

Primary Productivity & Carbon Assimilation Patterns in Tropical Marine Phytoplankton

J I GOES & V P DEVASSY

National Institute of Oceanography, Dona Paula, Goa 403 004

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Primary productivity values measured at 3 stations in the Lakshadweep Sea (Laccadive Sea) varied from 0.44 to 2.11 $\text{mg C m}^{-3} \text{h}^{-1}$ and were characterised by a distinct subsurface maxima. Over a diel cycle in the Minicoy Lagoon, the rates varied from 0.22 to 12.80 $\text{mg C m}^{-3} \text{h}^{-1}$ and closely paralleled the diurnal changes in solar irradiance levels. Studies on the distribution of assimilated carbon into the major end products of photosynthesis showed that the synthesis of polysaccharides is favoured at higher light intensities. There was a steady decline in the levels of polysaccharides synthesized with a decrease in levels of light available for photosynthesis. The synthesis of lipid/LMWM (low molecular weight metabolites) was also favoured at higher light intensities. A highly significant relationship between the percentage of lipid/LMWM synthesized and the productivity rates per unit chl *a* suggests that the appearance of newly fixed carbon within this fraction is strongly dependent on the rates of photosynthesis at any given time. A higher proportion of protein synthesized was observed under conditions of low light intensity. Regression analysis between the percentage of protein synthesized and the productivity rates per unit chl *a* showed an inverse relationship. Changes in the nature of the end products of photosynthesis in relation to available light in 2 clones of marine phytoplankton, viz. *Nitzschia closterium* and *Pleurosigma* sp., were in general agreement with those observed in natural populations.

Among the several factors that influence primary production, light is the most important, since it initiates and controls phytoplankton growth and primary production at all levels of availability¹. Quantity and quality of light at any given depth within the euphotic zone are functions of the intensity of the incident radiation, which varies diurnally, seasonally and with the quantity of suspended matter in the water column which generally and selectively absorbs light over various wave lengths. In a water column therefore, the algae can be expected to be subjected to different intensities and spectral characteristics of light, corresponding to the passage of the sun over the course of the day (diurnal photo period); to changes in season and to various depths in the water column. Consequently, the quality of end products of their carbon assimilation would be expected to vary as a function of available light for photosynthesis.

The present study examines the influence of different light intensities on the rates of photosynthesis and the distribution of assimilated carbon into the major end products of photosynthesis. These observations were made during the 102 cruise of *R V Gaveshani* (11-26 Jan. 1982), on natural phytoplankton samples drawn from different depths at 3 stations in the Lakshadweep Sea and over a diel cycle at one station in the Minicoy Lagoon. The results obtained therein have been discussed in conjunction with those ob-

tained in similar studies on 2 tropical species of marine diatoms in culture.

Materials and Methods

At the 3 stations in the Lakshadweep Sea i.e. sts 2328, 2330 and 2332, discrete water samples were collected from depths corresponding to 100, 60, 30, 16 and 1% of the incident light. Estimations of the euphotic depth were made from Secchi disc readings. At the station selected in the mid-lagoon at Minicoy Island (lat. 8°7'N, long. 73°18'E), water samples were collected at regular intervals of 3 h over a period of 24 h from depths corresponding to 100 and 60% of the surface incident light. Surface light intensity measurements were made using a light quanta meter.

Determinations of inorganic nitrate and phosphate were carried out in duplicate as per standard methods². Phytoplankton cell counts were made on settled aliquots of water samples using a Sedgwick rafter counting chamber. Chlorophyll *a* was determined fluorometrically².

Primary productivity measurements were made using the ¹⁴C technique³. Water samples were pre-filtered through a nylobolt net (300 μm) to prevent excessive zooplankton grazing and were dispensed into 125 ml glass stoppered bottles. The bottles were covered with appropriate neutral density filters (Kahlsico) to obtain the corresponding light intensities.

ies. The samples were inoculated with 5 μCi of $\text{NaH}^{14}\text{CO}_3$, incubated for 4 h in running seawater on the deck and then subsequently filtered on 0.45 μm membrane filters. The filters were rinsed with sterile seawater and the radioactivity on the filters was measured in a liquid scintillation counter using the channels ratio method for quench correction⁴.

In samples meant for studying the pattern of carbon assimilation into the major end products of photosynthesis, the differential solvent extraction technique⁵ was used. The labelled material retained on the filter paper was initially stored in 80% ethanol and subsequently treated to yield an ethanol soluble fraction containing lipid/low molecular weight metabolites (LMWM), a trichloroacetic acid fraction containing polysaccharides and a residual protein fraction. In calculating the proportion of carbon incorporated, the total radioactivity was determined as the sum of each fraction. Recovery values varied between 96 and 107% when total radioactivity determined as the sum of the 3 fractions was compared to that measured directly.

For studies on phytoplankton in culture, actively growing cells of *Nitzschia closterium* and *Pleurosigma* sp. were used. Aliquots of both these cultures (125 ml each) were transferred to glass stoppered bottles. Light intensities in the bottles were altered with neutral density filters to appropriate 100, 60, 30 and 16% light transmissions. The samples covered with the neutral density filters were allowed to acclimatize to the new conditions of light by pre-incubation for 4 h under constant illumination of 1.35×10^{17} quanta $\text{sec}^{-1} \cdot \text{cm}^{-2}$ and at ambient room temperature ($26^\circ \pm 1^\circ\text{C}$). The quantum of light intensity provided for these cultures was adapted so as to approximate the light conditions recorded for natural populations. On addition of 1 ml of 5 μCi $\text{NaH}^{14}\text{CO}_3$, the samples were incubated for 4 h and consequently filtered on to membrane filters (0.45 μm). The filters with the labelled material were rinsed with sterile seawater and the subsequent fractionation and measurement of radioactivity in each fraction were carried out as given above.

Results and Discussion

Inorganic nutrients—Inorganic $\text{NO}_3^- - \text{N}$ varied from 0.44 to 21.22 $\mu\text{g-at} \cdot \text{l}^{-1}$ whereas inorganic $\text{PO}_4^- - \text{P}$ varied from 0.31 to 2.09 $\mu\text{g-at} \cdot \text{l}^{-1}$ at the 3 stations in the Lakshadweep Sea (Table 1). High nutrient concentrations were recorded at depths corresponding to 1% of the surface irradiance. The proximity of this depth to the thermocline where nutrients generally diffuse upwards could explain the higher concentrations of nutrients recorded at those depths. Differences between surface and subsurface nu-

trient levels were not significant at the station selected in the Minicoy Lagoon (Fig. 1). $\text{NO}_3^- - \text{N}$ varied from 0.48 to 1 $\mu\text{g-at} \cdot \text{l}^{-1}$ whereas $\text{PO}_4^- - \text{P}$ values recorded varied from 0.19 to 0.65 $\mu\text{g-at} \cdot \text{l}^{-1}$. Nutrient levels were comparatively low during the day but gradually increased towards the hours of darkness. It has been indicated that phytoplankton show diurnal patterns

Table 1 – Surface Light Intensity, Depth-wise Distribution of Nutrients, Phytoplankton Biomass and Rates of Photosynthesis at 3 Stations in Lakshadweep Sea

Irradiance* %	Conc. $\mu\text{g-at} \cdot \text{l}^{-1}$			Rate of photo-synthesis
	$\text{NO}_3^- - \text{N}$	$\text{PO}_4^- - \text{P}$	Chl <i>a</i>	
St 2328 (lat. 10°N, long. 68°E)				
100	0.44	0.31	0.03	33.45
60	0.71	0.48	0.16	20.40
30	0.44	0.45	0.29	6.80
16	1.93	0.54	0.19	4.69
1	21.22	2.09	0.29	3.69
St 2330 (lat. 10°N, long. 67°E)				
100	0.50	0.42	0.03	35.84
60	0.50	0.46	0.13	11.26
30	1.00	0.42	0.03	56.75
16	0.35	0.37	0.45	3.39
1	8.48	1.23	0.03	2.16
St 2332 (lat. 10°N, long. 65°E)				
100	0.75	0.65	0.45	4.36
60	0.75	0.42	0.03	58.70
30	0.50	0.49	0.03	42.17
16	0.75	0.37	0.06	28.08
1	1.48	1.92	0.16	6.22

*Surface irradiance (quanta. $\text{sec}^{-1} \cdot \text{cm}^{-2}$) at sts 2328, 2330 and 2332 respectively was 1.35×10^{17} , 1.45×10^{17} and 2.2×10^{17}

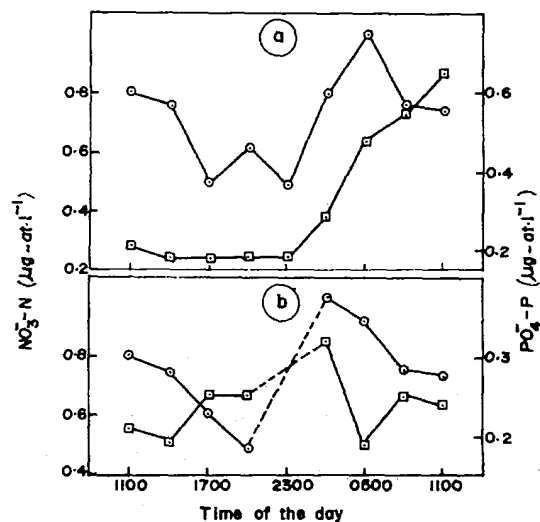


Fig. 1 – Diel variation in inorganic nitrate (⊙) and phosphate (⊠) in surface (a) and subsurface (b) waters.

in the uptake of nutrients^{6,7}. During the day due to active photosynthesis, nutrients tend to get depleted at a faster rate. Harris⁸ reported that zooplankton themselves excrete a large quantity of nitrogen during the night. A substantial quantity of nutrients may also be added by way of phytoplankton cell breakage during grazing by zooplankton. A large standing stock of zooplankton was recorded during the hours of darkness⁹, which could have contributed significantly to the enhanced levels of nutrients observed during the night.

Phytoplankton biomass—Phytoplankton standing stock measured as chl *a* varied from 0.03 to 0.45 $\mu\text{g}\cdot\text{l}^{-1}$ at the 3 stations in the Lakshadweep Sea. At sts 2328 and 2330 highest chl *a* concentrations were recorded at the subsurface, whereas at st 2332 chl *a* concentration was highest at the surface (Table 1).

Variations in chl *a* concentrations at the surface and subsurface in Minicoy Lagoon are presented in Fig. 2. Chl *a* varied from 0.45 to 3.92 $\mu\text{g}\cdot\text{l}^{-1}$ at the surface and from 0.64 to 1.73 $\mu\text{g}\cdot\text{l}^{-1}$ at the subsurface. The steady decline in chl *a* levels towards the hours of darkness could have resulted from the active grazing of zooplankton during this period. A sharp increase in chl *a* levels was however noticed in the subsurface waters at 2300 hrs. This increase was not paralleled by a similar increase in the concentration in surface waters where chl *a* values appear to remain steady during the morning hours.

Cell counts—Diatoms were distinctly the most abundant group of phytoplankton at sts 2328, 2330 and 2332. At st 2328, *N. closterium* and *Coscinodiscus* sp. were the dominant forms, whereas *Chaetoceros* sp. and *Thalassiosira* sp. were dominant at sts 2330 and 2332 respectively. *Nitzschia seriata* and *Nitzschia* sp. were present at all 3 stations. The dinoflagellate population at all 3 stations consisted mainly of *Prorocentrum* sp. and *Ceratium* sp. Blue green algal forms were present at all stations and were generally confined to the subsurface waters. A few microflagellates were also present but were either too small or ill-defined for species identification. Total counts expressed as cells $\cdot\text{l}^{-1}$ varied from 1.65 to 5.25 $\times 10^3$. The relationship of total cell numbers with chl *a* concentrations was not significant. It is presumed that the addition of formaldehyde as a preservative to phytoplankton would have led to a rupture and sizeable loss of phytoplankton cells¹⁰.

The standing crop of phytoplankton at both the surface and subsurface in the Minicoy Lagoon was dominated by chain forming diatoms such as *Chaetoceros lauderi* and *Chaetoceros difficilis*. The dinoflagellate *Gonyaulax* sp. was also present in substantial numbers and was the dominant form in samples at 1400 hrs. A computation of Shannon-Weaver divers-

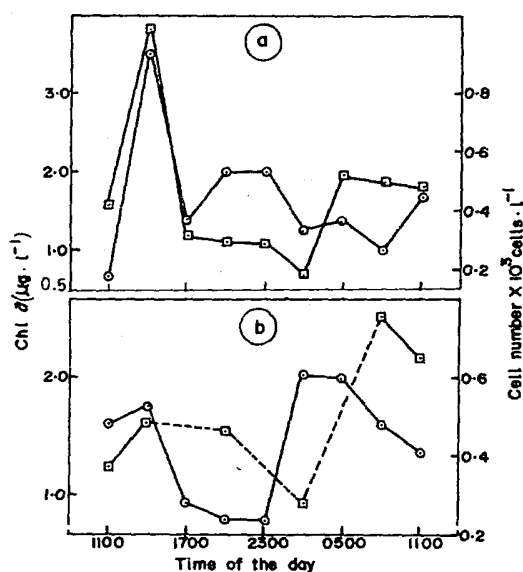
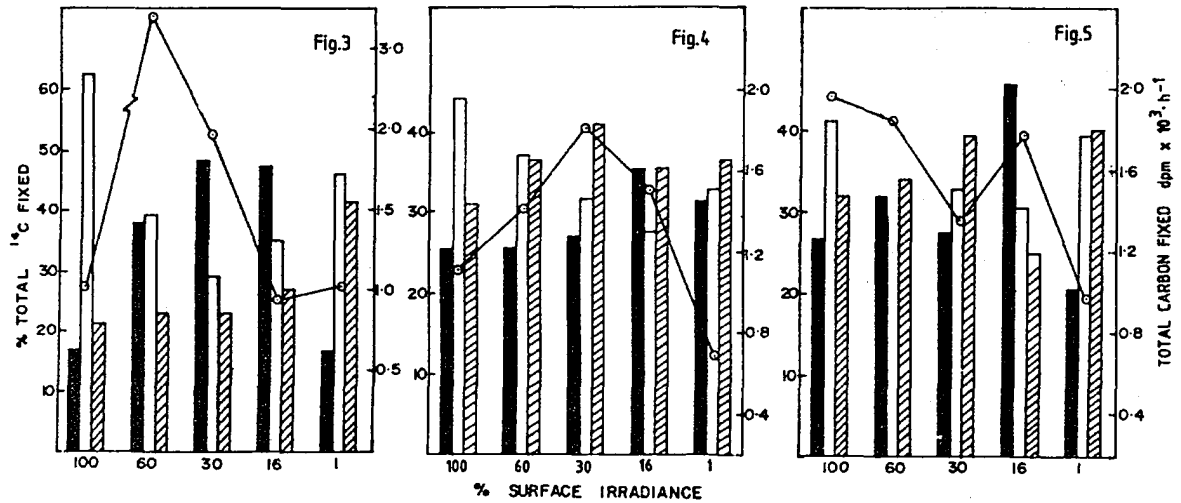


Fig. 2—Diel variation in chl *a* (⊙) and phytoplankton cell number (⊠) in surface (a) and subsurface waters (b) at Minicoy Lagoon.

ity index indicated that the phytoplankton population was more diverse at night than during the day-light hours in both surface and subsurface waters. Total cell counts plotted in Fig. 2b vary from 0.18 to 1.12 $\times 10^3$ cells $\cdot\text{l}^{-1}$ at the surface and from 0.28 to 0.77 $\times 10^3$ cells $\cdot\text{l}^{-1}$ in the subsurface waters. A steady decline in cell number observed during the hours of darkness may also suggest increased grazing pressure. Total cell counts correlated significantly with chl *a* values ($r=0.73$).

Primary productivity—Photosynthetic rates at the 3 stations varied from 0.44 to 2.11 $\text{mg C}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$ and were characterised by a distinct subsurface maxima (Figs 3-5). When expressed per unit chl *a* the surface phytoplankton populations at st 2332 appear to be strongly photoinhibited. Over large areas of the Arabian Sea, maximum production rates are found in the subsurface layers than at the surface¹¹. These low production rates at the surface have been attributed to the prevalent conditions of intense solar radiation which leads to photoinhibition and consequently low production rates.

In the Minicoy Lagoon, variations in primary productivity rates over the course of the day were significantly high i.e. from 0.22 to 12.80 $\text{mg C}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$ (Figs 6b and 7b) and closely paralleled the diurnal changes in levels of solar irradiance. Diurnal fluctuations in primary productivity rates have often been related to fluctuations in phytoplankton biomass estimated on the basis of chl *a*¹². In this study however, the low productivity values recorded during the first observation



Figs 3 to 5 – Depthwise variation in rates of photosynthesis (\odot) and proportion of (^{14}C) bicarbonate incorporated into lipid/LMWM (\blacksquare), polysaccharide (\square) and protein (\boxplus) fractions at sts 2328 (3), 2330 (4) and 2332 (5).

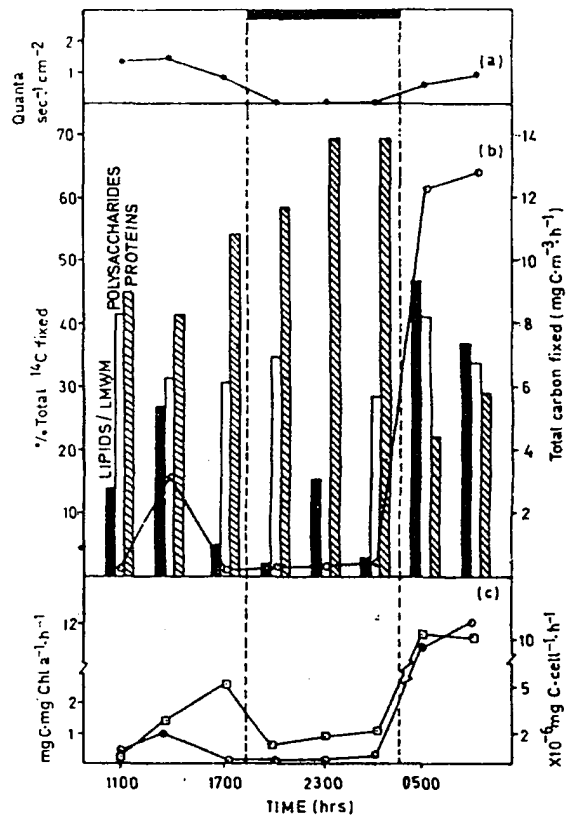


Fig. 6 – Diel variation in rates and patterns of photosynthetic carbon assimilation in surface phytoplankton at Minicoy Lagoon [(a) Changes in surface irradiation ($-\bullet-$), (b) variations in rates of photosynthesis ($-\odot-$) and proportion of (^{14}C) bicarbonate incorporation into lipid/LMWM, polysaccharide and protein fractions, (c) rate of photosynthesis expressed per unit, chl a (\odot) and per cell (\square)].

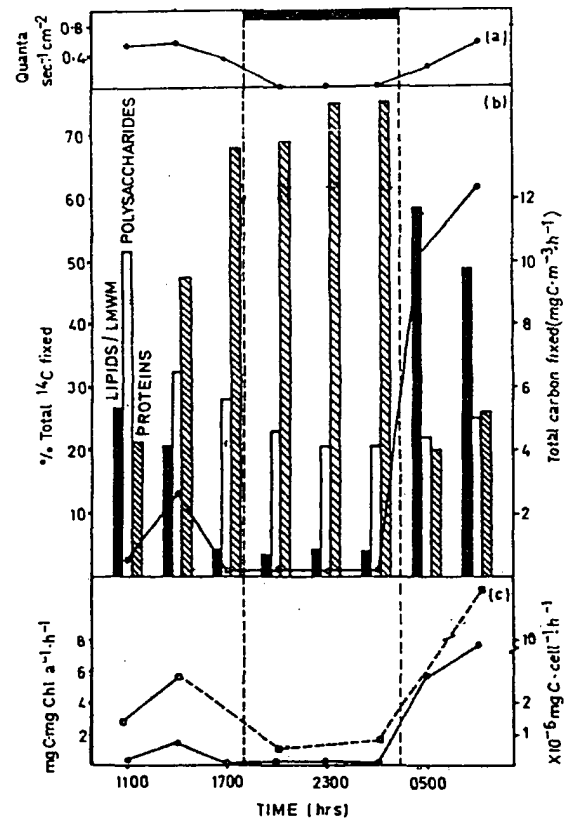


Fig. 7 – Diel variation in rates and patterns of photosynthetic carbon assimilation in subsurface phytoplankton [Other details as in Fig. 6].

at 1100 hrs, cannot be wholly explained on the basis of the low chl *a* values observed at this time of the day. It is more likely that the cells at this time of the day were photoinhibited, since higher photosynthetic rates per unit chl *a* were observed at the subsurface where the photoinhibitory effects of solar radiation were less pronounced. These observations are supported by the fact that the photosynthetic rates per unit chl *a* showed an initial increase after 1100 hrs, when solar irradiance levels declined. Rates of photosynthesis were highest during the early morning hours. Increase in photosynthetic rates after a period of darkness has often been attributed primarily to increased chl *a* synthesized during the dark period^{13,14}. However, in these studies, the photosynthetic rates when expressed per unit chl *a* (Figs 6c and 7c) were higher in the early morning hours than those recorded during other times of the day. It may be inferred, that this increase could not be wholly due to the increased levels of chl *a* as observed in this study, but due to favourable exogenous factors like light and nutrient levels prevalent at this time of the day.

Photosynthetic carbon assimilation patterns— Variations in the appearance of labelled carbon into the lipid/LMWM, polysaccharide and protein fractions within the euphotic zone at sts 2328, 2330 and 2332 are presented in Figs 3, 4 and 5 respectively. At all stations, the proportion of polysaccharides synthesized was highest at surface light intensities. A steady decline in the proportion of polysaccharides synthesized was noted with a decrease in the levels of light available for photosynthesis. This trend was not observed at depths corresponding to 1% light intensities. Barlow¹⁵ has reported that phytoplankton populations photosynthesizing under conditions of high light intensity and low nitrate concentrations tend to accumulate newly fixed carbon into polysaccharides. Although in theory, polysaccharide synthesis may not be linked to the availability of nitrate, as this nutrient is incorporated into the amino acids of the protein, it is likely that at low nitrate concentrations, the continued assimilation of carbon is channeled into the polysaccharide fraction rather than the protein fraction. The discrepancy observed in the proportion of polysaccharide synthesized at depths corresponding to 1% light intensities could have resulted from the high nitrate concentrations at these depths.

The proportion of protein synthesized in general shows a concomitant increase with increasing depth within the water column. Wallen and Geen¹⁶ working on natural populations from different depths in the euphotic zone have primarily related such increases in the proportion of protein synthesized to the increasing percentage of blue light within the euphotic column. A similar finding was however attributed to a

decrease in levels of light intensity within the water column. In these studies it may be pointed out that although the samples were drawn from different depths within the water column, only changes in light quantity rather than quality could be simulated using the neutral density filters. The increased levels of protein synthesized could thus be attributed more to the declining light levels rather than an increase in the levels of blue light.

The appearance of labelled carbon in the lipid/LMWM fraction showed no definite trend, although higher percentages were observed at the subsurface level.

Diel variations in the patterns of carbon assimilation in surface and subsurface populations (Figs 6b and 7b respectively) in the Minicoy Lagoon appear to follow a similar trend. A one-way analysis of variance was used to test the hypothesis that there is no difference between the surface and subsurface populations in the appearance of label into either the lipid/LMWM, the polysaccharide or protein fractions over the course of the day. Variance ratios are not significant and hence the hypothesis was accepted.

Around mid-day when light intensities recorded were the highest, assimilation of labelled carbon within the cell was characterized by higher percentage incorporation of carbon into the polysaccharide fraction. The proportion of label incorporated into the polysaccharide fraction thereafter shows a steady decline with declining light intensities and the declining rates of photosynthesis. After the period of darkness, with an increase in the rates of photosynthesis, there is an increase in the proportion of label appearing in the polysaccharide fraction. Morris and Skea¹⁷ have observed that protein synthesis in the dark is fueled by cellular reserves of polysaccharides. Fluctuations in polysaccharide synthesis have also been reported by Holm-Hansen¹⁸ during studies on adenosine triphosphate levels in phytoplankton cells, photosynthesizing in a light/dark cycle. He explained such changes on the basis of the shift from photophosphorylation in the light to oxidative phosphorylation in the dark which is essential to maintain ATP levels in cells constant.

Diel variations in the incorporation of labelled material in the lipid/LMWM fraction are significantly high and closely parallel the levels of solar irradiance. A regression analysis between the percentage of label appearing in the lipid/LMWM fraction and the primary productivity rates per unit chl *a* yields *r* values of 0.83 and 0.89 ($P < 0.001$) for the surface and subsurface respectively, suggesting that the synthesis of this fraction is closely dependent on the rates of photosynthesis at any given time. High levels of lipid synthesis in phytoplankton have normally been associated

with stressful conditions of nutrient deficiency¹⁹. Lipids may also be accumulated when phytoplankton are photosynthesizing at low temperatures²⁰. In this study, the nutrient concentrations were reasonably high to make nutrient deficiency unlikely and the temperatures recorded were also never significantly low, so as to favour lipid synthesis. It is thus probable, that the radioactivity in this fraction was located in the LMWM component rather than the lipid component of this fraction.

The appearance of labelled carbon in the protein fraction also showed a distinct pattern of variation over the course of the day. During the day light hours, the percentage of protein synthesized was low as compared to the percentage synthesized during the hours of darkness. A regression analysis between the percentage of protein synthesized and the rates of photosynthesis per unit chl *a* shows an inverse relationship between these 2 variables [$r = -0.86$ and -0.71 ($P < 0.001$) for surface and subsurface populations respectively].

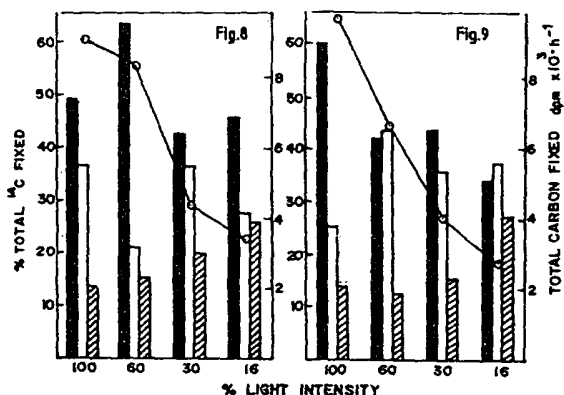
An analysis of covariance between percentages of protein and that of lipid/LMWM synthesized, suggests that a strong increase in the amounts of protein synthesized is correlated with a strong decrease in lipid/LMWM synthesized ($r = -0.99$ and -0.91 for surface and subsurface populations respectively). Li and Harrison²¹ suggested that growth and cell division in phytoplankton were more closely dependent on the synthesis of protein. As such, only when protein synthesis was optional, storage products viz. polysaccharides and lipids were accumulated. It is probable thus, that, when the photosynthetic rates were low during the night and the proportion of low molecular weight precursors was not sufficiently high to maintain a continued rate of protein synthesis, storage products were rapidly broken down to provide energy for a continued rate of protein synthesis. This may explain the higher percentage of labelled carbon observed in the protein rather than the polysaccharide or lipid/LMWM fractions during the hours of darkness. These results are in agreement with those of Morris *et al.*⁵ who indicated that photosynthesis at low light intensities favours protein synthesis at the cost of polysaccharide synthesis. Morris and Skea¹⁷ have also observed that the consumption of polysaccharide and LMWM intermediates for continued synthesis of protein during the dark would result in increased percentages of ¹⁴C appearing in the protein fraction.

Studies on phytoplankton in culture— In both the forms studied, viz. *N. closterium* and *Pleurosigma* sp., the photosynthetic rates were highest at 100% light intensity and declined steadily with decreasing light intensities (Figs 8 and 9).

Variations in the appearance of carbon into the major photosynthetic end products at different light intensities are also presented in Figs 8 and 9. The results do show that light has a significant effect on the patterns of carbon assimilation within the cells. In *N. closterium* there was a greater percentage of carbon incorporated into the lipid/LMWM at 100 and 60% than at 30 and 16% light intensities. In *Pleurosigma* sp., the highest percentage of radioactivity in the lipid/LMWM fraction was observed at 100% light intensities. As reported for natural populations it is likely that the higher percentage of radioactivity observed in the lipid/LMWM fraction was due to incorporation of labelled carbon in the LMWM rather than the lipid component as the nutrient concentrations and temperatures were both optimal.

The amount of labelled carbon appearing in the polysaccharide fraction shows no definite trend with changing light intensities. In *N. closterium* higher percentages of polysaccharide synthesized were observed at 60 and 30% light intensities, whereas in *Pleurosigma* sp., higher percentages were recorded at 60, 30 and 16% light intensities. These observations are not in agreement to those made at sts 2328, 2330 and 2332 wherein higher rates of polysaccharide synthesized were observed at greater light intensities. As pointed out, the synthesis of polysaccharides was favoured at these stations because of the prevalent conditions of high light intensity and in addition, the low nitrate concentrations in the upper layers of the euphotic column. Nitrate levels in both the cultures were sufficiently high and this could perhaps explain this discrepancy.

The percentage of label appearing in the protein fractions showed a definite trend in both the forms studied. With a decrease in light available to the cells, there was a concomitant increase in the percentage of



Figs 8 and 9 - Effect of light intensity on the rate photosynthesis (○) and on proportion of (¹⁴C) bicarbonate incorporation into lipid/LMWM (■), polysaccharide (□) and protein (▨) fractions in *Nitzschia closterium* (8) and *Pleurosigma* sp. (9).

protein synthesized. These results are in conformity with those observed in these studies on natural populations, wherein it was found that in cells photosynthesizing under conditions of low light intensity, a greater proportion of newly fixed carbon appears in the protein fraction.

The results presented above suggest that phytoplankton populations in general do exert a great deal of control in the partitioning of carbon within the cells in relation to the quantity of incident light at any given time. It is possible that cellular control of the partitioning of carbon within the cell is capable to a considerable degree of evening out the fluctuations in a turbulent environment so as to produce a near constant growth rate as possible.

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References

- 1 Jerlov N G, in *Marine ecology*, edited by O Kinne (John Wiley, New York) 1970, 95.
- 2 Strickland J D H & Parsons T R, *A practical handbook of sea water analysis: Bull Fish Res Bd Canada*, **167** (1968) 310 pp.
- 3 Steemann-Nielsen E, *J Cons Perm Int Explor Mer*, **18** (1952) 117.
- 4 Hendee WR (Ed.), *Radioactive isotopes in biological research* (John Wiley & Sons, New York) 1973, 356 pp.
- 5 Morris I, Glover H E & Yentsch C S, *Mar Biol*, **27** (1974) 1.
- 6 Eppley R W, Rogers J N, McCarthy J J & Sourmia A, *J Phycol*, **7** (1971) 150.
- 7 Toetz P W, *Hydrobiologia*, **49** (1976) 49.
- 8 Harris E, *Bull Bingham Oceanogr Coll*, **17** (1959) 31.
- 9 Goswami S C, personal communication.
- 10 Desikachary T V, personal communication.
- 11 Radhakrishna K, Devassy V P, Bhargava R M S & Bhattathiri P M A, *Indian J Mar Sci*, **7** (1978) 271.
- 12 Glooschenko W A, Curl H & Small L F, *J Fish Res Bd Canada*, **29** (1972) 1253.
- 13 Stross R G, Chisholm S W & Downing T A, *Biol Bull*, **145** (1973) 100.
- 14 Anderson R S, *Oecologia (Berl)*, **14** (1974) 1.
- 15 Barlow R G, *J Exp Mar Biol Ecol*, **63** (1982) 1.
- 16 Wallen D G & Geen G H, *Mar Biol*, **10** (1971) 34.
- 17 Morris I & Skea W, *Mar Biol*, **47** (1978) 303.
- 18 Holm-Hansen O, *Plant and Cell Physiol Tokyo*, **11** (1970) 689.
- 19 Collyer D M & Fogg G E, *J Exp Bot*, **6** (1955) 256.
- 20 Smith A E & Morris I, *Science*, **207** (1980) 197.
- 21 Li W K W & Harrison W G, *Mar Biol*, **72** (1982) 175.
- 22 Fisher R A & Yates F (Eds), *Statistical tables for biological, agricultural and medical research* (Oliver & Boyd, Edinburgh) 1963, 146pp.