6 Isotope Composition of Organic Matter in Seawater

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6.1 INTRODUCTION

Marine organic matter in seawater is one of the most active carbon reservoirs on the earth surface and plays an important role in earth's climate system. Marine organic matter is also a key component in the exchange among the biosphere, hydrosphere, and geosphere (Hedges, 1992). Therefore, knowledge of the cycling of organic matter in the marine environments is indispensable for understanding of the biogeochemistry of a variety of elements, function of ecosystems, and impact of human activities on global climate changes. Isotopic signatures, such as those of stable isotope ratios of C, N, and S, as well as radiocarbon, have been widely applied as powerful tools to study biogeochemical cycling of organic matter in marine environments (Eadie et al., 1978; Sigleo and Macko, 1985; Peterson and Fry, 1987; Altabet, 1988; Altabet et al., 1991; Cifuentes et al., 1988; Sackett, 1989; Rau et al., 1990, 1991a; Druffel and Williams, 1992; Druffel et al., 1992; Benner et al., 1997; Raymond and Bauer, 2001a; Tanaka et al., 2004; Knapp et al., 2005; Chen et al., 2006; Tagliabue and Bopp, 2008). Interactions of organic carbon with inorganic carbon in marine environments and their interchange with atmospheric CO_2 are always accompanied by variations in carbon isotope signals (Tagliabue and Bopp, 2008). Therefore, isotopic measurements provide a baseline for assessment of sources, sinks, transport, and transformation of marine organic matter, as well as the geochemical processes controlling the distribution of organic matter in the oceans (Druffel and Williams, 1992; Tumbore and Druffel, 1995; Raymond and Bauer, 2001).

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Marine organic matter is heterogeneous in terms of chemical composition (Mayer, 1994; Benner et al., 1997; Bergamaschi et al., 1997; Guo and Santschi, 1997a), isotopic signature (Druffel et al., 1992; Santschi et al., 1995; Guo et al., 1996; Wang et al., 1998; Eglinton et al., 1996; Pearson and Eglinton, 2000; Wang and Druffel, 2001), and bioavailability (Amon and Benner, 1994; Hunt et al., 2000). Organic matter in seawater includes three major forms based on its size spectrum (Figure 6.1) (Sharp, 1973; Guo and Santschi, 1997b): dissolved organic matter (DOM), colloidal organic matter (COM), and particulate organic matter (POM). These different organic matter phases are operationally defined based on specific filtration/separation techniques.

Micro- and macrofiltration, *in situ* pumps, and sediment traps have been successfully used to collect particulate organic matter, including suspended POM, phytoplankton, and sinking POM from marine environments for chemical and isotopic measurements (Bishop et al., 1978; Knauer et al., 1979; Altabet, 1990; Fry et al., 1991; Hedges et al., 2001; Peterson et al., 2005). Ultrafiltration, depending on pore size cutoffs of filters or membranes, has been proven to be effective for isolating colloidal organic matter (Benner et al., 1992; Guo and Santschi, 1997b; Guo and Santschi, 2007). However, sampling of DOM from seawater for chemical and isotopic characterization remains a challenging task, although recent development in methodologies has allowed direct measurements of $DO^{13}C$ and $DO^{15}N$ in seawater (Druffel et al., 1992; Clercq et al., 1998; Gandhi et al., 2004;

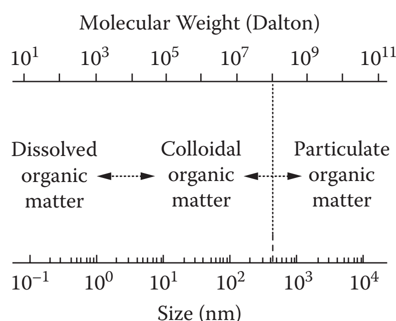

FIGURE 6.1 Size spectrum of marine organic matter, including dissolved organic matter (DOM), colloidal organic matter (COM), and particulate organic matter (POM) in seawater.

Knapp et al., 2005; Bouillon et al., 2006; Beaupre et al., 2007; Osburn and St.-Jean, 2007; Lang et al., 2007).

Biogeochemical cycling of organic matter in the ocean is complex and has not been fully understood (Hedges, 2002; Lee and Wakeham, 1992; Mopper et al., 2007), partially due to the relatively poor characterization in chemical and isotopic compositions of marine organic matter (Benner et al., 1992; Guo and Santschi, 1997b; Hansell and Carlson, 2002). This is largely caused by the difficulty in separating trace amounts of dissolved and colloidal organic matter from seawater. In general, the concentration of DOC in seawater is less than 0.5–1 mg-C/L, variable with water depth and ocean basins (Guo et al., 1995; Hansell and Carlson, 1998), while sea salt concentrations are usually in the order of 35,000 mg/L (Chester, 2003) (~35,000 times higher than those of bulk DOM in seawater).

Recent advances in separation and isolation techniques for DOM have greatly improved our ability to understand the cycling of DOM and COM and associated trace elements in marine environments (Hansell and Carlson, 2002; Guo and Santschi, 2007; Mopper et al., 2007). Indeed, a growing body of literature has reported the measurements of stable isotope and radiocarbon, especially for COM or high molecular weight DOM samples from the marine environments (Guo and Santschi, 2007, and references therein). While many DOM separation techniques/methods have been used, depending on specific research purposes, the most common methods are cross-flow ultrafiltration (Benner et al., 1992; Buesseler et al., 1996; Guo and Santschi, 2007), solid phase extraction (e.g., Louchouarn et al., 2000; Kim et al., 2003; Dittmar et al., 2008), and reverse osmosis coupled with electrodialysis (Koprivnjak et al., 2006; Vetter et al., 2007).

Major elements of marine organic matter include carbon, nitrogen, phosphorus, sulfur, oxygen, and hydrogen. So far, most stable isotope data of marine organic matter are for carbon, and measurements of N, S, and O isotopes are still scarce, especially for the DOM pool (Peterson et al., 1985; Hoefs, 2004; Fry, 2006). In this chapter, we will first describe the basic concept on isotopic fractionation, the stable isotopes of C, H, N, O, and S, as well as radiocarbon and their isotopic composition in natural marine organic matter. The focus will be shifted to sampling procedures of DOM, COM, and POM, and treatment/preparation of organic matter samples for bulk isotope, compound classes, and compound-specific isotope analyses.

6.2 STABLE ISOTOPES AND RADIOCARBON

Table 6.1 lists the common isotopes of carbon, nitrogen, sulfur, oxygen, and hydrogen, along with their isotopic mass, natural abundance (%), and standard/reference materials for isotopic measurements. Similar to carbon, nitrogen, and sulfur, phosphorus is one of the important elements that build biochemical molecules. However, phosphorus has only one stable isotope (^{31}P). Although phosphorus has many radioactive isotopes, most of them with a half-life within minutes or seconds, only ^{32}P and ^{33}P (both of them are beta-emitters) could be useful oceanic tracers (Benitez-Nelson, 2000, and references therein) due to their relatively longer decay half-lives. Therefore, phosphorus is considered to be a monoisotopic element and is not listed in Table 6.1.

In addition to traditional stable isotopes (C, N, S, H, and O), numerous stable isotope tracers with applicability to marine biogeochemical cycles have emerged in recent years. These nontraditional stable isotopes, such as Li, B, Si, Ca, Fe, Cu, Zn, Se, Mo, and Cd, can be measured via new analytical techniques, such as multiple-collector inductively coupled plasma-mass spectrometry (MC-ICP/MS), but their isotopic variations are generally very small. These nontraditional stable isotopes are not discussed in this chapter.

6.2.1 δ-Notation

The δ-notation is a means to express isotopic composition of a sample relative to a standard (Hoefs, 2004). The isotopic ratios in natural samples usually show a very small variance, sometimes in the range of the third to fifth decimal place. Therefore, measurements of stable isotopes at or near

TABLE 6.1
A List of Commonly Used Isotopes of Carbon, Nitrogen, Sulfur, Hydrogen, and Oxygen

Element	Isotope	Isotopic Mass	Abundance (%)	Isotope Standard
Carbon	^{12}C	12.0000	99.985	Pee Dee Belemnite (PDB)
	^{13}C	13.00335	0.015	
	^{14}C[a]	14.0	10^{-10}	Oxalic acid/1890 wood
Nitrogen	^{14}N	14.00307	99.63	Atmospheric N_2
	^{15}N	15.0001	0.37	
Sulfur	^{32}S	31.97207	95.00	Canyon Diablo meteorite (CDT)
	^{33}S	32.97145	0.76	
	^{34}S	33.96786	4.22	
	^{36}S	35.96708	0.014	
Hydrogen	^{1}H	1.00794	99.985	Standard Mean Ocean Water (SMOW)
	^{2}H	2.0141	0.015	
Oxygen	^{16}O	15.9949	99.758	PDB or SMOW
	^{17}O	16.9991	0.037	
	^{18}O	17.9991	0.204	

[a] ^{14}C is a radioactive isotope with a half-life of 5,730 ± 40 years.

natural abundance levels are usually reported in the delta (δ) notation, whereby δ is a value given in parts per thousand or per mil (‰). The delta value is dimensionless. The δ-notation for an isotope of an element X can be expressed as

$$\delta X\ (‰) = [(R_{sample} - R_{standard})/R_{standard}] \times 1{,}000 \quad (6.1)$$

or

$$\delta X\ (‰) = [(R_{sample}/R_{standard} - 1)] \times 1{,}000 \quad (6.2)$$

where R_{sample} is the relative abundance of the heavy to the light isotope of element X in the sample, and $R_{standard}$ is the isotopic ratio of standard materials (e.g., Pee Dee Belemnite (PDB) for stable carbon isotopes and atmosphere-N_2 for nitrogen isotopes). Using C isotopes as an example, the $\delta^{13}C$ is calculated as

$$\delta^{13}C = [(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{standard} - 1] \times 1{,}000 \quad (6.3)$$

In addition to the δ-notation, other measurement notations include atom percentage and atom percent excess, among others (Hoefs, 2004).

For radiocarbon measurements, $\Delta^{14}C$ is usually used in addition to $\delta^{14}C$ (Broecker and Peng, 1982):

$$\Delta^{14}C = \delta^{14}C - 2\ (\delta^{13}C + 25.0)\ (1 + \delta^{14}C/1{,}000) \quad (6.4)$$

The value of $\Delta^{14}C$ here is a normalized value of $\delta^{14}C$, taking the fractionation between ^{14}C and ^{13}C into consideration.

6.2.2 Isotope Fractionation

Isotope fractionation includes equilibrium fractionation, kinetic fractionation, and fractionation through physical processes. In equilibrium fractionation, the fractionation is temperature dependent and related to differences in thermodynamic properties (e.g., bonding properties) of molecules

with different isotopes. The fractionation is normally larger at lower temperatures and declines with increase in temperature. The higher the temperature the less fractionation occurs. Heavy isotopes are typically enriched in the more condensed phases in equilibrium fractionation (Hoefs, 2004, and references therein).

Kinetic fractionation occurs as a unidirectional process that separates stable isotopes from each other by their mass. This applies to most biogeochemical reactions. Molecules with lighter isotopes react more readily. For example, lighter C and N isotopes (that is, ^{12}C and ^{14}N) are preferentially incorporated into phytoplankton cells during photosynthesis in surface ocean compared to ^{13}C and ^{15}N (Farquhar et al., 1989; Needoba et al., 2003). Meanwhile, fractionation is also related to both temperature and availability of dissolved CO_2 or inorganic N in surface ocean. For ^{14}C isotope, the assumption is that the fractionation of ^{14}C relative to ^{12}C is twice that of ^{13}C, reflecting the difference in atomic mass (Stuiver and Polach, 1977). Other fractionations include those occurring during trophic transfers or food web dynamics, and other mass-independent fractionation, such as the case for sulfur isotopes (Hoefs, 2004).

6.2.3 Isotope Mixing between End-Member Organic Components

Delta (δ) values of stable isotopes of a natural sample can usually be added linearly from different end-member components, making isotope mass balance equations straightforward (Fry and Sherr, 1984; Kwak and Zedler, 1997; Raymond and Bauer, 2001b). For example, measured isotope values (δ_S) of a specific sample, which may be composed of several different end-member sources, can be expressed as

$$\delta_{\text{sample}} = \Sigma f_i \delta_i \tag{6.5}$$

or

$$\delta_{\text{Sample}} = f_1 \delta_1 + f_2 \delta_2 + f_3 \delta_3 + \cdots \tag{6.6}$$

where f_i is the mass or molar fraction of component i, and δ_i is the isotope ratio of component i. This equation can also be used for blank corrections. According to the concept of mass balance,

$$f_1 + f_2 + \cdots = 1 \tag{6.7}$$

For a two-component system, one can calculate the contribution of each component based on the measured isotopic values of sample and components 1 and 2. For example,

$$f_1 = (\delta_{\text{sample}} - \delta_2)/(\delta_1 - \delta_2) \tag{6.8}$$

Using multiple isotopes (e.g., $\delta^{13}C$ and $\Delta^{14}C$; $\delta^{34}S$ and $\delta^{36}S$), one can use multiple equations to solve multiple unknown parameters in a multiple-component system (Bauer et al., 2001).

6.2.4 Carbon Isotopes

Carbon, along with other bioactive elements, is a building block of all biochemical molecules. Among the isotopes listed in Table 6.1, carbon isotopes are the most commonly used and measured for tracing the biogeochemical cycling of marine organic matter. There are three natural carbon isotopes: ^{12}C, ^{13}C, and ^{14}C. Their average natural abundances are approximately 98.89%, 1.11%, and 10^{-10} %, respectively (Table 6.1). The combined measurements of ^{13}C and ^{14}C provide a more complete picture of dynamic cycling of marine organic carbon (Hedges, 1992; Trumbore and Druffel, 1995). Both ^{13}C and ^{14}C are extensively employed to study the sources, reactivity, and

fates of dissolved organic carbon (DOC) and particulate organic carbon (POC) in riverine (Hedges et al., 1986; Peterson and Fry, 1987; Guo and Macdonald, 2006; Raymond et al., 2007), estuarine (Cifuentes et al., 1988; Hedges and Keil, 1999; Raymond and Bauer, 2001a, 2001b; Fry, 2006), coastal, and marine environments (Bauer et al., 1992, 2001; Druffel and Williams, 1992; Druffel et al., 1992, 1996; Santschi et al., 1995; Guo et al., 1996, 2003; Wu et al., 1999; Loh et al., 2004). In addition, the carbon isotopic signatures tend to be less affected by photochemical degradation and structural modification processes than other organic biomarkers. Thus, ^{13}C and ^{14}C are preferentially applied to study carbon biogeochemical cycles over the other organic biomarkers. However, the signatures of $\delta^{13}C$ and $\Delta^{14}C$ in organic matter can also be altered by the bacterial activities during mixing of water bodies (Raymond and Bauer, 2001a).

The use of isotopes $\delta^{13}C$ as fingerprints to trace the contribution from different sources relies on the fact that each source has a distinct $\delta^{13}C$ signal in a DOC or POC pool. Organic matter from marine, terrestrial, and marsh environments has a $\delta^{13}C$ range from –35‰ to –5‰ (Coffin et al., 1994). Typically, organic matter from a terrestrial source (C_3 plants) is relatively depleted in ^{13}C (–35‰ to –25‰), C_4 marsh macrophytes are enriched in ^{13}C (–18‰ to –8‰), and the $\delta^{13}C$ values of C_3 marine phytoplankton are intermediate (Fry and Sherr, 1984; Hedges et al., 1986; Tan, 1987). Two major factors are responsible for the large variation in $\delta^{13}C$ of various organic matter: (1) the pathway of carbon metabolism in plants (Fry, 2006) and (2) the isotopic composition of their carbon source. In the process of carbon fixation during the Calvin cycle (C_3 pathway), the key carboxylating enzyme (ribulose bishposphate carboxylase) discriminates further against the heavier carbon (^{13}C) than the enzyme (phosphoenolpyruvate carboxylase) produced in the Hatch-Slack cycle (C_4 pathway), resulting in a more negative $\delta^{13}C$ in the C_3 plants than in C_4 plants. On the other hand, land plants use atmospheric CO_2 ($\delta^{13}C$ = –7‰) as their carbon source, while marine plants assimilate HCO_3^- ($\delta^{13}C$ = ~0‰) from seawater (Libes, 1992). Other factors also contribute to the fractionation of ^{13}C and ^{14}C in plants, including temperature (Rau et al., 1982), partial pressure of CO_2 (Rau et al., 1991b), growth rate (Laws et al., 1995), and community structure of the primary producer (Falkowski, 1991).

Because the ^{14}C is radioactive, one can use it to measure apparent ages of organic matter in marine environments (Williams and Druffel, 1987; Bauer et al., 1992; Santschi et al., 1995). The presence of ^{14}C in organic materials is the basis of the radiocarbon dating method. The half-life of ^{14}C is 5,730 ± 40 years. Therefore, radiocarbon can be used to trace marine biogeochemical processes with a timescale of ~50,000 years. The relationship between $\Delta^{14}C$ and the age of carbon-containing materials can be expressed as

$$\text{Radiocarbon age (years BP)} = -8033 \times \ln\left[1 + (\Delta^{14}C/1{,}000)\right] \tag{6.9}$$

where –8,033 represents the mean lifetime of ^{14}C and ln represents the natural logarithm. Table 6.2 shows examples of the radiocarbon composition of marine organic matter. One fundamental assumption for radiocarbon dating is that there is a negligible exchange of organic carbon between the target reservoir and its environment. The application of ^{14}C as a proxy of the age of organic matter is, however, complicated and ambiguous (Bauer, 2002; Mortazavi and Chanton, 2004), mainly due to mixing processes. Single-compound dating could better reflect the true age of the source material.

6.2.5 Nitrogen Isotopes

Nitrogen has two stable isotopes, ^{14}N and ^{15}N, and their natural abundances are 99.63% and 0.37%, respectively. The average abundance of ^{15}N in the atmosphere ($\delta^{15}N$ = 0‰) is relatively constant at 0.366%. Atmospheric N_2 is thus used as the standard for reporting $\delta^{15}N$ values. The natural variation of the N isotopic signal of different source materials is useful for understanding food web dynamics and organic matter cycling in ecosystems, especially for tracing the source of nitrogen to aquatic systems (Schell et al., 1998; Sigman, 2000; Knapp et al., 2005; Fry, 2006). While C isotopes

TABLE 6.2
Examples of Stable Isotope Composition of Marine Organic Material

Location	Organic Fraction	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Reference
Marine	DOC	–21.8	—	Eadie et al. (1978)
Pacific	DON	—	4–11	Gedeon et al. (2001)
Atlantic	DON	—	3.35–4.68	Knapp et al. (2005)
Patuxent estuary	COM	–24.8	8.5–10.8	Sigleo and Macko (1985) Sigleo (1996)
Pacific Ocean	COM	–21.63 ± 0.17	7.93 ± 0.69	Benner et al. (1997)
Atlantic Ocean	COM	–22.0 ± 0.26	8.0 ± 1.2	Benner et al. (1997)
Chesapeake Bay	COM	–24.08 ± 0.42	8.83 ± 0.19	Sigleo and Macko (2002)
San Francisco Bay	COM	–26.70 ± 0.72	7.92 ± 0.84	Sigleo and Macko (2002)
Gulf of Mexico	COM	–21.67 ± 0.76	3.87 ± 0.65	Guo et al. (2003)
Middle Atlantic Bight	COM	–21.2 ± 0.15	5.49 ± 0.77	Guo et al. (2003)
Boston Harbor	COM	–30.1 to –23.7	2.8–5.7	Zou et al. (2004)
Delaware/Chesapeake Bay	COM	–25.1 to –23.1	4.4–8.9	Zou et al. (2004)
San Francisco Bay	COM	–26.1 to –23.1	5.1–6.4	Zou et al. (2004)
Mississippi River plume	COM	–22.6 ± 1.0	4.3 ± 0.5	Guo et al. (submitted)
Atlantic	POM	—	4–11	Altabet (1988)
North Pacific	POM	–23.20 ± 0.62	3.06 ± 1.16	Wu et al. (1999)
Atlantic	POM	—	–0.8–5.4	Mino et al. (2002)
Seawater	Plankton	–21.3 ± 1.1	8.6 ± 1.0	Peterson et al. (1985)
Arctic	Zooplankton	–20.2 to –25.6	5.8–14.2	Schell et al. (1998)

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Note: DOM, dissolved organic matter in <0.45 or 0.7 μm bulk fraction; COM, colloidal organic matter in the size fraction between 1 kDa and 0.2 or 0.7 μm; POM, particulate organic matter in the >0.7 μm or sediment trap samples.

vary little during trophic transfers, N isotopes undergo significant changes between trophic levels in marine environments (e.g., Schell et al., 1998). Thus, the use of multiple stable isotopes can provide insights into the biogeochemical cycling of marine organic matter.

Examples of N-isotope measurements for marine organic matter are given in Table 6.2. Most environmental $\delta^{15}N$ values vary within the range from –10 to +20‰ (Peterson and Fry, 1987). Although N isotopic measurements have been applied to study the effects of denitrification and assimilation in the ocean (Liu and Kaplan, 1984; Sigman, 2000), measurements of N isotopes in marine organic matter so far have been carried out mainly on phytoplankton, suspended and settling particles, and sediment samples (Wada and Hattori, 1976; Altabet, 1988; Saino and Hattori, 1987; Cifuentes et al., 1988; Benner et al., 1997; Schell et al., 1998; Chen et al., 2006). Measurements of $\delta^{15}N$ on marine dissolved organic matter are still scarce, although there are increasing reports on COM and $DO^{15}N$ measurements (Sigleo and Macko, 1985; Feuerstein et al., 1997; Guo et al., 2003; Knapp et al., 2005; Miyajima et al., 2005).

6.2.6 Sulfur Isotopes

Sulfur has four stable isotopes, ^{32}S, ^{33}S, ^{34}S, and ^{36}S. Their abundances are 95.02%, 0.76 %, 4.22%, and 0.02%, respectively (Table 6.1). Canyon Diablo meteorite is used as a standard for sulfur isotope measurements, which are reported relative to troilite in the Canyon Diablo iron meteorite (CDT).

Sulfur is normally a minor component of natural organic matter. On average, sulfur to organic carbon ratios range from 0.014 for humic substance (Aiken et al., 1985; Thurman, 1985) to 0.037 for marine organic matter (Guo et al., 1999). In marine sediments, the organic S/C ratio varied

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TABLE 6.3
Examples of Radiocarbon Composition of Marine Dissolved, Colloidal, and Particulate Organic Matter

Location	Organic Matter Type	$\Delta^{14}C$ (‰)	Apparent ^{14}C Age (Year BP)	Reference
Pacific	DOC	–150 to –540	1,300 to 5,710	Williams and Druffel (1987)
Gulf of Mexico	COM	–126 to –432	1,076 to 4,538	Santschi et al. (1995)
Atlantic	COM	–89 to –403	751–4,143	Guo et al. (1996)
Arctic	COM	–87 to –379	680–3,770	Benner et al. (2004)
Pacific	POC	43–139	> Modern	Druffel and Williams (1990)
Pacific	Sinking POC	99–136	> Modern	Druffel and Williams (1990)

from 0.01 in surface sediments to 0.11 in deeper or older sediments (Lückge et al., 2002). Natural variation in sulfur isotopes can be used for tracing sediment diagenesis, organic matter cycling, and natural and anthropogenic solute sources. However, few measurements of sulfur isotopes in marine organic matter have been made. Available data of sulfur isotopic composition are for organic matter in suspended particulate matter, sinking particles, and marine organisms (Peterson and Fry, 1987). Since the concentration of inorganic sulfate in seawater far outweighs the concentration of organic matter, separation and purification of organic sulfur from sea salts and sulfate is the first step for the measurement of its isotopes in marine organic matter, especially dissolved organic matter. Adding to this difficulty, sulfur content in organic matter is much lower than the contents of carbon and nitrogen (Guo et al., 1999); therefore, the measurements of sulfur isotopes require a much larger sample size than C and N isotope analysis.

6.2.7 Hydrogen Isotopes

Hydrogen has three isotopes, two stable isotopes, ^{1}H and ^{2}H (D for deuterium), and one radioactive isotope (^{3}H or T for tritium). The radioactive isotope tritium (half-life $t_{1/2}$ = 12.3 years) can be used for tracing water mixing with a timescale within 100 years. Although most measurements of δD or D/H ratio are used to trace water sources and hydrological processes, the D/H ratio has been widely used to study the diagenesis of sedimentary organic matter, organic geochemistry, and climate and environmental changes, since D/H ratios of organic hydrogen can preserve quantitative information about paleoenvironmental and paleoclimate conditions. In recent years, measurements of D/H ratios of carbon-bound hydrogen in individual organic compounds have considerably advanced our knowledge on climate and environmental changes (Sauer et al., 2001; Huang et al., 2004). However, measurements of hydrogen isotopes of marine organic matter are still scarce despite their great potential to trace terrestrial input to the marine environment, link food web and hydrology, and extend the carbon cycling studies (Malej et al., 1993; Fry, 2006).

6.2.8 Oxygen Isotopes

Oxygen has three stable isotopes, ^{16}O, ^{17}O, and ^{18}O. Natural variation of the oxygen isotopic composition can be used for determining water mixing, precipitation sources, and evaporation effects, as well as for deciphering paleoceanographic, paleoclimate, and paleohydrologic records. Similar to hydrogen isotopes, measurements of oxygen isotopes in marine samples have been mostly made for water samples and sediment core samples. Few measurements of oxygen isotopes have been made for marine dissolved organic matter, and little is known about how oxygen isotopes are incorporated into marine organic matter.

6.3 SAMPLING OF MARINE DISSOLVED ORGANIC MATTER

The IR-MS system coupled with elemental analyzer and combustion chamber has become a routine procedure for stable isotope measurements of bulk organic carbon and nitrogen. Many commercial stable isotope facilities or organizations/agencies have provided services for routine analyses of $\delta^{13}C$ and $\delta^{15}N$ in water samples at affordable prices (e.g., http://stableisotopefacility.ucdavis.edu/). Similarly, advances in accelerator mass spectrometry (AMS) have reduced the sample size for radiocarbon analysis to micrograms of carbon (e.g., Tuniz et al., 1998). However, direct measurements of stable isotope composition of marine dissolved organic matter remain difficult except for carbon isotopes (Beaupre et al., 2007; Osburn and St.-Jean, 2007; Lang et al., 2007). Sampling of marine organic matter is a limiting step in the measurements of isotope composition, especially for N and S isotope analyses. The development in sampling technique of particulate organic matter (suspended and sinking particles, plankton and sediment samples) has provided sufficient amounts of particulate material for the measurement of isotope composition. In this section, focus will be on the sampling of DOM and COM from seawater for stable isotope analysis.

6.3.1 Bulk DOM for $\delta^{13}C$ and $\Delta^{14}C$ Measurements

There are several factors causing measurements of $\delta^{13}C$ and $\Delta^{14}C$ in seawater DOC to be difficult. First, the DOC concentrations in seawater are very low, ranging from 35–45 μM in deep waters to 60–80 μM in surface waters (Guo et al., 1995; Hansell and Carlson, 1998; see also Chapter 2). In addition, there are different ways to oxidize the DOC, including high-energy UV irradiation (Williams and Gordon, 1970; Williams and Druffel, 1987; Bauer et al., 1992; Beaupre et al., 2007), wet chemical oxidation (St.-Jean, 2003; Osburn and St.-Jean, 2007), high-temperature sealed tube oxidation of the lyophilized DOC/salt mixture (Peterson et al., 1994; Fry et al., 1996), combined UV-persulfate oxidation (Bauer et al., 1992; Clercq et al., 1998; Bouillon et al., 2006), and high-temperature catalytic oxidation of seawater DOC (Lang et al., 2007). However, some uncertainties exist in the quantitative oxidation of DOC and subsequent conversion to CO_2 (see Chapter 2).

Measurements of isotopic composition ($\delta^{13}C$ and $\Delta^{14}C$) of dissolved organic carbon (DOC) in the ocean have been reported since the 1960s (Williams et al., 1969; Williams and Gordon, 1970). In recent years, UV oxidation and high-temperature combustion are the most used techniques to convent DOC into CO_2 for isotope analysis (Bauer et al., 1992; Druffel et al., 1992; Beaupre et al., 2007; Lang et al., 2007). For low-DOC oceanic waters, a traditional vacuum line is needed to carry out large-volume seawater DOC oxidation and CO_2 purification using either UV oxidation (Figure 6.2) or high-temperature catalytic oxidation (Figure 6.3).

Seawater samples are filtered through precombusted quartz filters or glass fiber filters (GF/F, 0.7 μm) or prerinsed polycarbonate Nuclepore filters (0.45 μm) to remove particulate organic matter (see Section 2.3.1). Such pore size of filters cannot remove all bacteria from the seawater, and the DOM in samples can be decomposed microbially and photochemically. Thus, samples should be preserved or frozen immediately after sample filtration to prevent bacterial utilization of DOC (see Section 2.3.2). However, many laboratories do not recommend preservation with $HgCl_2$.

Precombusted quartz or glass bottles are used for DOC sampling and storage. Based on recommendations by the JGOFS program and GEOTRACES studies, high-density polyethylene (HDPE) bottles can also be used for DOC sampling (Dickson et al., 2007) and have been adopted by commercial stable isotope laboratories. One advantage of using HDPE bottles is that samples are safer during sample freezing and transportation than those in quartz or glass bottles.

Due to low DOC concentration in seawater, at least 50 ml of filtered seawater is needed to yield a total of 20–50 μg of DOC for C isotope analysis. With an improved low-blank UV oxidation and extraction system, this sample size is now sufficient for both $d^{13}C$ and $\Delta^{14}C$ measurements (Beaupre et al., 2007), which could not be measured previously.

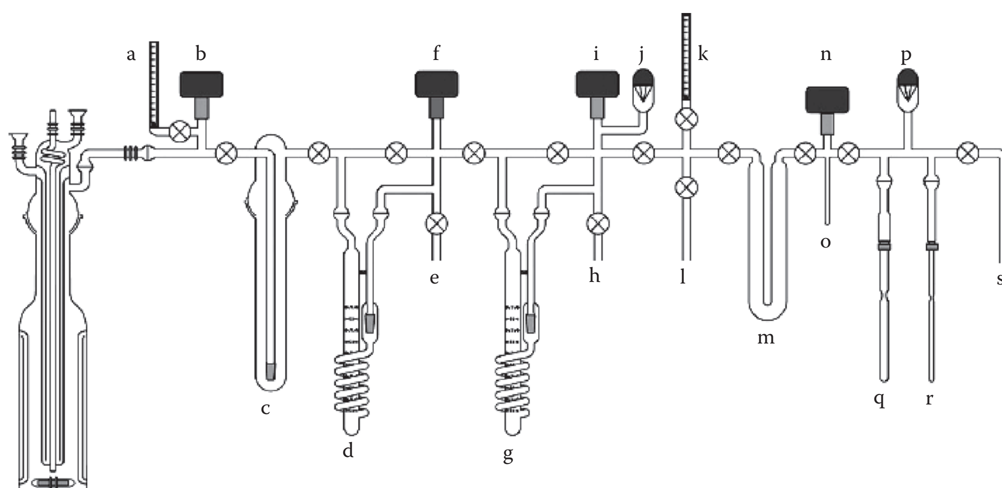

FIGURE 6.2 A schematic showing traditional vacuum line for purifying CO_2 from the oxidation of DOC in seawater. Individual components are connected by spherical joints with Viton o-rings. (A) Flow meter 1, (K) flow meter 2,(B, F, I, N) capacitance manometers, (J, P) thermocouple pressure gauge sensors, (C) KI solution trap, (D) modified Horibe trap 1 (dry ice/isopropanol slush bath), (G) modified Horibe trap 2 (liquid nitrogen bath), (M) U-tube trap, (O) calibrated volume with a 7 mm OD, 7 cm long cold-finger, (Q, R) break-seal tubes for 14C and 13C splits, respectively, secured by internally threaded o-ring adapters with Teflon bushings, (E, H, L, S) conduits of a manifold (not shown for clarity) leading to the vacuum pump. (From Beaupre et al., 2007; with kind permission of American Society of Limnology and Oceanography.)

6.3.2 Bulk DOM for Stable N Isotope Analysis

Measurements of N isotopes have been widely conducted for nitrate or dissolved inorganic nitrogen (DIN) pools in seawater (Liu and Kaplan, 1984, 1989; Sigman et al., 2001, 2005) and marine particulate organic matter samples (Altabet, 1988; Wu et al., 1997; Schell et al., 1998; Waser et al., 2000). However, procedures for direct measurements of N isotopes in the DON pool are still lacking. Currently used methods involve multiple measurements and a number of complex preparation

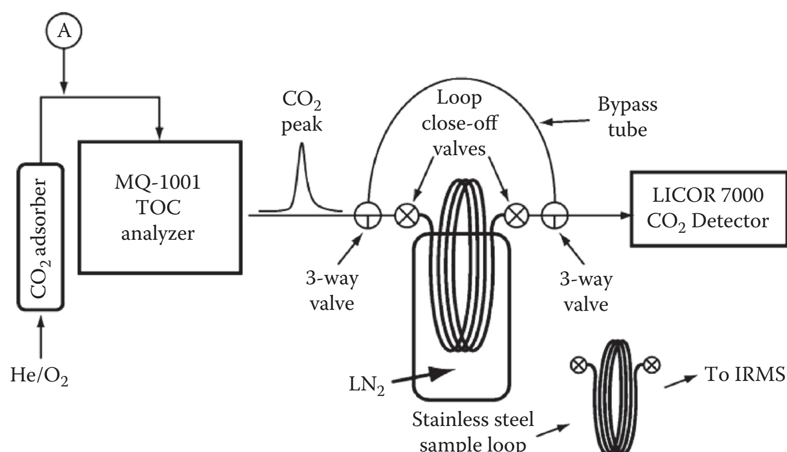

FIGURE 6.3 Schematic of oxidation and trapping system using high-temperature combustion and liquid N_2 for CO_2 trapping. (From Lang et al., 2007, with kind permission from Elsevier Limited.)

processes prior to mass spectrometric determination of the $^{15}N/^{14}N$ ratio. Recently, Miyajima et al. (2005) used gas chromatography/negative-ion chemical ionization mass spectrometry (GC-NICI-MS) to determine ^{15}N enrichment of DON in natural waters after derivatization of NO_3 with pentafluorobenzyl bromide. Knapp et al. (2005) have reported $\delta^{15}N$ isotope values for bulk DON in seawater, based on the measurements of N isotopes in the nitrate (NO^{3-}) pool coupled with the commonly used persulfate oxidation method to convert total dissolved nitrogen (TDN) into nitrate (see Section 9.4.1.2), followed by the denitrifier method for isotopic analysis of NO_3. Similar to the measurements of DON, this method also involves multiple procedures and measurements, but it provides reasonable values of $\delta^{15}N$ of bulk DON pool in seawater for the first time.

Based on Knapp et al. (2005), 12 ml of filtered seawater is used for the conversion of TDN to NO_3^-, with the addition of 2 ml of a persulfate oxidizing reagent (Solorzano and Sharp, 1980), followed by autoclaving for 55 minutes on a slow vent setting. The reagent blank is based on the measurements of NO_3^- and $\delta^{15}N$ in 12 ml of the persulfate oxidizing reagent. The concentration and isotopic composition of DON are calculated by mass balance from the analyses of $\delta^{15}N$ of TDN and NO_3^- in filtered seawater samples.

6.3.3 Seawater Dissolved Organic Sulfur for Isotope Analysis

All four stable isotopes of sulfur (^{32}S, ^{33}S, ^{34}S, and ^{36}S) can be measured at the nanomole level (Ono et al., 2006). However, there are no reports for direct measurements of S isotope composition in marine dissolved organic matter samples. This is largely due to the low organic sulfur concentration and high sea salt content in seawater, although measurements of stable S isotopes in the sulfate pool have been reported previously (Rees et al., 1978).

6.4 SAMPLING OF HMW DISSOLVED ORGANIC MATTER FROM SEAWATER

As discussed in the previous section, measurements of stable isotopic composition in marine dissolved organic matter have been hampered by the difficulty in the quantitative extraction of DOM from seawater. Once DOM is isolated from sea salt and lyophilized into powdered DOM samples, measurements of stable isotopes will become routine.

As shown in Figure 6.1, dissolved organic matter also contains a significant or considerable portion of high molecular weight (HMW) or colloidal organic matter that can be further isolated using a number of separation and isolation techniques, such as cross-flow ultrafiltration, solid phase extraction, and reverse osmosis coupled with electrodialysis. Marine DOM is heterogeneous and contains different organic components with different molecular weights and isotope signatures, which makes the sampling of size-fractionated DOM and the measurement of their isotopes even more meaningful (Santschi et al., 1995, 1998; Guo et al., 1996; Loh et al., 2004; Wang et al., 2006). The solid phase extraction method has been proved to be simple and efficient for extracting DOM from seawater, although it requires adjustments of the pH of samples before extraction (Dittmar et al., 2008). In this section, the ultrafiltration method, solid phase extraction, and the newly developed reverse osmosis/electrodialysis method will be discussed.

6.4.1 Ultrafiltration Method for Isolating HMW-DOM

Cross-flow ultrafiltration has been widely applied to extract DOC from seawater due to its capability in processing large volumes of seawater, and has proven to be an effective and efficient tool for deciphering chemical and isotopic composition of marine dissolved organic matter (Benner et al., 1992; Moran and Buesseler, 1992; Santschi et al., 1995; Guo et al., 1996; Guo and Santschi, 2007, and references therein). A typical ultrafiltration system is composed of a prefiltration component and ultrafiltration component (Figure 6.4). The prefiltration system is usually composed of a peristaltic pump, tubing, and a prefilter with a pore size ranging from 0.2 to 1 μm, depending on research purposes, while the ultrafiltration system consists of ultrafiltration cartridges with a molecular weight

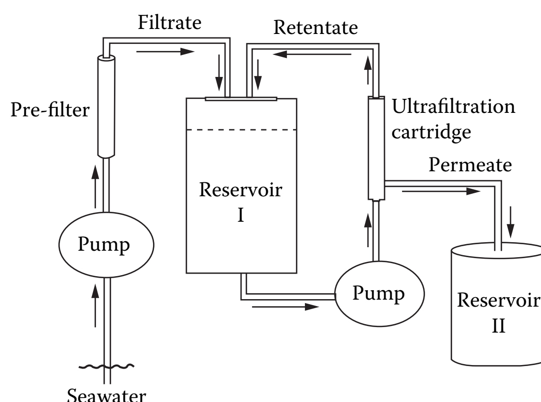

FIGURE 6.4 A diagram showing components of a typical cross-flow ultrafiltration system, including prefiltration and ultrafiltration. The prefiltration system is usually composed of a peristaltic pump, a prefilter with a pore size ranging from 0.2 μ to 1 μm, and Teflon tubing, while the ultrafiltration system consists of ultrafiltration cartridge(s), reservoirs for prefiltered seawater and permeate, pump, tubing, and other accessories.

cutoff ranging from 0.5 to 200 kDa, reservoirs for prefiltered seawater and permeate, a pump, Teflon tubing, and other accessories (Figure 6.4).

To avoid contamination, surface seawater is peristaltically pumped through a prerinsed cartridge prefilter (in-line setup) into an ultrafiltration reservoir. The first several tens of liters of filtrate should be discarded and used for rinsing reservoirs. Deep water collected by Niskin bottles is transferred to an enclosed reservoir by closed in-line tubing and then pumped peristaltically through a prerinsed filter cartridge into an ultrafiltration reservoir. Filtered seawater should be ultrafiltered immediately after sample collection (Guo et al., 1996).

New ultrafiltration cartridges should be thoroughly cleaned with laboratory detergent (e.g., Micro solution), NaOH, HCl solution, and large volumes of ultrapure water before sampling. Concentrations of each chemical are determined based on manufacturer recommendations for specific ultrafiltration membranes. Between cleaning solutions, large volumes of ultrapure water should be used to rinse the cartridges. To enhance the efficiency during cartridge cleaning, a recirculation mode is used for cleaning with chemical solutions (e.g., Micro, NaOH, and HCl), while a flushing mode or once-through mode (both retentate and permeate lines going into a waste) is used during ultrapure water rinsing/cleaning. Each chemical is recycled for 20–30 minutes under normal operating conditions and allowed to soak for another 20–30 minutes. Between each solution, large volumes of ultrapure water are then flushed through the ultrafiltration system, and after chemical cleaning, more ultrapure water is flushed again under normal ultrafiltration operating conditions. Once the cartridges are cleaned, a small volume of prefiltered seawater is used to condition the cartridges before ultrafiltration (Guo et al., 2000). Used ultrafiltration membranes are usually preserved in NaN_3 solution, so they should be cleaned before sampling based on the same cleaning procedure for new cartridges.

Cartridges are cleaned between samples (or sampling stations) with NaOH and HCl solutions. All ultrafiltration cartridges should be calibrated and checked for their integrity before use with macromolecules of known molecular weights based on specific cartridge cutoffs. The standard macromolecules used for calibration should have a molecular weight slightly higher than the membrane's molecular cutoff (e.g., >1 kDa standard molecule vs.1 kDa membrane, 14 kDa standard molecule vs. 10 kDa membrane, etc.). Further details about cartridge calibration procedures are described in Guo et al. (2000).

The HMW-DOC fraction isolated by cross-flow ultrafiltration is largely dependent on membrane pore size (usually ranging from 0.5 to 200 kDa), membrane materials (hydrophobic vs. hydrophilic),

and operational conditions (e.g., concentration factor used). A significant fraction of low molecular weight (LMW) DOC can be retained during ultrafiltration (Guo and Santschi, 1996; Guo et al., 2000). Therefore, a high concentration factor is recommended, although the retained LMW-DOC may be flushed out during the diafiltration process (Guo et al., 2000, 2001). After ultrafiltration, the retentate of the water sample, which is highly concentrated in DOC, is desalted using diafiltration with large volumes of ultrapure water. Then, the sample is freeze-dried at –45°C to become powdered DOM for further analysis (Guo and Santschi, 1996). Due to the low concentrations of DOC in seawater and high concentrations of sea salts, diafiltration (desalting) is a necessary step in isolating COM for isotopic and chemical characterization. About 20 L of ultrapure water is sufficient for the diafiltration of a retentate solution (Guo and Santschi, 1996). After desalting, the isolated HMW-DOC may represent 5–45% of the bulk DOC in seawater, depending on initial DOC concentration and ultrafilter cutoffs used in ultrafiltration.

6.4.2 Solid Phase Extraction Method

Solid phase extraction has been used in isolating DOM samples from seawater for chemical and isotopic characterization (Louchouarn et al., 2000; Dittmar et al., 2008, and references therein). Louchouarn et al. (2000) used C18–solid phase extraction (SPE) cartridges to determine the DOM extraction efficiency from seawater and the reusability of the C18-SPE cartridges. They found that the DOM recovery rate by the C18-SPE cartridges was comparable to that by the ultrafiltration method and higher at lower pH (1.5–4.0) conditions, while the extraction efficiencies were independent of flow rate.

Recently, Dittmar et al. (2008) presented a protocol for efficient extraction of DOM from seawater using commercially prepacked cartridges. They found a much higher DOM recovery using styrene divinyl benzene polymer type sorbents (PPL) compared to the C18 cartridges. Based on results from NMR, C/N, and $\delta^{13}C$ measurements, they found that styrene divinyl benzene polymer type sorbents (PPL) could extract up to 62% of DOC as salt-free extracts, while the C18 extracts about 40% of bulk DOC. Figure 6.5 shows the seawater DOM extraction procedures using styrene

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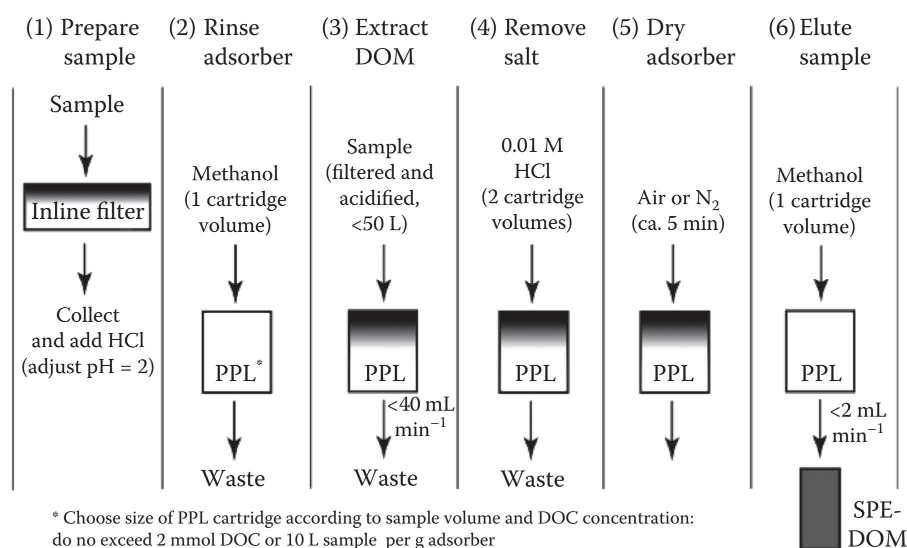

FIGURE 6.5 Procedures of DOM solid phase extraction using commercially prepacked cartridges, styrene divinyl benzene polymer type sorbents (PPL). (From Dittmar et al., 2008, with kind permission of the American Society of Limnology and Oceanography.)

divinyl benzene polymer type sorbents based on Dittmar et al. (2008). After seawater samples are filtered through precombusted GF/Fs, filtrates are acidified to pH 2 with HCl for the extraction of organic acids and phenols. To avoid overloading, less than 10 L of sample or a total of 2 mmol of DOC for 1 g of sorbent is used for extraction. The cartridges are rinsed with one column volume (CV) of methanol before extraction. To extract DOM, the seawater samples are passed through the cartridges with a peristaltic pump or gravity at a flow rate of 40 ml min^{-1} (Figure 6.5, step 3). Before DOM elution, the cartridges are rinsed with at least two CV of 0.01 M HCl to remove residual salts. DOM is eluted with one CV of methanol at a flow rate of <2 ml min^{-1} into muffled glass ampoules (Figure 6.5, step 6). The elutes are stored at –20°C until further processing and analysis.

6.4.3 Reverse Osmosis/Electrodialysis Method

Vetter et al. (2007) used the coupled reverse osmosis (RO)/electrodialysis (ED) system to separate DOC from seawater samples (Figure 6.6). This new separation method can recover up to 64–93% of marine DOC and has greatly improved the recovery efficiency of marine DOM. This system could be used on board research vessels. Immediately after sample collection, seawater is filtered through a 0.45 µm filter to remove particulate matter and transferred to the tank of the RO/ED system. The RO/ED system includes a commercial RO module (Dow FilmTec TW30-4021) from the Dow Chemical Company (Midland, Michigan), a Standex Procon CMP-7500 SS pump (Procon, Murfreesboro, Tennessee), and stainless steel tubing and fittings (Figure 6.6). The permeate fluxes are about 0.5–2 L min^{-1} under 200–210 PSIG. The electrodialysis component is composed of membranes (Neosepta AMX and CMX, from Ameridia) and electrodialysis stacks (Type 100, DeukumGmbH, Frickenhausen, Germany; 50 and 100 cell pairs, respectively). The anode is platinized titanium mesh, while the cathode is a stainless steel plate.

The RO system is operated at 200 PSIG pressure with a feed flow rate of 400 L per hour to the module. The flow rate through the seawater circuit of the ED system is about 640 L per hour. The flow rate in the ED concentrate circuit was roughly equal to that in the seawater circuit, while the flow rate in the ED electrode rinse was adjusted to avoid pressure gradients to the concentrate and seawater circuits. Before sampling, the RO/ED system, tanks, and tubing are first rinsed with filtered seawater. After every run, the RO module is rinsed with NaOH solution (0.01 M) manually by slowly filling and draining the RO pressure vessel several times. Electrodialysis is performed in the constant electrical current/variable voltage mode.

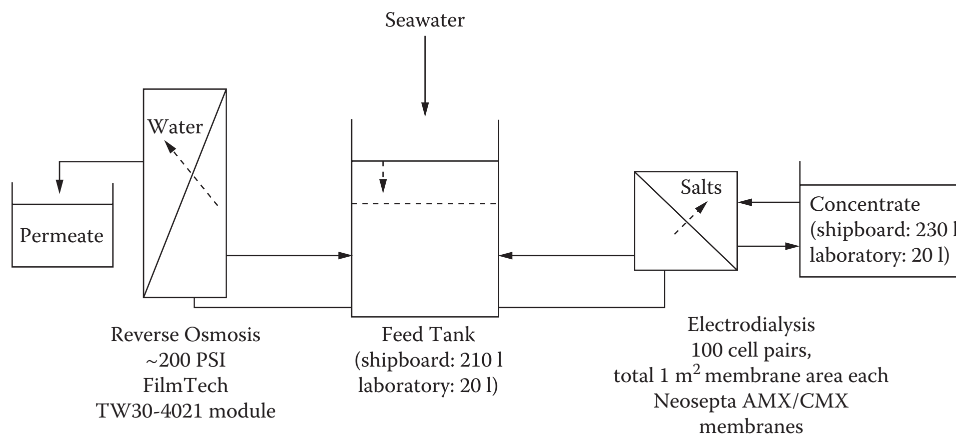

FIGURE 6.6 System for DOM separation from seawater combining reverse osmosis and electrodialysis techniques. (from Vetter et al., 2007, with kind permission from Elsevier Limited.)

6.5 SAMPLING OF PARTICULATE ORGANIC MATTER FROM SEAWATER

Conventional gravity and vacuum filtration methods have been widely used for collecting POM samples for stable isotope analysis (Sharp, 1973; Druffel and Williams, 1990). However, there are potential problems related to small-volume filtration, such as blank and DOC sorption on filters (Moran et al., 1999; Liu et al., 2005). Other practical methods for collecting marine POM samples include (see also Chapter 13):

- Multiple-unit large-volume *in situ* filtration system (MULVFS): This *in situ* filtration system can collect up to 100–200 mg of particulate samples (e.g., 1–53 μm or >1 μm) for organic and isotopic analyses, depending on membrane pore size, type of filters, and pumping time (Bishop and Edmond, 1976).
- McLane filtration system: This filtration system can collect particulate sample onto a 142 mm membrane filter (either GF/F or other membrane types) by drawing ambient seawater through the filter holder with a rotary pulse pump. This is a single-event filtration system, capable of pumping up to 25,000 L per deployment, and requires multiple systems for multiple-layer sampling (see also http://www.mclanelabs.com).
- Sediment traps: This device can collect sinking particles *in situ* by differential settling in a cylindrical settling trap. It could also separate different particle size fractions with different settling velocities (Peterson et al., 2005; Buesseler et al., 2007). While some preservatives used in sediment trap deployment may affect measurements of stable isotopes and other organic components, short-term sediment trap deployments may provide sufficient amounts of POM for isotope analysis.

In addition to these methods, continuous flow centrifugation, Go-Flo rosette filtration, including direct filtration (see Figure 8.3) and offline filtration of seawater subsample, aggregate sampling devices (e.g., Lunau et al., 2004), and other *in situ* filtration devices are being used for collecting POM samples from seawater.

6.6 SAMPLE PREPARATION

6.6.1 Sample Processing for Bulk Organic Samples

Sample processing for direct measurements of stable C and N isotopes in bulk DOM samples is discussed in Sections 6.3.1 and 6.3.2. For isolated COM and POM samples, carbonates are first removed before organic carbon and its isotope analyses (Guo and Macdonald, 2006). This is done through either acid fuming by using concentrated HCl in a closed desiccator or acid treatment using diluted HCl solution. Recent studies recommend the use of acid fuming for the removal of inorganic carbon from organic samples for stable isotope and radiocarbon analyses (Komada et al., 2008; see Section 2.3.3 for detailed procedure). While there is a difference in OC content and its isotope composition between acid fumigation and direct acidification, N and its isotope composition show little difference between these two removal processes.

For isolated COM samples, most of the DIC, DIN, and sulfate can be removed during diafiltration processes, and only DOM components are retained after diafiltration. Thus, there will be little inorganic substances in the desalted COM samples. However, measurements on COM samples without proper desalting or diafiltration could bias the isotope signatures of COM due to the influence of residual DIC, DIN, and sulfate in isolated COM samples.

6.6.2 Organic Compound Class Separation for Stable Isotope Analysis

Using stable C isotope as an example, methods for separating total lipid, total hydrolyzable amino acids (THAAs), total carbohydrates (TCHOs), and other organic residue fractions have been

developed and successfully used in many previous studies on COM, POM, and SOM (Wang et al., 1996, 1998, 2006; Wang and Druffel, 2001; Loh et al., 2004; Zou et al., 2004). Sample size for compound class isotopic analysis can be as little as a few milligrams of organic carbon, which can be separated into several organic fractions, with 97–103% recovery for organic C using standard compounds (Wang et al., 1996, 1998). The blank effects on $\Delta^{14}C$ and $\delta^{13}C$ measurements during sample processing and combustion are negligible (Wang et al., 1998). Separation procedures for total lipids, THAAs, TCHOs, and organic residue are briefly described below.

6.6.2.1 Lipid Extraction

Isolated marine organic matter samples are first extracted for total lipids with a 2:1 v/v mixture of methylene chloride:methanol (both high-purity grade). The extraction is repeated four times, and the combined extracts are dried by rotary evaporation. The dried sample is transferred with methylene chloride into a precombusted quartz tube and then dried with a high-purity N_2 gas stream. The extracted residue left in the centrifuge tube after lipid extraction is dried at room temperature for THAA and TCHO extractions (Wang et al., 1998).

6.6.2.2 Total Hydrolyzable Amino Acids Isolation

Isolation of the THAA fraction from marine DOM and POM requires acid hydrolysis to break down the polymer forms, such as proteins (or peptides), to the free amino acids (McCarthy et al., 2004). After lipid extraction, half of each dried DOM/POM sample is weighed directly into a 50 ml glass centrifuge tube and hydrolyzed with 6 M HCl (Ultrex pure) under N_2 at 100°C in an oven for 19 hours. After hydrolysis, samples are centrifuged and the supernatants are transferred into 100 ml pear-shaped glass flasks. The remaining solid material is rinsed twice with ultrapure water, centrifuged, and the supernatants combined with the acid hydrolysate. The hydrolysate is dried by either rotary evaporation or freeze drying.

lhe dried THAA fraction is dissolved in 2 ml of ultrapure water and desalted using cation exchange column chromatography, with AG 50 W-X8 resin (100–200 mesh, analytical grade, BioRad) that had been soaked in 6 M, double-distilled HCl for at least 1 week and rinsed with ultrapure water several times. Free amino acids are collected in a 1.5 N NH_4OH elute and dried by rotary evaporation or freeze drying. The dried sample is transferred with ultrapure water to a precombusted quartz tube and dried again in a desiccator *in vacuo* for final combustion (Wang et al., 1998).

6.6.2.3 Total Carbohydrate Isolation

The extraction and isolation of total carbohydrates (TCHOs) is based on the method used by Cowie and Hedges (1984). Another half of the residue in the centrifuge tube after lipid extraction is hydrolyzed with H_2SO_4 at 100°C for 3 hours. After hydrolysis, finely ground $Ba(OH)_2 \cdot 8H_2O$ is added to neutralize the acid, and the resulting $BaSO_4$ is removed by centrifuging. The solution is adjusted to a pH of 6–7 with 1.5 N NH_4OH. The hydrolysate containing free sugars is desalted on a 20 ml mixed cation/anion column packed with mixed (1:1 v/v) cation AG 50W-X8 (100–200 mesh) and anion AG 1-X8 (100–200 mesh) resins. The volume of TCHO fraction collected by elution with ultrapure water is reduced to about 2 ml by rotary evaporator at 50°C, then transferred into a precombusted quartz tube for final combustion (Wang et al., 1998). Santschi et al. (1998) used an alcohol extraction/precipitation method to extract and purify polysaccharides from marine HMW-DOM samples for radiocarbon measurements.

6.6.2.4 Acid-Insoluble Fraction

The material left in the centrifuge tube after removal of the supernatant and rinses is defined as the acid-insoluble fraction. This solid material is transferred into a precombusted quartz tube (with 1 ml

ultrapure water, three or four times), and the sample is dried in a desiccator *in vacuo* for later combustion.

6.6.3 Procedures for Specific Organic Compound Separation

6.6.3.1 Fatty Acid Separation

Aliquots of freeze-dried isolated DOM, COM, or POM samples (about 10–90 mg of organic matter) are first extracted with 10 ml of methanol, followed by 3 × 10 ml of methylene chloride:methanol (2:1 v/v). The extracted lipids are further saponified at 100°C with 0.5 M KOH in methanol/H_2O (95:5 v/v) for 2 hours. Neutral lipids are first extracted from the solution at pH ~13, while fatty acids are extracted at pH < 2 after addition of HCl to the solution. Fatty acids are methylated with BF_3-methanol at 100°C for 2 hours to form fatty acid methyl esters (FAMEs). The FAMEs are quantified by capillary gas chromatography (GC) with an on-column injector and a flame ionization detector. Prior to GC analysis, a known amount of internal standard (nonadecanoic acid methyl ester) is added to each extract to aid quantification. FAMEs are analyzed on a 30 m × 0.25 mm i.d. HP-5 column coated with 5% diphenyl–95% dimethylsiloxane copolymer (0.25 μm film thickness). The following GC temperature program is implemented: 60 to 150°C at 10°C min^{-1}, then 150 to 310°C at 4°C min^{-1} and isothermally at 310°C for 5 minutes. FAMEs are identified from mass spectra obtained from a GC-MS system. The operating conditions for GC-MS are helium as carrier gas; mass range 50–610 kDa with a 0.4-second scan interval; 70 eV ionizing energy; GC temperature program 50 to 150°C at 20°C min^{-1} followed by 150 to 310°C at 4°C min^{-1} and a 5-minute hold at 310°C.

Fatty acid molecular stable carbon isotopic compositions are measured using a GC combustion system interfaced with an isotope ratio mass spectrometer (IR-MS). Compounds are separated with a 30 m × 0.25 mm i.d. column, and the GC temperature is programmed for 50 to 170°C at 20°C min^{-1}, followed by 170 to 300°C at 4°C min^{-1} and a 15-minute hold at 300°C. Peaks eluting from the GC column are combusted to CO_2 over CuO/Pt wires at 850°C and online transported to the IR-MS. The isotopic composition of CO_2 peaks is measured by the IR-MS operated at 10 kV acceleration potential and by magnetic-sector mass separation. To obtain actual fatty acid isotope ratios, the $\delta^{13}C$ of FAMEs is corrected for the carbon added during methylation (Zou et al., 2006).

6.6.3.2 Amino Acids

Individual amino acid isotopic analyses on isolated DOM, COM, or POM samples are made after acid hydrolysis with 6 M HCl at 100°C for 20 hours using isopropyl-TFA derivatives. Samples are hydrolyzed in duplicate, derivatized, and analyzed on a GC-IR-MS. McCarthy et al. (2004, 2007) reported the results of $\delta^{13}C$ measurements on specific amino acid species, including alanine (Ala), aspartic acid + asparagine (Asp), glutamic acid + glutamine (Glu), glycine (Gly), valine (Val), leucine (Leu), isoleucine (Ile), lysine (Lys), and phenylalanine (Phe). Isotope variability of individual amino acid species is dependent on the sample size of marine organic matter. For example, total variability of individual AA isotope composition is usually lower in biological and particulate samples but higher in isolated DOM and COM samples (McCarthy et al., 2004). Similarly, individual AA species, which can be well separated chromatographically from other AA compounds, have a lower analytical variability.

6.6.3.3 Other Organic Compounds

Eglinton et al. (1996) first used automated preparative capillary gas chromatography (PCGC) coupled with a fraction collector to separate and recover sufficient quantities of individual target compounds for natural abundance ^{14}C analysis by accelerator mass spectrometry (AMS). Based on the PCGC approach, the natural radiocarbon abundance can be accurately determined on specific compounds in marine organic samples, which are complex and heterogeneous.

Repeta and Aluwihare (2006) measured compound-specific natural abundance radiocarbon of seven neutral sugar species in HMW-DOM from the North Pacific Ocean. The isolated HMW-DOM is hydrolyzed to release neutral sugars, which are then purified by high-pressure liquid chromatography and analyzed for radiocarbon content. The hydrolysis is conducted with 4 M HCl at 108°C for 4 hours. Following the hydrolysis, acid is removed by freeze drying and the residue is dissolved in ultrapure water and desalted using Biorex 5 anion exchange resin. The resin column is washed with 30 ml of pure water to elute the carbohydrate fraction. The carbohydrate fraction is freeze-dried, dissolved in ultrapure water, and further purified by HPLC with refractive index detection using two cation exchange columns. Monosaccharides are collected in three fractions (glucose/rhamnose, galactose/mannose/xylose, and fucose/arabinose). After freeze drying, each fraction is dissolved in ultrapure water, diluted with acetonitrile, and further purified by reverse phase HPLC (Repeta and Aluwihare, 2006). Purified sugars are collected, evaporated to dryness, dissolved in ultrapure water, and transferred to a precombusted quartz tube containing 300 to 500 mg of copper oxide. After freeze drying, the sealed tubes are combusted at 850°C to convert sugars into carbon dioxide for natural abundance radiocarbon analyses using AMS.

6.7 INSTRUMENT FOR THE MEASUREMENTS OF STABLE ISOTOPES AND RADIOCARBON

6.7.1 Isotope Ratio Mass Spectrometer

The isotope ratio mass spectrometer (IR-MS) is the instrument used for the precise measurement of stable isotope ratios. Traditionally, isotope analysis has been carried out using offline preparation systems followed by automated analysis. Recent advances using fully automated online preparation systems allow routine high-throughput measurements of small amounts of marine organic matter samples (a few micrograms). The mass spectrometer can be configured in both continuous-flow and dual-inlet modes, providing high precision for the determination of H/D, $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, $^{34}S/^{32}S$ (from SO_2 and SF_6), and other isotope ratios. The IR-MS instrument has the following basic components (Figure 6.7): ion source, magnet, and collectors (usually an array of Faraday cups). It can measure reference and sample at the same time and provide simultaneous detection of multiple isotopes.

An elemental analyzer is used to combust organic matter in filter, powdered, or liquid samples. Evolved gases (e.g., CO_2, N_2, H_2O vapor) are carried through an oxidizing furnace tube, a reducing furnace tube, and a water trap. The mixed gases are separated based on their different affinities for the chromatography column, with nitrogen eluting from the column before carbon dioxide. The gases are sequentially transported with the helium stream to the mass spectrometer, where they are analyzed by continuous flow mode. The coupling of IR-MS and an elemental analyzer allows

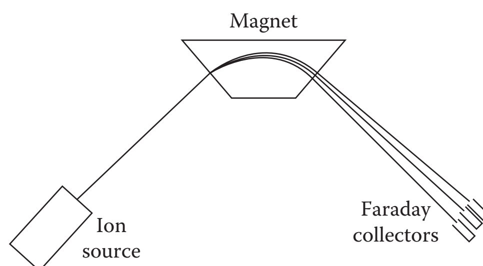

FIGURE 6.7 A diagram showing components in a typical isotope ratio mass spectrometer (IR-MS). (From Karl von Reden, Woods Hole Oceanographic Institution, with kind permission of Media Relations, Woods Hole Oceanographic Institution.)

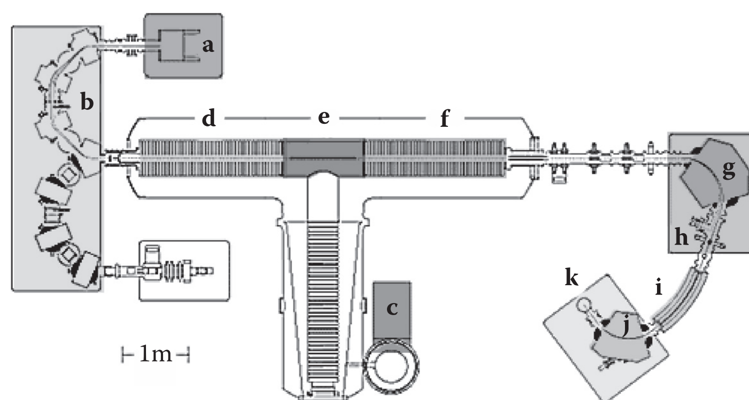

FIGURE 6.8 A diagram showing component of an accelerator mass spectrometer (AMS).

routine $\delta^{15}N$ and $\delta^{13}C$ analyses for organic samples. Although it is not as precise as the dual inlet, an elemental analyzer makes sample preparation much easier, and both $\delta^{15}N$ and $\delta^{13}C$ are measured on the same sample.

Furthermore, the IR-MS can also be interfaced with a gas chromatograph (GC) and a combustor, called GC-C-IR-MS. The GC-C-IR-MS allows measurement of isotope composition of individual compounds, such as fatty acid and amino acids (see also Section 6.6.3).

6.7.2 Accelerator Mass Spectrometer

Radiocarbon can be measured by either beta counting or accelerator mass spectrometry (AMS) techniques (see Chapter 13). AMS has an advantage in the measurements of natural abundance radiocarbon in organic matter samples, when sample size is too small (e.g., marine DOM samples) to be accurately measured by beta counting (e.g., Tuniz et al., 1998). Components of the AMS system include ion source, injector magnet, tandem accelerator, analyzing and switching magnets, electrostatic analyzer, and gas ionization detector (Figure 6.8). The strength of AMS is its power to separate a rare isotope (e.g., ^{14}C, with an abundance in parts per trillion) from other abundant isotopes (e.g., ^{12}C, with a abundance in 98.89%). This makes it possible to detect many naturally occurring, long-lived radionuclides that can be used as tracers for organic carbon cycles, such as ^{14}C and ^{129}I (see Chapter 13). Therefore, AMS techniques, where all the ^{14}C atoms are counted directly, can outperform the decay counting techniques, where only those decaying during the counting interval are counted. Especially for isotopes with a long half-life, decay counting techniques become much disadvantaged compared to AMS techniques. The isotopic fractionation on the $^{14}C/^{12}C$ ratio is about two times that on the $^{13}C/^{12}C$ ratio due to the difference in mass. To account for differences in isotopic fractionation between ^{14}C and ^{12}C, values of $\delta^{13}C$ are needed. Depending on the detection limit and decay half-life of ^{14}C, the timescales that can be resolved using radiocarbon are higher than 50 years (the standard deviation may be larger than the age obtained for recent or young samples), but less than 50,000–60,000 years. This maximum ^{14}C age limit is encountered when the ^{14}C activity in a sample is too low to be determined compared to the background ^{14}C activity. Thus, no age is reported greater than 60,000 years.

6.7.3 GC-IR-MS

The coupling of a conventional GC with a specialized isotope ratio mass spectrometer allows the interface to convert all organic matter continuously and quantitatively into a single molecular form for isotopic measurements (e.g., Eglinton et al., 1996; Sessions, 2006). In general, if a compound of

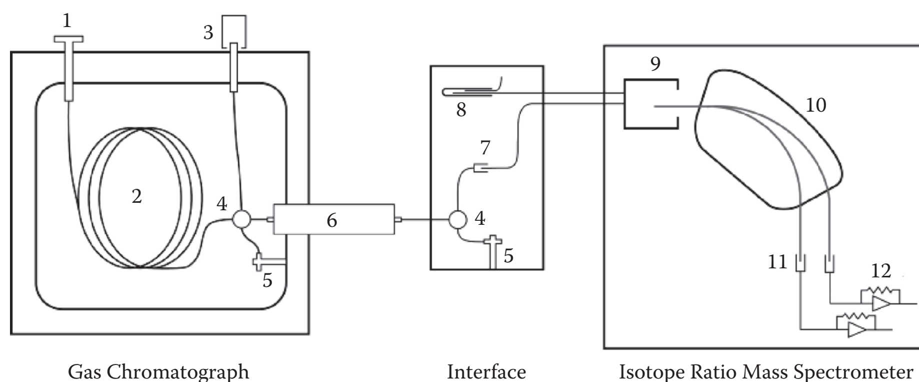

FIGURE 6.9 Schematic of a typical GC-IR-MS system configured for D/H analysis. Major components are: (1) injector, (2) analytical column, (3) FID, (4) unions, (5) back flush valves, (6) pyrolysis reactor, (7) open split, (8) reference gas injector, (9) electron impact ionization source, (10) magnetic-sector mass analyzer, (11) Faraday detectors, and (12) analog electrometers. (From Sessions, 2006, with kind permission from Elsevier Limited.)

interest can be analyzed by GC or GC-MS, the ^{13}C isotope ratio can be measured using a specific GC column and oven program.

Figure 6.9 shows a typical GC-IR-MS system configured for D/H analysis (Sessions, 2006). Major components include: (1) injector, (2) analytical column, (3) flame ionization detector, (4) unions, (5) back flush valves, (6) pyrolysis reactor, (7) open split, (8) reference gas injector, (9) electron impact ionization source, (10) magnetic-sector mass analyzer, (11) Faraday detectors, and (12) analog electrometers. Isotopes of C and N are analyzed, respectively, as CO_2 and N_2, derived from combustion of sample residues, while H and O are analyzed as H_2 and CO produced by pyrolysis.

6.8 QUALITY ASSURANCE

Common quality assurance and quality control (QA/QC) should be performed on a daily basis. Before sample analysis, standard reference materials are analyzed to verify instrument performance. If the values of the standard materials do not fall within the expected range, samples are not analyzed until the expected performance has been established. During sample analysis, blank samples, including procedural, reagent, and instrument blanks, and reference standard samples are analyzed with the samples frequently for the purpose of quality assurance and quality control. Furthermore, replicate measurements should be done for each sample to determine the reproducibility and to verify the performance of the IR-MS system. By evaluating the data from reference materials, blanks, reproducibility, and instrument drift, the data quality can be evaluated. If a problem is detected with either drift or poor performance, the samples should be reanalyzed. The standard deviation of IR-MS analyses (about ±0.4‰) can be obtained based on internal standard measurements over a few days' operation. Precision is normally 0.1‰ for $\delta^{13}C$ and 0.2‰ for $\delta^{15}N$.

6.9 FUTURE RESEARCH

Stable isotopes and radiocarbon composition of organic matter can provide useful information on biogeochemical cycles of DOM, COM, and POM in estuarine, coastal, and oceanic environments.

A combination of multiple isotope measurements enhances the power to understand the complicated systems and the interlinked processes. Further research is needed to focus on:

- Improving the efficiency of DOM isolation and separation from seawater for multiple isotope analyses
- Analysis of sulfur isotopic composition in marine organic samples with low sulfur abundance, and improving direct measurements of DON-$\delta^{15}N$ in seawater samples
- Improving online coupling between compound separation techniques such as GC and HPLC, and specialized instruments for isotope and radiocarbon analyses, such as IR-MS and AMS
- Finding a specific organic compound in the marine organic matter pool that has a distinctive stable isotope signature in terrestrial, marine, atmospheric, and anthropogenic organic sources, so that the relative contribution of autochthonous and allochthonous sources can be quantified in the marine environment
- Coupling of organic size fractionation with compound class and compound specific separation techniques for the measurements of isotopic composition of marine organic matter

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REFERENCES

Aiken, G. R., D. M. McKnight, R. L. Wershaw, and P. MacCarthy. 1985. *Humic substances in soil, sediment, and water: Geochemistry, isolation, and characterization*. New York: John Wiley & Sons.

Altabet, M. A. 1988. Variations in nitrogen isotopic composition between sinking and suspended particles: Implications for nitrogen cycling and particle transformation in the open ocean. *Deep Sea Research* 35:535–54.

Altabet, M. A. 1990. Organic C, N, and stable isotopic composition of particulate matter collected on glass-fiber and aluminum oxide filters. *Limnology and Oceanography* 35:902–9.

Altabet, M. A., W. G. Deuser, S. Honjo, and C. Stiene. 1991. Seasonal and depth-related changes in the source of sinking particles in the North Atlantic. *Nature* 354:136–39.

Amon, R. M. W., and R. Benner. 1994. Rapid cycling of high-molecular-weight dissolved organic matter in the ocean. *Nature* 369:549–52.

Bauer, J. E., E. R. M. Druffel, D. M. Wolgast, and S. Griffin. 2001. Sources and cycling of dissolved and particulate organic radiocarbon in the northwest Atlantic continental margin. *Global Biogeochemical Cycles* 15:615–36.

Bauer, J. E., E. R. M. Druffel, D. M. Wolgast, and S. Griffin. 2002. Temporal and regional variability in sources and cycling of DOC and POC in the northwest Atlantic continental shelf and slope. *Deep Sea Research II* 49:4387–419.

Bauer, J. E., P. M. Williams, and E. R. M. Druffel. 1992. ^{14}C activity of dissolved organic carbon fractions in the northcentral Pacific and Sargasso Sea. *Nature* 357:667–70.

Beaupre, S. R., E. R. M. Druffel, and S. Griffin. 2007. A low-blank photochemical extraction system for concentration and isotopic analyses of marine dissolved organic matter. *Limnology and Oceanography: Methods* 5:174–84.

Benitez-Nelson, C. R. 2000. The biogeochemical cycling of phosphorus in marine systems. *Earth Science Reviews* 51:109–35.

Benner, R., J. D. Pakulski, M. McCarthy, J. I. Hedges, and P. G. Hatcher. 1992. Bulk chemical characteristics of dissolved organic matter in the ocean. *Science* 255:1561–64.

Benner, R., B. Benitez-Nelson, K. Kaiser, and R. M. W. Amon. 2004. Export of young terrigenous dissolved organic carbon from rivers to the Arctic Ocean. *Geophysical Research Letters* 31, L05305, doi: 10.1029/2003GL019251.

Benner, R., B. Biddanda, B. Black, and M. McCarthy. 1997. Abundance, size distribution, and stable carbon and nitrogen isotopic compositions of marine organic matter isolated by tangential-flow ultrafiltration. *Marine Chemistry* 57:243–66.

Bergamaschi, B. A., E. Tsamakis, R. G. Keil, T. I. Eglinton, D. B. Montlucon, and J. I. Hedges. 1997. The effect of grain size and surface area on organic matter, lignin and carbohydrate concentration, and molecular compositions in Peru Margin sediments. *Geochimica et Cosmochimica Acta* 61:1247–60.

Bishop, J. K. B., and J. M. Edmond. 1976. A new large volume filtration system for the sampling of oceanic particulate matter. *Journal of Marine Research* 34:181–98.

Bishop, J. K. B., D. R. Ketten, and J. M. Edmond. 1978. The chemistry, biology and vertical flux of particulate matter from the upper 400 m of the Cape Basin in the southeast Atlantic Ocean. *Deep Sea Research* 25:1121–61.

Bouillon, S., M. Korntheuer, W. Baeyens, and F. Dehairs. 2006. A new automated setup for stable isotope analysis of dissolved organic carbon. *Limnology and Oceanography: Methods* 51:216–26.

Broecker, W. S., and T.-H. Peng. 1982. *Tracers in the sea*. Palisades, NY: Eldigio Press.

Buesseler, K. O., A. N. Antia, M. Chen, S. W. Fowler, W. D. Gardner, O. Gustafsson, K. Harada, A. F. Michaels, M. R. van der Loeff, M. Sarin, D. K. Steinberg, and T. Trull. 2007. An assessment of the use of sediment traps for estimating upper ocean particle fluxes. *Journal of Marine Research* 65:345–416.

Buesseler, K. O., J. Bauer, R. Chen, T. Eglinton, O. Gustafsson, W. Landing, K. Mopper, S. B. Moran, P. H. Santschi, R. Vernon-Clark, and M. Wells. 1996. An intercomparison of cross-flow filtration techniques used for sampling marine colloids: Overview and organic carbon results. *Marine Chemistry* 55:1–32.

Chen, M., L. Guo, Q. Ma, Y. S. Qiu, R. Zhang, E. Lv, and Y. P. Huang. 2006. Zonal patterns of $\delta^{13}C$, $\delta^{15}N$ and ^{210}Po in the North Pacific Ocean. *Geophysical Research Letters* 33:L04609, doi: 10.1029/2005GL025186.

Chester, R. 2003. *Marine Geochemistry*. 2nd ed. New York: Blackwell Publishing.

Cifuentes, L. A., J. H. Sharp, and M. L. Fogel. 1988. Stable carbon and nitrogen isotope biogeochemistry in the Delaware estuary. *Limnology and Oceanography* 33:1102–15.

Clercq, M., J. Van der Plicht, and H. A. J. Meijer. 1998. A supercritical oxidation system for the determination of carbon isotope ratios in marine dissolved organic carbon. *Analytica Chimica Acta* 370:19–27.

Coffin, R. B., L. A. Cifuentes, and P. M. Elderidge. 1994. The use of stable carbon isotopes to study microbial processes in estuaries. In *Stable isotopes in ecology and environment science*, ed. K. Lajitha and R. H. Michener. Oxford: Blackwell Scientific.

Cowie, G. L., and J. I. Hedges. 1984. Determination of neutral sugars in plankton, sediments, and wood by capillary gas chromatography of equilibrated isomeric mixtures. *Anaytical Chemistry* 56:504–10.

Dickson, A. G., C. L. Sabine, and J. R. Christian. 2007. *Guide to best practices for ocean CO_2 measurements*. PICES Science Report 34.

Dittmar, T., B. Koch, N. Hertkorn, and G. Kattner. 2008. A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. *Limnology and Oceanography: Methods* 6:230–35.

Druffel, E. R. M., S. Griffin, J. E. Bauer, D. Wolgast, and X. Wang. 1996. Distribution of particulate organic carbon and radiocarbon in the water column from the upper slope to the abyssal NE Pacific Ocean. *Deep Sea Research II* 45:667–87.

Druffel, E. R. M., and P. M. Williams. 1990. Identification of a deep marine source of particulate organic carbon using bomb ^{14}C. *Nature* 347:172–74.

Druffel, E. R. M., and P. M. Williams. 1992. Importance of isotope measurements in marine organic geochemistry. *Marine Chemistry* 39:209–15.

Druffel, E. R. M., P. M. Williams, J. E. Bauer, and A. J. Ertel. 1992. Cycling of dissolved and particulate organic matter in the open ocean. *Journal of Geophysical Research* 97:15639–59.

Eadie, B. J., L. M. Feffrey, and W. M. Sackett. 1978. Some observations on the stable carbon isotope composition of dissolved and particulate organic carbon in the marine environment. *Geochimica et Cosmochimica Acta* 42:1265–69.

Eglinton, T. I., L. I. Aluwihare, J. E. Bauer, E. R. M. Druffel, and A. P. McNichol. 1996. Gas chromatographic isolation of individual compounds from complex matrixes for radiocarbon dating. *Analytical Chemistry* 68:904–12.

Falkowski, P. G. 1991. Species variability in the fractionation of ^{13}C and ^{12}C marine phytoplankton. *Journal of Plankton Research* 13:21–28.

Farquhar, G. D., J. R. Ehleringer, and K. T. Hubick. 1989. Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* 40:503–37.

Feuerstein, T. P., P. H. Ostrom, and N. E. Ostrom. 1997. Isotopic biogeochemistry of dissolved organic nitrogen: A new technique and application. *Organic Geochemistry* 21:363–70.

Fry, B. 2006. *Stable isotope ecology*. New York: Springer.

Fry, B., H. W. Jannasch, S. J. Molyneaux, C. O. Wirsen, and J. A. Muramoto. 1991. Stable isotope studies of the carbon, nitrogen and sulfur cycles in the Black Sea and the Cariaco Trench. *Deep Sea Research* 38 (Suppl. 2A):S1003–19.

Fry, B., E. T. Peltzer, C. S. Hopkinson, A. Nolin, and L. Redmond. 1996. Analysis of marine DOC using a dry combustion method. *Marine Chemistry* 54:191–201.

Fry, B., and E. B. Sherr. 1984. $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contributions to Marine Science* 27:13–47.

Gandhi, H., T. N. Wiegner, and P. H. Ostrom. 2004. Isotopic (C-13) analysis of dissolved organic carbon in stream water using an elemental analyzer coupled to a stable isotope ratio mass spectrometer. *Rapid Communication in Mass Spectrometry* 18:903–6.

Gedeon, M. L., P. H. Ostrom, N. E. Ostrom, and H. Gandhi. 2001. Simultaneous determination of dissolved organic nitrogen concentrations and isotope values in the subtropical North Pacific. In *ASLO 2001 Aquatic Science Meeting*, February 12–16, 2001, Albuquerque, NM, p. 57.

Guo, L., B. J. Hunt, and P. H. Santschi. 2001. Ultrafiltration behavior of major ions (Na, Ca, Mg, F, Cl, SO_4) in natural waters. *Water Research* 35:1500–8.

Guo, L., and R. W. Macdonald. 2006. Source and transport of terrigenous organic matter in the upper Yukon River: Evidence from isotope (^{13}C, ^{14}C and ^{15}N) composition of dissolved, colloidal and particulate phases. *Global Biogeochemical Cycles* 20:GB2011, doi: 10.1029/2005GB002593.

Guo, L., and P. H. Santschi. 1996. A critical evaluation of the cross-flow ultra-filtration technique for sampling colloidal organic carbon in seawater. *Marine Chemistry* 55:113–27.

Guo, L., and P. H. Santschi. 1997a. Isotopic and elemental characterization of colloidal organic matter from the Chesapeake Bay and Galveston Bay. *Marine Chemistry* 59:1–15.

Guo, L., and P. H. Santschi. 1997b. Composition and cycling of colloids in marine environments. *Review of Geophysics* 35:17–40.

Guo, L., and P. H. Santschi. 2007. Ultra-filtration and its applications to sampling and characterization of aquatic colloids. In *Environmental colloids and particles*, ed. K. Wilkinson and J. Lead. New York: John Wiley & Sons.

Guo, L., P. H. Santschi, and T. S. Bianchi. 1999. Dissolved organic matter in estuaries of the Gulf of Mexico. In *Biogeochemistry of Gulf of Mexico estuaries*, ed. T. S. Bianchi, J. R. Pennock, and R. R. Twilley. New York: John Wiley & Sons.

Guo, L., P. H. Santschi, L. A. Cifuentes, S. Trumbore, and J. Southon. 1996. Cycling of high molecular weight dissolved organic matter in the Middle Atlantic Bight as revealed by carbon isotopic (^{13}C and ^{14}C) signatures. *Limnology and Oceanography* 41:1242–52.

Guo, L., P. H. Santschi, and K. W. Warnken. 1995. Dynamics of dissolved organic carbon in oceanic environments. *Limnology and Oceanography* 40:1392–403.

Guo, L., N. Tanaka, D. M. Schell, and P. H. Santschi. 2003. Nitrogen and carbon isotopic composition of high-molecular-weight dissolved organic matter in marine environments. *Marine Ecology Progress Series* 252:51–60.

Guo, L., L. Wen, D. Tang, and P. H. Santschi. 2000. Re-examination of cross flow ultra-filtration for sampling aquatic colloids: Evidence from molecular probes. *Marine Chemistry* 69:75–90.

Guo, L., D. M. White, C. Xu, and P. H. Santschi. 2008. Chemical and isotopic composition of colloidal organic matter from the Mississippi River plume. *Marine Chemistry*, submitted. AU: Please update.

Hansell, D. A., and C. A. Carlson. 1998. Deep-ocean gradients in the concentration of dissolved organic matter. *Nature* 395:263–66.

Hansell, D. A., and C. A. Carlson. 2002. *Biogeochemistry of marine dissolved organic matter*. New York: Academic Press.

Hedges, J. I. 1992. Global biogeochemical cycles: Progress and problems. *Marine Chemistry* 39:67–93.

Hedges, J. I. 2002. Why dissolved organic matter? In *Biogeochemistry of marine dissolved organic matter*, ed. D. A. Hansell and C. Carlson. New York: Academic Press.

Hedges, J. I., J. A. Baldock, Y. Gélinas, C. Lee, M. Peterson, and S. G. Wakeham 2001. Evidence for non-selective preservation of organic matter in sinking marine particles. *Nature* 409:801–4.

Hedges, J. I., J. R. Ertel, P. D. Quay, P. M. Grootes, J. Richey, A. H. Devol, and G. W. Farwell. 1986. Organic carbon-14 in the Amazon River system. *Science* 231:1129–31.

Hedges, J. I., and R. G. Keil. 1999. Organic geochemical perspectives on estuarine processes: Sorption reactions and consequences. *Marine Chemistry* 65:55–65.

Hoefs, J. 2004. *Stable isotope geochemistry*. Berlin: Springer Verlag.

Huang Y., B. Shuman, Y. Wang, and T. Webb III. 2004. Hydrogen isotope ratios of individual lipids in lake sediments as novel tracers of climatic and environmental change: A surface sediment test. *Journal of Paleolimnology* 31:363–75.

Hunt, A. P., J. D. Parry, and J. Hamilton-Taylor. 2000. Further evidence of elemental composition as an indicator of the bioavailability of humic substances to bacteria. *Limnology and Oceanography* 45:237–41.

Kim, S., A. J. Simpson, E. B. Kujawinski, M. A. Freitas, and P. G. Hatcher. 2003. High resolution electrospray ionization mass spectrometry and 2D solution NMR for the analysis of DOM extracted by C18 solid phase disk. *Organic Geochemistry* 34:1325–35.

Knapp, A. N., D. M. Sigman, and F. Lipschultz. 2005. N isotopic composition of dissolved organic nitrogen and nitrate at the Bermuda Atlantic time-series study site. *Global Biogeochemical Cycles* 19:GB1018, doi: 10.1029/2004GB002320.

Knauer, G. A., J. H. Martin, and K. W. Bruland. 1979. Fluxes of particulate carbon, nitrogen, and phosphorus in the upper water column of the northeast Pacific. *Deep Sea Research* 26A:97–108.

Komada, T., M. R. Anderson, and C. L. Dorfmeier. 2008. Carbonate removal from coastal sediments for the determination of organic carbon and its isotopic signatures, $d^{13}C$ and $\Delta^{14}C$: Comparison of fumigation and direct acidification by hydrochloric acid. *Limnology and Oceanography: Methods* 6:254–62.

Koprivnjak, J. F., E. M. Perdue, and P. H. Pfromm. 2006. Coupling reverse osmosis with electrodialysis to isolate natural organic matter from fresh waters. *Water Research* 40:3385–92.

Kwak, T. J., and J. B. Zedler. 1997. Food web analysis of southern California coastal wetlands using multiple stable isotopes. *Oecologia* 110:262–77.

Lang, S. Q., M. D. Lilley, and J. I. Hedges. 2007. A method to measure the isotopic (13C) composition of dissolved organic carbon using a high temperature combustion instrument. *Marine Chemistry* 103:318–26.

Laws, E. A., B. N. Popp, R. R. Bidigare, M. C. Kennicutt, and S. A. Maco. 1995. Dependence of phytoplankton carbon isotopic composition on growth rate and $[CO_2]_{aq}$: Theoretical considerations and experimental results. *Geochimica et Cosmochimica Acta* 59:1131–38.

Lee, C., and S. G. Wakeham. 1992. Organic matter in the water column: Future research challenges. *Marine Chemistry* 39:95–118.

Libes, S. M. 1992. *An introduction to marine biogeochemistry*. New York: John Wiley & Sons.

Liu, K. K., and I. R. Kaplan. 1984. Denitrification rates and availability of organic matter in marine environment. *Earth and Planetary Science Letters* 68:88–100.

Liu, K. K., and I. R. Kaplan. 1989. The eastern tropical Pacific as a source of 15N-enriched nitrate in seawater off southern California. *Limnology and Oceanography* 34:820–30.

Liu, Z. F., G. Stewart, J. K. Cochran, C. Lee, R. A. Armstrong, D. I. Hirschberg, B. Gasser, and J. C. Miquel. 2005. Why do POC concentrations measured using Niskin bottle collections sometimes differ from those using in-situ pumps? *Deep Sea Research I* 52:1324–44.

Loh, A. N., J. E. Bauer, and E. R. M. Druffel. 2004. Variable ageing and storage of dissolved organic components in the open ocean. *Nature* 430:877–80.

Louchouarn, P., S. Opsahl, and R. Benner. 2000. Isolation and quantification of dissolved lignin from natural waters using solid-phase extraction and GC/MS. *Analytical Chemistry* 72:2780–87.

Lückge, A., B. Horsfield, R. Littkeand, and G. Scheeder. 2002. Organic matter preservation and sulfur uptake in sediments from the continental margin off Pakistan. *Organic Geochemistry* 33:477–88.

Lunau, M., A. Sommer, A. Lemke, H. P. Grossart, and M. Simon. 2004. A new sampling device for microaggregates in turbid aquatic systems. *Limnology and Oceanography: Methods* 2:387–97.

Malej, A., J. Faganeli, and J. Pezdi. 1993. Stable isotope and biochemical fractionation in the marine pelagic food chain: The jellyfish *Pelagia noctiluca* and net zooplankton. *Marine Biology* 116:565–70.

Mayer, L. M. 1994. Relationships between mineral surfaces and organic-carbon concentrations in soils and sediments. *Chemical Geology* 114:347–63.

McCarthy, M. D., R. Benner, C. Lee, and M. L. Fogel. 2007. Amino acid nitrogen isotopic fractionation patterns as indicators of heterotrophy in plankton, particulate, and dissolved organic matter. *Geochimica et Cosmochimica Acta* 71:4727–44.

McCarthy, M. D., R. Benner, C. Lee, J. I. Hedges, and M. L. Fogel. 2004. Amino acid carbon isotopic fractionation patterns in oceanic dissolved organic matter: An unaltered photoautotrophic source for dissolved organic nitrogen in the ocean? *Marine Chemistry* 92:123–34.

Mino, Y., T. Saino, K. Suzuki, and E. Maranon. 2002. Isotopic composition of suspended particulate nitrogen in surface waters of the Atlantic Ocean from 50 degrees N to 50 degrees S. *Global Biogeochemical Cycles* 16:GB 1059, doi: 10.1029/2001GB001635.

Miyajima, T., Y. Tanaka, and I. Koike. 2005. Determining ^{15}N enrichment of dissolved organic nitrogen in environmental waters by gas chromatography/negative-ion chemical ionization mass spectrometry. *Limnology and Oceanography: Methods* 3:164–73.

Mopper, K., A. Stubbins, J. D. Ritchie, H. M. Bialk, and P. G. Hatcher. 2007. Advanced instrumental approaches for characterization of marine dissolved organic matter: Extraction techniques, mass spectrometry, and nuclear magnetic resonance spectroscopy. *Chemical Reviews* 107:419–42.

Moran, S. B., and K. O. Buesseler. 1992. Short residence time of colloids in the upper ocean estimated from ^{238}U-^{234}Th disequilibria. *Nature* 359:221–23.

Moran, S. B., M. A. Charette, S. M. Pike, and C. A. Wicklund. 1999. Differences in seawater particulate organic carbon concentration in samples collected using small- and large-volume methods: The importance of DOC adsorption to the filter blank. *Marine Chemistry* 67:33–42.

Mortazavi, B., and J. P. Chanton. 2004. Use of Keeling plots to determine sources of dissolved organic carbon in nearshore and open ocean systems. *Limnology and Oceanography* 49:102–8.

Needoba, J. A., N. A. Waser, P. J. Harrison, and S. E. Calvert. 2003. Nitrogen isotope fractionation in 12 species of marine phytoplankton during growth on nitrate. *Marine Ecology Progress Series* 255:81–91.

Ono, S., B. Wing, D. Rumble, and J. Farquhar. 2006. High precision analysis of all four stable isotopes of sulfur (^{32}S, ^{33}S, ^{34}S and ^{36}S) at nanomole levels using a laser fluorination isotope-ratio-monitoring gas chromatography–mass spectrometry. *Chemical Geology* 225:30–39.

Osburn, C. L., and G. St.-Jean. 2007. The use of wet chemical oxidation with high-amplification isotope ratio mass spectrometry to measure stable isotope values of dissolved organic carbon in seawater. *Limnology and Oceanography: Methods* 5:296–308.

Pearson, A., and T. I. Eglinton. 2000. The origin of n-alkanes in Santa Monica Basin surface sediment: A model based on compound-specific Δ^{14}C and δ^{13}C data. *Organic Geochemistry* 31:1103–16.

Peterson, B. J., and B. Fry. 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* 18:293–320.

Peterson, B., B. Fry, M. Hullar, and S. Saupe. 1994. The distribution and stable carbon isotopic composition of dissolved organic carbon in estuaries. *Estuaries* 17:111–21.

Peterson, B. J., R. W. Howarth, and R. H. Grarritt. 1985. Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. *Science* 227:1361–63.

Peterson, M. L., S. G. Wakeham, C. Lee, M. A. Askea, and J. C. Miquel. 2005. Novel techniques for collection of sinking particles in the ocean and determining their settling rates. *Limnology and Oceanography: Methods* 3:520–32.

Rau, G. H., C. W. Sullivan, and L. I. Gordon. 1991a. δ^{13}C and δ^{15}N variations in the Weddell Sea particulate organic matter. *Marine Chemistry* 35:355–69.

Rau, G. H., R. E. Sweeney, and I. R. Kaplan. 1982. Plankton ^{13}C/^{12}C ratio changes with latitude: Differences between northern and southern oceans. *Deep Sea Research* 29:1035–39.

Rau, G. H., T. Takahashi, and D. J. DesMarais. 1991b. Latitudinal variations in plankton δ^{13}C: Implications for CO_2 and productivity in past oceans. *Nature* 341:516–18.

Rau, G. H., J. I. Teyssie, F. Rassoulzadegan, and S. W. Fowler. 1990. ^{13}C/^{12}C and ^{15}N/^{14}N variations among size-fractionated marine particles: Implications for their origin and trophic relationships. *Marine Ecology Progress Series* 59:33–38.

Raymond, P. A., and J. E. Bauer. 2001a. Use of ^{14}C and ^{13}C natural abundances for evaluating riverine, estuarine, and coastal DOC and POC sources and cycling: A review and synthesis. *Organic Geochemistry* 32:469–85.

Raymond, P. A., and J. E. Bauer. 2001b. DOC cycling in a temperate estuary: A mass balance approach using natural ^{14}C and ^{13}C isotopes. *Limnology and Oceanography* 46:655–67.

Raymond, P. A., J. W. McClelland, R. M. Holmes, A. V. Zhulidov, K. Mull, B. J. Peterson, R. G. Striegl, G. R. Aiken, and T. Y. Gurtovaya. 2007. Flux and age of dissolved organic carbon exported to the Arctic Ocean: A carbon isotopic study of the five largest arctic rivers. *Global Biogeochemical Cycles* 21:GB4011, doi: 10.1029/2007GB002934.

Rees, C. E. 1978. Sulphur isotope measurements using SO_2 and SF_6. *Geochemica et Cosmochemica Acta* 42:377–81.

Repeta, D. J., and L. I. Aluwihare. 2006. Radiocarbon analysis of neutral sugars in high-molecular-weight dissolved organic carbon: Implications for organic carbon cycling. *Limnology and Oceanography* 51:1045–53.

Sackett, W. M. 1989. Stable carbon isotope studies on organic matter in the marine environment. In *Handbook of environmental isotope geochemistry*, ed. P. Fritz and J. Ch. Fontes. Amsterdam, The Netherlands: Elsevier Science.

Saino, T., and A. Hattori. 1987. Geographical variation in the water column distribution of suspended particulate organic nitrogen and its ^{15}N natural abundance in the Pacific and its marginal seas. *Deep Sea Research I* 34:807–27.

Santschi, P. H., E. Balnois, K. Wilkinson, J. Buffle, and L. Guo. 1998. Fibrillar polysaccharides in marine macromolecular organic matter as imaged by atomic force microscopy and transmission electron microscopy. *Limnology and Oceanography* 43:896–908.

Santschi, P. H., L. Guo, M. Baskaran, S. Trumbore, J. Southon, T. S. Bianchi, B. D. Honeyman, and L. Cifuentes. 1995. Isotopic evidence for contemporary origin of high-molecular weight organic matter in oceanic environments. *Geochimica et Cosmochimica Acta* 59:625–31.

Sauer, P. E., T. I. Eglinton, J. M. Hayes, A. Schimmelmann, and A. L. Sessions. 2001. Compound-specific D/H ratios of lipid biomarkers from sediments as a proxy for environmental and climate conditions. *Geochimica et Cosmochemica Acta* 65:213–22.

Schell, D. M., B. A. Barnett, and K. A. Vinette. 1998. Carbon and nitrogen isotope ratios in zooplankton of the Bering, Chukchi and Beaufort Sea. *Marine Ecology Progress Series* 162:11–23.

Sessions, A. L. 2006. Isotope-ratio detection for gas chromatography. *Journal of Separation Science* 29:1946–61.

Sharp, J. H. 1973. Size classes of organic carbon in seawater. *Limnology and Oceanography* 18:441–47.

Sigleo, A. C. 1996. Biochemical components in suspended particles and colloids: Carbohydrates in the Potomac and Patuxent estuaries. *Organic Geochemistry* 24:83–93.

Sigleo, A. C., and S. A. Macko. 1985. Stable isotope and amino acid composition of estuarine dissolved colloidal material. In *Marine and estuarine geochemistry*, ed. A. C. Sigleo and A. Hattori. Chelsea, MI: Lewis Publishers.

Sigleo, A. C., and S. A. Macko. 2002. Carbon and nitrogen isotopes in suspended particles and colloids, Chesapeake and San Francisco estuaries, USA. *Estuarine, Coastal and Shelf Sciences* 54:701–11.

Sigman, D. M. 2000. The δ^{15}N of nitrate in the Southern Ocean: Nitrogen cycling and circulation in the ocean interior. *Journal of Geophysical Research—Oceans* 105:19599–614.

Sigman, D. M., K. L. Casciotti, M. Andreani, C. Barford, M. Galanter, and J. K. Bohlke. 2001. A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater. *Analytical Chemistry* 73:4145–53.

Sigman, D. M., J. Granger, P. J. DiFiore, R. Ho, C. Cane, and A. van Geen. 2005. Coupled nitrogen and oxygen isotope measurements of nitrate along the eastern North Pacific margin. *Global Biogeochemical Cycles* 19:GB4022.

Solorzano, L., and J. H. Sharp. 1980. Determination of total dissolved nitrogen in natural waters. *Limnology and Oceanography* 25:754–58.

St.-Jean, G. 2003. Automated quantitative and isotopic (^{13}C) analysis of dissolved inorganic carbon and dissolved organic carbon in continuous flow using a total organic carbon analyzer. *Rapid Communications in Mass Spectrometry* 17:419–28.

Stuiver, M., and H. A. Polach. 1977. Discussion: Reporting of ^{14}C data. *Radiocarbon* 19:355–65.

Tagliabue, A., and L. Bopp. 2008. Towards understanding global variability in ocean carbon-13. *Global Biogeochemical Cycles* 22:GB1025, doi: 10.1029/2007GB003037.

Tan, F. C. 1987. Discharge and carbon isotope composition of particulate organic carbon from the St. Lawrence River, Canada. In *Transport of carbon and minerals in major world rivers*, ed. E. T. Degens, S. Kempe, and W. Gan, 64, 301–10. Mitt. Geol-Paläont. Inst. Univ. Hamburg, SCOPE/UNEP Sonderbd.

AU: Please spell out.

Tanaka, T., L. Guo, C. Deal, N. Tanaka, T. Whitledge, and A. Murata. 2004. Nitrogen deficiency in a well oxygenated cold bottom water over the Bering Sea Shelf: Influence of sedimentary denitrification. *Continental Shelf Research* 24:1271–83.

Thurman, E. M. 1985. *Organic geochemistry of natural waters*. Dordrecht, The Netherlands: Kluwer Academic.

Tumbore, S. E., and E. R. M. Druffel. 1995 Carbon isotopes for characterizing sources and turnover of non-living organic matter. In *Role of nonliving organic matter in the earth's carbon cycle*, ed. R. G. Zepp and Ch. Sonntag. New York: John Wiley & Sons.

AU: Please provide page numbers.

Tuniz, C., J. R. Bird, D. Fink, and G. F. Herzog. 1998. *Accelerator mass spectrometry. Ultrasensitive analysis for global science*. Boca Raton, FL: CRC Press.

Vetter, T. A., E. M. Perdue, E. Ingall, J.-F. Koprivnjak, and P. H. Pfromm. 2007. Combining reverse osmosis and electrodialysis for more complete recovery of dissolved organic matter from seawater. *Separation and Purification Technology* 56:383–87.

Wada, E., and A. Hattori. 1976. Natural abundance of $\delta^{15}N$ in particulate organic matter in the North Pacific Ocean. *Geochimica et Cosmochimica Acta* 40:249–51.

Wang, X. C., J. Callahan, and R. F. Chen. 2006. Variability in radiocarbon ages of biochemical compound classes of high molecular weight dissolved organic matter in estuaries. *Estuarine, Coastal and Shelf Science* 68:188–94.

Wang, X.-C., and E. R. M. Druffel. 2001. Radiocarbon and stable carbon isotope compositions of organic compound classes in sediments from the NE Pacific and Southern Oceans. *Marine Chemistry* 73:65–81.

Wang, X. C., E. R. M. Druffel, S. Griffin, C. Lee, and M. Kashgarian. 1998. Radiocarbon studies of organic compound classes in plankton and sediment of the Northeast Pacific Ocean. *Geochimica et Cosmochimica Acta* 59:1787–97.

Wang, X. C., E. R. M. Druffel, and C. Lee. 1996. Radiocarbon in organic compound classes in particulate organic matter and sediment in the deep Northeast Pacific Ocean. *Geophysical Research Letters* 23:3583–86.

Waser, N. A., W. G. Harrison, E. J. H. Head, B. Nielson, V. A. Lutz, and S. E. Calvert. 2000. Geographic variations in the nitrogen isotope composition of surface particulate nitrogen and new production across the North Atlantic Ocean. *Deep Sea Research I* 47:1207–26.

Williams, P. M., and E. R. M. Druffel. 1987. Radiocarbon in dissolved organic matter in the central North Pacific Ocean. *Nature* 330:246–48.

Williams, P. M., and L. I. Gordon. 1970. Carbon-13:carbon-12 ratios of dissolved and particulate organic matter in the sea. *Deep Sea Research* 17:19–27.

Williams, P. M., H. Oeschger, and P. Kinney. 1969. Natural radiocarbon activity of dissolved organic carbon in north-east Pacific Ocean. *Nature* 224:256–58.

Wu, J., S. E. Calvert, and C. S. Wong. 1997. Nitrogen isotope variations in the subarctic northeast Pacific: Relationships to nitrate utilization. *Deep Sea Research I* 44:287–314.

Wu, J., S. E. Calvert, C. S. Wong, and F. A. Whitney. 1999. Carbon and nitrogen isotopic composition of sedimenting particulate material at Station Papa in the subarctic northeast Pacific. *Deep Sea Research II* 46:2793–832.

Zou, L., M. Y. Sun, and L. Guo. 2006. Temporal variations of organic carbon inputs into the upper Yukon River: Evidence from fatty acids and their stable carbon isotopic compositions in dissolved, colloidal and particulate phases. *Organic Geochemistry* 37:944–56.

Zou, L., X. C. Wang, J. Callahan, R. A. Culp, R. F. Chen, M. A. Altabet, and M. Y. Sun. 2004. Bacterial roles in the formation of high-molecular-weight dissolved organic matter in estuarine and coastal waters: Evidence from lipids and the compound-specific isotopic ratios. *Limnology and Oceanography* 49:297–302.

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