

NITROGEN UPTAKE BY PHYTOPLANKTON AND ZOOXANTHELLAE IN A CORAL ATOLL

L'ABSORPTION DE L'AZOTE PAR LE PHYTOPLANKTON ET LES ZOOXANTHELLES DANS UN ATOLL

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ABSTRACT

This paper describes the nitrogen uptake, measured by assimilation of ^{15}N - labelled nitrate, ammonium, urea and ^{14}C - labelled urea, by phytoplankton, intact corals and freshly isolated zooxanthellae from the Kavaratti Atoll of the Lakshadweep Archipelago (Indian Ocean). This paper also presents the ambient concentrations of these nitrogenous nutrients in the lagoon and surrounding waters. In addition, the excretion of these nitrogen compounds by major coral species of the atoll was also measured. Based on these data, and in conjunction with microbiological studies carried out, nitrogen dynamics in the coral atoll ecosystem located in the nitrogen-impooverished oceanic waters is discussed.

NH_4 is the principal form in which N is excreted by corals, and the rates varied from 223 to 775 ng-at (mg coral tissue) $^{-1}\text{h}^{-1}$ in 4 species. Urea excretion forms about 5% of total N excreted. N balance (NH_4 , urea, NO_3) calculated from 4 species of corals shows that zooxanthellae can derive all their N requirements from coral excretion alone, and that about 80% of the N taken up by zooxanthellae is translocated to corals in 4 hours.

RESUME

Nous présentons ici les résultats sur l'absorption de l'azote par le phytoplancton, les coraux en place et les zooxanthelles fraîchement isolées, de l'atoll de Kavaratti (archipel de Lakshadweep, Océan Indien). Ces études ont été menées en mesurant l'assimilation des composés azotés tels que les nitrates, l'ammoniaque et l'urée marqués au ^{15}N et ^{14}C . Nous présentons également les données sur les concentrations ambiantes de ces sels nutritifs dans les eaux du lagon et autour de l'atoll. Nous avons également mesuré l'excrétion de ces composés par les principales espèces de coraux de l'atoll. En se basant sur ces données, et en liaison avec les études microbiologiques menées parallèlement, nous discutons de la dynamique de l'azote dans les écosystèmes coralliens situés au milieu des eaux océaniques oligotrophes, donc pauvres en azote immédiatement disponible.

NH_4 est le composé principal excrété par les coraux et les taux varient entre 223 et 775 natg (mg tissu corallien N) $^{-1}\text{h}^{-1}$ pour 4 espèces. L'excrétion de l'urée représente environ 5% de l'azote total excrété. Le bilan d'azote (NH_4 , NO_3 , urée) calculé pour 4 espèces de coraux montre que les zooxanthelles peuvent satisfaire tous leurs besoins en azote à partir de l'excrétion corallienne, et que 80% de l'azote absorbé par les zooxanthelles sont transférés dans les coraux en 4 heures.

INTRODUCTION

Nutrient flux studies in coral reefs have received much attention because of the apparent contrast between the high primary productivities of coral reefs, and the low nutrient content of the surface waters surrounding the reef. Very often, dissolved inorganic N and P in these waters are not even adequate to sustain phytoplankton production, let alone the benthic production which is about two orders of magnitude higher. This suggests that the high productivity of coral reefs can be sustained only by an efficient recycling of nutrients within the ecosystem. Such a mechanism exists between corals and zooxanthellae for P (Pilson and Betzer, 1973), and so would for N also, since corals with zooxanthellae excrete very little or no NH_4 , than those without zooxanthellae (Kawaguti, 1953; Szmant-Froelich and Pilson, 1977; Muscatine and D'Elia, 1978), presumably because of the uptake of NH_4 by zooxanthellae.

In this communication, we report our results on the flux of N, in the form of NO_3 , NH_4 and urea, in the Kavaratti atoll. In addition to NO_3 , we selected NH_4 and urea also, because both these are taken up in preference to NO_3 as N source by primary producers (McCarthy et al., 1977), and there are several species which prefer urea above NH_4 and NO_3 (Rensen et al., 1974). Secondly, nitrogen in marine invertebrates is excreted principally as NH_4 and to a certain extent as urea, both of which are potential N source for primary producers. Hence our studies were on ^{15}N uptake for NO_3 , NH_4 and urea, and ^{14}C -urea decomposing activity by phytoplankton, freshly isolated zooxanthellae (hereafter referred to as FIZ) and intact corals, and also N release rates of corals.

MATERIAL AND METHODS

The present study was carried out during a cruise on board R.V. Gaveshani to the Kavaratti atoll ($10^\circ 33' \text{N}$; $72^\circ 36' \text{E}$) of the Lakshadweep Archipelago in the Arabian sea. Kavaratti is a perfect atoll (Fig. 1) and has an island of 3.45 sq. km.

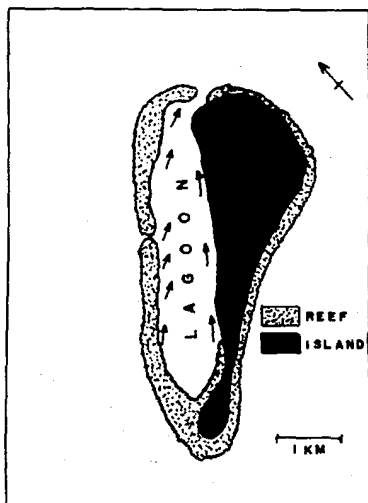


Fig. 1. Map of Kavaratti atoll (Lakshadweep).

The lagoon is about 4500 m long and 1200 m wide. The reef width ranges from 250 to 400 m. Currents in the lagoon follow a unidirectional flow through

the year, accelerating enroute from the SW corner to the entrance (Fig. 1).

Ambient NO_3 , NO_2 , NH_4 and urea concentrations were determined on 25 water samples collected from the oceanic waters close to the reef and 12 samples in the lagoon in 3 transects.

Phytoplankton uptake of N was measured by the ^{15}N method (Dugdale and Goering, 1967). Two litres of water samples were added with $^{15}\text{NaNO}_3$ (97.4 atom %), $^{15}\text{NH}_4\text{Cl}$ (99.1 atom %) and $^{15}\text{NH}_4\text{CO}_3$ (99.2 atom %) in quantities previously calculated so as to approximate 10% of the ambient concentration of a nutrient, and incubated.

Zooxanthellae for ^{15}N uptake studies were isolated from the coral *Pocillopora damicornis*. Pieces of the coral were crushed in a porcelain mortar and homogenized with few ml of Millipore (0.45 μ) filtered sea water (MFSW). The homogenate was strained through a 100 μ Nitex net, centrifuged for 3 minutes at 2000 RPM, and the zooxanthellae were resuspended in MFSW. This procedure was repeated several times until a clear suspension of zooxanthellae was obtained. Equal aliquots of this suspension were then added to MFSW (2 l), and incubated with $^{15}\text{NO}_3$, $^{15}\text{NH}_4$ and ^{15}N -urea, at approximately 10% of the ambient concentrations. Subsamples of zooxanthellae suspension were taken for chl *a* measurements and cell counts.

For ^{15}N uptake by corals, four major species, *P. damicornis*, *Acropora digitifera*, *A. sp.* and *Porites murrayensis*, in the Kavaratti reef were selected. Small pieces of these corals were placed in MFSW (2 l) to which $^{15}\text{NO}_3$, $^{15}\text{NH}_4$ and ^{15}N -urea were added at about 10% of the ambient concentration. At the end of the incubation, the corals were killed by quick-freezing. Zooxanthellae from these corals were separated as above, suspended in 100 ml of MFSW, from which aliquots were taken for ^{15}N assay, chl *a* and cell counts.

The urea decomposing activity was determined as follows: to one litre seawater samples and zooxanthellae suspensions, 1 ml of ^{14}C -urea (56 ng-at N and 0.1 μC) was added. And the end of the incubation, the samples were filtered on 0.45 μ Millipore filter. Aliquots of the filtrate were acidified and bubbled to chase off $^{14}\text{CO}_2$, and 0.5 ml of the filtrate was taken for radioactivity assay. The filters and filtrates were added with 5 ml of NE 220, extracted overnight, and assayed for radioactivity in a liquid scintillation counter. With the 4 species of corals, urea decomposing activity was determined in a similar way (incubation volume 1 l), but the zooxanthellae were separated and recovered after incubations, as was done with ^{15}N experiments.

All incubations - with phytoplankton, FIZ, corals - for both ^{15}N and ^{14}C -urea experiments, were run in triplicate. Incubations were always for 4 hours, from 1000 h to 1400 h and were carried out in tubes filled with running seawater and placed under natural sunlight. All GF/C filters used for the recovery of the particulate organic matter with ^{15}N were previously ignited overnight at 400°C in a muffle furnace.

Excretion of N was measured in the same four species of corals as used for ^{15}N and ^{14}C -urea studies. Small branches and colonies of these corals were kept in dark in running seawater for about 15 hours, then transferred to MFSW in dark, and the N release rates were measured after a 2 h incubation. Preconditioning of the corals to such a long period in dark was necessary to minimize the dark uptake of ammonium (Muscatine and D'Elia, 1978). For each species, 5-10 specime-

ns were tested, and all incubations were run with appropriate controls. At the end of the incubation, coral pieces were recovered and kept frozen pending coral tissue N analyses. These were later homogenized in NH_4 -free distilled water, the homogenate was made up to a standard volume, and aliquots of $1/5$ it were taken for tissue N analyses.

The ^{15}N enrichment and total nitrogen content (PON) of the particulate fraction were determined simultaneously by mass spectrometry using a VG Micromass Model 601 instrument (Winsford, England) equipped with a double collector and coupled with an appropriate nitrogen gas preparation line (Slawyk et al., unpublished).

Nitrate and nitrite were measured following the methods given by Strickland and Parsons (1972) ammonium by the indophenol method of Koroleff (1970), and urea by the diacetylmonoxime method of Newell et al. (1967). Chl *a* from particulate matter and corals were extracted with 90% acetone and measured fluorometrically in a Turner designs fluorometer. N in coral tissue homogenate was converted to NH_4 by the Kjeldahl method (Strickland and Parsons, 1972), and measured by the indo-phenol method as above.

RESULTS

In situ concentrations:

The average concentrations of NO_3 , NH_4 , urea and PO_4 in the open ocean close to the reef and the lagoon waters of Kavaratti atoll are shown in Table 1.

Table 1. Concentration of nutrients ($\mu\text{g-at N l}^{-1}$) in oceanic and lagoon waters of Kavaratti atoll

Nutrient	NO_3	NH_4	Urea	PO_4^*
Oceanic	0.46 ± 0.22 n = 22	1.74 ± 0.94 n = 22	1.36 ± 1.05 n = 25	0.34 ± 0.03 n = 9
Lagoon	0.53 ± 0.19 n = 12	0.77 ± 0.12 n = 11	0.91 ± 0.45 n = 10	0.23 ± 0.09 n = 18

* from Sankaranarayanan, 1973

The differences in the concentrations of NH_4 , urea and PO_4 between the oceanic and lagoon waters are clearly significant, demonstrating a removal of these nutrients as the oceanic water passes over the reef. In the case of NO_3 , however, there could probably be a slight export from the reef to the lagoon.

^{15}N uptake:

a. Oceanic waters: Data on ^{15}N uptake by oceanic phytoplankton and related parameters are shown in Table 2. Clearly NH_4 is the most preferred form as evident from the specific and absolute uptake rates for NH_4 which are one or two orders of magnitude greater than those for urea or NO_3 , and from the RPI indices which show a preference (>1) for NH_4 and rejection (<1) for urea and NO_3 . The high PON/Chl *a* ratio (161) shows that the phytoplankton forms only a fraction of the particulate organic matter, which was composed largely of or-

ganic aggregates that were clearly visible to the naked eye.

b. Lagoon waters: Contrary to in oceanic waters, NH_4 uptake was not evident in lagoon waters, instead urea appeared to be the preferred form, followed by NO_3 (Table 2). Compared to oceanic waters, urea uptake rate was 10 times lower and NO_3 uptake, 4 times. This demonstrates an unhealthy phytoplankton in the lagoon waters in spite of the higher chl *a* concentration, and also the presence of a very large fraction of detritus. Average PON/Chl *a* ratio in the lagoon waters (549) was about 3.5 times greater than in the oceanic waters.

c. by freshly isolated zooxanthellae: The most interesting feature in the nitrogen uptake by freshly isolated zooxanthellae is their preference to urea over NH_4 and NO_3 (Table 3), as shown by uptake rates and RPI index, even though NH_4 was more abundant than either urea or NO_3 .

d. by whole corals: Nitrogen uptake by whole corals (Table 4) demonstrates, on the other hand, a preference for NH_4 followed by urea and NO_3 , in 3 of the 4 species tested, and in the case of *A. sp.*, NO_3 ranks first. Comparison of the sum of uptake of all the 3 forms of N between FIZ and corals show that the nitrogen uptake in corals is 3-9 times lower than with isolated zooxanthellae.

^{14}C -urea uptake:

In the method we have followed, quantity of labelled urea not degraded at the end of the incubation is calculated from the radioactivity of

the filtrate after $^{14}\text{CO}_2$ is driven off, and then, by comparison with the initial concentration of ^{14}C -urea added, the quantity of urea degraded is calculated. In the experiments with seawater samples from open ocean, lagoon and zooxanthellae suspensions, after a routine 4 h incubation, we could not find any residual activity in the filtrate. This demonstrates that all the ^{14}C -urea added has been degraded completely and, as the tracer added was $56 \text{ ng-at urea l}^{-1}$, the rate of urea decomposition was $14 \text{ ng-at l}^{-1} \text{ h}^{-1}$ (Table 5). This is only a minimal estimate and could be still higher if all the tracer urea has been degraded much before the end of the 4 h incubation.

Mean urea assimilation rates (% of radioactivity on the filter/total radioactivity added) of oceanic phytoplankton were 5.6 in light and 4.2 in dark. In the lagoon waters, these rates were 3.9 and 2.7, and with zooxanthellae were

Table 2. Nitrogen uptake by phytoplankton of Kavaratti atoll

Nutrient	$V h^{-1}$	$\mu\text{ng-at } l^{-1} h^{-1}$	% availability	Relative Preference Index	C:N ratio by atoms	PON_1 $\mu\text{g } l^{-1}$	$\text{Chl } a_1$ $\mu\text{g } l^{-1}$
<u>Oceanic waters</u>							
NH_4	0.137 ± 0.033	264.3 ± 31.4	60	1.54	8.1	27.3 ± 6.4 (n = 15)	0.17 ± 0.06 (n = 7)
Urea	0.011 ± 0.002	17.0 ± 8.2	32	0.18	7.6		
NO_3	0.003 ± 0.0004	5.6 ± 0.3	7	0.25	7.8		
<u>Lagoon waters</u>							
NH_4	n.d.	n.d.	21	-	7.3	148.1 ± 23.5 (n = 9)	0.27 ± 0.05 (n = 8)
Urea	0.004	1.80	29	2.00			
NO_3	0.0003 ± 0.0001	1.3 ± 0.5	50	0.84			

Table 3. Nitrogen uptake by freshly isolated zooxanthellae

Nutrient	$V h^{-1}$	μN $\text{ng-at } (\mu\text{g Chl})^{-1} h^{-1}$	% availability	RPI
NH_4	0.023 ± 0.004	6.9 ± 1.0	46	0.59
Urea	0.035 ± 0.005	18.2 ± 1.6	33	2.14
NO_3	0.0007 ± 0.0003	0.3 ± 0.1	21	0.08

3.9 and 5.4.

In the case of incubation of corals with ^{14}C -urea, 10-30% of the added ^{14}C -urea was recovered undegraded in the filtrate. The decomposition rates calculated from these data (Table 5) were 20-50% lower than with isolated zooxanthellae. The assimilation rates were also several times lower than with the isolated zooxanthellae.

Nitrogen excretion by corals:

The average NH_4 and urea excretion rates of the four coral species are shown in Table 6. Variations in NH_4 excretion rates were upto 3 times between the species, but urea excretion rates were reasonably consistent. In spite of the variations in NH_4 excretion rates, urea excretion was generally about 5% of the N excreted, except in *A. digitifera* where it was about 15%.

In most of our experiments, we have observed an increase in NO_3 also in the incubation medium. Szmant-Froelich and Pilson (1977, 1984) have come across a similar situation in their study on NH_4

excretion by *Astrangia danae*, attributed the NO_3 increase to nitrification of NH_4 by bacteria living on the coral colonies, and have adjusted the NH_4 excretion rates for the NO_3 increase. We have also made similar corrections but it is worth mentioning here that the NO_3 increase was usually significant, at times as high as 60-70% of the NH_4 excreted. This would suggest high rates of nitrifying activity at the level of the coral heads.

Chl content of zooxanthellae:

Chl a per million cells of zooxanthellae varied from 1.73 μg in *A. digitifera* to 3.1 μg in *P. murrayensis* (Table 4). These are lower than those reported for other reef corals (4.8-9.6 μg Titlyanov, 1981), *Tridacna* (4.9 μg Smilie, 1976) and a temperate coral (4.22 μg Szmant-Froelich and Pilson, 1984). The low chl content may be because of the strong illumination in the shallow waters (1-2 m) of the lagoon from where these corals were collected.

Table 4. Nitrogen uptake by corals

Nutrient	$V h^{-1}$	ρN ng-at ($\mu g \text{ Chl}$) $^{-1} h^{-1}$	% availability	RPI	Chl a μg (million cells) $^{-1}$
<u>P. damicornis</u>					
NO_3	0.0002	0.5	15	0.99	2.07 ± 0.7 (n = 8)
NH_4	0.0011	1.9	71	0.79	
Urea	0.0004	1.0	14	2.04	
<u>A. digitifera</u>					
NO_3	0.0003	0.5	5	4.09	1.73 ± 0.52 (n = 9)
NH_4	0.0008	1.3	39	1.19	
Urea	0.0007	1.0	56	0.63	
<u>A. sp.</u>					
NO_3	0.0016	2.1	38	1.37	1.87 ± 0.55 (n = 10)
NH_4	0.0010	1.7	20	2.05	
Urea	0.0003	0.3	42	0.17	
<u>P. murrayensis</u>					
NO_3	n.d.	n.d.	52	-	3.10 ± 0.3 (n = 9)
NH_4	0.0027	4.1	30	3.04	
Urea	0.0015	5.0	18	0.58	

DISCUSSION

N uptake by phytoplankton:

Consistent with the results from a wide variety of environments (see Collos and Slawyk, 1980) NH_4 is the most preferred form of N taken up by phytoplankton in oceanic waters, followed by urea and NO_3 . NH_4 accounted for 92% of the N uptake, urea for 6% and NO_3 for 2%. The rates of NH_4 uptake in our study are much higher than in other tropical and subtropical oceanic waters (Dugdale and Goering, 1967; MacIsaac and Dugdale, 1969; Eppley et al., 1973), and might be related to the high ambient NH_4 concentration ($2.1 \mu g\text{-at N l}^{-1}$). Again, this high concentration might explain the low NO_3 and urea uptake even though their concentrations (0.3 and $1.15 \mu g\text{-at N l}^{-1}$) were not negligible. NH_4 at concentrations greater than $1 \mu g\text{-at N l}^{-1}$ suppresses urea and NO_3 uptake (McCarthy and Eppley, 1972; McCarthy et al., 1977); NH_4 concentrations in the sea close to the reef were always above $1 \mu g\text{-at N l}^{-1}$, and as often, between 2 and $3 \mu g\text{-at N l}^{-1}$.

With the lagoon phytoplankton, we found very

low nitrogen uptake, even though the chl a concentration here was higher than in the open sea, which suggests an unhealthy phytoplankton population in the lagoon.

Nitrate flux:

The average nitrate concentration in the sea near the reef was $0.46 \mu g\text{-at N l}^{-1}$, higher than elsewhere ($0.33 \mu g\text{-at N l}^{-1}$) in the Lakshadweep sea (Wafar et al., 1986), and in the lagoon, it was $0.53 \mu g\text{-at N l}^{-1}$. These suggest that the seawater while passing from the open ocean to the lagoon gets enriched with nitrate. The most probable way this could happen in the shallow waters is through nitrification. Our results on N excretion by corals show that significant nitrification occurs in the vicinity of coral colonies. In all our experiments, we have observed increase in NO_3 , in addition to NH_4 and urea, and when the increases in different experiments were averaged, the NO_3 increase is a fairly uniform percentage of the total N increase - 42% in *P. damicornis* and *A. sp.*, 46% in *P. murrayensis* and 48% in *A. digitifera*. In absolute values, these were equivalent to 190-432 $ng\text{-at } NO_3\text{-N increase}$

Table 5. ^{14}C -urea uptake by phytoplankton, zooxanthellae and corals. Values in brackets are turn-over times calculated from urea-N transport rates.

Sample	Decomposition rate $\text{ng l}^{-1} \text{h}^{-1}$	Ambient urea concentration nM l^{-1}	Assimilation rate	Turn-over time (days)
<u>Open Ocean</u>				
Light	7	575	5.6	3.4 (2.8)
Dark	7	575	4.2	
<u>Lagoon</u>				
Light	7	600	3.9	3.6 (27.8)
Dark	7	600	2.7	
<u>Zooxanthellae</u>				
	$\text{nM } (\mu\text{g chl})^{-1} \text{h}^{-1}$	$\text{nM } (\mu\text{g chl})^{-1}$		
Light	0.52	34.65	3.9	2.8 (2.2)
Dark	0.52		5.4	
<u><i>P. damicornis</i></u>				
Light	0.42	34.37	1.34	3.4 (2.9)
Dark	0.49		0.06	
<u><i>A. digitifera</i></u>				
Light	0.42	31.07	0.93	3.1 (2.6)
Dark	0.64		0.08	
<u><i>A. sp.</i></u>				
Light	0.32	30.45	0.73	4.0 (8.5)
Dark	0.36		0.13	
<u><i>P. murrayensis</i></u>				
Light	0.28	30.39	0.19	4.5 (0.5)

$\text{h}^{-1} \text{l}^{-1}$ of water immediately surrounding a coral colony with a biomass of 1 mg N. We have not measured coral biomass on the reef, but even at a very conservative estimate of 1 mg coral N l^{-1} of the water over the reef, the nitrification rates on the reef would be one to two orders of magnitude higher than those known for oceanic waters (Kaplan, 1983).

The organisms responsible for a major removal of nitrate from reef waters would be the corals themselves. There is evidence to show that symbiotic corals can take up NO_3 from the seawater (Franzisket, 1974; D'Elia and Webb, 1977) and that the symbiotic algae exhibit assimilatory nitrate reductase activity (Crossland and Barnes, 1977). Our own results show that NO_3 is taken up by FIZ (though only at an insignificant rate compared to NH_4 and urea uptake) and also by corals. It is of particular interest to compare the percentage of NO_3 taken up as the percentage of $\text{NH}_4 + \text{NO}_3 + \text{urea}$ taken up, by FIZ and by corals. In FIZ, it was only 1%, but in the intact corals, it was 14% (*P. damicornis*), 18% (*A. digitifera*) and 51% (*A. sp.*). The greater uptake of NO_3 in intact corals may reflect the continuous production of NO_3 in the incubation medium which attained several $\mu\text{g-at l}^{-1}$

at the end of the incubation than in the experiments with isolated zooxanthellae. This confirms the conclusions of D'Elia et al. (1983), that the capacity for NO_3 uptake can possibly be inductively increased by exposure to high levels of NO_3 . Incidentally the $V\text{-NO}_3$ and $\rho\text{-NO}_3$ both in zooxanthellae and corals, were an order of magnitude lower than V_{max} and ρ_{max} for NO_3 calculated for freshly isolated as well as cultured zooxanthellae (D'Elia et al., 1983); Domotor and D'Elia, 1984).

The coral N:Chl ratio was 50 ± 4 ($n=5$) in *P. murrayensis* i.e. 1 mg of coral N will contain 20 μg Chl. Assuming that this is valid for the other 3 species of corals also, and using the NO_3 uptake rates of zooxanthellae, ratios of the NO_3 uptake rate by unit coral N, and NO_3 increase in incubation medium per unit coral N, can be calculated. These ratios (Table 7) suggest that only a fraction of NO_3 produced would be taken up by the corals. Since the flow is from the reef to the lagoon, any NO_3 produced but remaining unused, in and around coral colonies, will be exported to the lagoon, which may explain higher NO_3 average there. Within the lagoon, the organisms responsible for removal of NO_3

Table 6. NH_4 and urea excretion rates ($\text{ng-at (mg coral tissue N)}^{-1} \text{ h}^{-1}$) in 4 species of corals.

Species	<u>P. damicornis</u>	<u>A. digitifera</u>	<u>A. sp.</u>	<u>A. murrayensis</u>
NH_4	389 + 135 (n = 7)	223 + 82 (n = 6)	554 + 186 (n = 3)	775 + 61 (n = 3)
Urea	23 + 9 (n = 5)	40 + 8 (n = 5)	32 + 17 (n = 3)	33 + 8 (n = 3)

Table 7. Nitrogen balance of reef corals

Species	Uptake/release ratio			N translocated from zooxanthellae to coral $\text{ng-at } (\mu\text{g chl})^{-1} \text{ h}^{-1}$	Total N excretion $\text{ng-at } (\mu\text{g chl})^{-1} \text{ h}^{-1}$	% Basal N requirement satisfied by translocation	
	NO_3	NH_4	urea				
<u>P. damicornis</u>	0.02	0.4	15.8	22.0	87	20.6	107
<u>A. digitifera</u>	0.03	0.8	9.1	22.6	89	13.2	172
<u>A. sp.</u>	0.01	0.3	11.4	21.3	84	29.3	73
<u>P. murrayensis</u>	0.02	0.4	11.0	16.3	64	40.4	40

would be the seagrasses Thalassiosira hemprichii and Cymodocea isoetifolia; the gross production of the seagrass bed at Kavaratti lagoon was $11.97 \text{ gC m}^{-2} \text{ d}^{-1}$ (Qasim and Bhattathiri, 1971) whereas that of the reef was $6.15 \text{ gC m}^{-2} \text{ d}^{-1}$, and of the lagoon floor (excluding seagrass beds) was $3.84 \text{ gC m}^{-2} \text{ d}^{-1}$ (Qasim et al., 1972).

NH_4 flux:

The average concentration of $1.74 \mu\text{g-at } \text{NH}_4 \text{ l}^{-1}$ near the reef is similar to the level of NH_4 reported from coral reefs (Muscatine, 1980). However, this is about 7 times higher than the average levels of NH_4 elsewhere in the euphotic zone of the Lakshadweep sea (Wafar et al., 1986). The reasons for the increase in NH_4 near the reef can either be due to a NH_4 excretion by corals and organisms on the reef slope or the high urea decomposing activity (see urea flux). N excretion of corals clearly show that NH_4 is the principal form of N excreted, and varied from 223 (A. digitifera) to 775 $\text{ng-at (mg coral tissue N)}^{-1} \text{ h}^{-1}$ (P. murrayensis). These are an order of magnitude lower than the NH_4 excretion in the temperate coral A. danae (Szmant-Froelich and Pilson, 1984). We do not have comparable data on reef-building corals, except the study of Muscatine and D'Elia (1978) but their data are not normalised to coral biomass. Our measurements of NH_4 excretion can be underestimated in that there could have been a retention of a fraction of NH_4 within the corals. Muscatine and D'Elia (1978) showed that corals can

take up NH_4 at similar rates in dark as well as in light, and that a dark period of 19 h was necessary to measure release of NH_4 . Corals in our experiments were kept in dark for about 15 h, but this may not mean that there was no retention of NH_4 at all.

Assimilation of NH_4 by corals, FIZ as well as cultured zooxanthellae are fairly well-documented (Kawaguti, 1953; Muscatine and D'Elia, 1978; Burris, 1983; D'Elia et al., 1983; Domotor and D'Elia, 1984; Szmant-Froelich and Pilson, 1984). Muscatine and D'Elia (cf. Szmant-Froelich and Pilson, 1984) report uptake rates of 1.5 to 3.36 $\text{ng-at N } (\mu\text{g chl a})^{-1} \text{ h}^{-1}$ in 3 coral species at NH_4 concentrations of 1 to 3 $\mu\text{g-at N l}^{-1}$. These are in the range of the uptake rates measured in the 4 coral species (Table 4) at similar ambient concentrations. With FIZ from a variety of hosts, D'Elia et al. measured ϕ_{max} of 6.6 to 22.4 $\text{ng-at } (\mu\text{g chl a})^{-1} \text{ h}^{-1}$. In cultured zooxanthellae ϕ_{max} are in the range of 15 to 41 $\text{ng-at } (\mu\text{g chl a})^{-1} \text{ h}^{-1}$ (Domotor and D'Elia, 1984). The ϕ_{NH_4} measured in our studies are lower than these. Likewise, V_{NH_4} in zooxanthellae and corals are also much lower than the V_{max} of 0.04 to 0.12 h (D'Elia et al., 1983). These low rates may probably be because of the lower ambient concentrations of NH_4 in our experiments.

Ratios of NH_4 uptake by zooxanthellae to NH_4 release by corals, are, as for NO_3 , less than 1 (Table 7). These suggest that the release of am-

monium by a coral can easily meet the NH_4 demand by its zooxanthellae at ambient concentrations of NH_4 normally encountered in the reefs. Nevertheless, zooxanthellae can remove NH_4 at rates higher than we have measured (see above) which might explain the removal of NH_4 as the seawater passes over the reef.

Urea flux:

The average urea concentration of $1.36 \mu\text{g-at l}^{-1}$ near the reef is much lower than the average of $2 \mu\text{g-at l}^{-1}$ in the euphotic zone of the Lakshadweep sea (Wafar et al., 1986). This substantial decrease would be due to a high urea-decomposing activity observed in the waters near the reef and the lagoon. Urea decomposition rates were consistently $14 \mu\text{g-at l}^{-1} \text{ h}^{-1}$ and the turnover times (3.4 - 3.6 days) were very short compared to the 59 and 98 days found for oceanic waters (Remsen et al., 1974), demonstrating a high efficiency of biological activity. The high rate of urea decomposition is in conformity with the high biomass of urea-hydrolyzing bacteria. Their average MPN (most probable number) was 209 ml⁻¹ in the oceanic waters near the reef and 243 ml⁻¹ in the lagoon waters, and their proportion in the total number of heterotrophs varied from 40 to 97% in oceanic waters and 32 - 96% in lagoon waters (Chandramohan and Ramaiah, in preparation). The MPN of urea hydrolysers at Kavaratti atoll are an order of magnitude higher than in estuarine and nearshore waters (Rajendran et al., 1980), 2-3 orders of magnitude higher than in oceanic waters (Remsen et al., 1974) and are close enough to those measured in a polluted estuary (Remsen et al., 1972). These results demonstrate clearly that the biomass and activity of urea hydrolysers near the coral reef might assume a high significance in urea flux.

Previous studies on nutrient uptake kinetics (D'Elia et al., 1983) show that zooxanthellae take up ammonium at much faster rates than NO_3 . However, our results show that the zooxanthellae prefer urea more than NH_4 or NO_3 , and assimilate urea N at more than double the rate for NH_4 -N. The preference for urea is reinforced by the fact that NH_4 was available at higher proportion than urea in the incubation medium (Table 3). With intact corals, though, urea uptake was not seen in preference, except in *A. digitifera* where V-urea was as high as V- NH_4 . It is probable that the continuous release of NH_4 by the polyps at rates several times higher than for urea, could have inhibited urea uptake. In phytoplankton, such an effect exists (McCarthy and Eppley, 1972).

Urea release by corals was only 4-5% of the total N excreted, except in *A. digitifera* where it was 15%. Comparisons of urea uptake rates by zooxanthellae and its release rate by corals, gave ratios ranging from 9.1 to 15.8 (Table 7). These suggest that zooxanthellae can derive only 10% of its nitrogen requirements from urea excretion by corals.

Nitrogen balance:

From our data, it would be possible to evaluate whether zooxanthellae can obtain all their N requirements from corals and how far the latter derive benefit from the organic nitrogen synthesized by its zooxanthellae. The sum of NO_3 , NH_4 -1 and urea uptake by FIZ is $25.4 \text{ ng-at } (\mu\text{g chl a})^{-1} \text{ h}^{-1}$. One mg of coral N of *P. murrayensis* contains $20 \mu\text{g chl a}$. Assuming that this is valid for the other 3 species also, the measured total N excretion in Table 6 can be converted to give rates of N excretion $(\mu\text{g chl a})^{-1} \text{ h}^{-1}$. These vary from

13.2 to $40.4 \text{ ng-at } (\mu\text{g chl a})^{-1} \text{ h}^{-1}$ (Table 7). Comparison of these with the N uptake by zooxanthellae shows that they, under symbiotic conditions, can obtain all their N requirements from coral excretion alone. Zooxanthellae in our experiments were most likely taking up N at near their maximum rate, because the total N uptake of $25.4 \text{ ng-at } (\mu\text{g chl a})^{-1} \text{ h}^{-1}$ is close to the maximum values of P_{max} of $22.4 \text{ ng-at } (\mu\text{g chl a})^{-1} \text{ h}^{-1}$ in FIZ (D'Elia et al., 1983) and $24 \text{ ng-at } (\mu\text{g chl a})^{-1} \text{ h}^{-1}$ in cultured zooxanthellae (Domotor and D'Elia, 1984). Exception was *A. digitifera*, but it is possible that our measurements of N excretion on this species may be underestimates. Besides, NH_4 uptake concurrent with release cannot be completely ruled out.

Translocation of a substantial fraction of the photosynthesized carbon (Muscantine and Cerniichiari, 1969) and nitrogen (Burris, 1983) from zooxanthellae to host in intact corals is known. The magnitude of this translocation can be calculated, albeit roughly, from our data, assuming that the isolated zooxanthellae and those in corals take up N at the same rate and that the differences in N incorporated between the two is proportional to N translocation. These show that, with the exception of *P. murrayensis* (64%), the % of N translocated in all 3 corals is nearly the same (84 to 89%) (Table 7). This amount, when compared with the N excretion rate, shows that N translocated can satisfy from 40 to 100% of the basal N requirements of corals; total N requirements of reef corals, though, are not known.

These results, though preliminary and obviously with some limitations, nevertheless show that the recycling of N between corals and zooxanthellae is very efficient, and this tight coupling can explain the high primary productivity observed in coral atolls in the midst of oligotrophic waters. While zooxanthellae can obtain all their N requirements from the hosts, the corals may have to depend additionally on external sources of N. One such source known is the zooplankton in the waters passing over the reef and this could be of importance to coral nutrition in the Lakshadweep atolls, since the biomass of zooplankton in the lagoon was consistently lower than in the sea outside the reef (Tranter and Gean, 1969; Wafar, 1977).

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