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Urea as Nitrogen Source for Phytoplankton Production in Coastal Waters of Goa

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Annual variation of urea in coastal waters off Goa is 0 to 2.92 μg-at N.l^{-1} and 0 to 4.69 μg-at N.l^{-1} in adjacent estuarine waters of Mandovi. Peaks of phytoplankton production accompanied with the decrease in urea in June and October, when ammonia and nitrate were high, indicate that urea is utilised or decomposed by phytoplankton organisms. Seasonal fluctuation of urea is between 7 and 55% of total utilizable nitrogen, which is a large reserve of nitrogen for phytoplankton growth. Assimilation ratio (ΔN:ΔP) indicates that nitrate is in short supply. However, urea available in these waters can substitute nitrogen deficiencies for the high rate of phytoplankton production.

Nitrate and phosphate are known to be limiting nutrients in the tropical marine environment. High levels of autotrophic productivity observed[1] in these waters suggest that the demand for these nutrients should be more than that available. Further, to understand the production process in tropical waters it is necessary to investigate other forms of nitrogen and phosphorus readily available for phytoplankton. The important distinction between nitrate and ammonia uptake by phytoplankton as sources of new and regenerated production respectively, has been advanced by Dugdale and Goering[2]. The nitrate consumed by phytoplankton in the euphotic zone is replenished by vertical convection of deep waters rich in nitrate, whereas ammonia within the euphotic zone is produced through regeneration process mediated by zooplankton and bacteria. Since urea, like ammonia, is also a product of regeneration by heterotrophs[3], phytoplankton growth supported by urea can also be classified as that belonging to regenerated production. Occurrence of this compound in significant quantities in the coastal and offshore waters[4-9] indicates its importance as N source for phytoplankton. However, not much is known about the changes in urea-nitrogen in relation to primary production during a complete annual cycle, especially in tropical coastal environments. Investigations have therefore been undertaken on primary production and nutrient content with special emphasis on the distribution of urea nitrogen in Goa waters.

Materials and Methods

In order to study changes in productivity and nutrient content, a reference station (RS) was established in the coastal waters 9-10 km off the Aguada Bay, Goa at a depth of 30 m (Fig. 1). Observations at this station were made every month from Feb. 1980 to March 1981. Simultaneously, another series of stations (1 to 6), were also worked out in the Mandovi estuary starting from Aguada Bay (estuarine mouth) up to the fresh water zone in the Mandovi river, covering a distance of about 70 km (Fig. 1). In the shallow estuarine region (av. depth 6-10m) only 2 (surface and near bottom) depths were sampled, while at RS samples were obtained from 3 depths (surface, near bottom and mid-depth). During the monsoon (June-Sept.) season due to the formation of a sand bar at Aguada mouth sampling was not possible at RS. Water samples were obtained using Van Dorn sampler. For the measurement of primary production, water samples in duplicate (125 ml) were inoculated with ^{14}C (4 μ Ci) and incubated on board under simulated *in situ* conditions using neutral density filters to adjust the light level[10]. After incubation for 6 h the samples were filtered through Sartorius membrane filters (0.45 μm pore size),

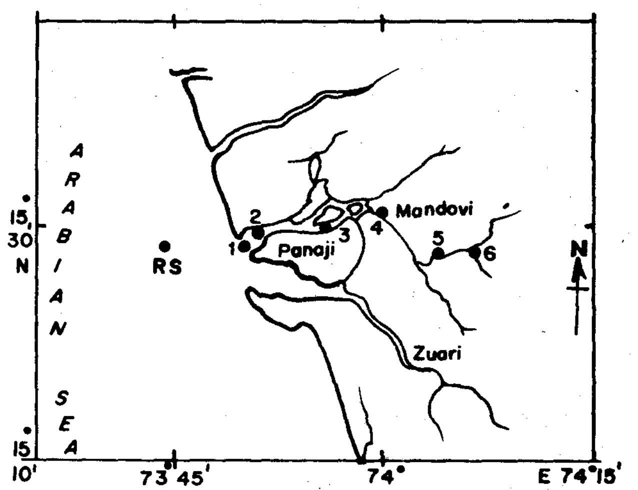

Fig. 1—Position of reference station (RS) and transect survey stations

exposed to acid fumes and dried. These filters were extracted in liquid scintillation cocktail and the radioactivity assayed by liquid scintillation counter[10]. Chlorophyll was extracted in 90 % acetone and the fluorescence measured using Turner Designs Fluorometer (USA). Urea nitrogen was analysed using diacetyl monoxime method[11]. The other nutrients were analysed by the standard procedures given by Grasshoff[12].

Results

Concentrations of urea varied from 0 to 2.92 μg-at $N.l^{-1}$ in the coastal waters and from 0 to 4.60 μg-at $N.l^{-1}$ in the estuarine region. Urea concentration at RS (Fig. 2a) was low in Oct. but it increased quite steadily towards a peak in Jan.-Feb. Annual cycle of urea in the estuarine region showed 3 peaks (Fig. 2 b and c). The concentration remained high throughout the monsoon season when physical processes like precipitation and land drainage were active. Further the urea values increased also towards the upstream portion of the estuary. The changes during the rest of the period were almost similar to those in the coastal waters. Vertical distribution of urea indicated that the concentration remained high in the bottom water both in estuarine and coastal waters.

Integrated mean values of urea at RS and mean values for the 3 estuarine stations (sts 1 to 3) for different months are shown in Fig. 3, along with the other nutrients, chlorophyll *a* and ^{14}C assimilation. Unlike urea, ammonia concentrations were highly fluctuating, with a mean range of 0.38 to 4.04 μg-at $N.l^{-1}$, in the estuarine and coastal waters. On the other hand, the nitrate concentrations were steady (av. range : 0.01 to 13.86 μg-at $N.l^{-1}$) showing a sharp increase during the monsoon period. Total organic nitrogen values excluding urea fluctuated between a monthly mean of 0.78 to 26.62 μg-at $N.l^{-1}$. Mean chlorophyll values in the euphotic zone varied from 0.27 to 4.66 mg m^{-3} whereas the primary productivity varied between 380.5 and 1231.3 mg C m^{-2} d^{-1} at the RS during post (Oct. to Jan.) and premonsoon (Feb. to May) periods. In the estuarine region the monthly mean values of chlorophyll ranged from 0.54 to 11.34 mg m^{-3} in surface waters. The primary productivity of the water column varied from 0.9 to 1205.5 mg C m^{-2} d^{-1} during the annual cycle. Chlorophyll and primary productivity remained high during postmonsoon period in the estuarine and coastal waters (Fig. 3). Sharp peaks of chlorophyll and primary productivity were also noticed at the beginning of monsoon season in June and at the cessation of monsoon in Oct. But the production remained quite low during peak monsoon months (July and Aug.).

Discussion

Utilisation and decomposition of urea associated with phytoplankton activity were reported[4-7]. The reports confirm the importance of regenerated nitrogen for phytoplankton production, a theory advanced by Dugdale and Goering[2]. Occurrence of urea in significant quantities both in estuarine and coastal waters of Goa indicates the important contribution of this compound to regenerated production. But in an area like the present study, where the standing crop of phytoplankton is high together with temporal and spatial variability in nutrient concentration (Figs 2 and 3), it would be important to observe the *in situ* rate of utilisation of a single nitrogenous nutrient when other forms are also present in a wide range of concentrations. McCarthy and Eppley[13] using phytoplankton cultures found that ammonia at elevated concentrations could be used in preference to urea, urea-nitrogen and nitrate being utilised simultaneously over a wide range of concentrations. McCarthy *et al.*[14] found high preference in phytoplankton for ammonia and urea over nitrate and nitrite.

In the present study urea decreased suddenly to a minimum of 0.1 μg-at $N.l^{-1}$ in Oct. in the surface water (Fig. 2 a and b) when ammonia and nitrate were high (Fig. 3). ^{14}C assimilation and chlorophyll concentration were also high during this month (Fig. 3), indicating bloom formation by phytoplankton. Similarly decrease in the surface urea concentration in June was accompanied by the outburst of planktonic activity although urea nitrogen was expected to increase during this period, due to land runoff. At RS, while ammonia and nitrate were depleted in Feb. urea concentration remained high. But in March even urea was reduced to low levels. Studies on phytoplankton growth with urea, as the only source of nitrogen[15] indicated that the phytoplankton species, viz. *Asterionella japonica*, *Synechocystis* sp. and *Chlorella* sp., isolated from coastal waters of Goa showed maximum growth at concentrations of 1, 2 and 1 μg-at $N.l^{-1}$ respectively. Laboratory experiments as well as field observations showed that decrease in urea concentration was associated with high phytoplankton production chiefly in Oct., June and Feb. - March, indicating that urea was utilised by phytoplankton during these months.

Uptake of urea by phytoplankton is not the only way whereby urea concentration could be reduced in the environment, since degradation of urea to carbon dioxide and ammonia through heterotrophic activity might also be responsible for reducing the urea content in the water. This could be observed in the estuarine water particularly when the decrease in urea in Feb. was accompanied by an increase in the ammonia

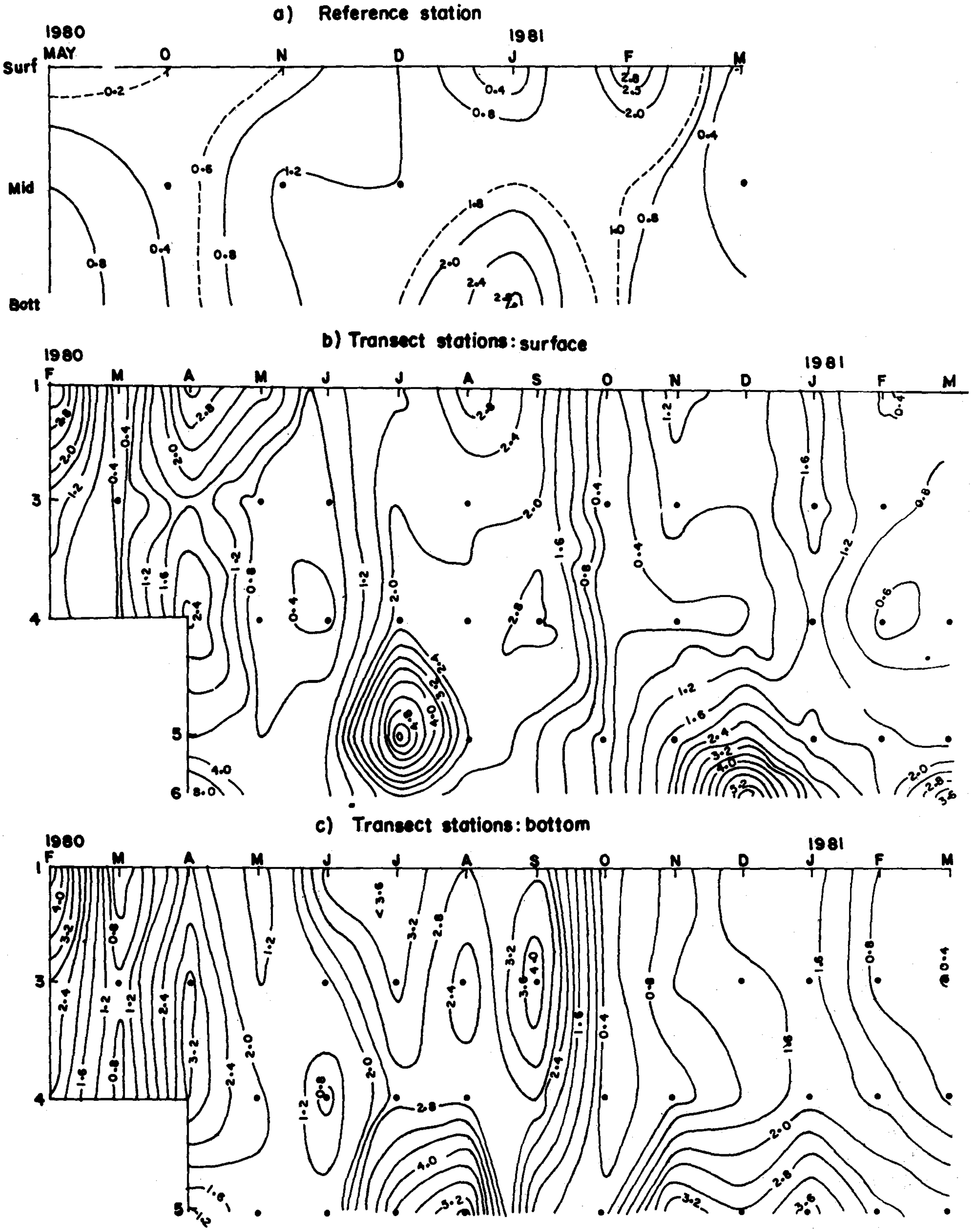

Fig. 2—Urea distribution at reference station (a) and transect survey stations (b and c)

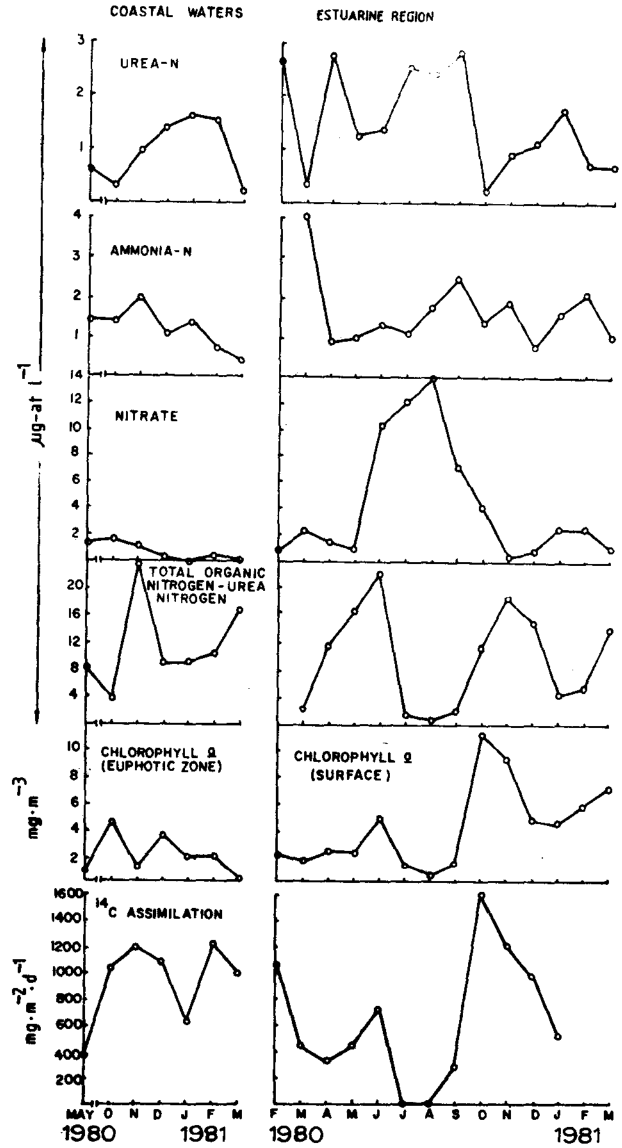

Fig. 3—Seasonal variation of integrated mean values of different parameters in coastal and estuarine waters

content. Studies on bacteria distribution indicated that urea decomposers constituted 15 to 22% of the total bacterial population in the coastal waters of Goa[16]. Many studies revealed that phytoplankton also plays an important role in urea decomposition in addition to bacteria[3,17]. Since phytoplankton production usually remained high in these waters, the sharp decrease in urea concentration encountered during June, Oct., Feb.-March could be either due to utilisation or decomposition by phytoplankton. Further investigations on *in situ* utilisation/decomposition are however needed to decide the actual fate of urea in these waters.

Nevertheless in view of the above observations, it could be stated that unlike organic nitrogen of high molecular weight, urea is one of the readily utilisable forms of nitrogen in tropical waters. Therefore urea in combination with ammonia and nitrate could be considered as rapidly recycled nitrogen potential available for phytoplankton production. For convenience this combination of nitrogeneous compounds is termed here as utilisable nitrogen. The integrated mean values of urea as percentage of utilisable nitrogen content at the RS are shown in Table 1. During the post and premonsoon periods, urea concentration fluctuated from 7 to 55% of the total utilisable nitrogen. Earlier studies on urea distribution in the coastal waters of the west coast of India[8] indicated that urea formed more than 10% of utilisable nitrogen in the euphotic zone of 0 to 100 m depth.

However, increase in urea concentration in the upstream portion of the estuary, especially during monsoon and high concentrations of this nutrient seen in bottom waters suggested that the major sources of urea to the coastal waters were land drainage and decomposition of organic matter[3].

It would be clear from the above observations that urea formed a significantly large proportion of nitrogen in these waters. Availability of nitrogeneous nutrients to the phytoplankton could be further examined by the nitrate : phosphate ratio. It is seen

Table 1—Urea as Percent of Utilisable Nitrogen Content at Reference Station

	Utilisable N (μg-at l^{-1})	Urea N (μg-at l^{-1})	Urea as % of utilisable N
May 1980	3.95	0.62	15.8
Oct.	4.26	0.29	6.9
Nov.	4.60	0.94	21.6
Dec.	3.42	1.38	40.5
Jan. 1981	3.08	1.58	51.2
Feb.	2.78	1.53	55.0
March	0.83	0.23	27.7

Table 2—NO_3^- : PO_4^{3-} Ratio in Coastal and Estuarine Waters

	Coastal	Estuarine
March 1980	—	2.9
April	—	2.2
May	2.1	1.5
June	—	24.5
July	—	56.6
Aug.	—	12.0
Sept.	—	6.7
Oct.	0.8	3.9
Nov.	1.4	0.9
Dec.	1.3	1.9
Jan. 1981	0.1	7.2
Feb.	1.6	6.3
March	0.1	3.1

from Table 2 that this ratio varied from 0.89 to 7.2 in the estuarine stations during the stable post and premonsoon periods (Oct. to May). The linear regresssion of nitrate and phosphate values for estuarine and coastal waters taken together during the post and premonsoon periods gave an assimilation ratio ($\Delta N:\Delta P$) of 11. Both the concentration and assimilation ratios were low and indicated that nitrate was in short supply in these waters. These deficiencies of nitrogen could necessarily be supplemented by urea[5,13,15] which in turn was found to occur in sufficiently large concentrations, along with ammonia.

In conclusion it may be stated that urea is activily involved in the process of nitrogen supply to maintain high rates of phytoplankton production in the coastal waters of Goa.

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