show no change in metabolic rate during the experimental period. The energy expenditure is calculated from the oxy-caloric coefficient. While the control animals spend only 5.56 cal in 1 hr, the animals exposed for 6 and 12 hr need 7.49 and 8.4 cal respectively. The post-exposure energy demand of 1.2 cal for 6 hr exposed mussels and 2.04 cal for 12 hr exposed mussels show clearly the strain on the animal in relation to the duration of exposure.

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Effect of Urea on Growth of Marine Phytoplankters

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Growth rates of 3 phytoplankters—Asterionella japonica, Synechocystis sp., and Chlorella sp.—were studied as a function of urea by enriching the nutrient-depleted water with varying concentrations (0 to 50 µg-at N/litre) of urea; growth rate was calculated from the increase in chlorophyll a content of the organisms. Maximum growth of these phytoplankters was observed between concentrations 1 and 2 µg-at urea-N/litre.

UREA in sea water is probably similar to ammonium ions, produced as a result of excretion of animals, and plants consume it during photosynthesis. Phytoplankters of estuaries and coastal waters use urea as a nitrogen source at times of nitrate deficiency. It may also form a source of nitrogen in oligotrophic waters where it is found in sufficiently high quantities, but within the biological limits during certain seasons.

In the present study, growth experiments have been conducted with different concentrations of urea using phytoplankton species, Asterionella japonica, Synechocystis sp. and Chlorella sp., isolated from the Goa waters and maintained as unialgal cultures. Increase in chlorophyll a (chl. a) content has been used as an index of growth rate. Earlier studies on growth and uptake experiments are confined mostly to ammonia, nitrate and phosphate.

The importance of urea as a source of nitrogen for the phytoplankton growth has been emphasized only recently, after the standard methods of urea determination have been worked out.

For each set of experiments, 2 l of sea water, filtered through GF/C pads, were taken in a series of flasks. Urea was added in different concentrations (0 to 50 µg-at N/litre). All the flasks were then inoculated with equal quantities of healthy (but not bacteria free) algal cells. The flasks were kept under alternating periods of light (8 hr of fluorescent illumination) and darkness. The initial level of nitrate present in the sea water was 0.86 µg-at/N litre.

Aliquots of 300 ml were drawn from each flask at intervals of 1 or 2 days, filtered through GF/C pads, and chl. a from the filters was extracted with 90% acetone and measured on a spectrophotometer. Samples for nitrate and chl. a were analysed by standard methods. Growth rate of each organism was calculated from:

\[ \mu = \frac{\ln \text{Chl.}\text{a}_{t}}{\text{Chl.}\text{a}_{0} \left( \frac{1}{t_{n}} \right)} \]

where chl. a\text{t} and chl. a\text{0} were chl. a concentrations at times \text{t} and \text{0} respectively.

Urea was estimated using diacetyl spectrophotometric method of Newell et al.

Growth rate in A. japonica was low initially at almost all concentrations of urea. It increased steadily and reached the peak from 6th to 10th day and decreased thereafter. However, in the control experiment with no urea, the growth remained more or less steady (Fig. 1). In Synechocystis sp. also the growth for the first few days was low in all urea concentrations. Peak growth reached from 3rd to 9th day and then decreased (Fig. 1). Chlorella sp. also showed a slow rate of growth at all urea concentrations in the beginning. It increased from 10th day till the last day of the experiment. At higher concentration of (50 µg-at urea-N/litre) the growth remained low throughout (Fig. 1).

Maximum growth rate (µ) values of phytoplankton cells recorded under different urea concentrations are given in Table 1 for different species. Maximum growth rate for A. japonica, Synechocystis sp. and Chlorella sp. was respectively at concentrations 1, 2
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Fig. 1 — Ratio of chl. at/chl. ao as an index of growth for 3 phytoplankton species at different urea concentrations

[Urea concentrations (μg-at N/litre): a, 2; b, 10; c, 20; d, 50; e, 1; f, 0.5; and g, 0 (control)]

<table>
<thead>
<tr>
<th>Species</th>
<th>Concentration (μg-at urea-N/litre)</th>
<th>Growth Rate (μ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. japonica</td>
<td>0, 0.5, 1, 2, 10, 20, 50</td>
<td></td>
</tr>
<tr>
<td>Synechocystis sp.</td>
<td>0.22, 0.76, 0.88, 0.605, 0.54</td>
<td></td>
</tr>
<tr>
<td>Chlorella sp.</td>
<td>0.45, —, 0.605, 1.14, 1.003, 0.86, 0.83</td>
<td></td>
</tr>
</tbody>
</table>

Table 1 — Maximum Growth Rate (μ) for A. japonica, Synechocystis sp., and Chlorella sp. in Different Urea Concentrations

and 1 μg-at urea-N/litre. There was a gradual decrease in the growth rate with increasing concentrations of urea.

Carpenter et al.1 have shown the importance of urea as a nitrogen source for estuarine and neritic species of phytoplankters. Hulbert8 has observed that the Chlorella sp. was a more predominant species of phytoplankton in the surface waters of New York Bight, where variable amounts of urea were present. Dayis et al.9 have also shown that most Chlorella sp. are able to use urea as a nitrogen source. These observations agree well with the present findings that Chlorella grows well in low concentrations of urea. McCarthy10 has indicated that Skeletonema costatum can utilize urea at concentrations less than 1 μg-at N/litre. He has also observed that not all phytoplankters can use urea as a chief source of nitrogen. For example, P. Micans has failed to grow in the laboratory with urea as a main source of nitrogen.

In the coastal waters of Goa, urea concentration varies from 1.5 to 7.5 μg-at N/litre, but in the Velsao Bay (Goa), the variation in the urea concentration is from 10 to 120 μg-at N/litre. Such high concentrations of urea are due to the effluent discharge from a fertilizer factory located in the vicinity of Velsao Bay.

Several authors have reported different urea concentrations from the surface waters of the sea. Newell11 and Ramen12 have found values ranging
Mass Mortality of Fish in the Visakhapatnam Harbour

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Mass mortality of fish was observed in the Visakhapatnam Harbour, which receives both industrial and domestic effluents from installations located in its environs, on 4 different occasions which was preceded by sudden climatic and hydrographical changes in the environment. Mortality was localized in one arm of the harbour which receives the effluents from an oil refinery and a fertilizer factory. Mortality might have been caused by the sudden discharge of large quantities of acids and sulphur dioxide which brought out conditions favourable for emanation of free CO₂ in abnormal quantities. The mortality was also believed to have been caused by asphyxiation due to lowered oxygen tension and presence of abnormal quantities of free CO₂, which was supported by the data collected on one occasion (20 Sept. 75).

From 0.5 to 5 μg-at urea-N/litre, using diacetyl method of urea estimation, whereas McCarthy has reported values from 0 to 0.67 μg-at urea-N/litre using urease technique. The urea concentration, therefore, present in the environment can be a significant source of available nitrogen for the phytoplankton in coastal waters of the tropics, perhaps when nitrate and ammonia levels in the euphotic layer are low. These findings also suggest that abnormally high concentrations of urea in any locality, like the Velsao Gay (Goa), may prove to be harmful to the algae.

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