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Seasonal patterns of urea regeneration by size-fractionated microheterotrophs in well-mixed temperate coastal waters

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Urea regeneration by size-fractionated plankton was measured over an annual cycle at a coastal station in the permanently well-mixed waters of the western English Channel. Rates of urea regeneration in the <200 μm fraction varied from 0.6 to 20.6 nmol N L^{-1} h^{-1}. Regeneration rates were lowest in winter and highest in summer. The ratio of the rates of regeneration to uptake of urea was close to 1 on all time (seasonal and nycthemeral), and space (vertical) scales indicating that regeneration by microheterotrophs supplied the totality of urea used by phytoplankton. On an annual basis, urea regenerated by the microheterotrophs (0.98 mol N m^{-2} $year^{-1}$) was equivalent to ~33% of the total regenerated N (urea + ammonium). The major part of urea regeneration was due to the nanoplankton (51%) and microplankton fractions (36%). Regeneration of urea in the picoplankton was detectable only from April to October and represented, on an average, 25% of the total urea regenerated during this period. Urea regeneration in micro- and nanoplankton fractions was mainly associated with ciliates and in the picoplancton fraction with bacteria.

INTRODUCTION

A large number of studies have shown that urea is an important source of N for a great variety of marine phytoplankton, ranking often in importance as much as, or greater than, nitrate (Wafar *et al.*, 1995; Kudela and Cochlan, 2000). Coastal waters can be supplied with urea through terrestrial and atmospheric inputs (Remsen *et al.*, 1974; Timperley *et al.*, 1985; Middelburg and Nieuwenhuize, 2000; Glibert *et al.*, 2001; Lomas *et al.*, 2002) and transfer from sediments (Pedersen *et al.*, 1993; Lund and Blackburn, 1989; Therkildsen and Lomstein, 1994; Rysgaard *et al.*, 1998). *In situ* biological sources of urea are excretion by macrozooplankton and regeneration by microheterotrophs (protozoans and bacteria). However, macrozooplankton provides only a minor fraction of the urea N taken up by phytoplankton (Båmstedt, 1985; Harrison *et al.*, 1985; Miller and Glibert, 1998; Conover and Gustavson, 1999). On the other hand, regeneration by microheterotrophs can provide an important fraction of N needed by phytoplankton (Price *et al.*, 1985; Hansell and Goering, 1989; Slawyk *et al.*, 1990; Cho *et al.*, 1996; Bronk *et al.*, 1998; Lomas *et al.*, 2002). Most of the data on urea regeneration by microheterotrophs, however, are from discrete spatial and temporal measurements except those of Bronk *et al.* (Bronk *et al.*, 1998) and Lomas *et al.* (Lomas *et al.*, 2002) from the Chesapeake Bay where measurements were made in different seasons.

The permanently well-mixed waters of the western English Channel are characterized by high rates of primary production (Boalch *et al.*, 1978; Wafar *et al.*, 1983). Measurements with ^{15}N at a coastal station (Station Astan, Fig. 1) showed that a substantial fraction of total primary production [new and regenerated production, *sensu* (Dugdale and Goering, 1967)] is in the form of regenerated production (L'Helguen *et al.*, 1996) fuelled by intense regeneration of ammonium (Le Corre *et al.*, 1996). Along with ammonium, regeneration of urea was also studied at this station. In this article, we report an annual cycle of *in situ* rates of urea regeneration in three size fractions (<1, 1–15 and 15–200 μm) of plankton measured with a isotope-dilution method. With these

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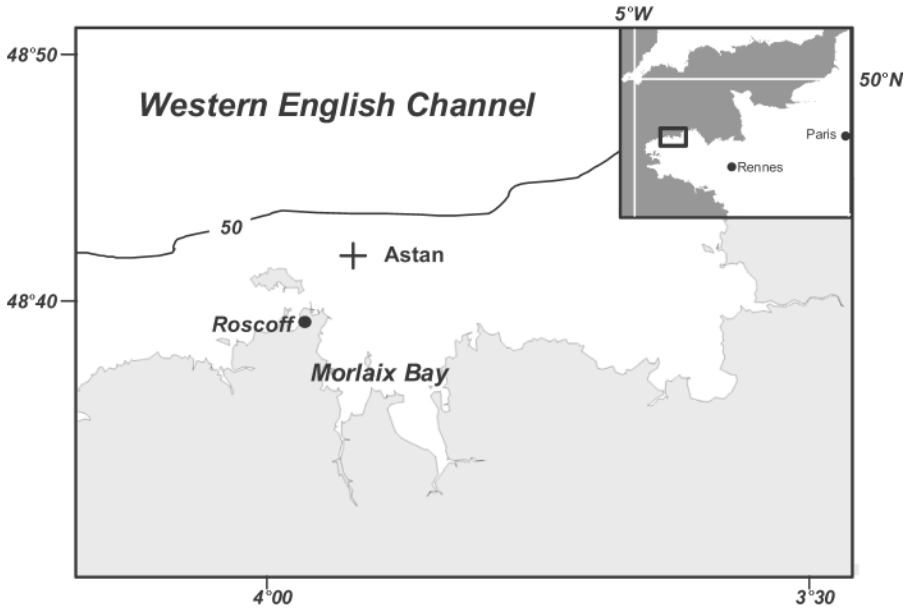

Fig. 1. Location of sampling station.

data, we evaluate the characteristics and importance of urea regeneration and the role of microheterotrophs in this process.

METHOD

Study area and sampling

The study site (48°45′ N; 3°58′ W) was in the north-western part of the Morlaix Bay in the well-mixed waters of the western English Channel (Fig. 1), in a sector little influenced by terrestrial inputs (Wafar *et al.*, 1983; L'Helguen *et al.*, 2000). The mean depth over the station was 45 m. Water samples for hydrographic parameters, nutrients and measurements of N fluxes were collected on 22 field trips, each one at around a neap tide, between January and December, 1988. On each trip, uptake and regeneration of urea were measured on three size fractions (<200, <15 and <1μm) of samples collected from the 50% light-penetration depth. Samples were gravity-filtered through Nytex (bolting silk) nets to get the <200 and <15 μm fractions and vacuum-filtered (<100 mm of Hg) through 1-μm Nuclepore filters to obtain the <1 μm fraction. Regeneration and uptake rates of urea were obtained directly from the <1 μm fraction and indirectly (by difference) from the 15–200 μm and 1–15 μm fractions. In addition to routine measurements at 50% light depth, urea regeneration and uptake rates were measured in unfractionated samples (<200 μm) from five light-penetration depths (100, 50, 14.5, 3.5 and 0%) in February, April, May, June and September. On these occasions, urea fluxes were also measured during the night on samples collected from 50% light-penetration depth.

Chemical and biological parameters

Concentrations of urea were measured in a Technicon Auto-Analyser II following the procedure described by Aminot and Kérouel (Aminot and Kérouel, 1982), with a precision of ±10 nmol N L^{-1} and a detection limit of 20 nmol N L^{-1}. Particulate material for organic nitrogen (PON) measurements was recovered on pre-combusted (450°C, 4 h) Whatman GF/F filters and analysed in a Perkin-Elmer model 240 elemental analyser using acetanilide as the standard. The coefficient of variation of these measurements at concentrations from 0.1 to 5 μmol N L^{-1} was 5%. Water samples fixed with acid Lugol were used for counting nanoflagellates (auto- and heterotrophic forms were not separated) and ciliates. Samples for bacterial counts were fixed in formalin, stained with acridine–orange and filtered onto blackened 0.2-μm Nuclepore filters (Hobbie *et al.*, 1977). Counts were done under epifluorescence in an Olympus BH2 microscope. On a routine basis, bacterial counts were obtained from each size fraction. Ciliates and nanoflagellates were counted from unfractionated samples (<200 μm) once a month in winter and each sampling date after that. On several occasions, counts of nanoflagellates and ciliates were also made on fractionated samples so that the distribution of heterotrophic

Table I: Abundances (average ± SD) and % contribution (average ± SD) of ciliates (n = 8), nanoflagellates (n = 8) and bacteria (n = 22) in micro- (15–200μm), nano- (1–15μm) and picoplankton (<1 μm) fractions

	Size fraction		
	15–200 μm	1–15 μm	<1 μm
Ciliate			
cells L^{-1}	310 ± 490	1060 ± 840	0
%	19 ± 22	81 ± 21	–
Nanoflagellate			
10^3 cells L^{-1}	339 ± 492	373 ± 225	8 ± 8
%	35 ± 21	58 ± 22	7 ± 7
Bacteria			
10^5 cells mL^{-1}	0.38 ± 0.57	0.52 ± 0.33	2.97 ± 1.63
%	9 ± 12	17 ± 12	74 ± 14

and bacterial populations in different size fractions could be determined (Table I).

Rates of urea uptake and regeneration

Uptake and regeneration of urea were measured with the ^{15}N tracer. The isotope dilution method described by Slawyk *et al.* (Slawyk *et al.*, 1990) was used to measure regeneration rates. Each size class (4 L in polycarbonate bottles) was inoculated with 50 nmol N L^{-1} of ^{15}N-labelled urea (95 atom %, CEA, France). Half of the sample was immediately filtered onto pre-combusted GF/F filters so as to determine initial enrichment in ^{15}N of the particulate organic and dissolved fractions. The initial enrichment of the urea pool varied from 19 to 55 atom % excess of ^{15}N. The other half of the sample was incubated in 2-L polycarbonate bottles, under simulated *in situ* conditions for 4 h. All the incubations began 2 h before local noon. At the end of the incubation, the samples were filtered onto pre-combusted GF/F filters under a vacuum <100 mm of Hg. Filters were then oven-dried and stored until isotopic analyses. The filtrate was frozen (–20°C) pending measurements of concentration and ^{15}N enrichment in the urea fraction.

The urea in the filtrate was extracted following the diffusion method described by Slawyk *et al.* (Slawyk *et al.*, 1990). The procedure involves in sequence, remove of initial ammonium from the water sample, hydrolysis of urea with urease and concentration of the liberated ammonium by diffusion. To 200 ml of filtrate, placed in a 500-ml Erlenmeyer flask, 100 mg of MgO to raise the pH (>9) and 2 μmol N of unlabelled urea (as a carrier) were added. A strip of pre-combusted GF/C filter wetted with 50 μl of 0.5 N H_2SO_4 was suspended above the sample. The flask was then capped tightly and left for 1 week at 60°C in an oven. Percent removal of ammonium present in the filtrate, including ammonium of sample and of carrier added, using this diffusion process was 95.9 (coefficient of variation = 1.1%). After discarding the GF/C strip, urease was added to the filtrate. A new acidified GF/C strip was suspended over the filtrate, and the capped flask was incubated for a further week at 60°C, enabling hydrolysis of urea and diffusion of the ammonium produced into the filter strip. At the end of the incubation, the strip was oven-dried at 60°C and stored until isotopic analysis. Urea hydrolysis was >90%, and its recovery by diffusion averaged 91%.

The ^{15}N:^{14}N isotope ratio of particulate matter and of urea recovered from the filtrate was determined by emission spectrometry in a GS1 optical spectrometer (SOPRA, France) following the procedures given by Guiraud and Fardeau (Guiraud and Fardeau, 1980). The coefficient of variation was 1% at a range of 0.5–1.5 atom % excess of ^{15}N. Atom % excess ^{15}N in the urea pool of the aqueous fraction was calculated following the equations given by Slawyk *et al.* (Slawyk *et al.*, 1990) where the measured atom % ^{15}N in the extracted-urea pool was corrected for the amount of unrecovered ammonium of sample and of carrier added. The coefficient of variation, calculated for the experimental determination of ^{15}N enrichment in the urea N pool was 4.5%.

Absolute uptake rates (ρ) were calculated with the equation given by Dugdale and Wilkerson (Dugdale and Wilkerson, 1986) where PON concentration is the one measured at the beginning of the incubation. Urea-uptake rates were corrected for isotope dilution (Glibert *et al.*, 1982); the correction factor varied from 1 to 1.2 (average: 1.05). Urea regeneration rates (R) were calculated from the equation of Laws (Laws, 1984) or that of Glibert *et al.* (Glibert *et al.*, 1982), depending upon whether there were measurable changes in urea concentration during the course of the experiment or not. The precision of uptake and regeneration measurements was estimated on separate incubation bottles ($n = 5$) of the same sample. It includes all the steps of the ^{15}N experiment (inoculation, incubation, filtration and preservation) and the analytical precision of the different parameters required for the rate calculations (urea and PON concentration, ^{15}N enrichment in the urea and particulate N pools). The coefficient of variation was 4 and 3.2% for uptake and regeneration rates, respectively, at the level of 5 nmol N L^{-1} h^{-1}. Mass balance of ^{15}N tracer for the experiments showed almost complete recovery of the ^{15}N added: 99.2% ±5% was accounted for in the combined PON and urea pools at the end of the incubations. Model II regressions were used in all statistical analyses in this article.

RESULTS

Urea concentrations and microheterotroph abundances

Concentrations of urea were an average of 110 nmol N L^{-1} (±50 nmol N L^{-1}) and vertically homogeneous during the course of the seasonal cycle (Fig. 2). Ciliate density varied from 0 to 2.9 × 10^3 cells L^{-1} over the year with a large maximum during spring and summer. A major proportion of the ciliates (81%) was in the 1–15 μm fraction and the remainder in the 15–200 μm fraction (Table I). Nanoflagellate density ranged from 0.14 to 23.3 × 10^5 cells L^{-1} and was generally ~5 × 10^5 cells L^{-1} except in spring when it exhibited a major peak in May. On average, ~58% of the nanoflagellates were in the 1–15 μm fraction, 35% in the 15–200 μm fraction and only 7% in the <1 μm fraction (Table I). Bacteria numbers varied from 1.9 to 8 × 10^5 cells mL^{-1}. They increased during spring and showed a large maximum extending from May to early August followed by an other peak in September. About 74% of the bacteria were present in the <1 μm fraction (Table I).

Urea regeneration rates

Seasonal variations

Rates of urea regeneration ranged from 0.6 to 20.6 nmol N L^{-1} h^{-1} (average: 5.0 ± 4.6 nmol N L^{-1} h^{-1}). Rates were low (average: 1.6 ± 0.9 nmol N L^{-1} h^{-1}) in winter, but increased during spring to a maximum at the end of June (Fig. 3). Subsequently, they remained at relatively high levels (average: 8.7 ± 1.6 nmol N L^{-1} h^{-1}) until September and declined then to low rates in winter. Rates of urea regeneration followed the seasonal patterns observed for urea-uptake rates and were also comparable to the variation of ciliates and bacteria abundances. The ratio between regeneration and uptake rates of urea (R/ρ) was almost close to 1 during the whole seasonal cycle (Fig. 4).

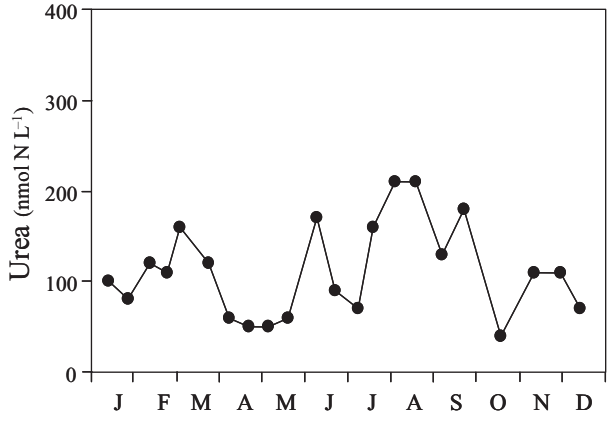

Fig. 2. Seasonal changes of urea concentrations. The depth distribution was homogeneous and the data are averages from three depths.

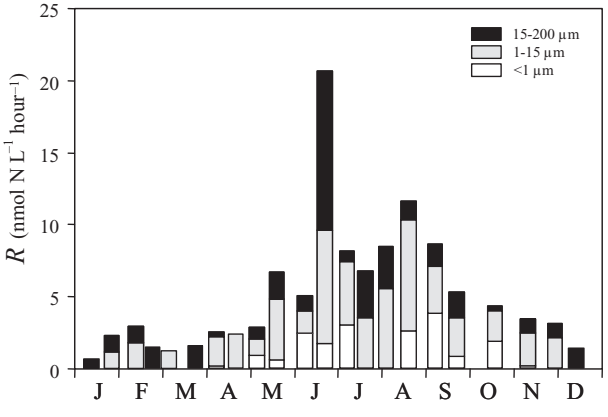

Fig. 3. Seasonal changes of urea regeneration rates in micro- (black bars), nano- (grey bars) and picoplankton (empty bars) fractions at 50% light-penetration depth.

Vertical variations

Rates of urea regeneration were substantially higher within the euphotic zone than below (Fig. 5), usually with a surface or sub-surface maximum. Profiles of regeneration rates were comparable with those of urea-uptake rates. Regeneration rates were generally similar to those of uptake rates both at discrete depths (average

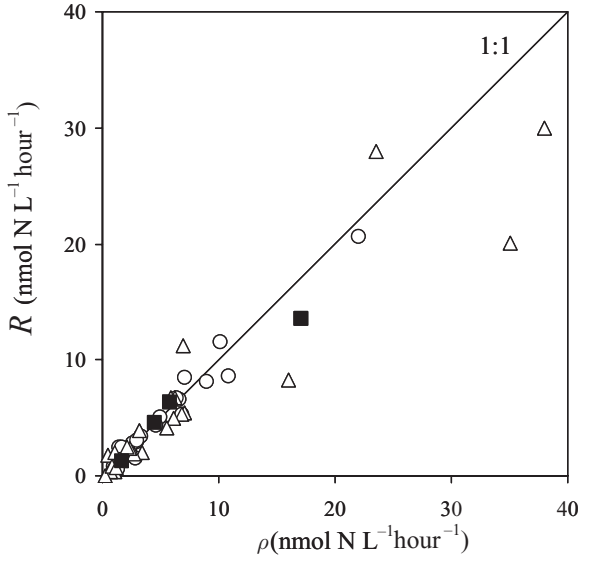

Fig. 4. Relationship between regeneration (R) and uptake rates (ρ) of urea at 50% light-penetration depth during the seasonal cycle (○), at different depths in the water column (Δ) and during the night (■) ($R = 0.81 \times \rho - 0.53$, $r = 0.94$, $n = 51$, $P < 0.01$). The line represents the 1:1 ratio.

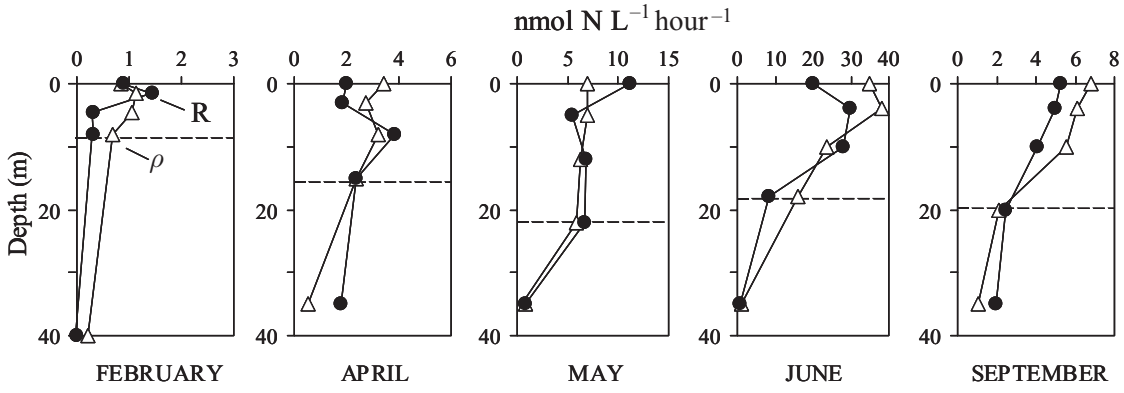

Fig. 5. Vertical profiles of regeneration (●) and uptake (Δ) rates of urea during selected months along the seasonal cycle. The dashed line represents the 1% light-penetration depth.

R/ρ : 1.02 ± 0.62, Fig. 4) and integrated over the water column (average R/ρ : 0.88 ± 0.27).

Diel variations

Rates of urea regeneration measured in the night were 3–55% lower than those measured during the day (Table II). Variations of urea regeneration rates during the course of a day–night cycle were similar to those of uptake; for example, in June, a sharp decrease in regeneration during the night is paralleled by a similar sharp decrease in uptake. The R/ρ ratio for the night time (0.92) was close to the one for day time (0.99) (Table II, Fig. 4).

Size classes

Major part (51% on an average) of urea regeneration (0–7.9 nmol N L^{-1} h^{-1}; average 2.7 ± 2.2 nmol N L^{-1} h^{-1}) was in the 1–15 μm fraction (Fig. 3). The 15–200 μm fraction accounted for about one-third of the total regeneration of urea (0–11.0 nmol N L^{-1} h^{-1}; average: 1.7 ± 2.3 nmol N L^{-1} h^{-1}). In these two size classes, ciliates and nanoflagellates predominated the heterotrophic community (Table I). Regeneration in the 1–200 μm fraction was correlated significantly with the number of ciliates (r = 0.56, $P < 0.05$) but not with the one of nanoflagellates. The average rate of urea regeneration in the <1 μm fraction was 1.0 ± 1.2 nmol N L^{-1} h^{-1} (range: 0–3.8 nmol N L^{-1} h^{-1}). Regeneration of urea in this fraction was detectable only from April to October (Fig. 3), with a maximum in summer. During this period, regeneration in the <1 μm fraction ranged from 4 to 47% (average: 25 ± 16%) of the total urea regenerated. More than 70% of the bacteria enumerated were in this latter size class, whereas nanoflagellates were scarce (5% of the total numbers) and ciliates absent (Table I). During spring and summer, changes in rates of urea regeneration in the <1 μm fraction related well with those of bacterial numbers (r = 0.81, P <0.01).

Table II: Day and night regeneration (R) and uptake rates (ρ) of urea during selected months along the seasonal cycle

	R			*ρ*			*R/ρ*	*R/ρ*
	Night (nmol N L^{-1} h^{-1})	Day (nmol N L^{-1} h^{-1})	N/D	Night (nmol N L^{-1} h^{-1})	Day (nmol N L^{-1} h^{-1})	N/D	Night	Day
February	–	1.5	–	0.7	1.1	0.64	–	1.36
April	1.3	2.4	0.54	1.7	2.7	0.63	0.76	0.89
May	6.4	6.6	0.97	5.8	6.6	0.88	1.10	1.00
June	13.6	30	0.45	17.1	35.1	0.49	0.80	0.85
September	4.6	5.3	0.87	4.5	6.1	0.74	1.02	0.87
Mean			0.71			0.67	0.92	0.99
SD			0.25			0.15	0.17	0.21

N/D is the Night:Day ratio.

Annual budget

Using the relationship between column-integrated urea regeneration rates (R_{Int}) and those measured at 50% light-depth ($R_{50\%Io}$) ($R_{Int} = 14.95 \times R_{50\%Io} + 0.06$, $r = 0.94$, $P < 0.05$) obtained from five sets of data (see above), rates of urea regeneration obtained from 50% light-depth during the seasonal cycle were converted to column rates and further corrected for nighttime regeneration rates with a night/day ratio of 0.71 (Table II). Integration of these monthly rates gave an annual urea production of 0.98 mol N m^{-2} $year^{-1}$. The annual utilization of urea, calculated in a similar way using the relationship between column-integrated urea-uptake rates (ρ_{Int}) and those measured at 50% light-depth ($\rho_{50\%Io}$) ($\rho_{Int} = 15.98 \times \rho_{50\%Io} + 0.03$, $r = 0.99$, $P < 0.01$) and a night/day ratio of 0.67 (Table II), amounted to 0.94 mol N m^{-2} $year^{-1}$.

DISCUSSION

Quantification of planktonic urea regeneration in earlier studies was carried out mainly with macroplankton assemblages or individual species as a function of their physiological status (Conover and Gustavson, 1999). The importance of microheterotrophs, though suspected (Caron and Goldman, 1990), has only been evaluated in a few studies. Using a mass balance approach in which urea concentration changes and ^{15}N-labelled-urea uptake where measured, Price *et al.* (Price *et al.*, 1985) found urea regeneration rates up to 54 nmol N L^{-1} h^{-1} and up to 94 nmol N L^{-1} h^{-1} for frontal and stratified coastal waters, respectively. Using a dual isotopic method (^{14}C and ^{15}N), Hansell and Goering (Hansell and Goering, 1989) measured regeneration rates between 37 and 109 nmol N L^{-1} h^{-1} at two stations in the north-eastern Bering sea. Applying their ^{15}N isotope dilution technique, Slawyk *et al.* (Slawyk *et al.*, 1990) obtained urea regeneration rates ranging from 0 to 46 nmol N L^{-1} h^{-1} at an oceanic station off Galapagos islands. However, much higher rates of urea regeneration prevail in estuarine waters: up to 400 nmol N L^{-1} h^{-1} (Bronk *et al.*, 1998) and between 480 and 1630 nmol N L^{-1} h^{-1} (Lomas *et al.*, 2002) were obtained from studies in Chesapeake Bay. Rates of urea regeneration measured in our study are comparable to those obtained in coastal (Price *et al.*, 1985) and oceanic waters (Hansell and Goering, 1989; Slawyk *et al.*, 1990). However, it is difficult to compare urea regeneration rates without taking into account microheterotroph abundance. Unfortunately, among the few studies in which urea regeneration rates are available, only Price *et al.* (Price *et al.*, 1985) report microheterotroph abundances. Both urea regeneration rates and ciliates counts obtained by the latter are comparable with our data. In addition, seasonal changes of urea regeneration rates in our study followed the patterns of ciliates and bacterial counts suggesting that changes in microheterotroph communities could, at least in part, explain changes in urea regeneration. The seasonal cycle of urea regeneration also followed the patterns described for ammonium regeneration (Le Corre *et al.*, 1996) as well as for nitrogen (nitrate, nitrite, ammonium and urea) uptake (L'Helguen *et al.*, 1996). The seasonal cycle of these latter parameters reflect the distinctive features of primary production in the western English Channel compared to other coastal systems, i.e. a summer maximum related to the strong vertical mixing of these coastal waters (Wafar *et al.*, 1983).

The R/ρ ratio is close to 1 on all time and space scales (Fig. 4) demonstrating that regeneration by microheterotrophs provides the totality of urea taken up. This is different from what was known till now where regeneration of urea by the microheterotrophs was generally lower than urea utilization (Price *et al.*, 1985; Slawyk *et al.*, 1990; Bronk *et al.*, 1998). However, urea concentrations measured in our study as well as those found earlier in the English Channel (Newell, 1967; Aminot and Kérouel, 1982) are lower than urea concentrations generally reported for coastal waters where they exceed frequently 0.5 μmol N L^{-1} (Remsen, 1971; Kristiansen, 1983; Price *et al.*, 1985; Turley, 1986; Pettersson, 1991; Kudela and Cochlan, 2000). The near-uniform low concentrations of ambient urea throughout the year (Fig. 2) and the close coupling observed between uptake and regeneration (Fig. 4) suggest that others sources of urea such as excretion by macrozooplankton or terrestrial and benthic inputs are unimportant in the well-mixed waters of the western English Channel. The low concentrations limited the urea uptake during the major part of the year since the ratios between the maximum uptake rates of urea (ρmax) and the uptake rates at trace ^{15}N addition (ρ) were consistently higher than 1 (L'Helguen *et al.*, 1996). Urea recycled by microheterotrophs is not sufficient to sustain maximum uptake rate; it is totally consumed and therefore does not accumulate in the water column.

On an annual basis, urea regenerated by microheterotrophs (0.98 mol N m^{-2} $year^{-1}$) corresponded to ~50% of the ammonium regenerated (1.98 mol N m^{-2} $year^{-1}$, Le Corre *et al.*, 1996) and therefore to ~33% of the total regenerated N (urea + ammonium) in the study area. Thus, urea is an important source of regenerated nitrogen in the well-mixed waters of the western English Channel. The proportion of nitrogen regenerated as urea was generally high during the whole seasonal cycle but showed seasonal variations, ranging from about half in winter and autumn to about a third in spring and summer. Similar results were shown by Bronk *et al.* (Bronk *et al.*, 1998) in the Chesapeake Bay

where regeneration of urea represented ~50% of the total regenerated N by the microheterotrophs in May but only ~25% in August and none in October

More than 80% of regeneration of urea was associated with the 1–15 and 15–200 μm fractions (Fig. 3) where ciliates and flagellates were the major components of microheterotrophs (Table I) suggesting an important role of protozoans. Though protozoans are known as principal producers of ammonium in the marine environment (Glibert, 1982; Wheeler and Kirchman, 1986; Probyn, 1987; Ferrier and Rassoulzadegan, 1991; Maguer *et al.*, 1999) their role in the regeneration of urea is not well known yet. Our results show that the protozoans also regenerate nitrogen in the form of urea and confirm the important role played by this group of heterotrophs in the regeneration of this compound (Johannes, 1965; Caron and Goldman, 1990; Glibert, 1993). Regeneration in the 1–200 μm fraction was correlated significantly with the number of ciliates but not with the one of flagellates, an important fraction of which could be autotrophs (Le Corre *et al.*, 1996). This suggests that ciliates are the main organisms responsible for the regeneration of urea in the well-mixed waters of the western English Channel. Ciliates are also the principal group of microheterotrophs involved in the regeneration of ammonium in these coastal waters (Le Corre *et al.*, 1996). However, although our study, based on ^{15}N isotope dilution methodology, bore clear evidence of urea production by microheterotrophs, we are not able to specify the importance of each group of protozoans to urea regeneration. This kind of information would need laboratory studies of urea excretion targeted on protozoan species similar to those done by Nagata and Kirchman (Nagata and Kirchman, 1991) on excretion of other organic compounds.

During spring and summer, a significant fraction of urea regeneration (~25% on an average) occurred in the <1 μm fraction (Fig. 3). This could have been mainly mediated by bacteria since (i) they were practically the only heterotrophic organisms in this size class (Table I) and (II) changes in their number related significantly with those of rates of urea regeneration in this fraction during spring and summer. Since a non-negligible fraction of bacteria (26%, Table I) was present in the >1 μm fraction, the contribution of bacteria to urea regeneration could still be higher. Taking into account the total number of bacteria and their specific regeneration rate (nmol N bacteria^{-1} h^{-1}) estimated in the <1 μm fraction, one may evaluate the total urea production by bacteria: it would reach during spring and summer an average of 1.9 nmol N L^{-1} h^{-1} (±1.4) representing 30% of total regeneration of urea by microheterotrophs. The important role of bacteria in the production of urea has already been shown in estuarine waters [up to 100 nmol N L^{-1} h^{-1} (Cho *et al.*, 1996)]. Urea represents one of the organic forms that are readily remineralized during the growth of bacteria (Jørgensen *et al.*, 1993; Kroer *et al.*, 1994), and its production could be related to the existence of easily degradable organic substrate (Satoh, 1980; Lomstein *et al.*, 1989; Therkildsen and Lomstein, 1994). The presence of high concentrations of dissolved organic nitrogen during the periods of high primary production (spring and summer) in the well-mixed waters of the western English Channel (Wafar *et al.*, 1984) thus may have favoured bacterial production of urea.

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