Black Sea methane geochemistry

William S. Reeburgh,* Bess B. Ward,†‡ Stephen C. Whalen,* Kenneth A. Sandbeck,*§ Katherine A. Kilpatrick†‖ and Lee J. Kerkhof†

Abstract—Methane concentrations and oxidation rates were measured in the water column and sediments of the Black Sea at a central station during leg 5 of the 1988 U.S.–Turkey Black Sea Expedition. Methane concentrations were 10 nM in the upper 100 m, increased to 11 μM at 550 m, and were uniform to the bottom. Water column methane oxidation rates were measured using two independent radiotracer techniques: tracer level additions of ^{3}H–CH$_4$ and non-tracer level additions of ^{14}C–CH$_4$. The methods agree within a factor of two. Methane oxidation rates were low in the surface 100 m and increased to relatively uniform values of 0.6 μM y^{-1} below 500 m. Sediment methane concentration and oxidation rate distributions showed that shelf and slope sediments were methane sources, while deep basin sediments were methane sinks.

These measurements were used to construct a methane budget for Black Sea waters. Microbially mediated anaerobic methane oxidation is the dominant water column methane sink, followed by evasion to the atmosphere, abyssal plain sediment consumption and outflow at the Bosporus. The source of methane appears to be anoxic, high deposition rate shelf and slope sediments. The water column oxidation rate measurements suggest a short (5–20 year) residence time for methane in the Black Sea, indicating a higher geochemical activity than previously believed. The quantity of carbon participating in the Black Sea methane cycle is equivalent to about 0.5% of the primary production.

INTRODUCTION

Methane is a radiatively active trace gas that is presently undergoing an atmospheric concentration increase of about 1% per year (Khalil and Rasmussen, 1983; Steele *et al.*, 1987; Blake and Rowland, 1988). Since methane could be important in global warming, the global methane budget and general methane geochemistry are subjects of active investigation.

Although large inventories of methane are present in anoxic marine sediments and anoxic water columns, the ocean is a minor source (1.9%) in the global methane budget

---

*Institute of Marine Science, University of Alaska–Fairbanks, Fairbanks, AK 99775-1080, U.S.A.

†Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92093, U.S.A.

‡Present address: Marine Sciences Program, University of California–Santa Cruz, Santa Cruz, CA 95064, U.S.A.

§Present address: Dept. of Microbiology, SC-42, University of Washington, Seattle, WA 98195, U.S.A.

‖Present address: Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, FL 33149, U.S.A.

(CICERONE and OREMLAND, 1988), indicating that processes capable of consuming methane and modulating the flux to the atmosphere are functioning within the ocean. Methane is present throughout the Black Sea water column. The Black Sea is the largest surface water reservoir of dissolved methane and contains 96 Tg, a quantity equivalent to the annual methane emission to the atmosphere by wetlands or rice culture (CICERONE and OREMLAND, 1988).

The Black Sea, the world's largest anoxic basin, has been regarded as a "type" environment (GLENN and ARTHUR, 1985). The deep water of the Black Sea is believed to have become anoxic following introduction of sea water to a fresh-water lake at the end of the last glaciation and low stand of sea level. Previous methane measurements in the Black Sea followed the introduction and application of gas chromatography to dissolved gas analysis in marine chemistry (SWINNERTON *et al.*, 1962). The first measurements were reported by HUNT (1974), and are presented as pooled data, so no single station profile is available. A limited number of water column methane measurements were performed by ATKINSON and RICHARDS (1967). The measurements of SCRANTON (1977) are most recent and define the methane distribution in the Black Sea best. All of these previous measurements were performed in shore laboratories on stored samples. Black Sea sediment hydrocarbon measurements (including methane) were reported by HUNT (1974) and HUNT and WHELAN (1978), but limited information on methane depth distributions is available. Clathrate hydrates of methane were reported in Black Sea sediments by YEFREMOVA and ZHIZHCHENKO (1974).

Methane has been regarded as a fairly recalcitrant molecule in nature. Photochemical oxidation in the stratosphere by the OH radical is considered to be the major methane sink, but organisms capable of metabolizing methane are known to exist in soils and aquatic environments. Recent studies have shown that microbially mediated methane oxidation occurs in aerobic as well as anaerobic environments. The organisms responsible for aerobic methane oxidation in fresh-water systems have been isolated and are well-characterized (ANTHONY, 1982). A single marine methylotroph has been isolated (SIEBURTH *et al.*, 1987), but the organisms involved in oceanic methane metabolism are not well known. Marine nitrifiers, which are morphologically and physiologically similar to aerobic methane oxidizers, may be involved in methane oxidation in oxic marine environments (WARD, 1987).

Anaerobic methane oxidation has been studied in the water columns of the Cariaco Trench (REEBURGH, 1976; WARD *et al.*, 1987; SCRANTON, 1988) and Saanich Inlet (WARD *et al.*, 1989). Anoxic marine sediments have received more attention as the locus of anaerobic methane oxidation because the oxidation rates are highest and the methane concentration changes are largest there. A strong geochemical case has been advanced for anaerobic methane oxidation, involving model (REEBURGH, 1976; ALPERIN, 1988), rate (REEBURGH, 1980; ALPERIN and REEBURGH, 1984, 1985) and stable isotope (ALPERIN *et al.*, 1988) studies in anoxic marine sediments. The organisms responsible for anaerobic methane oxidation have not been isolated, and their requirements are not understood. Anaerobic methane oxidation in anoxic marine sediments is an important sink in the global methane budget, consuming between 20 Tg y^{-1} (HENRICHS and REEBURGH, 1987) and 100 Tg y^{-1} (REEBURGH, 1989), and limiting net transport of methane to the water column and to the atmosphere.

Our goals in this study were to measure detailed profiles of methane concentration and methane oxidation rates in the Black Sea water column and sediments using techniques

developed since the last Black Sea studies in 1977. We performed these analyses at a central station with a view toward assembling a methane budget for the Black Sea and comparing Black Sea methane geochemistry with that of other anoxic basins.

METHODS AND MATERIALS

Sampling

One station, BSK-2 (43°05'N, 34°00'E, 2200 m depth), was emphasized during leg 5 (13–29 July 1988). A box core was collected at a basin slope station, BS5-2 (41°37.86'N, 28°58.37'E, 600 m depth), and sediment methane distributions were measured in sub-cores. The locations of both stations are shown in Fig. 1.

Water column samples were obtained with 30-l Niskin bottles mounted on a CTD rosette and equipped with Teflon-coated springs and silicone O-rings. A slight nitrogen overpressure was used in sampling low-oxygen waters. Sediments were collected with a 0.25-m^2 box corer. Subcores were taken by gently inserting 6-cm diameter core liners.

Methane concentration measurements

Methane analyses were made at sea using a Shimadzu Mini-2 gas chromatograph with a flame ionization detector. The instrument was equipped with a 1 m × 3.2 mm o.d. molecular sieve 5A column, which was operated at 70°C. Samples were introduced through an external stripping/trapping manifold similar to that described by BULLISTER
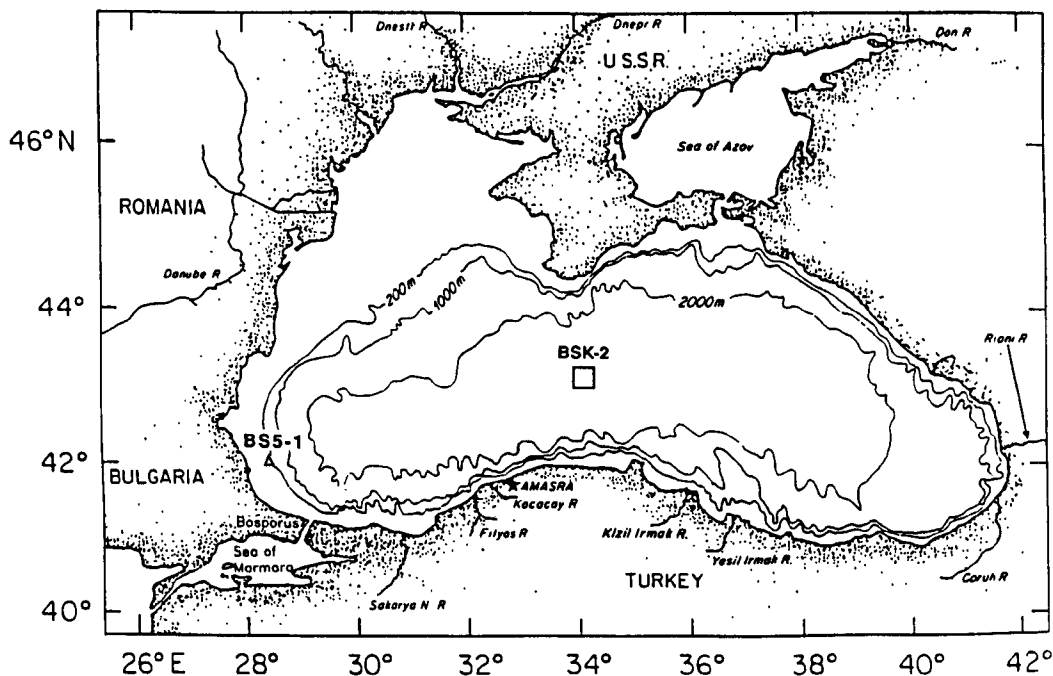

FIG. 1.   Station locations. The water column and sediment CH$_4$ concentration and oxidation rate measurements were conducted at Sta. BSK-2 (43°05'N, 34°00'E). Sediment methane concentrations were measured at Sta. BS5-1 (41°35.5'N, 28°56.92'E, 170 m).

(1984). Samples of 5–7 cm^3 were introduced to the stripper by syringe and were stripped for 7 min at a flow rate of 30 cm^3 min^{-1}. The stripped gases were trapped at $-68°C$ (isopropanol slush) in a U-trap containing Carbosphere (60/80 mesh). Following stripping, the Carbosphere trap was isolated with appropriate valves on the manifold and heated to 100°C. The trapped sample was introduced to the gas chromatograph by switching the carrier flow through the trap.

Glass syringes (Becton–Dickinson, 50 cm^3) were used for collection and introduction of water samples to the stripper. The syringes were filled from the Niskin samplers with a thick-walled tygon tube attached to the Luer fitting. The piston was removed from each syringe barrel and several volumes were allowed to overflow before re-inserting the piston. The syringes were cleaned to insure that all surfaces wetted and were checked following filling to insure that no bubbles were trapped. The syringes were sealed with Luer caps and were stored in a cold room at in situ temperature prior to analysis. Sample volumes delivered were determined by measurements of syringe piston area and the piston travel following sample injection. The syringes were modified by fitting machined lucite blocks to the syringe piston and barrel with silicone sealant. Shaft locks fashioned from drilled-through Swagelok tube caps were attached to the syringe barrel blocks and were used to immobilize guide shafts attached to the syringe piston blocks during the piston travel measurements. Piston travel was measured with a digital micrometer using the parallel sides of the lucite blocks as measuring surfaces; sample volumes are known to 0.001 cm^3.

Overall precision of the methane determinations was estimated by replicate measurements and varied with concentration as follows: <10 nM, 4.3%; 10–100 nM, 2.0%; >100 nM, 0.8%. Working standards checked against laboratory standards traceable to NBS standards were used in the field.

Sediment methane concentrations were measured using a headspace technique. Sediment cores were collected from box cores with pre-drilled liners whose holes were covered with electrical tape. Horizontal subcores were collected sequentially, as the electrical tape covering the holes was removed, using cut-off 3-cm^3 syringes as horizontal subcorers. The syringe contents were injected into helium-flushed 30-cm^3 serum vials containing 10 cm^3 of distilled water. The vials were capped with butyl rubber stoppers and aluminum crimp seals. Following equilibration, syringe samples of the headspace gas were injected into a sample loop and were analysed for methane.

Rate measurements

Water column methane oxidation rates were measured with independent methods employing two different radiolabeled methane tracers, $^{14}CH_4$ and C^3H_4. The $^{14}CH_4$ measurements were emphasized in the oxic zone, while the C^3H_4 measurements emphasized deep anoxic waters. Although methane oxidation rates were measured with both tracers at the same nominal depths, no parallel determinations on samples from the same bottle were made.

$^{14}CH_4$ tracer. This tracer has been employed previously in water column methane oxidation rate measurements (WARD et al., 1987, 1989), and is based on recovery and quantification of product $^{14}CO_2$ and labeled particulate carbon. The $^{14}CH_4$ was produced

biogenically using the procedure of DANIELS and ZEIKUS (1983); specific activity (23.5 mCi mmol^{-1}) was determined by scintillation counting of methane (ZEHNDER *et al.*, 1979). A thick-walled Teflon tube attached to the Niskin samplers was used to fill several 160-cm^3 serum bottles, avoiding bubbles and allowing approximately 2 vol. to overflow. The serum bottles were sealed without bubbles using solid black butyl rubber stoppers (boiled in dilute NaOH to reduce potential toxicity) and aluminum crimp seals. Tracer gas was injected through the stopper using a second needle to release displaced sample. Tracer additions varied from 10 to 200 μl, depending on the experiment, and formed a small bubble in the incubation bottle. Final dissolved methane concentrations were calculated using the corrected (1975b) equation of RUDD and HAMILTON (1975a). Methane concentrations after tracer addition in oxic layer experiments were about 1 μM, which was substantially above the ambient concentrations (nM). Experimental concentrations in samples from the anoxic layer were within 20%, and usually within 10%, of the ambient methane concentration. Incubations were terminated after approximately 12 h by addition of three drops of 10 N NaOH and were processed immediately for labeled particulates by filtration through 0.3-μm pore size Millipore filters. Methane oxidized to $^{14}CO_2$ was recovered by acidification of the filtrate and capture on phenethylamine-soaked wicks (80% recovery efficiency) as previously described by WARD *et al.* (1989). Zero-time experiments were performed at intervals to provide a blank; these were filtered and treated as above immediately after tracer addition. All samples were incubated in a dark, temperature-controlled incubator near *in situ* temperature; they were shielded from light during all manipulations. Filters containing ^{14}C-particulates and recovered $^{14}CO_2$ were assayed by liquid scintillation counting on board ship with a Beckman LS-100 liquid scintillation counter; quench corrections were determined with external standard ratios. Methane oxidation rates were calculated by summing conversion of $^{14}CH_4$ to radiolabeled CO_2 and particulate matter. Kinetic experiments (performed on samples from the oxic layer or interface region) showed a linear relationship between oxidation rate and methane concentration. Results from the single end-point experiments were corrected for altered substrate concentration due to label addition based on this linear relationship. Measured oxidation rates were found to be constant for at least 12 h in previous experiments (WARD *et al.*, 1987, 1989), resulting in linear label accumulation over time. We did not perform time-course experiments here, but increased the total number of depths studied using replicate single end-point experiments.

C^3H_4 *tracer.* The rate measurements based on C^3H_4 involved incubating water samples in sealed bulbs and measuring the product of oxidation, ^3H_2O, following removal of the unreacted C^3H_4. Tubular glass gas sampling bulbs fitted with a straight bore stopcock at each end were filled from the Niskin bottles. The bulbs were filled avoiding bubbles and were overflowed 2–3 vol. The gas sampling bulbs were calibrated gravimetrically; their volumes ranged between 150 and 180 cm^3 and were known to ±0.02 cm^3. The C^3H_4 was prepared from [methyl ^{3}H] methylamine hydrochloride with transiently starved methanogenic bacteria (SANDBECK and REEBURGH, 1989). Two batches with specific activities of 2.81 and 3.44 Ci mmol^{-1} were prepared and used. The C^3H_4 tracer (50 μl O_2-free N_2 containing 0.2 μl C^3H_4) was introduced into the bulb body by opening one of the stopcocks and threading a cannula attached to a gas-tight syringe through the bore. The outboard stub end of the gas bulb stopcock permitted overflow following additions and remained filled with sample due to capillarity. The sample-filled stub ends served as an

effective barrier to contamination by atmospheric O_2, and permitted open-air manipulations. Single end-point experiments at true tracer levels ($\ll 1\%$ of CH_4 pool) were used in the C^3H_4 oxidation rate measurements. Two bulbs were filled at each depth; one was killed prior to tracer injection by addition of 1 ml saturated $HgCl_2$ (final concentration >1 mM). The experimental and killed control bulbs were incubated near *in situ* temperature for 24 h. Aliquots of water from the killed control and experimental bulbs were drawn into stripping vessels (Alperin, 1988), where natural and tracer methane were removed by stripping with water-saturated helium. Aliquots of stripped experimental and killed control samples containing product and background ^3H_2O, respectively, were counted as an Aquasol gel. The samples were counted initially on shipboard. These samples and archived samples were counted on return to Fairbanks with a Beckman LS-3801 liquid scintillation counter employing H-factor quench correction.

Sediment methane oxidation rates. Measurements of methane oxidation rates were made using techniques identical to those of Alperin and Reeburgh (1985). Sediment cores were collected from box cores with pre-drilled liners whose holes were covered with electrical tape. Horizontal subcores were collected sequentially, as the electrical tape covering the holes was removed, with Alperin–Reeburgh syringes. The sediment-filled syringes were sealed with black butyl rubber stoppers and aluminum crimp seals. Tracer quantities of $^{14}CH_4$ were injected into the syringes, which were incubated 12 h at *in situ* temperatures. Following incubation, the syringes were frozen at $-30°C$ to stop biological activity. The frozen samples were injected into a stripped 0.1 N NaOH solution, which retained CO_2 and permitted stripping of CH_4. Methane and $^{14}CH_4$ were stripped from the stirred slurry with He and were oxidized to CO_2 by passage through a stainless steel combustion tube filled with CuO and heated to 800°C. The contents of the stripping vessel were subsequently acidified with 7.2 N H_2SO_4 and CO_2 and $^{14}CO_2$ were trapped in a Harvey trap containing Woeller's (1961) solution. The $^{14}CO_2$ was counted as above.

RESULTS

Water column methane distribution

Water column methane concentration measurements from this study are shown in Figs 2 and 3. Figure 2 shows the entire profile, while Fig. 3 shows an expanded-scale plot of the upper 120 m. This figure shows the location of the methane concentration increase at the top of the anoxic zone, and also shows methane saturation relative to the atmosphere. The saturation line in Fig. 3 was calculated using an atmospheric methane concentration of 1.7 ppm (Cicerone and Oremland, 1988) and the sea water methane solubility data of Yamamoto *et al.* (1976), as summarized in IUPAC (1987). The oxic surface waters of the Black Sea are 2- to 5-fold supersaturated relative to the atmosphere. Figure 4 compares our water column methane data with measurements reported by Atkinson and Richards (1967) and Scranton (1977). The Atkinson and Richards measurements were among the first ocean water column methane measurements reported; they were made on stored samples and used a relatively low-sensitivity thermal conductivity detector. Scranton's measurements were also made on stored samples, but used a flame ionization detector. Scranton noted loose stoppers in her 735 and 1764 m samples and deleted these data. Despite Scranton's reservations that all of her measurements could be low as a result of
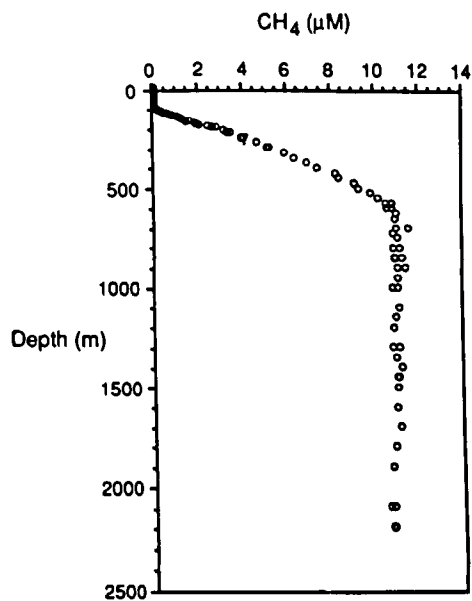
CH$_4$ (µM)

Depth (m)

Fig. 2.   Water column methane concentration profile at Sta. BSK-2. This profile is a composite of samples collected from 19–26 July 1988.
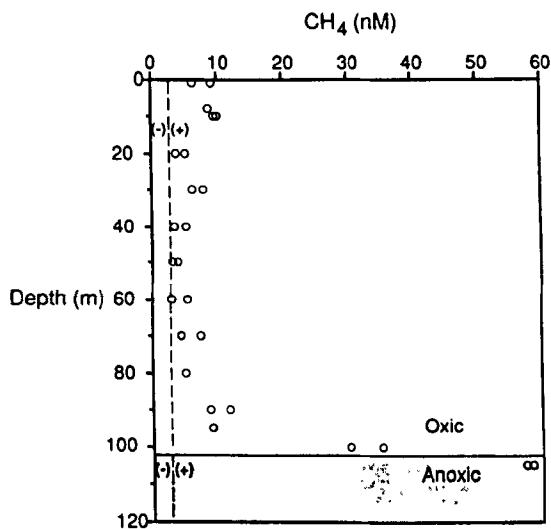
CH$_4$ (nM)

Depth (m)

Oxic

Anoxic

Fig. 3.   Surface Black Sea water column methane distribution. The atmospheric equilibrium line was calculated using an atmospheric CH$_4$ concentration of 1.7 ppm and the seawater Bunsen coefficients for CH$_4$ of YAMAMOTO et al. (1976).
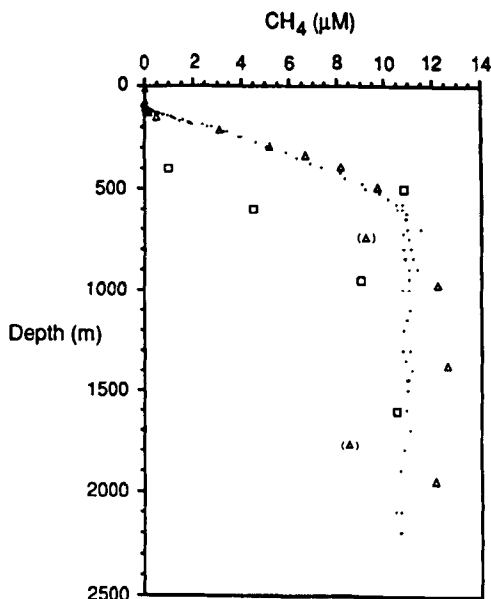
Fig. 4. Comparison of Black Sea water column CH₄ distributions from this study (●) with previous measurements by ATKINSON and RICHARDS (1967) (□) and SCRANTON (1977) (△). Scranton considered the two measurements shown in parentheses uncertain.

leakage during storage, they agree very well with ours at depths above 500 m. Given this agreement, we cannot exclude the possibility that Scranton's higher concentrations below 500 m are real and that a change in Black Sea deep water column methane has occurred between 1975 and 1988.

Sediment methane distributions and oxidation rates

Sediment methane distributions were measured at three stations; results from two of the stations are reported here. Station BS5-1 (41°35.5'N, 28°56.92'E, 170 m) (Fig. 5a) was located on the shelf; the sediment:water interface was well within the anoxic zone. Station BS5-10 (43°04.82'N, 33°58.88'E, 2212 m) (Fig. 5b) was located on the abyssal plain in the vicinity of BSK-2, the station emphasized during leg 5. We had difficulty obtaining turbidite-free cores at this station. The sediment methane concentration distribution shown in Fig. 5b and the methane oxidation rate measurements shown in Fig. 5c were made on varved, turbidite-free sediments. Radioisotopes were not used on leg 5 until a sediment trap mooring at Sta. BSK-2 had been recovered and deployed; no oxidation rate measurements were made at Sta. BS5-1.

There are distinct differences in the shelf and abyssal plain sediment methane distributions. The continental shelf station (BS5-1) sediment methane distribution is concave up and has millimolar methane levels at depth. This distribution is a typical marine distribution (see ALPERIN and REEBURGH, 1984, for review). In contrast, CH₄ concentrations in the abyssal plain (BS5-10) cores are lower than methane concentrations in the adjacent overlying water, and decrease with depth in the core. The sediments in core BS5-10 are consuming methane from the adjacent overlying water. Thus, the continental shelf
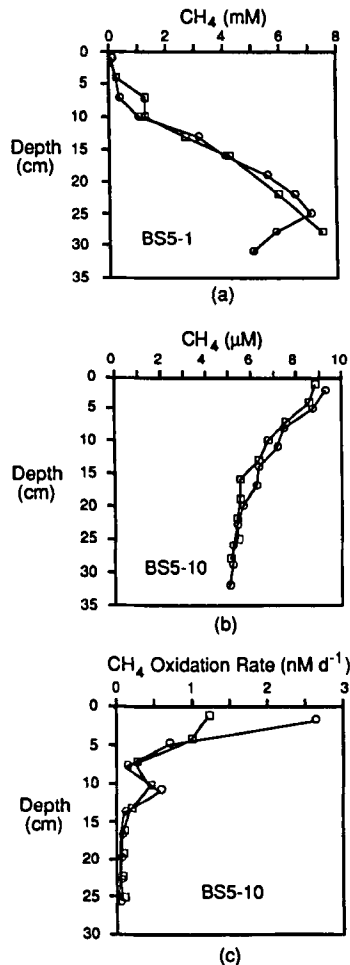
Fig. 5. Methane concentrations and oxidation rates in Black Sea sediments. (a) Methane concentration distributions from two subcores at Sta. BS5-1 (41°35.5'N, 28°56.92'E, 170 m). This station was located below the oxycline on the shelf. Methane concentrations at the sediment surface exceeded those in the adjacent overlying water. Note mM concentration scale. (b) Methane concentration distributions at Sta. BS5-10 (43°04.82'N, 33°58.88'E, 2212 m). Note μM concentration scale. This core was varved throughout. Cores collected in this area containing turbidites had slightly higher methane concentrations in the turbidite interval. All deep basin sediment methane concentrations are lower than those in the adjacent overlying water. (c) Methane oxidation rate distributions ($^{14}CH_4$ tracer) measured in two subcores from Sta. BS5-10. The concentration (b) and oxidation rate (c) profiles indicate that deep Black Sea sediments are consuming water column methane.

station sediment represents a methane source, while the abyssal plain sediment can only be a sink.

Water column oxidation rate distributions

The $^{14}CH_4$-derived water column methane oxidation rate measurements are shown in Fig. 6. Figure 6a shows all measurements and is plotted with a log rate scale to cover the
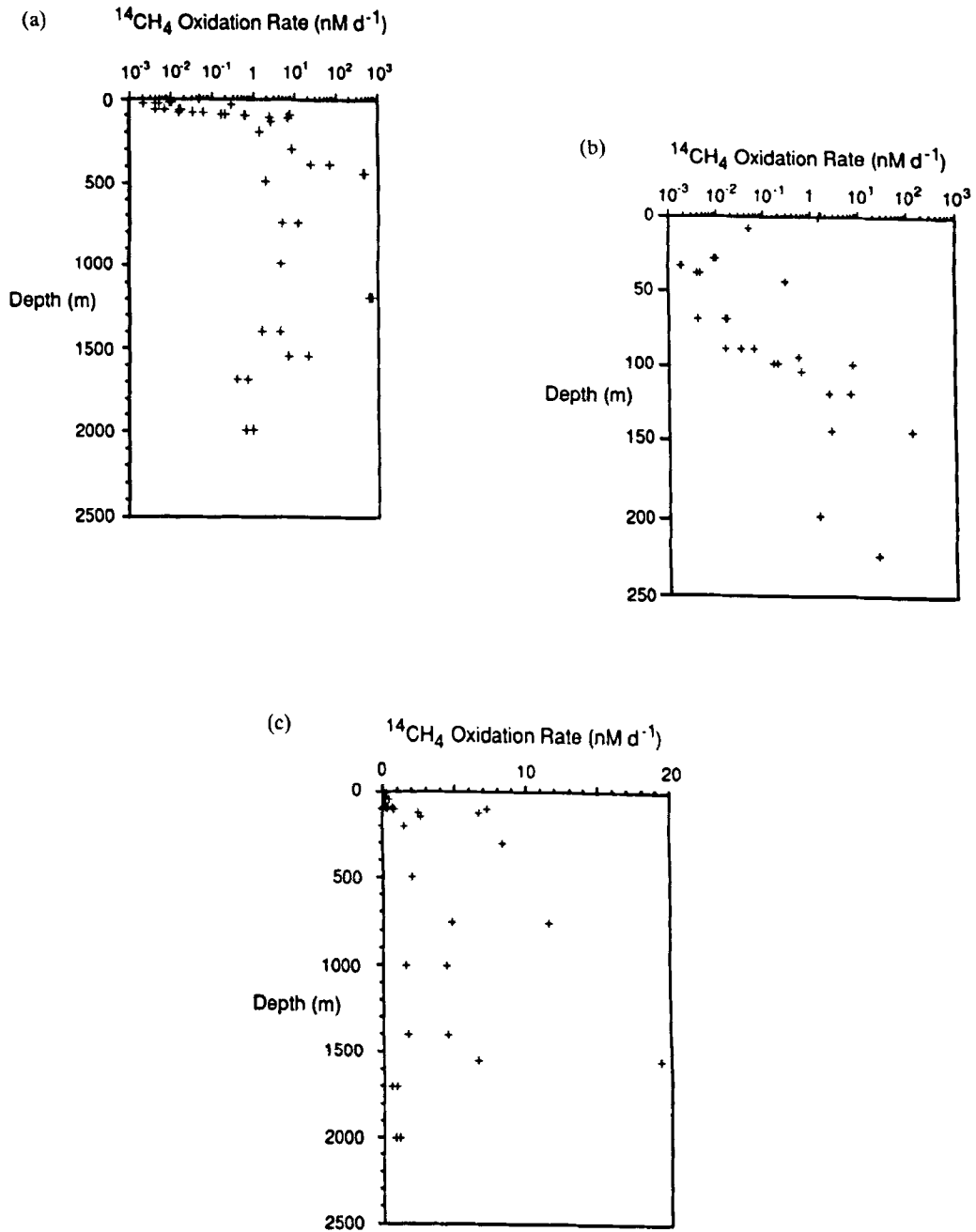
(a) $^{14}CH_4$ Oxidation Rate (nM d^{-1})

(b) $^{14}CH_4$ Oxidation Rate (nM d^{-1})

(c) $^{14}CH_4$ Oxidation Rate (nM d^{-1})

Fig. 6. Black Sea water column oxidation rate measurements using $^{14}CH_4$ tracer. (a) Plot of all $^{14}CH_4$-based measurements over the entire water column. Note log rate scale. The highest rate measurements at 500 and 1200 m were replicated. (b) Expanded scale plot (log rate scale) of $^{14}CH_4$-based methane oxidation rate measurements in upper 250 m. Note that methane oxidation rates in the upper 100 m are 10^2-fold lower than those below the methane concentration increase at 100 m. (c) Plot of $^{14}CH_4$-based rate measurements (linear rate scale) excluding all rates greater than 20 nM day^{-1}.
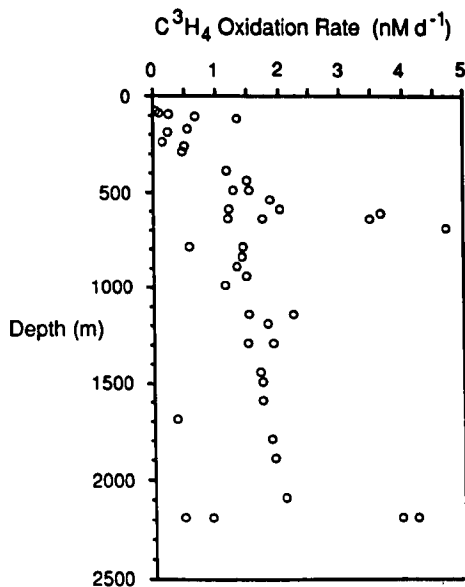
Fig. 7. Black Sea water column methane oxidation rate measurements using C^3H_4 tracer. Note linear rate scale.

range of rates, approximately 5 orders of magnitude, between the oxic and anoxic rates. The $^{14}CH_4$-based rate measurements emphasized the oxic layer and oxic/anoxic interface regions; rate measurements from the upper 250 m are plotted in Fig. 6b, again using a log rate scale to span the range of rates encountered. Methane oxidation rates in the oxic layer were low, about 10^{-3} nM d^{-1}, and increased to values of a few nanomoles per day at depths below 100 m, the depth at which the methane and H_2S concentrations begin increasing. Figure 6c excludes four points with methane oxidation rates greater than 20 nM h^{-1} and shows the same results plotted on a linear rate scale. The C^3H_4-derived water column methane oxidation rate measurements are shown in Fig. 7. These measurements were concentrated in the anoxic layer, and do not show the same range in rates as the $^{14}CH_4$-derived rates. The $^{14}CH_4$ and C^3H_4 data sets are compared in Fig. 8, which has a log oxidation rate scale.

DISCUSSION

Black Sea hydrography

Hydrographic conditions in the Black Sea are covered in detail by several papers in this issue (MURRAY et al., 1991; CODISPOTI et al., 1991; BUESSELER et al., 1991). This brief discussion highlights the relationships of the methane distributions to hydrographic features at Sta. BSK-2, which is removed from the influence of the Bosporus inflow. The main features are the oxic:anoxic interface, the depth interval influenced by entrainment and lateral mixing of Cold Intermediate Layer water (centered at about 50 m) and the Mediterranean inflow, and the benthic layer.
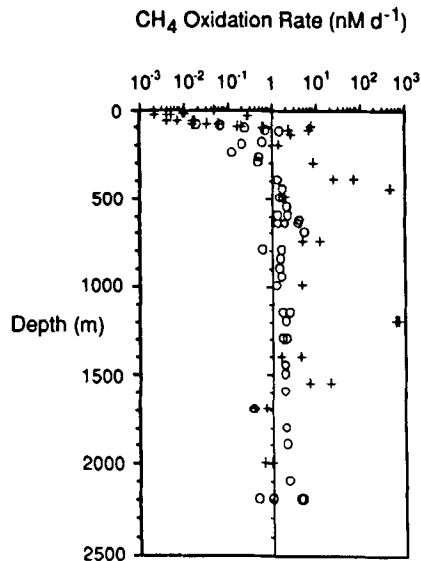
CH₄ Oxidation Rate (nM d⁻¹)

CH$_4$ Oxidation Rate (nM d^{-1})

Fig. 8. Comparison of Black Sea water column methane oxidation rates (log rate scale) using $^{14}CH_4$ (+) and C^1H_4 (O) tracers

Small quantities (10 nM) of methane are present in the oxic and sub-oxic portions (upper 50 m; 50–100 m) of the water column. Methane concentrations increase, as do H_2S and NH_4^+ concentrations (FRIEDERICH *et al.*, 1990), at 100–110 m. No unusual overlaps are evident. Methane concentrations reach 11 μM at depths of 550–600 m and are fairly uniform at 11 μM from this depth to the bottom. Scatter greater than analytical precision is evident in the methane profile from 550 to 1700 m and could be an entrainment-related feature. Variations in Bosporus inflow and entrainment appear to influence the water column from 200 m to as deep as 1700 m. Distributions of ^{14}C and tritium in the Black Sea water column suggest different rates and depths of deep water renewal. ÖSTLUND (1974) reported uniform radiocarbon ages of 1000 y for Black Sea waters between 300 and 1700 m and ages of 2000 y for waters below 2000 m. ÖSTLUND and DYRSSEN (1986) indicate that waters below 2000 m have an apparent age of 2000 y, and that the water age gradually decreases to 800 y at 300 m. TOP and CLARKE (1983) reported that tritium decreased to below detection at 900 m and increased to above zero in samples from 1745 and 1939 m. The benthic layer is identified by a density step at about 1750 m (MURRAY *et al.*, 1991), and has uniform properties.

Rate measurements

The agreement between models and measurements using $^{14}CH_4$ in measurements of anaerobic methane oxidation in sediments (ALPERIN and REEBURGH, 1984) indicates that this tracer gives reliable results with high (mM) methane concentration systems. Since $^{14}CH_4$ can be prepared with a maximum specific activity of ~60 mCi mmol^{-1}, use of this tracer in water column methane oxidation rate measurements involves tracer additions

that frequently exceed the ambient water column pool size (nM to μM levels). Corrections based on further kinetic experiments are needed to calculate *in situ* rates. Tritiated methane has a much higher theoretical specific activity (86 Ci mmol^{-1}) and was developed as a tracer here in an attempt to avoid exceeding ambient pool sizes.

The use of C^3H_4 raises several questions that must be addressed. First, tritium-labelled compounds are notorious isotope exchangers, and it is necessary to insure that rapid hydrogen isotope exchange between methane and water does not occur. Studies on hydrogen isotopes in natural methane from a variety of environments (WHITICAR *et al.*, 1986; WHITICAR and FABER, 1985) show that differences in hydrogen isotope ratios in methane and formation waters are preserved over geologic time, indicating that hydrogen isotope exchange between the tracer C^3H_4 and surrounding water should cause no problems in short-term tracer experiments. Second, it is difficult to avoid ^3H_2O contamination of the tracer C^3H_4. We took the following measures to limit ^3H_2O contamination and its effects. We avoided use of ^3H_2O in the synthesis of C^3H_4. Tritium-labeled methane can be prepared using *Methanobacterium thermoautotrophicum*, the same organism used to prepare $^{14}CH_4$, by substituting ^3H_2O for water in the medium (DANIELS and ZEIKUS, 1983; DANIELS *et al.*, 1980). However, to obtain high C^3H_4 specific activities, milliliter quantities of high specific activity ^3H_2O must be present; avoiding ^3H_2O contamination is almost impossible. We reduced ^3H_2O contamination by using a different approach in preparation of C^3H_4. We used a transiently starved co-culture of methanogens to deaminate [methyl-^3H] methylamine hydrochloride (SANDBECK and REEBURGH, 1989). The much smaller quantities of ^3H_2O present result only from oxidation of the methyl-amine, and were further decreased by equilibration with tritium-free water. We accounted for the remaining ^3H_2O contamination by performing a killed control experiment with each rate measurement.

Comparison of C^3H_4 *and* $^{14}CH_4$ *rate measurements.* Both water column methane oxidation rate distributions show an increase in rate across the oxic:anoxic interface. Both data sets show substantial variability, ranging from 2 (C^3H_4) to 5 ($^{14}CH_4$) orders of magnitude. A large part of this variability results from the very low rates in the upper 100 m. The most obvious difference in the two data sets is the presence of several very high rate values in the $^{14}CH_4$ data. Since these high rate values were replicated (two measurements at 500 m, three at 1200 m), it is difficult to exclude these high values as artifacts. No simultaneous $^{14}CH_4$ and C^3H_4 rate measurements were made on the same water samples, so we are unable to assess the degree of agreement or disagreement between the two data sets directly.

There is no obvious explanation for the variability, but the most likely possibility is related to the location of Sta. BSK-2 and possible changes in water characteristics during leg 5. Station BSK-2 is located between two main gyres which dominate Black Sea surface circulation (ZENKEVICH, 1963). The main gyres break up into smaller, more complex gyres in the boundary region. High-resolution water column profiles (CODISPOTI *et al.*, 1991) and distributions of ^{134}Cs and ^{137}Cs resulting from the 1986 Chernobyl power station accident (BUESSELER *et al.*, 1991) indicate that isopycnal mixing leads to relatively rapid horizontal transport, with interleaving layers bearing different chemical signatures. This interleaving is particularly evident in comparisons of pump profiles at Sta. BSK-2 over periods of days and weeks (CODISPOTI *et al.*, 1991; FRIEDERICH *et al.*, 1990). These two factors, complex circulation patterns between the gyres and horizontal mixing, may combine to introduce

temporal variability into our data, which were collected on independent casts during a period of over a week. The relatively small sample sizes used in the rate measurements could also contribute to the scatter. Both sets of rate measurements were made on samples smaller than $200 \, cm^3$. Clearly, larger samples have a higher probability of containing methane oxidizers. However, this argument is not supported by the observed replication of the high $^{14}CH_4$ oxidation rates.

Inspection of Fig. 8 shows that the bulk of our rate measurements lie within a band covering approximately a factor of two. The mean ($\pm 1\sigma$) of the C^3H_4 rate measurements ($n = 42$) is $1.56 \pm 1.07 \, nM \, day^{-1}$. Excluding measurements in the surface 100 m and values exceeding $20 \, nM \, day^{-1}$, the mean of the $^{14}CH_4$ rate measurements ($n = 20$) is $3.96 \pm 4.65 \, nM \, day^{-1}$. The overall average ($n = 62$) is $2.33 \pm 2.97 \, nM \, day^{-1}$. The accuracy of these rate measurements rests on the determination of tracer specific activity. Although specific activities were determined by different methods, it seems unlikely that systematic errors larger than the precision are possible. Given the number of manipulations and the potential for random errors, the agreement between the $^{14}CH_4$ and C^3H_4 data sets is remarkably good. Despite the absence of a true comparison on the same samples, the grouping of the two independent rate data sets suggests there are no major systematic errors in the two rate determinations, and permits use of a single rate term in a methane budget.

Methane budget

Several observations suggest that conditions necessary for use of a steady-state vertical advection–diffusion model (CRAIG, 1969) for interpreting these results are not met in the Black Sea water column. The oxic:anoxic interface in the Black Sea has shoaled since the last studies in 1967 (MURRAY *et al.*, 1989). Continuous CTD data collected during the 1988 Black Sea expedition show no linear T–S mixing zone (MURRAY *et al.*, 1991), as previously observed with discrete samples in the ~100–400 m depth interval during the 1969 R.V. *Atlantis II* expedition (SPENCER and BREWER, 1971). Distributions of ^{14}C and tritium in the Black Sea water column suggest different rates and depths of deep water renewal, possibly the result of variations in entrainment and injection depths (MURRAY *et al.*, 1991). MURRAY *et al.* (1991) have identified a benthic layer below a density step at 1750 m. Finally, the sediment methane profiles indicate that the sediments undergo a transition from methane source to methane sink at some depth along the continental slope, complicating the methane source:sink relationships.

We compiled a simple methane budget for Black Sea waters to gain perspective on the magnitudes of the various source and sink terms. We assume Black Sea waters are a homogeneous reservoir and consider the following methane sinks: evasion to the atmosphere, water column oxidation, sediment consumption and losses at the Bosporus. We consider sediments within the anoxic zone and underlying high productivity margins of the Black Sea, sediments from 100 m (the base of the anoxic zone) to 1500 m (the base of the continental slope), as the methane source. Area:volume relationships are taken from fig. 3 of DEUSER (1974). Each of the budget terms is discussed below; a summary is shown in Table 1.

Evasion at the air:sea interface. Wind velocity data (EROL IZDAR, personal communication) for the central Black Sea indicate winds ranging between 2 and 5 $m \, s^{-1}$ for 40–50%

Table 1. *Black Sea methane budget*

Sinks	mol CH_4 y^{-1}
Evasion at air:sea interface rate: 9.7 mmol m^{-2} y^{-1} extent: 4.23×10^{11} m^2	4.1×10^9
Water column oxidation Upper 100 m (aerobic/anaerobic) rate: 0.36 nM y^{-1} extent: 5.3×10^{16} l	1.9×10^7
Below 100 m (anaerobic) rate: $0.6\,\mu$M y^{-1} extent: 4.8×10^{17} l	2.9×10^{11}
Oxidation by abyssal sediments rate: 0.1 mmol m^{-2} y^{-1} extent: 2.3×10^{11} m^2	2.3×10^7
Outflow at Bosporus rate: 1.9×10^{14} l y^{-1} extent: 10 nM	1.9×10^6
Source	mol CH_4 y^{-1}
Shelf/slope sediment production (100–1500 m) extent: 1.9×10^{11} m^2 rate: 1.53 mol m^{-2} y^{-1}	2.9×10^{11}

of each month. Winds ranging between 0 and 5 m s^{-1} occur during 50–75% of each month. We used the following equation (HARTMAN and HAMMOND, 1985) to estimate the air–sea methane flux:

$$\text{Flux} = K_L(C_{\text{measured}} - C_{\text{equilibrium}}),$$

where K_L is the gas transfer coefficient (3.8 m day^{-1} at a wind speed of 8.5 m s^{-1}), and C_{measured} and $C_{\text{equilibrium}}$ are measured and equilibrium methane concentrations (Fig. 3c). Evasion of methane from the Black Sea is estimated to occur at a rate of 9.7 mmol m^{-2} y^{-1}. The area of the Black Sea is 4.23×10^{11} m^2, and the calculated methane loss is 4.1×10^9 mol y^{-1}.

Water column consumption. We used a methane oxidation rate of 1.6 nM day^{-1} (0.6 μM y^{-1}) as representative of methane oxidation in the Black Sea water column. We consider that anaerobic methane oxidation is active at depths below 100 m. Water column oxidation occurs over 90% of the Black Sea volume (4.8×10^{17} l) and represents a sink of 2.9×10^{11} mol y^{-1}. This is the dominant term in the Black Sea methane budget. Methane oxidation in the Black Sea surface 100 m layer is a negligible term (1.9×10^7 mol y^{-1}) in this budget because of the low methane oxidation rates (10^{-1} to 10^{-3} nM day^{-1}) and the small volume (5.3×10^{16} l) of the oxic layer.

Sediment consumption. The sediment methane oxidation rate measurements (Fig. 5c) were graphically integrated with depth to obtain an estimate of the magnitude of this sink.

The average of the two sediment rate distributions is $100\,\mu$mol m^{-2} y^{-1}. The methane flux into the sediments (Fig. 5b) averages $550\,\mu$mol m^{-2} y^{-1}. This sink consumes 2.3×10^7 to 1.3×10^8 mol y^{-1} when applied to the Black Sea area below depths of 1500 m (55%, 2.3×10^{11} m^2).

Losses at the Bosporus. Outflow at the Bosporus is estimated to be 190 km^3 y^{-1} (1.9×10^{14} l y^{-1}) (SERPOIANU, 1985). We assume that the methane concentration of the Bosporus outflow is 10 nM (Fig. 3), so the Bosporus outflow represents a low of 1.9×10^6 mol y^{-1}.

Methane source. We consider that sediments below the oxic:anoxic interface and above the continental slope–abyssal plain transition, 1500 m, are the source of Black Sea water column methane. This depth interval covers 45% of the Black Sea area (1.9×10^{11} m^2). A source of 2.9×10^{11} mol y^{-1}, equivalent to the dominant sink term (water column consumption), requires an average flux of 1.5 mol m^{-2} y^{-1}. The methane flux calculated from the methane distributions in the only core available for this estimate (BS5-1), is 0.2 mol m^{-2} y^{-1}. For comparison, SCRANTON's (1988) time-dependent model for the Cariaco Trench requires a sediment methane flux of 0.125–0.175 mol m^{-2} y^{-1} to maintain the observed water column distribution. The high carbon deposition sediments of the northwest shelf and Danube fan are expected to be important sources of Black Sea water column methane.

We assume that water column methane production is not an important term in the Black Sea methane budget. Studies on methane in anoxic marine sediments (MARTENS and BERNER, 1974; CRILL and MARTENS, 1983) show that low-sulfate, high organic carbon conditions accompany high rates of methanogenesis. Laboratory studies (ABRAM and NEDWELL, 1978; THOMPSON *et al.*, 1983) suggest that sulfate-reducing bacteria are able to outcompete methanogens for both hydrogen and acetate, resulting in a spatial separation of sulfate reduction and methanogenesis. These sediment observations suggest that large-scale water column methane production is unlikely. A methane maximum located at the base of the mixed layer is a common open-ocean feature (BURKE *et al.*, 1983) that appears to result from reactions accompanying grazing. The location and magnitude of this process cannot account for anoxic basin methane concentrations. Anoxic sediments must be the major methane source.

Methane oxidation or production (2.9×10^{11} mol y^{-1}) divided by the Black Sea area (4.23×10^{11} m^2) results in a basin-wide rate of 0.7 mol m^{-2} y^{-1} or 0.5 g C m^{-2} y^{-1}. Primary production is known to vary widely, but overall estimates (in g C m^{-2} y^{-1}) for the Black Sea have been made by SOROKIN (1964), 109 ave, SHIMKUS and TRIMONIS (1974), 71–118, and GLENN and ARTHUR (1985), 90–270. DEUSER (1970) suggests that Sorokin's estimate is best overall, so the quantity of carbon participating in the Black Sea methane cycle is equivalent to approximately 0.5% of the primary production.

Black Sea:Cariaco Trench comparison. There are several striking differences in Black Sea and Cariaco Trench methane distributions. The Cariaco Trench has a surface mixed layer methane maximum (WARD *et al.*, 1987), which was not observed at the Black Sea station emphasized in this study. The methane distributions in the anoxic portions of the water columns differ, reflecting differences in circulation and sediment methane sources. Methane distributions in the Cariaco Trench show a linear methane increase with depth to

Table 2. *Methane residence times in the Cariaco Trench and Black Sea*

	Cariaco Trench		Black Sea
A. *Methane concentration (μM)*			
	Increasing to 7 (WIESENBURG, 1975)		Surface 100 m: 10^{-2}
	Increasing to 12.5 (WARD et al., 1987)		Below 500 m: 11
B. *Methane consumption rate (μM y^{-1})*			
modeled:	0.0011–0.0153 (REEBURGH, 1976)		0.015 (SCRANTON, 1977)
	0.15–0.3 (SCRANTON, 1988)		
measured	0.0129–0.160 (WARD et al., 1987)		Surface 100 m: 0.36×10^{-3}
			Below 100 m: 0.6
C. *Methane residence time (y)**			
	(3.5 μM)	(6.25 μM)	
modeled: (Reeburgh rates)	228–3200	400–5700	73
(Scranton rates)	23–40	21–40	
measured: (Ward et al. rates)	22–480		3.6–18
D. *Water residence time (y)*	1900		900 (deep)
			2000 (bottom) (ÖSTLUND, 1974)
			500 (MURRAY et al., 1991)

*Cariaco Trench methane residence times calculated using average water column concentrations from A (3.5 μM, 6.25 μM) and modeled and measured rates from B.

values of 7–12.5 μM, while Black Sea methane distributions increase to a value of 11 μM at 500 m and are uniform below that. Entrainment associated with the Mediterranean inflow to the Black Sea (MURRAY et al., 1991), injection of this water at a range of depths, and rapid lateral mixing (LIVINGSTON et al., 1990) decrease Black Sea deep water residence times and homogenize distributions. Similar processes are apparently less important in the Cariaco Trench, but HOLMEN and ROOTH (1990) point out that episodic injection of warm hypersaline shelf waters is required to explain tritium observations there. Incomplete information is available regarding the sediments as methane sources for both environments. This study shows that shelf/slope sediments can be sources and that abyssal sediments are capable of consuming water column methane. This situation probably results from more direct deposition of primary production on the Black Sea shelf. The source/sink crossover depth is not known, but is presumed to occur at some depth on the basin slope. Sediment methane measurements are only available in the deep basins of the Cariaco Trench. REEBURGH'S (1976) vertical advection–diffusion model treatment of the Cariaco Trench methane distributions considered that deep basin sediments were the methane source, while SCRANTON'S (1988) time-dependent model treatment considers sediments at all depths to be a uniform methane source. Clearly, a better understanding of methane sources in both environments is needed. Transects from shelves to deep basins involving determination of sediment methane fluxes from depth distributions and oxidation rates are needed in both environments to clarify methane sources.

Residence or turnover times for methane (concentration/consumption rate) based on model results and recent rate measurements in the Cariaco Trench and the Black Sea are given in Table 2. Since Cariaco Trench water column methane distributions are not uniform, mean concentrations reflecting average conditions (3.5, 6.25 μM) in the WIESENBURG (1975) and WARD et al. (1987) studies were used. These concentrations were divided

by the modeled and measured methane oxidation rates from the REEBURGH (1976), SCRANTON (1988) and WARD *et al.* (1987) studies to obtain the residence time values shown in section C of Table 2. The recent oxidation rate measurements indicate that the residence time for methane is much shorter in anoxic basins than previously believed, much shorter than water replacement times. Although large quantities of methane are present in anoxic waters and sediments, methane is effectively recycled within the ocean by anaerobic oxidation in anoxic sediments and water columns and by aerobic oxidation in oxic waters. Methane is emerging as a small but very active participant in the carbon cycle, even in anoxic environments. The ocean is a small atmospheric methane source because of this activity.

The notion that anoxic basins are long-term steady state systems has been questioned recently for deep waters of the Cariaco Trench (SCRANTON, 1988; SCRANTON *et al.*, 1987) and for surface waters of the Black Sea (MURRAY *et al.*, 1989). Possible variations in entrainment (MURRAY *et al.*, 1991) and rapid lateral mixing (BUESSELER *et al.*, 1991) suggest that a large depth interval of the Black Sea water column may be subject to episodic additions and mixing, further eroding the steady-state assumption. HOLMEN and ROOTH (1990) point out that enclosed deep basins are extremely sensitive to externally forced bottom ventilation. More information on ventilation and mixing processes is needed to reconcile our observed uniform water column methane concentrations and oxidation rates (which imply rapid mixing and uniform sources) with sediment methane sources and water column mixing and renewal processes that appear to decrease toward the Black Sea bottom. A major question is how such uniform methane distributions and oxidation rates are maintained in the deepest parts of the Black Sea.

CONCLUSIONS

(1) A detailed methane distribution was measured at a central station in the Black Sea. Methane concentration was uniform at 10 nM in a 100 m surface layer, increased to 11 μM at 550 m, and remained uniform to the bottom. Methane distributions in slope and abyssal sediments indicate that shelf/slope sediments are water column methane sources. Methane distributions and rate measurements in abyssal sediments indicate they can be a methane sink.

(2) Independent radiotracer techniques based on C^3H_4 and $^{14}CH_4$ tracers were used to measure methane oxidation rates in the Black Sea water column. Results from the two techniques agree within a factor of two for the bulk of the measurements. Methane oxidation rates were relatively uniform at 0.6 μM y^{-1} in the anoxic Black Sea water column. Methane oxidation rates in the upper 100 m were 10^2-fold lower than those in anoxic waters.

(3) A simple methane budget for the Black Sea was compiled from the above measurements. The budget balances evasion at the air:sea interface, water column consumption, consumption by abyssal sediments and outflow at the Bosporous against input from shelf and slope sediments. Anaerobic methane oxidation is the dominant methane sink in the Black Sea, and is 10^2-fold larger than the next sink term, evasion at the air:sea interface.

(4) The methane oxidation rate measurements suggest a much shorter residence time for CH$_4$ in anoxic basins than previously believed. Additional work is needed to reconcile the uniform methane distributions and uniform oxidation rate distributions, which

indicate short methane residence times, with source functions and mixing processes that appear to decrease with depth.

Future work

A set of water column methane samples was collected for stable isotope (δ^2H–CH_4 and δ^{13}C–CH_4) analysis. Since isotope fractionation accompanying anaerobic oxidation is large (ALPERIN *et al.*, 1988), results from these analyses should shed light on the extent of water column methane oxidation, methane sources, vertical and lateral mixing rates, and possible methane production in the water column. A series of cross-shelf/slope transects involving measurements of sediment methane distributions and oxidation rates is needed to identify the CH_4 source : sink crossover point and quantify the sediment source term.

REFERENCES

ABRAM J. W. and D. B. NEDWELL (1978) Inhibition of methanogenesis by sulphate reducing bacteria competing for transferred hydrogen. *Archiv für Microbiologie*, **117**, 89–92.
ALPERIN M. J. (1988) The carbon cycle in an anoxic marine sediment: concentrations, rates, isotope ratios and diagenetic models. Ph.D. dissertation, University of Alaska–Fairbanks, 241 pp.
ALPERIN M. J. and W. S. REEBURGH (1984) Geochemical observations supporting anaerobic methane oxidation. In: *Microbial growth on C-1 compounds*, R. L. CRAWFORD and R. S. HANSON, editors, American Society for Microbiology, pp. 282–289.
ALPERIN M. J. and W. S. REEBURGH (1985) Inhibition experiments on anaerobic methane oxidation. *Applied and Environmental Microbiology*, **50**, 940–945.
ALPERIN M. J., W. S. REEBURGH and M. J. WHITICAR (1988) Carbon and hydrogen isotope fractionation resulting from anaerobic methane oxidation. *Global Biogeochemical Cycles*, **2**, 279–288.
ANTHONY C. (1982) *The biochemistry of methylotrophs*. Academic Press, London.
ATKINSON L. P. and F. A. RICHARDS (1967) The occurrence and distribution of methane in the marine environment. *Deep-Sea Research*, **14**, 673–684.
BLAKE D. R. and F. S. ROWLAND (1988) Continuing worldwide increase in tropospheric methane, 1978 to 1987. *Science*, **239**, 1129–1131.
BUESSELER K. O., H. D. LIVINGSTON and S. A. CASSO (1991) Mixing between oxic and anoxic waters of the Black Sea as traced by Chernobyl cesium isotopes. *Deep-Sea Research*, **38** (Suppl.), S725–S745.
BURKE R. L. JR, D. F. REID, J. M. BROOKS and D. LAVOIE (1983) Upper water column methane geochemistry in the eastern tropical north Pacific. *Limnology and Oceanography*, **28**, 19–32.
BULLISTER J. L. (1984) Atmospheric chlorofluoromethanes as tracers of ocean circulation and mixing: measurement and calibration techniques and studies in the Greenland and Norwegian Seas. Ph.D. dissertation, University of California, San Diego, 172 pp.
CICERONE R. J. and R. S. OREMLAND (1988) Biogeochemical aspects of atmospheric methane. *Global Biogeochemical Cycles*, **2**, 299–327.

CODISPOTI L. A., G. E. FRIEDERICH, J. W. MURRAY and C. SAKAMOTO (1991) Chemical variability in the Black Sea: Implications of data obtained with a continuous vertical profiling system that penetrated the oxic/anoxic interface. *Deep-Sea Research*, **38** (Suppl.), S691–S710.

CRAIG H. (1969) Abyssal carbon and radiocarbon in the Pacific. *Journal of Geophysical Research*, **74**, 5491–5506.

CRILL P. M. and C. S. MARTENS (1983) Spatial and temporal fluctuations of methane production in anoxic coastal marine sediments. *Limnology and Oceanography*, **28**, 1117–1130.

DANIELS L. and J. G. ZEIKUS (1983) Convenient biological preparation of pure high specific activity [14] C-labelled methane. *Journal of Labelled Compounds and Radiopharmaceuticals*, **20**, 17–24.

DANIELS L., G. FULTON, R. W. SPENCER and W. H. ORME-JOHNSON (1980) Origin of hydrogen in methane produced by *Methanobacterium thermoautotrophicum*. *Journal of Bacteriology*, **141**, 694–698.

DEUSER W. G. (1970) Organic carbon budget of the Black Sea. *Deep-Sea Research*, **18**, 995–1004.

DEUSER W. G. (1974) Evolution of anoxic conditions in the Black Sea during Holocene. In: *The Black Sea—geology, chemistry and biology*, E. T. DEGENS and D. A. ROSS, editors, American Association of Petroleum Geologists, Memoir 20, pp. 133–136.

FRIEDERICH G. E., L. A. CODISPOTI and C. A. SAKAMOTO (1990) Bottle and pumpcast data from the 1988 Black Sea expedition. Technical Report No. 90-3, Monterey Bay Aquarium Research Institute, Pacific Grove, CA.

GLENN C. R. and M. A. ARTHUR (1985) Sedimentary and geochemical indicators of productivity and oxygen contents in modern and ancient basins: the Holocene Black Sea as the "type" anoxic basin. *Chemical Geology*, **48**, 325–354.

HARTMAN B. and D. E. HAMMOND (1985) Gas exchange in San Francisco Bay. *Hydrobiologia*, **129**, 59–68.

HENRICHS S. M. and W. S. REEBURGH (1987) Anaerobic mineralization of marine sediment organic matter: Rates and the role of anaerobic processes in the oceanic carbon economy. *Geomicrobiological Journal*, **5**, 191–237.

HOLMEN K. J. and C. G. H. ROOTH (1990) Ventilation of the Cariaco Trench, a case of multiple source competition? *Deep-Sea Research*, **37**, 208–225.

HUNT J. M. (1974) Hydrocarbon geochemistry of Black Sea. In: *The Black Sea—geology, chemistry and biology*, E. T. DEGENS and D. A. ROSS, editors, American Association of Petroleum Geologists, Memoir 20, pp. 499–504.

HUNT J. M. and J. K. WHELAN (1978) Dissolved gases in Black Sea sediments. *Initial Reports of the Deep Sea Drilling Project*, **42**, 661–665.

IUPAC (1987) Solubility Data Series, Methane, Vol. 27/28, H. L. CLEVER and C. L. YOUNG, volume editors, Pergamon Press, Oxford.

KHALIL M. A. K. and R. A. RASMUSSEN (1983) Sources, sinks and seasonal cycles of atmospheric methane. *Journal of Geophysical Research*, **88**, 5131–5144.

LIVINGSTON H. D., K. O. BUESSELER and S. A. CASSO (1990) Mixing of oxic and anoxic waters of the Black Sea. *Eos*, **71**, 139.

MARTENS C. S. and R. A. BERNER (1974) Methane production in the interstitial waters of sulfate-depleted marine sediments. *Science*, **185**, 1067–1069.

MURRAY J. W., H. W. JANNASCH, S. HONJO, R. F. ANDERSON, W. S. REEBURGH, Z. TOP, G. E. FRIEDERICH, L. A. CODISPOTI and E. IZDAR (1989) Unexpected changes in the oxic/anoxic interface in the Black Sea. *Nature*, **338**, 411–413.

MURRAY J. W., Z. TOP and E. OZSOY (1991) Temperature and salinity distributions in the Black Sea. *Deep-Sea Research*, **38** (Suppl.), S663–S689.

ÖSTLUND H. G. (1974) Expedition "Odysseus 65": Radiocarbon age of Black Sea water. In: *The Black Sea—geology, chemistry and biology*, E. T. DEGENS and D. A. ROSS, editors, American Association of Petroleum Geologists, Memoir 20, pp. 127–132.

ÖSTLUND H. G. and D. DYRSSEN (1986) Renewal rates of Black Sea water. In: *The chemical and physical oceanography of the Black Sea, Reports of the Chemistry of the Sea* XXXIII, University of Göteborg, Göteborg, Sweden.

REEBURGH W. S. (1976) Methane consumption in Cariaco Trench waters and sediments. *Earth and Planetary Science Letters*, **28**, 337–344.

REEBURGH W. S. (1980) Anaerobic methane oxidation: rate depth distributions in Skan Bay sediments. *Earth and Planetary Science Letters*, **47**, 345–352.

REEBURGH W. S. (1983) Rates of biogeochemical processes in anoxic sediments. *Annual Review of Earth and Planetary Sciences*, 11, 269–298.

REEBURGH W. S. (1989) Interaction of sulphur and carbon cycles in marine sediments. In: *Evolution of the global biogeochemical sulphur cycle*, P. BRIMBLECOMBE and A. YU. LEIN, editors, SCOPE 39, Wiley, New York, pp. 125–159.

RUDD J. W. M. and R. D. HAMILTON (1975a) Measurement of microbial oxidation of methane in lake water. *Limnology and Oceanography*, 19, 519–524.

RUDD J. W. M. and R. D. HAMILTON (1975b) Factors controlling rates of methane oxidation and the distribution of methane oxidizers in a small stratified lake. *Archive für Hydrobiologie*, 75, 522–538.

SANDBECK K. A. and W. S. REEBURGH (1989) Microbiological preparation of ^{3}H-labelled methane. *Journal of Labelled Compounds and Radiopharmaceuticals*, 27, 1285–1291.

SCRANTON M. I. (1977) The marine geochemistry of methane. Ph.D. thesis, W.H.O.I./M.I.T. Joint Program, Woods Hole, 251 pp.

SCRANTON M. I. (1988) Temporal variations in the methane content of the Cariaco Trench. *Deep-Sea Research*, 35, 1511–1523.

SCRANTON M. I., F. L. SAYLES, M. P. BACON and P. G. BREWER (1987) Temporal changes in the chemistry and hydrography of the Cariaco Trench. *Deep-Sea Research*, 34, 945–963.

SERPOIANU G. (1985) Les exchanges d'leau par le Bosphore. *Oceanologica Acta*, 8, 373–378.

SHIMKUS K. M. and E. S. TRIMONIS (1974) Modern sedimentation in Black Sea. In: *The Black Sea—geology, chemistry and biology*, E. T. DEGENS and D. A. ROSS, editors, American Association of Petroleum Geologists, Memoir 20, pp. 249–278.

SIEBURTH M. McN., P. W. JOHNSON, M. A. EBERHARDT, M. E. SIERACKI, M. LIDSTROM and D. LAUX (1987) The first methane-oxidizing bacterium from the upper mixed layer of the deep ocean: *Methylomonas pelagica* sp. nov. *Current Microbiology*, 14, 285–293.

SOROKIN Y. I. (1964) On the primary production and bacterial activities in the Black Sea. *Journal du Conseil, Conseil permanent International pour l'exploration de la Mer*, 29, 41–60.

SPENCER D. W. and P. G. BREWER (1971) Vertical advection–diffusion and redox potentials as controls on the distribution of manganese and other trace metals in waters of the Black Sea. *Journal of Geophysical Research*, 76, 5877–5892.

STEELE L. P., P. J. FRASER, R. A. RASMUSSEN, M. A. K. KHALIL, T. J. CONWAY, A. J. CRAWFORD, R. H. GAMMON, K. A. MASARIE and K. W. THONING (1987) The global distribution of methane in the troposphere. *Journal of Atmospheric Chemistry*, 5, 125–171.

SWINNERTON J. W., V. J. LINNENBOM and C. H. CHEEK (1962) Determination of dissolved gases in aqueous solutions by gas chromatography. *Analytical Chemistry*, 34, 483–485.

THOMPSON L. A., D. B. NEDWELL, M. T. BALBA, I. M. BANAT and E. SENIOR (1983) The use of multiple-vessel, open flow systems to investigate carbon flow in anaerobic microbial communities. *Microbial Ecology*, 9, 189–199.

TOP Z. and W. B. CLARKE (1983) Helium, neon and tritium in the Black Sea. *Journal of Marine Research*, 41, 1–7.

WARD B. B. (1987) Kinetic studies on ammonia and methane oxidation by *Nitrosococcus oceanus*. *Archiv für Microbiologie*, 147, 126–133.

WARD B. B., K. A. KILPATRICK, P. C. NOVELLI and M. I. SCRANTON (1987) Methane oxidation and methane fluxes in the ocean surface layer and deep anoxic waters. *Nature*, 327, 226–229.

WARD B. B., K. A. KILPATRICK, A. E. WOPAT, E. C. MINNICH and M. E. LIDSTROM (1989) Methane oxidation in Saanich Inlet during summer stratification. *Continental Shelf Research*, 9, 65–75.

WHITICAR M. J. and E. FABER (1985) Methane oxidation in sediment and water column environments—isotope evidence. *Organic Geochemistry*, 10, 759–786.

WHITICAR M. J., E. FABER and M. SCHOELL (1985) Biogenic methane formation in marine and freshwater environments: CO_2 versus acetate fermentation—isotope evidence. *Geochimica et Cosmochimica Acta*, 50, 693–709.

WIESENBURG D. A. (1975) Processes controlling the distribution of methane in the Cariaco Trench, Venezuela. M.S. thesis, Old Dominion University, 106 pp.

WOELLER F. H. (1961) Liquid scintillation counting of $^{14}CO_2$ with phenethylamine. *Analytical Biochemistry*, 2, 508–511.

YAMAMOTO S., J. B. ALCAUSKAS and T. E. CROZIER (1976) Solubility of methane in distilled water and seawater. *Journal of Chemical Engineering Data*, 21, 78–81.

YEFREMOVA A. G. and B. P. ZHIZHCHENKO (1974) Occurrence of crystal hydrates of gases in the sediments of modern marine basins. *Dokl. Akad. Nauk. S.S.S.R. Earth Sci. Sect.*, **214**, 219–220.

ZEHNDER A. J. B., B. HUSER and T. D. BROCK (1979) Measuring radioactive methane with the liquid scintillation counter. *Applied and Environmental Microbiology*, **37**, 897–899.

ZENKEVICH L. A. (1963) *Biology of the seas of the U.S.S.R.* Interscience, New York.