

NUMERICAL CONTRIBUTION OF PHYTOPLANKTONIC CELLS, HETEROTROPHIC PARTICLES AND BACTERIA TO SIZE FRACTIONATED POC IN THE CANANÉIA ESTUARY (25°S 48°W), BRAZIL

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Synopsis

Oxidable POC, at two stations in the Cananéia estuary, was found to be largely dependent upon the smallest size POC. The correlation factors between POC and the numerical abundance of cells, heterotrophic particles and bacteria, in each of the size categories studied, were generally low and non-significant for both stations, with a few exceptions. At St. I the number of heterotrophic particles seems to account for some of the POC variation over the year. At St. II, the only significant correlation found was between the number of the largest and intermediate size classes bacteria and the equivalent size classes POC. At this station the importance of the detritus component is suggested. The differences found between the stations, concerning the numerical contribution of cells, particles and bacteria to total POC, have been attributed to the differential hydrodynamic conditions acting upon material coming from land, due to diverse location of the stations. Sampling date and the collection of different water masses have also been considered as factors that may greatly affect the relationships studied.

Descriptors: Size distribution, Particulate organic carbon, Phytoplankton, Particle counters, Bacteria, Estuaries, Cananéia: SP, Brazil.

Descritores: Fracionamento, Carbono orgânico particulado, Fitoplâncton, Número de partículas, Bacteria, Estuários, Cananéia: SP.

Introduction

Studies comprising differential filtration of suspended particulate organic matter into different sizes is a way to contribute for the understanding of the relative importance of parts of a planktonic community.

In the Cananéia estuary water sample fractionation have been carried out in previous studies, most of them concerned to phytoplankton and primary production (Teixeira, 1963; Teixeira, Tundisi & Santoro Ycaza, 1967; Tundisi & Texeira, 1968; Tundisi, 1969, 1971). The present report constitutes the first investigation undertaken in this environment, concerning to POC, heterotrophic particles and bacteria size fractionation, and is an integrant part of an investigation into the relationships between POC and some biotic environmental variables. Some of the results have already been reported in Mesquita (1983).

Material and methods

The present study was carried out at surface water samples taken from St. I
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and St. II (Fig. 1), using a 9ℓ Van Dorn bottle, in the Cananéia estuary (25°S 48°W), Brazil, over 1976. A preliminary filtration through a 100 μm nylon netting was carried out on the samples to remove the larger particles and grazers. The 100 μm sample was then size-fractionated, and the size fractions were gently concentrated by a reverse flow system based in Dodson & Thomas (1964). The mesh size of the screens for the size fractionation were 50 μm and 10 μm, and the finer filter was a 0,45 μm Gelman Acropor membrane. Each filter was glued to a PVC cylinder 25 cm height and 12 cm wide. The maximum average value of the concentration factor was 3,4 X for the smallest size categorie, 6,0 X for the 50-10 μm size class and 7,0 X for the largest size class. The average time required for concentrating the 10-0,45 μm and the 50-10 μm size categories was two and half an hour (2h 30m), and for the largest size class (100-50 μm) was 5 minutes. The total time spent for the whole sample processing (screening and concentration) was at most 3 hours.

Plankton particle number (phyto-

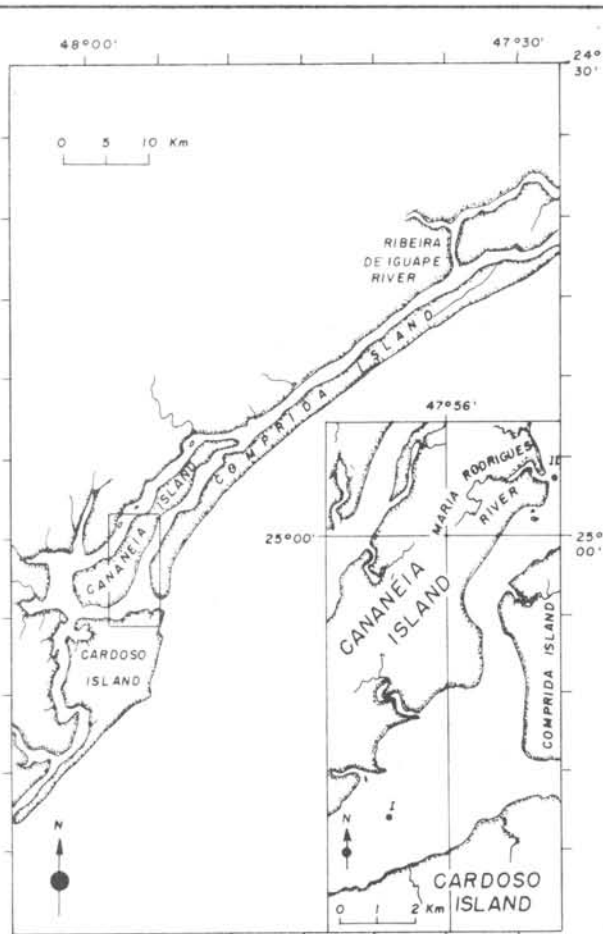


Fig. 1. Sampling stations at the Cananéia estuary: I, Trapandê; II, Maria Rodrigues.

planktonic cells, heterotrophic particles and bacteria) and POC were monthly determined for 3 size classes: 100-50 μm , 50-10 μm and 10-0,45 μm (Tabs 1-2). Cell counting was undertaken in a 20 μl aliquot of the concentrate of each size categorie, using the fluorescence technique of Wood (1962). Cells showing bright red fluorescence under the fluorescent light (an HBO 200 mercury lamp) were considered as "phytoplanktonic cells" and their number was reported as "phytoplankton cell counting". After staining the particles with acridine orange dye (final concentration 1:20,000), those particles displaying bright green color and distinct from bacterial cells by their size and morphology were designated "heterotrophic particles" and their number "heterotrophic particle counting". This group consists of a miscellaneous assemblage of particles, of difficult classification by the present methodology. The green fluorescence these particles

show may be caused either by the uptake of the acridine orange dye or by their autofluorescence. Spores, fecal pellets, mineral material, cells or organisms without photosynthetic pigments may be included in this group.

Bacterial cells were considered as all the particles, distinct from the heterotrophic particles, that have shown bright green fluorescence after using the acridine orange dye.

A hundred ml (100 ml) aliquot of the concentrates of each size categorie, were strained through Whatman GF/C filters and, the carbon of the particulate matter retained was determined by oxidation using dichromic acid. Blanks were obtained by repeating the analysis on GF/C filters previously wetted with sea water and rinsed with 25 ml of 0,6M Na_2SO_4 to remove halides. The method was calibrated against a standard glucose solution. The standard deviation of the analytical determination of the particulate organic carbon (POC) was 50 $\mu\text{g C.l}^{-1}$ or, approximately, 15% of the mean.

Temperature and salinity data are also presented at Tables 1 and 2.

To have an indication of the correlation structure of the variable on Tables 1 and 2, the correlation coefficient for the following pairs of variable was calculated:

- 1) total POC and each of the three size classes POC;
- 2) phytoplankton cell number and the POC for each of the three size classes POC;
- 3) heterotrophic particle number and the POC for each of the three sizeclasses POC and,
- 4) bacterial cell number and the POC for each of the three size classes POC.

The coefficient of determination (r^2) was calculated for each one of the situations described above, to show the percentage of variation of POC that can be attributed to the others variables. The results are shown in Table 3.

For each one of the variables the percentage of each size classe with respect to the total was calculated (Tabs 1-2).

The lower limit of significance for the correlation factors obtained was chosen as 0,708 for the 1% level.

Table 1. Some hydrological data, cell and particle densities, particulate organic carbon concentrations and size classes percentage of the total at Station 1, over 1976. T = water temperature; S = salinity; Phy = phytoplankton; H = heterotrophs; B = bacteria; POC = particulate organic carbon; % = size classes percentage of the total

Date	T (°C)	S (‰)	Size class (µm)	Phy		H		B		POC	
				cell no. ($\times 10^6 \cdot l^{-1}$)	%	part no. ($\times 10^6 \cdot l^{-1}$)	%	cell no. ($\times 10^6 \cdot l^{-1}$)	%	($\mu g \cdot l^{-1}$)	%
21/01/76	27.8	18.10	100-50	350.683	39	307.725	3	596.939	17	250	12
			50-10	214.637	24	2145.470	19	565.190	16	470	22
			10-0.45	333.531	37	8540.493	78	2396.991	67	1400	66
17/02/76	26.0	17.36	100-50	151.023	40	686.192	9	263.025	4	190	13
			50-10	116.074	31	1962.386	27	786.042	14	430	29
			10-0.45	111.703	29	4673.353	64	4745.050	82	870	58
24/03/76	25.8	15.13	100-50	45.876	11	676.969	36	87.111	14	470	52
			50-10	81.319	20	221.642	12	55.403	9	300	33
			10-0.45	280.965	69	984.544	52	496.864	78	140	15
22/04/76	25.0	20.16	100-50	37.531	22	26.141	14	12.984	6	290	38
			50-10	87.202	51	85.956	46	68.902	30	160	21
			10-0.45	45.462	27	74.999	40	145.582	64	320	42
18/05/76	22.2	21.58	100-50	6.716	11	137.346	17	78.202	28	140	17
			50-10	32.528	55	469.122	58	86.127	31	320	38
			10-0.45	19.785	34	201.605	25	117.388	42	380	45
22/06/78	19.5	21.00	100-50	8.987	14	60.514	13	8.011	8	150	19
			50-10	23.864	37	69.711	15	27.779	28	160	20
			10-0.45	31.344	49	331.484	72	64.051	64	470	60
20/07/76	18.5	17.46	100-50	3.701	5	189.386	47	11.421	15	70	11
			50-10	22.869	32	50.169	12	28.870	39	200	32
			10-0.45	44.532	63	161.125	40	34.231	46	360	57
24/08/76	18.5	24.30	100-50	34.108	27	57.498	12	33.717	11	150	14
			50-10	66.782	53	176.843	38	170.177	57	460	44
			10-0.45	26.036	20	234.846	50	95.932	32	430	41
14/09/76	19.5	23.44	100-50	4.654	6	21.261	10	11.928	5	100	16
			50-10	29.592	36	74.743	36	82.756	35	250	39
			10-0.45	47.142	58	110.625	53	139.899	60	290	45
20/10/76	22.0	26.60	100-50	6.620	16	16.670	12	12.408	7	0	0
			50-10	15.023	36	41.155	29	62.213	38	160	44
			10-0.45	20.476	49	84.900	59	90.262	55	200	56
23/11/76	25.8	26.46	100-50	84.677	34	45.783	10	31.917	10	150	7
			50-10	91.625	37	138.782	29	87.480	28	530	26
			10-0.45	72.985	29	291.348	61	90.319	61	1380	67
14/12/76	28.0	19.24	100-50	0.315	2	30.292	20	25.953	20	80	13
			50-10	11.664	76	50.506	34	75.048	57	210	34
			10-0.45	3.341	22	66.660	45	30.936	23	320	52
Annual mean	23.2	20.90	100-50	61.241	29	188.007	10	97.801	10	170.00	17
			50-10	66.107	31	457.216	23	174.674	18	304.16	30
			10-0.45	86.525	40	1313.182	67	712.204	72	546.66	54

Table 2. Some hydrological data, cell and particle densities, particulate organic carbon concentrations and size classes percentage of the total at Station 11, over 1976. Further details as in legend to Table 1

Date	T (°C)	S (‰)	Size class (µm)	Phy		H		B		POC	
				cell no. ($\times 10^4 \cdot l^{-1}$)	%	part no. ($\times 10^4 \cdot l^{-1}$)	%	cell no. ($\times 10^6 \cdot l^{-1}$)	%	(µgC.l ⁻¹)	%
21/01/76	27.8	18.10	100-50	350.683	39	307.725	3	596.939	17	250	12
			50-10	214.637	24	2145.470	19	565.190	16	470	22
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			50-10	29.592	36	74.743	36	82.756	35	250	39
			10-0.45	47.142	58	110.625	53	139.899	60	290	45
20/10/76	22.0	26.60	100-50	6.620	16	16.670	12	12.408	7	0	0
			50-10	15.023	36	41.155	29	62.213	38	160	44
			10-0.45	20.476	49	84.900	59	90.262	55	200	56
23/11/76	25.8	26.46	100-50	84.677	34	45.783	10	31.917	10	150	7
			50-10	91.625	37	138.782	29	87.480	28	530	26
			10-0.45	72.985	29	291.348	61	90.319	61	1380	67
14/12/76	28.0	19.24	100-50	0.315	2	30.292	20	25.953	20	80	13
			50-10	11.664	76	50.506	34	75.048	57	210	34
			10-0.45	3.341	22	66.660	45	30.936	23	320	52
Annual mean	23.2	20.90	100-50	61.241	29	188.007	10	97.801	10	170.00	17
			50-10	66.107	31	457.216	23	174.674	18	304.16	30
			10-0.45	86.525	40	1313.182	67	712.204	72	546.66	54

Results

Particulate organic carbon (POC) ($\mu\text{g C.l}^{-1}$)

At St. I except for March and August, the 10 μm fraction contributed over the year at least 42% of the total organic carbon. The annual mean was 50%.

At St. II the average annual percentage of the 10 μm -0,45 μm fraction was 43%.

The carbon found in both the 50 μm -10 μm categorie and the 10 μm -0,45 μm size class comprised 84 and 79% of the total POC, at Sts I and II, respectively.

At both stations there was a highly significant and positive correlation, at 1% level, between total POC and the POC of the 10 μm -0,45 μm fraction ($r_{\text{St.I}} = 0,95$; $r_{\text{St.II}} = 0,74$) (Tab. 3). In general, it seems that the C contribution of the smaller particles to total POC, dominates over the contribution of larger ones at both stations. The POC smallest size categorie seems to be relatively more important at St. I than at St. II, regarding its contribution to total POC.

Standing-crop measurements:

Phytoplankton cell counting (cells. l^{-1})

At St. I and on an annual basis the cell concentration in the 10 μm -0,45 μm size class comprised 40% of the total cell number. On the same annual basis the 50 μm -10 μm size group contribution to total cell number was 31%. Therefore, at St. I and regarding their contribution to the total cell number the 50 μm -10 μm and 10 μm -0,45 μm size categories dominate over the 100 μm -50 μm size class.

At St. II, for part of the year the 50 μm -10 μm group dominate over the other size classes. This group, averaged annually 36% of the total. On an annual basis, the <10 μm > 0,45 μm size class comprised 26% of the total. Counts of particles larger than 50 μm were relatively high-39% of the total, annually (Tab. 2).

The phytoplankton cell counting in the size categorie between 50 and 0,45 μm was, on an annual basis, 71% of the total at St. I and 62% at St. I.

Therefore, it seems that small phytoplanktonic cells are, on an average, numerically important at both stations.

At St. I a significant positive correlation was found between POC and

the phytoplankton counting in the 50 μm -10 μm size class, at 5% level. Phytoplankton cell counting in this size class accounted, over the year, for 43% (r^2) of the POC variation in the same size categorie.

The phytoplankton cell counting in the 50 μm -0,45 μm fraction (the sum of the number of cells in the < 50 μm > 0,45 μm and < 10 > 0,45 μm size classes), was significantly correlated with POC of the same size group at 5% level and, accounted for 34% (r^2) of its variation throughout the year.

At St. II no significant association was found between POC and phytoplanktonic cell number in any of the size groups considered.

Heterotrophic particle counting (particle no. l^{-1})

At St. I the relative contribution of the 10 μm -0,45 μm size group to total heterotrophic count ranged from 25 to 78% over the year, averaging annually 67% of the total.

The heterotrophic particle number in the size class between 10 μm and 0,45 μm has shown a highly significant and positive correlation with POC and accounted for 90% (r^2) of its variation in the same size group (Tab. 3).

Table 3. Correlation coefficient (r) and coefficient of determination (r^2) between POC and phytoplankton cell no. (Phy), heterotrophic particles number (H), bacterial number (B) and total POC - for each size class, at St. I and II, over 1976 $n = 10$

Variables	Size classes (μm)	Station I		Station II	
		r	r^2	r	r^2
Phy (cell no. l^{-1})	100-50	0.33	0.11	0.45	0.20
	50-10	0.66	0.43	0.44	0.19
	10-0.45	0.43	0.18	0.13	0.01
	50-0.45	0.58	0.34	0.05	0.00
H (particle no. l^{-1})	100-50	0.64	0.41	0.60	0.36
	50-10	0.55	0.30	0.29	0.08
	10-0.45	0.95	0.90	0.25	0.06
	50-0.45	0.67	0.45	0.53	0.28
B (cell no. l^{-1})	100-50	0.30	0.09	0.76	0.58
	50-10	0.56	0.31	0.71	0.50
	10-0.45	0.47	0.22	0.42	0.18
	50-0.45	0.51	0.26	0.75	0.56
Total POC ($\mu\text{g C.l}^{-1}$)	100-50	0.31	0.09	0.71	0.50
	50-10	0.87	0.76	0.76	0.58
	10-0.45	0.95	0.90	0.74	0.55
	50-0.45	0.98	0.96	0.97	0.94

A significant and positive correlation at 5°/∞ level between these variables was found in the 100 µm-50 µm size categorie (Tab. 3).

The number of heterotrophic particles in the size group between 50 µm and 0,45 µm comprised on an annual basis 90% of the total and accounted for 45% of the POC variation over the year in the equivalent size categorie.

At St. II, throughout the year, the heterotrophic particle counting in the 10 µm-0,45 µm size class constituted on an annual basis 43% of the total. In the 50 µm-0,45 µm size group, the heterotrophic particles comprised annually 79% of the total (Tab. 2).

At this station the only significant correlation found between POC and heterotrophic particles was at 5% level, in the 100 µm-50 µm categorie.

Bacterial cell counting (cells. l⁻¹)

At St. I the bacterial number in the 10 µm-0,45 µm size fraction contributed during the year with 72% for the total bacterial number.

At St. II the 10 µm-0,45 µm size class constituted on an annual average 46% of the total bacteria counts. Throughout the year, the smallest size categorie generally dominated over the largest groups, except for January, August and September, when the 50 µm-10 µm size group showed higher contribution to the total number of bacteria (Tab. 2).

Comparatively, at St. II was found a higher retention of bacteria in the larger size classes than at St. I. Awhile at St. I, on an average, 28% of the total number of bacteria, was found in the 100 µm-10 µm size group, at St. II this size categorie comprised 54% of the total bacterial number (Tabs 1, 2).

At St. II correlation factors of bacterial number with POC, were positive and significant at 1% level only in the 50 µm-10 µm and 100 µm-50 µm size categories. Bacteria accounted for 50% and 58% of the POC variation over the year, respectively in the sizes classes between 50 µm and 10 µm and, 100 µm and 50 µm (Tab. 3).

Bacterial number in the 50 µm-0,45 µm size group comprised on an annual basis, 90% of the total at St. I and 80% at St. II (Tabs 1, 2).

At St. II, bacterial number was positively correlated (at 1% level) with POC, in the size class between 50 µm and 0,45 µm, accounting for 56% of the POC variation in this size group.

Discussion

In the present work a few methodological difficulties should be mentioned. Although the aliquot for POC determination and the one for countings, were equally concentrated their volume were alike. Concentration of samples favors its representativity concerning the number, type and size of particles. Therefore, concentration minimizes the artefacts introduced by volume differences of the aliquots, though it does not cancel them. Accordingly, an over-representation of small particles in the smallest aliquot shall not be dismissed. On the other hand, counting is always a less precise way of assessing the standing-crop of particles than the analytical method used in the present work.

Another questionable point is concerned to the diverse retention characteristics of the Whatman GF/C, used for filtering sub-samples for POC determination, and the Acropor Gelman membrane filters, used for sample size-fractionation, in relation to the smallest size particles. Sheldon (1972) has shown that the retention characteristics of the GF/C and the Millipore filters were very similar. The stated pore size of the Millipore and the Acropor Gelman membrane filters is 0,45 µm. However, admitting a loss of material through the GF/C filter, and considering the precision of the analytical procedure used to be 50 µg C.l⁻¹ it seems doubtful that this material would be detected by the wet oxidation technique used in the present work. On the other hand, for the microscope particle counting a 20 µl aliquot was used awhile for the POC assessing 100 ml aliquots of the same concentrate was utilized. In other words, for the POC measurement a 5000 X greater aliquots were used.

With all these methodological limitations in mind and for the sake of data interpretation, the lower limit of significance for the correlation factor found, was chosen as 0,708 for the 1% level.

It is apparent from the present results that the largest proportion of the total POC measured is concentrated in the size class between 0,45 μm and 50 μm . 96 and 94% of the total POC variation over the year, respectively at St. I and St. II, is attributed to POC variation in the smallest size groups.

Most of the phytoplanktonic cells, heterotrophic particles and bacteria were also found in the smallest size groups. Numerically, heterotrophic particles and bacteria, were more important than phytoplankters, at both stations.

The present results agree with many reports on the numerical dominance of the smallest size particles over the largest ones in marine environments (Teixeira, 1963; Hobson, 1967; Beers & Stewart, 1969; Gordon, 1970a; Malone, 1971; Zeitzschel, 1970; Lenz, 1972; Beers, Reid & Stewart, 1975). Mullin (1965) has shown the preponderance of smallest size POC in surface waters of the Indian Ocean. The results of Gordon (1970b), on POC size classes in the North Atlantic Ocean were inconclusive.

The implications of such results on food chain studies have been discussed in the literature (Mullin, 1965; Jorgensen, 1966; Odum, 1968; Lenz, 1972; Poulet, 1973, 1974; Parsons & Le Brasseur, 1970; Richman, Heinle & Huff, 1977).

Generally, the correlations factor between POC concentration and the numerical abundance of the organic groups in each of the size classes studied, were low and non significant for both stations. The lack of correlation of POC with number of cells and particles in marine environments, have been pointed out by Gordon (1970b) and Zeitzschel (1970).

At St. I, in the smallest size group, a highly significant correlation, was found between total POC and the smallest size group POC, and of this with heterotrophic particle number suggesting the numerical importance of this group in its contribution to total POC.

Phytoplankton and bacteria does not appear to be numerically important at this station. At St. I, Mesquita (1983) has shown that phytoplankton carbon constitutes only a small fraction of the suspended particulate organic carbon and has suggested that the POC levels may

have a relative high contribution of heterotrophs, most them depending indirectly upon the phytoplankton.

At St. II, the quantities of phytoplanktonic organisms and heterotrophic particles seem to be of minor significance as a factor affecting the levels of total POC. The numerical abundance of bacteria seems to be associated with POC in the largest size group and accounts for some of its variation over the year. The significant correlation found between total POC and POC of the largest size group, would suggest bacteria as an important factor affecting the POC levels in the water. Apparently, bacteria would also be numerically important in the 50 μm -10 μm size group, on account of its significant correlation with POC of the same size. However, for most of the year, bacteria in the smallest size group (10 μm -0,45 μm) dominates numerically over the other size classes studied. However, no significant correlation was found between bacterial densities and POC in the smallest size class. Relatively to St. I, this station shows greater predominance of detritus-attached bacteria over free-bacteria (Mesquita, 1978). The occurrence of large quantities of detritus attached-bacteria at St. II, may be an explanation for the relative higher retention of bacteria in the size classes between 10 μm and 100 μm , and for its numerical dominance over the smallest size categories, a few times during the year.

The abundance of detritus-attached bacteria, the relative high retention of bacteria in the size classes greater than 10 μm and, the lack of significant correlation between POC and bacterial number, in the smallest size class (where bacteria is most abundant), may suggest that it was not bacteria that would be carbon rich, but the detritus where it was found attached.

The POC measured has higher contribution of heterotrophic particles at St. I than at St. II. At St. II bacteria does not seem to be as important as the detritus that harbored them. In a previous study (Mesquita, 1983) the detritus quantities in the Canania estuary was shown to be greater at St. II than at St. I.

These differences found between stations, concerning the numerical importance of the organic categories

studied and their contribution to the POC pool, may be due to collection of diverse water masses as suggested by the salinity data, and considering that samples were taken at different days. However, it seems also likely that carbon, from sources that have not been considered in the present work, would interfere on the relationships between the variables.

In the Cananéia region, at the fringe of a tidal creek similar to St. II in the present work, the annual litter fall from the mangrove trees, was estimated to be 478 g dry weight/m² (Schaeffer-Novelli *et al.*, 1981), a figure approximately half of the majority of the litter fall values found for the most productive areas (Odum, McIvor & Smith, 1982) studied up to now. It is known that in the Cananéia region, the material fallen does not accumulate on the forest floor, on account of the tidal flux. Therefore, a significant part of this material is exported to the estuary. Lugo *et al.* (1980) estimate that mangroves supply 32% of the organic carbon input to Rookery Bay, in south Florida, a place where mangroves are important but not the dominant source of carbon.

The differences found between the stations considered in the present report shall not be due to differential terrestrial inputs into the 2 stations, for both are within the typical mangrove region (Besnard 1950a,b; Gerlach, 1958). St. II is closer to land and shows smaller depth than St. I. Besides, St. I is under larger influence of oceanic waters and St. II, of seepage ground water. Because of these, the circulation pattern at each of these stations may be different. Probably, St. II has better hydrodynamic conditions for retaining greater amounts of material coming from land than does St. I.

On the other hand, it should be mentioned the complex chemical effects that may occur in waters of varying and low salinities as those found at St. II. Colloidal particles are stabilised in fresh water by repulsion between their electric charges, but in sea water these charges are neutralised causing flocculation (Phillips, 1972). The characteristic yellow colour of the water coming from the mangrove catchment area suggest the significant occurrence of

dissolved humic substances. Mostly of the terrestrially derived humic material precipitates out within the estuary or close inshore (Sieburth & Jensen, 1968).

Parsons (1975) stated that "the bulk of the particulate organic carbon in the immediate vicinity of some coastal areas or within the influence of large river is of terrestrial origin". Although the two stations considered in the present account are both within a coastal area, it is apparent the differential interference of allochthonous organic material in the relationships studied. At St. II the POC measured may be constituted by a significant fraction of alien organic material while at St. I it may represent better the general productivity of the water analysed.

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