Urea is one of the major nitrogen (N) sources for primary production in the ocean and a large contributor to the oceanic 'regenerated production' (Eppley & Peterson 1979, Harrison et al. 1985). In order to understand the regulation of N regeneration, it is important to find out which organisms are responsible for urea production in the euphotic zone and what mechanisms regulate urea production. The general perception has been that urea is mainly produced by metazoa. For instance, McCarthy & Whittedge (1972) concluded, based on extrapolation of laboratory measurements to field conditions, that most of the urea demand of phytoplankton could be explained by urea production by zooplankton and fish. In a field study in the central North Pacific gyre, Eppley et al. (1973) concluded that urea excretion by zooplankton could explain 40 to 110% of urea utilization by phytoplankton. However, recently Harrison et al. (1985) reported that dominant macrozooplankton species in the eastern Canadian

Arctic supplied only ca 3% of the urea-N requirements of the phytoplankton. The possible contribution of microorganisms to the observed urea production rates was not examined in these studies.

More recent studies indicate that microorganisms may be quite important in urea production. Price et al. (1985) measured urea production rates in the Strait of Georgia (Vancouver, Canada) in intact seawater samples. They found that N regeneration processes generated urea at rates similar to (frontal region) or 2- to 5-fold larger than (stratified waters) ammonium regeneration rates. They proposed a model which included (dissolved organic nitrogen) DON → urea as an important pathway of N flux but they did not identify the organisms responsible for mediating this regenerative flux. Hansell & Goering (1989) and Slawyk et al. (1990) used isotope dilution methods to measure *in situ* urea production rates. They found that planktonic assemblages <333 μm (Hansell & Goering 1989) or <200 μm (Slawyk et al. 1990) regenerated urea rapidly enough to replenish the urea pool in less than 1 d. While these studies implied that bacteria were, in part, responsible for urea production, they did not quantify the contribution of bacteria to urea production. Bacterial metabolism of purines, pyrimidines, argi-

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nine and hypoxanthine is known to result in urea production (Turley 1985, Mobley & Hausinger 1989). It is therefore possible that the utilization of these DON components by natural assemblages of bacteria in seawater produces urea at rates which are significant in total urea regeneration.

The goal of this study was to test whether bacteria in the planktonic microbial assemblages are significant producers of urea in surface waters.

MATERIALS AND METHODS

Study area and sample collection. Surface seawater samples were collected with a sterile 1 l polycarbonate (Nalgene®) flask at the end of the Scripps pier in California, USA (32° 53' N, 117° 15' W) and in the estuarine system of the Mankyung and Dongjin rivers (MD estuary), Korea (Fig. 1). The MD estuary is a shallow (mostly <10 m) and tidally mixed estuary (for further description see Cho & Shim 1992). The salinity at the sampling stations ranges from 18 to 32‰. Additional samples were collected at 4 stations during cruises in the Southern California Bight (the Bight) in October

1985, September 1988, and February 1992. Subsurface samples were taken with a Niskin bottle.

Urea production. In order to measure bacterial production of urea dissociated from urea uptake by phytoplankton, the seawater was fractionated through 0.6 or 0.8 μm Nuclepore filters (Bight samples) or GF/C filters with nominal pore-size of 1.2 μm (MD estuary). The reason for using different filters for size-fractionation was that the same pore-size filters were not available on all field trips. These filters permit a major fraction of bacteria to pass but retain most of the phytoplankton. One bottle or duplicate bottles of the filtrates (volume of 1 to 2 l) were incubated at *in situ* temperature in the dark for 10 to 24 h, and the abundance of bacteria and urea concentration were measured periodically. Filtrates added with neutral buffered formalin (final conc. of 1%) served as blanks for the MD estuary samples. In one experiment (Scripps pier sample, December 1985), we tested whether bacteria rather than non-bacterial contaminants (protozoa and picophytoplankton) were responsible for urea production. We inoculated 0.2 μm filtered seawater with the natural assemblages of bacteria in 0.6 μm filtrate (2% v/v) and incubated (seawater culture, SWC; Ammerman et al. 1984) the SWC in the dark at *in situ* temperature. Bacterial abundance and urea concentration were determined periodically. In 5 experiments, the time-course of urea accumulation was monitored (Fig. 2) while in the other 5 samples only the starting and the end-point concentrations of urea were determined (see Table 1). Production rates of urea for the size-fractionated samples were calculated using linear regression for the whole incubation period (Fig. 2A, B) or for the initial (6 h) linear part of the incubation (Fig. 2C, D). The production rate of urea for the SWC was calculated for the incubation period from 18 to 53 h (Fig. 2E). Rates of bacterial cell production were estimated in all experiments over the same time intervals as those of urea production.

Urea decomposition. Measurements of urea decomposition activity were followed with added ^{14}C -urea, essentially by the method of Remsen et al. (1974). In most experiments, 50 ml of seawater (0.6, 0.8 or 1.2 μm and 183 μm size-fractionated samples or whole seawater) was amended with 0.2 μm filter-sterilized ^{14}C -urea (54 to 58 mCi mmol^{-1} , NEN) to attain 0.25 to 0.62 μCi per sample. The resulting concentration of added urea was 86.2 to 252.5 nM (except in experiments to study concentration-dependence of urea decomposition; below). For some samples this addition could not be regarded as true tracer, but it did not substantially increase the rates as the bacterial urease systems were near saturation at the natural urea concentrations (see 'Discussion'). Samples were incubated under white-fluorescence light, *in situ* light, or in the dark. One bottle was fixed with neutral buffered formalin (final conc.

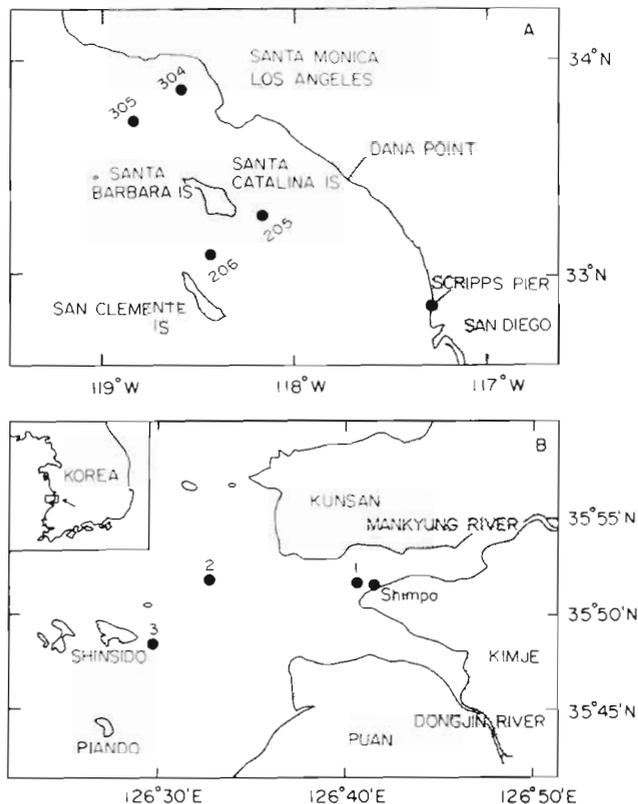


Fig. 1. Sampling stations in (A) the Southern California Bight, USA and (B) an estuarine system of the Mankyung and Dongjin rivers in Korea

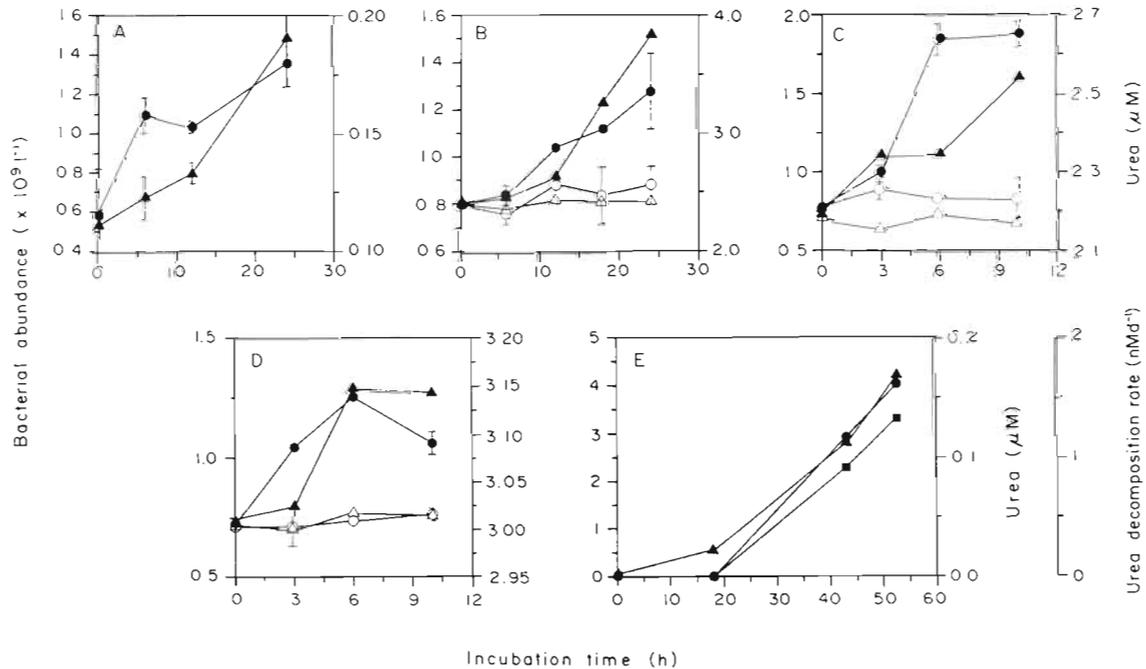


Fig. 2. (A) Changes of mean values of bacterial abundance (▲) and urea (●) in duplicate bottles of 0.6 μm filtrates from the Scripps pier (January 1989). (B–D) Those in single bottle of GF/C filtrates from the Mankyung and Dongjin rivers estuary [(B) November 1992; (C, D) June 1993]. (Δ , \circ) Formalin-killed blanks. (E) Changes of bacterial abundance (▲), urea (●), and urea decomposition activity (■) in seawater culture (December 1985). All samples were from the surface. Error bars are 1 SD

of 1%) and served as a control. After incubation with ^{14}C -urea for 1 or 2 h, 1 ml of 2N H_2SO_4 was added to the samples placed in a gas-tight container. Liberated $^{14}\text{CO}_2$ was collected in 0.2 ml of phenethylamine and radioassayed by liquid scintillation spectrometry using the external standard ratio method. Urea decomposition rates were calculated as: $(f/t) \times (\textit{in situ} \text{ urea concentration})$, where f = fraction of the trace ^{14}C converted to $^{14}\text{CO}_2$ and t = duration of incubation. All measurements were done at least in duplicates. The coefficient of variation among replicates was usually <7%.

The concentration-dependence of urea decomposition was determined in 1.0 μm pore-size Nuclepore filtrates (bacterial fraction) for 2 samples taken in the Bight, one from 150 m at Stn 206 and other at the end of the Scripps pier. Addition of mixtures of ^{14}C -urea and unlabeled urea was made to achieve final concentrations of added urea from 17 to 1600 nM (Scripps pier) or 9 to 4300 nM (Stn 206). The initial rates of decomposition of the added urea to $^{14}\text{CO}_2$ were determined in 30 min incubations following the procedure described above. A modified Eadie-Hofstee plot (Azam & Hodson 1981) was used to determine V_{max} and $K_t + S_n$ (where S_n is the natural concentration of urea).

Other analyses. Urea was measured by the urease method of McCarthy (1970) on the day of experiment for the Bight samples between December 1985 and

1989. Bight samples from 1992 and samples from the MD estuary were frozen and brought to the laboratory to measure urea concentration by the method of Price & Harrison (1987). We did not observe any significant changes in urea concentration for the MD estuary samples analyzed within a week (not shown). Ammonium was measured by the method of Strickland & Parsons (1972) or Grasshoff et al. (1983). Bacteria were enumerated by epifluorescence microscopy of acridine orange stained samples (Hobbie et al. 1977).

RESULTS

Urea production

The rate of accumulation of urea in the filtrates varied from undetectable to 139 nM d^{-1} in the Bight and from 264 to 1176 nM d^{-1} in the MD estuary (Table 1). The increase in urea roughly paralleled the increase in bacterial abundance. Formalin killed blanks showed no changes in urea concentration (Fig. 2B, C, D). The seawater culture (SWC) showed an increase in urea concentration in parallel to the increase in bacterial abundance (Fig. 2E). The rate of urea production of 112 nM d^{-1} was linear for the incubation period of 18 to 53 h. Per-cell urea production rates ranged from 40 to 1348 $\text{amol cell}^{-1} \text{d}^{-1}$ (Table 1).

Table 1. Initial urea concentration, initial bacterial abundance in filtrates (<0.8 μm Nuclepore or GF/C filtrates), production rates of urea and bacteria in seawater culture and filtrates, and per-cell urea production rates. Samples were from Stn 305 and the Scripps pier in the Southern California Bight (Bight), USA and from Stn Shimpo in the estuarine system of the Mankyung and Dongjin (MD estuary) rivers, southwest coast of Korea

Location	Date	Depth (m)	Urea (nM)	Bacterial abundance ($\times 10^9 \text{ l}^{-1}$)	Production rates of:		Per-cell urea production rates ($\text{amol cell}^{-1} \text{ d}^{-1}$)
					Urea (nM d^{-1})	Bacteria ($\text{cells l}^{-1} \text{ d}^{-1}$)	
Bight							
Stn 305	Sep 1988	20	166 ± 27	1.3	38	–	–
		40	179 ± 12	–	139	–	–
	Feb 1992	10, 30	– ^a	–	nd ^b	–	–
Scripps pier	Jan 1989	0	115 ± 5	0.5	56	9.6×10^8	58
	Dec 1985 ^c	0	nd ^b	1.1	112	28.2×10^8	40
MD estuary							
	Nov 1992	0	2400 ± 150	0.8	984	7.3×10^8	1348
	Feb 1993	0	697 ± 231	0.3	264	6.4×10^8	413
	Jun 1993	0	2210 ± 13	0.8	1176	13.9×10^8	846
		0	3005 ± 70	0.7	528	21.1×10^8	250

^aRaw data were acquired by absorptivity measurements which showed negligible urea production, but the data were lost before being recorded
^bnd: below detection limit. ^cSeawater culture

Urea decomposition

Urea decomposition rate in the actively growing SWC was negligible ($0.3 \text{ amol cell}^{-1} \text{ d}^{-1}$ or 1.1 nM d^{-1} ; Fig. 2E) in comparison with the urea accumulation rate of 112 nM d^{-1} (above).

The bacterial fractions (<0.8 μm) in the Bight samples showed low urea decomposition rates in compari-

son with the <183 μm filtrable fraction (0 to 22%; Table 2). Bacterial fractions (GF/C filtrates) in the MD estuary samples also showed low urea decomposition rates in comparison with the whole seawater (0 to 10%; Table 2) with one exceptionally high value of 64%. The per-bacterium rates of urea decomposition calculated for the bacterial fraction (on the assumption that all urea decomposition in these filtrates was due to bacteria) ranged from 0 to $8 \text{ amol cell}^{-1} \text{ d}^{-1}$ (Table 2).

The kinetic data of urea decomposition by natural assemblages of marine bacteria in 1.0 μm filtrate yielded a linear fit on a modified Eadie-Hofstee plot (Fig. 3). The sample off Scripps pier had a $K_i + S_n$ of 26 nM and a V_{max} of 11 nM d^{-1} . The corresponding values for Stn 206 were 33 nM and 3 nM d^{-1} . Per-cell V_{max} was $13 \text{ amol cell}^{-1} \text{ d}^{-1}$ for the sample from Stn 206. Bacterial abundance data was not determined for the Scripps pier sample. If we assume it was 10^9 l^{-1} in the bacterial fraction (Cho 1988), then the per-cell V_{max} for the pier sample was $11 \text{ amol cell}^{-1} \text{ d}^{-1}$. Thus, the per-cell V_{max} values for the 2 samples, one from the euphotic and the other from the aphotic zone, would be similar.

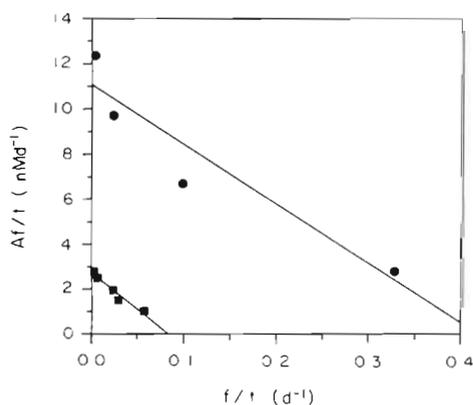


Fig. 3. Modified Eadie-Hofstee plots of urea decomposition activity data for samples from the <1.0 μm fraction from the Southern California Bight. (●) Samples from Scripps pier; (■) samples from 150 m at Stn 206. f/t : fraction of total urea pool decomposed per day. Slope = $-(K_i + S_n)$; y -intercept = V_{max} ; x -intercept = $1/T_i$ (K_i = decomposition constant; S_n = ambient concentration; V_{max} = maximum decomposition rate; T_i = turnover time at the ambient concentration). Regression equations: Scripps pier, $y' = 10.8 - 25.9f/t$; Stn 206, $y' = 2.8 - 33.3f/t$; where y' is multiplication of added urea concentration (A) and f/t

DISCUSSION

Urea production by bacteria

The urea production rates which we ascribed to bacteria were obtained for operationally defined 'bacterial size-fractions' (<0.6, <0.8 μm and GF/C filtrate). The interpretation of the SWC experiment is the least

Table 2. Bacterial abundance in $<0.8 \mu\text{m}$ filtrate, *in situ* urea concentration, urea decomposition in $<0.8 \mu\text{m}$ filtrates and $<183 \mu\text{m}$ filtrates, ratio of urea decomposition rates in $<0.8 \mu\text{m}$ filtrates to $<183 \mu\text{m}$ filtrates, and per-cell urea decomposition rate in the euphotic zone of the Southern California Bight (Bight). In the MD estuary, GF/C filtrates and whole seawater were used for the measurements of urea decomposition

Stn	Date	Depth (m)	Bacterial abundance ($\times 10^9 \text{ l}^{-1}$)	Urea conc. (nM)	Urea decomposition rates		Ratio of $<0.8 \mu\text{m}/<183 \mu\text{m}$	Per-cell urea decomposition rates ($\text{amol cell}^{-1} \text{ d}^{-1}$)
					$<0.8 \mu\text{m}$ (nM d^{-1})	$<183 \mu\text{m}$ (nM d^{-1})		
Bight								
305	Sep 1988	15	0.5	323 ± 117	0.2	6.4	0.03	0.4
		30	0.7	269 ± 11	0.0	6.3	0.00	0.0
	Sep 1988	15	1.1	393 ± 66	2.7	38.8	0.07	2.5
		30	1.2	nd ^a	nd ^a	nd ^a	–	–
	Oct 1985	50	0.5	35 ± 7	0.2	0.9	0.22	0.4
		50	0.5	nd ^a	nd ^a	nd ^a	–	–
304	Oct 1985	17	0.5	100 ± 14	0.1	21.4	0.01	0.2
		49	0.6	30 ± 10	0.2	1.7	0.12	0.3
205	Oct 1985	50	0.7	350 ± 79	0.8	13.4	0.06	1.2
MD estuary								
Shimpo	Nov 1992	0	0.8	2400 ± 150	6.1 ^b	9.6	0.64	7.6
	Jun 1993	0	1.2	2210 ± 13	0.0	141.6	0.00	0.0
	Jun 1993	0	1.5	4330 ± 65	10.5	105.1	0.10	7.0
1	Feb 1994	0	0.5	1560 ± 210	1.3	45.9	0.03	2.6
2	Feb 1994	0	0.5	670 ± 10	0.9	22.9	0.04	1.8
3	Oct 1992	0	1.1	1120 ± 130	0.0	23.5	0.00	0.0
^a nd: below detection limit								
^b Incubations under <i>in situ</i> light condition showed the same rates of urea decomposition as in the dark, suggesting that it was mainly bacterial ureolytic activity								

ambiguous. The inoculum consisted of 2% (v/v) of $0.6 \mu\text{m}$ Nuclepore filtrate and was therefore expected to exclude phytoplankton and protozoa and include bacteria (and unavoidably, viruses as well). In examining the samples for bacterial counts (2 to 5 ml samples; 10 to 20 microscope fields at $1250\times$) we did not observe any phytoplankton or protozoa. The seawater culture generated urea in parallel with the accumulation of bacteria beyond the 18 h time point (Fig. 2E). Abiotic urea production was considered negligible since it was undetectable between 0 and 18 h (Fig. 2E). We therefore conclude that the urea accumulation rate (112 nM d^{-1} for the period between 18 h and 53 h) was largely due to bacteria.

The urea concentrations in our study (below detection limit to 393 nM in the Bight, 670 to 4330 nM in the MD estuary) were within the range of previous studies in the Bight (McCarthy 1970, McCarthy & Kamykowski 1972, Cho & Azam 1995) and MD estuary (Shim et al. 1994). Our data of urea concentrations were also comparable to the studies done in other coastal areas (Steinmann 1976, Turley 1985) and estuaries (Remsen et al. 1972, Webb & Haas 1976).

The urea measurements in the 'bacterial size-fractions' (Nuclepore and GF/C filtrates) were made to

obtain conservative estimates of the *in situ* rates of urea production due to bacteria. The estimates are conservative because at our sampling sites generally $\sim 10\%$ of bacteria are retained by the filters. There was no obvious contamination of the filtrates with phytoplankton and protozoa as judged on the basis of samples examined for bacterial counts. We cannot rule out that some protozoa passed the filters we used for size-fractionation, but their populations remained too low to be detected in the samples we examined.

The mean rate of bacterial urea production in the Bight was $58 \pm 58 \text{ nM d}^{-1}$ (mean \pm SD, $n = 6$, equivalent to $1.6 \mu\text{g N l}^{-1} \text{ d}^{-1}$) while higher rates ($738 \pm 417 \text{ nM d}^{-1}$, mean \pm SD, $n = 4$) were found in the MD estuary. Since primary production data and historical data on urea demand in phytoplankton is available for the Bight, we can use the Bight data to evaluate if the urea production rates contributed significantly to the phytoplankton N demand and phytoplankton urea-N demand. The phytoplankton N demand in the Bight was estimated to be 1.8 to $4.6 \mu\text{g N l}^{-1} \text{ d}^{-1}$ as follows: during our study the primary production was 883 to 2315 nM C d^{-1} or 126 to 331 nM N d^{-1} (assuming that C/N was 7 and the euphotic depth 50 m). Thus, our mean rate of urea production was equivalent to 35 – 91% of the estimated

phytoplankton N demand. McCarthy (1972) found that 28% of the primary production in the Bight could be supported by urea. For the calculation we used the mean value of the urea production rates. Individual values are highly variable (above) and they would result in bacterial urea production being equivalent to 0% to well over 100% of the phytoplankton N demand. Thus, it seems that bacterial production of urea at times could contribute significantly to the urea dynamics, and supply a large fraction of the urea demand of the phytoplankton. Literature data show that urea and ammonium may be equally important as N source for phytoplankton. Except in high nitrate waters, where urea contributes only 4% of total N uptake by phytoplankton (Kokkinakis & Wheeler 1987), urea usually comprises from 19 to 80% of total N uptake by phytoplankton in marine environments (Kristiansen 1983, Harrison et al. 1985, Sahlsten 1987, Probyn 1988, Sahlsten et al. 1988, Hansell & Goering 1990, Glibert et al. 1991). Thus, bacterial production of urea could be a significant source of urea-N to phytoplankton in marine environments.

There are no previous measurements in our study areas to which we can compare our rates of bacterial production of urea. Production rates in the Bight are considerably lower than those for total seawater in the Strait of Georgia (648 to 1128 nM d⁻¹; Price et al. 1985), in Mikawa Bay (624 nM d⁻¹; Mitamura & Saijo 1975), for the <333 µm size fraction of seawater in the Bering Sea (600 to 1176 nM d⁻¹; Hansell & Goering 1989) and for the <200 µm filtrable fraction off the Galapagos Islands (1100 nM d⁻¹; Slawyk et al. 1990). Bacterial urea production rates in the MD estuary (528 to 1176 nM d⁻¹), however, were similar to the values reported above. These regional differences could be due to differences in factors such as dissolved organic nitrogen and/or bacterial activity.

Per-cell urea production rates were in the range of 40 to 1348 amol cell⁻¹ d⁻¹ (1.1 to 37.7 fg N cell⁻¹ d⁻¹; Table 1). Per-cell urea production rates were much faster in the MD estuary than in the Bight (Table 1). Bacterial abundance and bacterial production were not related to per-cell urea production rates in this study. Per-cell urea production rates were in most cases close to or larger than the daily bacterial N demand for growth, assuming a bacterial N content of 3.4 to 11.6 fg cell⁻¹ for the range of small natural marine bacteria (0.036 µm³; Simon & Azam 1989) and large growing bacteria in seawater culture (0.25 µm³; Ammerman et al. 1984). These results indicate that bacteria would excrete excess N in an organic form such as urea, consistent with recent views that urea seems to be a major organic N compound released during bacterial growth (Jørgensen et al. 1993, Kroer et al. 1994).

Urea decomposition by bacteria

The characteristics of bacterial urea hydrolysis (Fig. 3) indicated the presence of a high affinity (K_1+S_n values of 26 and 33 nM) but low capacity system. Even if we treat the K_1+S_n values as upper limits of K_m , the values are much lower than the concentrations of urea in typical seawater samples. If the K_m values for 2 samples are applicable to the other samples then the urea hydrolysis should be close to V_{max} . Only in 1 sample (MD estuary, June 1993), however, was urea decomposition rate in the bacterial fraction close to V_{max} of 11 nM d⁻¹ (Table 2). Bacterial ureolytic activity in marine surface waters might not be fully expressed (see below).

The rates of urea decomposition in the SWC experiment were low (0.9 and 1.3 nM d⁻¹ at 42 and 53 h time points, respectively; Fig. 2E) compared to the corresponding rates of urea production (112 nM d⁻¹). Thus, the bacterial assemblages in the SWC were net producers of urea. Bacterial fractions (<0.8 µm Nuclepore filtrates) of a number of samples taken at stations in the Bight also showed very low rates of urea decomposition, ranging from zero to 3 nM d⁻¹. By comparing this range of urea decomposition rates with rates of urea production in the Bight (58 ± 58 nM d⁻¹, mean ± SD) we conclude that the predominant role of the bacteria was to produce rather than decompose urea. The eutrophic MD estuary (chlorophyll *a*: >1 to <20 µg l⁻¹; NH₄⁺: undetectable to 47 µM; Shim et al. 1994) showed higher urea decomposition rates (0 to 10.5 nM d⁻¹; Table 2). However, since the urea production rate was proportionately high (Table 1), the decomposition rate was only 0 to 4% of the production rate. Further, in 2 samples (November 1992 and June 1993; Tables 1 & 2) in which both measurements of bacterial production and decomposition of urea were made, urea production rates were over 2 orders of magnitude higher than the bacterial urea decomposition. Thus, the conclusion that bacteria are net producers of urea appears to be valid for the examined mesotrophic and eutrophic environments.

Earlier studies have also found that planktonic bacterial assemblages play only a trivial role in urea decomposition (Remsen et al. 1974, Turley 1985). Good correlations between chlorophyll and urea decomposition, results of size-fractionation experiments similar to ours, and the fact that there is no energetic advantage for bacteria in using urea as an N source led Turley (1985) to conclude that 'bacteria rather ignore urea' in surface seawater samples. Although the relationship between chlorophyll *a* and urea decomposition was not studied here, our data are consistent with Turley (1985). Bacterial fractions (0.8 µm filtrates in the Bight and GF/C filtrates in the MD estuary) usually showed

<12% of total activity. In one exceptional case, in the MD estuary, 64% of the total urea decomposition activity was in the GF/C filtrate. Dark and light incubations showed no differences in activity, indicating that most of the activity might have been due to bacteria. Our results on per-cell urea decomposition by bacteria (Table 2) also support the conclusion that bacterial decomposition of urea is generally insignificant in surface waters: in the Bight samples, if N generated by urea decomposition (mean of $0.6 \text{ amol cell}^{-1} \text{ d}^{-1}$) was assumed to be utilized as the sole source of N for bacterial growth of $5.6 \text{ fg N cell}^{-1}$ (Lee & Fuhrman 1987), it would support mean bacterial doubling times of 357 d, which are 2 orders of magnitude longer than the observed doubling times of bacteria in the coastal waters of the Bight (on the order of 1 to 4 d; Cho & Azam 1988). In the MD estuary the bacterial doubling time which could be supported by urea decomposition (mean of $3.2 \text{ amol cell}^{-1} \text{ d}^{-1}$) was 63.1 d whereas the actual bacterial doubling time was <1.1 d (Fig. 2B, C, D). In SWC it was shown that the per-cell urea hydrolysis rate could vary broadly and reach values as high as $54 \text{ amol cell}^{-1} \text{ d}^{-1}$ (Cho 1988). It appears, therefore, that per-cell rates of ureolysis are subject to large variations and that bacterial hydrolysis of urea could at times contribute significantly to bacterial N demand.

In conclusion, bacteria appear to be net producers of urea in the euphotic zone of the coastal and estuarine environments studied here. Bacterial urea production could satisfy a significant fraction of the phytoplankton N demand. While urea decomposition by bacteria was generally insignificant relative to the bacterial N demand, it could be significant in N-limited conditions. Our results here and in an earlier study of the mesopelagic environment (Cho & Azam 1995) suggest that the role of bacteria in urea dynamics should be incorporated into models of oceanic N cycle.

Acknowledgements. We thank Dr G. Herndl for help in the field and laboratory experiments. We thank 3 anonymous reviewers for their constructive comments. The present studies were supported by NSF grants (F.A.) and by the Basic Science Research Institute program, Ministry of Education, 1995, Project No. BSRI-95-5409 (B.C.C. and J.H.S.).

LITERATURE CITED

- Ammerman JW, Fuhrman JA, Hagström Å, Azam F (1984) Bacterioplankton growth in seawater. I. Growth kinetics and cellular characteristics in seawater cultures. *Mar Ecol Prog Ser* 18:31–39
- Azam F, Hodson RE (1981) Multiphasic kinetics for D-glucose uptake by assemblages of natural marine bacteria. *Mar Ecol Prog Ser* 6:213–222
- Cho BC (1988) Significance of bacteria in biogeochemical fluxes in the pelagic ocean. PhD thesis, University of California, San Diego
- Cho BC, Azam F (1988) Major role of bacteria in biogeochemical fluxes in the ocean's interior. *Nature* 332:441–443
- Cho BC, Azam F (1995) Urea decomposition by bacteria in the Southern California Bight and its implications for the mesopelagic nitrogen cycle. *Mar Ecol Prog Ser* 122:21–26
- Cho BC, Shim JH (1992) Significance of estuarine mixing in distribution of bacterial abundance and production in the estuarine system of the Mankyung River and Dongjin River, Korea. *J Oceanol Soc Korea* 27:154–163
- Eppley RW, Peterson BJ (1979) Particulate organic matter flux and planktonic new production in the deep ocean. *Nature* 282:677–680
- Eppley RW, Renger E, Venrick E, Mullin MM (1973) A study of plankton dynamics and nutrient cycling in the central gyre of the North Pacific Ocean. *Limnol Oceanogr* 18:534–551
- Glibert PM, Garside C, Fuhrman JA, Roman MR (1991) Time-dependent coupling of inorganic and organic nitrogen uptake and regeneration in the plume of the Chesapeake Bay estuary and its regulation by large heterotrophs. *Limnol Oceanogr* 36:895–909
- Grasshoff K, Eberhardt M, Kremling K (1983) Methods of seawater analysis. Verlag Chemie, Weinheim
- Hansell DA, Goering JJ (1989) A method for estimating uptake and production rates for urea in seawater using [^{14}C]urea and [^{15}N]urea. *Can J Fish Aquat Sci* 46:198–202
- Hansell DA, Goering JJ (1990) Pelagic nitrogen flux in the northern Bering Sea. *Cont Shelf Res* 10:501–509
- Harrison WG, Head EJD, Conover RJ, Longhurst AR, Sameoto DD (1985) The distribution and metabolism of urea in the eastern Canadian Arctic. *Deep Sea Res* 32:23–42
- Hobbie JE, Daley RJ, Jasper S (1977) Use of Nuclepore filters for counting bacteria by epifluorescence microscopy. *Appl Environ Microbiol* 33:1225–1228
- Jørgensen NOG, Kroer N, Coffin RB, Yang XH, Lee C (1993) Dissolved free amino acids, combined amino acids, and DNA as sources of carbon and nitrogen to marine bacteria. *Mar Ecol Prog Ser* 98:135–148
- Kokkinakis SA, Wheeler PA (1987) Nitrogen uptake and phytoplankton growth in coastal upwelling regions. *Limnol Oceanogr* 32:1112–1123
- Kristiansen S (1983) Urea as a nitrogen source for the phytoplankton in the Oslofjord. *Mar Biol* 74:17–24
- Kroer N, Jørgensen NOG, Coffin RB (1994) Utilization of dissolved nitrogen by heterotrophic bacterioplankton: a comparison of three ecosystems. *Appl Environ Microbiol* 60:4116–4123
- Lee S, Fuhrman JA (1987) Relationships between biovolume and biomass of naturally derived marine bacterioplankton. *Appl Environ Microbiol* 53:1298–1303
- McCarthy JJ (1970) A urease method for urea in seawater. *Limnol Oceanogr* 15:309–313
- McCarthy JJ (1972) The uptake of urea by natural populations of marine phytoplankton. *Limnol Oceanogr* 17:738–748
- McCarthy JJ, Kamykowski D (1972) Urea and other nitrogenous nutrients in La Jolla Bay during February, March, and April 1970. *Fish Bull US* 70:1261–1274
- McCarthy JJ, Whitley TE (1972) Nitrogen excretion by anchovy (*Engraulis mordax*) and *E. ringens*) and jack mackerel (*Trachurus symmetricus*). *Fish Bull US* 70:395–401
- Mitamura O, Saijo Y (1975) Decomposition of urea associated with photosynthesis of phytoplankton in coastal waters. *Mar Biol* 30:67–72
- Mobley HLT, Hausinger RP (1989) Microbial ureases: significance, regulation, and molecular characterization. *Microbiol Rev* 53:85–108

- Price NM, Cochlan WP, Harrison PJ (1985) Time course of uptake of inorganic and organic nitrogen by phytoplankton in the Strait of Georgia: comparison of frontal and stratified communities. *Mar Ecol Prog Ser* 27:39–53
- Price NM, Harrison PJ (1987) A comparison of methods for the measurement of dissolved urea concentration in seawater. *Mar Biol* 92:307–319
- Probyn TA (1988) Nitrogen utilization by phytoplankton in the Namibian upwelling region during an austral spring. *Deep Sea Res* 35:1387–1404
- Remsen CC, Carpenter EJ, Schroeder BW (1972) Competition for urea among estuarine microorganisms. *Ecology* 53: 921–926
- Remsen CC, Carpenter EJ, Schroeder BW (1974) The role of urea in marine microbial ecology. In: Colwell RR, Morita RY (eds) *Effects of the ocean environment on microbial activities*. University Park Press, Baltimore, p 286–304
- Sahlsten E (1987) Nitrogenous nutrition in the euphotic zone of the Central North Pacific Gyre. *Mar Biol* 96:433–439
- Sahlsten E, Sorensson F, Petterson K (1988) Planktonic nitrogen uptake in the south-eastern Kattegat. *J Exp Mar Biol Ecol* 121:227–246
- Shim JH, Cho BC, Park MG (1994) Distributions of urea and urea decomposition rates in an estuarine system of Mankyung and Dongjin Rivers, Korea. *J Korean Soc Oceanogr* 29:402–413 (in Korean)
- Simon M, Azam F (1989) Protein content and protein synthesis rates of planktonic marine bacteria. *Mar Ecol Prog Ser* 51:201–213
- Slawyk G, Raimbault P, L'Helguen S (1990) Recovery of urea nitrogen from seawater for measurements of ^{15}N abundance in urea regeneration studies using the isotope-dilution approach. *Mar Chem* 30:343–362
- Steinmann J (1976) Untersuchungen über den bakteriellen Abbau von Harnstoff und Harnsäure in der westlichen Ostsee. *Botanica Mar* 19:47–58
- Strickland JDH, Parsons TR (1972) *A practical handbook of seawater analysis*, 2nd edn. Bull Fish Res Bd Can 167
- Turley CM (1985) Biological studies in the vicinity of a shallow-sea tidal mixing front. IV. Seasonal and temporal distribution of urea and its uptake by phytoplankton. *Phil Trans R Soc Lond Ser B* 310:471–500
- Webb KL, Haas LW (1976) The significance of urea for phytoplankton nutrition in the York River, Virginia. In: Wiley M (ed) *Estuarine processes*. Academic Press, New York, p 90–102

This article was submitted to the editor

Manuscript first received: June 27, 1995

Revised version accepted: April 24, 1996