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Studies of dissolved carbohydrates (or carbohydrate-like substances) in an estuarine environment*

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ABSTRACT

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Variations in concentrations of dissolved sugars were studied along the salinity gradient of a small estuary (Elorn, Bay of Brest, France) from February 1985 to January 1986. Total dissolved carbohydrate (TDCHO) and dissolved monosaccharides (MCHO) were measured by the methods of Burney and Sieburth and Johnson and Sieburth respectively. It must be noted that these methods cannot distinguish between carbohydrates and carbohydrate-like substances; consequently, these methods probably do not closely reflect biologically available pools of carbohydrates. In the river, TDCHO and MCHO values ranged from 230 to 970 $\mu\text{g C l}^{-1}$ and from 75 to 450 $\mu\text{g C l}^{-1}$ respectively. In the estuary, TDCHO and MCHO were usually lower; they varied respectively from 20 to 570 $\mu\text{g C l}^{-1}$ and from 0 to 180 $\mu\text{g C l}^{-1}$. In June, some TDCHO values were much higher, probably because some polysaccharide was produced at this time by phytoplankton excretion or lysis.

The relationship between $\text{Cl}\%$ and TDCHO was dependent on the seasons. TDCHO was conservative in autumn, winter and early spring. TDCHO and DOC concentrations were fairly well correlated during the same period. Similar results have been previously reported by various workers. MCHO and TDCHO concentrations were well correlated throughout this study except in June. From these results, it was concluded that most dissolved carbohydrates were linked to a conservative fraction of the organic matter in periods of low biological activity, whereas newly biosynthesised carbohydrates were responsible for non-conservative behaviour.

INTRODUCTION

There is a growing interest in the study of dissolved organic matter (DOM) in seawater as it becomes increasingly obvious that DOM interacts with many biological and chemical phenomena.

*This paper forms part of the thesis (Thèse de Doctorat) of W. Senior.

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Although carbohydrates are important constituents of DOM (usually 10–20%), relatively little is known about them, especially in estuarine and coastal ecosystems, mainly because analytical techniques cannot be used in routine work. Some methods have been used to determine either the total carbohydrate content or the monomeric sugar composition of seawater, but these methods are difficult and time consuming. (Further information is given in the following reviews: Mopper (1980), Dawson and Liebezeit (1981a,b) and Liebezeit and Dawson (1982).)

Sugars, in the total dissolved carbohydrate (TDCHO) pool, are present as monosaccharides (MCHO) or polysaccharides (PCHO); the latter should be divided between biogenic polysaccharides and carbohydrates bound to humic substances (HS). However, it is not easy to distinguish between these last fractions and they are usually measured together as PCHO.

From the data collected in various earlier studies, the following features appear:

(1) TDCHO and MCHO concentrations vary, in seawaters and brackish waters, from 30 to 1300 $\mu\text{g C l}^{-1}$ and from 2 to 300 $\mu\text{g C l}^{-1}$ respectively. A few higher values have also been reported (Sumitra et al., 1972).

(2) The MCHO fraction usually accounts for a significant part of TDCHO (Burney and Sieburth, 1977; Ittekkot et al., 1981; Sakugawa and Handa, 1985); sometimes for more than half (Burney et al., 1979; Harvey, 1983).

(3) Diurnal fluctuations of TDCHO may occur in periods of biological activity (Walsh, 1965; Burney et al., 1981a,b; Harvey, 1983; Eberlein et al., 1983), but the MCHO concentrations are fairly constant over a 24-h period (Burney et al., 1981a,b; Burney, 1986a).

(4) In only a few studies have attempts been made to establish a relationship between sugar composition and other chemical, physical and biological parameters.

We report here results obtained from a study performed in a small estuary located in Brittany (Elorn Estuary, Bay of Brest, France; see Fig. 1.). Some preliminary results have been published elsewhere (Senior et al., 1987). To our knowledge, this is one of the few studies performed over such a long period of time in the same area. Seasonal variations and origins are studied; the nature of dissolved carbohydrates is discussed in the light of present and published data.

METHODS AND MATERIAL

Study area

Figure 1 shows the Elorn Estuary (Brittany, France) and the location of the seven sampling sites. Sites E_1 and E_2 are low-salinity sites, and they are

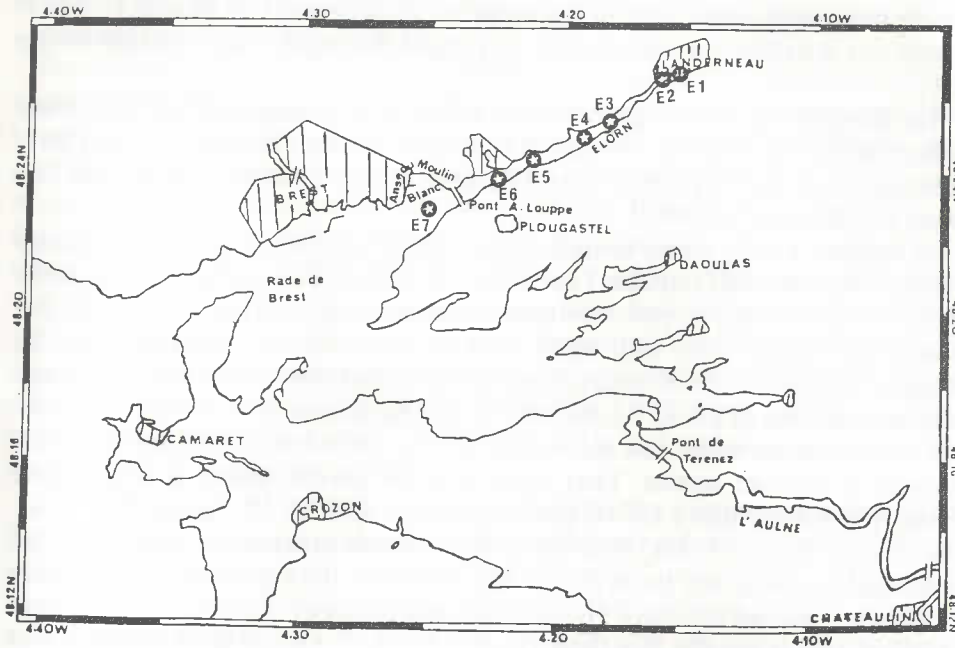


Fig. 1. Location of sampling sites in the Elorn Estuary.

considered to be located in the river. Elorn is a 12-km-long estuary. The depth ranges from 3.5 m at Landerneau to 12.5 m at Albert Louppe bridge.

During this study, the flow of the river varied between 1.3 and 18 m³ s⁻¹. A detailed record has been published earlier (Noureddin et al., 1987), together with the rainfall record. This estuary is strongly influenced by tide patterns; the renewal time of the water is between 2 and 4 days (L'Yavanc, 1984). Landerneau (see Fig. 1) is a medium-sized city of 20000 inhabitants. Two sewers discharge Landerneau's waste-water, after cleaning by a sewage-treatment system, slightly downstream from site E₂ (mean accumulated flux: 5000 m³ day⁻¹, mean DOC value 20 mg C l⁻¹). In the same area (between sites E₂ and E₃) there is also a seaweed-processing factory which extracts alginate from brown seaweeds (30000 t year⁻¹). Waste-water from this factory is discharged into the estuary in an erratic manner. Site E₃ is about 3 km downstream from this plant. In addition, upstream from site E₁, there is also a milk-processing factory, but its waste-water is treated. Lastly, at downstream sites E₆ and E₇, some organic matter may come from the main sewer that collects Brest waste-water which is discharged (after treatment) close to Moulin Blanc Bay (see Fig. 1).

Sampling

Water samples were collected from February 11, 1985, to January 8, 1986 (see Table 1). Samples were collected during the 2–3-h period around high

water; tide coefficients were in the range of 70–80 except on March 11 (98). It was not possible, for each cruise, to sample during the same period of the day.

The samples can also be grouped according to the season in which they were collected: spring (March 11–June 7) or early spring (March 11–April 24), summer (July and September) and autumn-winter (February 1985 and October 11–January 8, 1986).

At stations E₃–E₇, water sampling was carried out using an electrical pump (Lenze Discovertell Getriebe Type 11602, F.R.G.). This pump is a peristaltic pump with a synthetic polymer tube; consequently, there is no contact between mechanical parts and water, and no possibility of contamination by biogenic molecules; DOC measurements have also shown that there is no significant increase of DOC. At stations E₁ and E₂, a clean glass bottle was used. All samples were taken just below the surface and were filtered immediately through a 100- μm screen. They were kept for several hours at 5°C before being filtered through a GF/C precombusted (450°C, 4 h) glass fiber filter. For the few hours (3–4 h) between collection and processing, some bacterial degradation could not be avoided, but from the data published by Burney (1986a,b), we can estimate (in summer and in dark) that the TDCHO degradation rate is usually less than 10 $\mu\text{g C l}^{-1} \text{ h}^{-1}$. Ochiai et al. (1980) and Ochiai and Nakajima (1985) gave similar or lower decomposition rates in freshwater. At 5°C, the loss is probably less. Error introduced by keeping the water for a few hours did exist, but was of the same magnitude as the experimental analytical error (see analytical methods). For MCHO, bacterial utilization (for the same period) was probably significant. On the other hand, filtering with GF/C only partially removed bacteria. Consequently, some carbohydrates counted as TDCHO were indeed bacterial particulate carbohydrates, but probably only to a slight extent, because we also analysed 36 samples by HPLC and found bacterial sugars (rhamnose, fucose, ribose) only at low percentages (Senior, 1986). Filtrates were then kept in glass bottles after addition of 1 ml l⁻¹ of a saturated HgCl₂ solution. All glassware used throughout the analysis was precombusted (450°C, overnight).

Analytical methods

Analyses of DOC were carried out on a Dohrmann DC 80 analyser which performs photo-oxidation of samples in the presence of persulfate. Results are expressed in mg C l⁻¹. Chlorinity was measured by the Knudsen method according to the procedure described by Aminot (1983). Chlorophyll *a* and phaeophytin *a* were determined fluorometrically using a Turner III fluorometer, according to Lorenzen (1967), after extraction from the filters by an acetone–water mixture (90:10, v/v). However, we should point out that re-

sults obtained by this method may not be accurate for estuarine environments (Mantoura and Llewellyn, 1983).

For dissolved monosaccharides (MCHO), the method of Johnson and Sieburth (1977) was used, following the procedure given by Dawson and Liebezeit (1981a,b). Mannitol and glucose were used as standards. Molar concentrations were calculated from the control corrected mean absorbance data using the (glucose + mannitol)/2 regression line. Results are expressed in $\mu\text{g C l}^{-1}$ of glucose equivalent. In our hands, the precision of this method was not as high as stated by Dawson and Liebezeit (1981b). Measures were made in triplicate (three aliquots from the same sample bottle); standard deviation varied from 10 to 30 $\mu\text{g C l}^{-1}$ depending on the sample. Obviously, this method cannot distinguish between true monosaccharides and any substances which possess a terminal glycol function ($\text{CHOH}-\text{CH}_2\text{OH}$). Such a function is expected to be present in humic substances (HS), and consequently, interference with them cannot be avoided.

For total dissolved carbohydrates (TDCHO), the method of Burney and Sieburth (1977) was used. However, the conditions of hydrolysis were different (Ittekkot, 1982): 5-ml seawater samples with 1 ml of HCl (30%) were hydrolysed at 100°C for 3.5 h. After cooling, the acid was neutralized with concentrated NaOH (~1 ml). pH was checked with pH papers. Concentrations were corrected for dilutions. As for MCHO, HS interference occurs. However, a good correlation ($r=0.97$, $n=36$; slope=1.1) was obtained between the sum of individual sugars measured by HPLC (see above; Senior, 1986) and the result given by the MBTH method.

The concentration of dissolved polysaccharides (PCHO) is the difference between the concentration in TDCHO and the concentration in MCHO (Burney and Sieburth, 1977).

RESULTS AND DISCUSSION

Tables 1 and 2 list respectively the TDCHO and MCHO concentrations at each site during the period studied. The TDCHO concentrations were variable in the river and ranged from 230 to 970 $\mu\text{g C l}^{-1}$. This variability could be caused by some material being brought by tidal currents (sampling was performed at high water) from Landerneau's sewers or from the seaweed factory. In particular, the 970 $\mu\text{g C l}^{-1}$ value (100 $\mu\text{g C l}^{-1}$ more than the second-highest value) was abnormally high, because the tide coefficient (98) was significantly higher on this day than usual. Consequently, this value was not included in the calculations. For the remaining overall measurements, the mean value was 560 $\mu\text{g C l}^{-1}$. In autumn-winter, the average concentration in the river was higher (675 $\mu\text{g C l}^{-1}$) than in early spring (420 $\mu\text{g C l}^{-1}$). At downstream stations, the TDCHO concentrations were usually lower (20-

TABLE 1

TDCHO concentrations ($\mu\text{g C l}^{-1}$) in the Elorn Estuary

Date (1985)	Site						
	E ₁	E ₂	E ₃	E ₄	E ₅	E ₆	E ₇
February 11	860	530	350	340	140	40	20
March 11	970*	440	145	180	155	150	125
April 3	320	430	310	265	220	240	290
April 17	495	750	230	205	280	255	245
April 24	265	265	210	105	165	145	140
June 7	795	785	620	755	470	1080	395
July 1	550	480	325	185	270	265	165
September 20	305	230	395	385	320	195	205
October 11	765	755	330	295	295	305	215
November 25	545	835	120	115	145	90	100
December 9	700	715	400	315	295	265	245
January 8 (1986)	500	545	570	325	315	250	240

*This abnormally high value has been discarded.

TABLE 2

MCHO concentrations ($\mu\text{g C l}^{-1}$) in the Elorn Estuary

Date	Site						
	E ₁	E ₂	E ₃	E ₄	E ₅	E ₆	E ₇
February 11	290	200	110	130	0	0	0
March 11	390	180	70	60	55	90	40
April 3	245	225	180	165	115	135	170
April 17	450	400	95	80	80	105	75
April 24	165	185	170	100	150	90	95
June 7	105	165	90	75	75	430	130
July 1	325	305	120	60	105	110	35
September 20	105	75	145	100	115	80	90
October 11	325	335	125	105	110	70	65
November 25	260	385	45	30	35	35	10
December 9	285	305	125	130	100	55	65
January 8	185	230	130	95	75	55	40

$570 \mu\text{g C l}^{-1}$), with some notable exceptions in June ($1080 \mu\text{g C l}^{-1}$ at station E₆, for instance). The MCHO values were lower; they ranged from 75 to $450 \mu\text{g C l}^{-1}$ in the river and from 0 to $180 \mu\text{g C l}^{-1}$ at more downstream stations.

Relationship between chlorinity and TDCHO

TDCHO concentrations showed a relatively poor but significant relationship with chlorinity ($r=0.58$; $n=83$; $P<0.001$) for the overall measure-

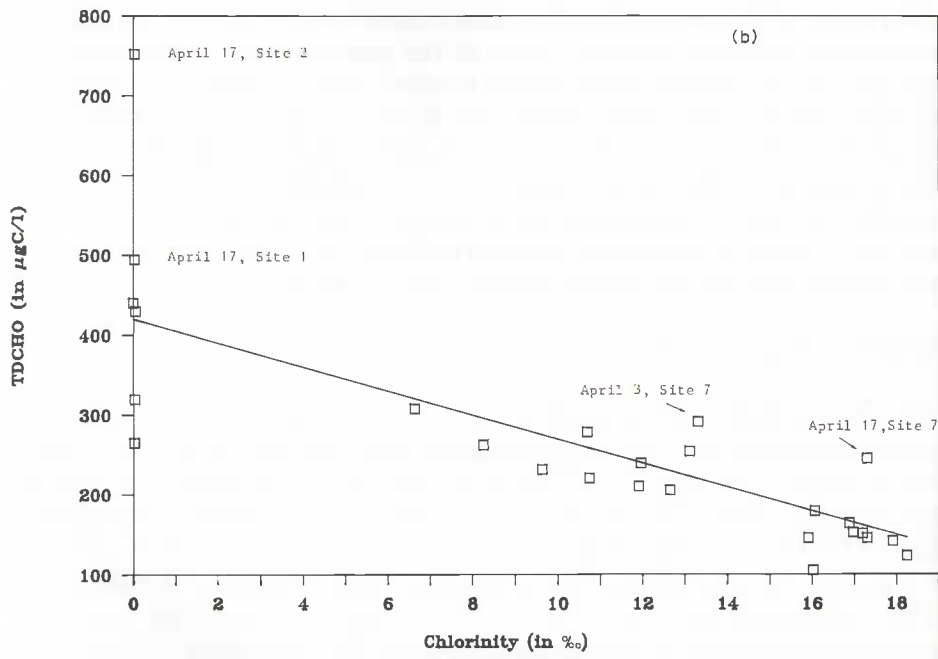
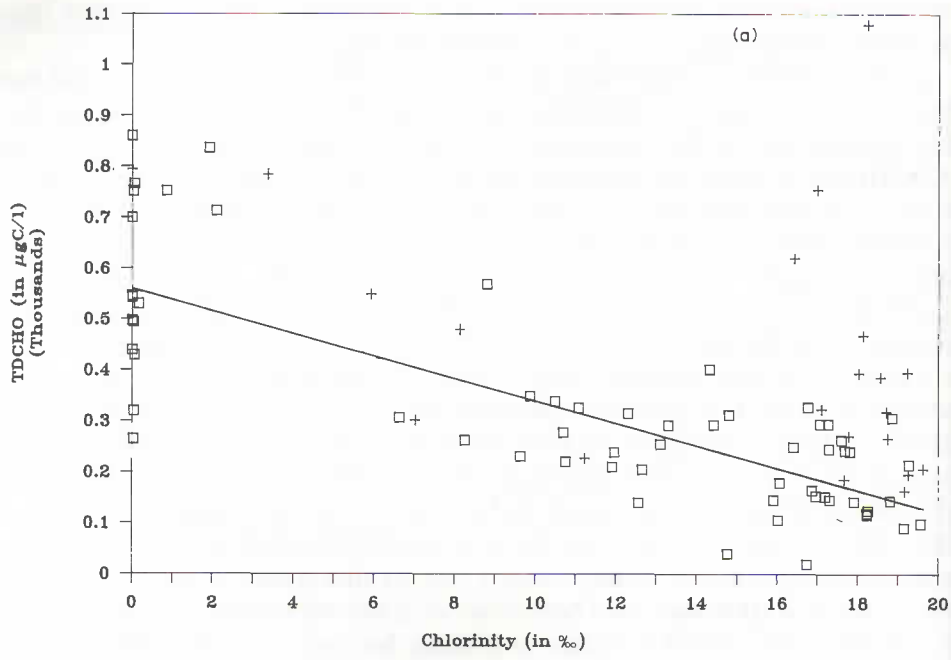
ments. If the values measured in June, July and September are not taken into account, a better relationship is obtained (see Fig. 2a).

If the data are split into early spring values (March 11–April 24) and autumn–winter values, two different regression lines are obtained (see Fig. 2). For this last period, the y -intercept and slope are higher, probably because the TDCHO input from the river was more important at this time, when terrestrial plants were decaying and when the land was more efficiently drained. It is worth pointing out that, without the river measurements, we obtained similar regression lines in early spring ($r=0.79$; $n=20$; $P<0.001$; $[\text{TDCHO}] = -13.5 (\pm 2.5) [\text{Cl}\text{‰}] + 390 (\pm 40)$) as well as in autumn–winter ($r=0.59$; $n=25$; $P<0.01$; $[\text{TDCHO}] = -23 (\pm 6.5) [\text{Cl}\text{‰}] + 600 (\pm 100)$). For both periods, y -intercepts (390 and 600 $\mu\text{g C l}^{-1}$) are acceptably close to the TDCHO mean values in the river (420 and 675 $\mu\text{g C l}^{-1}$; see above). This result suggests that the TDCHO concentration was widely variable in the river on a short time-scale, but the TDCHO flux introduced into the estuary by the river was more stable. These data also suggest that most of the TDCHO was coming from the river and was conservative in autumn–winter and early spring in this estuary and for this study. To our knowledge, this is the first time that such behaviour of dissolved carbohydrates has been established. This result is rather surprising, because a priori, carbohydrates should be a dynamic part of the DOM and should not be conservative.

During the period of higher biological activity (June–September), the TDCHO vs. $\text{Cl}\text{‰}$ relationship disappeared, probably because of some in situ production, especially in June (most of the points are above the regression line; see Fig. 2a). Surprisingly, during the early spring (March 11–April 24), the TDCHO vs. $\text{Cl}\text{‰}$ relationship was fairly strong ($r=0.77$; $n=27$), although there was some evidence of biological activity in the estuary (high concentrations in chlorophyll and phaeophytin on March 11 and April 3, for instance). Either the phytoplanktonic species of the spring bloom did not excrete any carbohydrates, or the excreted carbohydrates were very quickly used and did not build up any significant amount of TDCHO.

Relationship between TDCHO and phaeophytin

During the period of biological activity (June–September), there was a significant relationship ($r=0.76$, calculated without the abnormally high phaeophytin value ($10.3 \mu\text{g l}^{-1}$) of July 1 at station E_1) between TDCHO and phaeophytin (see Fig. 3). Burney et al. (1981a) also noted a correlation between TDCHO and phaeopigment fluctuations in a study of diel variations of TDCHO in a simulated estuarine ecosystem. Similarly, Ittekkot et al. (1981) measured maximum dissolved combined carbohydrate concentrations towards the end of a bloom in the North Sea. According to these workers, nutrient limitation leads to the release of large amounts of carbohydrates.



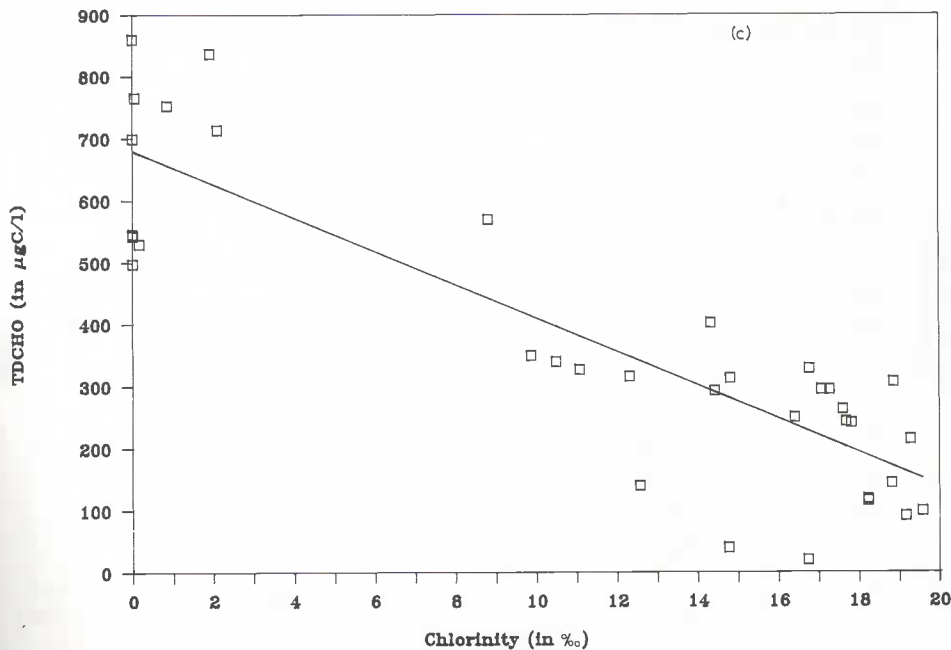


Fig. 2. The inverse relationship of TDCHO to Cl‰. (a) For the overall measurements, the regression line was $[\text{TDCHO}] = -18 (\pm 3) [\text{Cl}\text{‰}] + 560 (\pm 180)$ ($r=0.58$; $n=83$; $P<0.001$), but that shown here was calculated without the June–July–September values (+): $[\text{TDCHO}] = -22 (\pm 2) [\text{Cl}\text{‰}] + 560 (\pm 130)$ ($r=0.77$; $n=62$; $P<0.001$). (b) In spring, the regression line was $[\text{TDCHO}] = -15 (\pm 2.5) [\text{Cl}\text{‰}] + 420 (\pm 90)$ ($r=0.77$; $n=27$; $P<0.001$). (c) in autumn–winter, the regression line was $[\text{TDCHO}] = -27 (\pm 2.5) [\text{Cl}\text{‰}] + 680 (\pm 115)$ ($r=0.87$; $n=35$; $P<0.001$).

Usually, during a phytoplankton bloom, accumulation of dissolved carbohydrates is at a maximum in the stationary phase (Myklestad, 1977; Brockmann et al., 1979). Indeed, in Fig. 2a, it is obvious that large excretions of carbohydrates occurred in the bay in summer when nutrients were depleted. For instance, nitrate fell from $20 \mu\text{M l}^{-1}$ in April to $4 \mu\text{M l}^{-1}$ in June and $0 \mu\text{M l}^{-1}$ in July at station E₇ (Senior, 1986). Consequently, during this period, TDCHO were not only formed by refractory carbohydrates coming from the river, but also by newly biosynthesised sugars released by senescent algal cells or produced by zooplankton excretion (Burney et al., 1981a).

Relationship between dissolved carbohydrates (TDCHO or MCHO) and DOC

The TDCHO vs. DOC relationship calculated for the overall points was weak and the regression line aberrant (see Fig. 4a). However, if the June–July–September values, together with three additional abnormally high DOC values (station E₃ of February 11, stations E₂ of December 9; station E₇ of

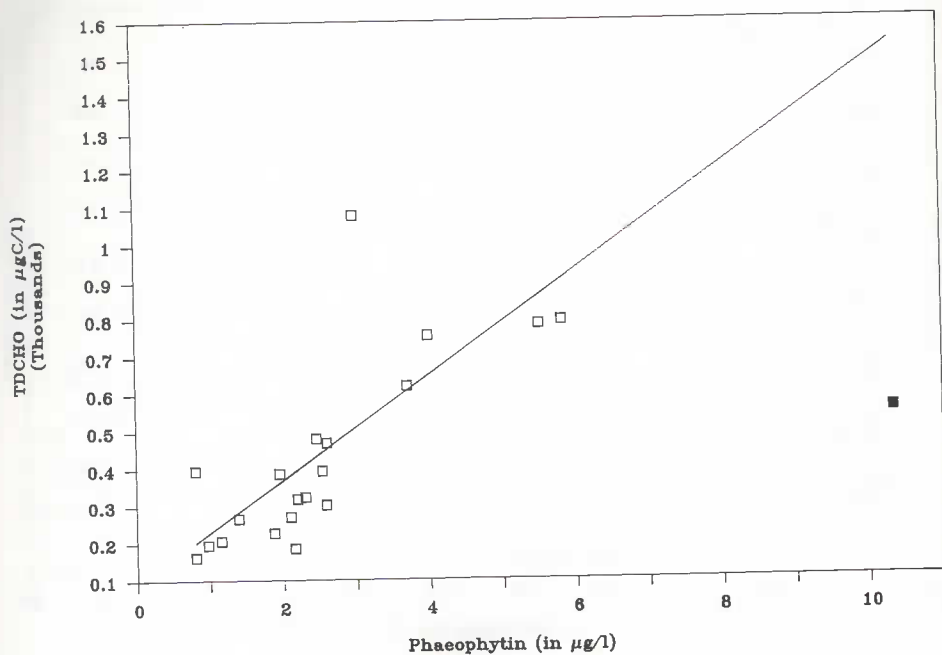
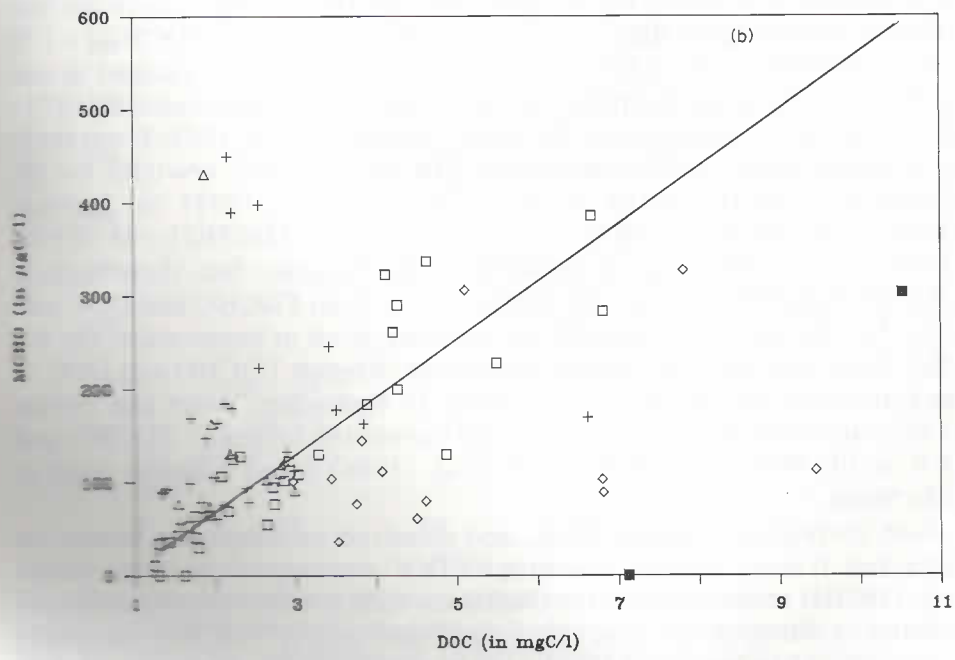
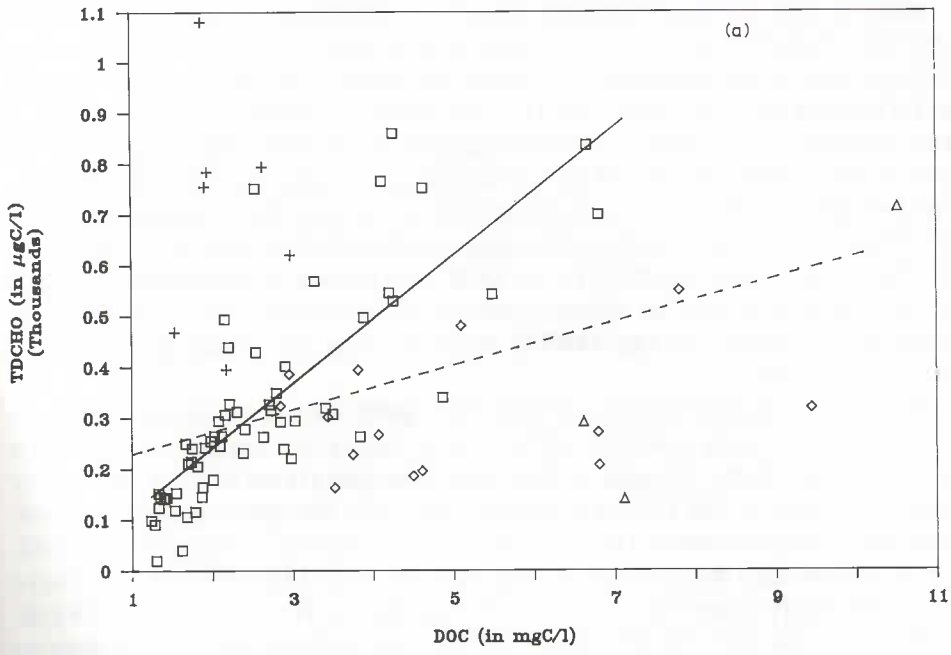


Fig. 3. The direct relationship between TDCHO and phaeophytin in June–July–September. The value for July 1 at site E₁ (■) is not included in the calculation of the regression line: $[\text{TDCHO}] = 140 (\pm 30) [\text{Phaeo}] + 90 (\pm 170)$ ($r = 0.76$; $n = 20$; $P < 0.001$).

April 3), are discarded, a much better relationship is obtained (see Fig. 4a). Very similar results have been found for MCHO. A moderately good correlation between MCHO and DOC may be calculated for the overall measurements ($r = 0.35$; $n = 84$; $P < 0.01$). The correlation coefficient is better if the same values as before are discarded ($r = 0.61$; $n = 60$; $P < 0.001$). The correlation becomes excellent in autumn–winter, with the exclusion of the corresponding abnormally high DOC values ($r = 0.89$; $n = 33$; $P < 0.001$, see Fig. 4b). Consequently, in the period of low biological activity, dissolved carbohydrates (TDCHO or MCHO) and DOC were correlated, and consequently had similar origins and evolutions. As the bulk of the DOM is known to be

Fig. 4. The direct relationship between dissolved carbohydrates and DOC. (a) TDCHO vs. DOC relationship: the broken regression line ($[\text{TDCHO}] = 38.5 (\pm 12) [\text{DOC}] + 230 (\pm 210)$; $r = 0.33$; $n = 83$; $P < 0.01$) was calculated from overall points. The solid regression line ($[\text{TDCHO}] = 126 (\pm 13) [\text{DOC}] - 10 (\pm 120)$; $r = 0.79$; $n = 59$; $P < 0.001$) was calculated without the June values (+) the July–September values (◇) and three additional high DOC values (△). (b) MCHO vs. DOC relationship: the regression line ($[\text{MCHO}] = 61 (\pm 6) [\text{DOC}] - 55 (\pm 50)$; $r = 0.89$; $n = 33$; $P < 0.001$) was calculated from only the autumn–winter measurements (□). ■: Winter measurements not included in calculation of the regression; +: early spring measurements; △: June measurements; ◇: July–September measurements.



refractory, this result implies that dissolved carbohydrates should be refractory too. However, we have to recognize that some usable dissolved carbohydrate may be lost, especially for the period preceding fixation (see Methods and Material section). For TDCHO, the probable losses ($15\text{--}30 \mu\text{g C l}^{-1}$) were low relative to TDCHO concentrations (only two values under $100 \mu\text{g C l}^{-1}$), and consequently did not invalidate our result. For MCHO, concentrations were lower and monosaccharides were used more rapidly (Ochiai and Nakajima (1985) measured a glucose degradation rate of $14 \mu\text{g C l}^{-1} \text{h}^{-1}$); consequently, the MCHO vs. DOC correlation is better than it should be. It is still true that, in autumn-winter, a significant part of the MCHO fraction (measured by the MBTH method) was not made up of usable monosaccharides.

When the biological activity is high, this correlation is no longer observed. For instance, in July and September, most points are under the regression lines (see Fig. 4a,b), because at this time, concentrations of DOC were very high, but those of MCHO or TDCHO were not. On the contrary, in June, there was a large excess of TDCHO (see Fig. 4a), mainly composed of PCHO.

It is noteworthy that similar correlations between TDCHO and DOC have been previously observed in various environments. Burney et al. (1979) reported that MCHO, PCHO and TDCHO showed significant correlations ($P < 0.01$) with DOC in a study performed in a large area on the continental shelf of North America and across the North Atlantic Ocean, with sampling at the surface or at depth. For instance, the TDCHO vs. DOC regression line calculated from their data ($r = 0.71$; $n = 84$; $P < 0.001$; $[\text{TDCHO}] = 170 (\pm 20)$ $[\text{DOC}] - 25 (\pm 30)$) is reasonably similar to that obtained in our study. The same group had previously published (Burney and Sieburth, 1977) data collected in Narragansett Bay from February to July 1975. From these data, a good linear correlation between TDCHO and DOC ($r = 0.83$; $n = 20$; $P < 0.001$; $[\text{TDCHO}] = 115 (\pm 20)$ $[\text{DOC}] - 70 (\pm 120)$) or between MCHO and DOC ($r = 0.87$; $n = 20$; $P < 0.001$; $[\text{MCHO}] = 48 (\pm 6)$ $[\text{DOC}] - 62 (\pm 45)$) can be deduced. In the Sargasso Sea, these authors (Burney et al, 1981b), observed similarities between TDCHO and DOC patterns, but unfortunately they did not give any detail or explanation. On the other hand, they did not find any correlation between TDCHO and DOC in the Caribbean Sea (Burney et al., 1982). In freshwater, Sweet and Perdue (1983) also noticed "a moderately good correlation between" TDCHO and DOC in the Williamson River. Satoh et al. (1986) found a similar result in Lake Suwa.

Such correlations between DOC and dissolved carbohydrates cannot be fortuitous. It would be very surprising for DOC and biogenic and even refractory TDCHO to follow similar evolutions in such a variety of environments. A better explanation could be that a significant part of TDCHO (especially in periods of low biological activity) is in a refractory form associated with

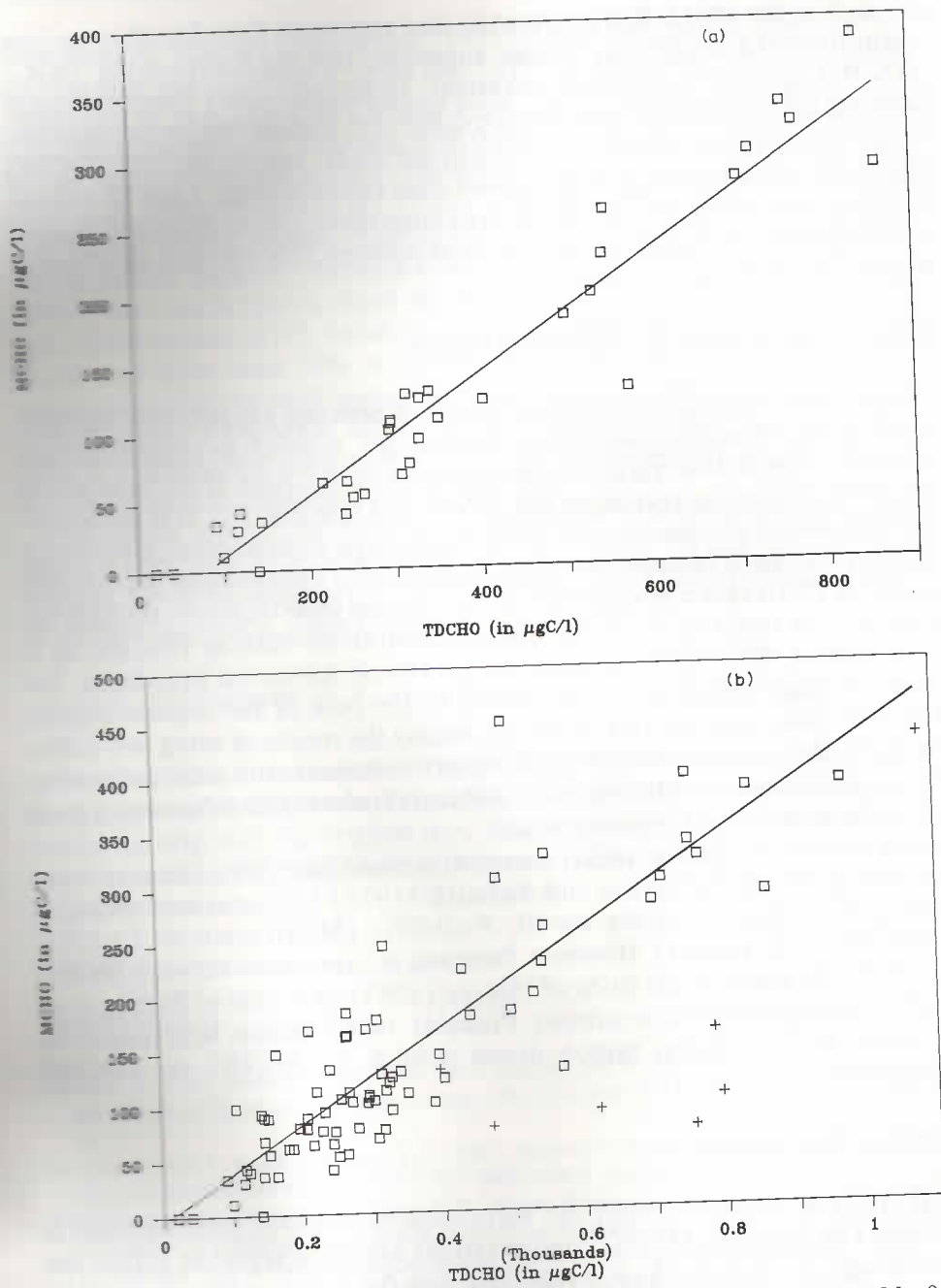


Fig. 5. The direct relationship of MCHO to TDCHO. (a) in autumn-winter: $[\text{MCHO}] = 0.44 (\pm 0.02)$ $[\text{TDCHO}] - 35 (\pm 30)$ ($r = 0.96$; $n = 35$; $P < 0.001$). (b) For overall points except those for June (+): $[\text{MCHO}] = 0.44 (\pm 0.03)$ $[\text{TDCHO}] - 5 (\pm 50)$ ($r = 0.87$; $n = 77$; $P < 0.001$).

the bulk of the DOM. It is noteworthy that regression lines found in various environments are relatively similar, suggesting that the nature of the 'DOC-TDCHO complex' is relatively unvariant. To this refractory fraction, freshly excreted polysaccharides may be added; they are responsible for the non-conservative behaviour observed in June in our study, and for the diel TDCHO variations often observed and reported (see Introduction). Lack of correlation may also result from production of large quantities of dissolved organic substances which disrupt the relationship between TDCHO and DOC (as in summer in the present study).

Relationship between MCHO and TDCHO

There was an excellent linear relationship between MCHO and TDCHO (see Fig. 5a) in autumn and winter. Surprisingly, most of the other points are relatively close to this regression line except those for June. If the June points are ignored, a similar regression line is obtained (see Fig. 5b). It is inconceivable that truly free monosaccharides and polymeric carbohydrates follow such similar patterns of release and uptake. It also seems unbelievable that MCHO as well as PCHO are not significantly used during their transport through the estuary (2-4 days) at relatively high concentrations such as $100-200 \mu\text{g C l}^{-1}$. Indeed, this result confirms the previously advanced hypothesis, because, if most carbohydrates are linked to the bulk of the organic matter, TDCHO values and MCHO values are simply the results of using two different methods (with and without hydrolysis) to measure the same parameter: the concentration of carbohydrates (and carbohydrate-like substances) present in the transformed organic matter.

It is noteworthy that a similar correlation could have been obtained from the data collected by Burney and Sieburth (1977) in winter and spring in Narragansett Bay ($r=0.96$; $n=20$; $P<0.001$; $[\text{MCHO}] = 0.39 (\pm 0.03)$ $[\text{TDCHO}] - 20 (\pm 25)$). However, there was no correlation between MCHO and TDCHO either in the study of Harvey (1983) or in that of Burney et al. (1979), both performed in summer. Probably, in this season, large quantities of newly biosynthesized carbohydrates disturb the MCHO vs. TDCHO relationship.

GENERAL DISCUSSION

As noted in the Introduction, our knowledge on dissolved carbohydrates is limited. For instance, MCHO concentrations are often reported within the $50-100 \mu\text{g C l}^{-1}$ range, which is relatively high for easily usable compounds. Glucose, the most dominant monosaccharide constituent of the MCHO fraction (Mopper et al., 1980), is measured by chemical methods at much higher concentrations than those expected from the $K_t + S_n$ values determined by mi-

crobiological methods (Gocke et al., 1981). These last workers supposed that glucose may exist either in a "truly free form" usable by heterotrophic microorganisms or in a "reversibly bound form" that is unusable. This last form could be associated, for instance, with organic compounds. Indeed, it is well known that carbohydrates are a constituent of humic substances (HS) (Wershaw et al., 1981; Wilson et al., 1983). The melanoidin theory even suggests that HS are mainly formed from carbohydrates. This theory has recently received further support (Benzing-Purdie and Ripmeester, 1983; Ikan et al., 1986), but little is known about the nature and the strength of bonds which link carbohydrates to HS. In fulvic acid (FA), De Haan and De Boer (1978) found that more than 50% of carbohydrates were not associated, or were loosely associated by weak acid-labile bonds to FA. Sweet and Perdue (1982) identified only 2.8% of the aquatic humus carbon as sugar carbon. Probably, there is a continuum between sugars loosely associated to HS and carbohydrate-like entities completely included in HS.

Our results support the view that a significant part of dissolved carbohydrates is associated with the refractory organic matter. To this TDCHO bound fraction, newly biosynthesized carbohydrates may be added, but the turnover of the new fraction is much quicker.

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