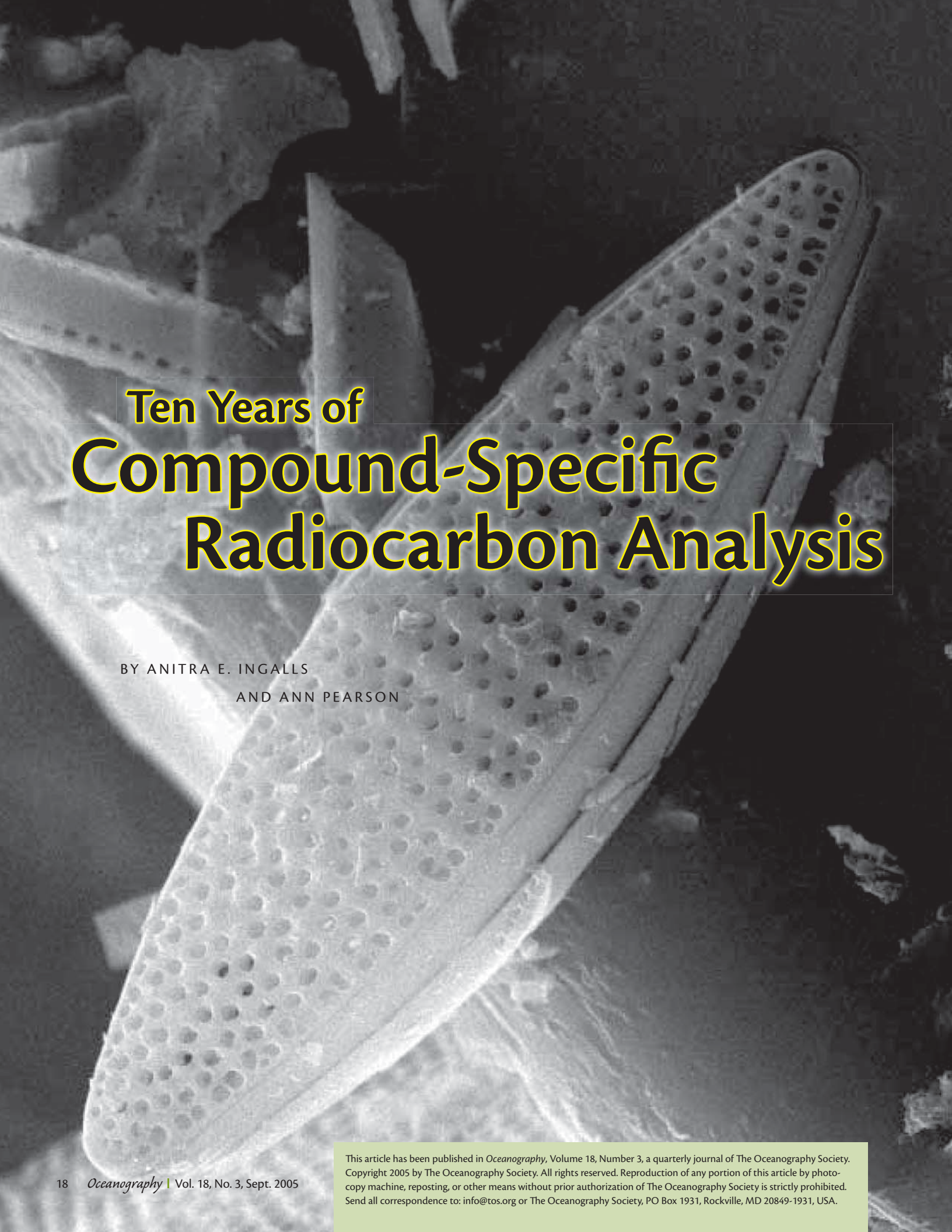

Ten Years of Compound-Specific Radiocarbon Analysis

BY ANITRA E. INGALLS
AND ANN PEARSON

SIXTY YEARS AGO, scientists demonstrated that cosmic rays react with nitrogen (^{14}N) in the atmosphere, substituting a neutron for a proton to produce ^{14}C (Libby, 1946; Libby et al., 1949). Soon it was discovered that ^{14}C is radioactive and that this isotope of carbon—better known as radiocarbon—would be useful as a clock for determining the age of materials on Earth (see Box 1). In particular, ^{14}C dating of the organic remains of living organisms has been an indispensable tool for archaeologists, paleontologists, historians, and geochemists, whose work fundamentally depends on determining when in the past an organism or whole ecosystem was alive.

In the atmosphere, ^{14}C combines with oxygen to form carbon dioxide, and this $^{14}CO_2$ is relatively uniformly distributed. Autotrophic organisms incorporate carbon dioxide into their tissues in the form of proteins, carbohydrates, lipids, and DNA. The radiocarbon content, or "^{14}C age," of an organism's tissue is the same as the age of its carbon dioxide source if it is an autotroph, or the same as that of the food source if it is a heterotroph. Once the organism dies, the only process affecting the ^{14}C content of the original tissue is radioactive decay. Often in archaeology and paleontology, the specimens, organisms, or artifacts are visually recognizable, allowing researchers to know the history of a given sample. Thus, the radiocarbon age of a sample can be tied directly to its provenance.

In contrast, oceanographers and many geochemists are faced with samples that contain organisms or components too small to identify or to separate physically, such as bacteria and the remains of decaying organisms. These heterogeneous, complex mixtures of living and dead organisms, together with their waste products, comprise the bulk of the organic matter in marine particles, seawater, and sediments. Radiocarbon dating of bulk marine organic and inorganic carbon reservoirs (dissolved inorganic carbon, dissolved organic carbon, particulate organic carbon, and sedimentary organic carbon of both terrestrial and marine origin) has allowed the average residence time of carbon in each of the respective pools to be calculated. The rate of exchange of carbon between organic and inorganic carbon reservoirs is an important control on the short- and long-term variability of the concentration of CO_2 in the atmosphere and therefore on Earth's climate. However, the complex nature of these bulk carbon reservoirs means that their ages inherently reflect not only radioactive decay, but also the average radiocarbon content of all of the compounds that are present and which may be derived from multiple sources.

Early investigations of organic carbon in the ocean revealed that the average ages of the heterogeneous reservoirs known as dissolved organic carbon (DOC) and sedimentary organic carbon (SOC) were "older" than samples of living autotrophic biomass in the ocean (e.g., phytoplankton) or on land (e.g., trees, grass). The offsets in age between these bulk organic carbon pools and their presumed major input sources, living biomass, suggested that many different processes control the molecular and isotopic make-up of heterogeneous organic materials.

Geochemists use organism-specific organic compounds called "biomarkers" to trace the fate of carbon originating from different biological sources in the marine environment. Following on the heels of compound-specific stable isotope analysis (GC-C-IRMS) (e.g., Freeman et al., 1990; Hayes et al., 1990), single-compound radiocarbon analysis was envisioned as a way to couple the diversity of carbon sources with the residence time of carbon in the respective source pools. The approach would add another dimension to studies of the molecular and isotopic composition of organic matter in the ocean and permit a better understanding of the dynamics of the global carbon cycle. Stable carbon and nitrogen isotopic signatures of biomarkers can be used to understand the metabolism(s) of the organism(s) that produced a particular compound, as well as the environment in which the organism(s) grew. Compound-specific radiocarbon measurements could be used in a similar way to determine the source of carbon used by an organism and the apparent age of the reservoir providing this source. As such, radiocarbon analysis of biomarkers could help define the metabolic pathways of organisms and quantify their contribution to the global cycle of organic carbon. In the mid-1990s, technical advances finally were made that could permit radiocarbon dating of individual biomarkers and initiate this new field (Eglinton et al., 1996; 1997; Pearson et al., 1998).

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HISTORICAL PERSPECTIVES

The transition from the early days of radiocarbon dating to the achievement of compound-specific radiocarbon analysis (CSRA) took several decades. The primary limitation was technological: numerous advances were required to pave the way for CSRA. In the early days of radiocarbon dating, beta counting was used to measure the disintegration of ^{14}C. This method required up to one gram of carbon and several days of counting time. The concentration of organic carbon in seawater (suspended plus dissolved) typically ranges from 40-150 µmol/L and the concentration of organic carbon in sediments typically ranges from 0.1-5.0 % by weight. As this would translate to a sample size requirement of 200 L of seawater

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BOX 1. CARBON ISOTOPE SYSTEMATICS

The relative proportion of carbon isotopes (stable ^{12}C and ^{13}C; and radioactive ^{14}C) in carbon-containing materials is used by oceanographers and geochemists to study the flow of carbon through the bio- and geosphere. Relative to $^{12}CO_2$, $^{14}CO_2$ is present in modern carbon at a concentration of about one part per trillion (10^{-12}). The 5730-year half life of ^{14}C makes it a convenient tracer of processes occurring on millennial time scales (less than about 50,000 years).

Radiocarbon data obtained by accelerator mass spectrometry (AMS) are reported as fraction modern (f_m), which is defined as the ratio of ^{14}C to ^{12}C in a sample relative to that in a standard. The f_m value must then be corrected for isotope fractionation that occurs during carbon fixation. For example, during photosynthesis, the lighter isotope of carbon, ^{12}C, is preferentially incorporated into biomass relative to the heavier isotope ^{13}C (and ^{14}C). This isotope fractionation reduces the amount of ^{13}C and ^{14}C in organisms relative to atmospheric carbon dioxide. All radiocarbon data measured on organic carbon must therefore be corrected for this isotope fractionation. A $\delta^{13}C$ value of -25 per mil (‰) is used for this correction (the $\delta^{13}C$ value to which all radiocarbon measurements are normalized). The data are then converted to $\Delta^{14}C$, which adjusts the ^{14}C content of the standard for decay since the year 1950. For a detailed description of reporting conventions see Stuiver and Polach (1977).

AMS data are also converted to radiocarbon ages (called conventional ^{14}C years or calendar years). For studies in which a ^{14}C age is desired, f_m can be corrected for biological fractionation and then converted to a conventional radiocarbon age using the Libby half-life of 5568 years according to the equation: $^{14}C\ Age = -8033 \ln f_m$

To convert radiocarbon years to calendar years, these values must be calibrated using a calibration curve that corrects the ^{14}C age to account for variability in the radiocarbon content of the atmosphere with time (Hughen et al., 2004; Guilderson et al., 2005). Variability in the ^{14}C content of atmospheric CO_2 arises from changes in Earth's magnetic field and the flux of cosmic rays, from changes in the rates of transfer of carbon between the many reservoirs of Earth's carbon cycle, and, presently, from the influence of nuclear bomb testing in the stratosphere during the late 1950s and early 1960s.

Oceanographers have one additional complication that must be corrected for in order to obtain an accurate age from ^{14}C. Inorganic carbon (CO_2) in the ocean is not in isotopic equilibrium with the atmosphere. Because the ocean mixes more slowly than the atmosphere, the inorganic carbon in the non-polar surface ocean is on average 400 years "older" than that in the atmosphere. This so called "reservoir effect" varies in the surface ocean. Variability in the reservoir age of water masses is due to mixing with subsurface waters that contain older ^{14}C ages due to the isolation of deep water from the atmosphere. Marine autotrophs form their biomass from this ^{14}C-depleted dissolved inorganic carbon pool. The radiocarbon content of marine organisms must be corrected for this reservoir effect.

or 100 g of sediment, it severely limited the kinds of studies that could be accomplished. Nevertheless, using these methods, Williams et al. (1969) determined that organic carbon dissolved in deep seawater had an average radiocarbon age of several thousand years. In addition, the organic carbon in sediments also is thousands of years older than expected from deposition of recently living phytoplankton from the overlying waters (e.g., Benoit et al., 1979).

Based on this early work, hypotheses to explain the old and variable age of organic carbon reservoirs were formulated, but those hypotheses could not easily be tested using available methods. The development of accelerator mass spectrometry (AMS) (Muller, 1977; Bennett et al., 1977; Nelson et al., 1977), which allowed direct counting of ^{14}C atoms (rather than waiting for them to decay) represented a major breakthrough in radiocarbon analysis. AMS reduced the required sample size by three orders of magnitude, to <1 mg C. This single technological advance revolutionized the field of radiocarbon dating, allowing measurements of the ^{14}C content of small amounts of materials to be made with unprecedented ease and speed.

During the two decades following the introduction of AMS, numerous studies of the radiocarbon content of bulk pools of organic carbon in the ocean were published. When combined with information about inorganic carbon, these studies resulted in a more complete picture of the average ages and residence

times of carbon throughout the global carbon cycle (Figure 1). For example, AMS analysis of dissolved organic carbon confirmed its age of several thousand years. New data also showed that the age of dissolved organic carbon increased with depth in the ocean (Williams and Druffel, 1987; Druffel et al., 1989). Sinking particles in the ocean, thought to primarily derive from phytoplanktonic detritus, appeared to have a young age due to their formation in young surface waters and a rapid settling rate (Druffel et al., 1986; Druffel et al., 1992). But, the radiocarbon content of *suspended* particulate organic carbon (POC) showed that these particles include a component either of old dissolved organic carbon or of old terrestrial organic carbon; this carbon is incorporated (if the former option) or increasingly exposed (if the latter option) during descent—the particles get somewhat "older" with depth (Druffel et al., 1986; Druffel et al., 1992; Druffel et al., 1996). This work revealed major gaps in our understanding of the production, transformation, transportation, and preservation of organic carbon on Earth.

Sample-size constraints still significantly limit the application of CSRA and make it difficult to investigate samples obtained from the water column, such as the DOC and POC pools mentioned above. However, radiocarbon analyses of compound classes (amino acid, lipid, and carbohydrate) extracted from water-

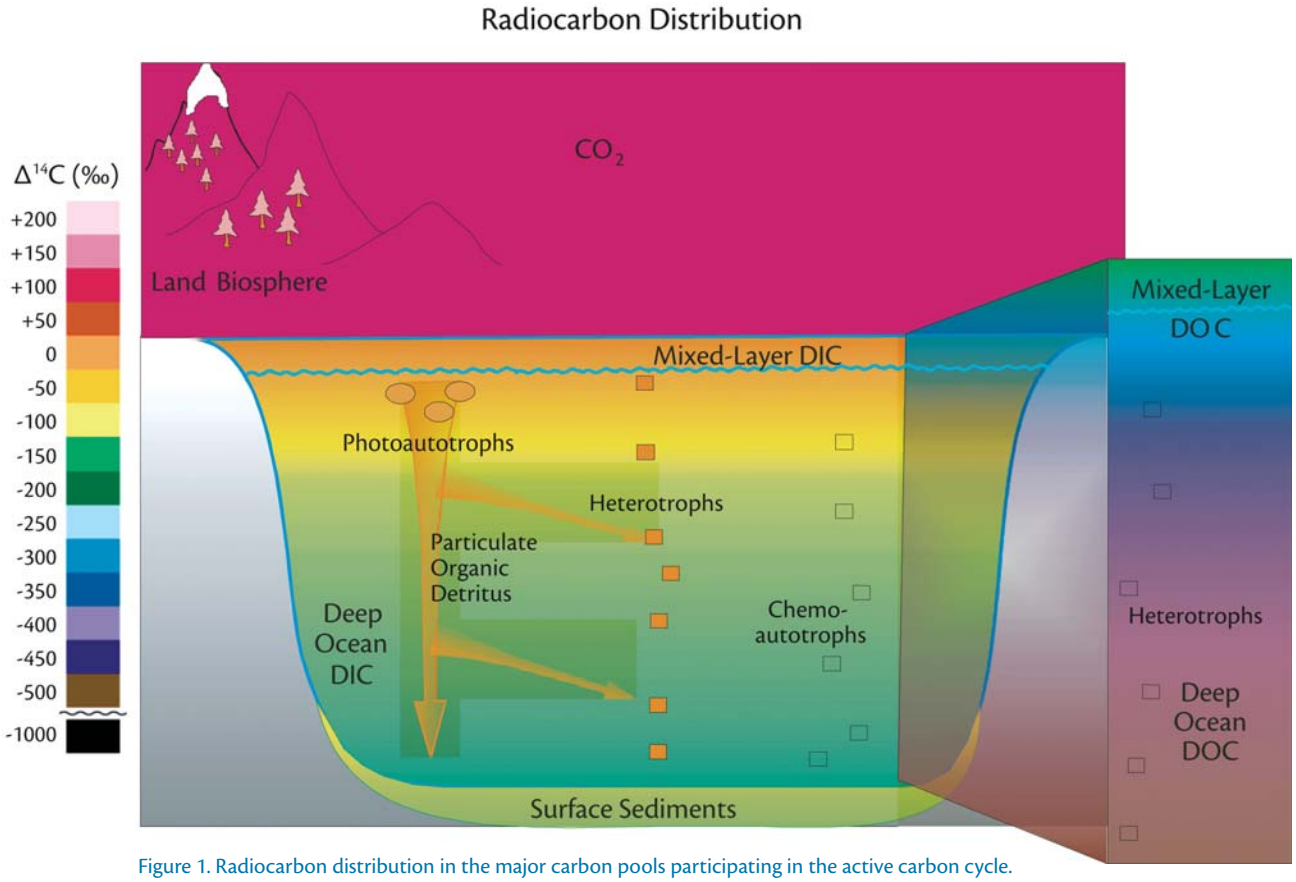

Figure 1. Radiocarbon distribution in the major carbon pools participating in the active carbon cycle. These $\Delta^{14}C$ values will vary depending on year of observation and location of mixed layer. The positive $\Delta^{14}C$ of the atmosphere is a result of the stratospheric testing of nuclear weapons that occurred in the late 1950s and early 1960s. This "bomb spike" has penetrated the surface ocean and is reflected in recently produced autotrophic biomass as well as in the heterotrophic consumers of this fresh organic matter. Particulate organic carbon (POC) that sinks rapidly through the water column retains this young component. Dissolved organic carbon (DOC) is older than dissolved inorganic carbon everywhere in the ocean. Like POC, DOC is heterogeneous and also contains young and old carbon. Illustration modified after Pearson (2000) by L. Aluwihare.

column materials currently are advancing our understanding of these pools of carbon (e.g., Wang et al., 1998; Aluwihare et al., 2002; Hwang et al., 2003). Initially, a more promising avenue was to develop CSRA to study biomarkers obtained from sediments, where typically much greater quantities of sample are available. CSRA as applied to marine sediments could be used to answer numerous important questions. For example, the radiocarbon age of individual compounds purified from sediments would allow a better understanding of the time-scales over which the CO_2 fixed into terrestrial biomass is transported to the ocean and buried in marine sediments. CSRA also would permit compound-specific reconstruction of sediment core chronologies, using biomarkers specific to marine sources. Thus, the chronologies of marine sediments, and of the geochemical proxies contained therein, could be investigated in ways not possible without CSRA. However, to realize these goals, the initial challenge was to devise a method by which individual organic biomarkers from known sources could be extracted and purified for radiocarbon analysis.

Beginning ten years ago, Tim Eglinton and others at the Woods Hole Oceanographic Institution pioneered the development of a method that allowed the measurement of the radiocarbon content of individual biomarker compounds. Individual compounds were separated from their original complex mixtures in quantities large enough for AMS. In order to allow the radiocarbon content of individual compounds to be analyzed, new developments in AMS technology were necessary. In particular, microscale AMS techniques that allowed samples containing <100 µg of carbon to be measured accurately were required to make CSRA routine (Pearson et al., 1998). These techniques would sufficiently reduce the sample size requirements to allow such small samples to be analyzed. The first data using this approach were published by Eglinton et al. (1997). In the following decade, a new field of study has blossomed: numerous new applications show great promise for advancing our understanding of the chemical, physical, and biological pathways and rates of organic carbon transformations that ultimately play an important role in regulating the global carbon cycle, and by extension, global climate.

CSRA is now entering the mainstream, and it continues to show promise as an approach for determining the pathways of carbon fixation by organisms, as well as the subsequent degradation, transport, and ultimate burial of that carbon. Oceanographic applications of CSRA include studies of the fixation, transformation, transport, and preservation of organic carbon; elucidation of microbial metabolic pathways; sources and reactivity of dissolved organic carbon; organic paleo-proxy dating; and development of improved sediment chronologies.

METHODS

CSRA requires the separation of complex mixtures into their individual components. This process usually involves a multiple-step purification procedure that culminates in the collection of a single compound in high purity (Figure 2). Initially, lipid biomarkers were targeted for CSRA studies due to their structural diversity, organism specificity, and ease of separation by preparative capillary gas chromatography (PCGC) (Eglinton et al., 1996). In these studies, samples are extracted with organic solvent and separated into bulk fractions based on polarity, using silica gel or Al_2O_3 column chromatography. These fractions then are derivatized to make them GC-amenable. Derivatized lipids are injected onto a gas chromatograph and the compounds are collected in cooled tubes as they elute from the column. Once collected, a purified compound is sealed in an evacuated quartz tube containing CuO as an oxidant. The tube is placed in an oven at ~850°C for several hours, converting the organic carbon into carbon dioxide. This carbon dioxide is then reduced to graphite, which is analyzed by AMS. Due to the success of the PCGC method, more recent work has aimed to broaden the types of compounds that are amenable to CSRA.

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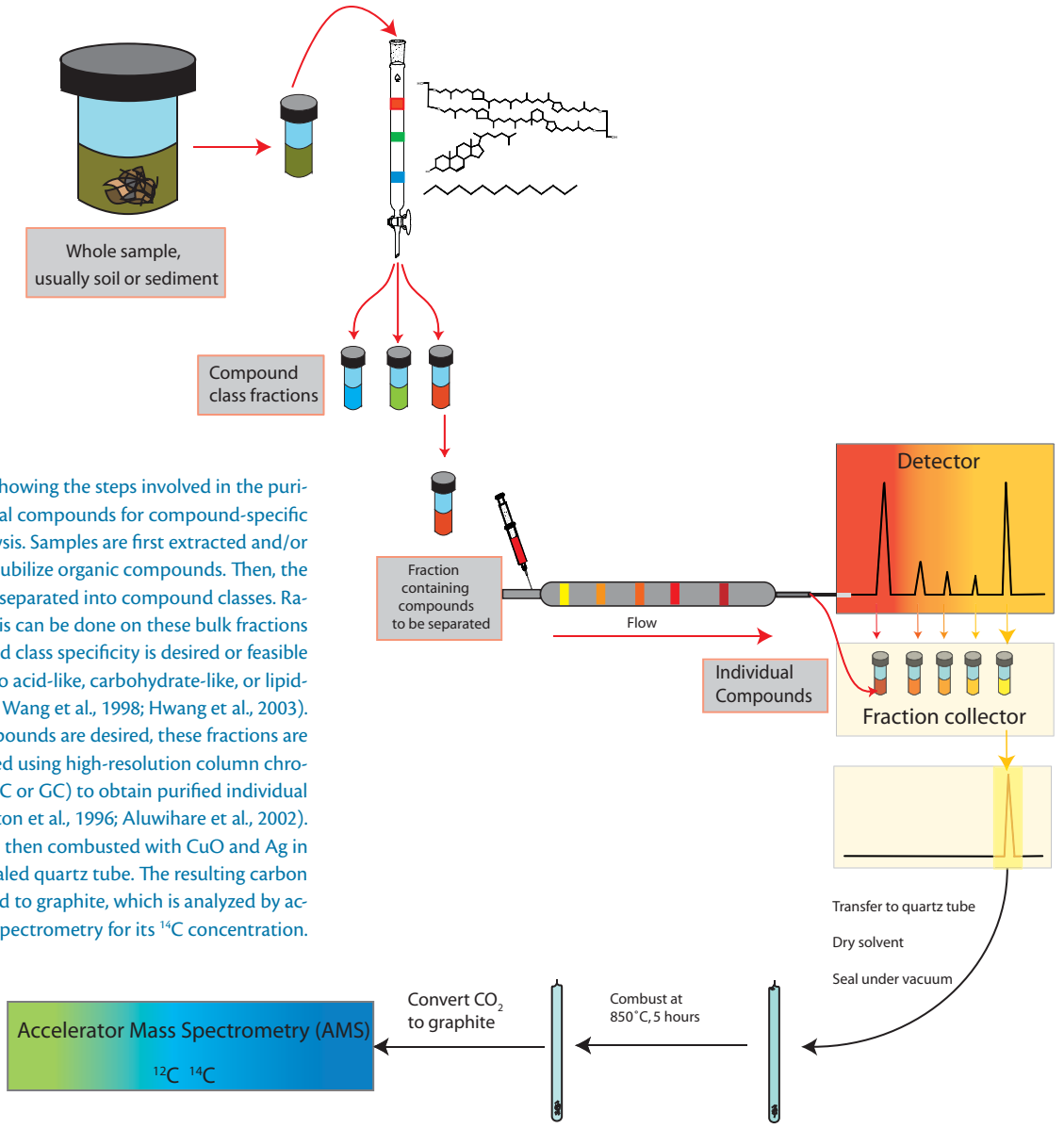


Figure 2. Scheme showing the steps involved in the purification of individual compounds for compound-specific radiocarbon analysis. Samples are first extracted and/or hydrolyzed to solubilize organic compounds. Then, the bulk extract is separated into compound classes. Radiocarbon analysis can be done on these bulk fractions if only compound class specificity is desired or feasible (alkenones, amino acid-like, carbohydrate-like, or lipid-like material) (e.g., Wang et al., 1998; Hwang et al., 2003). If individual compounds are desired, these fractions are further separated using high-resolution column chromatography (HPLC or GC) to obtain purified individual compounds (Eglinton et al., 1996; Aluwihare et al., 2002). Each compound is then combusted with CuO and Ag in an evacuated, sealed quartz tube. The resulting carbon dioxide is converted to graphite, which is analyzed by accelerator mass spectrometry for its ^{14}C concentration.

Currently, a variety of separation techniques are employed in CSRA studies (Figure 3). Both high-pressure liquid chromatography (HPLC) as well as various bulk separation techniques are being used for studies of polar compounds and macromolecules. While PCGC usually requires the derivitization of lipid compounds of interest, HPLC studies have focused on methods that eliminate the need for derivitization and focus on non-volatile polar compounds such as sugars, amino acids, polar lipids, and polyamines (Aluwihare, et al., 2002; Smittenberg et al., 2002; Ingalls et al., 2004; Quan and Repeta, 2005). Other methods have used low-pressure column chromatography to separate alkenones (Ohkouchi et al., 2002), and wet chemical techniques have been used to purify DNA (Cherrier et al., 1999). Each of these new methodological developments promises to yield a wealth of information.

SOME EXAMPLES OF THE APPLICATION OF CSRA

1. Archaeal Lipid ^{14}C and Prokaryotic Metabolic Pathways

One of the first applications of CSRA was to determine the extent of heterogeneity of the sources of lipid biomarkers found in marine sediments (e.g., Eglinton et al., 1997; Pearson and Eglinton, 2000, Pearson et al., 2001). From this work came an interesting application: investigation of the carbon sources used

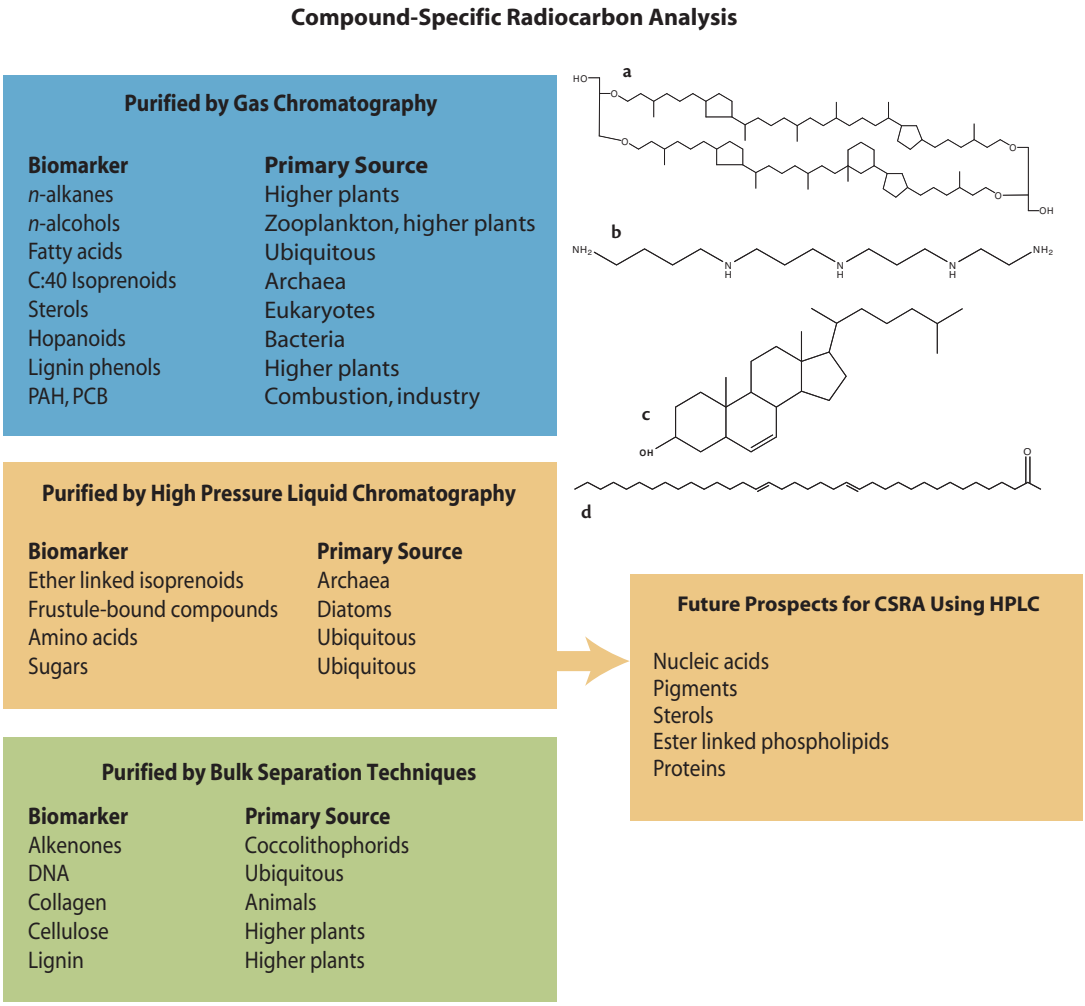


Figure 3. Compounds—and their primary sources—that currently are being used by compound-specific radiocarbon analyses (CSRA), as well as those that could be targeted for future studies. Selected structures from top to bottom: a) isoprenoidal glycerol dialkyl glycerol tetraether (GDGT); b) cholesterol; c) long-chain polyamine; and d) C_{37} alkenone.

by and metabolic pathways expressed by microorganisms in the environment. It has been estimated that <1% of all species of microbes from a given sample can be identified and grown in pure culture. Therefore, methods to assess the metabolism of natural consortia *in situ* are a valuable way to look at microbial groups or species and their role(s) in the global carbon cycle.

Samples of biomass-rich sediment from Santa Monica and Santa Barbara Basins, California, were studied by CSRA (Pearson et al., 2001). One interesting finding of this early work was the realization that virtually all of the biomass living at the sediment-water interface (~900 m depth) persisted by consuming only the "fresh" photosynthetic carbon raining down from surface waters (Figure 4). The lipids of bacteria (hopanols, odd-numbered and branched fatty acids), eukaryotes (sterols), and total community biomass (linear C_{16} and C_{18} fatty acids) were equal in ^{14}C content to the dissolved inorganic carbon (DIC) of surface waters. This occurred despite the "older" bulk radiocarbon age of the total organic carbon matrix from which these biomarkers were obtained. Significantly, all of the above-mentioned compounds contained measurable "bomb"-^{14}C, or $\Delta^{14}C$ values > 0‰ (Figure 1). Bomb-^{14}C refers to the increase in atmospheric ^{14}C concentration caused by above-ground nuclear weapons testing in the 1950s

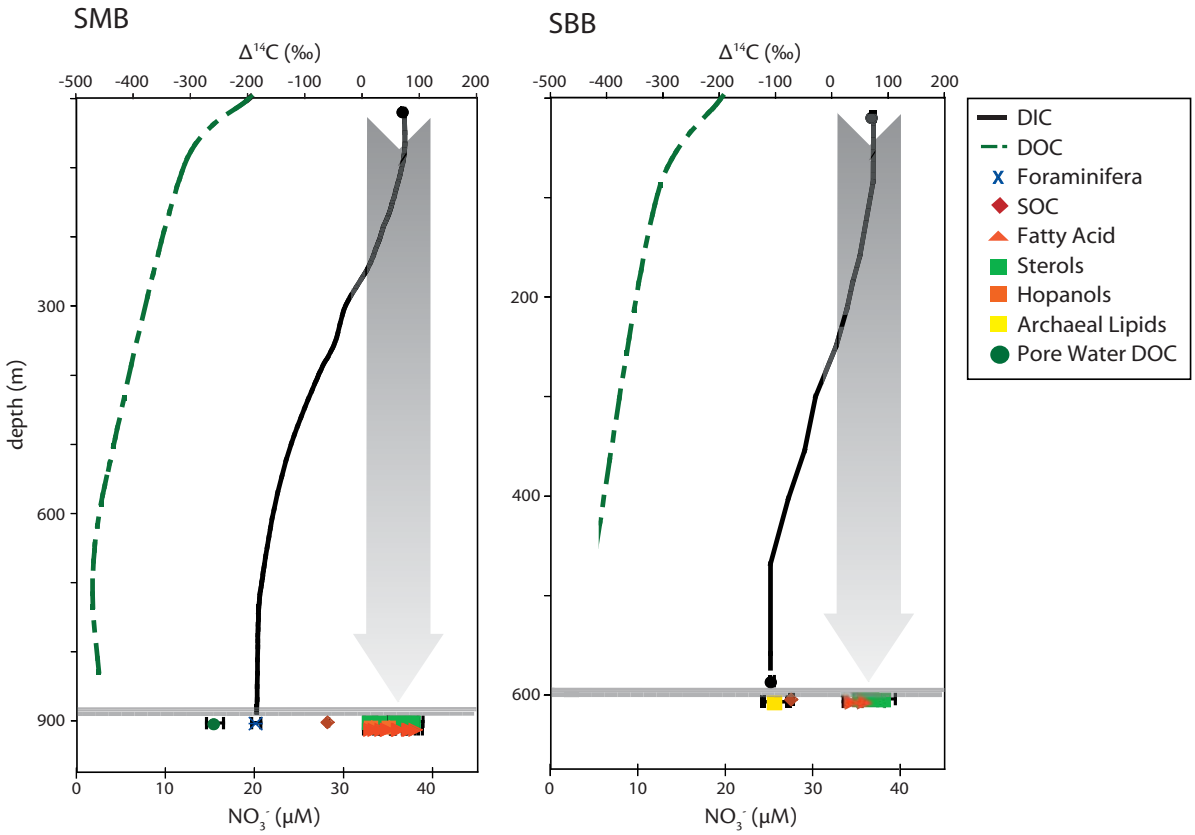


Figure 4. Compound-specific radiocarbon data for the sediment-water interfaces of Santa Monica and Santa Barbara Basins (adapted from data in Pearson et al., 2001). The large arrows show how the ^{14}C signatures of most biomarker classes correspond to the $\Delta^{14}C$ value of surface-water DIC. These biomarkers include fatty acids of bacteria and eukaryotes, bacterial hopanols, and eukaryotic sterols. In contrast, ether-linked lipids of archaea correspond to the ^{14}C composition of deep basin DIC. The bulk pools of organic carbon (total sedimentary organic carbon [SOC]) and dissolved organic carbon (DOC) are more ^{14}C-depleted than the biomarkers in both basins.

and 1960s. This artificial pulse of radiocarbon has invaded the modern surface ocean and terrestrial biota and allows the products of recent biological production to be identified easily. The modern surface ocean and terrestrial biosphere, and the biomass produced from CO_2 in contact with these reservoirs, presently contains this excess ^{14}C; in contrast, pre-1950s organic materials or biomass formed in locations physically removed from the influence of atmospheric CO_2 (i.e., the deep ocean) are distinguished by their lack of incorporation of bomb-^{14}C. The bomb-derived ^{14}C signal, therefore, was used as a diagnostic indicator for the products of surface-water marine autotrophy (Pearson et al., 2001).

The only exception to the above finding helped to reveal the metabolic pathway of the mysterious marine pelagic archaea. Free-living archaea (mostly Crenarchaeota) comprise up to 40 percent of all prokaryotic cells found below the photic zone (Karner et al., 2001), and the ether-linked isoprenoid lipids of these organisms are found widely in marine sediments (e.g., Schouten et al., 2000). Because these lipids are unusually enriched in ^{13}C compared to the typical ^{13}C-contents of marine lipids, it was speculated that the archaeal assemblage could be dominated by autotrophic species or perhaps by an unusual group of heterotrophs (Hoefs et al., 1997). The CSRA data from Santa Monica and Santa Barbara Basin sediments, which were measurements made on the cleaved isoprenoid alkane side-chains of the original lip-

ids, showed that the ^{14}C age of these organisms was identical to that of dissolved inorganic carbon (DIC) in the deep basin waters (Pearson et al., 2001). The results supported an autotrophic metabolism for the marine archaea. Such findings subsequently were confirmed by Wuchter et al., (2003), who showed uptake and incorporation of ^{13}C-DIC by archaea in a mesocosm incubation experiment carried out under ambient conditions and in the dark and by Herndl et al. (2005), who confirmed autotrophic incorporation of DIC throughout the water column.

2. Alkenone ^{14}C Age and Paleotemperature Proxy Records

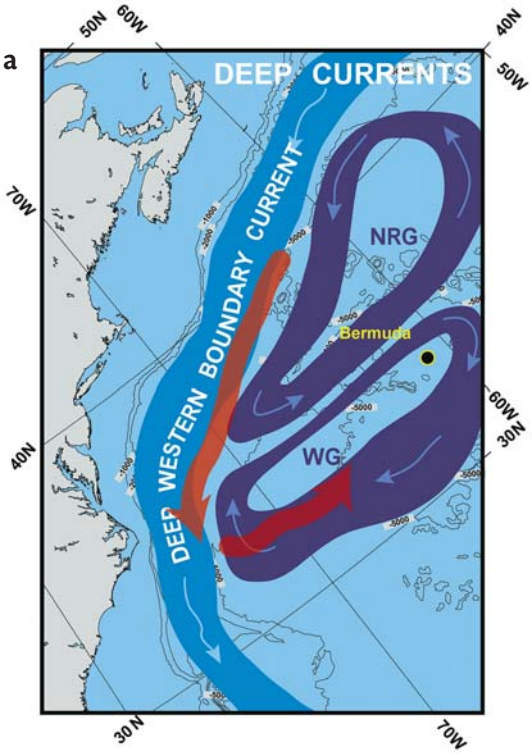
Alkenones are a class of lipid biomarkers that are produced exclusively by coccolithophorids, calcareous microphytoplankton that live in the surface ocean. Coccolithophorids produce abundant methyl- and ethyl-ketones, and the variable ratio of double bonds present in these compounds provides a record of the temperature of the water in which the organisms lived (Brassell et al., 1986). When coccolithophorids die and their remains sink to the seafloor, the composition of the double bonds in the alkenones is preserved. This so-called alkenone unsaturation index, or U^k_{37}, measured in marine sediment cores has been calibrated (Prahl and Wakeham, 1987) and used to determine the temperature of ocean surface waters in the past. Traditionally, alkenones from sediment cores are analyzed and the timing of the observed temperature changes is established using the radiocarbon content of calcareous tests of foraminifera from the same sediment sample. Many of these studies intentionally target drift deposits that have high sedimentation rates—such as those found in the subtropical Atlantic Ocean—in hope of obtaining high-resolution records of past temperature (Keigwin et al., 1984; Sachs and Lehman, 1999).

The alkenone paleotemperature proxy is a valuable tool for reconstructing ocean temperatures, and could be particularly valuable in sedimentary environments where foraminifera are scarce or absent. However, in a re-examination of the sediments of the Bermuda Rise drift deposit, compound-specific radiocarbon analysis of alkenones revealed that the alkenones were up to 7000 ^{14}C years older than co-occurring foraminifera (Ohkouchi et al., 2002). In addition, the calculated U^k_{37} of these samples corresponded to a sea surface temperature range of 7°C over the last 20,000 years, a much larger range than implied by other studies (Keigwin, 1996). Ohkouchi et al. (2002) suggested that these alkenones originally were produced during coccolithophorid blooms in the cold North Atlantic Ocean and initially were deposited on the Scotian Margin. These fine-grained sediments (and the alkenones they contained) were re-suspended by bottom currents, transported, and re-deposited in the sediments of the Bermuda Rise below warmer waters (Figure 5). This work gives pause to researchers carrying out paleoceanographic studies on the fine fraction of sedimentary deposits; follow-up to this initial study has resulted in important advances in our understanding of how sediment deposits record the past (Mollenhauer et al., 2003, 2005). The simultaneous analysis of the U^k_{37} and radiocarbon compositions of alkenones within the same sediment cores now makes it possible to determine the provenance of these important paleotemperature indicators in sediments and assess the integrity of the fine fraction of sediment cores for paleoceanographic work. This surely will improve the quality of paleotemperature reconstructions based on the alkenone temperature proxy.

3. Diatom ^{14}C and Sediment Core Chronologies

Diatoms are single-celled phytoplankton that live in the surface ocean and secrete amorphous, biogenic silica. The complex structures of these shells, or "frustules," are in part created by the diatom's use of a template of organic carbon. Like coccolithophorids, diatoms record the surface water environment in which they grew; upon death, diatom frustules sink through the water column and are deposited in sediments. As is the case with alkenone records, the timing of paleoproxy records obtained from the chemical constituents of sedimentary diatom frustules is often based on the ^{14}C-age of calcium carbonate in co-occurring shells of foraminifera.

Although many researchers have used the chemical and isotopic content of diatom frustules to understand the historical record of nutrient cycling and biological productivity, several factors have been shown to affect the reliability of diatom-derived records (Zheng et al., 2002; Robinson et al., 2004). First, the origin of diatomaceous deposits—particularly those with high accumulations rates—is not always known. Second, such deposits may not have foraminifera with which to establish ^{14}C-based core chronologies. Therefore, developing a radiocarbon clock based on carbon in-



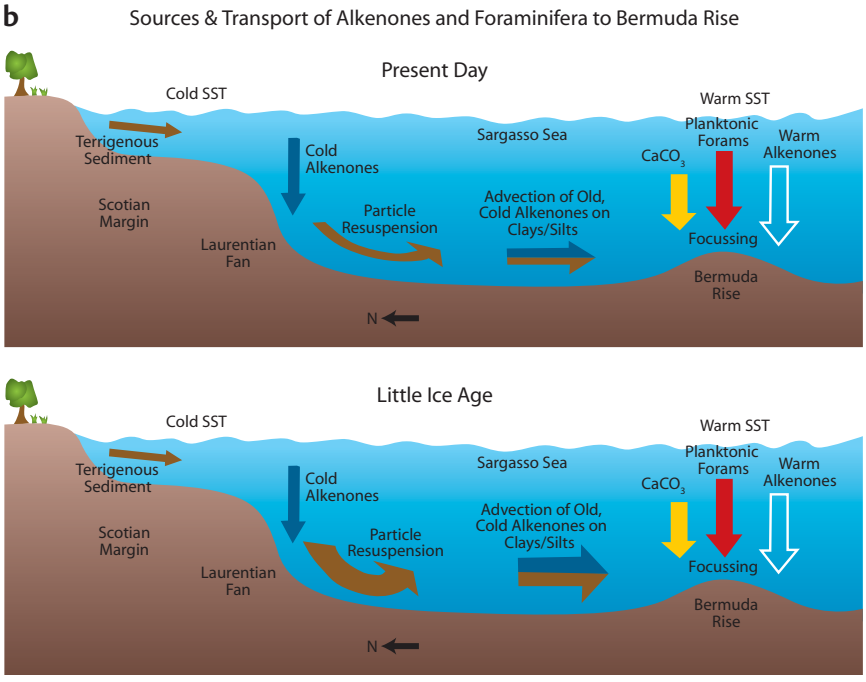


Figure 5. a) Map of the North Atlantic showing how deep currents can redistribute bottom sediments, particularly fine-grained material. These processes explain the old radiocarbon age of alkenones (temperature indicators) relative to foraminifera found in sediments of the Bermuda Rise (Ohkouchi et al., 2002). b) Schematic representation of how alkenones that are formed in high latitude cold locations are transported to lower latitude warm locations. During the Little Ice Age, lateral transport was more important than it is today. Paleoceanographic investigations that use proxies associated with fine-grained sediment constituents must consider the possibility that lateral advection could influence the sediment record. Figures contributed by T. Eglinton; illustration modified from L. Gathercole.

herent to the diatoms themselves is one approach for addressing these complicating factors. Organic compounds in frustules are thought to be biosynthesized by diatoms for the purpose of catalyzing the precipitation of biogenic silica during formation of frustules (Kröger et al., 1999). To date, long-chain polyamines and proteins containing polyamine-modified amino acids have been identified in diatom frustules (Kröger et al., 2000, 2002; Ingalls et al., 2004). Preliminary results suggest that additional compounds also are present (A. Ingalls, unpublished data). These compounds appear to be protected physically from degradation for long time scales, either due to their close association with the frustule (surface adsorption) or to encapsulation of the compounds in the mineral matrix (Shemesh et al., 1993; Sigman et al., 1999, Ingalls et al., 2003).

Promising results recently have been obtained from a new method for dating individual organic compounds purified from frustules (Ingalls et al., 2004). In this method, samples of diatoms are cleaned, dissolved in hydrofluoric acid and individual compounds are purified using preparative high-pressure liquid chromatography (HPLC) coupled to mass spectrometry. Early results suggest that some massive diatom deposits in the Southern Ocean are several thousand years older than the foraminifera from nearby sediment horizons (Figure 6). Therefore, these deposits must be sediment drifts resulting from the transport of relict sediments by bottom currents. These findings confirm earlier reports of substantial lateral transport and focusing of fine-grained material, including diatom frustules, in the Southern Ocean (e.g., Chase et al., 2003). However, it does not appear that laterally transported frustules are *always* an important component of Southern Ocean sediments. One sediment core showed relatively good agreement between the age of diatoms and foraminifera, suggesting the frustules in this core primarily were derived from

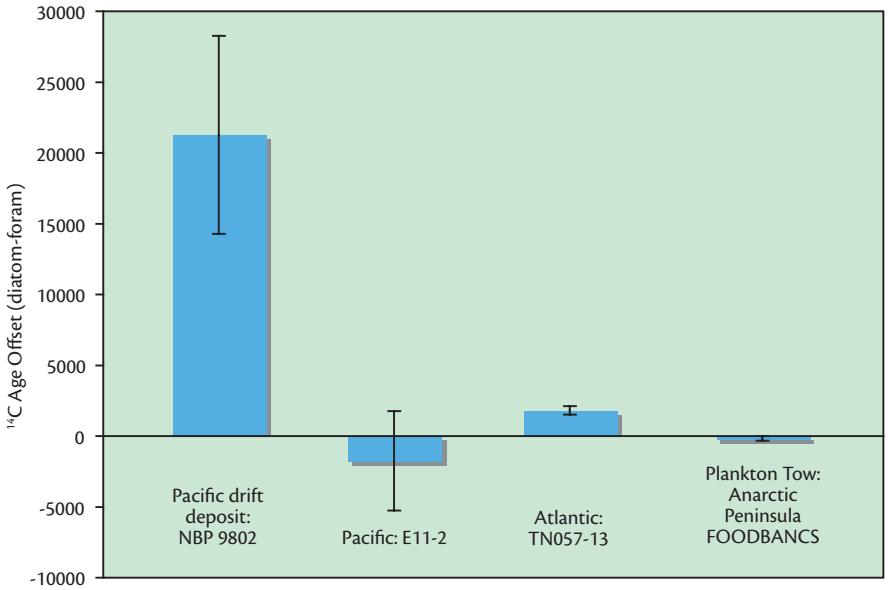


Figure 6. Plot showing the age difference between organic compounds in diatom frustules relative to that of foraminifera in three sediment cores. Large age differences exist at a drift deposit having a very high rate of sediment accumulation, suggesting extensive lateral transport. Small age differences are present in a core from the Atlantic sector of the Southern Ocean suggesting that most of the diatoms were deposited from overlying water. Analysis of diatom frustule-bound compounds and bulk carbon from a plankton tow collected in surface waters of the Antarctic Peninsula in the Southern Ocean show that these two pools of carbon are of similar age (data from Ingalls et al., 2004).

diatoms living in the overlying waters (Ingalls et al., 2004). This method holds promise for contributing to a better understanding of sediment transport and defining sediment core age models in the Southern Ocean and other diatom-rich areas that lack calcium carbonate.

4. Other Applications

The examples presented above represent only a fraction of the CSRA methods that are currently employed or under development. In addition to these examples, studies of fatty acids derived from phospholipids of bacterial origin have been used to follow the incorporation of amorphous carbon (kerogen) derived from ancient sedimentary rocks into microbial biomass (Petsch et al., 2001). This type of study is analogous to laboratory tracer experiments in which isotopically labeled compounds are introduced to microbial populations to trace the flow of carbon. Instead, Petsch et al. (2001) took advantage of the complete absence of ^{14}C in ancient kerogen to examine the utilization of this "refractory" organic matter by living microbes, and they found that it was indeed bioavailable.

Aluwihare et al. (2002) isolated and then hydrolyzed high-molecular-weight (HMW) dissolved organic carbon (compounds >1kDa) from seawater. The hydrolysate contained sugars that were subsequently purified by HPLC for ^{14}C dating by AMS. This work demonstrated that, despite the old average age of bulk DOC, there is a component of HMW DOC that is quite young. These individual sugars—and the oligosaccharides from which they were cleaved—likely were produced in surface waters by phytoplankton. Even in the deep waters of the Pacific, HMW DOC-derived sugars have a modern age suggesting that they must be transported to deep water in sinking particles where they are subsequently released into the dissolved pool.

In environmental science, CSRA is being used to investigate the origin of organic compounds of environmental concern that have industrial sources (Reddy et al., 2002, 2004). Polycyclic aromatic hydrocarbons and halogenated organic compounds often are assumed to originate from anthropogenic sources such as fossil fuel combustion or industrial processes. Radiocarbon dating of these compounds allows sources to be apportioned between natural (biomass burning and marine organisms) and industrial sources. The primary assumption is that most industrial sources are derived from petroleum and are devoid of radiocarbon, while naturally produced PAHs and halogenated compounds would have relatively modern signatures.

Finally, the introduction of atmospheric pressure chemical ionization for liquid chromatography mass spectrometry (LC-MS) has allowed the separation and analysis of intact polar lipids, including phospholipids of bacteria and ether-linked isoprenoid lipids of archaea. Archaeal lipids have been purified for

the purpose of obtaining sediment core chronologies (Smittenberg et al., 2004), as well as for studies of the metabolism of archaea in the water column (A. Pearson, unpublished data).

The variety of compounds that have now been purified for CSRA represent a wide spectrum of applications. The success of HPLC and bulk chemical separations (such as the alkenone method) suggests that numerous future possibilities for CSRA exist: future applications likely include using macromolecules such as proteins and nucleic acids, as well as pigments, to understand better the diversity of carbon sources used by living organisms in diverse environmental settings. All of the above examples deal with measurement of natural background values of ^{14}C, or for modern samples, with tracing the transport of ^{14}C derived from the atmospheric testing of nuclear weapons in the 1950s and 1960s. However, in addition to these "natural-abundance" approaches, CSRA is also being applied in the field of medicine. Very low-level addition of radiocarbon-labeled compounds is being used instead of the large quantities that typically are added to biological experiments that use ^{14}C-labelling. For example, small amounts of radiolabeled drugs and their metabolites can be followed in human subjects, allowing the development of more accurate models of drug metabolism in humans.

In the future, not only will new compounds be targeted for CSRA, but improvements in the efficiency of AMS ionization sources may permit samples as small as ~1 µg of carbon to be analyzed. With these new techniques available, it will be more important than ever to focus on reducing and quantifying the extent of background contamination and improving the quality of chromatographic separations used to isolate pure compounds for CSRA. Presently, even the combustion of samples in quartz tubes can result in up to ~1 µg of background carbon. For this reason, the field must move toward continuous flow techniques in which purification and combustion occurs online with AMS analysis. If samples can be combusted in a stream of effluent coming from a gas chromatograph, much like in the GC-C-IRMS method, sample handling and blanks could be minimized, further extending the utility of the growing field of CSRA and illuminating the carbon cycle in even more detail.

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