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Biological hot spots and the accumulation of marine dissolved organic matter in a highly productive ocean margin

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Abstract

Concentrations of dissolved organic carbon (DOC) and major biochemicals (amino acids and carbohydrates) were measured during five cruises (2009–2010) to the Louisiana margin in the northern Gulf of Mexico. Concentrations of amino acids and carbohydrates were elevated at mid-salinities and were indicative of plankton production of dissolved organic matter (DOM) in surface waters. Hot spots of two compositionally distinct types of labile DOM were identified based on the relative abundances of amino acids and carbohydrates. Amino acid-rich hot spots occurred sporadically in regions of high phytoplankton biomass and were mostly observed between dusk and dawn, reflecting a grazing source. In contrast, carbohydrate-rich hot spots were more widespread and were often found in nutrient-poor waters, indicating the production of carbon-rich DOM associated with nutrient limitation. Major biochemical indicators and bioassay experiments indicated labile DOM comprised a relatively small fraction of the DOC. Most DOM was degraded and had a semi-labile nature. Substantial accumulations of marine (plankton-derived) DOC were observed in surface waters, particularly at mid-salinities during the summer. Microbial alteration of marine DOC and nutrient limitation of microbial utilization of carbon-rich DOM appeared largely responsible for the accumulation of DOC. The reservoir of accumulated marine DOC in the shelf surface mixed layer ranged from 0.11 Tg C to 0.23 Tg C, with the lowest and highest values occurring during winter and summer. Substantial cross-shelf export of semi-labile marine DOM occurred during the summer and provided a major carbon and energy subsidy to microbial food webs in offshore waters.

Ocean margins account for $< 10\%$ of global ocean surface area but play a disproportionally large role in biological productivity, respiration, and carbon burial (Gattuso et al. 1998). Autotrophic and heterotrophic processes interact in a dynamic manner and are superimposed on strong physical forcing, promoting rapid and diverse biogeochemical processes. This topic has attracted considerable attention in the past two decades (Smith and Hollibaugh 1993; Bauer et al. 2013). The Louisiana margin in the northern Gulf of Mexico is a very dynamic system, with large inputs of nutrients and organic matter from the Mississippi–Atchafalaya River system that render this region among the world's most productive ocean margins (> 300 gC m^{-2} yr^{-1}) (Goolsby et al. 2001; Heileman and Rabalais 2008; Shen et al. 2012b). Heterotrophic bacteria respire a large amount of organic matter and exert a pronounced influence on air–sea CO_2 exchange and the development of hypoxic conditions in stratified bottom waters (Amon and Benner 1998; Rabalais et al. 2002; Green et al. 2006).

Studies of plankton activity on the Louisiana margin reveal spatial linkages among primary production, bacterial production, and remineralization processes in surface waters (Chin-Leo and Benner 1992; Gardner et al. 1994; Murrell et al. 2013). This is largely attributed to plankton production of labile dissolved organic matter (DOM) that fuels bacterial growth and activity (Amon and Benner 1998; Benner and Opsahl 2001). The rapid response of microorganisms to patches of labile DOM results in specific locations and time periods of enhanced biogeochemical processes, thereby

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creating hot spots and hot moments, respectively (McClain et al. 2003; Azam and Malfatti 2007; Stocker et al. 2008). The occurrence of hot spots and hot moments reveals the spatial and temporal heterogeneity of the system and is closely linked to the production of labile DOM.

Despite the well-documented high primary productivity, spatial and temporal distributions of labile DOM on the Louisiana margin have not been well characterized. Bioassay experiments with filtered water samples and associated microorganisms can be used to estimate the labile fraction of DOM (Søndergaard and Middelboe 1995). These authors defined labile DOM as the amount of dissolved organic carbon (DOC) decomposed within 1–2 weeks in bioassay experiments, and in a review of the literature they found that $19 \pm 12\%$ of the DOC in a variety of marine environments was labile. A study using shorter-term (2–5 d) bioassay experiments found similar variability ($11 \pm 7\%$) in the labile fraction of DOC in surface waters of the Louisiana margin (Gardner et al. 1994). Measurements of the biochemical components of DOM that can be rapidly utilized, such as amino acids and carbohydrates, can also be used to estimate the labile fraction of DOC (Benner 2003; Davis and Benner 2007). Benner and Opsahl (2001) used neutral sugars as biochemical indicators of plankton-derived DOM and found elevated production and consumption of carbohydrate-rich DOM at mid-salinity locations in plume waters.

Plankton production of labile DOM is strongly influenced by the highly variable nutrient and light conditions across the margin (Smith and Hitchcock 1994; Lohrenz et al. 1999; Turner and Rabalais 2013). Under nutrient limitation, phytoplankton growth is inhibited while photosynthesis still proceeds, resulting in preferential production of carbohydrates over proteins (Myklestad and Haug 1972; Jiang et al. 2012). Labile DOM is released into the environments through various processes, including extracellular release from phytoplankton and grazing-mediated release (Carlson and Hansell 2015). The former is active at high irradiance (Cherrier et al. 2015), whereas the latter can be active at night when zooplankton migrate to the surface to feed (Dagg 1995; Dagg et al. 1998). As a result, labile DOM produced under varying nutrient and light regimes can be compositionally different and have varying effects on bacterial activity and carbon cycling (Azam and Malfatti 2007; Buchan et al. 2014). The chemical composition of labile DOM can provide valuable insights about the interplay between primary and secondary production, but remains poorly characterized on the Louisiana margin.

Furthermore, although previous studies have measured high rates of primary production and community respiration, the net balance of these processes remains uncertain. Seasonal accumulation of DOC in surface waters has been reported in a wide range of aquatic environments and attributed to relatively low bioavailability of DOM and mineral nutrient limitation (Copin-Montégut and Avril 1993; Carlson et al. 1994; Williams 1995; Zweifel et al. 1995; Thingstad et al. 1997; Søndergaard et al. 2000). Bioavailable DOC that persists for periods of months to years is characterized as semi-labile DOM (Carlson and Hansell 2015), and it is likely that plankton-derived DOM on the Louisiana shelf includes semi-labile components that accumulate in surface waters. The highly variable nutrient conditions in the region can also promote the accumulation of semi-labile DOM. The estimation of marine DOC accumulation in river-influenced systems is challenging due to the confounding presence of terrigenous DOC (tDOC). Concentrations of tDOC were measured during this project (Fichot and Benner 2012, 2014), which facilitated the calculation of marine DOC concentrations in this ocean margin.

In this study, we identify hot spots of labile DOM on the Louisiana margin over an annual cycle using biochemical indicators (amino acids and carbohydrates) and examine how their spatial and temporal distributions reflect patterns of plankton production and nutrient limitation. The accumulation of marine DOC is estimated for each season, and the mechanisms controlling DOC accumulation are inferred from biochemical indicators and contemporaneous measurements of nutrients (Chakraborty and Lohrenz 2015). Furthermore, the mixed layer reservoirs of total and accumulated marine DOC on the Louisiana shelf are estimated and the implications are discussed.

Materials and Methods

Sample collection

Surface water samples were collected during five cruises to the Louisiana margin in the northern Gulf of Mexico on the R/V *Cape Hatteras* and the R/V *Hugh Sharp* in January, April, July, October–November 2009, and March 2010 as part of the GulfCarbon project. The study region is strongly influenced by the Mississippi and Atchafalaya River system (Fig. 1), which discharged 16,000–32,000 $m^3\ s^{-1}$ of freshwater into the margin during these sampling months (low in summer and high in spring; Shen et al. 2012b). About 50 stations were surveyed during each cruise (Fig. 1), except in January when 24 stations were sampled. A total of 222 surface water samples were collected from upper 3 m of water column across a salinity range of 0–37 with 10-L Niskin bottles mounted on a rosette with a conductivity–temperature–depth instrument. About 60–80% of the samples were collected during daylight hours. Immediately following collection, samples were gravity filtered through precleaned (450°C, 4 h) glass fiber (GF/F) filters (0.7-μm pore-size) and stored frozen (−20°C) in precleaned (450°C, 4 h) clear glass vials until analyses of DOC, amino acids, and neutral sugars were conducted in the home laboratory. Samples for measurements of neutral sugars were not collected during the April cruise. This study reports results within the salinity range of 20–37 ($n = 199$), which covers the vast

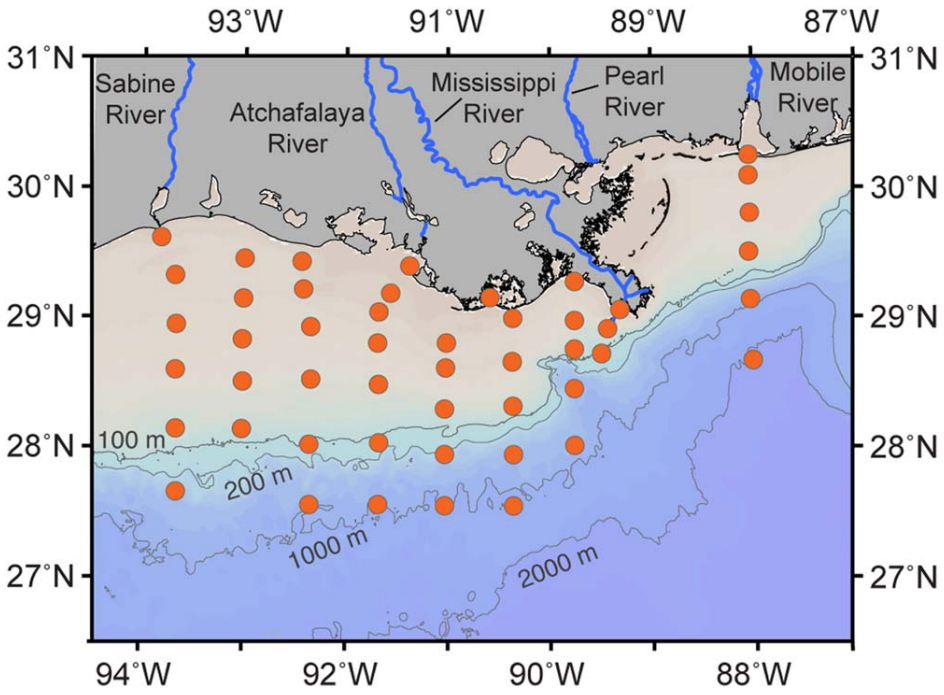

Fig. 1. Study area and sampling sites on the Louisiana margin in the northern Gulf of Mexico. Surface water samples were collected from 24 stations in January 2009 and from ~50 stations in April, July, October–November 2009, and March 2010. The sampling regions included the continental shelf (bottom depth ≤ 200 m) and slope (200 m < bottom depth ≤ 2000 m). The 100-, 200-, 1000-, and 2000-m isobaths are shown as thin grey lines.

majority of the ocean margin and excludes low salinity plumes dominated by river water.

Bioassay experiments

Shipboard bioassay experiments were conducted during the March 2010 cruise and used to determine the labile fraction of DOM, validate the use of amino acids and neutral sugars as biochemical indicators of labile DOM, and to examine the microbial utilization of amended labile substrates. Surface waters (salinity 23.3 and 27.8) from two locations were gravity filtered through precleaned GF/F filters. Filtered waters were divided into control treatments and treatments with additions (23–24 μmol DOC L^{-1}) of labile, plankton-derived DOM (Fichot and Benner 2012). The plankton-derived DOM was collected previously from a coastal diatom bloom, filtered (GF/F) and stored frozen (−20°C). The plankton-derived DOM was enriched in free and combined forms of amino acids and neutral sugars (~ 40% of the DOC), and it was added to the water samples at a ~ 1:500 dilution (v/v). The addition of inorganic nutrients from the plankton DOM amendment was minor. Each treatment was conducted in triplicate in precleaned (450°C, 4 h) 125-mL Kimax glass bottles. One set of replicates ($n = 3$) was immediately frozen at −20°C on day 0 (initial time point), and the other set ($n = 3$) was incubated onboard at ambient seawater temperatures (15–20°C) in the dark for 10–12 d. Water samples were frozen (−20°C) after incubation. All replicates were analyzed for concentrations of DOC, amino acids, and neutral sugars.

Chemical analyses

Concentrations of DOC were measured using high-temperature combustion and a Shimadzu total organic carbon (TOC) and total nitrogen (TN) analyzer equipped with an autosampler. Blank water (Milli-Q UV-Plus) and seawater reference standards were injected every 6th sample (Benner and Strom 1993). Blanks were negligible and the measured concentrations of reference standards were within the range of reported values (41–44 μmol L^{-1}). The coefficient of variation among four injections of a given DOC sample was typically ± 0.6%.

Amino acids were analyzed following the method of Kaiser and Benner (2005). Briefly, nitrogen-dried samples were hydrolyzed with 6 mol L^{-1} hydrochloric acid at 150°C for 32.5 min in a CEM Mars 5000 microwave and measured as *o*-phthaldialdehyde derivatives using an Agilent 1100 high performance liquid chromatography system equipped with a fluorescence detector (Excitation: 330 nm; Emission: 450 nm). A LiChrosphere RP18 column (4.6 × 150 mm, 5 μm) was used to separate the following 16 amino acids: aspartic acid + asparagine (Asx), glutamic acid + glutamine (Glx), serine (Ser), glycine (Gly), threonine (Thr), β-alanine (β-Ala),

arginine (Arg), alanine (Ala), γ-aminobutyric acid (γ-Aba), tyrosine (Tyr), valine (Val), phenylalanine (Phe), isoleucine (Ile), and lysine (Lys). Neutral sugars, the most abundant carbohydrates, were determined using a Dionex 500 high performance liquid chromatography system with a PA 1 column coupled with a pulsed amperometric detector (Skoog and Benner 1997; Kaiser and Benner 2009). Samples were hydrolyzed with 1.2 mol L^{-1} sulfuric acid, neutralized with a self-absorbed ion retardation resin, and desalted using a mixture of cation and anion exchange resins. Seven neutral sugars were quantified in the analysis: fucose (Fuc), rhamnose (Rha), arabinose (Ara), galactose (Gal), glucose (Glc), mannose (Man), and xylose (Xyl).

The concentration of each amino acid and neutral sugar was quantified using an external calibration curve generated with five concentrations of standards that bracketed the entire range of values observed in the samples. The final concentrations of total dissolved amino acids (TDAA) and neutral sugars (TDNS) were calculated as the sum of the sixteen amino acids and the seven neutral sugars, respectively. DOC-normalized yields of TDAA and TDNS were calculated as the percentages of DOC measured in TDAA and TDNS, respectively, as in Eq. 1 and Eq. 2:

$$\mathrm{TDAA(\%DOC)} = \frac{[\mathrm{TDAA-C}]}{[\mathrm{DOC}]} \times 100 \quad (1)$$

$$\mathrm{TDNS(\%DOC)} = \frac{[\mathrm{TDNS-C}]}{[\mathrm{DOC}]} \times 100 \quad (2)$$

where [DOC], [TDAA-C], and [TDNS-C] are concentrations of bulk DOC, and carbon measured in TDAA and TDNS, respectively. The two nonprotein amino acids (β-Ala and γ-Aba) are thought to be products of diagenetic alteration (Cowie and Hedges 1994), and were not included in the calculation of amino acid yields.

Statistical analyses

Statistical differences between variables were determined using the Mann–Whitney *U*-test (two-tailed, $\alpha = 0.05$) with SPSS software (version 20.0; IBM SPSS).

Results

Distributions and characteristics of DOM

Concentrations of DOC varied ~ 4.6-fold (63–290 μmol L^{-1}) over a salinity gradient of 20–37, with higher values occurring in mid-salinity (22–30) waters (Fig. 2a; Table 1). DOC concentrations were highly variable at mid-salinities and were more conservative at salinities > 30 (Fig. 2a). Average concentrations of DOC ranged from 114 μmol L^{-1} in January to 132 μmol L^{-1} in July (Table 1). In comparison, concentrations of amino acids and neutral sugars varied ~ 12-fold (173–2080 nmol L^{-1}) and ~ 8.5-fold (273–2319 nmol L^{-1}), respectively (Fig. 2b,c; Table 1). Concentrations of amino acids and neutral sugars were greatly elevated at salinities of 22–30 and decreased rapidly with increasing salinity. Remarkably high concentrations of neutral sugars were observed in July, when the values almost doubled those at similar salinities during other seasons (Fig. 2c).

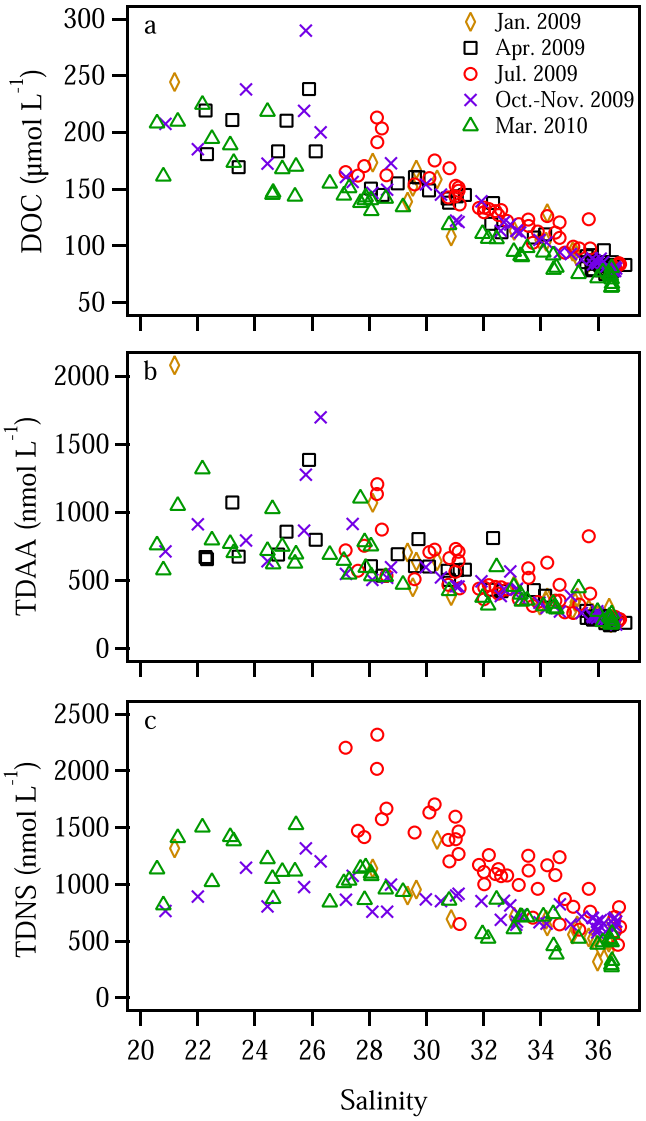

Fig. 2. Seasonal distributions and concentrations of **(a)** dissolved organic carbon (DOC), **(b)** total dissolved amino acids (TDAA), and **(c)** total dissolved neutral sugars (TDNS) across the salinity gradient (20–37). Note that concentrations of TDNS were not determined for the April 2009 cruise.

DOC-normalized yields of amino acids and neutral sugars were quite variable and displayed different distributions (Fig. 3). Yields of amino acids ranged over a factor of 5 from 0.7% to 3.5% of the DOC (avg.: 1.3 ± 0.4% DOC), but average values differed minimally among seasons (avg.: 1.2–1.4% DOC; Mann–Whitney *U*-test, $p > 0.01$; Table 1). Elevated yields of

Table 1. Average values and ranges for dissolved organic carbon (DOC), total dissolved amino acids (TDAA), and total dissolved neutral sugars (TDNS) in the mixed layer during the five cruises on the Louisiana margin.

Cruise	Salinity	Temp °C	DOC μmol L^{-1}	TDAA		TDNS		tDOC	$mDOC_{total}$	$mDOC_{acc}$	$mDOC_{acc}$ %DOC	n_{acc}/n_{total}
				nmol L^{-1}	%DOC	nmol L^{-1}	%DOC		μmol L^{-1}			
Jan 2009	33.1 ± 3.8	19 ± 3	114 ± 43	473 ± 415	1.3 ± 0.6	708 ± 293	3.5 ± 0.7	34 ± 42	81 ± 15	18 ± 16	13 ± 10	19/22
	(21.2–36.4)	(14–23)	(75–244)	(189–2080)	(0.8–3.5)	(319–1392)	(2.2–5.0)	(4–186)	(58–115)	(1–56)	(1–34)	
Apr 2009	32.2 ± 4.7	23 ± 1	123 ± 46	453 ± 280	1.2 ± 0.4	nd	nd	32 ± 36	91 ± 14	27 ± 21	18 ± 10	35/44
	(22.3–37.0)	(21–25)	(75–238)	(173–1387)	(0.7–2.3)			(2–125)	(72–142)	(1–86)	(1–36)	
Jul 2009	32.4 ± 2.7	30 ± 1	132 ± 33	513 ± 229	1.2 ± 0.4	1176 ± 426	5.1 ± 0.9	15 ± 15	117 ± 21	48 ± 26	34 ± 13	42/44
	(27.2–36.8)	(27–31)	(79–213)	(199–1207)	(0.7–2.3)	(467–2319)	(2.7–7.8)	(2–65)	(77–148)	(1–87)	(1–52)	
Oct-Nov 2009	32.0 ± 4.5	24 ± 2	126 ± 50	467 ± 310	1.2 ± 0.5	782 ± 172	3.8 ± 0.8	30 ± 39	96 ± 21	32 ± 26	22 ± 13	37/43
	(20.9–36.6)	(20–27)	(78–290)	(178–1697)	(0.7–3.4)	(578–1318)	(2.1–5.1)	(3–142)	(66–180)	(1–125)	(1–43)	
Mar 2010	30.0 ± 5.2	17 ± 1	125 ± 46	526 ± 273	1.4 ± 0.4	838 ± 339	3.9 ± 0.7	48 ± 46	76 ± 13	23 ± 15	17 ± 9	41/46
	(20.6–36.5)	(15–20)	(63–225)	(180–1319)	(0.8–2.9)	(273–1527)	(2.2–5.2)	(3–166)	(43–112)	(2–71)	(1–32)	
All cruises	31.8 ± 4.4	23 ± 5	125 ± 44	488 ± 291	1.3 ± 0.4	903 ± 369	4.2 ± 1.0	32 ± 38	93 ± 22	31 ± 24	22 ± 13	174/199
	(20.6–37.0)	(14–31)	(63–290)	(173–2080)	(0.7–3.5)	(273–2319)	(2.1–7.8)	(2–186)	(43–180)	(1–125)	(1–52)	

Temp, temperature; tDOC, terrigenous DOC; $mDOC_{total}$, marine DOC; $mDOC_{acc}$, accumulated marine DOC; nd, not determined. Data are reported as the average ± standard deviation; the range of values is presented in the parenthesis.
n_{acc}/n_{total}, number of stations where accumulation of marine DOC was observed, relative to the total number of stations sampled.

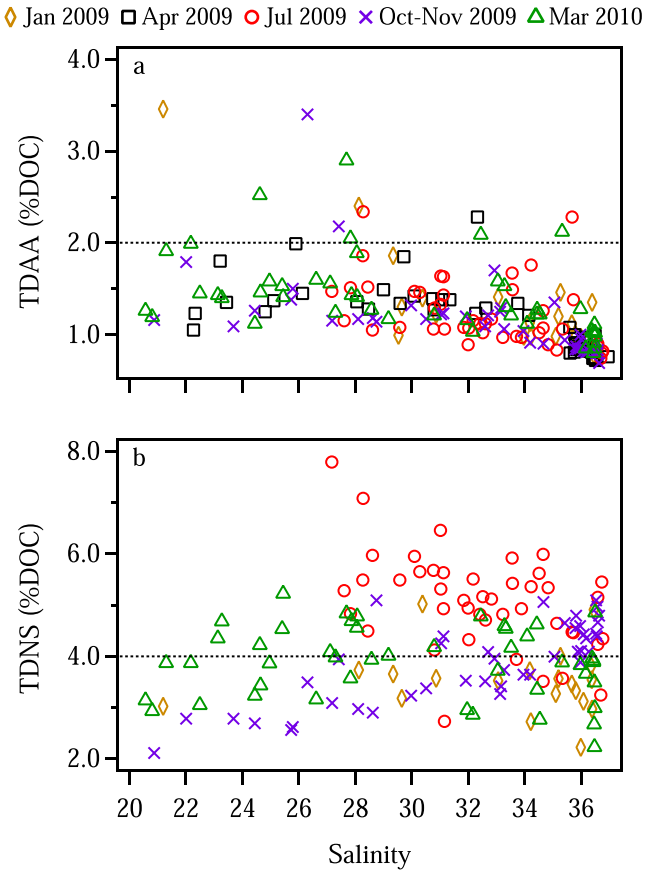

Fig. 3. Seasonal distributions of DOC-normalized yields of **(a)** total dissolved amino acids (TDAA, %DOC) and **(b)** total dissolved neutral sugars (TDNS, %DOC). The dashed line represents cutoff values used to detect the presence of labile DOM (TDAA: ≥ 2.0%; TDNS: ≥ 4.0%). Note that yields of TDNS were not determined for the April 2009 cruise.

amino acids (> 2% DOC) occurred largely in mid-salinity waters (Fig. 3a). Most yields were below 2% of the DOC and were scattered across the entire salinity range. In comparison, yields of neutral sugars ranged from 2.1% to 7.8% of the DOC (avg.: 4.2 ± 1.0% DOC) and showed strong spatial and temporal variability (Fig. 3b). The highest neutral sugar yields were observed in July across the 20–37 salinity range. In October–November, yields of neutral sugars remained low (e.g., < 3% DOC) in mid-salinity waters and were elevated above 4% DOC at salinities > 34. Yields of neutral sugars in March varied between 3% and 5% DOC and showed no clear spatial gradient (Fig. 3b). The spatial distributions of elevated yields varied between amino acids and neutral sugars, with most elevated amino acid yields at salinities < 29 and most elevated neutral sugar yields at salinities > 27.

Bioassay experiments

Two short-term (10–12 d) bioassay experiments were conducted with mid-salinity surface waters to determine the

labile fraction of DOC and to measure the yields of amino acids and neutral sugars in labile DOM. The unamended bioassay experiments showed that concentrations of labile DOC ranged from 2 μmol L^{-1} to 10 μmol L^{-1}, accounting for a small fraction (1–6%) of the total DOC (Table 2). In the amended treatments, the addition of plankton-derived DOM corresponded to a 14–17% increase (23–24 μmol L^{-1}) in DOC concentrations. After 10–12 d of incubation, the DOC concentrations decreased rapidly to values similar to those in the controls (Table 2), suggesting most or all of the added DOC was readily consumed and therefore labile. The utilization of labile DOC included the preferential removal of amino acids and neutral sugars, as revealed by the decreases in DOC-normalized yields of amino acids and neutral sugars (Table 2). These results corroborate previous findings showing that labile DOM is enriched in amino acids and neutral sugars (Amon et al. 2001; Davis and Benner 2007; Goldberg et al. 2009). The DOM with the lowest concentrations of labile DOC (~ 2 μmol L^{-1}) had an amino acid yield of 2.0% and a neutral sugar yield of 3.7%. All of the DOM experiments with higher initial yield values contained higher concentrations of labile DOC.

Table 2. Concentrations and compositions of dissolved organic matter (DOM) during the shipboard bioassay experiments.*

	Control		Amended	
	t = 0	*t* = final	*t* = 0	*t* = final
Exp 1 (salinity = 23.3)				
DOC (μmol L^{-1})	168 ± 1	158 ± 1	191 ± 1	160 ± 1
TDAA (nmol L^{-1})	897 ± 11	643 ± 14	2331 ± 18	949 ± 11
TDNS (nmol L^{-1})	1364 ± 38	730 ± 76	2524 ± 180	703 ± 20
TDAA (%DOC)	2.0 ± 0.0	1.6 ± 0.0	5.0 ± 0.0	2.4 ± 0.0
TDNS (%DOC)	4.7 ± 0.1	2.7 ± 0.3	7.8 ± 0.6	2.5 ± 0.1
Exp 2 (salinity = 27.8)				
DOC (μmol L^{-1})	134 ± 1	132 ± 1	157 ± 1	130 ± 1
TDAA (nmol L^{-1})	731 ± 35	670 ± 35	2142 ± 50	859 ± 11
TDNS (nmol L^{-1})	852 ± 12	713 ± 71	2067 ± 224	629 ± 30
TDAA (%DOC)	2.0 ± 0.1	1.9 ± 0.1	5.5 ± 0.1	2.5 ± 0.0
TDNS (%DOC)	3.7 ± 0.1	3.1 ± 0.3	7.7 ± 0.8	2.8 ± 0.1

*Plankton-derived DOM was added to the amended treatments. The incubation time was 12 d for experiment 1 (Exp 1) and 10 d for experiment 2 (Exp 2). Data are reported as the average ± standard deviation (n = 3). DOC, dissolved organic carbon; TDAA, total dissolved amino acids; TDNS, total dissolved neutral sugars.

In the amended experiments, the addition of labile plankton DOM resulted in a two to threefold increase in concentrations and yields of amino acids and neutral sugars (Table 2). After 10–12 d of incubation, concentrations and yields of neutral sugars decreased rapidly to levels comparable to those in the controls, whereas values of amino acids remained slightly elevated in the amended treatments (Table 2). These results indicate the microbial community was capable of rapidly utilizing labile DOM and probably produced some metabolites (e.g., D-amino acids) that were resistant to decomposition (Kawasaki and Benner 2006; Lechtenfeld et al. 2015).

Biochemical indicators of labile DOM

Based on the bioassay results, an amino acid yield higher than 2.0% DOC and a neutral sugar yield higher than 4.0%

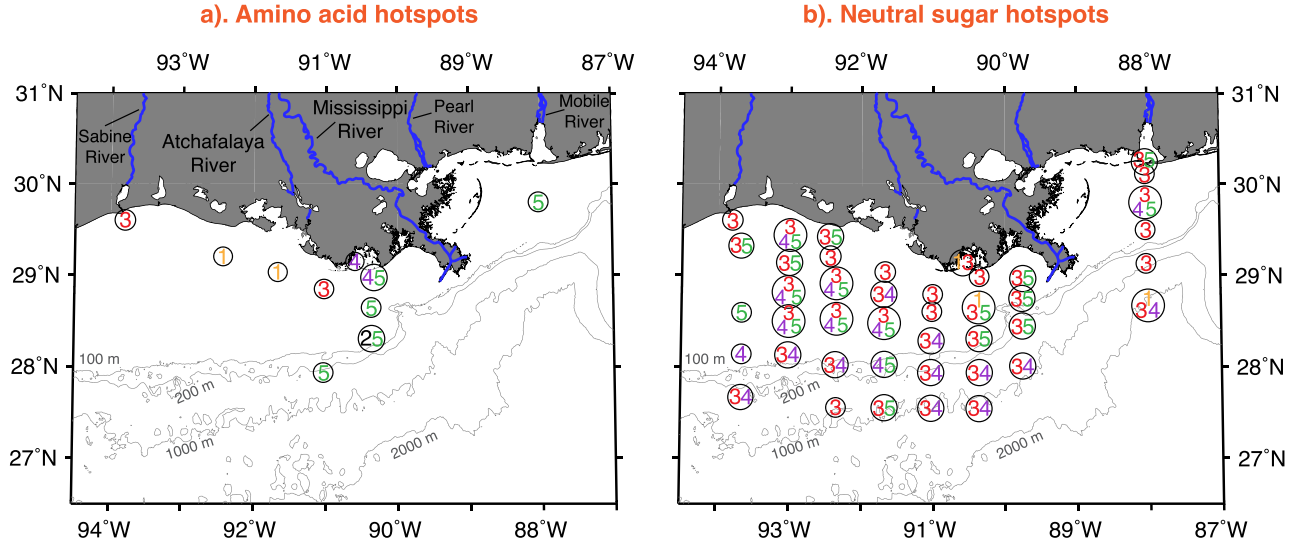

Fig. 4. Hot spots of labile DOM on the Louisiana margin. **(a)** Amino acid hot spots and **(b)** neutral sugars hot spots were regions where amino acid-rich labile DOM and neutral sugar-rich labile DOM were detected, respectively. The five cruises were denoted by numbers (1: January 2009; 2: April 2009; 3: July 2009; 4: October–November 2009; 5: March 2010), and as in Figs. 2, 3. Note that neutral sugar hot spots were not determined for the April 2009 cruise.

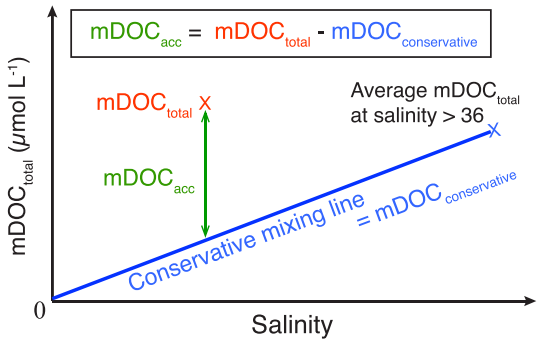

Fig. 5. Conceptual illustration showing the estimation of accumulated marine dissolved organic carbon ($mDOC_{acc}$). A conservative mixing line was drawn for concentrations of marine DOC ($mDOC_{total}$) between the river and marine endmembers. The $mDOC_{total}$ values lying above the mixing line indicate an accumulation of marine DOC. At a given salinity, the concentration of $mDOC_{acc}$ was calculated as the difference in concentration between $mDOC_{total}$ and the corresponding $mDOC_{conservative}$ value on the mixing line.

DOC were indicative of a significant amount (> 2 μmol L^{-1}) of labile DOM (Mann–Whitney *U*-test, $p < 0.05$). These yields were used as cutoffs for indicating the presence of labile DOM in the field samples. Labile DOM can be enriched in amino acids, neutral sugars or both. In this study, areas showing the occurrence of labile DOM were considered biological hot spots. Hot spots with labile DOM enriched in amino acids were identified in a relatively small number of stations ($n = 12$) that were largely located at mid-salinities (Fig. 3a). It is interesting to note that most amino acid hot spots ($n = 9$) were identified in water samples collected between dusk and dawn. The DOM in amino acid hot spots was significantly enriched in Glx, basic amino acids (Arg and Lys), and certain hydrophobic amino acids (Val and Phe), but depleted in Asx, Ser, Gly, Thr, and nonprotein amino acids (β-Ala and γ-Aba) (Supporting Information Fig. S1a). In comparison, hot spots with labile DOM enriched in neutral sugars were prevalent across a broad salinity gradient of 23–37, accounting for more than half of the samples ($n = 81$; Fig. 3b). Note that neutral sugars were not measured during the April cruise. The neutral sugar hot spots were identified in waters collected both during the day and at night. Less pronounced differences in compositions of neutral sugars were observed between neutral sugar hot spots and other areas (Supporting Information Fig. S1b). Glc was the dominant neutral sugar and was slightly enriched in hot spots, whereas Xyl, Man, and Ara were depleted in hot spots.

These compositionally different types of hot spots exhibited very different distributions in margin surface waters (Fig. 4). Amino acid hot spots occurred sporadically during most seasons and they were largely confined to nearshore regions. More amino acid hot spots were observed in March, with a few appearing over the shelf edge (Fig. 4a). In comparison, neutral sugar hot spots were prevalent over much larger areas

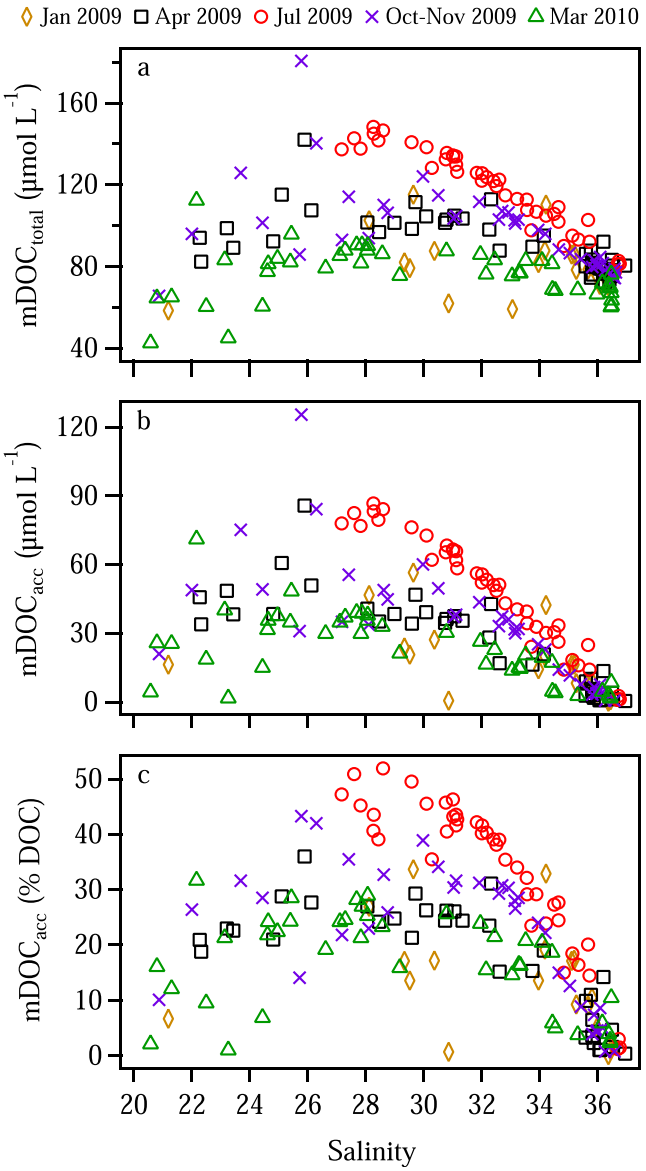

Fig. 6. Seasonal distributions and concentrations of **(a)** marine DOC ($mDOC_{total}$) and **(b, c)** accumulated marine DOC ($mDOC_{acc}$) in units of μmol L^{-1} and %DOC across the salinity gradient (20–37).

of the Louisiana margin, and their seasonal recurrence was evident at most locations (Fig. 4b). The hot spots were most prominent in July, with very high yields of neutral sugars occurring in nearly all shelf and slope waters sampled in this study. In October–November, neutral sugar hot spots shifted offshore and were present on the outer shelf and continental slope. Hot spots in March were largely absent in slope waters and were mostly located on the shelf (Fig. 4b). These results revealed large spatial and temporal variability of hot spots on the Louisiana margin and indicated the dynamic nature and heterogeneous composition of labile DOM, which was predominantly enriched in carbohydrates.

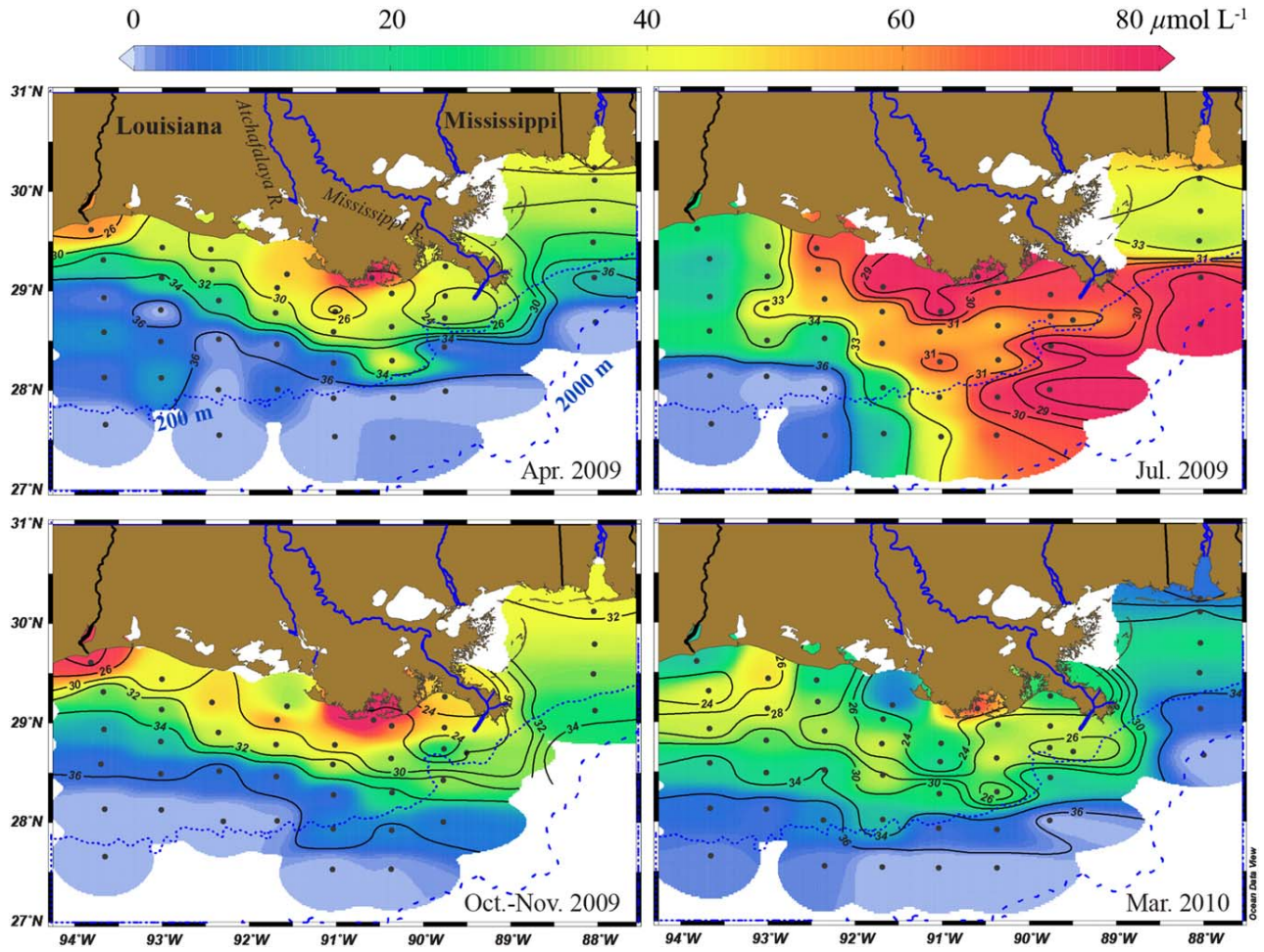

Fig. 7. Surface concentrations of accumulated marine DOC ($mDOC_{acc}$) on the Louisiana margin in April, July, October–November 2009, and March 2010. Solid dots represent sampling stations and solid lines depict the contour lines of salinity. The 200- and 2000-m isobaths are shown as dashed lines.

Estimation and distribution of accumulated marine DOC

The accumulation of marine-derived DOC during each season was investigated by accounting for the inputs of tDOC from rivers. The concentration of tDOC (Fichot and Benner 2012, 2014) was subtracted from the total DOC concentration to yield a calculated concentration of marine DOC ($mDOC_{total}$) as in Eq. 3,

$$mDOC_{total} = DOC - tDOC \quad (3)$$

A conservative mixing line of $mDOC_{total}$ across the salinity gradient from the river to marine end-members was used to estimate concentrations of accumulated marine DOC ($mDOC_{acc}$) (Fig. 5). Positive deviations from conservative mixing indicate an accumulation of marine DOC. The concentration of $mDOC_{total}$ is zero at the river endmember and the average concentration of $mDOC_{total}$ at salinity > 36 was used as the marine endmember value for each cruise (Supporting Information Table S1). The concentration of $mDOC_{acc}$ at a given salinity was calculated by subtracting the value on the conservative mixing line ($mDOC_{conservative}$) from the observed concentration of $mDOC_{total}$ as in Eq. 4

$$mDOC_{acc} = mDOC_{total} - mDOC_{conservative} \quad (4)$$

The small variability in $mDOC_{total}$ at the marine endmember (1–7%; Supporting Information Table S1) had a minor impact on the calculated concentrations of $mDOC_{acc}$.

The accumulation of marine DOC was observed during all five cruises and at 174 of the 199 stations spanning the 20–37 salinity range (Table 1; Fig. 6). Concentrations of $mDOC_{acc}$ ranged from 1 μmol L^{-1} to 125 μmol L^{-1} with an average value of 31 ± 24 μmol L^{-1}, accounting for as much as 52% of the total DOC (avg.: 22 ± 13%; Table 1). The concentrations of $mDOC_{acc}$ varied substantially among seasons, with lowest concentrations in January (18 ± 16 μmol L^{-1}), increasing concentrations in April (27 ± 21 μmol L^{-1}), highest concentrations in July (48 ± 26 μmol L^{-1}), and declining

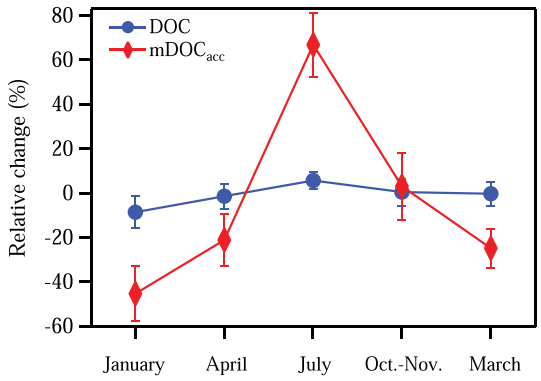

Fig. 8. Relative changes in average concentrations of dissolved organic carbon (DOC) and accumulated marine DOC (mDOC$_{acc}$) during each cruise. The relative change (%) is calculated as $(X_{each} - X_{all})/X_{all} \times 100$, where X_{each} and X_{all} are the average concentrations of DOC (or mDOC$_{acc}$) during each cruise and all five cruises, respectively. Results are reported as the average ± standard error.

concentrations in October–November (32 ± 26 μmol L^{-1}) and March (23 ± 15 μmol L^{-1}). Concentrations and percentages of mDOC$_{acc}$ were elevated at intermediate salinities (26–32) during all seasons. There was >30 μmol L^{-1} of mDOC$_{acc}$ at these mid-salinities and the mDOC$_{acc}$ accounted for over 20% of the total DOC, with a few exceptions in January and March (Fig. 6). The observed patterns of mDOC$_{acc}$ generally followed those of primary production (Redalje et al. 1994; Lohrenz et al. 1997).

Spatial distributions of surface mDOC$_{acc}$ varied seasonally on the Louisiana margin (Fig. 7). In April and October–November, mDOC$_{acc}$ was largely confined to the shelf and higher concentrations of mDOC$_{acc}$ were observed in nearshore waters of intermediate salinity. In July, a strikingly large area of surface waters was enriched in mDOC$_{acc}$. The mDOC$_{acc}$-rich waters spread much further to the east and south. They crossed the shelf break and were present over much of the continental slope, in stark contrast to the confined distributions in other months. Concentrations of mDOC$_{acc}$ in these waters were typically higher than 60 μmol L^{-1} and closely paralleled the distribution of salinity (Mann–Whitney *U*-test, $p < 0.001$). In March, waters with elevated concentrations of mDOC$_{acc}$ (e.g., > 30 μmol L^{-1}) mostly resided on the shelf, but they showed a broader distribution across the Louisiana–Texas shelf compared with those in spring and fall (Fig. 7).

Concentrations of bulk DOC and mDOC$_{acc}$ revealed contrasting seasonal variability (Fig. 8). Average concentrations of DOC varied by less than 10% and were not significantly different among the five cruises (Mann–Whitney *U*-test, $p > 0.05$). In comparison, average concentrations of mDOC$_{acc}$ varied two to threefold among seasons. Compared with the average values for all cruise data, mDOC$_{acc}$ concentrations were 45% and 20% lower in January and April, respectively, and were 67% higher in July (Fig. 8). The elevated production of marine DOC in summer was offset by the concurrently enhanced removal of tDOC (Fichot and Benner 2014), making the changes in total DOC concentrations less discernible during productive seasons. These results are consistent with previous studies in other ocean margins (Davis and Benner 2005; Mathis et al. 2007; Shen et al. 2012a), demonstrating that ecosystem productivity is reflected in the composition and bioavailability of DOM but not in bulk DOC concentrations.

The seasonal reservoirs of DOC, marine DOC, and accumulated marine DOC were quantified in the surfaced mixed layer of the Louisiana shelf following the approach used in Fichot and Benner (2014) (Table 3). The reservoir of DOC varied from 0.70 Tg C to 1.51 Tg C, and was dominated by marine DOC (0.50–1.32 Tg C). The reservoir of accumulated marine DOC was substantial (0.11–0.23 Tg C) and accounted for 13–31% of marine DOC on the Louisiana shelf, with the highest fraction occurring during the summer.

Discussion

Plankton production and hot spots of labile DOM

The Louisiana margin receives large nutrient loads from the Mississippi–Atchafalaya River system and is among the world's most productive ocean margins (Goolsby et al. 2001; Heileman and Rabalais 2008). This margin is characterized by highly variable environmental conditions (e.g., river discharge, solar irradiance, nutrients, and currents) that drive large spatial and temporal gradients in plankton community

Table 3. Surface mixed layer reservoirs of dissolved organic carbon on the Louisiana shelf.*

Cruise	Mixed layer volume (km^3)	DOC (Tg C)	mDOC$_{total}$ (Tg C)	mDOC$_{acc}$ (Tg C)
Apr 2009	920	1.13	0.95	0.13
Jul 2009	586	0.82	0.75	0.23
Oct–Nov 2009	1285	1.51	1.32	0.17
Mar 2010	562	0.70	0.50	0.11
AVG ± SD	838 ± 340	1.04 ± 0.36	0.88 ± 0.34	0.16 ± 0.05

*Ordinary kriging was used to interpolate discrete field measurements over the shelf (62,068 km^2; Fichot and Benner 2014). Calculations were not performed for the January 2009 cruise due to insufficient data. mDOC$_{total}$, marine dissolved organic carbon; mDOC$_{acc}$, accumulated marine dissolved organic carbon. 1 Tg = 1 × 10^{12} g. AVG ± SD, average ± standard deviation.

structure and productivity (Lohrenz et al. 1999; Chakraborty and Lohrenz 2015; Cherrier et al. 2015). Maximal phytoplankton biomass and productivity commonly develop at mid-salinities due to lower chromophoric DOM and suspended sediment loads and elevated nutrients, and they can exceed 30 mg chlorophyll m^{-3} and 10 gC m^{-2} d^{-1}, respectively, during spring and summer (Lohrenz et al. 1999; Lehrter et al. 2009; Chakraborty and Lohrenz 2015). Despite the high nutrient inputs, phytoplankton growth on the margin is often limited by nitrogen (N), phosphate (P), or co-limitated by N and P depending on season and location (Smith and Hitchcock 1994; Turner and Rabalais 2013). Phytoplankton biomass remains low ($<$ 1 mg chlorophyll m^{-3}) in nutrient-depleted offshore waters, where primary production (0.5–1.0 gC m^{-2} d^{-1}) is dominated by small species, such as cyanobacteria (Lehrter et al. 2009; Chakraborty and Lohrenz 2015). Grazers, such as copepods and protozoa, are widespread on the margin, and they can release a large fraction of phytoplankton production as labile DOM (Strom et al. 1997; Liu and Dagg 2003). In this study, DOM directly released from phytoplankton as well as DOM released during grazing and viral lysis is collectively referred to as plankton DOM. Bacterial respiration and production are tightly linked to plankton DOM, and maximal rates are often observed at mid-salinities during summer in waters with elevated concentrations of carbohydrates (Chin-Leo and Benner 1992; Benner and Opsahl 2001). Nutrient enrichment experiments also indicate the potential for N or P limitation of bacterial growth in margin surface waters (Chin-Leo and Benner 1992; Pomeroy et al. 1995).

The observed distributions of DOC, amino acids and neutral sugars were consistent with previous investigations demonstrating major production of plankton DOM at intermediate salinities on the Louisiana shelf (Benner and Opsahl 2001; Wang et al. 2004). High rates of primary production and zooplankton grazing are characteristic at mid-salinities (Redalje et al. 1994; Liu and Dagg 2003), and both processes can release DOM that is enriched in proteins, peptides, and carbohydrates (Myklestad and Haug 1972; Strom et al. 1997). Concentrations of amino acids and neutral sugars, the most abundant carbohydrates, were greatly elevated at mid-salinities and the values were much higher than those in adjacent marine waters. High concentrations of these biochemicals provide clear molecular evidence of plankton-derived DOM in mid-salinity waters, which was most prominent during the summer when concentrations of carbohydrates were highest.

Plankton DOM includes labile components that can rapidly trigger the formation of hot spots with high rates of microbial growth, respiration, and nutrient regeneration (Pakulski et al. 1995; Azam and Malfatti 2007; Stocker et al. 2008). Bioassay experiments are commonly used to determine the labile fraction of DOM (Søndergaard and Middelboe 1995; Lønborg et al. 2009), but this approach alters environmental conditions and is limited in spatial and temporal coverage. Amino acids and carbohydrates are common components of labile DOM and their relative abundance can reflect the inherent bioavailability of DOM. These biochemicals have been used as indicators of the presence of labile DOM across broad spatial and temporal scales (Amon et al. 2001; Benner 2003; Davis and Benner 2007; Goldberg et al. 2009). Bioassay experiments quantify the abundance of labile DOM and measure its rate of biological utilization under specific environmental conditions. Biochemical indicators can also quantify the abundance of labile DOM, but they do not provide information about the rate of DOM biological utilization, which is dependent on physicochemical conditions and microbial community composition (Carlson and Hansell 2015). As used in this study, measurements of biochemical indicators identify the presence of labile DOM that can be utilized within 2 weeks under favorable environmental conditions. The bioassay experiments in this study demonstrated that labile DOM was present in water samples when amino acids or carbohydrates comprised a minimum of 2% or 4% of the DOC, respectively. These values are similar to those observed after labile DOM was consumed in other bioassay experiments (Amon et al. 2001; Davis and Benner 2007), and they were used in this study as baseline values for the detection of labile DOM. Labile DOM can be enriched in amino acids, carbohydrates, or both, and the locations where labile DOM was detected were designated as biological hot spots.

A large number of hot spots were identified in margin surface waters and they exhibited substantial spatial and temporal variability, reflecting the productive and dynamic nature of this ecosystem. Hot spots frequently occurred at mid-salinities (26–29) on the inner shelf, where high phytoplankton biomass and production are often observed during most seasons (Redalje et al. 1994; Chakraborty and Lohrenz 2015). Interestingly, unlike neutral sugar hot spots, amino acid-rich hot spots were mostly identified in waters collected during low- and no-light time periods, suggesting diel variability in the source of labile DOM. Extracellular release from phytoplankton is active at high solar irradiance (Cherrier et al. 2015), preferentially releasing carbohydrates (Myklestad et al. 1989). In comparison, grazing releases a potpourri of cell contents that include amino acids and other biomolecules (Carlson and Hansell 2015), and can vary with the diel vertical migration of zooplankton. Previous diel measurements of gut-pigments in copepods in surface waters of the Louisiana shelf showed elevated values at night (Dagg 1995). It appears that grazing in surface waters at night is an important source of amino acid-rich labile DOM.

Hot spots with labile DOM enriched in amino acids showed distinct spatial distributions from those with carbohydrate-rich labile DOM, indicating compositional variability in labile plankton DOM. Amino acid-rich hot spots were found at relatively few locations, and they mostly occurred on the inner shelf where concentrations of nutrients and chlorophyll were high (Chakraborty and Lohrenz 2015). A few amino acid hot spots appeared further south near the shelf-edge in March,

coinciding with a widespread nutrient-rich plume that resulted in elevated concentrations of chlorophyll across outer shelf and slope waters (Huang et al. 2013; Chakraborty and Lohrenz 2015). Amino acid hot spots appeared to reflect areas of high phytoplankton biomass. A similar pattern was observed in productive waters of the Chukchi Sea where concentrations of dissolved and particulate amino acids were strongly correlated with chlorophyll concentrations (Davis and Benner 2005).

In contrast, carbohydrate-rich hot spots were prevalent over a large area of the Louisiana margin and appeared to reflect widespread nutrient limitation of phytoplankton growth (Smith and Hitchcock 1994; Turner and Rabalais 2013). Under nutrient limitation, phytoplankton growth is inhibited while light-driven photosynthesis still proceeds, resulting in enhanced release of extracellular carbohydrates, particularly when nitrate: phosphate ratios are high (Myklestad and Haug 1972). Low concentrations of phosphate (< 0.2 μmol L^{-1}) and chlorophyll (< 2 mg m^{-3}), and high ratios of dissolved inorganic nitrogen to soluble reactive phosphate (DIN: SRP > 22) were measured in shelf and slope waters in July (Chakraborty and Lohrenz 2015). Carbohydrate hot spots were most pronounced during July, providing strong evidence for plankton production of carbon-rich labile DOM that was produced under conditions of nutrient limitation. Additional evidence is gained from observations in October–November when carbohydrate hot spots largely shifted to the continental slope and corresponded with low concentrations of DIN (0.4 ± 0.1 μmol L^{-1}), SRP (0.05 ± 0.02 μmol L^{-1}) and chlorophyll (0.11 ± 0.08 mg m^{-3}) (Chakraborty and Lohrenz 2015).

The widespread occurrence of hot spots during the summer cruise is consistent with general observations of elevated rates of plankton production during the summer. In comparison, plankton production is much lower during winter, resulting in a very limited number of hot spots in January. Overall, the amino acid and carbohydrate biochemical indicators highlighted areas of high biological production and activity in a spatially and temporally dynamic ocean margin.

Accumulation of semi-labile marine DOC

Large accumulations of marine DOC (0.11–0.23 Tg C) were observed in the shelf mixed layer, and most of the accumulated marine DOC appeared to be of semi-labile reactivity despite the widespread occurrence of labile DOM hot spots. The average yields of amino acids (1.3 ± 0.4% DOC) and carbohydrates (4.2 ± 1.0% DOC) on the Louisiana margin were comparable to those in oceanic surface waters and were indicative of semi-labile DOM (Davis and Benner 2007; Goldberg et al. 2009; Kaiser and Benner 2009). In addition, the average amino acid and neutral sugar composition of the DOM was indicative of degraded and altered material of a semi-labile nature. Most of the accumulated DOM on the Louisiana margin is composed of semi-labile molecules that have turnover times of months to years and accumulate in surface waters, which have a 2- to 3-month residence time on the Louisiana shelf (Dinnel and Wiseman 1986; Fichot and Benner 2014). The most prominent accumulation of marine DOC occurred at mid-salinities during the summer, corresponding to the spatial and temporal patterns of plankton production and heterotrophic activity (Benner and Opsahl 2001; Dagg et al. 2007). Most plankton DOM has undergone substantial microbial alteration, which reduces its bioavailability and contributes to its accumulation.

It appears that nutrient limitation promotes the accumulation of marine DOM on the Louisiana margin as it enhances phytoplankton production of carbohydrates and limits bacterial utilization of C-rich DOM (Myklestad and Haug 1972; Chin-Leo and Benner 1992; Thinstad et al. 1997; Skoog et al. 2002). There were relatively low concentrations of nutrients on the shelf and slope during summer (Chakraborty and Lohrenz 2015), when large accumulations of carbohydrate-rich DOM were observed. Concentrations of nutrients were elevated on the outer shelf and slope during March (Chakraborty and Lohrenz 2015), and this could have resulted in the relatively low concentrations of accumulated DOC at mid- to high-salinities at this time. Our results indicate there are multiple controls on the accumulation of DOC on the Louisiana margin, which is consistent with observations in other coastal ecosystems (Zweifel et al. 1995).

Marine DOM produced on the shelf is an important source of energy and key bioelements for microbial food webs (Chin-Leo and Benner 1992; Amon and Benner 1998; Green et al. 2006). The reservoir size of accumulated marine DOC (0.11–0.23 Tg C; 0.16 ± 0.05 Tg C) was similar to but lower than the seasonal estimates of net community production in the Mississippi River plume (0.11–0.51 Tg C) (Green et al. 2006; Guo et al. 2012). The semi-labile nature of $mDOC_{acc}$ facilitates its transport by eddies and wind-driven currents. Easterly winds are usually prevalent in all seasons but summer, when wind direction shifts to the south and triggers Ekman transport of shelf waters eastward toward the shelf break, promoting cross-shelf transport of shelf waters (Morey et al. 2003; Fichot et al. 2014). An impressive cross-shelf export of $mDOC_{acc}$ occurred during summer 2009. This event represents a major subsidy of shelf-derived marine DOM (> 60 μmol DOC L^{-1}) to offshore waters, which doubles the typical DOC concentrations in surface waters on the continental slope (60–70 μmol L^{-1}) (Benner and Opsahl 2001; Fichot and Benner 2014). Overall, these physical processes can deliver shelf-produced DOM and associated nutrients rapidly to offshore waters, thereby supporting microbial food webs in the open Gulf of Mexico.

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