

TABLE 3 — EFFECT OF NITROGENOUS CHEMICALS AND SOME POLLUTANTS ON CO<sub>2</sub> FIXATION BY THE BACTERIAL ISOLATE AT THE END OF 48 HR GROWTH

[Carbon source, egg 1%; chemicals, 1%; oil, 0.5%; and pesticide, 2 µmoles]

	A Alcohol soluble fraction (%)		B Alcohol insoluble fraction (%)		Total (%)	
	Incorporation	Inhibition	Incorporation	Inhibition	Incorporation (A + B)	Inhibition
Control	17.7	—	47.5	—	65.2	—
Ammonium Sulphate	17.2	2.8	45.9	3.4	63.1	3.2
Sodium Nitrite	0.2	98.9	0.5	99.0	0.7	99.0
Sodium nitrate	15.2	14.1	12.5	73.7	27.7	57.5
Sodium Azide	3.7	82.5	12.4	73.9	16.1	75.3
Dicyston	26.1	47.5	30.5	35.8	56.6	13.2
Thiophamine	12.6	28.8	30.4	36.0	43.0	34.1
Dasanit	4.7	73.4	13.2	72.2	17.9	72.6
Fenitrothione	1.8	89.8	3.3	93.1	5.1	92.2
Crude oil	3.6	80.0	9.6	79.8	13.2	79.8
Kerosene	10.2	42.4	8.7	81.7	18.9	71.0
Petroleum	4.6	74.0	0.8	98.3	5.4	91.7

other cases, except in sodium acetate, CO<sub>2</sub> incorporation in the alcohol soluble fraction was less than that of alcohol insoluble fraction.

In the presence of nitrogenous chemicals, CO<sub>2</sub> fixation was inhibited to varying degrees (Table 3). Of the 4 nitrogenous chemicals used in the present study, ammonium sulphate was found to inhibit less than the others (3.2%) and sodium nitrite had the maximum inhibitory effect (99%). Nitrites and azides, known for their toxicity, have inhibited the CO<sub>2</sub> fixation by 99 and 75.3% respectively. Ammonium sulphate does not affect the bacterial enzyme<sup>10</sup> which explains its minimum inhibition (3.2%) in the present isolate.

To study the effect of pesticides and oil (pollutants) on CO<sub>2</sub> fixation, different pesticides and hydrocarbons were used (Table 3). Inhibition was maximum in fenitrothione (92.2%) among pesticides and in petroleum (91.7%) among the hydrocarbons. Similar observations were made by Walker and Colwell<sup>11</sup>, who reported that petrohydrocarbons limit the growth of microbial flora of both estuarine and marine environment. This holds good in the case of Athiorhodaceae also where all the hydrocarbons have inhibitory effect on their growth and CO<sub>2</sub> fixation to varying degree.

These studies show that the pollutants, as in present study, are always present in sea water and their inhibitory effect on photosynthesis affects the productivity and will in turn lead to disturbed food chain.

The authors are grateful to Dr S. Z. Qasim, Director, for valuable suggestions. Thanks are due to Dr T. Ramakrishnan, Microbiology & Cell Biology Laboratory, Indian Institute of Science, Bangalore, for allowing one of the authors (N.G.K.K.) to carry out a part of the present work and to Drs V. N. Vasantarajan and C. Chitra for their help and suggestions. The authors (S.N. & P.A.L.) are thankful to CSIR for the award of Junior Research Fellowships, during the tenure of which the work was done.

#### References

1. WYNN WILLIAMS, D. D. & RHODES MURIEL, E., *J. appl. Bact.*, **37** (1974), 217.
2. PFENNING, N., *Ann. Rev. Microbiol.*, **21** (1967), 285.
3. GORE, P. S., *Curr. Sci.*, **41** (1972), 737.
4. RODINA, A. G., *Methods in aquatic microbiology*, edited by R. R. Colwell & M. S. Zambruski (University Park Press, Baltimore & Butterworth & Co. Ltd, London), 1972, 329.
5. JUNGALWALA, F. B. & CAMA, H. R., *Biochem. J.*, **95** (1975), 17.
6. GIBSON, K. D., NEUBERGER, A. & TAIT, G. H., *Biochem. J.*, **88** (1963), 325.
7. STANIEK, R. Y., DOUNGOROFF, M. & ADELBERG, E. A., *General microbiology* (Macmillan, India), 1974, 559.
8. STOKES, J. E. & HOARE, D. S., *J. Bacteriol.*, **100** (1969), 890.
9. ANDERSON, L. & FULLER, R. C., *Plant Physiol.*, **42** (1967), 487.
10. PAULSON, J. M. & LANE, M. D., *Biochemistry*, **5** (1966), 2350.
11. WALKER, J. D., SEEMAN, P. A. & COLWELL, R. R., *Marine Pollut. Bull.*, **5** (1974), 186.

#### Diel Variation in Phytoplankton Pigments & Primary Production off Bombay

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Received 24 January 1977; revised received 11 April 1977

Variations in biological parameters over 24 hr at 4 stations off Bombay indicated that chlorophyll *a* concentration, organic carbon production and particulate organic carbon (POC) were generally maximum during the day. Concentrations of pigments at any depth ranged from 0.09 to 1.74 (mg m<sup>-3</sup>). Similarly the production and POC varied from 1.15 to 86.12 (mgC m<sup>-3</sup> d<sup>-1</sup>) and 107.6 to 585.4 (mgC m<sup>-3</sup>). 'Smaller phytoplankton' contributed 50 to 100% to the pigment of the total phytoplankton. The maximum in assimilation number was found when NH<sub>4</sub>-N and NO<sub>3</sub>-N were maximum.

SHORT COMMUNICATIONS

SHORT term variations in the biological and chemical characteristics have been studied in various environments. Diel periodicity in photosynthesis of natural phytoplankton populations in oceanic waters has been investigated earlier<sup>1,2</sup>. These diel variations seem to be periodic even after appropriate corrections for the difference in population have been made<sup>3</sup>. Studies on the pigment variations made earlier have not given uniform results. Thus, it has been reported by a few workers that maximum *in situ* chlorophyll occurs at night<sup>4-8</sup> while others have found maximum pigment during the day<sup>2,9-12</sup>. It seems that the time of occurrence of the maximum pigment concentration in an environment depends on the previous light life history of phytoplankton, the cloudiness of the sky, etc<sup>4,8</sup>. Since the information available on the diel variation of biological parameters of the Arabian Sea is very little the present investigation has been undertaken.

The investigations were carried out on board R. V. *Gaeshani* during her 3rd cruise (21 March-1 April 1976). The station locations are: (1) 19°24.2' N, 71°23.3'E, (2) 19°21.6'N, 71°42'E, (3) 19°19.1'N, 72°01.1'E, and (4) 15°25'N, 73°48'E. Water sampling was carried out at 3 hr intervals from three depths. The sampling depths were 0, 10 and 50 m

for stations 1 and 2; 0, 15 and 30 m for station 3 and 0, 1 and 5 m for station 4. A known volume of water was filtered through Whatman GF/C glass filters for pigment estimations from whole plankton. Samples passed through a 64  $\mu$ m net were filtered through GF/C filter in order to estimate the pigment from 'smaller phytoplankton'. Primary production was measured at 6 hr interval by simulated *in situ* incubation with <sup>14</sup>C. After 24 hr incubations samples were filtered through a membrane filter of 0.45  $\mu$ m porosity. Particulate organic carbon (POC) was also estimated. Diurnal variations in pigment and other parameters were studied at all stations except station 2. At station 2, sampling and incubation were carried out only at sunset and sunrise. In the latter, one set was removed after 12 hr while the duplicate was kept for 24 hr incubation. Station 1, which was farthest from the shore was 75 m deep while station 4, closest to the shore had a depth of 15 m.

Chl. *a* was determined in a Turner Designs 10,000 fluorometer using the blue lamp F4T 5, excitation filter CS 5-60 and reference filter CS 3-66. Emission filters used were CS 70 and CS 16. The method adopted was as described earlier<sup>13</sup>. <sup>14</sup>C uptake was measured by counting the membrane filters in a thin window G.M. counter, the efficiency of which

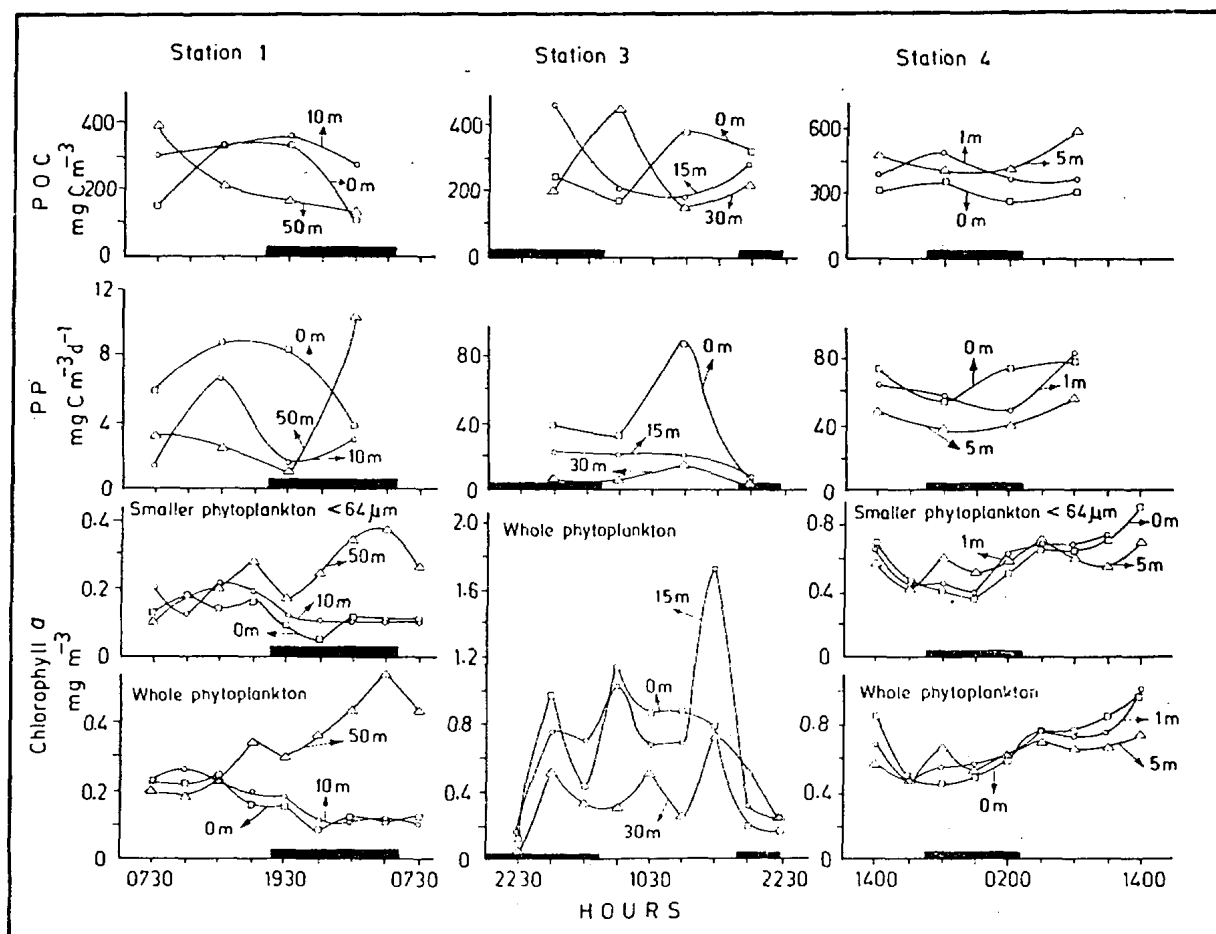


Fig. 1 — Diel variations in chl. *a*, primary production (PP) and particulate organic carbon (POC) at different depths

has been computed by comparison with a liquid scintillation system. POC was determined by wet acid digestion and colorimetry<sup>13</sup>.

At station 1 chlorophyll was maximum at the surface and at 10 m depth during the forenoon, while at 50 m depth the maximum was at night (Fig. 1). The ranges in pigment concentration ( $\text{mg m}^{-3}$ ) were: (i) 0 m, 0.09 to 0.25; (ii) 10 m, 0.1 to 0.26; (iii) 50 m, 0.2 to 0.55. Pigment from 'smaller phytoplankton' contributed 50 to 100% of that from whole plankton. The peak production from the upper layers showed maximum for the samples collected and incubated during the early afternoon while for 50 m it was with samples collected at night. The surface production ( $\text{mgC m}^{-3}\text{d}^{-1}$ ) varied from 3.77 to 8.81, while at 10 and 50 m the range in production was from 1.39 to 6.66 and 1.15 to 10.72. The assimilation number (AN) varied from 26 to 52 ( $\text{mgC d}^{-1}/\text{mg chl. } a$ ) at the surface. At other depths it was still lower. POC was maximum at the surface during the afternoon. At 50 m, it showed a decreasing trend.

As mentioned earlier, observations were made only twice at station 2. Chl. *a* concentration of the samples collected at sunset showed a variation

from 0.2 to 0.22 ( $\text{mg m}^{-3}$ ) at different depths. 'Smaller phytoplankton' contribution to the pigment from whole plankton was from 72 to 100%. The depthwise production ranged from 5.3 to 7.0 ( $\text{mgC m}^{-3}\text{d}^{-1}$ ). AN ranged from 34 to 40 at the surface. During the 2nd observation at sunrise the variation in the concentration of pigment at different depths was from 0.17 to 0.65 ( $\text{mg m}^{-3}$ ). <sup>14</sup>C uptake ranged from 5.2 to 5.8 ( $\text{mgC m}^{-3}\text{d}^{-1}$ ). But when the incubation was terminated after 12 hr the production ranged from 6.8 to 9.2.

Surface chl. *a* at station 3 was highest in the morning while at subsurface the maximum was late in the evening (Fig. 1). The lowest concentration was during night. The maximum concentrations at the 3 depths were 1.15, 1.74 and 0.77 ( $\text{mg m}^{-3}$ ) while the minimum values were 0.15, 0.17 and 0.05. The production was maximum for the samples collected just after midday. Lowest production was for the samples incubated at night. Thus, the ranges in production with depth were 6.15 to 86.12, 7.16 to 22.41 and 5.83 to 16.04 ( $\text{mgC m}^{-3}\text{d}^{-1}$ ). The ranges in POC at 0, 15 and 30 m were respectively 169 to 372, 169 to 456 and 152 to 445 ( $\text{mgC m}^{-3}$ ).

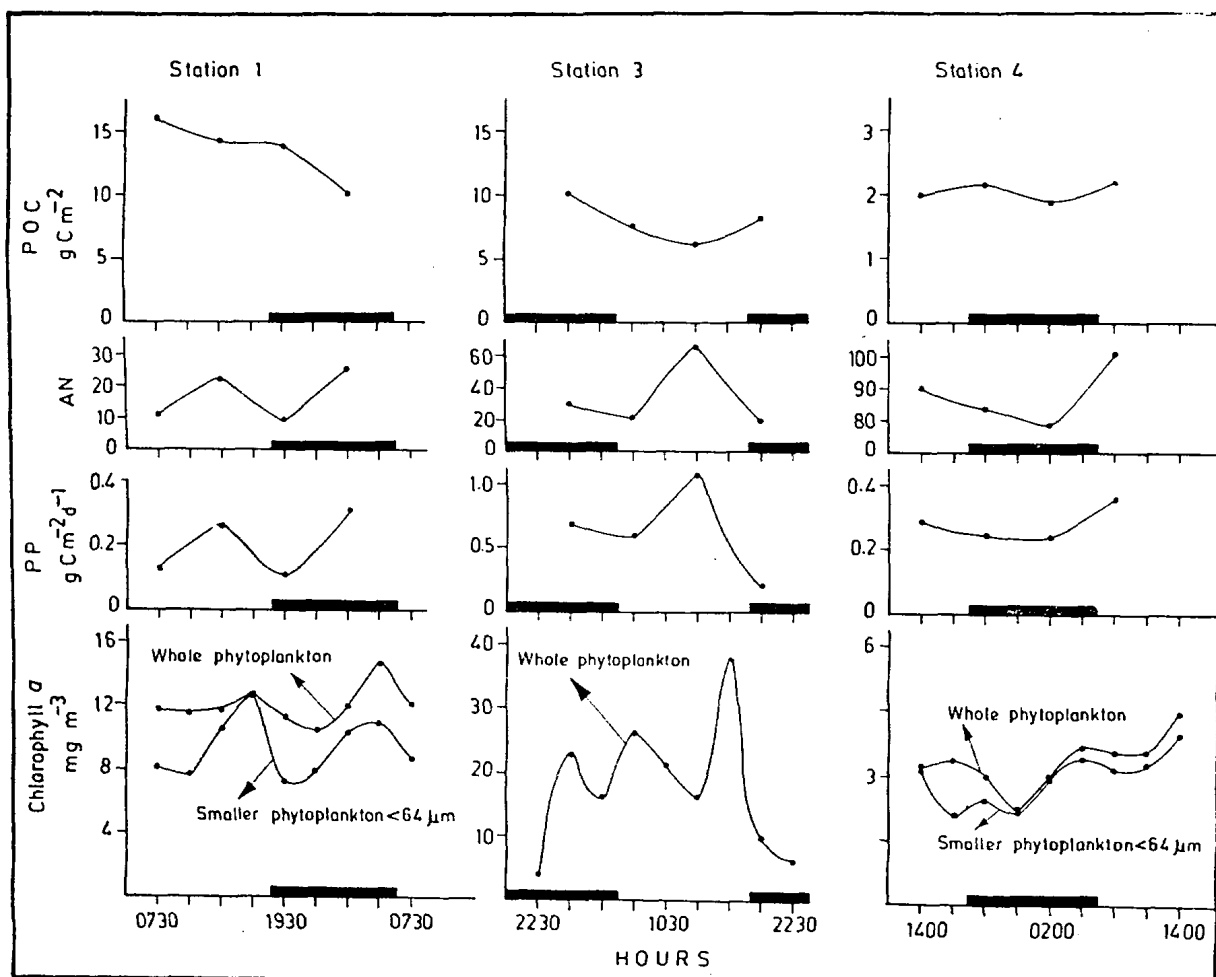


Fig. 2—Changes in column concentrations of chl. *a*, primary production (PP), assimilation number (AN) and particulate organic carbon (POC)

The pigment concentration at station 4 varied from 0.47 to 0.97, 0.47 to 1.01 and 0.47 to 0.73 ( $\text{mg m}^{-3}$ ) at 0, 1 and 5 m (Fig. 2). The maximum was found during the afternoon. Primary production was as follows: 0 m, 52.58 to 78.25; 1 m, 47.9 to 82.95; 5 m, 38.73 to 56.93 ( $\text{mgC m}^{-3} \text{d}^{-1}$ ). The minimum production was observed in samples collected and incubated during night at 0200 hrs. The highest production was shown by the sample collected during early sunlight hours.

Fig. 2 shows the column chl. *a*, primary production, POC and AN for stations 1, 3 and 4. At station 1, pigment from whole plankton and 'smaller phytoplankton' varied from 10.53 to 14.7 and 7.35 to 10.98 ( $\text{mg m}^{-2}$ ). Production and POC ranged from 0.11 to 0.31 ( $\text{gC m}^{-2} \text{d}^{-1}$ ) and 10 to 16.12 ( $\text{gC m}^{-2}$ ).

Samples from station 2 collected at sunset showed column chl. *a* and production as 10.38 ( $\text{mg m}^{-2}$ ) and 0.28 ( $\text{gC m}^{-2} \text{d}^{-1}$ ) whereas that collected at sunrise showed correspondingly 17.67 and 0.27. But in the latter case the production during daylight hours was 0.43 [ $\text{gC m}^{-2} (12\text{h})^{-1}$ ] indicating a loss of 37% during the dark hours.

At station 3 chl. *a*, production and POC showed variation from 4.04 to 37.74 ( $\text{mg m}^{-2}$ ), 0.20 to 1.08 ( $\text{gC m}^{-2} \text{d}^{-1}$ ) and from 6.31 to 10.41 ( $\text{gC m}^{-2}$ ). The pigment from whole and 'smaller phytoplankton' at station 4 were found to vary from 2.2 to 4.47 and 2.06 to 4 ( $\text{mg m}^{-2}$ ).  $^{14}\text{C}$  assimilation was between 0.24 and 0.36 ( $\text{gC m}^{-2} \text{d}^{-1}$ ). POC varied from 1.88 to 2.22 ( $\text{gC m}^{-2}$ ). The surface concentrations ( $\mu\text{g at/litre}$ ) of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  were found to range from 0.91 to 11.17 and 0.42 to 4.43 (station 1); 1.01 to 5.12 and 0.66 to 4.53 (station 3); 2.45 to 6.71 and 3.52 to 7.64 (station 4). AN (calculated on the basis of column production and chlorophyll *a*) was found to vary from 10 to 26, 20 to 66 and 79 to 101 respectively at the three stations.

The above results show that at station 1, the *in situ* chlorophyll was maximum during the day except for 50 m. Pigment from 'smaller phytoplankton' also showed the same trend. Similarly, samples collected and incubated during day time gave maximum production. These factors may not necessarily be repeated in other seasons. However, the present data showed similarity to the results reported by Ryther *et al.*<sup>10</sup> and Lorenzen<sup>11</sup>. The reduction of pigment during night is probably due to grazing by zooplankters and other herbivores<sup>12</sup> although marine diatoms are capable of synthesizing chl. *a* in the dark<sup>14</sup>. The diel periodicity in pigment is because of the synchronous cell division during the light period<sup>15</sup>.

AN was always more at the station closest to the shore. The maximum AN at stations 1 and 4 occurred when  $\text{NH}_4\text{-N}$  concentration was maximum while that at station 3 occurred when  $\text{NO}_3\text{-N}$  was maximum. Further, the concentrations of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  were higher at the nearshore location.

Diel variation occurring in phytoplankton is due to one of the reasons such as cellular characteristics, physiological aspects and mode of behaviour<sup>16</sup>. It has been found that photosynthesis, chlorophyll synthesis, excretion, nutrient uptake and cell division to have diel cycle<sup>1,2,9,16-20</sup>.

Variations observed in the photosynthetic carbon fixation can be explained by two hypotheses, viz. phasing and forcing<sup>20</sup>. The former is based on the inherent nature of algae such as the enhancement of processes in a light-dark cycle while the latter is on the assumption of a time dependent deficiency and destroying action causing the oscillation.

From  $^{14}\text{C}$  uptake studies carried out at station 2, it is clear that loss during dark is about 37% of the production per 12 hr. Similar loss in the Central North Pacific was about 50%<sup>21</sup>. Moreover, these authors found that such loss is dependent on the time of beginning of incubation. These observations if found true in other environments also, bring an element of error in the productivity estimate.

The short term cycle observed in the various parameters will significantly affect the analysis made at any particular instant since it may not give a true picture of the events happening. Thus, it has been found that estimates of diversity computed on the basis of pigment index is dependent on the sampling time<sup>22</sup>. Again, biomass estimated from pigment concentration will not give the true picture. Hence, it is suggested that the sampling procedures should be suitably modified in order to have a proper account of the pulsating events in the sea<sup>16</sup>.

The authors are thankful to Dr S. Z. Qasim, Director, for valuable comments. Thanks are due to Shri S. G. Prabhu Matondkar for help in some of the chemical analysis. Thanks are also due to Capt. S. D. Pimpalkhare, Master R. V. Gaveshani and his colleagues for help and cooperation.

#### References

1. DOTY, M. S. & OGURI, M., *Limnol. Oceanogr.*, **2** (1957), 37.
2. SHIMADA, B. M., *Limnol. Oceanogr.*, **3** (1958), 336.
3. DOTY, M. S., JAN NEWHOUSE & ROY, T. T., *Arch. Oceanogr. Limnol.*, **15** (1967), 1.
4. YENTSCH, C. S. & SCAGEL, R. F., *J. mar. Res.*, **17** (1958), 567.
5. STEEMANN NIELSEN, E. & JORGENSEN, E. G., *Arch. Hydrobiol.*, **58** (1962), 349.
6. EL-SAYED, S. Z. & MANDELLI, E. F., in *Biology of the Antarctic seas*, Vol. 2, edited by G. A. Lalne (American Geophysical Union Publication 1297, Washington DC), 1965, 87.
7. GLOOSCHENKO, W. A., *Quart. J. Fla. Acad. Sci.*, **33** (1970), 187.
8. GLOOSCHENKO, W. A., CURL, H. (Jr) & SMALL, L. F., *J. fish Res. Bd. Canada*, **29** (1972), 1253.
9. YENTSCH, C. S. & RYTHER, J. H., *Limnol. Oceanogr.*, **2** (1957), 140.
10. RYTHER, J. H., MENZEL, D. W. & VACCARO, R. F., *Limnol. Oceanogr.*, **6** (1961), 149.
11. LORENZEN, C. H., *Limnol. Oceanogr.*, **8** (1963), 56.
12. MCALLISTER, C. D., *Limnol. Oceanogr.*, **8** (1963), 289.
13. STRICKLAND, J. D. H. & PARSONS, T. R., *Bull. fish. Res. Bd. Canada*, **167** (1968), 310.
14. YENTSCH, C. S., *Deep Sea Res.*, **12** (1965), 653.
15. JORGENSEN, E. G., *Physiol. Plant.*, **19** (1966), 786.
16. SMAYDA, T., *Deep-Sea Res.*, **22** (1975), 151.
17. GOERING, J. J., DUGDALE, R. C. & MENZEL, D. W., *Limnol. Oceanogr.*, **9** (1964), 534.
18. EPPLEY, R. W., CARLUCCI, A. F., HOLM-HANSEN, O., KIEFER, D., MCCARTHY, J. J., VERRICK, E. & WILLIAMS, P. M., *Limnol. Oceanogr.*, **16** (1971), 741.
19. WALSH, G. E., *Limnol. Oceanogr.*, **10** (1965), 577.
20. STROSS, R. G., CHISHOLM, S. W. & DAVNING, J. A., *Biol. Bull.*, **145** (1973), 200.
21. EPPLEY, R. W. & SHARP, J. H., *Limnol. Oceanogr.*, **20** (1975), 981.
22. KROUT, J., *Pigment and pigment ratio (430 nm/665 nm) distribution in several marine environments*, M.S. thesis, University of Rhode Island, 1971.