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Preparation of natural methane samples for stable isotope and radiocarbon analysis

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Abstract

We describe procedures for preparing methane samples from natural waters or sediments for radiocarbon (^{14}C-CH_4) analysis. These procedures also allow the determination of $^{2}H/^{1}H$ and $^{13}C/^{12}C$ ratios of this methane. Methane is extracted from natural samples in the field using Lamont radon stripping boards (Mathieu et al. 1988) and trapped using liquid nitrogen-cooled U-traps filled with HiSiv 3000. The field procedures eliminate the need to return large samples to the laboratory. The U-traps are returned to the laboratory where a new two-stage purification-oxidation vacuum line is used to extract, purify, and oxidize the methane. The methane oxidation products (carbon dioxide and water) are analyzed for the natural content of ^{14}C, ^{2}H, and ^{13}C with standard procedures. These procedures were evaluated under the conditions that methane was extracted from between 19 to 114 L water or 0.07 to 0.35 L sediment. No isotope fractionation was observed and the blanks are small (0.52_8 ± 0.39 µmol methane with radiocarbon content $^{14}C/C$ = 96.1 ± 0.3 pMC [percent Modern Carbon] [Stuiver and Polach 1977]). Using ^{14}C-accelerator mass spectrometry (AMS), these procedures can produce a successful radiocarbon measurement when at least 1.81 µmol methane are collected. Thus, ^{14}C-CH_4 measurements can be made when the dissolved methane concentration is between saturation (mM) and 15.9 nM for the water column or 5.2 µM for the sediment.

Oceanic sediment methane, including methane clathrate hydrates (hydrates), is the Earth's largest global methane reservoir (Kvenvolden and Lorenson 2001) and is under investigation as a possible energy resource (Collett and Kuuskraa 1998). The extent to which oceanic methane participates in global climate change (Harvey and Huang 1995; Katz et al. 1999; Kennett et al. 2000) and the oceanic carbon and methane cycles (Kelley et al. 2005; Michaelis et al. 2002; Valentine et al. 2001) are topics of current research. Methane released from sediments may originate from seeps of petrogenic origin, decomposing hydrates, and diagenesis in recently deposited sediments. Stable isotope measurements of oceanic methane have been used to distinguish thermogenic and biogenic sources of methane (Kvenvolden 1995; Kvenvolden and Lorenson 2001; Schoell 1988; Whiticar 1999; Whiticar et al. 1986). These stable isotope conventions have shown that hydrates and seeps have both thermogenic or biogenic origins of methane (e.g., Kvenvolden 1995; Kvenvolden and Lorenson 2001; Michaelis et al. 2002; Winckler et al. 2002), while diagenetic sediment methane has biogenic origins (Alperin et al. 1988; Martens et al. 1999). Thus, stable isotope measurements of methane may not unambiguously determine if the source of methane to the water column and sediment is from seeps, decomposing hydrates, or sediment diagenesis (Table 1). Anaerobic and aerobic methane oxidation in the sediment and water column causes isotopic fractionation (Alperin et al. 1988; Martens et al. 1999), which further complicates source determination using stable isotopes. The stable isotopic content of water column methane can only be matched with its source if the extent of methane oxidation and the associated isotopic fractionation factors are known (Kessler 2005; Valentine et al. 2001).

Natural radiocarbon measurements of methane (^{14}C-CH_4) can uniquely determine different methane sources and, because radiocarbon results are normalized to ^{13}C (Stuiver and Polach 1977), are not affected by methane oxidation (Table 1). Methane formed at relatively shallow depths (0-50 cm) in the sediment can diffuse into the water column and likely con-

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Table 1. Examples of oceanic methane isotopic values

Methane type	Location	$\delta^{13}C\text{-}CH_4$ (‰)	$\delta^{2}H\text{-}CH_4$ (‰)	$^{14}C\text{-}CH_4$ (pMC)	
Sediment	Skan Bay, AK	–63.4 to –78.9	–83 to –224	88.5 to 97.1	Kessler 2005
Sediment	Cariaco Basin, Venezuela	–69.0 to –84.7	–116 to –194	64.8 to 86.4	Kessler et al. 2005; Kessler 2005
Water column	Cariaco basin, Venezuela	–47.9 to –57.5	–86 to –176	2.2 to 3.0	Kessler et al. 2005; Kessler 2005
Seep	Santa Barbara Basin, CA	–41.7 to –56.0	–188 to –211	0.08 to 0.21	Kessler 2005
Clathrate Hydrate	Hydrate Ridge, Cascadia Margin	–66.7 to –69.7	–180 to –193	0.22 to 0.29	Winckler et al. 2002

tains measurable amounts of radiocarbon (0 < pMC = 100; percent Modern Carbon (Stuiver and Polach 1977). In contrast, methane emitted from seeps or decomposing hydrates contains small amounts of radiocarbon or is radiocarbon-free (0 = pMC < 5) (Grabowski et al. 2004; Kessler 2005; Winckler et al. 2002) (Table 1). In any aquatic environment, radiocarbon measurements of sediment, seep, hydrate, and water column methane can be used with an isotope mass balance to determine the fraction of each of these sources to the water column. Here we present field and laboratory procedures that were designed primarily to prepare oceanic methane samples for natural radiocarbon analysis. These procedures also allow the parallel determination of the stable isotopic content of methane ($\delta^{2}H\text{-}CH_4$ and $\delta^{13}C\text{-}CH_4$) without isotopically fractionating the sample. Methane dissolved in waters and sediments as well as emitted from seeps and encapsulated by clathrate hydrates can be prepared for natural isotope analyses using these procedures. The procedures were evaluated under the conditions that methane was extracted from between 19 to 114 L water or 0.07 to 0.35 L sediment. The total carbon blank of the procedures is $0.52_8 \pm 0.39$ µmol with a radiocarbon content of 96.1 ± 0.3 pMC. Assuming a quantification limit of ± 5 pMC, these blanks permit $^{14}C\text{-}CH_4$ measurements on samples with a methane concentration ranging from saturation (mM) to 15.9 nM in water or 5.2 µM in sediment.

Conventional ^{14}C-accelerator mass spectrometry (AMS) requires 83 µmol carbon per measurement. In high methane concentration environments where greater than 83 µmol methane were collected, the sample was split so that a dual-inlet $\delta^{13}C$ isotope ratio mass spectrometry (IRMS) measurement could also be conducted. Sample sizes of the $\delta^{13}C\text{-}CH_4$ "splits" ranged from 40 to 100 µmol and contained a precision (± 1 σ) of 0.31‰. Dual-inlet IRMS $\delta^{2}H\text{-}CH_4$ measurements were also conducted on sample sizes of ca. 40 to 200 µmol methane giving a precision of 2.6‰.

A laboratory procedure has been designed at the Naval Research Laboratory to prepare methane samples dissolved in water or encapsulated by hydrates for AMS analysis (Pohlman et al. 2000). Their procedures require the water sample be returned to the laboratory, thus limiting the possible environments to those near methane saturation. Popp et al. (1995) and Sansone et al. (1997) presented systems using isotope-ratio-monitoring gas chromatography/mass spectrometry (irm-GC/MS) to measure $\delta^{13}C\text{-}CH_4$; their detection limit is 1 nM with a precision of < 0.8‰. For investigators interested in measuring only $\delta^{13}C\text{-}CH_4$, our methods do not improve upon these irm-GC/MS techniques. The methods presented here require ca. 2.5 h per sample compared with 15 to 30 min per sample for the irm-GC/MS techniques, and our blanks overwhelm the sample collected at dissolved methane concentrations in water of ≤ 5 nM. Continuous-flow IRMS systems have been developed to measure $\delta^{2}H\text{-}CH_4$ of atmospheric methane (e.g., Rice et al. 2001), however, these systems have not been adapted to make measurements on dissolved methane in water or sediment. The procedures presented here are most useful when $^{14}C\text{-}CH_4$ measurements are of interest in addition to the stable isotopes because all three isotopes are prepared simultaneously.

Materials and procedures

Shipboard methane extraction—Water and sediment collection: For extraction of methane dissolved in natural waters, 20 L glass carboys were fitted with neoprene stoppers equipped with a headpiece consisting of a drilled-through Female Run Tee (Swagelok Female Run Tee-NPT Thread, 1/2-inch × 1/2-inch), containing stainless steel inlet and outlet tubes. The inlet and outlet tubes were fitted with ball valves (Swagelok 40 Series Ball Valve, 1/4-inch) (Fig. 1-inset). The bottles were evacuated and filled directly from Niskin bottles with 19 L water.

Gravity or box cores were collected to sample methane with depth in sediments. To facilitate sediment transfer with minimal gas loss, sediment from cores or subcores was extruded into previously measured lengths (2 to 10 cm) of the same core tubing and isolated with stainless steel shims. The extruded sediment was transferred to a Mason jar (size: 0.946 L [1 quart]) by aligning the sample segment over the jar mouth and sliding the shim away, allowing the unconsolidated sediment to slip into the jar. The Mason jars were filled with 200 to 500 cc water degassed with ultrahigh-purity (UHP) helium and contained a magnetic stir bar to produce a strippable slurry. The Mason jars were sealed with regular Mason jar lids that were equipped with 2 drilled-through Swagelok O-seal male bulkhead connectors (1/8-inch) allowing insertion of stainless steel inlet and

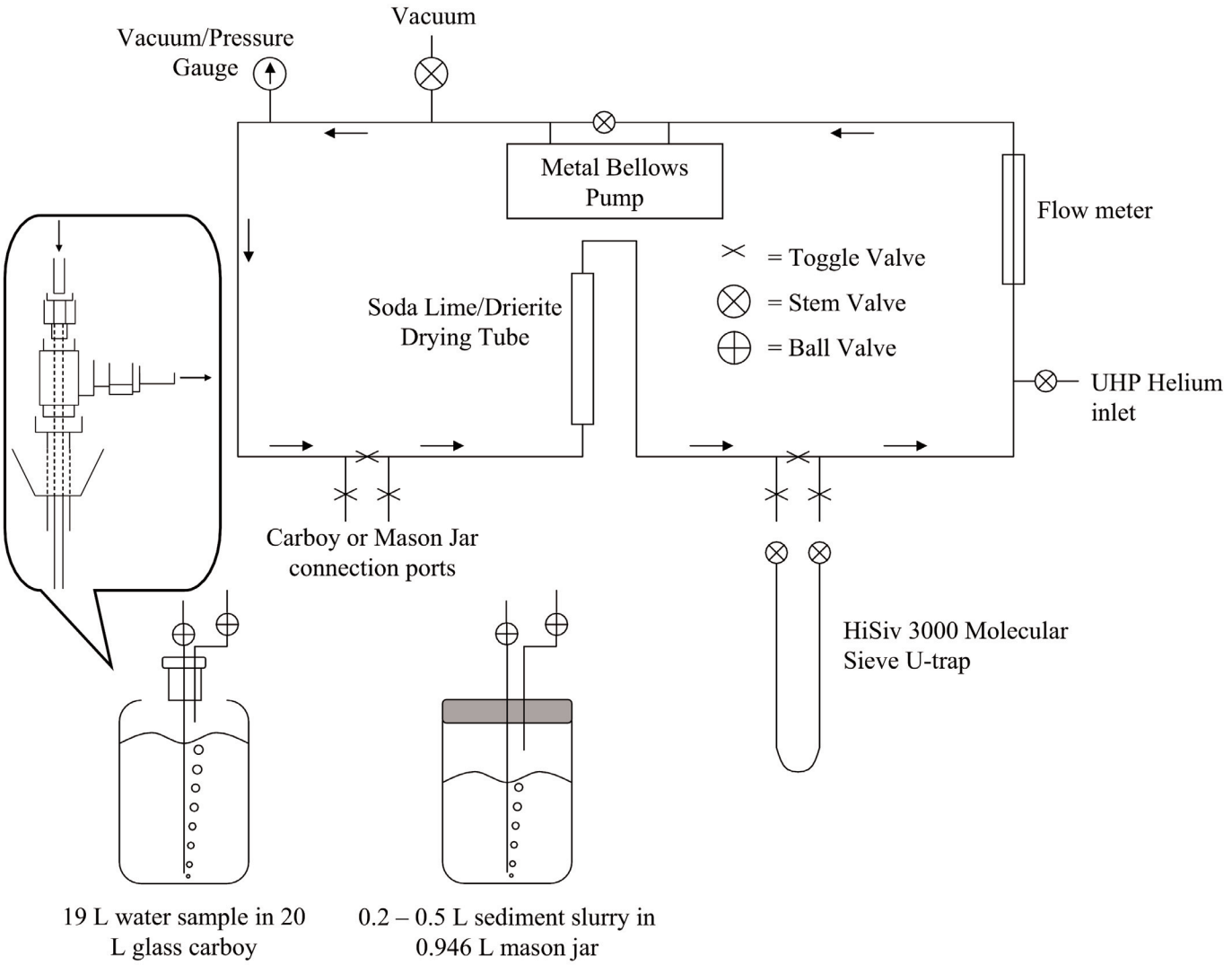

Fig. 1. Lamont radon stripping board (Mathieu et al. 1988). The arrows indicate the direction of gas flow. The stripping boards were modified by replacing the Nylaflow tubing with stainless steel tubing. The water carboy or sediment slurry Mason jar is attached to the stripping board with heavy-walled Nalgene tubing. The inset highlights the carboy headpiece. The U-traps are a quantitative trap for methane when cooled with liquid nitrogen.

outlet tubes. The inlet and outlet tubes were fitted with ball valves (Swagelok 40 Series Ball Valve, 1/8-inch) (Fig. 1).

Extraction system (Fig. 1): Lamont radon stripping boards (Broecker 1964; Mathieu et al. 1988) were used to extract and trap the methane from water and sediment. Each board was equipped with a circulating pump (Metal Bellows Corp., model MB-21) for circulating gas through the sample and traps. The stripping boards were modified by replacing the Nylaflow tubing with stainless steel tubing. A vacuum pump, UHP helium source, and U-trap were attached to the stripping board with Swagelok fittings. The water carboy or Mason jar containing a sediment slurry was attached to the stripping board with heavy-walled tubing (Nalgene 180 Clear polyvinyl chloride tubing, 1/8-inch wall, 1/2-inch outer diameter) equipped with polyethylene quick-disconnects.

Conventional AMS requires 83 µmol C, however the intrinsic AMS limit was determined to be 0.083 µmol modern C (Currie et al. 2000). At water column methane concentrations < 4 µM, additional carboys were connected to the stripping board in series to collect enough methane for ^{14}C-AMS measurement. We tested these extraction procedures with up to 6 carboys (114 L water) attached in series to the stripping board. Assuming blanks are nonexistent, extracting the methane from 114 L of 1 nM methane water would yield enough methane for ^{14}C-AMS measurement.

With the valves to the sample vessel closed, the system was evacuated and flushed with UHP helium 3 to 5 times. The system was then filled with 1 atmosphere of UHP helium. The sample vessel was opened to the stripping board, the sample bypass valve closed, and the circulation pump turned on. The helium

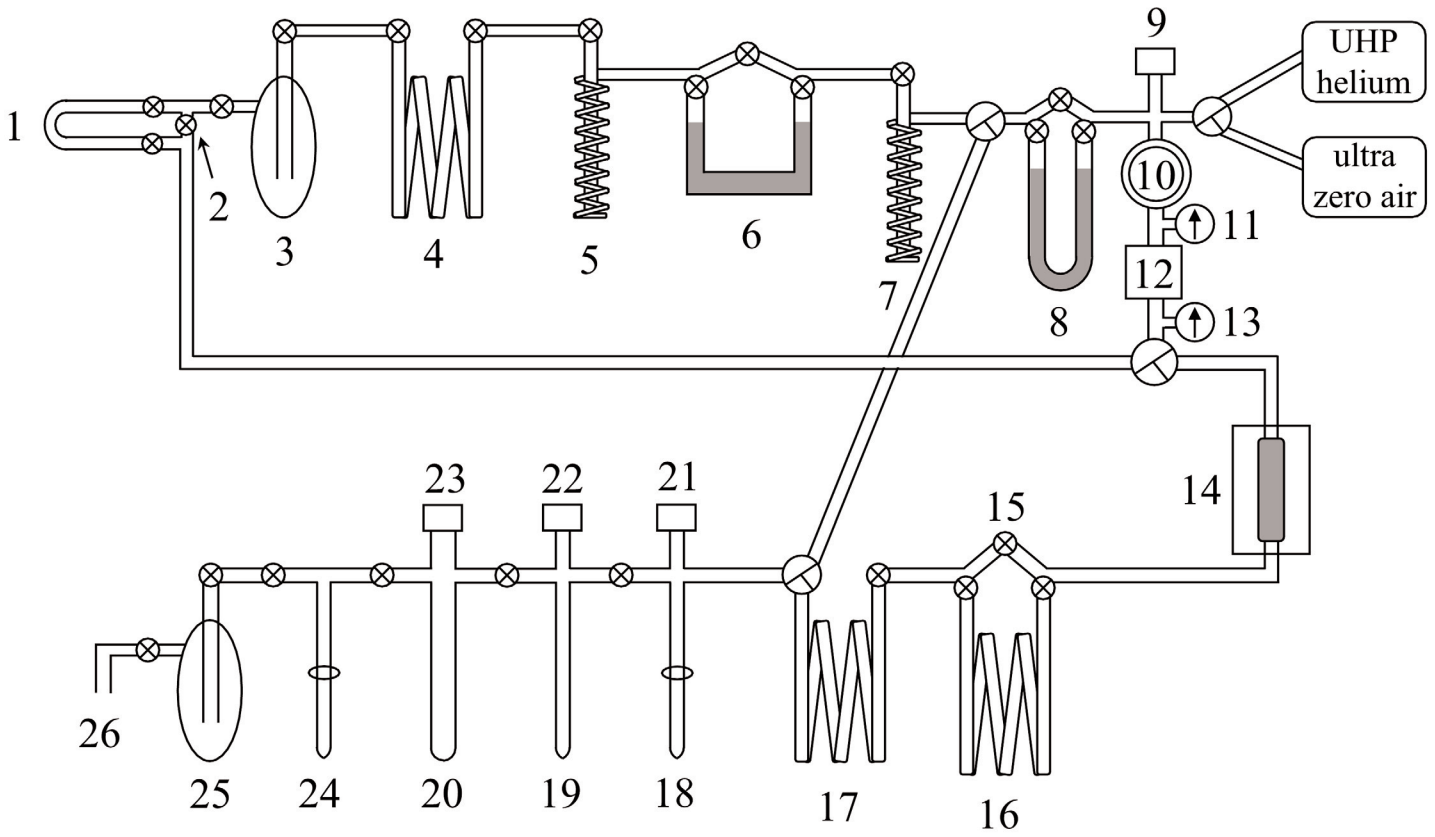

Fig. 2. Two-stage purification-oxidation vacuum line used to extract the methane from the U-trap, remove carbon and hydrogen impurities, oxidize the purified methane to CO_2 and H_2O, and quantify the amount of CO_2 collected. (1) U-trap containing sample, (2) U-trap bypass valve, (3) to (5) CO_2 and H_2O impurity traps cooled with Liquid Nitrogen (LN_2), (6) CuO oven (290°C) to oxidize non-methane hydrocarbons and CO to CO_2, (7) CO_2 and H_2O impurity trap cooled with LN_2, (8) HiSiv 3000 trap, (9) Baratron (MKS Inst.) digital pressure sensor (0.001 to 10.000 Torr), (10) metal bellows circulation pump (Metal Bellows Corp., model MB-21), (11) vacuum/pressure gauge, (12) mass flow controller, (13) vacuum/pressure gauge, (14) CuO Oven (975°C) to oxidize methane to CO_2 and H_2O, (15) waste trap bypass valve, (16) waste trap cooled with LN_2 to collect carbon and hydrogen impurities, (17) trap cooled with LN_2 to collect the CO_2 and H_2O from methane oxidation, (18) port to collect and remove H_2O for δ^2H measurement, (19) calibrated volume ($12.16_9 \pm 0.24_5$ cc), (20) calibrated volume ($17.57_5 \pm 0.04_2$ cc), (21) and (23) Baratron (MKS Inst.) digital pressure sensors (0.1 to 1000.0 Torr), (22) Baratron (MKS Inst.) digital pressure sensor (0.001 to 10.000 Torr), (24) Pyrex tube to remove sample, (25) vacuum pump trap, (26) vacuum pump, (⊗) valve, and (⊗) 3-way valve.

flow was monitored with a flow meter and kept at a rate of 2 to 4 L min^{-1} with a needle valve bypassing the circulation pump. The helium was circulated through the sample to strip the methane and was passed through a drying column filled with one-half soda lime and one-half drierite, removing the majority of carbon dioxide (CO_2) and water vapor. The methane was collected in a U-trap cooled with liquid nitrogen (LN_2) and the helium was recycled through the sample. (When LN_2 was not available in the field, we used a dry ice:ethanol slurry [–72°C] or portable immersion cooler that could refrigerate a Dewar of alcohol to –50°C.) The UHP helium was circulated through the sample for 2 h to quantitatively extract the methane. After 2 h, the valves to the U-trap were closed and the water or sediment sample was discarded. The U-traps were returned to the shore-based laboratory for methane purification and analysis.

U-traps (Fig. 1): The U-traps were fabricated from electropolished stainless steel tubing (0.9525 cm [3/8-inch] outer diameter, 60.96 cm [2 feet] long) bent in a U shape and equipped with nonrotating-stem needle valves with PEEK stem tips (Swagelok D-Series). The U-traps were filled with a molecular sieve (HiSiv 3000 in the 1/16-inch pellet form; formerly known as Silicalite [Flanigen et al. 1978]), which was chosen for its trapping efficiency, lack of isotope fractionation, and ability to quantitatively trap methane at LN_2 temperature. The U-traps are reusable and are reactivated between each use (helium flow at 0.5 L min^{-1}, 275°C, 2 h) with an oven designed to heat the traps without damaging the valves. After the traps are cleaned and reactivated, they are filled with helium slightly above atmospheric pressure so that any minor leaks result in helium diffusing out instead of air diffusing in. HiSiv 3000 is available from UOP Molsiv Adsorbents.

Laboratory operations—Vacuum line techniques (Fig. 2; numbers in parenthesis refer to the numbered parts of Fig. 2): A two-stage purification-oxidation vacuum line was devel-

Table 2. Efficiency and blanks of the procedures

	Efficiency (%)	Total CH_4 Blank (μmol)	Blank ^{14}C-CH_4 (pMC)
Vacuum line: High concentration samples	99.8 ± 0.9 (*n* = 33)	$0.110 \pm 0.04_6$ (*n* = 20)	84.2 ± 25.3 (*n* = 19)
Total process: 19-L sample	99.96 ± 0.01	$0.43_3 \pm 0.32$ (*n* = 4)	96.1 ± 0.3 (*n* = 12)
Total process: 114-L sample	87.1 ± 16.0 (*n* = 6)	$0.32_9 \pm 0.11$ (*n* = 4)	

oped to extract, purify, and oxidize the methane collected in the U-traps (Fig. 2). The purification stage of this vacuum line (1-13) is a continuous loop designed to extract and purify the sample methane from the U-traps. The sample U-trap (1) was attached to the vacuum line and heated to 275°C. The purification stage was evacuated and flushed with UHP helium 3 times before it was filled with ca. 500 Torr of UHP helium. The circulation pump (10) was activated, and the helium was continuously circulated for 10 min to remove trace impurities after which the U-trap (1) valves were opened and the U-trap bypass valve (2) was closed (*see* Safety Note). The helium circulated through the 275°C U-trap removing the trapped gases and transferring them into 3 LN_2 cooled traps (3-5) to remove H_2O and CO_2 impurities. The gases are then flowed into a 290°C CuO column (6), converting CO and nonmethane hydrocarbons to CO_2, followed by another LN_2 cooled trap (7) to collect the CO- and nonmethane hydrocarbons–derived CO_2. The purified helium/methane gas mixture was circulated through a final trap filled with HiSiv 3000 cooled with LN_2 (8) to trap the purified methane. The helium gas was recycled through the U-trap (1) to remove and purify any residual methane. This purification-circulation procedure was conducted for 15 min before the HiSiv 3000 trap containing the purified methane (8) was closed and the circulation pump (10) stopped.

The purification loop was closed and the adjoining oxidation loop was opened with the 3-way valves. This oxidation loop (8-17) continuously circulates the purified methane through a 975°C CuO furnace (14) to oxidize the methane to CO_2 and H_2O. The oxidation loop was evacuated and flushed 3 times with ultra zero air before the oxidation loop was filled with ca. 500 Torr of ultra zero air. (Ultra zero air was chosen because it contains an extra oxidant [O_2] with minimal carbonaceous impurities and does not pose the same hazards as using pure O_2.) The circulation pump (10) was started and the ultra zero air was circulated through the 975°C CuO furnace (14) and a LN_2 cooled waste trap (16) to remove any carbon and hydrogen impurities. After 10 min, the valves to the waste trap (16) were closed (*see* Safety Note), and the waste trap bypass valve (15) was opened. Next, the HiSiv 3000 trap with the purified methane (8) was opened (*see* Safety Note). The LN_2 was removed from the HiSiv 3000 trap (8) and it was heated to 275°C. Liquid nitrogen was placed on the trap (17) after the closed waste trap to collect the methane oxidation products CO_2 and H_2O. The oxidation loop was allowed to circulate for 20 min. After oxidation was complete, the air was slowly evacuated, retaining the methane oxidation products CO_2 and H_2O in the LN_2 cooled trap (17). The LN_2 on the trap containing the methane oxidation products (17) was replaced with a dry ice:ethanol slurry (–72°C) to continue trapping the H_2O, but liberate the CO_2. The CO_2 was quantified with a calibrated volume (19 or 20) and Baratron (MKS Inst.) digital pressure sensor (22 or 23).

If seep or hydrate gas is available, this two-stage purification-oxidation vacuum line can be used to isolate the pure methane component and oxidize it to CO_2 and H_2O. First, an aliquot of the seep or hydrate gas is statically condensed into a clean LN_2 cooled U-trap. Then, the U-trap is attached to the vacuum line and prepared as described above.

Isotope preparation: The H_2O produced from methane oxidation was collected and reduced to H_2 with activated zinc (Coleman et al. 1982) in a sealed Pyrex tube. The ratio of zinc to H_2O was kept constant at 50 mg zinc to 1 mg H_2O for all samples (Demény 1995). The H_2 gas was used for δ^2H analysis by dual-inlet IRMS at UC Irvine. An aliquot of the CO_2 produced from methane oxidation was used for $\delta^{13}C$ analysis by dual-inlet IRMS at UC Irvine. The remaining CO_2 was converted to graphite with hydrogen reduction catalyzed by iron (Vogel et al. 1984) and analyzed with ^{14}C-AMS at the Keck Carbon Cycle AMS facility at UC Irvine.

Assessment

A series of controlled experiments were conducted to test the trapping, purification and oxidation efficiencies, blanks (total carbon and ^{14}C-CH_4), and isotope fractionation associated with these procedures. The order of these controlled experiments was reverse to the analysis order of actual samples to isolate the characteristics of each step. Also, low methane concentration limitations were evaluated and experiments on actual samples were conducted in the UC Irvine San Joaquin Freshwater Marsh Reserve, Black Sea, Cariaco Basin, and Skan Bay, Alaska, for proof of concept.

Vacuum line techniques: Blanks, efficiency, isotope fractionation, and precision—First, the total carbon blank of the two-stage purification-oxidation vacuum line was tested by attaching clean U-traps and treating them as real samples. The total carbon blank of the vacuum line is $0.11_0 \pm 0.04_6$ μmol (*n* = 20) (Table 2).

Next, the efficiency, isotope fractionation, radiocarbon blank, and precision of the vacuum line were tested. An aliquot of pure radiocarbon-free methane (Scott Specialty Gases) was measured manometrically and statically condensed into a clean U-trap using LN_2. The U-trap was then attached to the two-stage purification-oxidation vacuum line

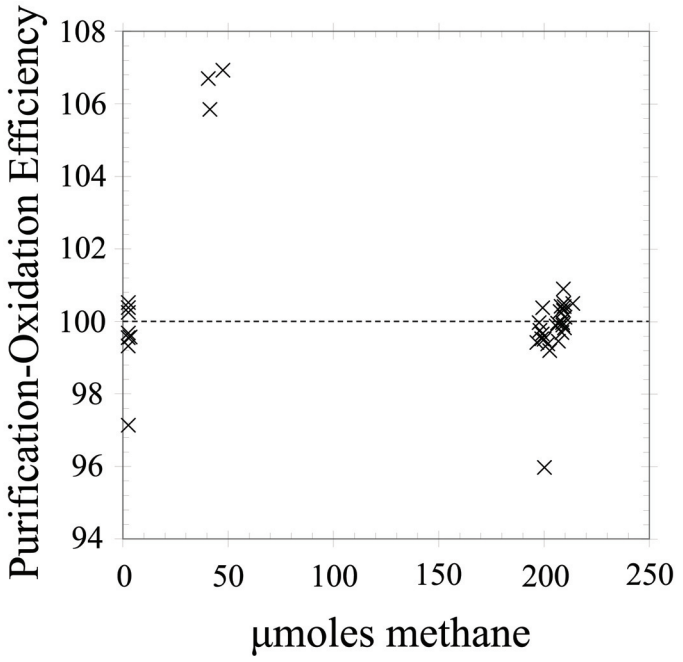

Fig. 3. Purification-oxidation vacuum line efficiency. The efficiency of the purification-oxidation vacuum line was found to be independent of sample size for samples ranging from 2.5 to 213.6 µmol methane. The dashed line represents 100% efficiency.

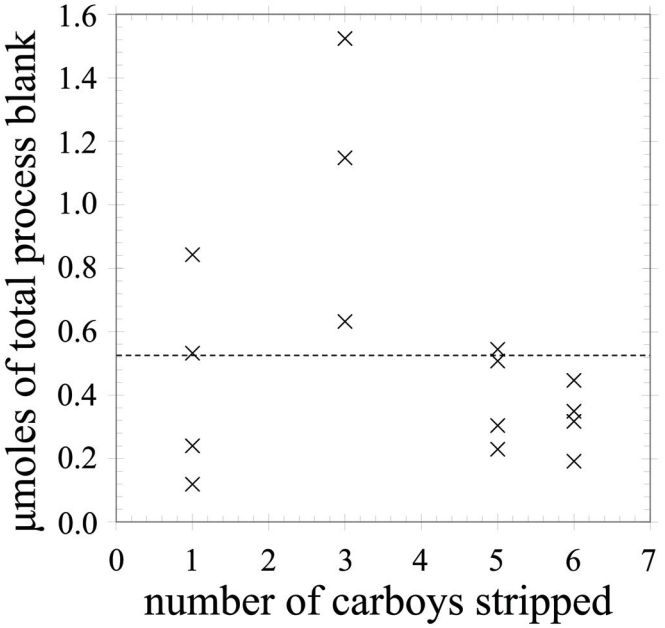

Fig. 4. Total process blanks. The blank of the total processes (0.52_8 ± 0.39 µmol [n = 15]) is independent of the number of water carboys stripped and is indicated by the dashed line.

and the sample was purified and oxidized with the procedures described above. The efficiency of this purification-oxidation technique is 99.8% ± 0.9% (n = 33) for methane samples ranging in size from 2.5 to 213.6 µmol (Fig. 3, Table 2). After the radiocarbon-free methane was subjected to the two-stage purification-oxidation vacuum line, its ^{2}H, ^{13}C, and radiocarbon contents were measured. The δ^2H value of the sample after the vacuum line techniques was -124.3_6‰ ± 2.6_3‰ (n = 24) and cannot be distinguished from the original standard value of -123.5_8‰ ± 2.8_5‰ (n = 32). The δ^{13}C value of the sample after the vacuum line techniques was –19.91‰ ± 0.21‰ (n = 26) and cannot be distinguished from the original standard value of –19.88‰ ± 0.13‰ (n = 9). The precision of the purification and oxidation technique is equivalent to that of the standard. An isotope mass balance (Eq. 1) was conducted determining the radiocarbon content of the vacuum line blank as 84.2 ± 25.3 pMC (n = 19, Table 2).

$$^{14}C_{Measured} = {}^{14}C_{sample} \times F + {}^{14}C_{Blank} \times (1-F)$$
$$F = \frac{\text{moles CH}_{4\text{ sample}}}{\text{moles CH}_{4\text{ sample}} + \text{moles CH}_{4\text{ blank}}} \quad (1)$$

Here, $^{14}C_{sample}$ is the radiocarbon-free methane standard which equals 0 pMC. The large variability of the ^{14}C-CH_4 blank is due to error propagation from the relatively large variability of the vacuum line total carbon blank.

Total process: Blanks, efficiency, isotope fractionation, and precision—A 20 L carboy was filled with 19 L Milli-Q water and connected to the stripping board along with a U-trap cooled with LN_2. Any methane originally dissolved in solution was stripped and trapped with procedures described above. This "waste" U-trap was replaced with a clean U-trap, and the stripping and trapping procedures were repeated. The amount of methane in this trap was quantified with the two-stage purification-oxidation vacuum line and considered the total processes blank. Additional carboys were connected in series (up to 6 carboys totaling 114 L water) and these procedures repeated. The total process blanks for 1, 3, 5, and 6 carboys are 0.43_3 ± 0.32 (n = 4), 1.10_1 ± 0.45 (n = 3), 0.39_6 ± 0.15 (n = 4), and 0.32_6 ± 0.11 (n = 4), respectively (Fig. 4); the total processes blank (0.52_8 ± 0.39 µmol [n = 15]) is independent of the number of carboys connected (Fig. 4, Table 2). The source of this blank may be from leaks in the stripping boards and/or vacuum line, impurities in the CuO oven, or CFC contaminants leaching from plastic tubing on the stripping board. The radiocarbon content of the total process blank was measured directly by forming a composite of 12 total process blanks. This composite indicated the radiocarbon content of the total process blank is 96.14 ± 0.31 pMC (Table 2).

With the methane-free Milli-Q water still attached to the stripping system, a clean U-trap was attached and a 211-µmol aliquot of pure radiocarbon-free methane (Scott Specialty Gases) was injected into the stripping system. After the methane was allowed to equilibrate to closely represent a real sample collected from a Niskin bottle with an evacuated car-

boy, it was stripped with the procedures described above. The quantity of methane trapped was determined with the two-stage purification-oxidation vacuum line. These procedures were tested at two trapping temperatures: LN_2 (–172°C) and the immersion cooler (–50°C). The extraction and recovery efficiency of the LN_2 and immersion refrigerator cooled U-traps are 98.82% ± 1.2% and 66.84% ± 2.2%, respectively.

After the radiocarbon-free methane was subjected to the stripping and vacuum line procedures, its δ^2H and $\delta^{13}C$ were measured. The procedures served to quantify the isotope fractionation and precision associated with the total process. All isotope results are independent of the cryogen used on the U-trap during stripping. The continuous loop design of the stripping boards recycles any isotopes that exit the U-trap back through the U-trap. This causes the isotopes to be homogenized in the U-trap during the 2 hour stripping process. The δ^2H value of the sample after the total process was -122.0_9‰ ± 1.6_8‰ (n = 5) and cannot be distinguished from the original standard value of -123.5_8‰ ± 2.8_5‰ (n = 32). The $\delta^{13}C$ value of the sample after the total process was –19.77‰ ± 0.31‰ (n = 5) and cannot be distinguished from the original standard value of –19.88‰ ± 0.13‰ (n = 9). These isotope results indicate that blanks do not influence the final stable isotope results at these sample sizes and isotopic fractionation is nonexistent. The overall precision of the extraction, purification, and oxidation techniques is equivalent to that of the standard.

The extent of methane breakthrough was assessed by locating Swagelok tees with one port containing a silicone rubber septum before and after the U-trap on the stripping board. Aliquots of gas were removed at selected times and the methane concentration was measured with gas chromatography (GC) and flame ionization detection (FID) (GC-Mini 2; Shimadzu Corp.) to monitor the stripping and trapping efficiency and to determine how much methane escaped the trap. For the LN_2 cooled U-trap, no measurable methane was evident in samples collected from the sample port following the U-trap (detection limit = 50 ppb). Samples collected at the septum upstream from the U-trap showed that 99.96% ± 0.01% of the methane was collected within the 2 h stripping and trapping. For the dry ice:ethanol slurry and immersion refrigerator-cooled U-trap experiments, significant amounts of methane were detected exiting the U-trap; after the 2 h stripping and trapping procedure, 95.48% ± 1.7% and 71.77% ± 4.8% of the methane injected was collected, respectively.

An equation was derived modeling the evolution of dissolved methane in the natural sample during shipboard extraction and trapping. This equation is a modification of equations derived by Flett et al. (1976) and Weiss and Craig (1973) and assumes LN_2 is the cryogen used on the U-trap (Eq. 2).

$$\frac{dm}{dt} = \frac{M \times F_R \times L_W^2 \times S_P}{(L_H + F_R \times T + L_W \times S_P)^2} - \frac{m[t]}{L_H} \times F_R \quad (2)$$

Here, dm/dt is the rate of change of the quantity of methane in the carboy or Mason jar, M is the concentration of methane in the sample (Molarity), F_R is the flow rate (L min^{-1}), L_W is the L of sample, Sp is the methane solubility with a pure methane atmosphere (Yamamoto et al. 1976), L_H is the L of headspace, T is time (min), and $m[t]$ is the moles of methane in the carboy or mason jar at time t. The model predicts the following extraction times are necessary to extract greater than 99.9% of the methane from 19 L of 15 µM methane water, 114 L of 1 nM methane water, and 0.71 L of a 3 mM methane sediment slurry at a flow rate of 2 L min^{-1}: < 20 min, 100 min, and < 5 min, respectively.

Low methane concentration limitations—For high methane concentration environments, such as the anoxic water columns and sediments of the Black Sea and Cariaco Basin, these procedures are able to make accurate and precise ^{14}C-CH_4 measurements due to the relatively low blanks, excellent efficiency, and lack of isotope fractionation (Kessler 2005; Kessler et al. 2005). However, as the concentration of methane in the water or sediment sample decreases, the precision of the final radiocarbon result decreases. Understanding how the methane concentration is related to the final precision is crucial to understanding what environments can produce reliable radiocarbon results.

What is the smallest amount of methane we can extract and still perform a useful ^{14}C-CH_4 measurement? If we know the blank very accurately and precisely, we can conduct any ^{14}C-CH_4 measurement as long as we collect approximately 0.083 µmol modern methane (Currie et al. 2000). However, the blank is relatively imprecise, so as the quantity of methane extracted approaches the size of the blank, the blank's imprecision causes the error in the final result to grow. To calculate the error in the final result, we begin with the isotope mass balance equation (Eq. 1). To calculate how the error of $^{14}C_{sample}$ is influenced by the blank's imprecision, we differentiate $^{14}C_{sample}$ with respect to *moles* CH_{4blank} and multiply by the error of *moles* CH_{4blank}. The result of this differentiation is displayed in Eq. 3.

$$\sigma_{^{14}C sample} = \left| \frac{^{14}C_{blank} - {}^{14}C_{Measured}}{\text{moles CH}_{4\text{ water}}} \right| \times \sigma_{blank} \quad (3)$$

Equation 3 displays maximum values when $^{14}C_{blank}$ = 100 and $^{14}C_{water}$ = 0, or vice versa. At low methane concentrations (< 0.4 µM), we extracted methane from 114 L water, which has a total methane blank value of $0.32_9 \pm 0.10_7$ µmol. Assuming errors larger than ± 5 pMC lead to results that are not interpretable, then at least 1.81 µmol methane must be collected corresponding to a methane concentration of 15.9 nM.

These procedures can be used in their current state to collect and prepare samples for natural radiocarbon analysis in natural water and sediment as long as 1.81 µmol methane can be collected. Thus we recommend measuring the methane concentration in a particular sampling site prior to implementing these procedures. Equipped with methane concentration data, an appropriate quantity of water or sediment can be collected yielding adequate methane to conduct a successful ^{14}C-CH_4 measurement.

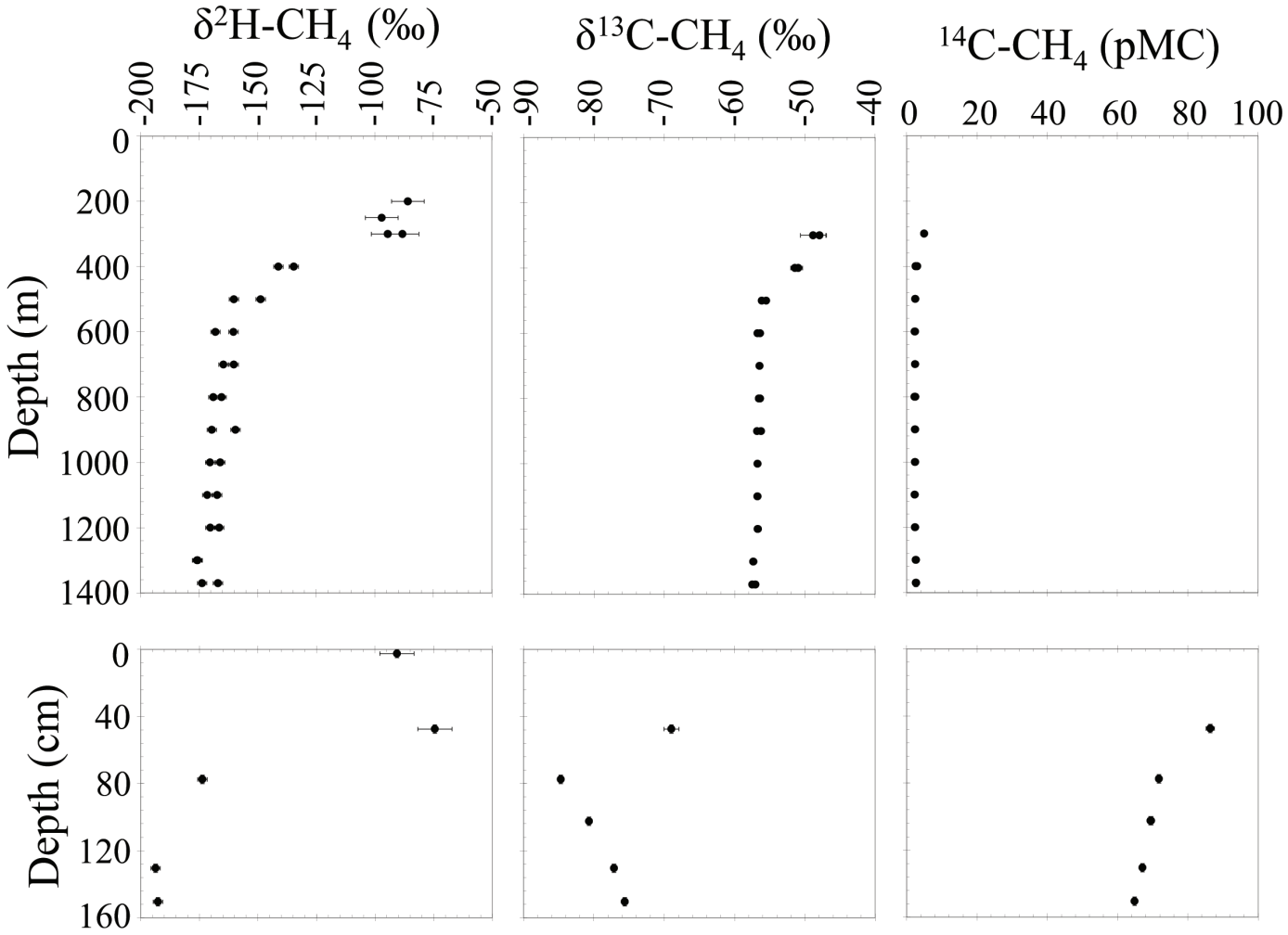

Fig. 5. Cariaco Basin water column and sediment isotope results. Note the difference in vertical scales between the water column (above) and sediment (below). Due to similar stable isotope (δ^2H-CH_4, δ^{13}C-CH_4) values in the water column and sediment as well as extensive methane oxidation in the water column and sediment, the stable isotopes do not unambiguously determine the source of the methane. The radiocarbon results clearly indicate sediment methane is not the source of methane to the water column and a dominant fossil source of methane is present.

Proof of concept: Applications to natural samples—Detection of non-methane impurities: Natural water samples were collected from the UC Irvine San Joaquin Freshwater Marsh Reserve and the Black Sea and subjected to our field and laboratory techniques. An aliquot of gas was removed from the two-stage vacuum line after the purification stage. Analysis of this purified gas with quadrupole mass spectrometry at the National Institute of Standards and Technology (Currie et al. 2000), showed no detectable traces of carbonaceous impurities ($m/z \leq 100$, detection limit was 100 ppm).

Cariaco Basin (Fig. 5): Water and sediment samples were collected from 21-24 January 2004 on board the B/O *Hermano Gines* in the deepest portion of the Eastern basin (10.5°N, 64.66°W, 1370 m) at the time-series station used by the CArbon Retention In A Colored Ocean (CARIACO) program (Astor et al. 2003; Scranton et al. 2001). Methane was extracted, trapped, purified, oxidized, and analyzed using the procedures described above. The anoxic waters of the Cariaco Basin contain μM methane concentrations, so at depths ≥ 300 m, methane was extracted from 19 L water. Sediment methane was extracted from gravity core segments with a volume 0.17 L sediment. The water column methane concentration was measured by a headspace equilibration technique incorporating a GC-FID (GC-Mini 2; Shimadzu Corp) (Kessler et al. 2005). The water column methane concentration calculated from the stripped and trapped methane, agreed with the GC methane concentrations to 3% on average below 300 m depth, providing additional evidence that our procedures are quantitative and the blanks are small. Methane oxidation occurs in the Cariaco Basin water column (Ward et al. 1987) causing signif-

icant isotopic fractionation (Alperin et al. 1988; Martens et al. 1999). The source of methane to the water column can be misinterpreted from the stable isotope data as being from sediment diagenesis. However, the ^{14}C-CH_4 results prove that the source of methane to the water column is not from sediments (Fig. 5). Between 400 and 1370 m depth, the Cariaco Basin water column methane is almost completely devoid of radiocarbon (2.5 ± 0.18 pMC) (Kessler et al. 2005). In contrast, the sediment methane contains significant amounts of ^{14}C (86.4 ± 1.2 pMC at 45 cm depth) (Kessler et al. 2005), challenging previous studies that methane diffusing from sediments is the water column methane source in the Cariaco Basin (Reeburgh 1976; Scranton 1988). A more complete interpretation of the Cariaco Basin isotope data can be found in Kessler et al. (2005) and Kessler (2005).

Skan Bay, Alaska: Skan Bay, Alaska, contains nM methane concentration seawater, which served as a test of the lower limit of these procedures. Water and sediment methane samples were collected from 28 August to 10 September 2003 on board the R/V Alpha *Helix* in Skan Bay, Alaska, which is located on the northern side of Unalaska Island (57°37′N, 167°03′W, 65 m). The water column methane concentration profile displayed a sub-surface methane maximum of 40 nM at 15 m depth that was apparently related to a vertically migrating population of euphausids. The low water column concentration required the extraction of methane from 114 L to obtain enough sample for radiocarbon measurement. Methane was also extracted from 0.07 to 0.35 L sediment by preparing slurries from core segments. The sediment methane was modern, with highest values in the surface 20 cm (97.06 ± 0.31 pMC). The water column ^{14}C-CH_4 results (68.1 ± 4.8 pMC) indicate that the source of methane to the water column is a mixture of modern and fossil (radiocarbon-free) methane.

Discussion

These procedures are currently leading to new insights. In the Cariaco Basin where the water column methane concentration is high (16.78 µM at 1370 m) and the methane turnover time is short (Kessler et al. 2005), previous studies were able to model the water column methane geochemistry with only a diffusive diagenetic sediment source (Reeburgh 1976; Scranton 1988). However, the radiocarbon results clearly show that water column (fossil) and sediment (modern) methane have different sources, challenging the results from these previous studies (Fig. 5). Since the Cariaco Basin is too warm (16.9°C) to form hydrates, these results indicate the presence of a previously unknown and dominant seep source of methane (Kessler et al. 2005).

The lower limit of these procedures is also leading to new insights in Skan Bay, Alaska, where the water column methane concentration approaches the open ocean methane concentration. The source of methane to the water column was suspected to be diffusing from the sediments and contain modern radiocarbon contents for several reasons: (1) this basin is relatively shallow, (2) there are high sedimentation rates, (3) no seeps have been discovered, and (4) the water column is flushed annually due to winter storms. The presence of non-modern water column ^{14}C-CH_4 values indicates that a portion of the water column methane has another source. The methane source to the water column may be from previously unknown seeps or particle microenvironments where the substrate for methane formation is non-modern terrestrial material.

Comments and recommendations

For high methane concentration water columns like the Black Sea and Cariaco Basin, as well as anoxic sediments with methane concentrations near saturation, the use of UHP helium in the shipboard stripping procedures may be substituted for a less pure helium. We recommend testing the blanks of this helium source before field experiment. For low methane concentration environments like the open ocean, stripping with UHP helium is essential to achieve the low blanks required to make a successful ^{14}C-AMS measurement.

These procedures have been successfully applied to the Black Sea (Kessler 2005) and Cariaco Basin (Kessler et al. 2005), where high methane concentration water columns allow the extraction of large quantities (> 200 µmol) of methane. Our Skan Bay water column results suggest that future work can involve studies of open ocean methane sources, where water column methane concentrations are ≥ 15.9 nM. However, many open ocean locations contain methane concentrations approaching 2 nM and radiocarbon would aid in determining the methane source. For example, the Eastern Tropical North Pacific (ETNP) is the largest open ocean pool of methane yet discovered and is suspected to have two different methane sources (Sansone et al. 2001). The subsurface waters seem to contain a biological source associated with the decomposition of detritus based on $\delta^{13}C$-CH_4 (< –35‰). The deeper water (below ca. 200 to 400 m) contains a $\delta^{13}C$-CH_4 (> –35‰) indicative of a coastal source transported along isopycnal surfaces that has undergone partial microbial oxidation (Burke et al. 1983; Sansone et al. 2001). By measuring water column ^{14}C-CH_4 with these new procedures, it can be determined if this isotopically heavy methane is of coastal origin.

To adapt these procedures to these low concentration methane environments, enough sample must be collected for ^{14}C-AMS with significantly low blanks. Since obvious leaks are not present in the stripping boards and vacuum-line techniques, the total process blanks are unlikely to decrease significantly. Stripping the methane from more than 6 carboys (114 L) may be necessary in the low concentration waters of the open ocean to collect enough methane for ^{14}C-AMS measurement. Since 1.81 µmol methane must be collected for AMS analysis, if the methane was extracted from 12 carboys (228 L), 8 nM seawater can be analyzed. We recommend determining the blanks associated with stripping the methane from greater than 6 carboys before application in the field.

Safety note—When traps filled with HiSiv 3000 are cooled with LN_2 (or to a lesser extent with a dry ice:ethanol slurry or the immersion cooler), they trap N_2, O_2, helium, CO_2, and H_2O as well as methane. When the cryogen is removed, these trapped gases expand and pressurize the traps. The steel U-traps were designed to withstand this pressurization. Glass traps containing HiSiv 3000 are not designed to withstand this pressurization and may explode. The cryogen should only be removed from glass traps filled with HiSiv 3000 when the traps are opened to a larger volume. This allows the condensed gases a larger volume to expand into and not increase the internal pressure above 1 atmosphere. Care must be taken when opening any trap filled with HiSiv 3000 and condensed gases even when still cooled with a cryogen. A large pressure release will occur, which may cause glass traps and vacuum line parts to explode. The vacuum line was designed to have enough volume so that the gas released from the U-traps would not cause the internal pressure to increase above 1 atmosphere. Since LN_2 can condense air without using a molecular sieve, the LN_2 must not be removed from the waste trap until the sample has been removed from the vacuum line and the contents of the waste trap evacuated away. More information on this safety hazard can be found in Wheeler et al. (2001).

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