ELSEVIER

Available online at www.sciencedirect.com
SCIENCE DIRECT®

Marine Chemistry 81 (2003) 119–135

MARINE CHEMISTRY

www.elsevier.com/locate/marchem

Distributions of carbohydrate species in the Gulf of Mexico

Chin-Chang Hung[a,*], Laodong Guo[b], Peter H. Santschi[a], Nicolas Alvarado-Quiroz[a], Jennifer M. Haye[a]

[a] Department of Oceanography, Texas A&M University, 5007 Ave. U, Galveston, TX 77551, USA
[b] International Arctic Research Center, University of Alaska Fairbanks, Fairbanks, AK 99775, USA

Received 6 June 2002; received in revised form 19 November 2002; accepted 20 January 2003

Abstract

In order to study the role of polysaccharides in the cycling of marine organic matter and transparent exopolymeric particles (TEP), the concentrations of total carbohydrates (p-TCHO), total uronic acids (URA) and total acid polysaccharides (APS) in suspended and sinking particles, as well as carbohydrates in the filter-passing "dissolved" phase (d-TCHO), were measured in vertical profiles along a N–S transect in the Gulf of Mexico, across a cold core (CCR) and a warm core (WCR) ring (eddy) during both July 2000 and May 2001. The concentrations of d-TCHO in 2000 ranged from 4 to 22 μM C, with a subsurface maximum, which was located slightly above the depth of chl *a* maximum, amounting to, on average, 34% of DOC in the CCR, and 13% in the WCR. The concentration of particulate carbohydrates (p-TCHO) in different size fractions (0.7–10, 10–53, and >53 μm) ranged from 0.04 to 1.1, 0.005 to 0.40, and 0.006 to 0.26 μM C, respectively, indicating that carbohydrates are mostly concentrated in small particles (0.7–10 μm). URA and APS were similarly concentrated in small particles, in which, on average, URA accounted for 87% and 57% of total URA, and APS for 92% and 88% of total APS in 2000 and 2001, respectively. URA accounted for 3–9% of carbohydrates in suspended particles, suggesting that URA are a minor component of the p-TCHO pool. Due to its surface-reactive nature, URA could play a major role in the coagulation of particles and macromolecules despite its relatively low abundance. While, on average, p-TCHO and total APS were more enriched in suspended particles than in sinking particles in both 2000 and 2001, the opposite was true for URA in both years. The greater contents of URA that are present in settling particles compared to suspended particles could indicate a mass flow in the direction of sinking particles, either caused by coagulation, by bacterial reworking of particulate and colloidal organic matter, or by their more refractory nature.

© 2003 Elsevier Science B.V. All rights reserved.

Keywords: Carbohydrates; Acid polysaccharides; TEP; DOM; Uronic acids; Seawater; Gulf of Mexico

1. Introduction

Carbohydrates are some of the most abundant biopolymers in the marine organic carbon pool, making up 10–70% of organic matter in plankton cells (Romankevich, 1984). Marine carbohydrates have been studied for several decades. Over the past decade, some major components have been identified in marine particles, including uronic acids (Mopper et al., 1995; Hung et al., 2001), amino sugars (Muldoon et al., 2001), aldoses (Skoog and Benner, 1998) and neutral sugars (Mopper et al., 1995; Borch and Kirch-

* Corresponding author. Tel.: +1-409-740-4772; fax: +1-409-740-4786.

E-mail address: hungc@tamug.tamu.edu (C.-C. Hung).

0304-4203/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0304-4203(03)00012-4

man, 1997). Polysaccharides have been recognized to play important roles in the production of biofilms and the formation of mucilaginous aggregates (Baldi et al., 1997; Leppard, 1997; Pettine et al., 1999), the destabilization of inorganic colloids through flocculation (Wilkinson et al., 1997), and complexation with trace metals (Jang et al., 1990, 1995). Moreover, exocellular polysaccharides, ubiquitous in colloidal organic matter in freshwaters (Buffle and Leppard, 1995) and marine systems (Santschi et al., 1998), play an important role in the formation of marine snow flocs (Alldredge and Passow, 1993; Passow et al., 1994).

In aquatic environments, extracellular acid polysaccharides (such as uronic acids) secreted by algae and bacteria in response to low nutrient or high metal stress (Costerton, 1984; Leppard, 1993) play a significant role in heavy metal detoxification (Leppard, 1997). Moreover, uronic acids function in the extracellular milieu by forming flocs (Alldredge and Silver, 1988; Mopper et al., 1995) and biofilms (Geesey et al., 1977; Leppard, 1997), binding extracellular enzymes in their active forms, scavenging trace metals from the water, immobilizing toxic substances, altering the surface characteristics of suspended particles, and modifying the solubility of associated molecules (Costerton, 1984; Leppard, 1993, 1997). Due to the strong metal binding capacity of acid polysaccharides (Quigley et al., 2002, and references therein), the scavenging flux of Th(IV) and other metals becomes closely related to the flux of organic matter (e.g., Coale and Bruland, 1987; Baskaran et al., 1996; Santschi et al., 1999; Guo et al., 2002). Most recently, an acid polysaccharide with molecular weight $\sim$13 kDa and a pK_a of $\leq$3 has been identified as the main binding agent for Th(IV) (Quigley et al., 2002).

While the distribution of dissolved and particulate polysaccharides has been well studied, the source functions and individual relationships between neutral and acid polysaccharides in particulate size fractions to those of phytoplankton species are still poorly understood. One important reason for the scarcity of reliable acid polysaccharide data is that many methods used for acid polysaccharide analysis are associated with substantial uncertainties. Also, most methods use acid hydrolysis, during which lactones irreproducibly generated. However, lactones mostly escape conventional carbohydrate analytical methods (Fazio et al., 1982; Filisetti-Cozzi and Carpita, 1991; Hung and Santschi, 2001; Hung et al., 2001).

The objectives of this research are to (1) improve the quantitative assessment of both total acid polysaccharides (APS) and uronic acids (URA); (2) investigate the quantitative aspect of abundance and distribution of polysaccharides, APS and URA compounds in different size fractions (0.7–10, 10–53, and >53 μm) of suspended and of sinking particles; and (3) better understand the role that polysaccharide species play in the cycling of organic carbon.

In this work we present (1) the spatial distribution and phase partitioning of particulate carbohydrates, including URA and total APS from the coastal to the oligotrophic open Gulf of Mexico; (2) vertical distributions of carbohydrates at deep stations, including the CCR and WCR stations; and (3) investigations into relationships between POC, p-TCHO, APS and URA concentrations as well as the comparison between p-TCHO, APS and URA concentrations in suspended and sinking particles.

In companion papers, we present (1) the relationships between the distribution and speciation of carbohydrates with those of phytoplankton species (Hung et al., in press) that allows us to make inferences about the source functions of APS compounds; and (2) the relationships between the distribution and speciation of carbohydrates as well as phytoplankton speciation with POC/^{234}Th ratios (Santschi et al., 2003). From such relationships, we were able to relate the variability in the distributions of POC/^{234}Th to those of carbohydrates and plankton species.

2. Materials and methods

2.1. Study area

Water column samples from the Gulf of Mexico were taken aboard the R/V Gyre during 1–10 July, 2000 and 17–25 May 2001, along a N–S transect, covering a Cold Core Ring (CCR) and a Warm Core Ring (WCR, Table 1), extending from the coastal area near Galveston to the oligotrophic open Gulf region. In the first expedition (July 2000) to the Gulf of Mexico, the study area can be separated into four different regions: (1) stations 1–4, which were located on the continental shelf; (2) station 5, which was

Table 1
Sampling locations, water depth, surface water temperature, and salinity at Gulf of Mexico stations in 2000 and 2001

Station no.	Date of collection	Latitude	Longitude	Water depth (m)	Temperature (°C)	Salinity
2000						
1	2 July 2000	28°51′N	94°19.8′W	25	28.85	35.289
2	2 July 2000	28°50.3′N	94°59.6′W	20	29.69	35.400
3	2 July 2000	28°28.4′N	95°24.95′W	25	29.42	35.751
4	3 July 2000	28°00′N	95°26′W	75	28.87	36.100
5 (CCR)	4 July 2000	27°30′N	95°11′W	985	28.81	36.322
6	5 July 2000	26°55′N	95°16′W	1600	28.89	36.556
7 (WCR)	8 July 2000	26°00′N	95°20′W	1790	29.40	36.680
2001						
1	18 Mar 2001	28°52′N	94°59′W	17	25.92	27.841
2	18 Mar 2001	28°29′N	94°59′W	30	26.24	28.954
3	19 Mar 2001	27°59′N	95°00′W	81	25.86	34.451
4 (CCR)	19 Mar 2001	27°38′N	94°59′W	638	26.04	33.640
5	21 Mar 2001	27°00′N	94°59′W	1400	26.34	34.998
6 (WCR)	22 Mar 2001	26°19′N	95°00′W	1632	26.50	36.420

CCR: cold core ring; WCR: warm core ring.

located in the center of a CCR, with a surface water temperature of 28.81 °C; (3) station 7, which was located in a WCR, with a surface water temperature of 29.40 °C; (4) station 6, which was located in the boundary region between the CCR and WCR. From the satellite image of dynamic height and the sea surface temperature, the positions of the CCR and WCR could be easily distinguished (Table 1, see also http://www-ccar.Colorado.EDU/~ realtime/gom-real-time_ssh/). The concentrations of nitrate in surface waters were mostly near the detection limit, except at station 4, where the nitrate concentration was at 0.4 μM.

In May 2001, the study area was close to that of the 2000 cruise including the relative positions of CCR (station 4 with a surface temperature 26.04 °C) and WCR (station 6 with surface temperature 26.50 °C), even though the sampling locations were slightly different from those in 2000. The hydrographic features, given in Table 1, include three different water mass characteristics, analogous to those in 2000.

2.2. Sampling and analytical methodology

Large volumes (1000–3500 l) of seawater were collected for filtration from different water depths using a submersible pump or a Rosette sampling assembly with 12 × 20 l Niskin bottles. Sub-samples were collected for the determination of nutrients, dissolved oxygen (DO), chlorophyll *a* (chl *a*), dissolved monosaccharides (d-MCHO), polysaccharides (d-PCHO) and total carbohydrates (d-TCHO). Dissolved carbohydrate species were not measured in 2001. Nutrients were analyzed by a flow-injection spectrophotometric method (Grasshoff et al., 1983), DO was analyzed by a Winkler titration, and chl *a* was determined by HPLC following acetone extraction (Pinckney et al., 2001). Dissolved organic carbon (DOC) was determined by a high temperature catalytic combustion Shimadzu TOC 5000 Analyzer (Guo et al., 1994). Dissolved carbohydrates, including d-MCHO, d-PCHO and d-TCHO, were analyzed using modification (Hung et al., 2001) of the 2,4,6-tripyridyl-*s*-triazine (TPTZ) method (Myklestad et al., 1997).

Suspended particles were filtered through a series of filters of different size, i.e., a 53-μm Nitex mesh, a 10-μm Nitex mesh, and a 0.7-μm GF/F filter. Nitex mesh filters were further processed by removing the particles from the Nitex to a beaker by a two-stage ultrasonification procedure in 10 ml M.Q. water for 1 min. The first and second particle slurry wash solutions were combined and re-filtered through a GF/F filter. The GF/F filter was then dried and cut into several pie pieces from the same filter. Subsequently, the pie pieces were weighed to determine the aliquot percentage of the filtered sample for the determination of particulate organic carbon (POC), total carbohy-

drate (p-TCHO), total uronic acid (URA) and total acid polysaccharide (APS) concentrations. During the re-filtering and ultrasonification procedure, a fraction of particulate organic matter may have been released as dissolved carbohydrates and other DOC fractions. In the first year, the reported concentrations were not corrected for this loss, but the loss was assessed in the second year, and the data were then corrected only for that year. For instance, an average 9.4 ± 5.5% of total POC in all samples was lost during the two-step ultrasonification procedure in 2001. Similar amounts of dissolved carbohydrates were solubilized during ultrasonification, with a 8.5 ± 5.4% loss in terms of total carbohydrates. However, the dissolved uronic acid loss of 18 ± 13% was considerably higher than that of carbohydrates and POC, likely due to their fibrillar nature (Santschi et al., 1998).

Sinking particles were collected by floating sediment traps attached to a surface buoy (cylindrical plastic core tubes of 6.8-cm diameter and 1:10 aspect ratio), exposed for 24 h, at depths of 75 and 120 m at stations 5, 6, and 7 in 2000, as well as at depths of 65, 90, 120 m at stations 4 and 6 in 2001. Sinking particles were filtered through a GF/F filter and dried for the determination of POC, p-TCHO, URA, and APS concentration. POC concentrations were determined using a Perkin Elmer CHNS/O analyzer (PE 2400 Series II). The concentrations of p-TCHO were analyzed by the TPTZ method after H_2SO_4 hydrolysis (Myklestad et al., 1997; Pakulski and Benner, 1992; Burdige et al., 2000). Briefly, particulate samples were placed in an ampoule, after which 4 ml of 1.2 M H_2SO_4 solution was added to the ampoule. The samples were hydrolyzed at 100 °C for 3 h in an oven. After cooling, the solution was neutralized with $NaOH/Na_2CO_3$, and total carbohydrates were measured spectrophotometrically with the TPTZ method at 595 nm.

The concentration of particulate URA was analyzed according to Filisetti-Cozzi and Carpita (1991), as modified by Hung and Santschi (2001). In short, particulate samples were placed into a vial and 0.4 ml of Nanopure water was added. Then, 40 µl of 2 M sulfamic acid was added and the solution was stirred on a vortexer. Subsequently, 2.4 ml of 75 mM sodium tetraborate in concentrated sulfuric acid was added to the vial and heated at 100 °C for 10 min in a boiling water bath. After cooling, 30 µl of 0.15% *m*-hydroxydiphenyl was added, and the absorbance was measured at 525 nm. Glucuronic acid was used as a standard compound for measuring uronic acid concentrations. A plain GF/F filter was used as a procedural blank.

The concentration of total APS in the particulate phase was measured by the alcian blue stain method of Passow and Alldredge (1995). In 2000, the APS samples were collected by GF/F filters while collected by polycarbonate filters in 2001. Briefly, particulate acid polysaccharides were stained by alcian blue for 2 s and washed with Nanopure water to remove the excess alcian blue dye. The stained particles were dissolved in 4 ml of 80% sulfuric acid for 2 h using ultrasonification. Finally, the supernatant solution was measured at 787 nm in a 1-cm cuvette. Total APS standards, e.g., alginic acid (from *Macrocystis pyrifera*), xanthan, or carrageenan, were prepared as a standard solution in seawater (< 0.45 µm), in order to match the ionic strength and composition of the samples. The analytical precision was about 10–15% for six duplicate samples. The concentrations of total APS were then expressed as µM C alginic acid equivalents.

3. Results

3.1. Correlation of APS standard

Measurement of transparent exopolymer particles (TEP) by the alcian blue method has been extensively used since the introduction of this method to determine the concentration of TEP in marine environments (Passow and Alldredge, 1995). However, some results suggested that apparent concentrations of TEP-carbon measured by the alcian blue method might have a greater variability than POC itself, but also account for a greater percentage of POC-C (see later sections). Such a high variability and percentage might be a result of the weight normalization, the choice of the standard, or filter type, and/or other limitations of the alcian blue method. Here we report a modification of the method that allows one to estimate the carbon content of acidic polysaccharides in TEP.

Different APS standard solutions were processed by an ultrasonic technique or ground up by a tissue grinder to form gel-like APS. Total APS standards

retained by the GF/F filters were measured for their net weight (A1 and A2, Table 2) as well as carbohydrate or organic carbon content (A3 and A4, Table 2). Corrections based on particulate carbohydrate or organic carbon (POC) analysis were deemed much more satisfactory than corrections based on weight difference because the weight method appears to be prone to artifacts, possibly due to the water content and its variability (Table 2). Weight normalization would result in a higher apparent concentration of total APS, given the preparation methods used. The main reason is that APS are just one of the polysaccharide fractions, as polysaccharides are composed of neutral and acidic polysaccharides and amino sugars, etc. (Borch and Kirchman, 1997). Therefore, it is unlikely that the total APS-C content can be equal or higher than that of the total CHO-C.

With no application in mind, it would be difficult to choose a proper standard compound, due to the relatively large differences in slopes. Passow and Alldredge (1995) chose bacterially produced xanthan as the standard. However, results given in Table 2 indicate an order of magnitude variability of the slopes for this standard, with results strongly depending on sample pre-treatment, demonstrating that they are highly sensitive to the method of preparation. Alginic acid slopes, however, were much less variable, and were nearly independent of sample pretreatment. Results show that the slopes of the alginic acid standards measured by the carbohydrate analysis method were significantly higher than those measured

Table 2
Slope of different carbohydrate standard compounds that bind to alcian blue

Carbohydrates	A1	A2	A3	A4
Alginic acid	0.00075	0.00261	0.00206	0.00204
Gum Xanthan	0.00312	0.00076	0.00896	0.00083
Carrageenan (IV)	n.d.	0.00110	0.00938	0.00547
Lipopolysaccharides	n.d.	0.00138	0.00222	0.00168
Lipoteichoic acid	n.d.	0.00028	0.00267	0.00159

A1: processed by tissue grinder and measured by weight. A2: processed by ultrasonication and measured by weight. A3: processed by tissue grinder and measured by carbohydrate or POC analysis. A4: processed by ultrasonication and measured by CHO or POC analysis.

Standards were prepared in seawater and unit of the slope is absorbance μg C^{-1} (R.S.D. = 2–13%, n = 4). Salts on the filter were removed by D.W.

n.d.: not detectable.

by the weight method (Table 2). Furthermore, alginic acid, processed as gel-like particles by either a tissue grinder or an ultrasonic technique, gave very similar results (A3 = 0.00206 and A4 = 0.00204), making it a much more robust standard. Therefore, it was decided that sodium alginate was the most appropriate and representative total APS standard.

Alginic acid is considered here to be a representative uronic acid, and characteristic of the six-carbon sugar extracellular polysaccharides (EPS) produced by bacteria (Rehm and Valla, 1997) and algae (Decho, 1990). For example, EPS from marine bacteria contain about 20–50% of their polysaccharides as uronic acids (Kennedy and Sutherland, 1987; Decho, 1990).

Other APS standards, however, showed large variations, such as Gum Xanthan (A3 = 0.00896, A4 = 0.00083, Table 2). Gum Xanthan is one of the most surface-active polysaccharides, can cause filtration artifacts as it often causes filter clogging, and has seldom been reported to be a component of marine microbial exopolymeric particles.

Thus, a more appropriate selection of an APS standard compound should improve the semiquantitative acid polysaccharide analysis, even though it is still relatively difficult to obtain homogenous gel-like particles by a tissue grinder or even by the ultrasonic technique. In addition, because the composition of APS in marine environments is variable, the quantitative aspect of each result needs to be assessed separately, based on what is known about the prevailing phytoplankton assemblage. It is clear, however, that the analytical quantitation of APS compounds will depend on the availability of appropriate APS standards, regardless of the general methodology used. In addition, the alcian blue method needs to be modified if other non-TEP particles are present, such as detrital and non-organic matter, as they may influence the staining of APS. Furthermore, high turbidity from the presence of large amounts of suspended matter may also affect the absorption measurement itself (Passow, 2002a). Recently, using GC/MS techniques (Doco et al., 2001), our preliminary results for suspended particles collected from the CCR in 2001, indicated that the sum of galacturonic, mannuronic acid, and a number of unknown APS compounds, accounted for 4–68% of URA (with an average of 27%) and 1–14% of APS (average of 5%). These concentrations thus confirm the magnitude of

our APS determinations, thus making sure that total APS concentrations (as alginic acid C-equivalent) are realistic APS concentrations in the ocean. The quantitative analysis of individual APS compounds will be published elsewhere (Hung et al., in preparation).

3.2. General hydrography

Depth profiles of nitrate, dissolved oxygen (DO), DOC and chl *a* concentrations at stations 5, 6, and 7 in 2000 are shown in Fig. 1. At station 6, the boundary between the CCR and the WCR, the strong nitracline extended from 123 to 190 m, and the oxygen maximum was located at 80 m. DO concentrations then decreased to a minimum level at a depth of 500 m. This low oxygen zone was also observed in the WCR (station 7) at a depth of about 500 m. However, the oxygen profile inside the CCR (station 5) was not much different from those at stations 6 and 7, especially in the oxygen minimum layer, which ranged from 100 to 500 m, suggesting that bacterial activity within the low oxygen zone in the open Gulf waters was not significantly influenced by the presence of CCR or WCR in the upper water column. Because surface waters and deep waters do not move simultaneously and congruently with time, bacteria in deeper waters decompose detritus from any surface water masses regardless of the presence of CCR and WCR in the euphotic zone. DOC concentrations in our study area ranged from 40 to 96 μM C, with lower values in deeper waters and elevated concentrations in surface waters. The DOC distribution in the Gulf of Mexico was similar to that reported by Guo et al. (1994). Lower chl *a* concentrations were observed in the surface layer of the CCR, and the phytoplankton biomass increased with depth to a maximum value of 0.37 μg chl *a* l^{-1} at around 85 m near the bottom of the euphotic zone. The depth of the chl *a* maximum layer at stations 6 and 7 was located at 110 and 120 m, respectively, slightly deeper than at station 5, but their maximum values were only about 50% of the value in the CCR. This indicates that because nutrient-rich water upwelled closer to the surface in the CCR, the resulting phytoplankton biomass was also higher.

In the second year, the hydrographic setting in the study area was generally similar to that in first year, with some exceptions. The high nitrate concentrations (~ 0.7 μM) were prevalent in the open Gulf surface waters and the nitracline was shallower than that in 2000 (Fig. 2). This indicates that more nutrients were brought to the surface layer in 2001. The vertical profile of DO in the WCR (station 6) was different from that in the CCR (Station 4), where a DO minimum (~ 2.9 ml/l) zone was observed near the bottom of the euphotic zone at ~ 100 m. This suggests stronger respiration activities than at the WCR station, where DO was 3.92 ml/l at ~ 100 m, likely due to higher POC fluxes in the CCR. The DO minimum zone in 2001 was much shallower than in 2000, suggesting that the sampling time in 2001 might have been during or right after a period of high productivity. Similarly, the maximum chl *a* concentrations (0.48 μg/l) at the CCR station in 2001 was also slightly higher than the maximum value (0.37 μg/l) in 2000 at the same station, demonstrating that primary production was also likely higher in 2001 than in 2000. The DOC concentrations did not reflect such difference. As presented in Hung et al. (in press), the main phytoplankton species during the July 2000 cruise were Haptophytes, Prochlorophytes and Cyanobacteria, with Cyanobacteria peaking at about 75 m, while both Prochlorophytes and Haptophytes peaked at about 120 m water depth. During May 2001, the main phytoplankton species consisted of Prymnesiophytes, Prasinophytes, Prochlorophytes, Pelagophytes, and Dinoflagellates.

3.3. Distributions of d-MCHO, d-PCHO and d-TCHO concentrations

The horizontal distributions of dissolved monosaccharides (d-MCHO), polysaccharides (d-PCHO) and total carbohydrates (d-TCHO) in surface waters from the 2000 expedition are shown in Fig. 3. In short, d-TCHO concentrations were consistently high, at near 20 μM C, at stations 1–6, while at station 7, the concentrations of d-TCHO dropped to about 8 μM C. When the concentrations of d-MCHO in the surface waters (stations 1–6) decreased from nearshore to offshore, those of d-PCHO increased toward the open Gulf stations (Fig. 3). At station 5, inside the CCR, the d-MCHO concentrations showed a minimum, and d-PCHO concentration a maximum. d-PCHO concentrations were also high at station 6, which was at the boundary between the CCR and WCR. At station 7 inside the WCR station, both d-MCHO and d-PCHO

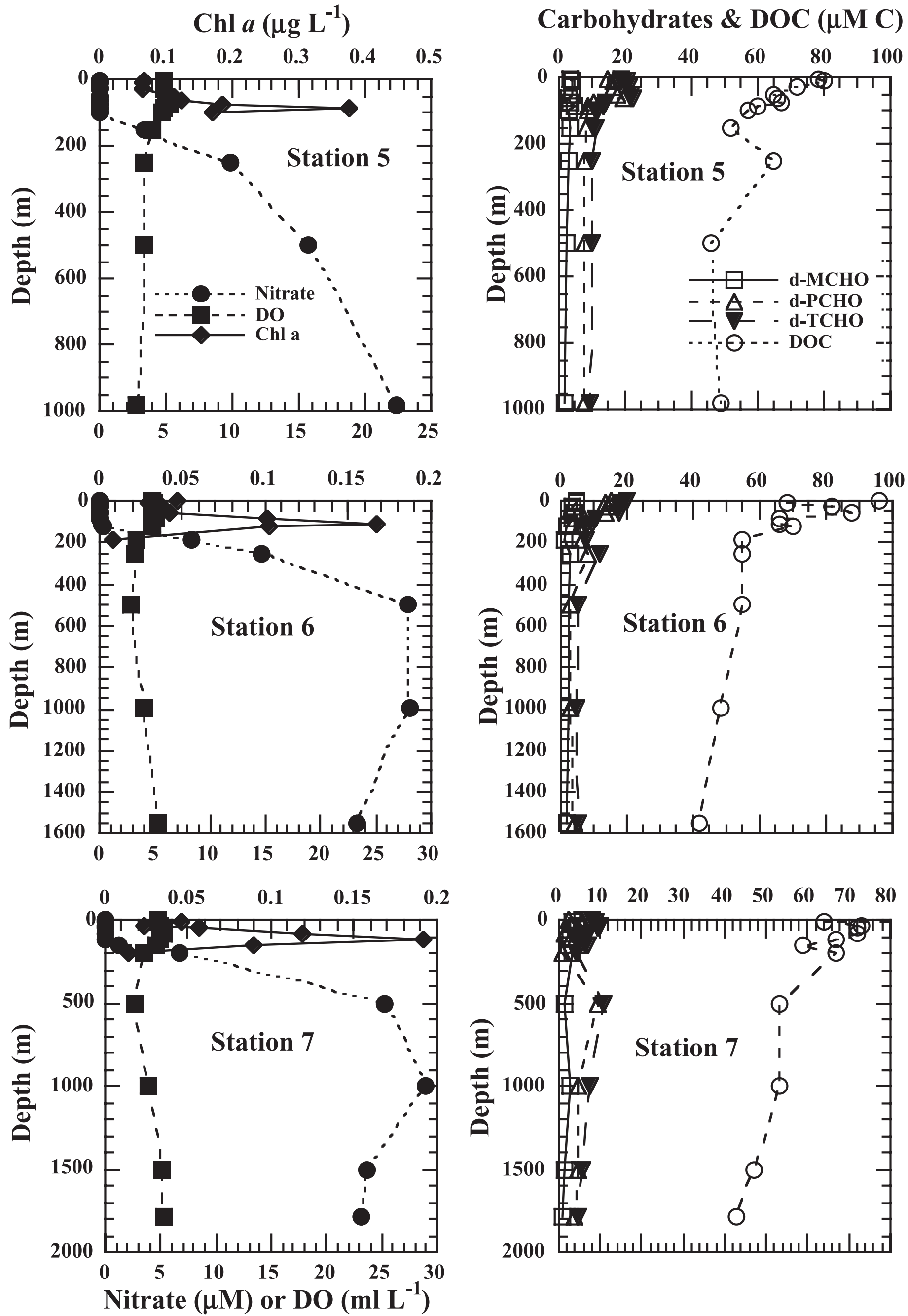

Fig. 1. Vertical profiles of nitrate, dissolved oxygen (DO), chlorophyll *a* (Chl *a*), dissolved organic carbon (DOC), dissolved d-MCHO, d-PCHO, and d-TCHO concentrations at stations 5, 6 and 7 in 2000.

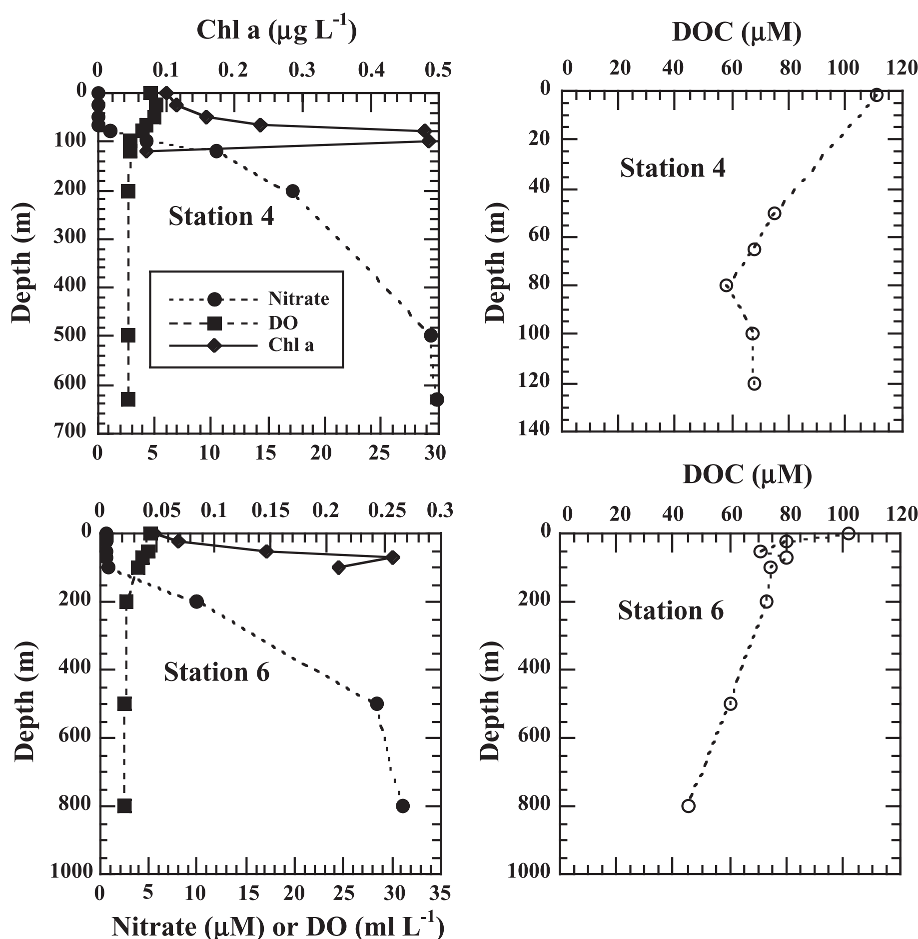

Fig. 2. Vertical profiles of nitrate, dissolved oxygen (DO), chlorophyll *a* (Chl *a*), and dissolved organic carbon (DOC) concentrations at stations 4 and 6 in 2001.

concentrations were relatively low. Furthermore, d-TCHO, as a percentage of DOC, was significantly lower in surface water (2 m) at station 7 (9% of DOC) than at station 5 (24% of DOC) and station 6 (21% of DOC).

The depth profiles of d-MCHO, d-PCHO and d-TCHO concentrations at stations 5, 6, and 7 in 2000 are shown in Fig. 1. The d-MCHO concentrations at these stations were almost constant with depth, except for one higher value just below the oxygen maximum. d-PCHO concentrations at station 5 showed a subsurface maximum, slightly above the chl *a* maximum layer (85 m), and then decreased with depth to a constant level. At station 6, d-PCHO concentrations were higher than those of d-MCHO, and showed a surface maximum and secondary mid-water maximum. Depth profiles of dissolved d-PCHO and d-TCHO concentrations at the WCR station (station 7) are distinctly different from those at the CCR station (station 5). The calculated average d-MCHO, as a percentage of DOC, did not show large variations in samples from the euphotic zone (0–120 m) from stations 5, 6 and 7, (averaging 5% of DOC, with values ranging from 3% to 8% of DOC). However, the average d-PCHO, as a percentage of DOC, in the euphotic zone of stations 5, 6 and 7 were rather variable (averaging 20%, 13% and 5% of DOC, respectively). At stations 5 and 6, d-TCHO, on average, amounted to 22% of DOC and at station 7, 11% of DOC. Overall, a good correlation between d-

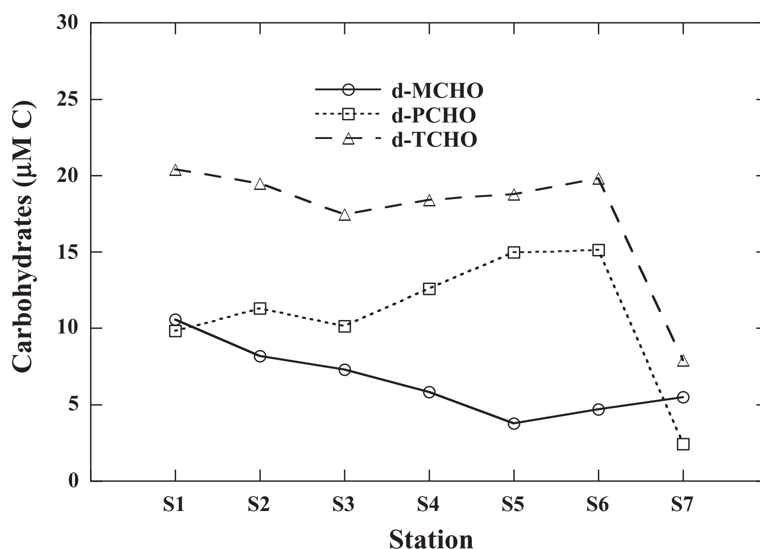

Fig. 3. Horizontal distribution of dissolved d-MCHO, d-PCHO, and d-TCHO concentrations from stations 1 to 7 in 2000.

TCHO and DOC ([d-TCHO] = 0.21 × [DOC], $r = 0.68$, $p < 0.001$, figure not shown) was found at stations 5 and 6 suggesting that d-TCHO is a major fraction of DOC. Pakulski and Benner (1994) reported that the concentration of d-TCHO in Gulf of Mexico surface waters ranged from 24 to 32 μM C, values that are close to our measured values, despite the fact that their sampling locations were different from ours.

3.4. Concentrations of POC and particulate carbohydrate species

The size fractionation data of POC, particulate carbohydrate species (p-TCHO, URA and APS) in the surface waters in 2000 are plotted in Fig. 4 and shown in Table 3. The concentrations of total POC in all three size fractions generally decreased from nearshore to offshore towards the open Gulf stations (Fig. 4A), even for small particles. A comparison of the POC concentrations of the >53, 10–53, and 0.7–10 μm particles in surface samples from stations 1 to 7 shows that the small particles (0.7–10 μm) are the major components of the POC pool, while the large particles (>53 μm) are only minor components of POC (Fig. 4A). If all samples in 2000 are included in the average, small particles accounted for 79–96% of POC, with an average value of 87% of POC. In 2001, POC concentrations in small particles had large variations (Table 4), ranging from 43% to 90% of POC, with an average value of 76%, which was a little (10%) lower than that in 2000.

The surface distribution of p-TCHO in different size fractions in 2000 is plotted in Fig. 4B, and shows an analogous distribution to that of POC, with a decreasing trend from nearshore to the open Gulf stations, and an enrichment in the small size fraction (83–95% of p-TCHO, with an average value of 89% of p-TCHO). In 2001, p-TCHO in small particles showed a wide variation, ranging from 33% to 93% of p-TCHO. At the chl *a* maximum layer in CCR (e.g., station 4, 80 m), p-TCHO in medium-sized particles (10–53 μm) was even higher than that in small particles, likely due to differences in phytoplankton speciation (see later section).

For 2000, the distributions of particulate URA and total APS in the surface waters are shown in Fig. 4C–D and detailed data are listed in Table 3. Total URA and APS distributions were similar to those of POC, with the highest values in the nearshore stations, and decreasing to the low values at the WCR station 7. Small particles were the major size fraction for URA and APS, comprising, on average, 87% of total particulate URA and 92% of APS. In 2001, APS presented mainly in small particles (56–97%, with an average of 88% of APS), but URA in small particles accounted for only 50% of total particulate URA,

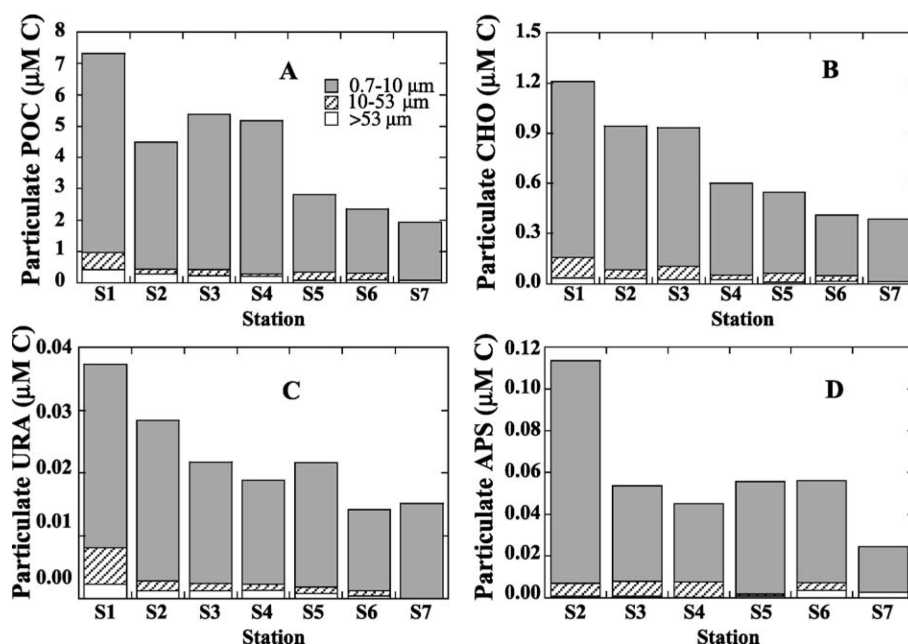

Fig. 4. The size fractionation (0.7–10, 10–53, and >53 μm) of (A) POC, (B) CHO, (C) URA and (D) total APS concentrations in the surface water at stations 1–7 in 2000. APS concentration is expressed as alginic acid equivalents (μM C).

ranging from 18% to 75%. Both URA and total APS, produced in situ by different phytoplankton or bacterial species, were most abundant in the smallest size fraction, especially in open Gulf waters (in both CCR and WCR). Furthermore, both URA and total APS concentrations in the CCR were significantly higher

Table 3

Particulate organic carbon (POC), carbohydrates (p-TCHO), acid polysaccharides (APS) and uronic acid (URA), in different size fractions in 2000

Station no.	Depth (m)	POC 0.7–10 μm	POC 10–53 μm	POC >53 μm	p-TCHO 0.7–10 μm	p-TCHO 10–53 μm	p-TCHO >53 μm	APS 0.7–10 μm	APS 10–53 μm	APS >53 μm	URA 0.7–10 μm	URA 10–53 μm	URA >53 μm
1	2	6.35	0.55	0.42	1.05	0.123	0.035	n.d.	n.d.	n.d.	0.0292	0.0058	0.0023
2	2	4.05	0.17	0.28	0.86	0.052	0.031	0.1065	0.0061	0.00087	0.0256	0.0016	0.0012
3	2	4.96	0.19	0.23	0.83	0.077	0.026	0.0456	0.0074	0.00084	0.0193	0.0012	0.0012
4	2	4.90	0.08	0.21	0.55	0.027	0.024	0.0374	0.0072	0.00046	0.0165	0.0010	0.0013
5	2	2.47	0.26	0.08	0.48	0.053	0.011	0.0535	0.0011	0.00090	0.0198	0.0010	0.0008
	10	1.98	0.36	0.12	0.34	0.050	0.012	0.0711	0.0011	0.00050	0.0068	0.0018	0.0003
	30	2.11	0.62	0.11	0.40	0.064	0.017	0.0765	0.0054	0.00052	0.0128	0.0029	0.0003
	65	2.04	n.d.	0.08	0.35	n.d.	0.017	0.0446	n.d.	0.00036	0.0148	n.d.	0.0003
	73	2.41	0.49	0.14	0.69	0.069	0.022	0.0627	0.0029	0.00005	0.0144	0.0019	0.0006
6	2	2.03	0.22	0.10	0.36	0.033	0.016	0.0488	0.0036	0.00372	0.0129	0.0008	0.0004
7	2	1.85	n.d.	0.083	0.37	n.d.	0.012	0.0217	n.d.	0.00273	0.0149	n.d.	0.0002
	10	1.27	n.d.	0.050	0.41	n.d.	0.013	0.0265	n.d.	0.00218	0.0103	n.d.	0.0002
	35	1.30	n.d.	0.066	0.33	n.d.	0.012	0.0441	n.d.	0.00120	0.0122	n.d.	0.0003
	45	1.47	n.d.	0.075	0.39	n.d.	0.008	0.0475	n.d.	0.00023	0.0097	n.d.	0.0003
	75	2.28	n.d.	0.058	0.40	n.d.	0.006	0.0447	n.d.	0.00065	0.0055	n.d.	0.0002

POC, p-TCHO and URA are expressed in μM C.

n.d.: not detectable.

APS was collected by GF/F filter (0.7 μm) and its concentration is given in alginic acid equivalents (μM C).

Table 4
Particulate organic carbon (POC), carbohydrates (p-TCHO), acid polysaccharides (APS) and uronic acid (URA), in different size fractions in 2001

Station no.	Depth (m)	POC 0.7–10 μm	POC 10–53 μm	POC >53 μm	p-TCHO 0.7–10 μm	p-TCHO 10–53 μm	p-TCHO >53 μm	APS 0.4–10 μm	APS 10–53 μm	APS >53 μm	URA 0.7–10 μm	URA 10–53 μm	URA >53 μm
1	2	10.91	1.70	4.37	1.12	0.122	0.257	0.148	0.0155	0.0012	0.082	0.027	0.020
2	2	8.05	0.74	0.74	0.84	0.067	0.049	0.211	0.0047	0.0011	0.040	0.013	0.004
3	2	3.57	0.38	0.36	0.52	0.029	0.040	0.135	0.0028	0.0008	0.019	0.006	0.006
4	2	3.53	0.46	0.36	0.52	0.038	0.021	0.091	0.0022	0.0003	0.018	0.009	0.006
	25	2.02	0.24	0.11	0.31	0.015	0.010	0.089	0.0024	0.0007	0.006	0.004	0.001
	50	2.37	0.44	0.14	0.28	0.036	0.014	0.050	0.0028	0.0005	0.008	0.008	0.002
	65	3.23	1.02	0.24	0.33	0.130	0.022	0.086	0.0092	0.0014	0.048	0.005	0.003
	80	5.03	5.48	1.13	0.33	0.385	0.079	0.089	0.0624	0.0081	0.022	0.083	0.016
	100	1.75	0.62	0.26	0.24	0.053	0.027	0.066	0.0071	0.0006	0.015	0.014	0.005
	120	2.08	0.53	0.53	0.04	0.023	0.055	0.032	0.0038	0.0121	0.008	0.003	0.005
5	2	15.13	2.72	1.77	1.07	0.404	0.151	0.116	0.0372	0.0271	0.100	0.019	0.031
6	2	3.79	0.19	0.30	0.30	0.014	0.031	0.094	0.0018	0.0011	0.015	0.002	0.004
	25	2.88	0.19	0.25	0.22	0.013	0.019	0.098	0.0013	0.0023	0.015	0.002	0.003
	50	2.79	0.17	0.15	0.22	0.012	0.012	0.114	0.0024	0.0014	0.013	0.003	0.003
	72	2.76	1.07	0.66	0.34	0.103	0.047	0.082	0.0051	0.0040	0.009	0.014	0.009
	100	2.07	0.19	0.10	0.18	0.005	0.010	0.053	0.0031	0.0002	0.007	0.002	0.003

POC, p-TCHO and URA are expressed in μM C.
n.d.: not detectable.
APS was collected by polycarbonate filter (0.4 μm) and its concentration is given in alginic acid equivalents (μM C).

than those in the WCR in both 2000 and 2001, likely reflecting the difference in microorganism biomass, as judged by the chl *a* concentrations.

4. Discussion

4.1. Dissolved carbohydrate species

Phytoplankton, prokaryotes, and macroalgae can excrete specific exocellular polysaccharides, such as acid polysaccharides during their growth (Decho and Herndl, 1995; Hoagland et al., 1993; Myklestad, 1995; Biddanda and Benner, 1997). Thus, the depth distribution of dissolved carbohydrate compounds should reflect their relative sources. The production of d-TCHO in the surface layer (0–100 m) was likely from phytoplankton, which showed maximum concentrations in the euphotic zone (Fig. 1), while the production of d-TCHO in the secondary mid-water maximum might have been from bacterial degradation of sinking particles. An interesting feature is that d-TCHO concentrations exhibited a deep water maximum at about 500 m at the WCR station 7, located in the oxygen minimum layer, which likely was caused by bacterial decomposition processes. Ogawa et al. (2001) reported that the production of some refractory carbohydrates may be the result of excretion by specific bacterial strains. Based on Biersmith and Benner (1998), dissolved heteropolysaccharides in the surface ocean may be mainly produced by releases of extracellular material by phytoplankton. d-PCHO in the euphotic zone of productive waters (e.g., station 5) were much higher than that in waters with low productivity (e.g., WCR, station 7), which agrees with the laboratory observations by Benner and co-workers. In 2000, the lower value at station 7 may have been the result of nutrient- and phytoplankton-deficient waters, and the faster degradation rates of bioactive dissolved carbohydrates.

4.2. Composition of particulate carbohydrate species

The percentage of total p-TCHO (sum of three fractions, 0.7–10, 10–53, >53 μm) in the POC pool in 2000 ranged from about 12% to 32% with an average of 18% of POC (Fig. 5A). However, the average ratio of total p-TCHO/POC in 2001, was lower, i.e., only about 50% of that (e.g., 9% of POC) in 2000. It is not obvious what factors caused

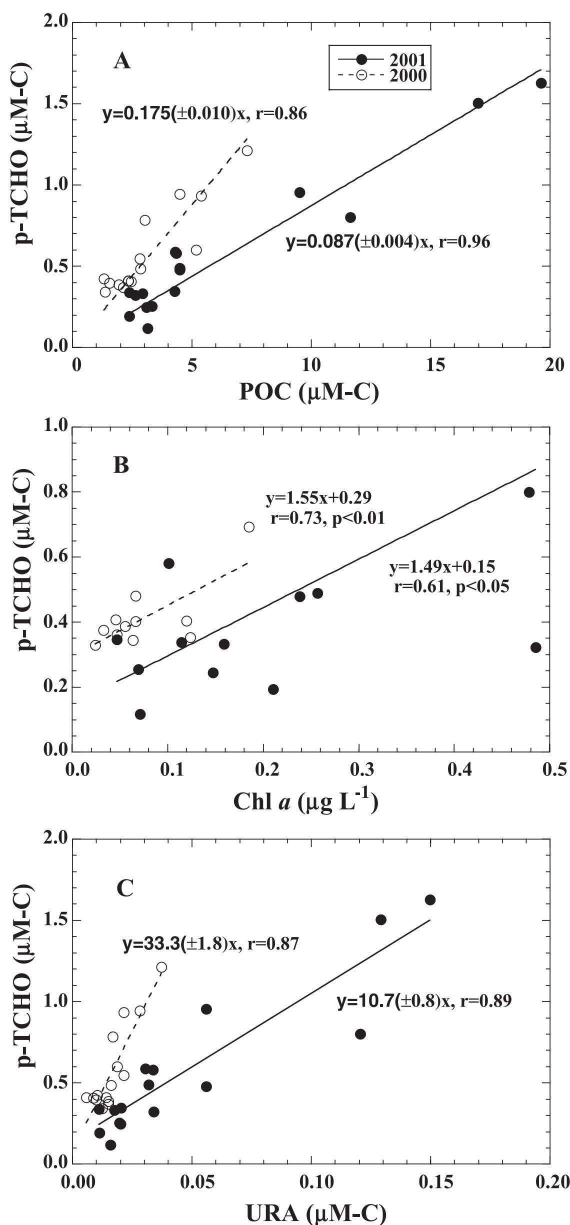

Fig. 5. (A) Relationship between p-TCHO and total POC in 2000 and 2001. (B) Relationship between p-TCHO and total Chl *a* concentrations in 2000 and 2001. (C) Relationship between total URA and total p-TCHO in 2000 and 2001.

the lower carbohydrate content in 2001. Morris (1981) suggested that the content of carbohydrates in phytoplankton is affected by different nutrient concentrations and that low nutrients cause higher carbohydrate production. During the 2000 expedition, nutrient concentrations in the surface layers of the euphotic zone were almost near the detection limit. Conversely, nutrient concentrations (e.g., nitrate + nitrite) in the surface waters in 2001 ranged from 0.7 to 0.9 μM, consistently higher than the respective values in 2000. Hecky et al. (1973) found that the p-TCHO content of cell walls for different diatom species showed large variations. Phytoplankton species composition also showed large differences between the 2 years, with Cyanobacteria controlling the APS distribution in upper water column in 2000, and Prymnesiophytes in 2001 (Hung et al., in press; Santschi et al., 2003), which could be the cause of additional variability in p-TCHO contents of particles. Furthermore, the ratio of total p-TCHO/POC might also be different for different phytoplankton growth stages. Biddanda and Benner (1997) reported, for example, that more p-TCHO were produced during the stationary stage than during the other stages. Thus, the differences in total p-TCHO/POC ratios between the 2 years are likely due to nutrient limitation and differences in phytoplankton species composition.

Although the phytoplankton assemblages were different between 2000 and 2001, a good positive correlation between p-TCHO and chl *a* was found for the open Gulf water stations (excluding the near shore stations) in both 2000 and 2001 expeditions (Fig. 5B). The slopes for both data sets (2000 and 2001) are indistinguishable, suggesting that the composition of p-TCHO of the natural phytoplankton assemblages were very similar. Parsons et al. (1961) reported that the ratio of p-TCHO/chl *a* in living phytoplankton is about 17, which is very similar to what we observed here (18 = average slope (1.5) × 12). The intercept in 2000 is not equal to zero (0.29 ± 0.04 μM C), suggesting a non-living carbohydrate contribution, but the intercept in 2001 is, within the error, equal to zero (0.14 ± 0.15 μM C). These results thus demonstrate the presence in 2000 of carbohydrates in dead particles, such as detritus, fecal pellets, marine snow. As mentioned above, nutrient concentrations in the euphotic zone were very low in 2000, suggesting that sampling occurred after a high productivity event, while nutrient concentrations in 2001 were higher, suggesting that sampling occurred prior to or during high primary production. Therefore, p-TCHO in detritus and non-living particles in 2000 was more abun-

dant than in 2001. In addition, the fact that the intercept in 2001 was close to zero implies that most of the p-TCHO are from living microorganisms.

Generally, total carbohydrates are composed of monosaccharides, polysaccharides, acid polysaccharides, amino sugars and other alcohol sugars. Total APS include all anionic polysaccharides, such as carboxylic, sulfated and phosphated polysaccharides. URA are acid polysaccharides with carboxylic groups, such as mannuronic acid, glucuronic acid and galacturonic acid. Although the distribution of p-TCHO has been relatively well studied, little is known about the relative composition of total APS and URA in different particle size fractions. The composition of p-TCHO, total APS, and URA in the 0.7–10, 10–53, and >53 μm batches are shown in Tables 3 and 4. The relative percentage of URA in p-TCHO in the 0.7–10, 10–53 and >53 μm size fractions in 2000 did not show marked variations, ranging from 1.4% to 4.5%, with an average value of 3.0%, 3.1%, and 3.2% for the three fractions. The relative concentrations of total APS in p-TCHO in 0.7–10 μm (5.5–20.7%, average 11.4%), 10–53 μm (2.2–27.3%, average 9.6%) and >53 μm (0.2–23.7%, average 8.1%) are considerably higher than URA in the p-TCHO. Similar compositions were also observed for total URA concentrations, showing that URA only accounted for 2.9% of p-TCHO and 8.6% of p-TCHO (Fig. 5C) in 2000 and 2001, respectively.

The URA determination method used here does not measure sulfated APS such as carrageenan or phosphorus containing APS, which suggests a need to develop an improved methodology to measure all functional groups of total APS. A phosphate analysis of the radiolabeled total APS fraction separated by isoelectric focusing/gel electrophoresis (Quigley et al., 2002)revealed that phosphate in the APS fraction of low pH (<4), extracted from organic matter during the 2000 expedition (Santschi et al., 2003), was present, strongly suggesting the presence of phosphorus containing lipopolysaccharides that may be abundant in the acid-polysaccharide-rich mucilage or cell envelope of Gram-negative cyanobacteria. The alcian blue method selected for total APS detection is measuring phosphated APS at similar rates as alginic acids (Table 2). Total URA and total APS (sum of three fractions, i.e., 0.7–10, 10–53, and >53 μm) normalized to POC, ranged from about 0.24% to 0.91% and from 0.9% to 3.3% of POC, respectively. Because data of URA and total APS in suspended particles are scarce, it is difficult to directly compare our data with previous data. Most recently, Hamanaka et al. (2002) determined surface active carbohydrates (extracted by methanol/water) in the euphotic zone and reported that the average surface active carbohydrates ranged from 0.6% to 2.3% of POC, which is very similar to the APS values from our investigation.

4.3. Comparison of CHO, URA and total APS in suspended and sinking particles

The average POC-normalized carbohydrate (p-TCHO) content in suspended particles in 2000, within the euphotic zone, was 19% of POC in the CCR and 24% of POC in the WCR, with an average of 22% of POC overall (Fig. 6A). p-TCHO in sinking particles ranged from 6.3% to 13.3% of POC, with an average value of 9.2% of POC. Similarly, the average POC-normalized p-TCHO content (9.4%) in suspended particles was higher than that in sinking particles (8.8% of POC) in 2001 (Fig. 6B). Hernes et al. (1996) surveyed carbohydrate content, using molecular-level analysis (e.g., GC/MS method) in sediment trap materials collected at 100 m water depth from the central equatorial Pacific, and reported that p-TCHO in POC ranged from 4.9% to 17.8% of POC. Bergamaschi et al. (1999), using the GC/MS method, reported total carbohydrate concentrations from Dabob Bay sediment trap materials, which varied from 6.1% to 23.6% of POC. Hamanaka et al. (2002) measured the export of carbohydrates from the euphotic zone and found that the total carbohydrate export by sediment traps at 30 m depth was 13.8% of that of POC. The percentage of p-TCHO in terms of POC in our samples is little higher than that measured by Hernes et al. (1996) and Bergamaschi et al. (1999). Since their p-TCHO values contain only neutral sugars, while our p-TCHO values contain all carbohydrates, it seems reasonable that our values are slightly higher. Regardless of the difference in methodology, our total p-TCHO values are close to those from the literature.

An interesting phenomenon is that the average p-TCHO content in suspended particles was higher than that in sinking particles in both 2000 and 2001. Two factors may have contributed to this difference in p-

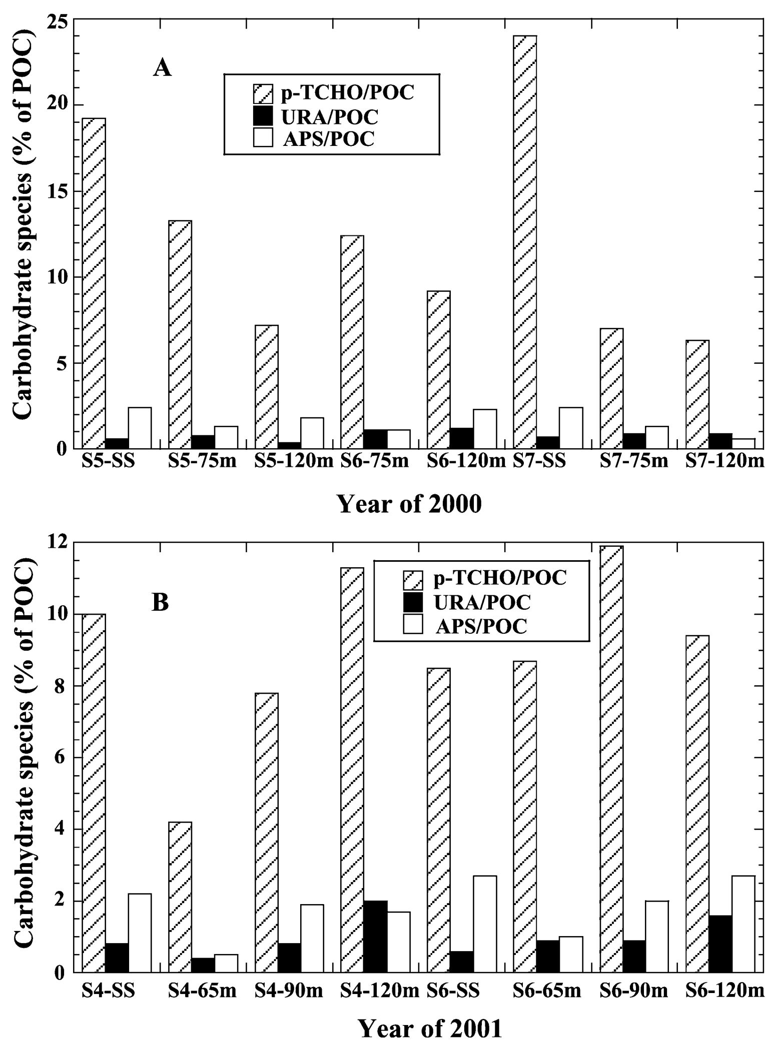

Fig. 6. (A) Comparison of p-TCHO, URA and APS, normalized to POC in suspended and sinking particles in 2000. SS in S5-SS and S7-SS represents the average carbohydrate species in suspended particles and numerical after stations such as S5-75 m represent the depth of sinking particles where collected. (B) Comparison of p-TCHO, URA and APS, normalized to POC in suspended and sinking particles in 2001. APS concentration is expressed as alginic acid equivalents (μM-C). Symbols are the same as those in panel (A).

TCHO content: (1) Since suspended particles consist of detritus and living microorganisms, suspended particles with a considerable amount of living phytoplankton should contain more carbohydrates than degraded particles. Since, as has been mentioned before, the calculated ratio of p-TCHO/chl *a* in our particulate samples from both years was similar to that of living plankton, living phytoplankton could have been a significant fraction in our samples during that time. When this ratio of p-TCHO/chl *a* was used to calculate p-TCHO concentrations at 75 m from both stations 5 and 7 in 2000, the estimated p-TCHO concentration originating from living phytoplankton should be 0.26 (about 33% of p-TCHO) and 0.17 μM C (41% of p-TCHO) at station 5 and 7, respectively. This comparison suggests that living phytoplankton in suspended particles could make up a significant fraction in our samples. (2) Sinking particles are mainly composed of fresh fecal pellets, dead plankton cells and marine snow. It is well recognized that the

fecal pellets and dead plankton cells are fresher, i.e., less degraded, than marine snow. However, it is difficult to distinguish the relative percentage of fecal pellets, dead cells and marine snow in a sinking particle sample. In any case, these sinking particles are further along in the degradation or decomposition process than suspended particles.

Values of URA, normalized to POC, in sinking particles in 2000 and 2001 were 0.88% and 1.1% of POC, accounting for only a small fraction of POC (Fig. 6A and B). Average URA concentrations in suspended particles within the euphotic zone were 0.64% and 0.7% of those of POC in 2000 and 2001. Bergamaschi et al. (1999) reported uronic acid data from Dabob Bay sediment trap materials, which ranged from 0.3% to 1.2% of POC. Hamanaka et al. (2002), while investigating the export of surface-active carbohydrates by sediment traps at 30 m depth, stated that uronic acids were present at about 0.7% of POC. Despite the differences in sampling location and depth, our values of URA, normalized to POC, are very comparable to previously reported data. Unlike p-TCHO, average URA concentrations in sinking particles were higher than those in suspended particles in both 2000 and 2001. Cowen (1992) reported that the abundance of attached bacteria in sinking particles is significantly greater than in suspended particles. Kenne and Lindberg (1983) and Decho (1990) reported that certain uronic acids, mainly mannuronic acid and glucuronic acid, are principle components of bacterial exopolymers. The higher amounts of URA that are present in settling particles compared to suspended particles could thus reflect a mass flow in the direction of sinking particles, either caused by bacterial reprocessing of particulate and colloidal organic matter, or by coagulation. Most importantly, the relationship between POC normalized polysaccharide fractions (p-TCHO, URA and APS) and ^{234}Th/POC were significant in both large suspended and sinking particles, suggesting that they play an important role in Th scavenging (Santschi et al., 2003).

The relative differences of APS in suspended vs. sinking matter are similar to those of total carbohydrates, i.e., higher in suspended particles and lower in sinking particles (Fig. 6). Because small particles should have a higher surface area, compared to large particles, APS, as the surface-active carbohydrate fraction, should thus also be higher, provided the POC content in both small particles and large particles (e.g., sinking particles) is similar. Besides excretion of APS by microorganisms under normal growth conditions (Passow, 2002b), APS are also secreted by phytoplankton and bacteria in response to high metal concentrations (Leppard, 1997) or low nutrient stress conditions (Strycek et al., 1992). The reasons for the difference of APS in suspended and sinking particles could also be related to the relative importance of production and degradation rates. The fraction of APS in suspended particles was higher than APS in sinking particles. This could be caused by the relative differences in encymatic degradation rates of attached bacteria in suspended and sinking particles. There is no simple explanation to our observations regarding the difference of APS between suspended and sinking particles, however, as neither bacterial biomass nor individual uronic acids such as galacturonic, glucuronic and mannuronic acids, were measured in sinking particles. In order to resolve these questions, it might be necessary to simultaneously measure bacterial biomass and temporal variation of individual APS compounds collected from both suspended and sinking particles from field experiments in the future.

5. Conclusions

From our investigation of carbohydrate speciation and size distribution in the Gulf of Mexico in 2000 and 2001, we arrive at the following conclusions:

(1) Although we developed a way to estimate the carbon content of acidic polysaccharides in CHO and/or TEP, further improvements to measure individual APS compunds and other polysaccharides, such as lipopolysaccharides or carregeenans, are still needed.
(2) Small particles (0.7–10 μm) are the most important fraction of POC, p-TCHO, URA and APS, making up, on average, 81% of POC, 83% of p-CHO, 71% of URA and 90% of APS.
(3) URA (3–9% of p-CHO) and APS (10–15% of p-TCHO) only account for a minor fraction of p-TCHO, suggesting that the APS content is significantly lower than previously reported and that a large fraction of APS is not characterized.

(4) Greater amounts of URA are found in sinking particles relative to suspended particles, likely due to coagulation; however, the opposite was true for APS, likely due to relative differences in production and enzymatic degradation rates.

A better understanding of the relative importance of URA and other APS compounds in the carbohydrate pool of suspended and sinking particles will require knowledge of the sources and degradabilities of individual URA and APS compounds.

Acknowledgements

We would like to thank Kent Warnken, Kathy A. Schwehr, Chris Knoll, Jane Vidas, Ian Walsh, and crew members of the R/V Gyre for their assistance in sample collection, Amber Bevard and Kim Roberts for their help with sample processing in the laboratory, Associate Editor, Dr. Jim Bauer, and three anonymous reviewers for valuable comments on the manuscript. This work was funded, in parts, by NSF (OCE-9906823), and the Texas Institute of Oceanography.

Associate editor: Dr. James Bauer.

References

Alldredge, A.L., Silver, M.W., 1988. Characteristics, dynamics and significance of marine snow. Prog. Oceanogr. 20, 41–82.

Alldredge, A.L., Passow, U., Logan, B.E., 1993. The abundance and significance of a class of large, transparent organic particles in the ocean. Deep-Sea Res. I 40, 1131–1140.

Baldi, F., Minacci, A., Saliot, A., Mejanelle, L., Mozetic, P., Turk, V., Malej, A., 1997. Cell lysis and release of particulate polysaccharides in extensive marine mucilage assessed by lipid biomarkers and molecular probes. Mar. Ecol. Prog. Ser. 153, 45–57.

Baskaran, M., Santschi, P.H., Guo, L., Bianchi, T.S., Lambert, C., 1996. ^{234}Th:^{238}U disequilibria in the Gulf of Mexico: the importance of organic matter and particle concentration. Cont. Shelf Res. 16, 353–380.

Bergamaschi, B.A., Walters, J.S., Hedges, J., 1999. Distributions of uronic acids and *O*-methyl sugars in sinking and sedimentary particles in two coastal marine environments. Geochim. Cosmochim. Acta 63, 413–425.

Biddanda, B., Benner, R., 1997. Carbon, nitrogen, and carbohydrate fluxes during the production of particulate and dissolved organic matter by marine phytoplankton. Limnol. Oceanogr. 42, 506–518.

Biersmith, A., Benner, R., 1998. Carbohydrates in phytoplankton and freshly produced dissolved organic matter. Mar. Chem. 63, 131–144.

Borch, N.H., Kirchman, D.L., 1997. Concentration and composition of dissolved combined neutral sugars (polysaccharides) in seawater determined by HPLC-PAD. Mar. Chem. 57, 85–95.

Buffle, J., Leppard, G.G., 1995. Characterization of aquatic colloids and macromolecules: 2. Key role of physical structures on analytical results. Environ. Sci. Technol. 29, 2169–2175.

Burdige, D.J., Skoog, A., Gardner, K., 2000. Dissolved and particulate carbohydrates in contrasting marine sediments. Geochim. Cosmochim. Acta 64, 1029–1041.

Coale, K.H., Bruland, K.W., 1987. Oceanic stratified euphotic zone as elucidated by ^{234}Th:^{238}U disequilibria. Limnol. Oceanogr. 32, 189–200.

Costerton, J.W., 1984. . In: Klug, M.J., Reddy, C.A. (Eds.), Current Perspectives in Microbial Ecology. American Society of Microbiology, Washington, DC, pp. 115–123.

Cowen, J.P., 1992. Morphological study of marine bacterial capsules: implications for marine aggregates. Mar. Biol. 114, 85–95.

Decho, A.W., 1990. Microbial exopolymer secretions in the ocean environments their role(s) in food webs and marine processes. Oceanogr. Mar. Biol. Annu. Rev. 28, 73–153.

Decho, A.W., Herndl, G.J., 1995. Microbial activities and the transformation of organic matter within mucilaginous material. Sci. Total Environ. 165, 33–42.

Doco, T., O'Neill, M.A., Pellerin, P., 2001. Determination of the neutral and acidic glycosyl-residue compositions of plant polysaccharides by GC–EI–MS analysis of the trimethylsilyl methyl glycoside derivatives. Carbohydr. Polym. 46, 249–259.

Fazio, S.A., Uhlinger, D.J., Parker, J.H., White, D.C., 1982. Estimations of uronic acids as quantitative measures of extracellular and cell wall polysaccharide polymers from environmental samples. Appl. Environ. Microbiol. 43, 1151–1159.

Filisetti-Cozzi, T.M.C.C., Carpita, N.C., 1991. Measurement of uronic acids without interference from neutral sugars. Anal. Biochem. 197, 157–162.

Geesey, G.G., Richardson, W.T., Yeomans, H.G., Irvin, R.T., Costerton, J.W., 1977. Microscopic examination of natural sessile bacterial populations from an alpine stream. Can J. Microbiol. 23, 1733–1736.

Grasshoff, K., Ehrhardt, M., Kremling, K., 1983. Methods of Seawater Analysis. Verlag Chemie, Weinheim. D-6940.

Guo, L., Coleman, C.H., Santschi, P.H., 1994. The distribution of colloidal and dissolved organic carbon in the Gulf of Mexico. Mar. Chem. 45, 105–119.

Guo, L., Hung, C.-C., Santschi, P.H., Walsh, I.D., 2002. ^{234}Th scavenging and its relationship to acid polysaccharide abundance in the Gulf of Mexico. Mar. Chem. 78 (2–3), 103–119.

Hamanaka, J., Tanoue, E., Hama, T., Handa, N., 2002. Production and export of particulate fatty acids, carbohydrates and combined amino acids in the euphotic zone. Mar. Chem. 77, 55–69.

Hecky, R.E., Mooper, K., Kilham, P., Degens, E.T., 1973. The amine acid and sugar composition of diatom cell walls. Mar. Biol. 19, 323–331.

Hernes, P.J., Hedges, J.I., Peterson, M.L., Wakeham, S.G., Lee, C.,

1996. Neutral carbohydrate geochemistry of particulate material in the central equatorial Pacific. Deep-Sea Res. 43, 1181–1204.
Hoagland, K.D., Rosowski, J.R., Retz, M.R., Roemer, S.C., 1993. Diatom extracellular polymeric substances: function, fine structure, chemistry and physiology. J. Phycol. 29, 537–566.
Hung, C.-C., Santschi, P.H., 2001. Spectrophotometric determination of total uronic acids in seawater using cation exchange separation and pre-concentration lyophilization. Anal. Chim. Acta 427, 111–117.
Hung, C.-C., Tang, D., Warnken, K.W., Santschi, P.H., 2001. Distributions of carbohydrates, including uronic acids, in estuarine waters of Galveston Bay. Mar. Chem. 73, 305–318.
Hung, C.-C., Guo, L., Schultz, G., Pinckney, J.L., Santschi, P.H., 2003. Production and flux of carbohydrate species in the Gulf of Mexico. Global Biogeochem. Cycles (in press).
Jang, L.K., Harpt, N., Grasmick, D., Vuong, L.N., Geesey, G.G., 1990. A two-phase model for determining the stability constants for interactions between copper and alginic acid. J. Phys. Chem. 94, 482–488.
Jang, L.K., Nguyen, D., Geesey, G.G., 1995. Selectivity of alginate gel for Cu vs Co. Water Res. 29, 307–313.
Kenne, L., Lindberg, B., 1983. Bacterial polysaccharides. In: Aspinall, G.O. (Ed.), The Polysaccharides, vol. 2. Academic Press, New York, pp. 287–363.
Kennedy, A.F.D., Sutherland, I.W., 1987. Analysis of bacterial exopolysaccharides. Biotechnol. Appl. Biochem. 9, 12–19.
Leppard, G.G., 1993. In: Rao, S.S. (Ed.), Particulate Matter and Aquatic Contaminants. Lewis, Chelsea, MI, pp. 169–195.
Leppard, G.G., 1997. Colloidal organic fibrils of acid polysaccharides in surface waters: electron-optical characteristics, activities and chemical estimates of abundance. Colloids Surf., A Physicochem. Eng. Asp. 120, 1–15.
Mopper, K., Zhou, J., Ramana, K.S., Passow, U., Dam, H.G., Drapeau, D.T., 1995. The role of surface-active carbohydrates in the flocculation of a diatom bloom in a mesocosm. Deep-Sea Res. 42, 47–73.
Morris, I., 1981. Photosynthetic products, physiological state and phytoplankton growth. Can. Bull. Fish. Aquat. Sci. 201, 83–102.
Muldoon, J., Shashkov, A.S., Senchenkova, S.N., Tomshich, S.V., Komandrova, N.A., Romanenko, L.A., Knirel, Y.A., Savage, A.V., 2001. Structure of an acidic polysaccharide from a marine bacterium *Pseudoalteromonas distincta* KMM 638 containing 5-acetamido-3,5,7,9-tetradeoxy-7-formamido-L-*glycero*-L-*manno*-nonulosonic acid. Carbohydr. Res. 330, 231–239.
Myklestad, S.M., 1995. Release of extracellular products by phytoplankton with special emphasis on polysaccharides. Sci. Total Environ. 165, 155–164.
Myklestad, S.M., Skanoy, E., Hestmann, S., 1997. A sensitive and rapid method for analysis of dissolved mono- and polysaccharides in seawater. Mar. Chem. 56, 279–286.
Ogawa, H., Amagai, Y., Koike, I., Kaiser, K., Benner, R., 2001. Production of refractory dissolved organic matter by bacteria. Science 292, 917–920.
Pakulski, J.D., Benner, R., 1992. An improved method for the hydrolysis and MBTH analysis of dissolved and particulate carbohydrates in seawater. Mar. Chem. 40, 143–160.
Pakulski, J.D., Benner, R., 1994. Abundance and distribution of carbohydrates in the ocean. Limnol. Oceanogr. 39, 930–940.
Parsons, T.R., Stephens, K., Strickland, J.D.H., 1961. On the chemical composition of eleven species of marine phytoplankton. J. Fish. Board Can. 18, 1001–1016.
Passow, U., 2002a. Transparent exopolymer particles (TEP) in aquatic environments. Prog. Oceanogr. 55, 287–333.
Passow, U., 2002b. Production of transparent exopolymer particles (TEP) by phyto- and bacterioplankton. Mar. Ecol. Prog. Ser. 236, 1–12.
Passow, U., Alldredge, A.L., 1995. A dye-binding assay for the spectrophotometric measurement of transparent exopolymer particles (TEP). Limnol. Oceanogr. 40, 1326–1335.
Passow, U., Logan, B.E., Alldredge, A.L., 1994. The role of particulate carbohydrate exudates in the flocculation of diatom blooms. Deep-Sea Res. I 41, 335–357.
Pettine, M., Patrolecco, L., Manganelli, M., Capri, S., Farrace, M.G., 1999. Seasonal variations of dissolved organic matter in the northern Adriatic Sea. Mar. Chem. 64, 153–169.
Pinckney, J.L., Richardson, T.L., Millie, D.F., Paerl, H.W., 2001. Application of photopigment biomarkers for quantifying microalgal community composition and in situ growth rates. Org. Geochem. 32, 585–595.
Quigley, M.S., Santschi, P.H., Hung, C.C., Guo, L., Honeyman, B.D., 2002. Importance of acid polysaccharides for ^{234}Th complexation to marine organic matter. Limnol. Oceanogr. 47, 367–377.
Rehm, B.H.A., Valla, S., 1997. Bacterial alginates: biosynthesis and applications. Appl. Microbiol. Biotechnol. 48, 281–288.
Romankevich, E.A., 1984. Geochemistry of Organic Mater in the Ocean. Springer, New York, pp. 32–34.
Santschi, P.H., Balnois, E., Wilkinson, K., Zhang, J., Buffle, J., Guo, L., 1998. Fibrillar polysaccharides in marine macromolecular organic matter, as imaged by atomic force microscopy and transmission electron microscopy. Limnol. Oceanogr. 43, 896–908.
Santschi, P.H., Guo, L., Quigley, M.S., Baskaran, M., Walsh, I., 1999. Boundary exchange and scavenging of radionuclides in continental margin waters of the Middle Atlantic Bight: implications for organic carbon fluxes. Cont. Shelf Res. 19, 609–636.
Santschi, P.H., Hung, C.-C., Schultz, G., Alvarado-Quiroz, N., Guo, L., Pinckney, J., Walsh, I., 2003. Control of acid polysaccharide production and ^{234}Th and POC export fluxes by marine organisms. Geophys. Res. Lett. 30 (2), 1044 (doi:10.1029/2003GL016046).
Skoog, A., Benner, R., 1998. Aldoses in various size fractions of marine organic matter: implications for carbon cycling. Limnol. Oceanogr. 42, 992–996.
Strycek, T., Acreman, J., Kerry, A., Leppard, G.G., Nermut, M.V., Kushner, D.J., 1992. Extracellular fibril production by freshwater algae and cyanobacteria. Microb. Ecol. 23, 53–74.
Wilkinson, K.J., Joz-Roland, A., Buffle, J., 1997. Different roles of pedogenic fulvic acids and aquagenic biopolymers on colloid aggregation and stability in freshwaters. Limnol. Oceanogr. 42, 1714–1724.