

Relationship between particulate and extracellular carbon compounds of phytoplankton photosynthesis in a tropical estuary

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ABSTRACT: The influence of seasonal environmental conditions on the formation and release of organic compounds by photosynthesizing phytoplankton was studied in a tropical estuary. The pattern of ^{14}C fixation into the various intracellular compounds changed from pre-monsoon to monsoon depending on the quality and intensity of light available during the 2 seasons as well as phytoplankton composition. During the pre-monsoon, carbon was largely incorporated into the intracellular carbohydrate fraction and during the monsoon, into the protein fraction. Quantitative analysis of some selected low molecular weight compounds present in the intracellular photosynthate pool and extracellular exudate pool suggested that the release of organic compounds is governed mainly by the rate of their production.

INTRODUCTION

A number of environmental factors including light, nutrient concentration and temperature are known to alter the quality as well as quantity of primary carbon compounds produced during photosynthesis both in culture and in natural marine environments. Thus, Wallen & Geen (1971) observed that a change in light quality from white to blue or green led to a channelling of carbon into proteins rather than carbohydrates. The same effect was noticed by Morris & Skea (1978) who studied the effects of light intensity on intracellular macromolecular synthesis. Smith & Morris (1980) suggested from their data that low temperatures result in higher production of lipids. Such variations in metabolic products could have significant effects on the growth characteristics of the phytoplankton community as well as on consumer populations.

It is now well established that extracellular release of photosynthetically produced organic compounds occurs in healthy, photosynthesizing phytoplankton in natural communities and that the release is somehow related to photosynthetic rates. The substances released consist of both low molecular weight (LMW) compounds such as glycolic acid and amino acids (Hellebust 1965, Watt 1966, Hammer & Eberlain 1981) and high molecular weight polysaccharides (Hellebust

1965, Iturriaga 1981, Chrost 1981). Fogg (1983) and Jensen (1984) have reviewed the nature and ecological significance of extracellular compounds.

The influence of different growth conditions on the excretion of carbon compounds has been studied in cultures as well as in nature (Ignatiades 1973, Ignatiades & Fogg 1973). However, to our knowledge, there are no reports relating the release of individual compounds to their formation in the cells under different growth conditions.

This paper reports the results of a study undertaken to determine the influence of seasonal environmental conditions on the formation and release of organic compounds during photosynthesis in an estuary, the Dona Paula Bay ($15^{\circ}27' \text{N}$; $73^{\circ}48' \text{E}$). Earlier studies in the area have indicated a high seasonal rate of extracellular production (Pant et al. 1977), the presence of glycolic acid (ca $540 \mu\text{g m}^{-3}$) in the waters (Pant 1979) and a seasonal trend in the pattern of intracellular distribution of assimilated carbon (Góes unpubl.). In view of the above observations, selected low molecular weight intermediates of the glycolate pathway (*viz.* glycolic acid, glycine and serine) and intermediates of carbohydrate and protein syntheses (e.g. glucose, aspartic acid and glutamic acid) were specifically analysed for their presence in the cells and in the exudate of naturally photosynthesizing phytoplankton from Dona Paula Bay.

MATERIALS AND METHODS

Experiments were conducted once every fortnight during the pre-monsoon months of April and May and the early monsoon month of June.

Water samples were collected from 0.5 m below the surface with Niskin bottles and pre-filtered through a 200 μm zooplankton net. Samples were pre-incubated with streptomycin ($30 \mu\text{g ml}^{-1}$) for 45 min in order to reduce bacterial activity. Preliminary experiments had shown that streptomycin at this concentration could inhibit bacterial activity by about 90 %, but had no significant effect either on the photosynthetic activity or extracellular production of phytoplankton. However, Møller Jensen (1983) had found 3 to 29 % inhibition of algal production in field experiments with streptomycin.

^{14}C -bicarbonate (Bhabha Atomic Research Centre, Trombay, India) was added ($40 \mu\text{Ci l}^{-1}$) after which the samples were distributed into 250 ml bottles and incubated for periods ranging from 30 min to 4 h under simulated *in situ* conditions. The incubated samples were filtered through glass fibre filter discs (Whatman GF/C) under a gentle vacuum ($<100 \text{ mm Hg}$). Filters in duplicate were exposed to fumes of HCl, placed in scintillation vials with 4 ml of scintillation fluid (NE 220, Nuclear Enterprises Ltd., U. K.) and radioassayed by liquid scintillation counting (Packard, Model Prias). Counting efficiency was determined by the external standard method. Another set of duplicate filter discs

was washed with filtered sea water and kept individually in 5 ml 80 % alcohol at -20°C until further analysis for cell constituents. One aliquot of 250 ml filtered at time zero and processed as above functioned as a blank. The combined filtrate was acidified to pH 2 and aerated for 30 min to remove excess inorganic ^{14}C . After aeration the pH was adjusted to between 8.5 and 9.0 and the filtrate stored saturated with chloroform at -20°C for analysis of extracellular compounds.

A flow-chart of the separation of intra- and extracellular compounds is shown in Fig. 1.

Separation of intracellular chemical compounds. Cell constituents were fractionated into hot ethanol-soluble (low molecular weight compounds and lipids), TCA-soluble (polysaccharides) and TCA-insoluble (proteins) components following the method of Morris et al. (1974).

The alcohol- and TCA-soluble fractions were concentrated by freeze-drying and the residue redissolved in about 1 ml of distilled water; 100 μl of the concentrates were used for measuring radioactivity. The rest of the alcohol-soluble fraction was passed successively through Dowex-50 and Dowex-1 cation- and anion-exchange resin in the H^+ and acetate form, respectively. Amino acids adsorbed on the cation-exchange resin were eluted with 2N NH_4OH while organic acids from the anion-exchange resin were eluted with 2N HCl. In a parallel experiment using artificial sea water containing ^{14}C -glycolic acid and ^{14}C -glucose the recovery of the labelled compounds from the resin

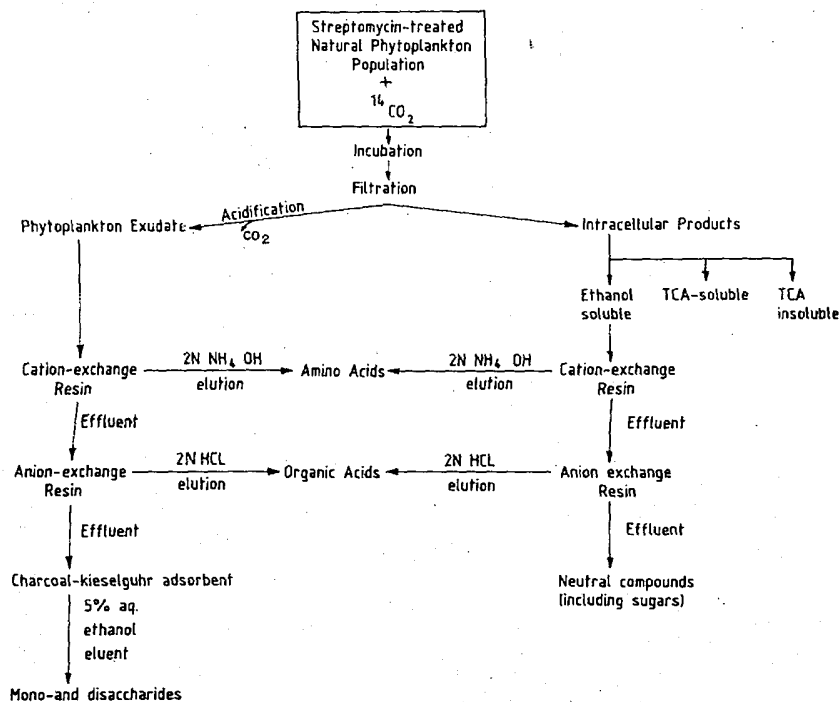


Fig. 1. Flow-chart showing procedure followed for the separation of intracellular and extracellular compounds

columns was determined as 90 to 95 %. The various eluates as well as the effluent (consisting of neutral compounds including sugars) were concentrated to dryness individually either in a rotary evaporator or in an oven at 40°C. (A loss of 20 to 25 % of radioactivity was observed when the latter method was used.) The residue of each concentrate was taken up in 1 ml distilled water, and 200 µl of the concentrated material was subjected to 1-dimensional paper chromatography on Whatman 3MM paper in the following solvent systems: ethanol-NH₃-water (78:12.5:9.5) for the separation of organic acids; butanol-acetic acid-water (4:1:1) for amino acids; and butanol-ethanol-water (52:33:15) for sugars. Before chromatography 200 µg of the appropriate non-radioactive carrier compounds were added to the samples. Authentic samples of glycolic acid, glucose, glycine, serine, aspartic acid and glutamic acid were co-chromatographed with the samples and their location on the chromatogram determined using appropriate spray reagents, viz. 1 % dichlorophenol indophenol in alcohol for organic acids, ninhydrin in acetone for amino acids, and alkaline KMnO₄ for reducing sugars. Spots on the chromatogram corresponding to the standard compounds were cut out, taken up in the scintillation cocktail and their radioactivity determined by LS-counting. The amino acids glycine and serine, which did not usually separate well in the butanol-acetic acid-water system were separated using acetone-water-diethylamine (16:3:1).

Separation of extracellular LMW compounds. Aliquots of 100 ml of the cell filtrate were passed successively through the same ion-exchange resins as before and the effluent, in turn, was passed through an activated-charcoal-kieselguhr (1:1) column to fractionate the saccharides. Combined mono- and disaccharides

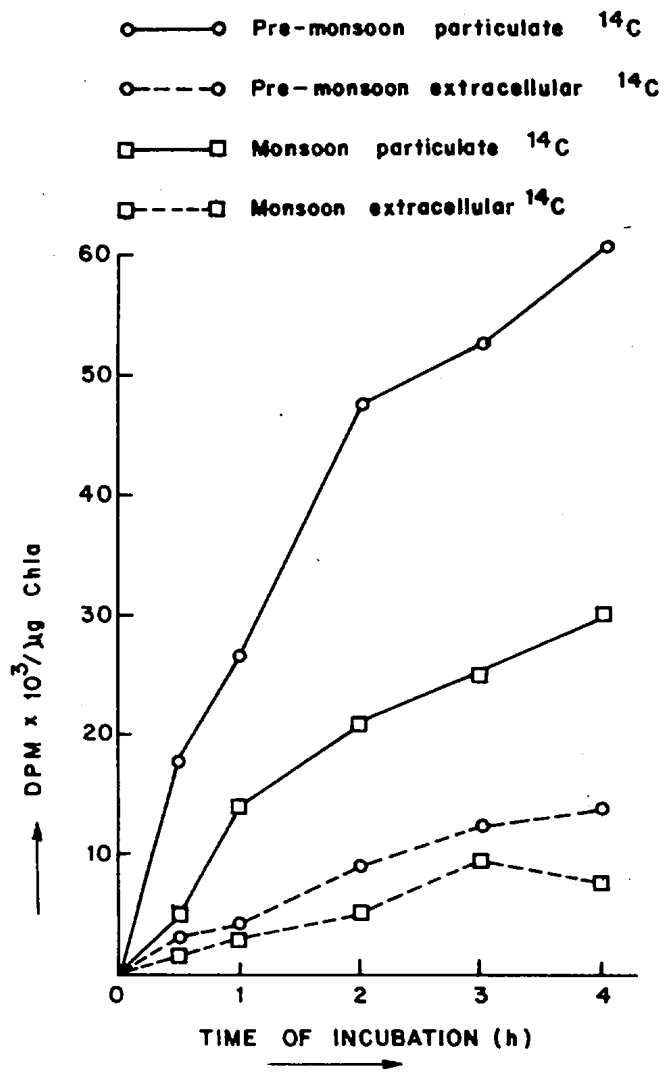


Fig. 2. Particulate fixation and release of ¹⁴C on 8 May 1984 (pre-monsoon) and 20 Jun 1984 (monsoon)

Table 1. Some physical and biological parameters of Dona Paula Bay

Date	Chl a (µg l ⁻¹)	Cell count (× 10 ³ l ⁻¹)	Dominant phytoplankton species and their abundance (%)	Temp.* (°C)	Salinity* (‰)	Incident solar radiation** (MJ m ⁻²)
Pre-monsoon						
10 Apr 84	2.5	71	<i>Chaetoceros</i> sp. (22%) <i>Bacteriastrium</i> sp. (29%)	30.6	35.8	12.43
24 May 84	1.7	77	Blue-green sp. (15%)			
8 May 84	2.9	63	All the above; <i>Coscinodiscus</i> sp. (19%)			
22 May 84	5.8	81	<i>Nitzschia bilobata</i> (21%)			
Monsoon						
14 Jun 84	0.9	20	<i>Navicula</i> sp. (23%)	29.4	23.0	2.96
20 Jun 84	2.0	34	<i>Nitzschia bilobata</i> (16%) <i>Skeletonema costatum</i> (16%) <i>Thalassiothrix</i> sp. (9%)			

* Mean seasonal value
** Total for the duration (4 h) of the experiment

were eluted with 5 % aqueous ethanol. All the eluates were concentrated and their components separated and estimated by paper chromatography and liquid scintillation as noted.

Hourly measurements of total incident solar radiation were made by the India Meteorological Department located 7 km away from the sampled station.

RESULTS AND DISCUSSION

Temperature, salinity, total incident solar radiation, chlorophyll *a*, major phytoplankton species and cell count values existing at the time of experiment are shown in Table 1.

Fig. 2 shows the time course of ^{14}C uptake and release as observed in 2 representative experiments in the pre-monsoon (8 May 1984) and monsoon (20 June 1984) seasons. The difference in the rates of extracellular release in the 2 seasons was statistically significant ($F = 12.67$; $p = 0.05$). Per cent extracellular release was also significantly different ($t = 4.10$; $p = 0.001$).

The pattern of ^{14}C assimilation into different cellular

compounds was found to differ with season (Table 2). While a larger proportion of the label was incorporated into carbohydrates during the pre-monsoon, incorporation into proteins increased significantly ($t = 5.41$; $p = 0.001$) and that into carbohydrates decreased ($t = 4.80$; $p = 0.001$) in June after the onset of the monsoon.

In the pre-monsoon, analysis of the intracellular LMW compounds showed a major portion of the label to be present in the neutral fraction comprising sugars whereas a relatively low proportion of the label was found in free amino acids (Table 3). This pattern was, however, reversed during the monsoon when more of the label was recovered from the amino acid fraction, especially from 2 of the principal amino acids tested, namely aspartate and glutamate. A clear change with season was noticed also in the cellular organic acid composition. Glycolic acid was the major organic acid incorporating radioactivity during the pre-monsoon months which are generally bright and calm. During the monsoon, label in the glycolic acid fraction was considerably reduced although the proportion of ^{14}C incorporated into the total organic acid pool was still high and comparable to the pre-monsoon value.

Table 2. Pattern of ^{14}C assimilation into cellular components

Date of sampling	Hours of incubation	^{14}C assimilation into cells ($\mu\text{g C l}^{-1}$)	Per cent ^{14}C assimilation into			
			Ethanol-soluble fraction	Carbohydrate	Protein	
10 Apr 84	0.5	51.5	48.2	39.4	29.4	
	1	64.7	51.2	33.4	21.6	
	2	95.6	43.4	30.3	21.1	
	4	130.0	46.7	35.0	18.2	
24 Apr 84	1	83.9	51.9	32.6	15.4	
	2	141.5	47.8	30.8	21.4	
	3	111.5	49.9	33.6	16.5	
	4	247.9	48.9	36.2	14.9	
8 May 84	0.5	62.7	55.1	42.1	16.7	
	1	104.0	44.0	37.4	18.6	
	2	185.5	46.6	37.1	16.3	
	3	183.8	51.9	29.9	18.0	
22 May 84	4	216.5	48.0	33.8	18.2	
	0.5	63.4	43.9	35.5	20.5	
	1	112.9	41.9	38.9	19.2	
	2	147.9	47.3	32.9	19.4	
14 Jun 84	3	162.7	50.8	32.9	19.0	
	4	168.6	53.9	30.0	16.1	
	0.5	7.9	40.9	25.9	33.1	
	1	11.6	45.9	26.9	27.1	
20 Jun 84	2	17.8	53.3	21.7	25.0	
	3	18.9	49.9	24.0	25.9	
	4	36.7	58.0	18.9	32.9	
	0.5	11.6	24.7	40.5	24.8	
	1	34.1	41.1	19.2	29.6	
	2	50.5	39.7	16.3	43.9	
	3	56.2	37.2	15.4	27.4	
	4	78.4	39.3	16.4	40.4	

Although the organic acids receiving the label were not characterized, there is reason to believe that the organic acid pool largely consisted of α -keto acids because aspartic acid and glutamic acid had concomitantly high amounts of the label.

The high level of radioactivity found in glycolic acid in the pre-monsoon suggests the occurrence of a significant phytoplankton photorespiration during the season. This is corroborated by the simultaneously high levels of ^{14}C found in glycine and serine, the 2 amino acid intermediates of glycolate metabolism via the glycolate pathway (Tolbert 1974). During the monsoon, label recovered from the glycolic acid fraction was low indicating that photorespiration was greatly reduced probably because of the lowered light intensity obtaining in the monsoon season.

The pattern of excretion of labelled LMW compounds by the phytoplankton was seen to follow the intracellular pattern closely (Table 4). During the pre-

monsoon months when carbon was largely entering the cellular carbohydrate fraction, the excreted LMW compounds consisted mainly of mono- and disaccharides (Table 4). Also, when conditions were optimal for photorespiration, as judged from the intracellular labelling pattern of glycolate, a high amount of labelled glycollate was noted among the excreted organic compounds. Calculated on the basis of radioactivity recovered from the glycolate fraction of the exudate, approximately $15\ \mu\text{g}$ of glycolate are released $\text{l}^{-1}\ \text{h}^{-1}$ (Table 4). The *in situ* levels of glycolic acid in the water estimated during the season by the method of Shah & Wright (1974) ranged from 160 to $180\ \mu\text{g}\ \text{l}^{-1}$ although, on an annual cycle, the levels were not found to correlate significantly with phytoplankton biomass determined as chlorophyll *a* (Jivexa Patel & A. Pant unpubl.).

It is not yet clear why during the pre-monsoon season there should be a simultaneous and extensive

Table 3. Pattern of ^{14}C assimilation into intracellular low molecular weight compounds

Date of sampling	Hours of incubation	Radioactivity in ethanol-soluble fraction* (DPM $\times 10^3$)	Radioactivity recovered in the ethanol-soluble fraction as								Glucose (% of NC)
			OA** (DPM $\times 10^3$)	Glycolate (% of OA)	AA** (DPM $\times 10^3$)	Gly	Ser (% of AA)	Asp	Glu	NC** (DPM $\times 10^3$)	
10 Apr 84	0.5	4.99	1.49	74.9	1.17	16.7	18.5	5.6	8.6	2.39	66.6
	1.0	8.41	2.74	76.9	2.45	16.1	19.2	6.1	7.8	4.22	75.2
	2.0	8.34	3.01	69.8	2.07	17.4	19.9	6.5	8.2	3.27	79.4
	4.0	14.41	4.73	71.3	4.05	17.2	20.0	8.2	10.3	5.63	71.4
24 Apr 84	1.0	7.55	2.29	81.2	2.14	18.9	19.9	6.3	8.4	3.87	78.3
	2.0	14.75	4.79	75.3	3.58	18.9	20.3	5.6	8.8	7.97	70.9
	3.0	10.86	3.30	68.0	2.25	17.3	21.3	7.9	8.7	5.40	71.8
	4.0	19.98	6.29	72.9	4.59	17.5	24.3	7.4	8.8	10.18	73.4
8 May 84	0.5	6.86	1.68	70.8	1.70	15.9	18.8	5.9	7.1	2.91	62.9
	1.0	9.32	3.20	62.9	2.34	18.6	21.2	6.0	8.8	3.32	69.2
	2.0	19.44	6.11	75.8	5.41	17.2	20.9	7.3	8.3	9.83	79.0
	3.0	17.27	5.32	79.4	4.47	19.1	23.4	6.7	7.4	6.51	68.0
	4.0	19.26	6.20	69.2	4.19	17.9	21.7	9.3	9.5	9.16	77.9
22 May 84	0.5	4.91	1.46	73.9	1.00	17.5	17.5	6.0	8.0	2.51	75.3
	1.0	10.19	3.22	74.5	2.38	18.3	18.9	6.1	8.6	5.26	71.6
	2.0	12.38	4.32	70.6	3.34	18.4	22.9	7.1	9.6	6.09	74.3
	3.0	17.04	5.52	68.8	4.48	19.9	16.7	6.9	8.8	8.55	71.6
	4.0	18.01	5.74	66.7	4.52	19.2	21.8	7.3	8.9	8.52	69.0
14 Jun 84	0.5	0.37	0.08	7.41	0.23	7.11	9.75	12.00	18.60	0.05	34.80
	1.0	0.99	0.29	7.25	0.82	9.45	10.81	12.75	20.75	0.12	37.23
	2.0	1.51	0.33	5.32	1.17	9.80	14.73	18.04	19.61	0.25	36.84
	3.0	1.89	0.60	6.88	1.29	11.63	14.25	21.44	25.80	0.33	36.95
	4.0	2.92	0.95	5.04	1.83	11.99	17.64	20.86	26.05	0.53	37.01
20 Jun 84	0.5	0.58	0.12	—	0.36	—	—	—	—	0.08	—
	1.0	2.82	0.80	8.75	2.31	7.79	12.77	19.04	20.99	0.34	36.76
	2.0	4.03	0.88	8.52	3.12	10.41	14.10	18.10	22.11	0.61	36.88
	3.0	4.19	1.27	5.12	2.85	—	—	—	—	0.72	36.11
	4.0	6.19	1.86	6.18	3.87	12.01	18.99	17.30	26.61	1.12	35.71

* From the particulate fraction of 250 ml water sample

** OA: organic acids; AA: amino acids; NC: neutral compounds

release of products of both photosynthesis and photorespiration. Inhibiting light intensity may result in such excretion or the response may be species-specific. The changes observed in the concentrations of some particulate and extracellular organic compounds with time suggest that the release is governed by the rates of production of the compounds in the cells and that it follows a pattern of diffusion occurring in the direction of decreasing chemical potential. The excreted compounds were almost entirely made up of LMW metabolites of carbohydrates and proteins.

The changes in the pattern of carbon assimilation and release occurring in the 2 seasons could be explained on the basis of variation in light in the 2 seasons (Table 1). Wallen & Geen (1971) have attributed the enhanced production of amino acids with depth to light quality rather than intensity. A similar situation may be operative during the monsoon when the cloud cover present can change the spectral quality of light to predominantly green (Neumann & Pierson

1966), quite apart from the decrease in total light intensity.

On the other hand, it is also possible that the observed changes in carbon assimilation and excretion were due to differences in the composition of phytoplankton since the basic chemical composition of algal species, especially diatoms, can vary considerably (Mykelstad 1974). Also, different algal species are known to release different products (Hellebust 1974, Jensen 1984). Effect of salinity on carbon fixation and release is not known but no effect of nutrient concentration is expected as nutrients are never seriously limiting in this environment (Goés unpubl.). Similarly, bacterial modification of exuded products could be ruled out as all our experiments were conducted in the presence of streptomycin which could reduce bacterial activity by more than 90%. Streptomycin does not affect the quality of extracellular release (Møller Jensen 1983).

In conclusion, seasonal variations in light and

Table 4. ¹⁴C-labelled extracellular low molecular weight compounds released during photosynthesis

Date of sampling	Hours of incubation	¹⁴ C excreted in the filtrate (μg C l ⁻¹)	Radioactivity present in the filtrate as								MDS* (DPM × 10 ³ l ⁻¹)	Glucose (% of MDS)
			OA* (DPM × 10 ³ l ⁻¹)	Glycolate (% of OA)	AA* (DPM × 10 ³ l ⁻¹)	Gly	Ser	Asp	Glu	(% of AA)		
10 Apr 84	0.5	19.9	5.68	86.8	3.52	32.3	40.8	7.0	7.0	7.84	71.6	
	1.0	13.7	3.80	77.6	1.80	30.5	38.9	8.3	11.1	4.76	80.0	
	2.0	31.1	7.32	80.1	3.60	31.9	38.8	8.3	6.9	8.92	79.2	
	4.0	37.4	10.08	70.1	5.40	34.2	37.0	7.4	-	14.36	78.7	
24 Apr 84	1.0	13.7	4.60	88.0	1.88	31.6	34.2	7.9	-	5.16	79.6	
	2.0	27.4	8.20	78.0	4.00	31.2	31.2	6.3	7.5	9.80	81.6	
	3.0	21.2	4.80	73.9	4.48	37.8	33.3	7.8	7.8	8.64	77.5	
	4.0	54.8	9.32	78.5	10.60	30.2	35.4	5.7	7.5	21.96	79.3	
8 May 84	0.5	14.9	4.20	69.0	2.64	32.1	35.8	5.6	5.7	4.96	72.7	
	1.0	21.2	5.96	81.5	3.28	28.8	36.4	7.6	9.1	7.92	85.5	
	2.0	44.8	13.76	83.6	7.44	31.5	32.9	6.0	9.4	16.32	80.0	
	3.0	61.0	16.32	75.2	8.76	32.0	30.3	8.0	5.1	22.40	75.2	
	4.0	70.9	7.24	74.5	6.84	29.9	37.9	5.1	8.0	30.24	74.5	
22 May 84	0.5	12.5	2.60	76.9	1.28	34.6	38.4	12.0	11.5	4.96	69.7	
	1.0	29.9	8.56	82.5	4.32	31.4	32.6	9.3	8.1	10.84	76.0	
	2.0	31.1	9.20	77.7	5.24	31.4	32.4	6.7	7.6	13.24	82.3	
	3.0	34.9	6.04	-	4.60	-	-	-	-	13.76	-	
	4.0	39.8	12.24	81.6	5.44	30.2	38.5	5.5	8.3	16.32	78.3	
14 Jun 84	0.5	4.9	1.04	-	3.32	8.9	5.9	23.8	31.3	0.96	68.4	
	1.0	3.7	0.96	15.8	2.88	12.1	8.6	32.8	34.5	0.84	70.5	
	2.0	7.5	2.12	9.5	7.08	8.5	9.9	17.6	37.3	2.40	64.6	
	3.0	8.7	2.36	12.7	6.88	13.8	10.9	26.1	36.9	2.20	68.2	
	4.0	19.9	3.92	11.5	12.00	12.5	9.2	21.3	35.8	4.36	66.7	
20 Jun 84	0.5	8.7	1.28	-	4.60	10.2	11.2	20.4	26.5	1.32	-	
	1.0	7.5	1.52	16.7	4.08	9.8	7.3	23.1	30.5	1.16	78.3	
	2.0	12.5	2.32	13.0	7.44	6.7	6.7	18.8	30.2	1.84	70.2	
	3.0	17.4	3.16	9.5	10.32	7.2	6.3	19.8	35.3	2.96	71.2	
	4.0	18.7	2.92	8.6	9.00	12.2	9.4	27.8	36.7	3.52	65.7	

* OA: organic acids; AA: amino acids; MDS: mono- and disaccharides. Blanks indicate samples lost during assay

species composition of phytoplankton are reflected in the changing pattern of carbon assimilation and release in this estuarine environment. The monsoons, as a series of episodic events, influence ecosystems like Dona Paula Bay. It may be possible to categorize these influences on phytoplankton by combining the traditional ^{14}C procedure to measure phytoplankton primary production with a chemical characterization of major products of photosynthesis.

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