

## NITROGENOUS NUTRIENTS AND PRIMARY PRODUCTION IN A TROPICAL OCEANIC ENVIRONMENT

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### ABSTRACT

Measurements of the concentrations of nitrogenous nutrients and primary production were made at 10 stations along 8°N and 10°N in the tropical oceanic Lakshadweep waters. Inorganic nitrogen (NO<sub>3</sub>, NO<sub>2</sub> and NH<sub>4</sub>) accounted for less than 10%, and dissolved organic nitrogen (DON) for more than 90%, of the total dissolved N in the euphotic zone. Urea formed a nearly constant fraction of total dissolved N (17-18%) and of DON (19-20%). Addition of NO<sub>3</sub>, NH<sub>4</sub> and urea as N source stimulated carbon fixation at all stations and at all depths. This, together with the low ambient concentrations of inorganic nitrogen compounds, demonstrates that the phytoplankton in these waters are nitrogen limited. Nitrate flux into the euphotic zone, computed from the vertical eddy diffusion coefficient and NO<sub>3</sub> gradient data, showed that the production associated with NO<sub>3</sub> assimilation varied from 11 to 60% (average 37%) of the observed primary production. Zooplankton regenerated NH<sub>4</sub> at an average rate of 0.59 μg-at N·(mg dry wt)<sup>-1</sup>·d<sup>-1</sup>. Assimilation of this quantity of NH<sub>4</sub> would account for 9-50% (average 23%) of the measured primary production. Stimulation of carbon fixation on addition of urea was, on an average, as much as with either NO<sub>3</sub> or NH<sub>4</sub> addition, demonstrating a potential for urea uptake by phytoplankton in these waters. Zooplankton regenerated urea at an average rate of 0.32 μg-at N·(mg dry wt)<sup>-1</sup>·d<sup>-1</sup>, providing nitrogen for 5-27% (average 13%) of the primary production. Excluding urea, the rest of the DON, though abundant, does not appear to be directly available for phytoplankton assimilation.

The concept that nitrate is the sole nitrogen source for primary production in the sea has changed during the last two decades. Ammonium has been recognized as an important alternative nitrogen source (Dugdale and Goering, 1967) and in most environments, may even be assimilated in preference to nitrate (Harvey and Caperon, 1976; McCarthy et al., 1977), accounting for as high as 80% of the inorganic nitrogen uptake in oligotrophic waters (MacIsaac and Dugdale, 1972). Similarly, the importance of urea to phytoplankton production has also been recognized. A large number of phytoplankton species isolated from estuarine and nearshore environments utilize urea effectively (Carpenter et al., 1972; Antia et al., 1975) and natural phytoplankton communities also assimilate urea, and show a preference for urea over nitrate, though ammonium is still the most preferred form (Harvey and Caperon, 1976; McCarthy et al., 1977).

Within the euphotic zone, ammonium and urea, derived mainly from excretion by zooplankton, form the bulk of the regenerated nitrogen. In temperate latitudes during summer, when nitrate is exhausted from the euphotic zone, ammonium regenerated by zooplankton excretion provides over 75% of the inorganic nitrogen requirements of phytoplankton (Wafar, 1981). To this must be added the contribution of urea to phytoplankton production, since urea accounts for a significant fraction of the total N excreted by zooplankton, and the phytoplankton can utilize ecologically significant concentrations of urea (McCarthy, 1972a). In southern California coastal waters, urea accounts for an average of 28% of the phytoplankton nitrogen productivity in summer (McCarthy, 1972b).

In tropical waters where nitrate is seldom abundant, contribution of regenerated nitrogen to primary production becomes even more important. In a tropical eutrophic environment, Harvey and Caperon (1976) found that the in situ regeneration of N was far larger than the new N added, and provided the primary

nitrogen source to support a large phytoplankton biomass and high productivity. Extending this, it can be argued that phytoplankton in tropical oceanic environments would depend almost exclusively on regenerated nitrogen.

In this paper, the distribution of nitrogenous nutrients, nitrate, nitrite, ammonium, urea and dissolved organic nitrogen (DON) in the euphotic waters and below, and primary production rates in unenriched seawater samples and in those enriched with phosphate, nitrate, urea and ammonium, at 10 stations in the Lakshadweep Sea (8°–14°N; 71°30'–74°E) are reported. Urea and ammonium regeneration rates by zooplankton have also been measured, and based on these results, the relative importance of regenerated and new nitrogen, and DON, to phytoplankton production in these tropical oligotrophic waters is discussed.

### MATERIALS AND METHODS

During the 116th cruise of R. V. GAVESHANI to the Lakshadweep Sea in February 1983, 10 stations in two transects (8°N; 72°–74°E and 10°N; 72°20'–74°E) were occupied. Light penetration was measured by a Secchi Disc and depths corresponding to 60% (12 m), 30% (30 m), 16% (55 m) and 1% (80 m) light penetration were calculated. Water samples from these depths, as also from the surface and 200 m depth, were obtained with 10-liter Van Dorn samplers. On each water sample, the following analyses were carried out.

*Nutrients.*—Nitrate, nitrite and phosphate were measured following the methods given by Strickland and Parsons (1972), ammonium by the indo-phenol method of Koroleff (1970), urea by the diacetylmonoxime method of Newell et al. (1967) and DON by persulfate oxidation to nitrate, the latter two methods being as given by Grasshoff (1976).

*Phytoplankton and Primary Production.*—Particulate matter of each water sample was separated onto a non-MgCO<sub>3</sub> treated GF/C filter pad and was used for the fluorometric determination of chlorophyll *a* and phaeopigments. Primary production was measured by incubating in duplicate 125-ml aliquots of water samples from each depth with 1 ml of Na<sub>2</sub>H<sup>14</sup>CO<sub>3</sub> (5 μCi). Each set of bottles was covered with pre-calibrated Khalsico neutral density filters to simulate appropriate submarine light intensity. All incubations were run for 24 h on the deck under natural sunlight in tubs filled with running seawater.

*Enrichment Experiments.*—At each station, additional subsamples were drawn from the sample obtained at the 60% light transmission depth and were dispensed into eight 125-ml glass bottles. To these, required volume of KH<sub>2</sub>PO<sub>4</sub> (50 μl) was added to obtain a final phosphate concentration of 0.6 μg-at P·liter<sup>-1</sup>. Two of these bottles were set aside as controls. To the rest, solutions of KNO<sub>3</sub> (500 μl), NH<sub>4</sub>SO<sub>4</sub> (50 μl) and urea (25 μl) each were added to two bottles to attain a final nitrogen concentration of approximately 5 μg-at N·liter<sup>-1</sup>. All the eight bottles were treated with <sup>14</sup>C in the form of Na<sub>2</sub>H<sup>14</sup>CO<sub>3</sub> (5 μCi) and were incubated for 24 h. The <sup>14</sup>C filters, both in the enrichment experiments and primary production measurements, were extracted in 5 ml of NE 220, and <sup>14</sup>C activity was measured in a Packard model liquid scintillation counter.

At one station, enrichment experiments were done for the entire euphotic column, i.e., all the samples from different light-depths were subsampled and enriched with nutrients as above. After the addition of nutrients and inoculation with <sup>14</sup>C they were covered with neutral density filters and were incubated on the deck. Thus it was possible to obtain control primary production profile (only PO<sub>4</sub> added) and experimental profiles (NO<sub>3</sub>, NH<sub>4</sub> and urea added) for the euphotic zone.

*Zooplankton Biomass and Excretion.*—Zooplankton was collected with a Héron-Tranter (HT) net (mouth area 0.25 m<sup>2</sup>; mesh size 300 μm) in vertical hauls from 200 m to surface, and biomass was estimated directly as dry wt. During a later cruise to the same area in November 1983, live zooplankton was collected by horizontal and vertical hauls for N excretion measurements. These were transferred to large glass troughs, and after removing the debris and dead zooplankton, were allowed to stand for 1–2 h. At the end of this period, the dead zooplankton was once again removed and the remaining were transferred gently to filtered sea water of known volume in glass troughs. Excretion of urea and ammonium was measured after a 2 h incubation in dark.

### RESULTS

Distribution of nitrate, phosphate and ammonium at the 10 stations along 8°N and 10°N is shown in Figure 1.

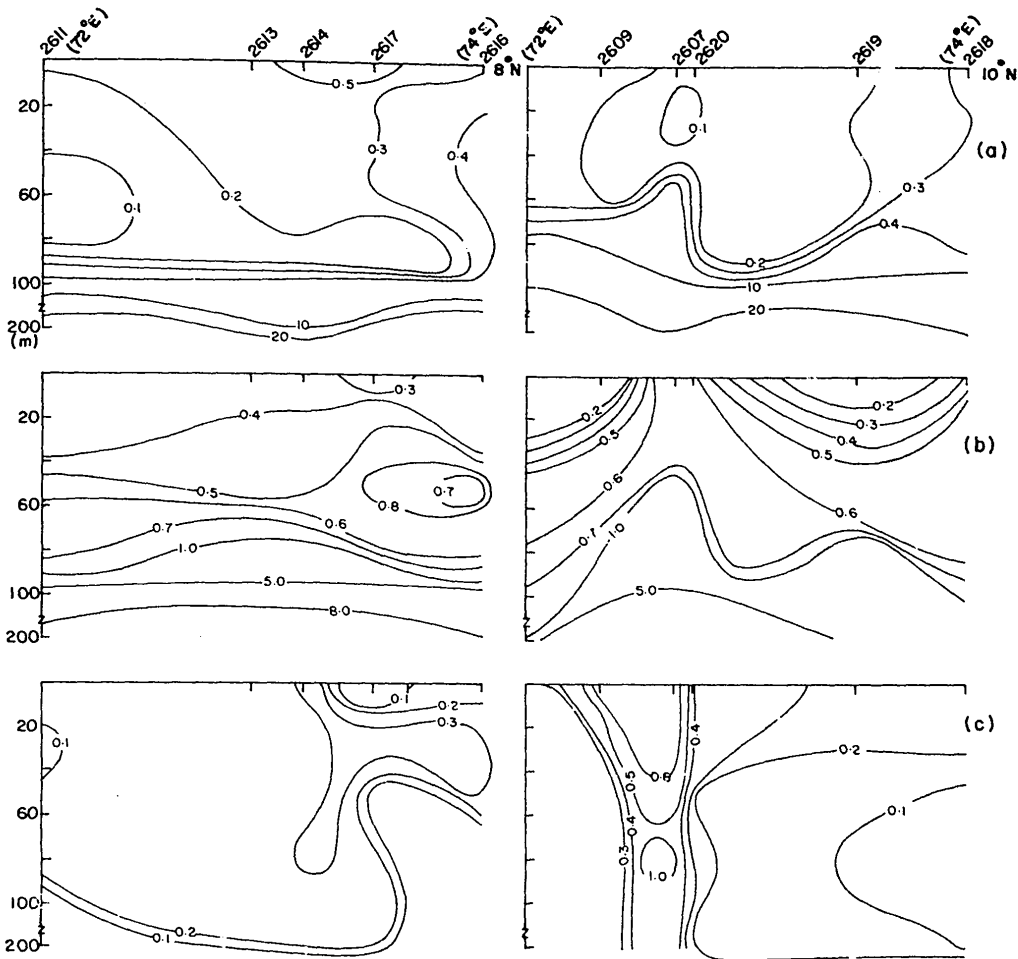


Figure 1. Vertical distribution of (a) nitrate, (b) phosphate and (c) ammonium. Isolines in  $\mu\text{g-at N}\cdot\text{liter}^{-1}$  and  $\mu\text{g-at P}\cdot\text{liter}^{-1}$ .

**Nitrate.**—Concentrations of nitrate were generally between  $<0.1$  and  $0.5 \mu\text{g-at N}\cdot\text{liter}^{-1}$  in the euphotic zone along both transects, with occasional values up to  $1 \mu\text{g-at N}\cdot\text{liter}^{-1}$ . At two stations along  $10^\circ\text{N}$ , nitrate varied between 4 and  $8 \mu\text{g-at N}\cdot\text{liter}^{-1}$  at 80 m depth (corresponding to 1% light intensity). The increased availability of nitrate at this depth resulted in an increased  $^{14}\text{C}$  assimilation at 1% light depth ( $1.8 \text{ mg C}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ ) over the 16% light depth ( $1 \text{ mg C}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ ) at stn 2607. Below the thermocline, nitrate increased rapidly to attain concentrations of  $20 \mu\text{g-at N}\cdot\text{liter}^{-1}$  and above at 200 m. Mean nitrate concentrations in the euphotic zone were  $0.33$  and  $1.05 \mu\text{g-at N}\cdot\text{liter}^{-1}$  along  $8^\circ$  and  $10^\circ\text{N}$ , equivalent to 2.9 and 9.4% of the total dissolved nitrogen.

**Phosphate.**—Phosphate ranged from  $0.2$  to  $0.7 \mu\text{g-at P}\cdot\text{liter}^{-1}$  in the euphotic zone along both transects, with the concentrations generally increasing with depth. At stn 2607, phosphate values were much higher than at other stations ( $0.68 \mu\text{g-at P}\cdot\text{liter}^{-1}$  down to 30 m depth and  $1 \mu\text{g-at P}\cdot\text{liter}^{-1}$  down to 80 m). At 200 m, phosphate concentrations were generally around  $8 \mu\text{g-at P}\cdot\text{liter}^{-1}$ .

**Ammonium.**—Ammonium varied from trace concentrations to  $0.35 \mu\text{g-at N}\cdot\text{liter}^{-1}$  in the euphotic zone and at 200 m, along both  $8^\circ\text{N}$  and  $10^\circ\text{N}$ . At stn 2607,

Table 1. Vertical distribution of urea and DON ( $\mu\text{g-at N}\cdot\text{liter}^{-1}$ )

Station		Depth (m)					
		0	12	30	55	80	200
2607	Urea	3.4	1.5	1.4	2.4	0.4	2.6
	DON	10.3	10.9	6.6	8.7	8.2	8.2
2609	Urea	2.2	0.9	1.1	2.1	3.0	1.9
	DON	8.2	11.2	10.7	12.4	22.5	42.4
2611	Urea	3.7	2.4	4.8	3.1	1.7	3.9
	DON	13.0	13.1	13.8	9.4	6.0	—
2614	Urea	1.9	1.4	2.7	1.8	2.5	1.5
	DON	7.7	11.5	—	12.2	16.4	18.4
2616	Urea	1.9	0.6	1.0	1.1	1.0	2.4
	DON	12.7	9.9	9.7	11.0	13.3	1.4
2617	Urea	1.5	1.4	1.8	2.0	2.7	3.3
	DON	8.4	8.8	9.0	8.6	11.5	—
2618	Urea	2.4	1.1	2.2	1.4	2.7	—
	DON	15.5	9.8	11.7	10.4	7.7	1.9
2619	Urea	4.9	2.6	1.8	1.4	1.6	1.7
	DON	8.5	9.5	6.9	7.3	6.4	—
2620	Urea	2.0	2.3	2.7	2.2	2.7	3.6
	DON	14.8	8.0	9.9	9.2	7.9	4.7

and down to 30 m at stn 2609, ammonium values were higher than  $0.5 \mu\text{g-at N}\cdot\text{liter}^{-1}$ , with a highest value of  $1.1 \mu\text{g-at N}\cdot\text{liter}^{-1}$  at 80 m depth at stn 2607. There were no marked variations in the ammonium concentrations above and below the thermocline. Mean ammonium concentrations in the euphotic zone were  $0.2$  and  $0.3 \mu\text{g-at N}\cdot\text{liter}^{-1}$ , representing 1.8 and 2.8% of total dissolved nitrogen, along  $8^\circ$  and  $10^\circ\text{N}$  respectively.

*Nitrite.*—Nitrite was generally not present in measurable quantities and when present, its concentration rarely exceeded  $0.1 \mu\text{g-at N}\cdot\text{liter}^{-1}$  except at the bottom of the euphotic zone, where it was between  $0.2$  and  $0.35 \mu\text{g-at N}\cdot\text{liter}^{-1}$ .

*Urea.*—Urea concentration varied from  $<0.1$  to  $5 \mu\text{g-at N}\cdot\text{liter}^{-1}$  (Table 1). Vertical distribution of urea showed wide fluctuations with depth at all stations; only at stn 2617 urea appeared to increase with depth. Such patchy distribution of urea is not an uncommon feature (Remsen, 1971; McCarthy, 1972b). However, in spite of the wide fluctuations, mean urea concentrations along  $8^\circ\text{N}$  ( $2.0 \mu\text{g-at N}\cdot\text{liter}^{-1}$ ) and  $10^\circ\text{N}$  ( $1.9 \mu\text{g-at N}\cdot\text{liter}^{-1}$ ) were remarkably similar. Interestingly, urea forms a nearly constant fraction of total dissolved nitrogen (18.2 and 17.3% along  $8^\circ$  and  $10^\circ\text{N}$ ), more particularly of DON (19.2 and 19.8%). This is remarkably close to the 21% calculated by Remsen (1971) for the continental shelf surface waters off Peru. As with ammonium, there were no differences in the urea concentrations above and below the thermocline.

*Dissolved Organic Nitrogen.*—Within the euphotic zone, DON concentrations ranged from  $6.4$  to  $22.4 \mu\text{g-at N}\cdot\text{liter}^{-1}$ , and as in the case of urea, showed wide fluctuations with depth (Table 1). The mean concentrations of DON in the euphotic zone ( $10.9$  and  $9.8 \mu\text{g-at N}\cdot\text{liter}^{-1}$  along  $8^\circ$  and  $10^\circ\text{N}$ ) were not significantly different. At 200 m depth, DON concentrations were several times lower than those measured in the euphotic zone.

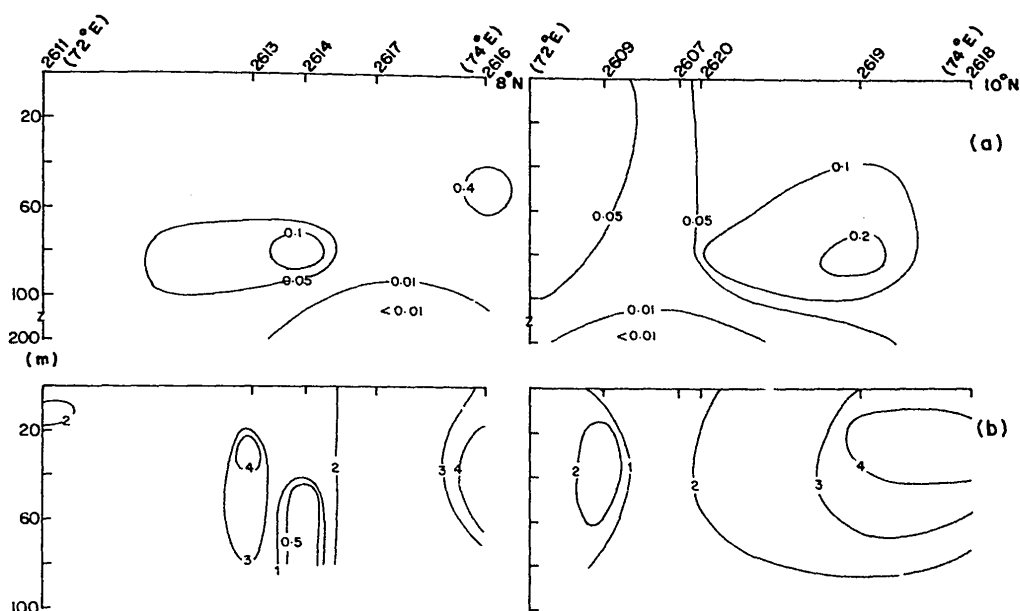


Figure 2. Vertical distribution of (a) chlorophyll *a* and (b) carbon assimilation. Isolines in  $\mu\text{g}\cdot\text{liter}^{-1}$  and  $\mu\text{g C}\cdot\text{liter}^{-1}\cdot\text{d}^{-1}$ .

DON was the major fraction of total dissolved nitrogen, accounting for 94.9% along  $8^\circ\text{N}$  and 87.5% along  $10^\circ\text{N}$ . Within the euphotic zone, its contribution to total dissolved nitrogen was always in excess of 90%; only below the thermocline did the proportion of DON decrease, to as low as 5–10%.

**Chlorophyll *a*.**—Concentrations of chl *a* were generally less than  $0.1 \mu\text{g}\cdot\text{liter}^{-1}$ , except for occasional values above 0.1 and up to  $0.5 \mu\text{g}\cdot\text{liter}^{-1}$  at certain depths (Fig. 2). Column chl *a* values were generally between 2 and  $5 \text{ mg}\cdot\text{m}^{-2}$ , except at stns 2619 ( $10^\circ\text{N}$ ) and 2616 ( $8^\circ\text{N}$ ) where they were 8 and  $13 \text{ mg}\cdot\text{m}^{-2}$  respectively. Mean chl *a* concentrations within the euphotic zone were the same ( $0.6 \text{ mg}\cdot\text{m}^{-3}$ ;  $4.8 \text{ mg}\cdot\text{m}^{-2}$ ) along both transects.

**Primary Production.**—Carbon fixation ranged from  $<1$  to  $5 \text{ mg C}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ , with maximum production at light intensities corresponding to 30–60% of the incident light (Fig. 2). Column production varied between 74 and  $306 \text{ mg C}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ . In spite of the variations in carbon assimilation rates at discrete depths and in the water column between stations, average rates of primary production along both the transects, like chl *a*, were quite close ( $2.5$  and  $2.7 \text{ mg C}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ ; 200 and  $216 \text{ mg C}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ ). A comparison with the earlier data on chl *a* and primary production from the same area during the same period (Bhattathiri and Devassy, 1979) showed that while mean chl *a* concentration was the same in both studies ( $4.8 \text{ mg}\cdot\text{m}^{-2}$ ), primary production in the present study was about 40% less than in the earlier ( $372 \text{ mg C}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ ).

**Enrichment Experiments.**—Addition of urea, ammonium and nitrate as N source increased carbon fixation higher than in controls, with the increase varying from 4% with addition of urea at stn 2619 to 168% with addition of ammonium at stn 2607 (Fig. 3). Individually, addition of nitrate increased carbon fixation from 16 to 104%, ammonium from 15 to 168%, and urea from 4 to 138%. When the increases in carbon assimilation as a result of enrichment at seven stations were

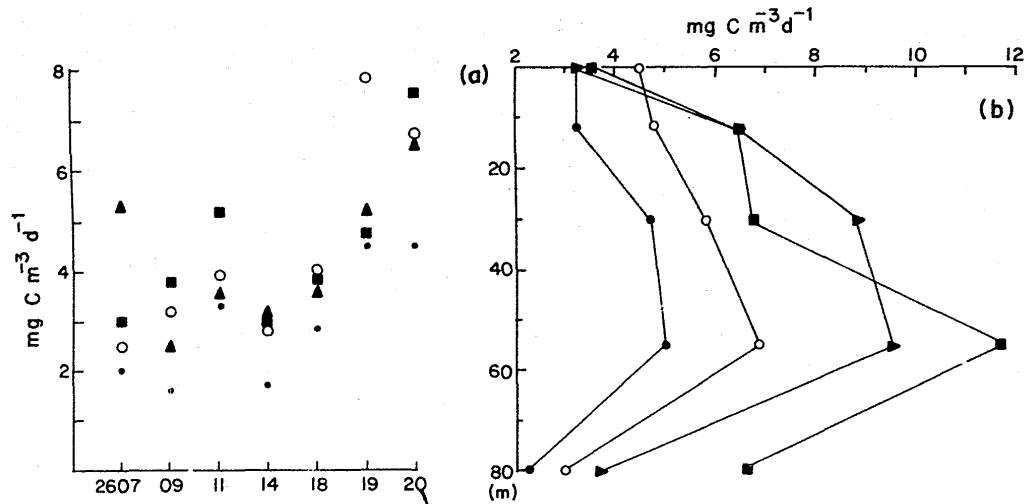


Figure 3. Increase in carbon assimilation on nutrient supplement: (a) at different stations and (b) at different depths. Closed circles—control; open circles—nitrate added; triangles—ammonium added; squares—urea added.

averaged, the mean increases were surprisingly close, i.e., 54, 59 and 60% with addition of nitrate, ammonium and urea, respectively.

Addition of urea resulted in a more significant increase in carbon assimilation than with either nitrate or ammonium at stns 2609, 2611 and 2620. Such effect was found with addition of ammonium and nitrate at stns 2607 and 2619, respectively. At stns 2614 and 2618, though there was increase in carbon assimilation with the addition of all these nitrogen sources, the increase caused by any of these was not significantly different from the others.

Carbon assimilation at all light intensities increased with the addition of nitrate, urea and ammonium (Fig. 3). Column primary production was twice as high with addition of ammonium ( $595 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ ) and urea ( $640 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ ) as with the unenriched samples ( $332 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ ); addition of nitrate enhanced primary production only to a lesser extent ( $433 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ ).

*Ammonium and Urea Excretion by Zooplankton.*—Release of ammonium nitrogen varied from  $0.15$  to  $1.09 \mu\text{g-at} \cdot (\text{mg dry wt})^{-1}$  of unsorted zooplankton  $\text{d}^{-1}$ , probably reflecting variations in the zooplankton species composition among the stations. Average excretory rate from 11 measurements was  $0.59 \mu\text{g-at} \cdot (\text{mg dry wt})^{-1} \cdot \text{d}^{-1}$ . The range and the average ammonium release rates do not differ much from those recorded earlier for unsorted zooplankton (Jawed, 1973; Mullin et al., 1975; Smith and Whitlege, 1977).

Urea nitrogen release varied from  $0.06$  to  $0.52 \mu\text{g-at} \cdot (\text{mg dry wt})^{-1}$  of unsorted zooplankton  $\text{d}^{-1}$ . Mean excretory rate from 10 measurements was  $0.32 \mu\text{g-at} \cdot (\text{mg dry wt})^{-1} \cdot \text{d}^{-1}$ . Unlike the case of ammonium, data on urea release rates of unsorted zooplankton are not abundant. Smith and Whitlege (1977) measured an average excretion rate of  $0.29 \mu\text{g-at} \cdot (\text{mg dry wt})^{-1} \cdot \text{d}^{-1}$  for the  $223\text{-}505 \mu\text{m}$  size fraction zooplankton from a coastal upwelling off northwest Africa, a value quite close to that obtained in the present study.

## DISCUSSION

Phytoplankton in the Lakshadweep Sea are inorganic nitrogen limited, regardless of whether it is in the form of nitrate or ammonium available for assimilation.

This is evident by the low ambient concentrations of nitrate and ammonium which fall in the range of half-saturation constants reported for their uptake by oceanic phytoplankton species (MacIsaac and Dugdale, 1969; Eppley et al., 1977), and from the stimulation of carbon fixation consequent to enrichment with inorganic nitrogen at all stations. The conventional method of detecting whether a nutrient is limiting or not, by an examination of  $\text{NO}_3\text{-N} : \text{PO}_4\text{-P}$  ratios, also supports this; N:P ratios were as a rule less than one and even when ammonium and nitrate were taken together to recalculate the ratios, they rarely exceeded two. Nitrite could hardly be a nitrogen source for phytoplankton since it was rarely present in measurable quantities and even the higher concentrations of nitrite at the bottom of the euphotic zone could only be a result of nitrite excretion by phytoplankton in the presence of excess nitrate and low light for photosynthesis.

Under nitrogen-limiting situations primary production would be regulated by the rate of input of nitrogenous nutrients into the euphotic zone. In the case of nitrate, it would be principally through a vertical transport from below the euphotic zone and the rate at which this occurs determines the level of production. King and Devol (1979) and Eppley et al. (1979) calculated this rate, the vertical eddy diffusion co-efficient ( $K_{zN}$ ), from  $^{15}\text{NO}_3$  uptake ( $Q_N$ ) and nitrate gradient ( $dN/dZ$ ) data by using the equation  $K_{zN} = Q_N/(dN/dZ)$  in the Pacific coastal and oceanic waters. Harrison et al. (1983) also made similar calculations, based on both  $^{15}\text{NO}_3$  and  $\text{H}^{14}\text{CO}_3$  uptake in the Atlantic shelf waters. In the present study, this coefficient was calculated using data on column  $^{14}\text{C}$  uptake, with and without nitrate enrichment, at station 2616. Integrated  $^{14}\text{C}$  assimilation in the euphotic zone was  $322 \text{ mg C}\cdot\text{m}^2\cdot\text{d}^{-1}$ , and with nitrate enrichment, it was  $419 \text{ mg C}\cdot\text{m}^2\cdot\text{d}^{-1}$ . As the ambient nitrate concentration was in traces, the enhanced carbon assimilation ( $97 \text{ mg C}\cdot\text{m}^2\cdot\text{d}^{-1}$ ) consequent to nitrate addition could be considered also to reflect simultaneous  $\text{NO}_3\text{-N}$  uptake in the presence of excess nitrate and the  $Q_N$  can therefore be calculated from this with a C:N assimilation ratio of 6.6 (atomic). This was equivalent to  $0.051 \text{ mg-at N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ . Nitrate gradient  $dN/dZ$  was  $0.218 \text{ mg-at N}\cdot\text{m}^{-4}$  and the vertical diffusivity calculated from these two sets of data was  $0.65 \text{ cm}^2\cdot\text{sec}^{-1}$ . This value is close to a majority of the values obtained in similar studies (King and Devol, 1979; Eppley et al., 1979; Harrison et al., 1983). However, this is likely to be an underestimate since it was assumed that there was no uptake of nitrate in the control bottles. If 50% of observed carbon assimilation is assigned to be due to nitrate uptake (Harrison et al., 1983) then the  $K_{zN}$  would be about  $1.08 \text{ cm}^2\cdot\text{sec}^{-1}$ .

By using the calculated  $K_{zN}$  and  $dN/dZ$  at other stations, nitrate flux into the euphotic zone was calculated by the Fickian expression for diffusion

$$\frac{dM}{dt} = -K_z \frac{dN}{dZ}$$

Data are shown in Table 2, along with equivalent new production values. These varied from 11.3% of the total production at stn 2607 to 59.7% at stn 2614. On an average, this accounted for about 37% of the total production. Simplicity of the calculation of  $K_{zN}$  and its application notwithstanding, the average 37% obtained in this tropical oceanic environment is quite similar to the average of 29% obtained for eastern tropical north Pacific (King and Devol, 1979).

In the case of ammonium, it is regenerated within the euphotic zone primarily through an excretion by zooplankton, and the phytoplankton production associated with ammonium uptake constitutes "regenerated" production. These, cal-

Table 2. Proportions of new and regenerated nitrogen in total production

Stn. No.	NO <sub>3</sub> gradient, dN/dZ (mg-at·m <sup>-2</sup> )	NO <sub>3</sub> flux,* dM/dt (mg-at N·m <sup>-2</sup> ·d <sup>-1</sup> )	Observed production† (mg-at N·m <sup>-2</sup> ·d <sup>-1</sup> )	% new production	Zooplankton biomass (g dry wt·m <sup>-2</sup> )	Nitrogen regenerated as NH <sub>4</sub> ‡ (mg-at N·m <sup>-2</sup> ·d <sup>-1</sup> ) (% regenerated production)	Nitrogen regenerated as urea‡ (mg-at N·m <sup>-2</sup> ·d <sup>-1</sup> ) (% regenerated production)
2607	0.034	0.165	1.466	11.3	1.724	0.509 (34.7)	0.276 (18.8)
2609	0.170	0.816	1.778	45.9	3.038	0.896 (50.4)	0.486 (27.3)
2611	0.169	0.811	1.394	58.2	1.552	0.458 (32.8)	0.248 (17.8)
2614	0.099	0.475	0.794	59.8	0.411	0.121 (15.3)	0.066 (8.3)
2616	0.218	1.046	3.314	31.6	1.433	0.423 (12.8)	0.229 (6.9)
2617	0.219	1.051	2.434	43.2	0.745	0.220 (9.0)	0.119 (4.9)
2618	0.100	0.480	3.094	15.5	1.198	0.353 (11.4)	0.192 (6.2)
2619	0.126	0.605	3.197	18.9	—	—	—
2620	0.211	1.013	2.093	48.4	1.222	0.360 (17.2)	0.196 (9.3)

\* Using  $K_{ZN}$  of 0.2 m<sup>2</sup>·h<sup>-1</sup>.

† Using a C:N ratio of 6.6.

‡ Using NH<sub>4</sub> and urea excretion rates of 0.59 and 0.32 mg-at N·(g dry wt)<sup>-1</sup>·d<sup>-1</sup> and calculating for 12 h residence time.

culated from zooplankton biomass at each station, an average excretion rate of 0.59  $\mu\text{g-at N}\cdot(\text{mg dry wt})^{-1}\cdot\text{d}^{-1}$ , and a zooplankton residence time of 12 h in the mixed layer, are shown in Table 2. Ammonium excreted by zooplankton would supply from about 9 to 50% (average 23%) of the total nitrogen requirements of phytoplankton. The proportion of nitrogen supplied for primary production through ammonium release by zooplankton in these waters is surprisingly similar to those observed in a coastal upwelling system off northwest Africa (28%—Herbland et al., 1973; 25%—Smith and Whitlege, 1977). In the central gyre of the north Pacific, ammonium released by zooplankton supplied about 40% of the total nitrogen demand by phytoplankton (Eppley et al., 1973).

Urea can be a significant nitrogen source for phytoplankton in these waters. Urea occurred at greater concentrations (average 2.0  $\mu\text{g-at N}\cdot\text{liter}^{-1}$ ) relative to inorganic nitrogen (average 0.9  $\mu\text{g-at N}\cdot\text{liter}^{-1}$ ), and addition of urea stimulated carbon fixation at all stations. On an average, addition of urea increased carbon assimilation as much as nitrate or ammonium addition did, and the column production almost doubled on urea supplement. This demonstrates a potential for urea uptake, and the urea regenerated by zooplankton would therefore be of importance in the nitrogen uptake by phytoplankton. Urea released by zooplankton would provide nitrogen for 5–27% (average 13%), and along with nitrate and ammonium, for 33–123%, of observed primary production (Table 2).

Nitrogen regeneration rates and the magnitude of regenerated production could be underestimated in that only macrozooplankton was considered. Excretion measurements in the present study were confined to only larger zooplankton, because the macrozooplankton constitute the dominant size fraction in offshore waters (Smith and Whitlege, 1977), contribute relatively more nitrogen in the shelf and oceanic waters (Jawed, 1973), and are of major importance in sustaining the nitrogen supply for primary production in the open ocean (Eppley et al., 1973).



However, Glibert (1982) has observed that even in oceanic waters, microheterotrophs, including microzooplankton, may be more important than macroheterotrophs in remineralizing ammonium. If the microheterotrophs are equally important in recycling N in oceanic waters (Harrison, 1980; Glibert, 1982), then the actual regenerated production in Lakshadweep waters can be twice as much as that estimated from macrozooplankton excretion alone (36%). Interestingly, this (72%), along with the average new production (37%), accounts for the whole primary production measured, which indirectly suggests that N recycling by microheterotrophs in these waters can indeed be an important process.

It is possible that a part of the regenerated ammonium and urea might be oxidized to nitrate by bacterial nitrification, but this process is unlikely to compete seriously with uptake by phytoplankton under nitrogen-limiting conditions. Heterotrophic bacteria generally occur in very low numbers in oligotrophic seas, their oxidation of ammonium to nitrate is a relatively slow process (Hattori, 1982), the ammonium consumption by bacterial nitrification is negligibly small compared to consumption by phytoplankton (Hattori et al., 1980), and in oceanic waters, the capacity for ammonium oxidation is two orders of magnitude lower than that for ammonium uptake by phytoplankton (Wada and Hattori, 1971). Similarly, phytoplankton seems to be responsible for the major part of urea decomposition in tropical oceanic waters (Herbland, 1976); the weakly exothermic hydrolysis reaction of urea does not allow ATP synthesis from ADP and phosphate, and hence it is less attractive for *sensu stricto* heterotrophic organisms than more complex organic compounds, whereas it is a nitrogen component easily hydrolysable for autotrophic organisms which are under nitrogen-limiting conditions.

It would be worthwhile to speculate on why the ambient concentrations of urea are much higher than those of inorganic nitrogen, if urea is taken up as efficiently as either nitrate or ammonium as a nitrogen source by phytoplankton; more so, when the  $K_s$  values for urea uptake, which are in the range of 0.4–1.7  $\mu\text{g-at N}\cdot\text{liter}^{-1}$ , imply that ecologically significant concentrations of urea can be utilized (McCarthy, 1972a). A possible explanation for this contrasting situation may be as follows: both ammonium and urea are primarily zooplankton regeneration products, and about 2 to 5 times more nitrogen is released as ammonium-N than as urea-N (present study; Smith and Whitley, 1977). Between these two sources of nitrogen, the former is the most preferred form (McCarthy et al., 1977), has an inhibitory effect on urea uptake (McCarthy and Eppley, 1972), exhibits an enhanced uptake capability greater than for urea and can affect, when present, the enhanced capability for uptake of urea (Horrigan and McCarthy, 1982). Exposed to zooplankton excretion products, phytoplankton growing in a nitrogen-limited environment would then take up ammonium more readily than urea. Under these conditions, as Horrigan and McCarthy (1982) have proposed, pulses of urea may persist for a longer time than pulses of ammonium. Whereas all marine phytoplankton species utilize ammonium efficiently, not all of them can do so with urea (McCarthy, 1972a), nor their distribution, and that of zooplankton, can be expected to be vertically uniform in the euphotic zone. This might explain the uniformly low concentrations of ammonium but higher and randomly distributed concentrations of urea in the euphotic zone, observed in the present study and elsewhere (Remsen, 1971), the latter reflecting an interaction of the abundance of zooplankton at a given depth and the ability of the phytoplankton at that depth to utilize urea.

Apart from urea, a major part of the DON does not seem to be utilized by phytoplankton. The average DON concentrations were 10.9 and 9.8  $\mu\text{g-at N}\cdot$

Table 3. Advection and regeneration of phosphorus and primary production

Stn. No.	PO <sub>4</sub> gradient, dP/dZ (mg-at·m <sup>-4</sup> )	PO <sub>4</sub> flux,* dM/dt (mg-at P·m <sup>-2</sup> ·d <sup>-1</sup> )	Observed production† (mg-at P·m <sup>-2</sup> ·d <sup>-1</sup> )	P flux as % of observed production	Phosphorus regenerated as PO <sub>4</sub> ‡ (mg-at P·m <sup>-2</sup> ·d <sup>-1</sup> )	% of observed production
2607	0.006	0.029	0.107	27	0.075	70
2609	0.046	0.221	0.129	171	0.132	102
2611	0.063	0.302	0.101	299	0.068	67
2614	0.068	0.326	0.058	562	0.018	31
2616	0.061	0.293	0.241	122	0.062	26
2617	0.062	0.298	0.177	168	0.032	18
2618	0.028	0.134	0.225	60	0.052	23
2619	0.027	0.130	0.232	56	—	—
2620	0.060	0.288	0.152	189	0.053	35

\* Calculated with  $K_{0.2}$  of 0.2 m<sup>2</sup>·h<sup>-1</sup>.

† Converted using C:P ratio of 106:1.

‡ Calculated using NH<sub>4</sub>-N:PO<sub>4</sub>-P excretion ratio of 6.8 (Mullin et al., 1975).

liter<sup>-1</sup>, and excluding urea, were 8.9 and 7.9  $\mu\text{g-at N}\cdot\text{liter}^{-1}$  along the 8° and 10°N transects. DON was the major fraction of total dissolved nitrogen, averaging about 90%, and N<sub>total</sub>:PO<sub>4</sub> ratios were rarely less than 15. These would suggest no nitrogen deficiency, yet the addition of inorganic nitrogen and urea caused stimulation of carbon fixation. This demonstrates that the bulk of DON is not readily available to phytoplankton, and as argued by Thomas et al. (1971), if this DON were available to phytoplankton then it would not be present in such high concentrations relative to inorganic nitrogen. Of the few characterized DON compounds, amino acids are certainly used as nitrogen source by several marine phytoplankton species (Antia et al., 1975; Fisher and Cowdell, 1982) but their concentrations, particularly in oceanic waters, are too low, often less than a hundred nM (Lee and Bada, 1977), to be of any significant use in phytoplankton production. The bulk of the DON, therefore, must be a refractory fraction, not readily available to phytoplankton (Thomas et al., 1971).

Phosphorus is unlikely to be a limiting nutrient for primary production in these waters. To begin with, phosphate was never below 0.3  $\mu\text{g-at P}\cdot\text{liter}^{-1}$ , a concentration which is well above the half-saturation constant for phosphate uptake by oceanic phytoplankton (0.14–0.18  $\mu\text{g-at P}\cdot\text{liter}^{-1}$ ; Perry and Eppley, 1981). Secondly, the carbon fixation rates were not significantly different in the unenriched samples in primary production measurements and in the control samples of the enrichment experiments where only phosphate was added. Thirdly, phosphate diffused across the thermocline into the euphotic zone alone would provide all the phosphorus required for primary production; at a majority of the stations, this was in excess of phytoplankton requirements (Table 3). In addition, zooplankton regenerate an average of 46% of the phosphorus required by phytoplankton (Table 3). Thus, diffusion and in situ regeneration would assure a phosphorus supply well above the phytoplankton needs at any given time. Further, oceanic phytoplankton exhibit physiological adaptations to low ambient phosphate concentrations; in the central northern Pacific Ocean, where phosphate was less than 0.04  $\mu\text{g-at P}\cdot\text{liter}^{-1}$ , the phytoplankton could alleviate phosphorus limitation by low  $K_m$  for phosphate uptake, the light-independence of uptake and the induction of cell-surface alkaline phosphatase activity (Perry and Eppley, 1981).

In conclusion, it can be stated that in these tropical oceanic waters, phytoplankton production is regulated by the supply of inorganic nitrogen. Calculated rates of replenishment of inorganic nitrogen through advection and macro-zooplankton

regeneration account on an average for about 60% of the nitrogen production. Urea is utilized, and can be as significant a nitrogen source as nitrate or ammonium, but the bulk of DON which constitutes the major fraction of total dissolved nitrogen is not directly available for phytoplankton assimilation.

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