

Contribution of Extracellular Production by Nannoplankton to $^{14}\text{CO}_2$ Fixation in a Tropical Estuary

A. PANT, T. BALASUBRAMANIAN, M. D. RAJAGOPAL, M. V. M. WAFAR & SUMITRA-VIJAYARAGHAVAN

National Institute of Oceanography, Dona Paula 403004, Goa

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During a 12-month investigation in Dona Paula Bay, nannoplankton (passing through a 60 μm mesh) was found responsible for almost 100% of CO_2 fixation except during 3 months. In Feb. 1975 and Jan. and Feb. 1976 chain-forming net phytoplankton was dominant. Extracellular fixation in the *in situ* incubated samples varied from 0 to 22.1 $\text{mg C m}^{-3} \text{h}^{-1}$ (0 to 40% of particulate fixation). In Jan. and Feb. 1976 per cent extracellular release was higher in filtered than in unfiltered samples. No difference was found in these groups during the other months of study.

GENERALLY photosynthesizing unicellular algae liberate extracellular matter into their environment during CO_2 fixation and this represents a loss of energy. Such excretion underestimates total CO_2 fixation where standard radioactive techniques¹ are used to measure photosynthesis²⁻⁴. It has been suggested that phytoplankton produces a large part of the dissolved organic matter in the sea^{5,6} and that the release of extracellular matter forms an important link in the flow of energy from viable algal cells to bacteria in the water column^{7,8}. This would enable a greater production of bacterial biomass in the water column which, in addition to the algae, would be available as energy for the next trophic level.

To assess the total primary trophic level productivity it is necessary to have information about extracellular as well as particulate fixation. Determination of extracellular fixation rates for 1 yr would give some idea of the net production of dissolved organic matter through phytoplankton activity over a seasonal cycle. The importance of such metabolites has been discussed in terms of species succession and the exclusion of zooplankton by phytoplankton⁹.

Nannoplankton are important contributors to primary production in various aquatic environments¹⁰⁻¹². Earlier studies in Indian waters have shown that nannoplankton may be responsible for more than 60% primary production¹³⁻¹⁵, although their contribution to total biomass fluctuates seasonally¹⁶. If nannoplankton were found to be important contributors to particulate fixation in Goa waters, any extracellular fixation would be a consequence largely of the same organisms.

The null hypothesis set up for the first part of this investigation was that there is no difference in primary production or cell numbers between filtered and unfiltered samples ($H_0: \mu_{\text{filtered}} = \mu_{\text{unfiltered}}$). The second part consists of the estimation of rates of extracellular fixation in relation to environmental parameters.

Materials and Methods

Dona Paula Bay, a part of the estuary of the river Zuari was chosen for this study. Samples collected once a month from Jan. 1975 to March 1976 were filtered through a 60 μ mesh boiling nylon to separate the nannoplankton fraction. Filtered and unfiltered water was sub-sampled for cell counts and CO_2 fixation. Oxygen content and nutrient estimations were also carried out.

$^{14}\text{CO}_2$ fixation - Triplicate samples were taken for each set of filtered and unfiltered fractions resulting in a total of 6 light and 6 dark bottles each containing 125 ml of fluid. 4 μCi of $\text{NaH}^{14}\text{CO}_3$ was introduced into each bottle. A 100 ml aliquot from each bottle was filtered using a 0.45 μ membrane filter at the end of a 3 hr *in situ* incubation. The filtrate (50 ml) was acidified and aerated for 45 min to remove inorganic $^{14}\text{CO}_2$. Following neutralization to pH 9, a 100 μl aliquot was counted in a dioxan based scintillation cocktail at 84% efficiency. The filters were counted using a similar geometry.

An ampoule from the same batch was diluted with distilled water to a volume of 125 ml and a 50 ml aliquot was treated in the same way as the samples. Radioactivity fixed into extracellular products was corrected for both background and any organic ^{14}C present in the ampoules.

For cell counts, 250 ml samples were taken in duplicate from filtered and unfiltered water. Lugol's iodine (0.5 ml) was added to the samples prior to withdrawing a 1 ml aliquot into a Sedgewick-Rafter cell for counting. All counts were done on the day of sampling and each value presented in the tables is the average of 4 determinations.

Species diversity indices have been calculated according to the Shannon-Weaver equation. To facilitate comparison between water masses the Equitability Index (EI) of Heip¹⁷ was also obtained. This index is derived and varies between 0 and 1 depending on the dominance of species.

Among the nutrients, $PO_4\text{-P}$ was estimated according to Murphy and Riley¹⁸, $NO_2\text{-N}$ by the spectrophotometric method of Bendschneider and Robinson¹⁹ and $NO_3\text{-N}$ according to Koroleff²⁰. Other estimations were made following the methods in Strickland and Parsons²¹.

Results and Discussion

The null hypothesis that there is no difference between filtered and unfiltered samples is tested by analyses of variance using particulate fixation and cell count data (Fig. 1 and Table 1). Significant differences are found on only 3 occasions [Feb. 1975, Jan. and Feb. 1976 ($5\% < P < 1\%$)]. These results show that for the greater part of the year nannoplankton are responsible for almost all the primary production in this area. Only at certain times of the year, as mentioned above, net phytoplankton are dominant in the primary trophic level. Previous workers have also shown that nannoplankton are important contributors to organic production in both offshore and nearshore environments^{11,15}.

Lowest species diversity (Table 1) and EI are found during Feb. 1975 pulse of *Leptocylindrus minimus* and *Rhizosolenia* sp., the Jan. 1976 pulse of *Chaetoceros* spp and *Melosira* sp., and the Feb. 1976 bloom of *Chaetoceros* sp. alone. Except in these 3 instances there was no marked difference in filtered and unfiltered samples in so far as the occurrence of species was concerned. *Nitzschia longissima*,

Pleurosigma sp. and 2-celled filaments of *Skeletonema costatum* were found in filtered samples in addition to other more common species like *Tetraselmis gracilis*, *Cryptochrysis virescens*, *Chlorella* sp. and smaller diatoms such as *Navicula* spp, *Nitzschia seriata* and *Nitzschia closterium*. The March 1975 peak in cell counts (Table 1) comprised largely of *Nitzschia* and *Skeletonema costatum* and there was no significant difference between filtered and unfiltered samples in either cell counts or $^{14}CO_2$ fixation (Fig. 1 and Table 1). Unidentified flagellates less than 10 μm were present in all samples.

It is apparent from the data presented here that several species of nannoplankton exist concomitantly with net phytoplankton in populations dominated by the larger organisms. In Feb. 1976, for example, EI for the filtered sample is 0.42 indicating the presence of more species than in January (EI 0.306; Table 1) in a population dominated by *Chaetoceros* (EI 0.097 for the unfiltered sample). The same is true of the *Leptocylindrus/Rhizosolenia* bloom of the previous year (Feb. 1975) which is followed by the nannoplankton bloom of March 1975 (Table 1).

Lower nitrate values (Table 2) were associated with the presence of smaller-celled organisms during this study. From April to Aug. 1975 when $NO_3\text{-N}$ values were low, total phytoplankton populations were composed chiefly of nannoplankton organisms (Table 1). From Sept. to Nov. 1975 (Table 2) when nitrate values showed an increasing tendency,

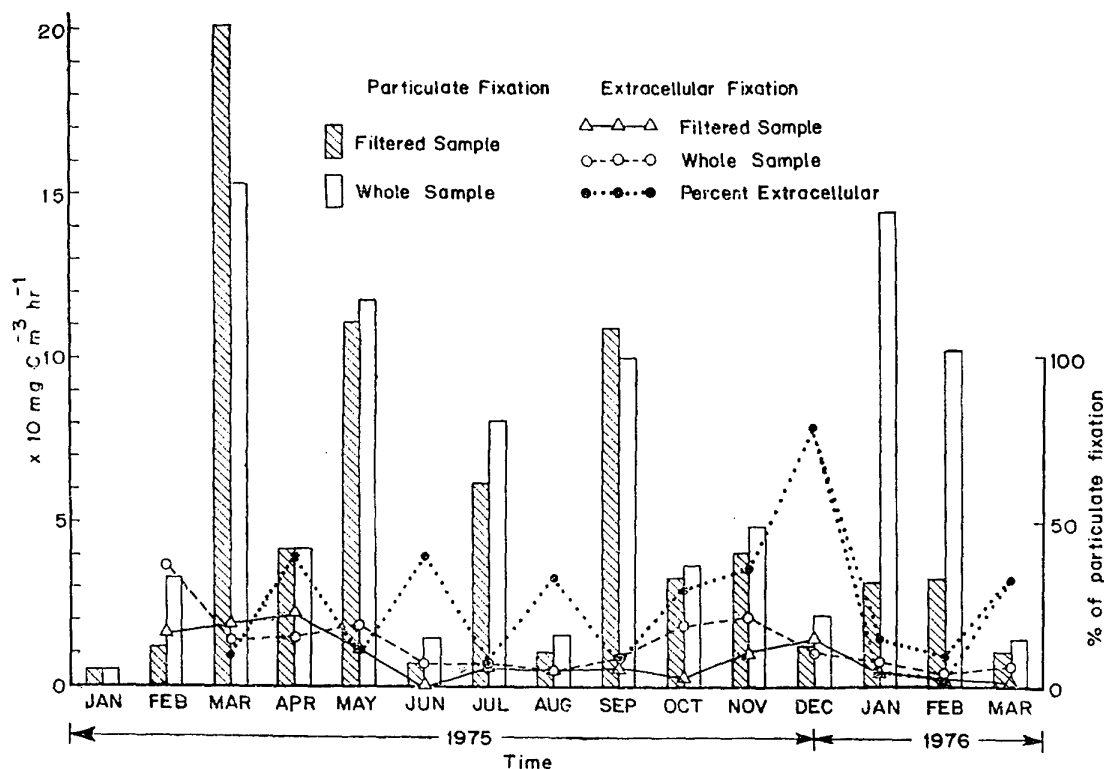


Fig. 1 — Particulate and extracellular organic fixation at Dona Paula (All values in $mg \text{ C m}^{-3} \text{ hr}^{-1}$)

TABLE 1 — SPECIES DIVERSITY INDICES AND CELL COUNTS, DONA PAULA BAY

(H₅ has been calculated according to Shannon and Weaver, the Equitability Index according to Heip¹⁷)

| Month of sampling | Cell counts × 10 ⁶ /litre | | Equitability | | | H ₅ | Dominant forms |
|-------------------|--------------------------------------|---------------------|--------------|-------|---------|----------------|---|
| | Filtered (mean ± %) | Whole (mean ± %) | Filtered | Whole | Average | | |
| 1975 | | | | | | | |
| Jan. | 0.25 ± 23 | 0.26 ± 23 | | | 0.52 | 2.8 | Several |
| Feb. | 1.8 ± 15 | 7.7 ± 40 | | | 0.19 | 1.8 | <i>Leptocylindrus</i> sp. <i>Rhizosolenia</i> sp. |
| March | 10.9 ± 42 | 9.7 ± 12 | | | 0.38 | 3.5 | <i>Nitzschia</i> sp. <i>Skeletonema costatum</i> |
| April | 1.5 ± 45 | 2.0 ± 9 | | | 0.45 | 3.3 | <10 μm, <i>Navicula</i> sp. <i>Nitzschia</i> sp. |
| May | 8.6 ± 30 | 1.2 ± 37 | | | 0.4 | 3.3 | Several |
| June | 0.3 ± 27 | 0.4 ± 35 | | | 0.69 | 2.2 | do |
| July | 0.8 ± 50 | 0.5 ± 32 | | | 0.56 | 2.7 | do |
| Aug. | 2.3 ± 46 | 1.7 ± 26 | | | 0.35 | 2.9 | do |
| Sept. | 2.5 ± 44 | 2.5 ± 44 | | | 0.28 | 2.1 | <i>Skeletonema costatum</i> , <10 μm |
| Oct. | 1.3 ± 34 | 0.7 ± 43 | | | 0.35 | 2.7 | <i>S. costatum</i> , <10 μm |
| Nov. | 2.7 ± 51 | 2.3 ± 17 | | | 0.38 | 2 | do |
| Dec. | 0.6 ± 53 | 1.1 ± 50 | | | 0.56 | 2.6 | Several |
| 1976 | | | | | | | |
| Jan. | 3.0 ± 34 | 7.0 ± 9 | 0.306 | 0.39 | 0.33 | 1.7 | <10 μm, <i>Chaetoceros</i> sp. |
| Feb. | 2.4 ± 36 | 7.9 ± 34 | 0.42 | 0.097 | 0.13 | 1.6 | <i>Chaetoceros</i> sp. |
| March | 0.3 ± 52 | 0.2 ± 40 | | | 0.69 | 2.2 | Several |

TABLE 2 — HYDROLOGICAL DATA AT DONA PAULA, GOA FOR THE DURATION OF STUDY

| Date | Temp. °C | Oxygen ml l ⁻¹ | pH | Salinity ‰ | μg-atom/litre | | | |
|----------|-------------|------------------------------|-----|---------------|--------------------|--------------------|--------------------|--------------------|
| | | | | | PO ₄ -P | NO ₂ -N | NO ₃ -N | NH ₄ -N |
| 1975 | | | | | | | | |
| 15 Jan. | 28 | 6 | 8.1 | 35.4 | 1.74 | 1.22 | 6.88 | 2.92 |
| 15 Feb. | 28 | 4.5 | 7.9 | 35.5 | 0.58 | 0.19 | 6.94 | 0.63 |
| 15 March | 29 | 5.8 | 7.9 | 35.7 | 0.97 | 0.67 | 3.99 | 1.86 |
| 15 April | 31 | 4.8 | 7.7 | 33.7 | 1.16 | 0.59 | 1.57 | 0.39 |
| 14 May | 32 | 3.9 | 7.9 | 32.6 | 1.05 | 0.65 | 1.88 | 2.72 |
| 15 June | 30.5 | 3.8 | 8.1 | 34.8 | 0.81 | 0.02 | 0.21 | 0.11 |
| 20 July | 28.2 | 4.5 | 8 | 10.7 | 0.27 | 0.19 | 1.32 | 0.69 |
| 15 Aug. | 28.5 | 4.6 | 7.9 | 10.4 | 1.13 | 0.32 | 1.94 | 1.14 |
| 15 Sept. | 28 | 4.8 | 7.9 | 6.9 | 0.85 | 0.37 | 2.11 | 1.79 |
| 15 Oct. | 27 | 6.2 | 8.2 | 20.5 | 0.63 | 0.32 | 2.94 | 0.88 |
| 15 Nov. | 26 | 3.7 | 7.9 | 29 | 1.42 | 3.4 | 2.44 | 3.94 |
| 15 Dec. | 26.5 | 5.9 | 8 | 32.1 | 1.83 | 0.66 | 4.14 | 6.14 |
| 1976 | | | | | | | | |
| 13 Jan. | 28.5 | 4.5 | 8 | 32.3 | 1.18 | 0.64 | 4.59 | 1.93 |
| 15 Feb. | 26 | 4.4 | 7.8 | 32.22 | 1.41 | 0.33 | 2.18 | 3.38 |
| 15 March | 29 | 3.7 | — | — | — | — | — | — |

Skeletonema costatum was found in fairly large numbers in the cell counts (7 to 15% of the total). The pulses of net phytoplankton such as *Leptocylindrus/Rhizosolenia* (Feb. 1975) and *Chaetoceros* (Feb. 1976) followed at higher NO₃-N concentrations. The work of Eppley *et al.*²² has shown that half-saturation constants of nitrate uptake varied proportionately with cell size and inversely with specific growth rates. The differences in nitrate concentration in the environment may explain the shift in species composition over the year in the data presented here. In this connection it is interesting to note that the half-saturation constant experimentally determined by Eppley *et al.*²² for *Skele-*

tonema costatum (0.5) is numerically between the value for *Leptocylindrus* and *Rhizosolenia* (1.3, 1.7) on the one hand and the much smaller *Isochrysis galbana* and *Coccolithus* sp. (0.1) on the other. Qasim *et al.*²³ working with organisms isolated from the Cochin backwaters have also found that succession of species can be explained on the basis of the half-saturation constants of NO₃-N uptake.

The range of particulate ¹⁴CO₂ fixation in this study varied from 5 mg C m⁻³ hr⁻¹ in Jan. 1975 to 175 mg C m⁻³ hr⁻¹ in March 1975 (Fig. 1). Extracellular fixation ranged from 0 mg C m⁻³ hr⁻¹ in June 1975 to 22.1 mg C m⁻³ hr⁻¹ in Nov. 1975. Expressed as a percentage of the appropriate value for parti-

culate fixation, extracellular fixation thus varied from 0 to 40%. Routine estimates of particulate fixation in this area would, therefore, be expected to underestimate total primary production by 0 to 40%.

There was an inverse relationship between per cent extracellular release (Fig. 1) and phytoplankton abundance. Similar results have been reported from the Cochin backwaters⁴ and from other aquatic environments^{24,25}. For the data presented here higher cell numbers during the blooms of Feb. and March 1975 and Jan. and Feb. 1976 have probably resulted in lower production of extracellular matter. During May to Aug. 1975, whereas total cell counts were low, particulate fixation and relative extracellular fixation varied a great deal and values for extracellular fixation probably depended on light intensity in these monsoon months rather than cell numbers. That light plays a causal role in extracellular fixation is well documented^{2,26}. In samples exposed to surface light intensity there was an inverse relation between per cent extracellular release and instantaneous particulate fixation. Similar data were reported by Watt² and Thomas³.

The extent of extracellular fixation did not vary significantly between filtered and unfiltered samples over most of the year. During the diatom pulses of Jan. and Feb. 1976, however, relative extracellular fixation was slightly higher in filtered samples. This was probably a stress reaction by the nanoplankton to the removal of a large fraction of the total phytoplankton population by filtration. Where almost all the organisms were nanoplankton, as was the case over the rest of the year, gravity filtration would not materially affect cell density and, consequently, there would be no significant difference in the production of extracellular matter between filtered and unfiltered samples.

During the period of incubation of samples it is possible that a certain portion of liberated organic matter is heterotrophically consumed by bacteria. Thus, the values of extracellular fixation presented here are likely to be the difference between algal excretion and bacterial consumption, i.e. net extracellular fixation. Bacterial consumption would either be estimated as a part of particulate carbon retained on the filters or as inorganic ¹⁴C₂O₂ lost during acid treatment. Extracellular fixation by algae and the subsequent consumption of such products by bacteria may facilitate such bacterial growth in the water column. This biomass would be available as an energy source for the next trophic level. It is known that zoea stages and copepodites feed on bacteria as well as nanoplankton and such a bacterial biomass, in addition to detritus, may well support greater amounts of secondary and tertiary production than has hitherto been supposed on the basis of phytoplankton data and daylight zooplankton biomass values²⁷.

In the absence of data on the night-time vertical migration of zooplankton in Dona Paula Bay no

attempt could be made to compute tertiary production in the area. Such a computation would also require an estimate of the generation times of warm water zooplankton, their grazing and natural mortality rates and data regarding bacterial biomass and its contribution to the environment. The data presented here do, however, suggest that tertiary production calculated from photosynthetic phytoplankton data alone²⁸ is an underestimate as the contribution of bacteria to the primary trophic level has not been taken into account.

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