show no change in metabolic rate during the experimental period. The energy expenditure is calculated from the oxy-calorific coefficient. While the control animals spend only 5.56 cal in 11 hr, the animals exposed for 6 and 12 hr need 7.49 and 8.4 cal respectively. The post-exposure energy demand of 1.93 cal for 6 hr exposed mussels and 2.84 cal for 12 hr exposed mussels show clearly the strain on the animal in relation to the duration of exposure.

The author expresses his gratitude to Dr S. Z. Qasim, former Director of CMFRI, Cochin, for technical guidances. Thanks are due to Dr K. V. Sekharan, Senior Fishery Scientist, CMFRI, for his continued interest and guidance, to Dr M. N. Kutty, Reader, Madurai University, Madurai, for useful suggestions in setting up the experiments, to Mr T. Tholasilingam, Officer-in-Charge, CMFR Substation, Madras, for offering laboratory facilities and to Mr D. C. V. Easterson for useful suggestions. This work was carried out with financial assistance in part from Ministry of Education, Government of India, by offering a Research Training Scholarship.

References

- 1. PROSSER, C. L. & BROWN. F. A., Comparative animal physiology (Saunders, Philadelphia), 1961. KINNE, O., Marine ecology, Vol. 1 (Wiley Interscience),
- 1972
- GHIRETT, F., in Physiology of mollusca, edited by K. M. Wilbur 78 C. RI. Yonge (Academic Press, New York),
- 4. NEWELL, R. C., Biology of intertidal animals (Logos Press Ltd. London), 1970. BRUCE, J. N.. Biochem. J., 20 (1926). 829.
- 5.

6. BAYNE, B. L., Physiol. Zool. 40 (1967), 307.

- THOMPSON. R. J. & BAYNE. B. L., J. exp. mar. Biol. Ecol., 9 (1972), 111.

B. BROWN, B. E. & NEWELL, R. C., Mar. Biol., 16 (1972), 108.
 WIDDOWS, J., Mar. Biol., 20 (1973), 269.
 SHAFEE, M. S. & SUNDARAM. K. S., in Third All India symposium on estuarine biology (in press), 1975.

- 11. WEIGERT, E. G., Am. Zool., 8 (1968), 71. 12. MATHEW., C. V. & SUMITHRA. V.. Indian J. Fish., 20 12. MATHEW. (1973), 658.
- STRICKLAND, J. D. H. & PARSONS, T. R., A practical hand book of sea water analysis, Bull. No. 167 (Fish. Res. Bd, Canada), 1968.
 14. IVELEV, V. S., Zool. Zh., 18 (1939), 303.
 15. BOHLE, BJORN., J. exp. mar. Biol. Ecol., 10 (1972), 41.
 16. ZEUTHEN, E., Quart. Rev. Biol., 28 (1953), 1.
 17. HEMMINGSEN, A. M., Rep. Steno. Meml Hosp., 9 (1960). 1.

- SUNDARAM, K. S. & SHAFEE. M. S., in Third all India symposium on estuarine biology (in press), 1975.

Effect of Urea on Growth of Marine Phytoplankters

X. N. VERLENCAR

National Institute of Oceanography, Dona Paula 403004

Received 21 July 1975; revised received 9 December 1975

Growth rates of 3 phytoplankters – Asterionella japonica, Synechocystis sp. and Chlorella sp.- were studied as a function of urea by enriching the nutrientdepleted water with varying concentrations (0 to 50 µgat 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.

TREA in sea water is probably similar to ammonium ions, produced as a result of excretion of animals, and plants cotsume it during photosynthesis. Phytoplankters of estuaries and coastal waters use urea as a nitrogen source at times of nitrate deficiency¹. 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 seasons².

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^{3,4}. 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 ^{5,6}.

For each set of experiments, 2 1 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 (8hr 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 methods⁷. Growth rate of each organism was calculated from :

$$\mu = \ln \frac{\text{Chl.}a_t}{\text{Chl.}a_0} \left(\frac{1}{\text{t} \ln_2} \right)$$

where chl. *a*_t and chl. *a*, were chl. *a* concentrations at times t and 0 respectively⁴.

Urea was estimated using diacetyl spectrophoto-

metric method of Newell et al⁶.

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

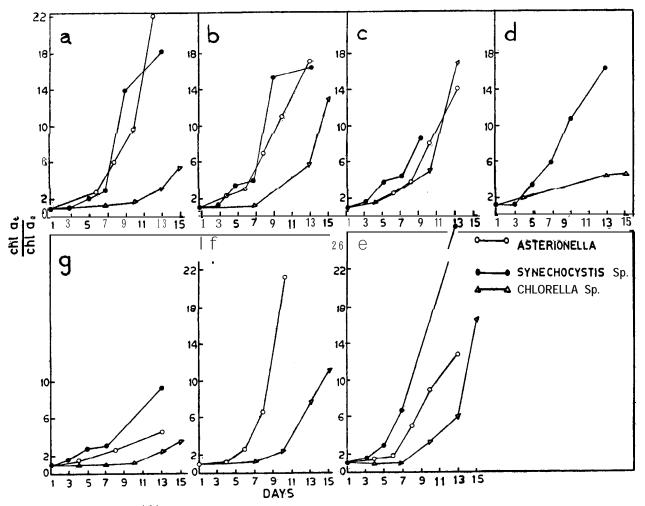


Fig. 1 – Ratio of chl. **at/chl.** ao as an index of growth for 3 phytoplankton species at different urea concentrations. [Urea concentrations (pg-at N/litre): a, 2; b 10; c 20; d, 50; e, 1; f, 0.5; and g, 0 (control)]

TABLE 1 -MAXIMUM GROWTH RATE (µ) FOR <i>A. japonica,</i> Synechocystis sp. AND Chlorella sp. IN DIFFERENT UREA CONCENTRATIONS						
Conc. pg-at urea-N/litre						
0 (Control)	0.5	1	2	10	20	50
A. japonica						
0.22	0.72	0.76	0.58	0.605	0.54	—
Synechocystis sp.						
0.45	_	0.605	1.14	1.003	0.86	0.83
Chlorella sp.						
0.351	0.579	0.741	0.377	0.617	0.635	0.283

and 1 pg-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. Davis *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 pg-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 pg-at N/litre, but in the Velsao Bay (Goa), the variation in the urea concentration is from 10 to 120 pg-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 in the sea. Newell¹¹ and Ramsen¹² have found values ranging from 0.5 to 5 μ g-at urea-N/litre, using diacetyl method of urea estimation⁶; whereas McCarthy⁵ has reported values from 0 to 0.67 μ g-at urea-N/ litre using urease technique⁵.

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.

The author is grateful to Dr S. Z. Qasim, Director, for his interest and encouragement. He is thankful to Dr M. G. Gogate, Professor of Physiology, Goa Medical College, Panaji, Shri P. M. A. Bhattathiri and Shri V. P. Devassy for their guidance and help.

References

- CARPENTER, E. J., REMSON, C. C. & WATSON, S. W., Limnol. Oceanogr., 17 (1972), 265.
 MCCARTHY, J. J. & KAMYKOWSKI, D., Fish. Bull., 70
- (1970), 1261. 3. EPPLEY, R. W., ROJERS, J. N. & MCCARTHY, J. J.,
- Limnol. Oceanogr., 14 (1969). 912. 4. QASIM, S. Z., BHATTATHIRI, P. M. A. & DEVASSY, V. P.,
- Mar. Biol., 21 (1973), 299. 5. MCCARTHY, J. J., Limnol. Oceanogr., 15 (1970), 309. 6. NEWELL, B. S., MORGAN, B. & CUNDY, J., J. mar. Res.,
- 25 (1967) 201.
- 7. STRICKLAND, J. D. H. & PARSONS, T. R., A manual of sea water analysis, Bull. No. 167 (Fish. Res. Bd, Canada),
- 8. HULBERT, E. M., cited by C. C. Remson, Limnol. Oceanogr., 16 (1971), 732.
- 9. DAVIS, E. A., DEDRIC, J., FRENCH, C. S., MILNER, H. W., MYERS, J., SMITH, J. H. C. & SPOEHR, H. A., in Algal cultures from laboratory to pilot plant, edited by H. S. Burlew (Carnegie Institution, Washington, Publ. 600), 1953. 105.
- MCCARTHY, J. J., Limnol. Oceanogr., 17 (1972), 738.
 NEWELL, B. S., J. mar. biol. Ass. U.K., 47 (1967), 271.
 RAMSON, C. C., Limnol. Oceanogr., 16 (1971), 732.

Mass Mortality of Fish in the Visakhapatnam Harbour

P. N. GANAPATI & A. V. RAMAN

Department of Zoology, Andhra University, Waltair 530003

Received 11 March 1976

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 (29 Sept. 75).

ASS mortality of fish has been reported from M ASS mortality of tish has been top the world, time to time, from different parts of the world, owing to some abnormal event in the aquatic environment. An appraisal of the extensive literature cited by Brongersma-Sanders¹ and more recently that of Korringa² indicates that fish mortalities result from a variety of causes, some of natural origin and some man-induced. Those from natural causes may result from such phenomena as tectonic earth or sea quakes, storms, salinity and temperature changes, noxious plankton blooms, decomposition of natural organic materials, bacterial or parasitic epidemics, etc. Man-induced fish-kills result from indiscriminate discharge of industrial and domestic wastes or to such other water manipulations as channel dredging, etc., that significantly alter the nearshore waters.

The present account is documentation of 4 such events of fish mortality, on a large scale, in the northwestern arm of the Visakhapatnam Harbour which receives the industrial effluents from a fertilizer factory and an oil refinery through a monsoon-fed stream, the Mehadrigedda. The mortalities occurred in November 1974 and in May, September and October 197.5, in the early hours of the morning, during low tide, and the reports were received a few hours later. Ganapati and Raman³ have already reported that the Mehadrigedda is the major source through which industrial effluents enter the harbour through the north-western arm from the fertilizer factory and the oil refinery. The industrial waste water, besides being acidic, contains appreciable amounts of toxic substances such as ammonia, fluorides, phenols and heavy metallic ions such as copper and lead.

The principal species of teleostean fish that succumbed were identified as Megalops cyprinoides, Nematalosa sp. and Mugil cephlaus of which Nematalosa sp. formed the bulk of the casualties. During the last 2 occasions of mortality (29.9.75 and 20.10.75) Nematalosa sp. alone was affected. Postmortem examination on this and the other affected species of fish showed no signs of any parasitic infection and their general appearance was found to be normal. Considering the number of fishes washed ashore during each event (average of about 350 individuals/100 m stretch of the canal), the fish-kills observed may be classified as 'Major Kills' involving more than a few thousands of fish. The mortalities were also found to be of an acute type occurring within a period of 6 hr, usually during the pre-dawn hours. In general, the size range of the fishes affected varied from about 5 to 15 cm.

While it was not possible to collect and analyse the water samples at the time when the mortality occurred, on all the 4 dates, some observations have been made on the weather conditions and the water quality collected at 0830 hr on 29.9.75 when the 3rd mortality was reported. There was fairly heavy rain (8.4 mm) and cloudy weather with little sunshine (1.3 hr) during the preceding 24 hr. The water temperature was 29°C, pH 5.5, free carbon dioxide 77 ppm and dissolved oxygen 1.2 ml/litre (29.0%). Extreme weather conditions of hot summer, with atmospheric temperature of 36°C and