



## Radiocarbon dating of diatom-bound organic compounds

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Received 31 October 2003; received in revised form 14 April 2004; accepted 30 June 2004

Available online 1 October 2004

### Abstract

Here we present a new method for obtaining radiocarbon dates for the organic compounds intrinsic to diatom frustules. This method will improve age models for sediment cores that lack calcium carbonate and improve current interpretations of diatom-based paleoproxies. In preparation for radiocarbon dating by Accelerator Mass Spectrometry, compounds intrinsic to diatom frustules are released from their opal matrix by dissolution in HF and then purified using preparative liquid chromatography-mass spectrometry (LC-MS). The method was applied to one sample from each of three cores (NBP9802 Station 7 GC2; TN057-13 PC4; E11-2) and a plankton tow (CRS 746, FOODBANCS) collected in the Southern Ocean. In each sample, radiocarbon ages of diatom-bound organic compounds differed from those obtained from foraminiferal CaCO<sub>3</sub>. Agreement between the foraminifera and compound-specific date was best in cores E11-2 and TN057-13. In contrast, compound-specific <sup>14</sup>C ages obtained from NBP9802 differed substantially from those measured for foraminiferal CaCO<sub>3</sub>. The influence of background contamination was assessed throughout all stages of the method and cannot be responsible for the discrepancy observed. Possible reasons for the disagreement between the ages of foraminifera and diatom-bound compounds are discussed in the context of sediment dynamics. These preliminary results suggest that our diatom-based dating method represents a major step forward in our ability interpret sediment records in the Southern Ocean and therefore our understanding of the role of the Southern Ocean in past climate.

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*Keywords:* Diatom-bound organic matter; Polyamines; Radiocarbon; Southern Ocean; LC-MS

### 1. Introduction

Our ability to reconstruct past ocean conditions from marine sediment cores depends on the accuracy

with which we can constrain core chronologies. Radiocarbon dating of foraminiferal calcium carbonate is a common method for determining the age of Holocene to late-Pleistocene age marine sediments. This method of dating relies on the presence of foraminifera in the sediment and the assumption that foraminiferal ages are equal to those of other sedimentary components of interest. Where possible, chronologies based on foraminifera have been applied to cores

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from the Southern Ocean (e.g., Hodell et al., 2001; Shemesh et al., 2002). However, many sedimentary deposits in the Southern Ocean, and elsewhere, lack calcium carbonate (e.g., Andrews et al., 1999). Thus, there is a need to develop an alternative to foraminifera-based  $^{14}\text{C}$  dating. Although some core chronologies have been improved using the radiocarbon age of bulk sedimentary organic carbon (acid insoluble organic matter, AIO), this is not an ideal material for dating sediments because it can derive from many different sources with different ages (Harris et al., 1996; Licht et al., 1998; Rabouille et al., 2002). Organic matter from different sources is also subject to a variety of post-depositional processes, resulting in age offsets among organic constituents and calcium carbonate (Eglinton et al., 1997; Ohkouchi et al., 2002).

Since AIO dating methods remain problematic, new approaches are necessary to improve paleoceanographic reconstructions in areas lacking calcium carbonate. The development of compound-specific radiocarbon dating methods (Eglinton et al., 1996) has led to new efforts to date individual fatty acids in sediment cores from the Ross Sea (Ohkouchi et al., 2003). While this approach looks quite promising, it requires large sample sizes (100–350 g dry weight of sediment). In addition, with the advent of proxies for past productivity and nutrient utilization that use diatom-bound organic matter (Shemesh et al., 1993; De La Rocha et al., 1998; Sigman et al., 1999; Rosenthal et al., 2000), it would be advantageous to know the age of sedimentary diatoms.

Like the shells of foraminifera, diatom frustules are denser than seawater; and despite their smaller size, they sink relatively rapidly. Instead of dating the mineral phase, as in methods based on  $\text{CaCO}_3$ , the source of carbon in the diatom method is the organic matrix locked within the diatom frustule during biomineralization. Like the shells of planktonic foraminifera, diatom biomass records the age of surface-water dissolved inorganic carbon at the time the organisms grew. Presumably the age of individual, frustule-bound compounds is equal to that of total diatom biomass.

Previous attempts to date diatom-bound organic carbon have focused on the total organic matter held within diatom frustules. These attempts have been unsuccessful, most likely due to adsorption of atmospheric  $\text{CO}_2$  and volatile organic compounds

onto frustule surfaces (Zheng et al., 2002). Incomplete cleaning may also result in erroneous ages. Recent work also demonstrates that even in the absence of analytical artifacts, the age of phytoplankton-derived organic compounds can differ from the age of co-occurring carbonate phases, including foraminifera (Mollenhauer et al., 2003). The favored explanation for this age offset invokes preferential redistribution of fine sediment relative to coarse sediment grains (e.g. foraminifera). This mixing may occur through the combined action of bottom currents and bioturbation, and the net effect is to deposit older, fine-grained material among the coarser foraminiferal deposits.

Our study attempts to remedy the analytical problems associated with dating bulk opal-intrinsic organic matter by developing a method to date specific compounds or groups of compounds from diatom frustules. Once accurate ages are available, the issue of sediment redistribution can be addressed. In the method presented here, individual compounds are isolated using a novel preparative liquid chromatography-mass spectrometry (LC-MS) method. These isolates are dated using the microscale approach to  $^{14}\text{C}$ -AMS analysis that was developed previously for measuring the  $\Delta^{14}\text{C}$  values of individual organic compounds isolated by preparative capillary gas chromatography (Eglinton et al., 1996; Pearson et al., 1998).

Many of the organic compounds associated with diatom frustules have already been characterized. Both proteins and long-chain polyamines are involved in the silicification process and are entrapped in the mineral phase during formation of frustules (Kröger et al., 1999, 2000, 2002). Amino acids are preserved in frustules for long time scales, suggesting that other organic components may also be preserved (Ingalls et al., 2003). Studies of the C/N ratio as well as the carbon and nitrogen isotopic composition of bulk diatom-bound organic matter also suggest that this organic matter is preserved on glacial/interglacial time scales (Singer and Shemesh, 1995; Sigman et al., 1999; Crosta et al., 2002).

In addition to providing a means to constrain the timing of past ocean events, the method presented here will lead to a better understanding of the carbon cycle of the Southern Ocean. By understanding the timing of diatom deposition and burial relative to other organic and inorganic sedimentary constituents,

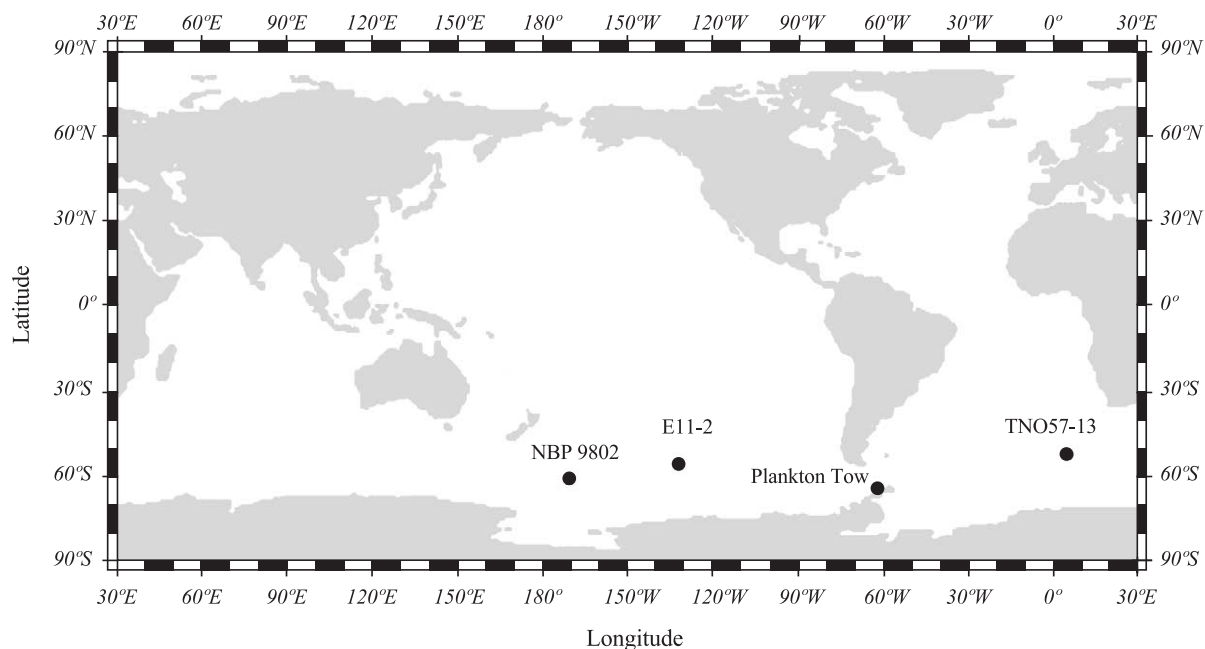


Fig. 1. Map of sample locations.

we will broaden our understanding of the role of diatoms in the long-term preservation of organic carbon. This work also expands on recent findings showing that organic matter in sediments derives from a variety of sources with a spectrum of ages that depend on source, carrier phase and subsequent physical processing (Eglinton et al., 1997; Pearson et al., 2001; Mollenhauer et al., 2003).

## 2. Methods

### 2.1. Sample locations

One sample from each of three cores was examined in this study (Fig. 1; Table 1): NBP9802 Station 7 GC2 (60.28°S 170°W), Pacific sector, 41–45 cm, collected during the Joint Global Ocean Flux

Table 1

Size, location and radiocarbon age of sediment cores and standard materials analyzed. Sediment ages are from foraminifera CaCO<sub>3</sub>. Plankton age is from bulk planktonic organic carbon

Sample name	Latitude	Longitude	Depth (cm)	<sup>14</sup> C Age <sup>a</sup>	Error (year)	Reference
NBP9802 St7 GC2	60.28°S	170°W	21–22	7370	55	this work
			129–134	6000	35	this work
TN057-13 PC4	53.2°S	5.1°E	448–450	9340	60	Hodell et al. (2001)
E11-2	56.04°S	115.06°W	174–176	27,420	320	Ninnemann and Charles (1997)
Plankton <sup>b</sup>	64°48.56'S	65°21'06W	surface water	1177	n/a	DeMaster
Lysine <sup>c</sup>	n/a	n/a	n/a	>modern		this work
Alanine <sup>d</sup>	n/a	n/a	n/a	infinite		Sigma
Charcoal	n/a	n/a	n/a	infinite		LLNL CAMS

n/a: not applicable.

<sup>a</sup> All ages are <sup>14</sup>C years (Libby half life) according to the convention of Stuiver and Polach (1977) with no reservoir correction.

<sup>b</sup> CRS 746 Food Bancs V, March 01, 2001.

<sup>c</sup> Lysine was from a modern source (Sigma).

<sup>d</sup> Alanine is a <sup>14</sup>C free standard (Sigma Lot # 57H0124).

Study aboard the N.B. Palmer in 1998; TN057-13 PC4 (53.2°S 5.1°E) Atlantic sector, 448–450 cm, collected in 1996 aboard the R.V. Thompson; and E11-2 (56.04°S 115.06°W) Pacific sector, 174–176 cm, collected in 1963 aboard the R.V. Eltanin. TN057-13 PC4 and E11-2 were stored refrigerated at 4 °C at Lamont Doherty and Florida State University in their Antarctic Marine Geology Research Facility, respectively. NBP9802 Station 7 GC2 was sectioned at sea and stored at room temperature at Lamont Doherty. A freeze-dried, Southern Ocean plankton tow (CRS 746, Food Bancs V, collected March 01, 2001 at 64°48.56'S, 65°21.06'W) was obtained from Dave DeMaster (North Carolina State University).

## 2.2. Sediment cleaning procedure and HF digestion

Sediment samples were subjected to a series of cleaning steps in order to remove non-diatom bound organic matter (Fig. 2). These cleaning steps were

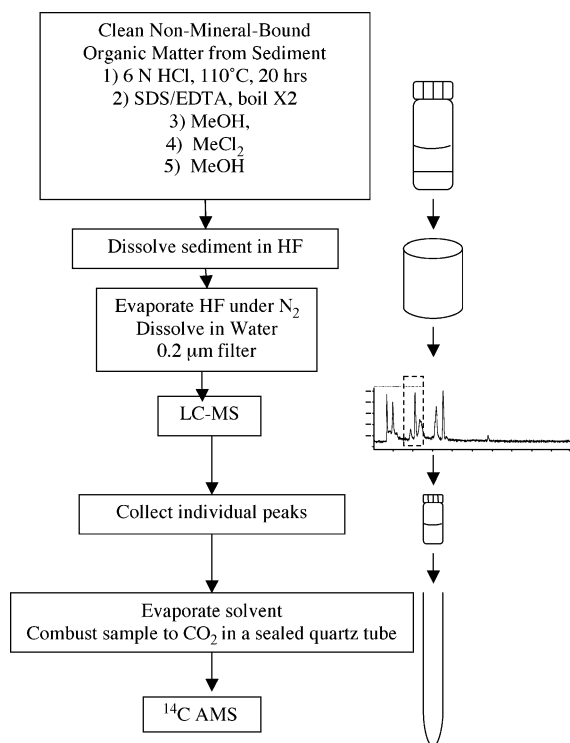


Fig. 2. Scheme for cleaning and analysis of diatom-bound organic matter from sediments.

carried out in Teflon centrifuge tubes and samples were centrifuged between each cleaning step to remove the reagent. Cleaning steps were as follows: hydrolysis in 6 N HCl for 20 h at 110 °C, boil in 2% SDS/100 mM EDTA solution 2× for 2 h (sodium dodecyl sulfate ethylene tetracetic acid (SDS/EDTA) solution is a metal chelator and anion pairing agent (surfactant) used to solubilize non-diatom-bound organic matter). Samples were then rinsed with milliQ water 7×, extracted with methanol 3×, extracted with methylene chloride 3×, extracted with methanol 3× and dry at 50 °C overnight. This cleaning procedure was compared with a similar procedure that lacked the SDS/EDTA step.

Clean dry sediment was weighed (~1–2 g) into a Teflon beaker, and cold HF (concentrated) was added to the beaker to dissolve diatom frustules. HF was removed by evaporation under a gentle stream of N<sub>2</sub>. The demineralized sample was then dissolved in water, filtered through a 0.2-μm Teflon syringe filter and subjected to LC-MS without further purification.

## 2.3. LC-MS

Compounds in HF extracts were separated and identified on an Agilent 1100 LC-MS equipped with a Zorbax Eclipse XDB-C8 column (4.6×150 mm) maintained at 50 °C. Compounds were eluted with a gradient of acetonitrile (Solvent A) and water (Solvent B) at a flow rate of 1 ml/min. The program ramped from 0%B to 20%B in 7 min, 50%B after 9 min and 80%B after 12 min, maintained at 80%B for 3 min and then equilibrated in 0%B for 10 min between injections.

Compounds were detected using electrospray ionization in positive ion mode. Spray chamber settings were as follows: Drying gas flow rate 13 ml/min, nebulizer pressure 45 psig, drying gas temperature 350 °C, capillary voltage 3500 V. Positive ion spectra were generated by scanning from 70 to 600 *m/z*.

During runs in which compounds were collected for isotopic analysis, an active splitter was used to divert 1% of the sample to the mass spectrometer and 99% of the sample to the fraction collector. In order to maintain an adequate solvent flow rate into the mass spectrometer, a make-up pump was used to deliver 0.5 ml/min of 50%A and 50%B throughout

the run. Individual chromatographic peaks were collected into 0.5-ml Teflon vials. Up to 12 injections of each sample were made and the fractions for each peak from all injections were pooled into Teflon centrifuge tubes and evaporated to a volume of <2 ml by heating (50 °C) under a stream of ultrahigh purity N<sub>2</sub>.

#### 2.4. Sample combustion and <sup>14</sup>C dating

Samples were transferred from Teflon centrifuge tubes to pre-combusted (850 °C, 5 h) quartz tubes (9 mm o.d.). The remaining solvent was freeze-dried on a vacuum line, CuO and Ag were added to each tube, and the tubes were flame-sealed under vacuum. Samples were combusted for 5 h at 850 °C to oxidize organic compounds to CO<sub>2</sub>. The quantity of CO<sub>2</sub> generated from combustion was quantified manometrically on a vacuum line. Splits for δ<sup>13</sup>C and Δ<sup>14</sup>C were collected cryogenically in 6 mm o.d. Pyrex tubes. CO<sub>2</sub> from combustion was converted to graphite targets and Δ<sup>14</sup>C was measured by accelerator mass spectrometry at the Center for AMS Research at Lawrence Livermore National Laboratory (LLNL). Dates are reported in conventional <sup>14</sup>C years with no reservoir correction. All reported values were corrected for the estimated size and Δ<sup>14</sup>C of the blank contribution according to the procedure outlined in the following section.

#### 2.5. Quantification and age estimate of process blank

Small amounts of contamination may have a large effect on the measured radiocarbon age of small samples, such as those analyzed here (<10 μmol C) (Eglinton et al., 1996; Brown and Southon, 1997; Pearson et al., 1998). Therefore, the influence of background carbon was estimated by determining the quantity and age of carbon contributed during sample preparation, and corrections were applied to the measured radiocarbon age of each sample using a mass balance equation. Several experiments were carried out in order to determine the size and age of background carbon.

First, we quantified the combustion blank by measuring the amount of CO<sub>2</sub> generated during combustion of evacuated, sealed quartz tubes containing only CuO and Ag. Next, we evaluated the age of the combustion blank by measuring the Δ<sup>14</sup>C of

CO<sub>2</sub> generated by combustion of radiocarbon free (Δ<sup>14</sup>C = -1000‰) charcoal (5.0 and 51.7 μmol C) and alanine (10.8 μmol C), and radiocarbon-modern (Δ<sup>14</sup>C > 0‰) lysine (20 μmol C) in sealed quartz tubes containing CuO and Ag.

The LC-MS method blank was quantified in two ways. First, effluent was collected from the LC-MS during blank injections. This procedure captured carbon bleeding off of the LC column during four separate time intervals spanning the range of solvent compositions from 0% to 80% acetonitrile in water. To test the size of the blank with respect to volume of effluent (or number of injections), material was collected from seven test runs representing 1, 5, 10, 15, 20, 35 and 49 injections. The amount of CO<sub>2</sub> produced after combustion of these blanks was quantified manometrically. Due to their small size (<0.58 μmol C), the age of the carbon in these blanks was not measured. Instead, we estimate the age of this LC-MS blank by analyzing the Δ<sup>14</sup>C value of a modern standard (lysine) and a radiocarbon dead standard (alanine), and compared these values to the values measured for unprocessed material by closed tube combustion. One lysine sample (5.8 μmol C) and four alanine samples (4.2–9.2 μmol C) were analyzed.

The age of the LC blank was also estimated by measuring the Δ<sup>14</sup>C of diatom frustule-bound compounds from the Southern Ocean plankton tow (CRS 746, FOODBANCS V), in which the bulk carbon had previously been dated by <sup>14</sup>C-AMS (D. DeMaster). Assuming that the Δ<sup>14</sup>C of the bulk carbon is the same as the diatom frustule-bound compounds and that the size of the blank is known, the Δ<sup>14</sup>C of the blank was estimated using a mass balance equation.

#### 2.6. δ<sup>13</sup>C analyses

CO<sub>2</sub> from combustions was analyzed for stable carbon isotope composition on a Micromass VG Optima isotope ratio mass spectrometer in the Laboratory for Geochemical Oceanography at Harvard University using a manifold-cracker inlet. Carbon isotope data are reported in the standard delta notation (δ<sup>13</sup>C), relative to the Pee Dee Belemnite standard, expressed in units of ‰. Sample sizes ranged from 0.42 to 5.0 μmol C.

### 3. Results and analyses

#### 3.1. Sample cleaning

Previous efforts by others to measure the radiocarbon age of bulk diatom-bound organic matter suggest that sedimentary diatom frustules are either very difficult to clean or that they are easily contaminated by exogenous sources of carbon during sample preparation (Zheng et al., 2002). Our work aims to avoid these problems by instead targeting individual, diatom-bound biomarkers for radiocarbon dating. To date, peptides and polyamines have been characterized from HF-digested frustules of cultured diatoms (Kröger et al., 1999).

Here we combined the cleaning methods used in two previous studies to minimize these concerns. We used a 6 N HCl hydrolysis to remove acid-soluble

compounds including amino acids (Ingalls et al., 2003). We also used an SDS/ETDA solution to clean samples. This method was borrowed from previous studies of frustule-bound compounds in fresh diatom cultures (Kröger et al., 1999). Solvent extractions were used as a final rinse to remove less polar compounds and to facilitate drying. We compared the results of this cleaning with those of a simple HCl hydrolysis followed by solvent extraction (Ingalls et al., 2003) and found that the composition and concentration of compounds were indistinguishable with the two methods.

None of the compounds isolated from the HF digests of diatoms (see below) were present in blank digests (HF without diatoms) or in the initial HCl hydrolysate of sediment prior to cleaning. These results suggest that our cleaning method did not introduce contaminants, but does not necessarily

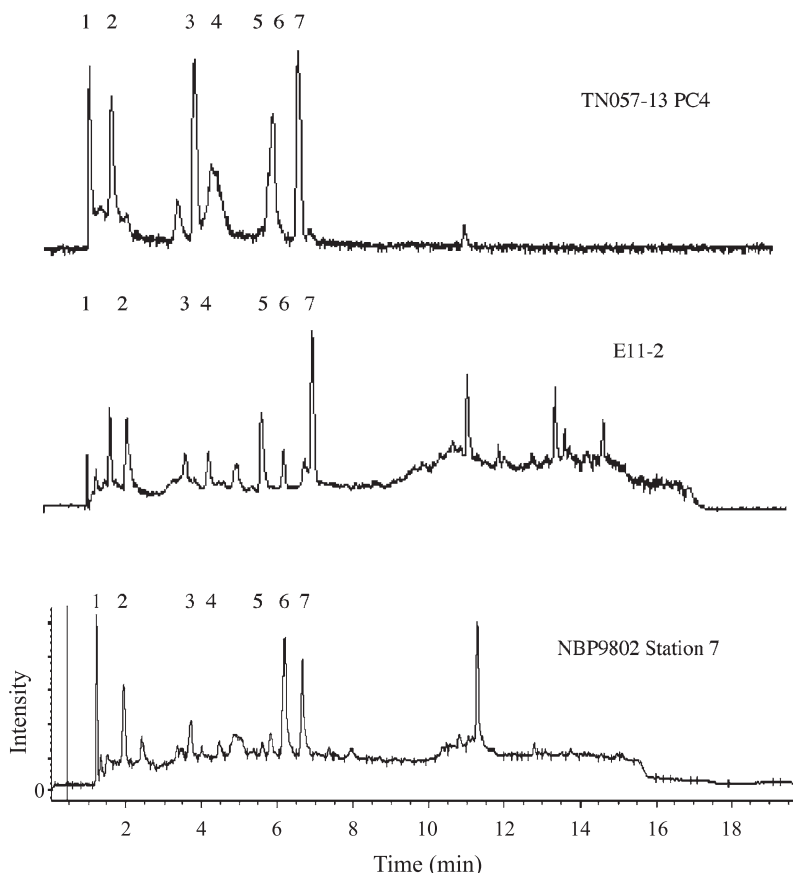


Fig. 3. LC-MS total ion chromatograms and peak assignments for TN057-13 PC4, NBP9802 Station 7 and E11-2.



demonstrate that the frustules were completely cleaned of all unbound organic matter. As an additional control on the thoroughness of the cleaning procedure, multiple compounds were collected for each sample to check for intra-sample variability.

### 3.2. LC-MS analysis

Several major organic compounds were detected by LC-MS positive ion electrospray mass spectrometry (Fig. 3). While each sample contained the same group of major compounds, the relative proportion of each compound differed. In NBP9802 Station 7, the first peak to elute contained a regular series of long-chain polyamines with  $m/z$  range of ~400–600 (Fig. 4) similar to those identified by Kröger et al. (1999). These compounds elute between 1.2 and 1.4 min, in agreement with their high polarity. This regular series of polyamines was not detected in plankton or in samples from TN057-13 or E11-2 (see

Discussion and conclusions). Instead, peak #1 in these samples contained an alternative set of unidentified compounds.

In all samples, a series of low molecular weight compounds eluted between 3 and 8 min (Table 2). The  $m/z$  of each peak is summarized in Table 2 and tentative structures have been assigned to these compounds based on their molecular weights and published reports of similar compounds in diatom frustules (Kröger et al., 1999, 2000; Poulsen et al., 2003). Each compound has a molecular weight that corresponds to a propylamine derivative of putrescine, ornithine, lysine, hydroxylysine or di- and tri-methyl-hydroxylysine. All of these compound classes have been identified in the hydrolysate of the protein silaffin 2, purified from the frustules of cultured diatoms (Poulsen et al., 2003). Electrospray collision-induced dissociation experiments and preliminary  $^1\text{H-NMR}$  analyses also suggest the presence of methyl, amine and carboxylic acid functional groups (Ingalls,

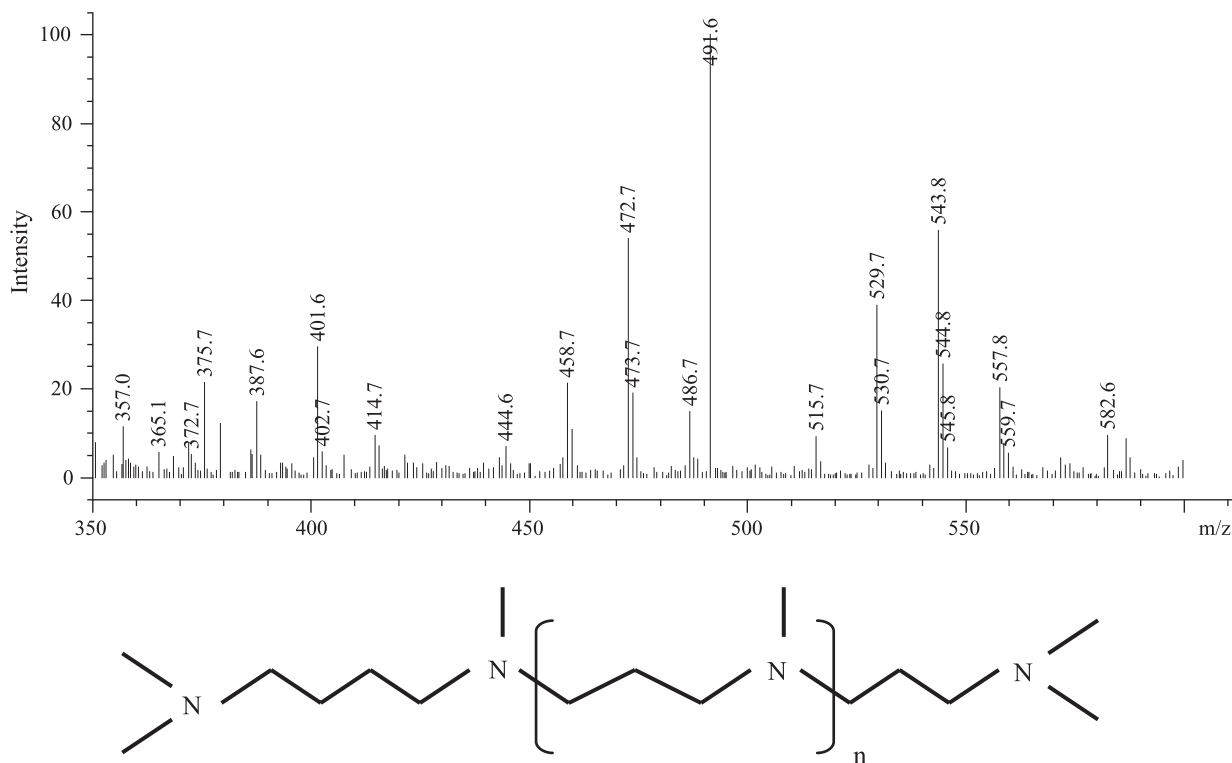


Fig. 4. Mass spectra of polyamines in peak 1 of the NBP9802 chromatogram in Fig. 3 (TN057-13 PC4). Polyamine structure is after Kröger et al. (2000).

Table 2  
Tentative identification of parent compounds containing propylamine derivatives

Peak #	<i>m/z</i>	Tentative identifications <sup>a</sup>
1	multiple	putrecine
2	147.2	lysine
2	347.3	orn; hyls; di/trimethyls
3	321.5	unknown
4	227.4	unknown
5	247.4	orn; di/trimethyls
6	335.5	unknown
7	261.4	orn; lys; trimethyls
7	305.5	hyls; dimethyls

<sup>a</sup> orn=ornithine, hyls=hydroxylysine, dimethyls=dimethylhydroxylysine.

unpublished data). Structural assignments as verified by MS/MS and NMR experiments will be published in a separate manuscript. The primary evidence that these compounds are native to the frustules is that they are only present in HF digests of diatomaceous sediments. Table 3 summarizes the peaks that were purified for <sup>14</sup>C analysis. See Fig. 3 for peak number assignments.

Table 3  
Sample size,  $\delta^{13}\text{C}$ ,  $\Delta^{14}\text{C}$  and radiocarbon ages of LC-MS fractions of diatom-bound organic compounds and standards (modern lysine, charcoal and <sup>14</sup>C free alanine)

Sample name	LC-MS peak #	Sample size ( $\mu\text{mol C}$ )	Measured $\delta^{13}\text{C}$ (‰) <sup>a</sup>	Measured $\Delta^{14}\text{C}$ (‰)	Instrument error (‰)	Corrected <sup>14</sup> C age range <sup>b</sup>
Plankton	1	4.2	-12.8	-154.5	7	1210–1430
	5–7	2.5	-10.2	-142.0	10	810–1130
NBP9802 St7 GC 41–45 cm	1	4.2	-24	-842	7.4	16,300–17,700
	1	8.3	-24	-851	3.7	16,000–16,700
	2	11.7	-24	-858	2.7	16,300–16,800
	5–7	1.7	-24	-814	18.8	20,900–34,900
TN057-13 PC4 448–450 cm	1	35.3	-25.3	-830	1.2	14,400–14,600
	3–7	5.3	-23.6	-714	6	10,800–11,400
E11-2 174–176 cm	1	15.9	-22.3	-907	2	20,000–20,600
	5–7	2.6	-21.5	-846	11	21,700–28,700
Charcoal	n/a	51.7	-23.9	-977.7	0.1	
	n/a	5.0	-23.9	-991.7	0.3	
Alanine	n/a	10.8	-23.9	-991.7	0.6	
Alanine (LC-MS)	1	4.2	-23.9	-949	1.1	
	1	6.7	-23.9	-959	3.7	
	1	7.5	-23.9	-973.6	0.4	
	1	9.2	-23.9	-982.0	0.8	
Lysine	n/a	20.0	-24	114	5	>modern
Lysine (LC-MS)	1	5.8	-24	79	5.9	>modern

n/a: not applicable.

<sup>a</sup> <sup>14</sup>C ages for NBP Station 7 GC2 and lysine were calculated using assumed  $\delta^{13}\text{C}$  values of -24‰.

<sup>b</sup> Incorporating all corrections for process blanks as discussed in text. <sup>14</sup>C years (Libby half-life) with no reservoir correction.

### 3.3. Quantification of blank correction

#### 3.3.1. Combustion blank

Combustion of empty tubes and tubes containing CuO and Ag consistently resulted in the production of  $\leq 0.117 \mu\text{mol}$  gas, and it is likely that some of this gas was water vapor (Pearson et al., 1998). The average combustion blank contained  $0.117 \pm 0.03 \mu\text{mol C}$  ( $1.4 \pm 0.4 \mu\text{g C}$ ). We assumed a combustion blank size of  $0.117 \pm 0.03 \mu\text{mol C}$ , the average size of all measured combustion blanks, in our calculations of the  $\Delta^{14}\text{C}$  of the combustion blank.

Combustion of charcoal resulted in an uncorrected  $\Delta^{14}\text{C}$  value of  $-997.7 \pm 0.1\%$  for a sample containing  $51.7 \mu\text{mol C}$ . Analysis of smaller samples,  $5.0 \mu\text{mol C}$  from charcoal and  $10.8 \mu\text{mol C}$  from <sup>14</sup>C-free alanine, both had  $\Delta^{14}\text{C}$  values of  $-991.7 \pm 0.3\%$  (Table 3). The modern lysine standard had an uncorrected  $\Delta^{14}\text{C}$  value of  $+114 \pm 5\%$  for a sample containing  $20.8 \mu\text{mol C}$ . In our calculations of the  $\Delta^{14}\text{C}$  of the combustion blank, we assume that charcoal and alanine had true  $\Delta^{14}\text{C}$  values of  $-1000\%$  and that the  $\Delta^{14}\text{C}$  value for modern lysine is equal to the average  $\Delta^{14}\text{C}$  of



atmospheric  $^{14}\text{C}$  over the last 15 years (assumes batch of lysine was prepared recently), or  $\Delta^{14}\text{C}=+118\text{‰}$  (Levin and Kromer, 1997).

Assuming that the further processing of  $\text{CO}_2$  to graphite followed by AMS analysis contributes no additional blank carbon, we can calculate a  $\Delta^{14}\text{C}$  value for the combustion blank. To do this, we took the slope of the line from a plot of  $\Delta^{14}\text{C}$  (‰) vs. 1/mass ( $\mu\text{g C}$ ) for all of the charcoal and alanine combustion blanks and calculated the  $\Delta^{14}\text{C}$  value of the blank according to the following equation:<sup>1</sup>

$$\text{slope} = m_b(\Delta^{14}\text{C}_b - \Delta^{14}\text{C}_s)$$

where  $m_b$  is the mass of the combustion blank (0.117  $\mu\text{mol C}$ );  $\Delta^{14}\text{C}_b$  is the  $\Delta^{14}\text{C}$  of the blank (unknown),  $\Delta^{14}\text{C}_s$  is the  $\Delta^{14}\text{C}$  of the sample ( $-1000\text{‰}$ ).

This results in a combustion blank with a  $\Delta^{14}\text{C}$  value of  $-530\text{‰}$  for an average blank of 1.4  $\mu\text{g C}$  (0.117  $\mu\text{mol C}$ ) and a range of  $-340\text{‰}$  to  $-630\text{‰}$  if the blank varies between 1.0 and 1.8  $\mu\text{g C}$  (0.083 and 0.15  $\mu\text{mol C}$ ). The  $\Delta^{14}\text{C}$  value of the combustion blank was also calculated (using a mass balance equation) from the single result for the analysis of modern lysine ( $\Delta^{14}\text{C}=+113\text{‰}$ ). This calculation suggested a  $\Delta^{14}\text{C}$  value of  $-640\text{‰}$  for a blank of 1.4  $\mu\text{g C}$  (0.117  $\mu\text{mol C}$ ) and range of  $-940\text{‰}$  to  $-470\text{‰}$  for a blank between 1.0 and 1.8  $\mu\text{g C}$  (0.083 and 0.15  $\mu\text{mol C}$ ), which is consistent with the results obtained for the  $^{14}\text{C}$ -dead standards. Because more data for the former provides a tighter constraint on the value of the blank, we used  $-530\text{‰}$  (range  $-340\text{‰}$  to  $-630\text{‰}$ ) for all combustion blank corrections to the data.

### 3.3.2. LC blank

The amount of carbon in the LC effluent from blank injections was proportional to the volume of effluent collected (Fig. 5). Regression of these data suggest that the theoretical minimum blank obtained from LC-MS preparation of 0 ml effluent, followed by combustion, is 2.67  $\mu\text{g C}$  or 0.223  $\mu\text{mol C}$  ( $y$ -intercept). Considering that the measured average size of the combustion blank is  $\sim 1.4 \mu\text{g C}$  (0.12  $\mu\text{mol C}$ ), this result suggests that there is some contamination associated with the process of collecting and transferring effluent to the combustion tube that is

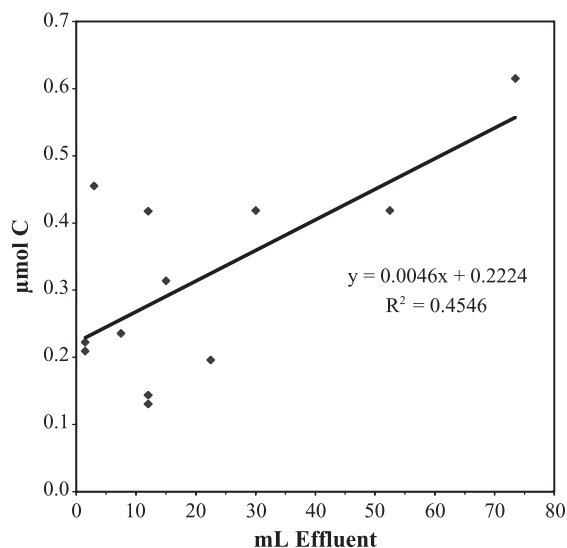


Fig. 5. Correlation between the volume of LC-MS effluent collected and the amount of carbon in the effluent.

independent of the volume collected. In addition to this background value, the contribution from effluent is,  $\sim 0.05 \mu\text{g C/ml}$  (0.0042  $\mu\text{mol C/ml}$ ). Using the equation for the regression in Fig. 5, a value for the total contribution from the LC system to each sample of purified compounds was calculated. In some cases, collected chromatographic peaks were combined after combustion to  $\text{CO}_2$  making the size of the blank larger due to the addition of 1.4  $\mu\text{g C}$  (0.12  $\mu\text{mol C}$ ) for each additional combustion tube. Therefore, the correction for total process blank was calculated individually for each sample; these corrections ranged from 2.7 to 5.7  $\mu\text{g C}$  (0.23–4.8  $\mu\text{mol C}$ ).

The  $\Delta^{14}\text{C}$  of the cumulative combustion plus LC-MS blank was assessed by purifying one sample of modern lysine and four samples of  $^{14}\text{C}$ -free alanine by LC-MS and measuring their  $\Delta^{14}\text{C}$  values. Lysine (5.8  $\mu\text{mol C}$ ) had a  $\Delta^{14}\text{C}$  value of  $+79\text{‰}$ . The  $\Delta^{14}\text{C}$  values of the alanine samples purified by LC-MS (4.2–9.2  $\mu\text{mol C}$ ) were proportional to the size of the sample injected and ranged from  $-949\text{‰}$  to  $-982\text{‰}$  (Table 3).

The  $\Delta^{14}\text{C}$  value of the LC-MS blank was calculated as done for the combustion blank, only the  $\Delta^{14}\text{C}$  values measured for alanine were plotted against 1/mass of the sample. To solve the expression,  $\text{slope} = m_b(\Delta^{14}\text{C}_b - \Delta^{14}\text{C}_s)$ , a cumulative blank of  $3.0 \pm 0.5 \mu\text{g C}$  ( $0.25 \pm 0.04 \mu\text{mol C}$ ) was used (calculated from Fig. 5, using the average effluent

<sup>1</sup> From rearrangement of the isotopic mass balance equation,  $\Delta_{\text{tot}}m_{\text{tot}} = \Delta_b m_b + \Delta_s m_s$ , into the form  $\Delta_{\text{tot}} = \Delta_s + (1/m_{\text{tot}})m_b(\Delta_b - \Delta_s)$ .

volume for collected alanine). Using this approach, the cumulative blank has a  $\Delta^{14}\text{C}$  value of  $-132\text{‰}$  and a range of values from  $-255\text{‰}$  to  $+42\text{‰}$  given the uncertainty in  $m_b$ . It is possible to check the reasonableness of these values by using a mass balance equation to calculate the  $\Delta^{14}\text{C}$  value of the LC component of the cumulative blank. Assuming that the total blank (0.25  $\mu\text{mol C}$  at  $-132\text{‰}$ ) is made up of 1.4  $\mu\text{g C}$  (0.12  $\mu\text{mol C}$ ) at  $-530\text{‰}$  (combustion blank) and 1.6  $\mu\text{g C}$  (0.13  $\mu\text{mol C}$ ) from the LC, the  $\Delta^{14}\text{C}$  value of the latter is  $+216\text{‰}$ . Since this value is greater than modern, the  $\Delta^{14}\text{C}$  value of the cumulative blank may be closer to its lower bound ( $-255\text{‰}$ ), which would yield a more reasonable value for the LC component ( $-14\text{‰}$ ).

As an additional constraint on the  $\Delta^{14}\text{C}$  value of the cumulative blank, we also applied individual mass balance calculations to each of the LC-MS purified standards (alanine and lysine) and to the sample of known age (plankton tow). Assuming a uniform blank size of 3  $\mu\text{g}$  (0.25  $\mu\text{mol C}$ ), the range of  $\Delta^{14}\text{C}$  values calculated for the cumulative blank was  $-644\text{‰}$  to  $+85\text{‰}$  with an average value of  $-260 \pm 270\text{‰}$ . This calculation suggests that the cumulative blank is indeed in the lower range of the  $\Delta^{14}\text{C}$  values as calculated by the slope method above.

Therefore, all reported  $\Delta^{14}\text{C}$  values for the samples of individual compounds collected by LC-MS are corrected according to:

$$\Delta^{14}\text{C}_s = [(\Delta^{14}\text{C}_{\text{measured}})(m_T) - (\Delta^{14}\text{C}_{\text{cb}})(m_{\text{cb}}) - (\Delta^{14}\text{C}_b)(m_b)]/m_s$$

where  $\Delta^{14}\text{C}_s$  = the  $\Delta^{14}\text{C}$  value of the sample;  $m_T$  = mass of the total sample analyzed,  $\Delta^{14}\text{C}_{\text{cb}}$  = the  $\Delta^{14}\text{C}$  value of the cumulative blank ( $-132\text{‰}$ , range  $-255\text{‰}$  to  $-132\text{‰}$ ),  $m_{\text{cb}}$  = total mass of blank accounting for the volume of LC effluent according to Fig. 5,  $(\Delta^{14}\text{C}_b)(m_b)$  = the contribution from additional combustion tubes, usually = 0,  $\Delta^{14}\text{C}$  values were calculated for the full range of the error corrected for  $\Delta^{14}\text{C}_{\text{cb}}$  ( $-255\text{‰}$  to  $-132\text{‰}$ ). The AMS instrument blank was then subtracted from the calculated minimum  $\Delta^{14}\text{C}$  values and added to the maximum  $\Delta^{14}\text{C}$  values to obtain the total range of possible  $\Delta^{14}\text{C}$  values; these values were then converted to conventional radiocarbon ages using the

Libby equation:  $\text{Age} = -8033 \ln(\Delta^{14}\text{C})$ , according to Stuiver and Polach (1977).

### 3.4. $^{14}\text{C}$ ages of samples

Results of all radiocarbon analyses are summarized in Table 3. Three LC-MS fractions (peak 1, peak 2 and peaks 5–7) were collected and dated from the core NBP9802 Station 7 GC2. Material collected from peak 1 was split into two subsamples and each was combusted and analyzed for  $^{14}\text{C}$  separately. The ages of peak 1 and peak 2 were similar (Table 3). The range in ages calculated for each of these samples was 16,000–17,700  $^{14}\text{C}$  years. Peaks 5–7 were significantly older than peaks 1 and 2 and had a range in ages from 20,900 to 34,900  $^{14}\text{C}$  years. These ages are all significantly older than the ages of 7,370 and 6000  $^{14}\text{C}$  years reported for foraminiferal  $\text{CaCO}_3$  in the 20–22- and 129–134-cm intervals (this work).

Two groups of compounds from TN057-13 PC4 were dated: peak 1 and peaks 3–7 (combined into one sample). The  $^{14}\text{C}$  ages of the two fractions were significantly different. Peak 1 had an older age (14,400–14,600  $^{14}\text{C}$  years) than peaks 3–7 (10,800–11,400  $^{14}\text{C}$  years). Co-occurring foraminifera from this core horizon were previously dated at 9340  $^{14}\text{C}$  years (Hodell et al., 2001).

Two fractions were dated from E11-2: peak 1 and peaks 5–7 (combined). The age of peak 1 was significantly younger (20,000–20,600  $^{14}\text{C}$  years) than the age of the later-eluting peaks (21,700–28,700  $^{14}\text{C}$  years). Co-occurring foraminifera from this core horizon were previously dated at 27,000  $^{14}\text{C}$  years (Ninnemann and Charles, 1997).

Blank corrections were also applied to the two peaks (1 and 5–7 combined) collected from the plankton sample. Peak 1 had an age of 1210–1430  $^{14}\text{C}$  years and peaks 5–7 had an age of 810–1130  $^{14}\text{C}$  years. AMS analysis of the bulk organic carbon from this sample resulted in an age of 1180  $^{14}\text{C}$  years (DeMaster, personal communication).

## 4. Discussion and conclusions

The method presented here represents a new tool for dating sediments that lack  $\text{CaCO}_3$ . Previous workers have attempted to develop methods to obtain

radiocarbon dates from sedimentary diatoms. In previous studies, the  $^{14}\text{C}$  ages of cleaned, combusted diatom frustules were measured and compared to ages obtained from co-occurring foraminiferal  $\text{CaCO}_3$ . In these studies, diatom frustules consistently contained sources of modern carbon, even when collected from sediments with infinite foraminiferal radiocarbon ages ( $\Delta^{14}\text{C} = -1000\text{‰}$ ) (Zheng et al., 2002). Our work suggests that, while contamination may be blamed for the inability to obtain radiocarbon dead frustules, other factors may also have contributed to discrepancies between the ages of sedimentary diatoms and foraminifera.

The method described here is a new application of compound-specific  $^{14}\text{C}$  analysis. Although compound-specific  $^{14}\text{C}$  methods were pioneered using preparative gas chromatography (Eglinton et al., 1996), our work suggests that aqueous-phase preparative LC can also be used to purify biomarkers for compound-specific isotope studies. Enough carbon can be collected from a minimum of five injections (here, five injections was typical); making sample preparation relatively rapid. Sizes of blanks can be measured and are routinely below  $5\ \mu\text{g C}$  ( $0.42\ \mu\text{mol C}$ ). Measurement of the size and  $\Delta^{14}\text{C}$  values of blanks allows correction for all of the carbon acquired by the sample during handling. The results of this study emphasize the importance of knowing the sources, sizes and  $\Delta^{14}\text{C}$  values of these process blanks.

In all three of the cores examined here, age offsets of varying ranges were observed between diatoms and foraminifera. These age offsets suggest that the two mineral phases do not record events on the same time-scale and/or that the shells are not deposited to the seabed by the same processes. In addition, diatoms also appear more likely to be affected by post-depositional processing, including bioturbation, resuspension and transport by bottom currents. Our findings are in agreement with recent work suggesting that the particle size distribution of organic sediments can play a role in determining the depositional age (Eglinton et al., 1997; Ohkouchi et al., 2002; Mollenhauer et al., 2003). Therefore, care should be used when interpreting diatom-based paleoproxies in sediment cores that have chronologies based on foraminiferal ages, as the two mineral phases may not sediment on the same timescale.

#### 4.1. TN057-13 PC4

TN057-13 PC4 is an ideal core for testing our diatom-based dating method because of its high sedimentation rate ( $\sim 50\ \text{cm}/10^3\ \text{cal year}$ ) and well-defined age model based on foraminiferal  $\text{CaCO}_3$ . Diatom based proxies ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$ ) have also been investigated previously and used to interpret leads and lags during the termination of the last glacial maximum (Hodell et al., 2001; Shemesh et al., 2002). Thus, knowledge of the age of diatoms in this core could significantly refine our understanding of the paleoceanography of the Southern Ocean.

Of the two diatom fractions analyzed from this sample, one (combined peaks 3–7) resulted in relatively good agreement between the ages of the purified compounds (10,800–11,400  $^{14}\text{C}$  years) and the co-occurring foraminifera (*N. pachyderma*,  $9340 \pm 60\ ^{14}\text{C}$  years) (Shemesh et al., 2002) (Table 3; Fig. 6). Thus, the minimum calculated offset in ages between the diatoms (peaks 3–7) and foraminifera is 1500 to 2000  $^{14}\text{C}$  years. This does not take into account other possible sources of uncertainty in the correction for processing blank, e.g., the potential for sample-to-sample variability in the combustion blank, which was averaged out of the correction. Although the blank for this sample is unusually large because each peak was collected individually and then combined after sample combustion (due to the small size of the individual fractions). Thus, the combustion blank was  $3\times$  that of other samples and any uncertainty in its magnitude is therefore amplified.

The calculated offset of up to 2000 years is similar to the difference between organic and inorganic components observed in previous studies of other locations (e.g., Ohkouchi et al., 2002; Mollenhauer et al., 2003). Together, these new data may have implications for the proposed leads and/or lags between Southern Ocean dynamics and atmospheric  $\text{CO}_2$  (Shemesh et al., 2002). Future analysis of this core will require larger samples to sufficiently reduce the uncertainty in the diatom ages.

The  $^{14}\text{C}$  age of peak 1 was significantly older (14,000–14,600  $^{14}\text{C}$  years) than the age reported for peaks 3–7 (Table 3, Fig. 6). One possible explanation for the discrepancy in the ages of the two peaks is

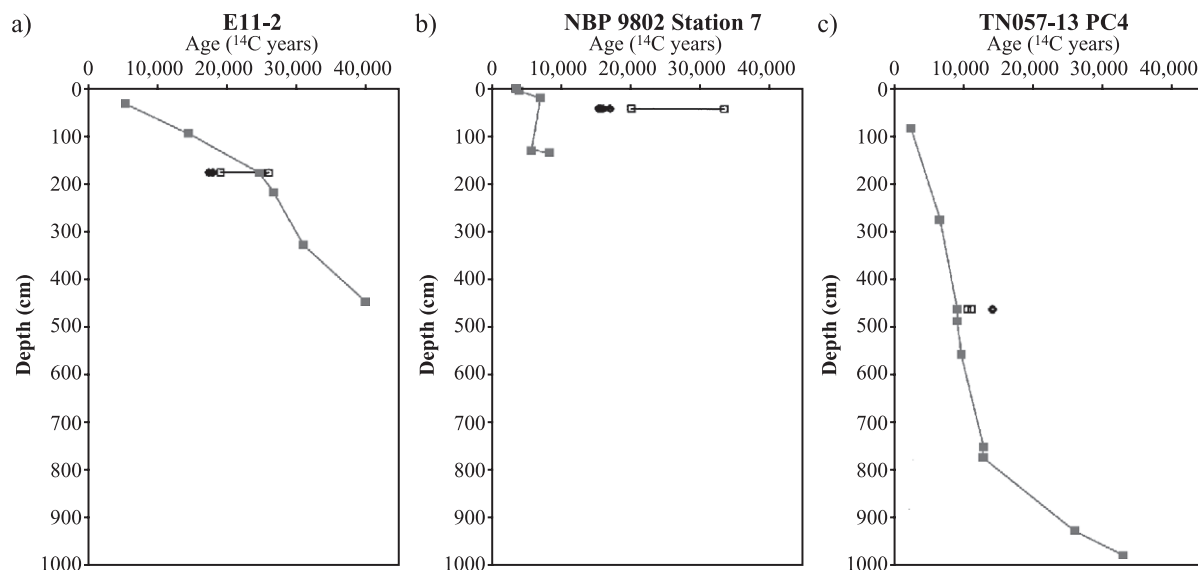


Fig. 6. Profiles of  $^{14}\text{C}$  ages in Southern Ocean sediment cores analyzed here. Grey closed squares are foraminifera ages (see Table 1 for references). Diamonds and open squares are blank corrected  $^{14}\text{C}$  ages of LC-MS purified compounds from cleaned dissolved sediment in peak 1 and peaks 3–7, respectively. (a) E11-2, (b) NBP9802 Station 7 GC2, (c) TN057 PC4.

that peak 1 contained compounds reflecting relict carbon that had not been thoroughly cleaned from the diatoms. Peak 1 was expected to contain polyamines similar to those found in the sample from NBP9802 Sta. 7 GC2. However, a regular series of polyamines was not detected in the mass spectrum of this fraction. Given the low retention time of peak #1, it contains highly polar materials such as amino acids and other unidentified compounds. Thus, it is possible that polyamines were present but that other compounds were of much higher relative abundance, resulting in a greatly reduced mass spectral signal for polyamines. In the absence of abundant polyamines, the majority of the material in this fraction may not be native to the diatom frustules and may not serve as a good material for dating. The peaks in the later fractions represent only a few major compounds and thus were of higher purity. Results of the plankton tow sample suggest that even in the upper water column, there are differences in the radiocarbon content of different organic components. Like TN057-13, peak 1 of the plankton tow was older than both bulk organic matter and peaks 5–7. The bulk organic carbon age reflected an intermediate value, perhaps suggesting that it contains component of both young and old age.

#### 4.2. NBP9802 Station 7 GC2

The chronology of the core from NBP9802 Station 7 GC2 is not as well defined as core TN057-13. Ages derived from  $\text{CaCO}_3$  are available from 0 to 22 cm and from 129 to 139 cm. Between 22 and 129 cm, the sediment consists of a massive layer of nearly pure diatoms. Our sample was taken from the 41- to 45-cm depth interval in the middle of the diatom layer. Because there is no  $\text{CaCO}_3$  anywhere in the massive sequence, it is not possible to obtain a foraminiferal age. In addition, the age of bulk  $\text{CaCO}_3$  at 22 cm is  $\sim 7370 \pm 55$   $^{14}\text{C}$  years and at 129 cm is  $6000 \pm 35$   $^{14}\text{C}$  years. One possible interpretation of this stratigraphy was that  $\sim 7370$  year ago the surface expression of the Antarctic Polar Front (APF) migrated over this site, creating a massive flux of opal to the sea floor through convergence and aggregation of diatoms at the front. This core was recovered near the modern location of the APF (Chase et al., 2003), and previous studies have suggested that convergent fronts are capable of generating massive layers of siliceous sediments by this mechanism (Kemp et al., 1995).

We measured the  $^{14}\text{C}$  content of three LC-MS fractions of diatom-bound organic matter from this sample (Table 3, Fig. 6). Peaks 1 and 2 from this

sample gave nearly identical ages ranging from 16,000 to 17,700  $^{14}\text{C}$  years suggesting that all of the compounds are likely to be from the same source (Table 3). The age of these samples exceeds the presumed foraminiferal age by  $\sim 10,000$   $^{14}\text{C}$  years.

The corrected age for the fraction representing peaks 3–7 was 20,900 to 34,900  $^{14}\text{C}$  years. While this result is possible, it seems unlikely that the compounds of high purity and known origin (peaks 3–7) would be older than the mixed assemblages found in peaks 1 and 2. Therefore, we suspect, that the reason for this old value is an analytical one: this sample is the smallest that was measured by  $^{14}\text{C}$ -AMS (20  $\mu\text{g}$  or 1.7  $\mu\text{mol C}$ ) and it had a large process blank (4.4  $\mu\text{g}$  or 0.37  $\mu\text{mol C}$ ). Therefore, uncertainty in the correction factor probably contributes to the older age. Because of the small size of the sample and the large blank, the  $\Delta^{14}\text{C}$  value of the blank has a large influence on the calculated age of this sample. This influence can be illustrated by substituting a  $\Delta^{14}\text{C}$  value for the cumulative blank of  $-644\text{‰}$  (the most negative value estimated for the cumulative blank using simple mass balance calculations) into the blank correction equation, instead of the consensus value of  $-255\text{‰}$  to  $-132\text{‰}$ . Using  $-644\text{‰}$  for the blank, the corrected age of this sample is 16,500  $^{14}\text{C}$  years which is more similar to the other fractions. Thus, for a sample of this size for which the blank represents a large contribution, the uncertainty is large.

Most significantly, none of the corrected values for diatom-derived compounds approach a sedimentary age of 7000  $^{14}\text{C}$  years regardless of the blank correction used. This indicates that the diatoms deposited within this siliceous layer must be older than the foraminifera-containing sediments above and below this massive deposit. Most likely, a major resuspension event transported sediment to this location, resulting in the massive diatom deposit. As noted above, the APF is located near this core site. High current velocities associated with the APF extend through the full depth of the water column as shown by AESOPS hydrographic sections. Therefore, we now believe that  $\sim 7000$  years ago the bottom expression of the APF passed near the core site, eroding older sediments and redepositing them at the site of the core. If this is the case, then the measured age represents the mean of the spectrum of ages exhibited throughout this massive deposit.

#### 4.3. E11-2

E11-2 has been used extensively for paleoceanographic reconstructions in the Southern Ocean (Ninnemann and Charles, 1997; Mashiotta et al., 1999; Rosenthal et al., 2000). The age model for the core is based on the abundance of the radiolarian *C. davisiana* and on  $\delta^{18}\text{O}$  values of foraminifera. Several  $^{14}\text{C}$  ages are also available from foraminifera (*Pachyderma*) and bulk  $\text{CaCO}_3$  (Chase et al., 2003). There are also Mg/Ca records (Mashiotta et al., 1999). Rosenthal et al. (2000) looked at the  $\delta^{13}\text{C}$  values of diatom-bound organic matter in the upper 300 cm.

In our samples, the carbon in peak #1 is between 20,000 and 20,600  $^{14}\text{C}$  years while the sample representing peaks 5–7 is 21,800 to 28,700  $^{14}\text{C}$  years. This latter value is in relatively good agreement with the measured foraminiferal age of  $\sim 27,000$   $^{14}\text{C}$  years. Although the small size of this sample (31  $\mu\text{g C}$  or 2.6  $\mu\text{mol C}$ ) also results in a relatively large error in the range of possible ages, the agreement between diatoms and foraminifera is good for peaks 5–7. In contrast, there is again an offset between the age obtained for peak 1 and the foraminiferal data: diatom data is 6500 years younger than expected. In general, the most likely way for the diatom fraction to be younger than the foraminiferal fraction would be effects associated with bioturbation. Bioturbation would selectively move surficial (young) diatoms into deeper sediment layers, depositing them near older foraminifera. There is some evidence that this type of bioturbation occurs in the Southern Ocean (Sayles et al., 2001). However, given a sedimentation rate of 6.5 cm/ky, and a bioturbation depth of  $\sim 10$  cm, the maximum offset that can likely be explained by this mechanism is  $\sim 2000$  years. Another partial explanation for the young age of fraction 1 may be the acquisition of modern contamination during the cores long storage time (Zheng et al., 2002) and subsequent incomplete cleaning by our method.

#### 4.4. Future prospects for radiocarbon dating diatom-bound organic matter

In summary, the use of peak 1 of our chromatographic separation of diatom-bound compounds for radiocarbon dating has yielded results that agree less reliably than data from later-eluting peaks. In the



future, only compounds in ranges of peaks 3–7 will be used for dating. In addition, we will continue to test the method developed here and hopefully gain knowledge of the history of the water column and past depositional events of the Southern Ocean. In particular, diatom-bound organic compounds will be dated from additional sediment depths in the TN057-13 PC4 core. This appears to be the most promising core available for further testing of this method.

With the acquisition of additional data, we will investigate the physical mechanisms responsible for the offsets between diatom and foraminiferal ages. Older ages of diatoms could reflect an aging of the whole diatom pool due to preferential resuspension of fine grains on the seabed, similar to what has been observed for alkenones (Mollenhauer et al., 2003). If this is the case,  $^{14}\text{C}$  is the proxy most affected by sedimentary mixing since it has a range of 0‰ to  $-1000\text{‰}$ , while  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  values have much smaller dynamic ranges. It is possible that taxonomic studies could shed light on this issue, but may not ever be able to quantify the effect. Nevertheless, radiocarbon dating of selected compounds purified from diatom frustules is the most promising approach to understanding sediment chronologies in the Southern Ocean, particularly in areas where foraminifera are absent. In addition, comparison of the ages of bulk organic matter and non-diatom-bound organic matter with the pure compound data will lead to new insights about how organic carbon is cycled and ultimately preserved in the Southern Ocean. Finally, this method will aid in the selection of ideal cores with well-defined diatom stratigraphies for paleoceanographic investigation.

### Acknowledgements

We thank T. Guilderson of LLNL CAMS for offering the  $^{14}\text{C}$  free alanine and charcoal and for AMS analyses. The staff at NOSAMS also provided advice and supplies for sample preparation. The plankton sample was generously provided by D. DeMaster.  $\delta^{13}\text{C}$  analyses were done with the help of D. Schrag and G. Eiseid. The manuscript benefited from the careful reviews of A. McNichol and one anonymous reviewer. This research was supported by Harvard University funds and OCE grant #0241363 to

A. Pearson and in part by a grants/cooperative agreement to R.F. Anderson and L.H. Burckle from the National Oceanic and Atmospheric Administration; NOAA Award No. NA77RJ0453. The views expressed herein are those of the author(s) and do not necessarily reflect the views of NOAA or any of its sub-agencies. Support from the core repository at the Antarctic Research Facility, Florida State University, is gratefully acknowledged. Support for the curating facilities of the LDEO Deep-Sea Sample Repository is provided by the National Science Foundation through Grant OCE0002380 and the office of Naval Research through Grant N00014-02-01-0073.

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