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Water column methane oxidation adjacent to an area of active hydrate dissociation, Eel River Basin

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Abstract—The role of methane clathrate hydrates in the global methane budget is poorly understood because little is known about how much methane from decomposing hydrates actually reaches the atmosphere. In an attempt to quantify the role of water column methane oxidation (microbial methane oxidation) as a control on methane release, we measured water column methane profiles (concentration and δ13C) and oxidation rates at eight stations in an area of active methane venting in the Eel River Basin, off the coast of northern California. The oxidation rate measurements were made with tracer additions of 3H-CH4.

Small numbers of instantaneous rate measurements are difficult to interpret in a dynamic, advecting coastal environment, but combined with the concentration and stable isotope measurements, they do offer insights into the importance of methanotrophy as a control on methane release. Fractional oxidation rates ranged from 0.2 to 0.4% of ambient methane per day in the deep water (depths >370 m), where methane concentration was high (20–300 nM), to near-undetectable rates in the upper portion of the water column (depths <370 m), where methane concentration was low (3–10 nM). Methane turnover time averaged 1.5 yr in the deep water but was on the order of decades in the upper portion of the water column. The depth-integrated water column methane oxidation rates for the deep water averaged 5.2 mmol CH4 m-2 yr-1, whereas the upper portion of the water column averaged only 0.14 mmol CH4 m-2 yr-1; the depth-integrated oxidation rate for deep water in the 25-km² area encompassing the venting field averaged 2 × 10^6 g CH4 yr-1. Stable isotope values (δ13C-CH4) for individual samples ranged from ~34 to ~52‰ (vs. PDB, Peedee belemnite standard) in the region. These values are isotopically enriched relative to hydrates in the region (δ13C-CH4 about −57 to −69‰), further supporting our observations of extensive methane oxidation in this environment.

1. INTRODUCTION

Methane (CH4) is a radiatively important trace gas in the atmosphere, and its concentration has increased dramatically because of anthropogenic activity. Past changes in CH4 concentration from glacial to interglacial epochs have contributed to the idea that CH4 may play an important role in modulating climate (Cicerone and Oremland, 1988). The oceans constitute a vast CH4 reservoir, mainly in the form of CH4 hydrates, although they are thought to play only a small role in the modern CH4 cycle (Cicerone and Oremland, 1988). In addition, little is known about the magnitude and fate of CH4 emissions from continuous venting systems (Hovland et al., 1993). However, evidence suggests that massive releases of oceanic CH4 may have played an important role in past climate change (Katz et al., 1999; Hesselbo et al., 2000; Kennett et al., 2000), leading to questions about the role of oceanic CH4 in future climate change.

Methane clathrate hydrates (hydrates) are solid, nonstoichiometric compounds formed from CH4 and water under conditions of low temperature and high pressure. Methane hydrates are only stable under limited conditions and are thought to be especially abundant in sediments along the world’s continental margins (Kvenvolden et al., 1993). The magnitude of the CH4 hydrate reservoir is estimated at up to 5 × 10^18 g (Collett and Kuuskraa, 1998), some of which may become destabilized in the event of ocean warming (MacDonald, 1990). The atmospheric CH4 budget includes hydrates as a source of CH4 to the atmosphere (5–10 Tg yr-1), but the actual contribution is not known, and the value is considered to be a placeholder (Cicerone and Oremland, 1988; Reeburgh, 1996). Because of the magnitude and potential instability of the hydrate reservoir, consideration of hydrate decomposition is important for predicting future atmospheric levels of CH4 as well as for understanding past changes.

Hydrate stability is governed by the chemical potential, and decomposition is driven primarily by changes in temperature, pressure, and methane supply (Dickens and Quinby-Hunt, 1994). Once CH4 is released from the hydrate matrix, it may be transported from the sediment to the water column as a dissolved gas by diffusion through the sediments (Alperin, 1989) or through advection of pore water (Linke et al., 1994); if pore waters become supersaturated, CH4 may also be transported by active bubbling and venting. Methane, entering the water column as bubbles, dissolves into the water within a few hundred meters of the sediment surface (Guinasso and Schink, 1973), except perhaps in the case of massive release by slumping events. Methane dissolved in the deeper water column remains in solution until the water mass equilibrates with the atmosphere.

Methanotrophy in sediments and waters is the primary control on release of hydrate-derived CH4 to the atmosphere.
Anaerobic CH₄ oxidation (also referred to sulfate-dependent CH₄ oxidation) is an important CH₄ removal process (Alperin and Reeburgh, 1984) and has been observed around hydrate-bearing regions in diffusion-dominated sediments (Kastner et al., 1998; Borowski et al., 1999; Whiticar et al., 1995; Valente et al., unpublished data), in advective seeps (Boetius et al., 2000), and in active vents (Hinrichs et al., 1999). Anaerobic CH₄ oxidation modulates the flux of CH₄ from sediment to the water column, quantitatively consuming CH₄ in most diffusion-dominated sediments (Reeburgh et al., 1993), and playing an important (but poorly quantified) role in seeps and vents. Methane that escapes the sediments and enters the water column is further subject to oxidation by aerobic methanotrophs in the waters surrounding dissociating hydrates (de Angelis et al., 1999; Suess et al., 1999). However, no studies have directly analyzed CH₄ oxidation in the water column overlying decomposing CH₄ hydrates. This article reports CH₄ oxidation above decomposing CH₄ hydrates in the Eel River Basin, off the coast of northern California.

2. MATERIALS AND METHODS

2.1. Study Site and Sampling

All samples were taken during an August 1999 cruise of the R/V *Melville* to the Eel River Basin, over the continental margin off the coast of northern California (Fig. 1). The cruise was part of a two-ship expedition run jointly between Scripps Institution of Oceanography and the Monterey Bay Aquarium Research Institute. Sampling focused on areas of active CH₄ venting identified with the ROV *Ventana*. Active CH₄ venting occurs in the region at the P-T phase boundary of CH₄ hydrate stability (520–530 m) and is probably indicative of active hydrate dissociation (Dickens and Quinby-Hunt, 1994). Two additional sites were also studied: a deep submarine canyon near active venting sites, and a station well removed from the vent area intended as a control. The control station was located further westward (~24 km) off the coast at 900-m water depth. Figure 2 presents temperature, salinity, and oxygen distributions typical of the stations occupied.

Water samples were collected at various depths in 30-L Niskin bottles attached to a Conductivity-Temperature-Depth (CTD)/Rosette. Four 125-mL crimp-top sample bottles were filled directly from each Niskin bottle. All sample bottles were flushed with two volumes of water, and care was taken to ensure that all were free of bubbles. After being capped with butyl rubber stoppers and crimped, the bottles were separated; one bottle was immediately transferred into a light-free incubator for rate studies, and the second bottle was used for CH₄ concentration analyses. The third bottle was set aside for shore-based CH₄ concentration analyses and the fourth bottle for δ¹³C-CH₄ analyses. All samples for shore-based analyses were treated with 0.1 mL of saturated mercuric chloride. Additional samples were taken for killed controls, duplicate rate measurements, and extended incubations. Additional samples were also taken for analysis of oxygen and other chemical components.

2.2. Analytical Methods

2.2.1. Concentration Analyses

Methane concentration analyses were performed with a gas chromatograph equipped with a flame ionization detector (GC 8A, Shimadzu Corp.). Measurements of water column CH₄ were performed at sea by introducing a 10-mL headspace into the 125-mL sample bottle by displacement with ultrahigh-purity N₂, vigorously shaking, equilibrating for 12 h, and analyzing the headspace in two 5-mL aliquots. Samples for shore-based CH₄ concentration analyses and for stable isotope analyses of δ¹³C-CH₄ were collected in a similar manner but were poisoned with 0.1 mL of saturated mercuric chloride. The concentration analyses were performed at Scripps Institution of Oceanography, and the δ¹³C-CH₄ analyses were performed in the isotope biogeochemistry laboratory at the University of Hawaii by use of the technique of Popp et al. (1995). Acetate measurements associated with the tracer synthesis were performed with high-pressure liquid chromatography (LC-600, Shimadzu Corp.) with an organic acids column (Alltech, I0A-1000) and an ultraviolet detector (SPD-6AV, Shimadzu Corp.) set at 210 nm. The mobile phase consisted of 0.5 mM H₂SO₄ set...
at a flow rate of 0.6 mL min⁻¹; a 0.2-mL sample loop was utilized. All radioactive samples were counted with a Beckman LS-3801 liquid scintillation counter employing H-factor quench correction. Cytoscan ES scintillation cocktail (ICN Inc.) was used for all counting.

### 2.2.2. Oxidation Rate Measurements

Water column CH₄ oxidation rate measurements were performed aboard ship by adding ³H-CH₄ to the fresh water samples, incubating samples in the dark for 24 h, removing the unreacted ³H-CH₄, and counting the ³H₂O product. Methane oxidation in the water column is a microbially mediated process in which oxygen acts as the oxidant (Eqn. 1).

\[
C\text{H}_4 + 2O_2 \rightarrow CO_2 + 2H_2O
\]

Sample bottles were removed from the incubator individually for addition of the tracer. The septum was first pierced with a needle to allow for displacement of water as the ³H-CH₄ tracer was added. A small bubble (100 μL, 9.5 μCi) of the ³H-CH₄ gas mixture was then added by syringe. The bottles were then shaken vigorously for 30 s to equilibrate ³H-CH₄ with the liquid phase. Calculations show that at equilibrium, >97% of CH₄ is in the liquid phase (Yamamoto et al., 1976). Sample bottles were then returned to the incubator; the entire label-addition process took less than 3 min. Samples were incubated for ~24 h in the dark near in situ temperature (4–7°C). After the incubations were complete, the caps were removed from the sample bottles, and 30 mL of liquid was removed. The sample bottles were sparged with He gas (~200 mL min⁻¹) for 30 min to remove residual ³H-CH₄, leaving the oxidation product (³H₂O) behind. Samples (1–4 mL) were taken from the bottles for preliminary shipboard liquid scintillation counting, and the remainder of each sample was stored for more precise measurement on shore. Methane oxidation rates (turnover rates, \(r_{CH_4}\)) were calculated in a fashion similar to Reeburgh et al. (1991) by multiplying the fraction of tritium-labeled CH₄ oxidized per unit time (fractional turnover rate, \(k\)) by the ambient CH₄ concentration (\(\text{CH}_4\)) assuming first-order kinetics (Eqn. 2). A minor correction (∼3%) was applied to the fractional turnover rate to account for methane remaining in the bubble during the incubation, and no corrections were applied for isotopic fractionation against tritium because this value is not known for the system.

\[
r_{CH_4} = k \times \text{CH}_4
\]

A detailed review of rate measurements has previously been published (Reeburgh, 1983). Oxidation rates were assumed to be constant during the 1-d incubation period, and a seawater blank was used for the initial equilibrium, \(C_i\), of the ³H-CH₄ gas mixture was then counted the ³H-H₂O product. Methane oxidation in the water column was calculated as the reciprocal of the fractional turnover rate times 0.7 to 50 yr and are only valid if the water mass remains in the area for long time periods and does not mix, which is unlikely.

### 2.3. Preparation and Use of Tritium-Labeled Methane

#### 2.3.1. Synthesis of ³H-CH₄

Tritium-labeled CH₄ used in this study was synthesized in the laboratory before being transported to the ship for use. Tritium-labeled CH₄ has previously been synthesized by transiently starving enrichment cultures of methyloptic methanogens and feeding them tritium-labeled trimethylamine (Sandbeck and Reeburgh, 1989). A new technique was developed for this study because tritium-labeled trimethylamine was not commercially available, and the original enrichment culture was lost. This technique uses a culture of the acetate utilizing methanogen, Methanosaeta thermophila strain CALS-1, to ferment (methyl)tritium-labeled acetate to CH₄ (Eqn. 3).

\[
C\text{H}_3\text{COOH} \rightarrow C\text{H}_4 + CO_2
\]

### Methanosaeta thermophila (DSMZ 3870) is an obligately acetoclastic, thermophilic methanogen (Zinder et al., 1987). A culture was obtained from S. Zinder (Cornell University) and was grown without agitation at 61°C and pH 6.6 by means of the technique of Hungate (1969). The culture media contained the following components (per liter): 0.5 g NH₄Cl, 0.4 g K₂HPO₄, 0.1 g MgCl₂·6H₂O, 0.05 g CaCl₂·2H₂O, 1.0 mg resazurin, 0.5 g NaHCO₃, 0.1 g coenzyme M, 0.04 mg biotin, 0.2 g Na₂S·9H₂O, 1.6 g NaCH₃CO₂, 5.0 mg sodium ethylenediaminetetraacetic acid dihydrate, 1.5 mg CoCl₂·6H₂O, 1.0 mg MnCl₂·4H₂O, 1.0 mg FeSO₄·7H₂O, 1.0 mg Na₂S·9H₂O, 1.0 mg K₂HPO₄, 0.1 mg AlCl₃·6H₂O, 0.3 mg NaCl, 0.5 mg Na₂CO₃, 0.2 mg CuCl₂·2H₂O, 0.2 mg NIST reagent 6H₂O, 0.1 mg H₂SeO₃, 0.1 mg H₂BO₃, and 0.1 mg NaMoO₄·2H₂O.

For the ³H-CH₄ synthesis, 8 mL of inoculum from the early exponential phase of growth was transferred into a custom-made (15 mL), centrifugeable, glass culture vial. Growth was monitored by analysis of headspace CH₄. When CH₄ production ceased, the culture was centrifuged (7300 g, 3 h) and the supernatant was decanted, leaving a pellet of cells in the culture bottle. The supernatant was removed and analyzed by high-pressure liquid chromatography; acetate levels were close to the detection limit of ~20 μM. The culture bottle containing the cell pellet was repeatedly purged with N₂ and CO₂ to remove CH₄. The cell pellet was resuspended in 2.6 mL of acetate-free culture media. Tritium-labeled acetate (ICN Inc., 2.6 mg of the 15°C material dissolved in 100 μL water instead of ethanol) was made anoxic by repeatedly purging the headspace with N₂ followed by the addition of 100 μL of a reducing solution (5 mM sodium sulfide and 5 mM cysteine). Immediately before transfer, a 1.0-mL syringe was filled with a 5 mM cysteine solution and the contents expelled before the syringe was used to transfer the tritium-labeled acetate into the culture bottle. The culture containing the tritium-labeled acetate was incubated at 61°C for 70 h to ensure that the majority of acetate was converted to CH₄. Liquid samples (20 μL) were removed from the culture bottle each day to follow the conversion of tritium-labeled acetate to product.

After the incubation was completed, the gas phase was transferred to a water-free vial for storage. A displacement technique involving two syringes was used to transfer the ³H-CH₄ from the culture bottle containing the tritium-labeled acetate into the culture bottle. A sodium hydroxide solution (1 mL at 1.0 mol/L) was first added to remove gas-phase CO₂. Additional sodium hydroxide solution was added into the culture bottle with one syringe while the gas headspace was simultaneously removed with another syringe. After all of the gas from the headspace had been transferred into the syringe, the syringe was withdrawn from the bottle and the gas immediately transferred through a Ag₂O/CaSO₄ trap (to remove water and carbon monoxide) and into an evacuated serum vial. The Ag₂O/CaSO₄ trap was made by removing the plunger from a 1.0-mL glass syringe and packing the barrel with silver (I) oxide and anhydrous calcium sulfate. The tip of the plunger was removed and packed into the top of the syringe for use as a septum. The trap was evacuated and the tip of the needle heated to the stopper of the evacuated serum vial containing ³H-CH₄ was pierced through the septum of the trap; then the needle of the trap was forced through the stopper of the evacuated serum vial; the vacuum in the serum vial drew the ³H-CH₄ through the trap into the vial. After all gas had been transferred out of the syringe, the needle was removed from the septum of the chemical trap and replaced with another syringe containing N₂ gas. The chemical trap was flushed with 1.0 mL of N₂ gas to ensure that all the label was transferred into the serum vial; then the chemical trap was separated from the serum vial. The serum vial was inverted, and 2.5 mL of liquid mercury was added to create a seal between the headspace and the rubber stopper.

The specific activity of ³H-CH₄ was determined in the laboratory by independently determining ³H-CH₄ activity and CH₄ concentration. A combustion line consisting of a CuO-filled column (maintained at 1000°C in a tube furnace) followed by a series of two water-filled Harvey traps was used to combust 20-μL samples of the ³H-CH₄ containing gas mixture and to trap the ³H₂O product. The gas samples were injected through a septum upstream of the combustion line, and compressed zero air was used as the carrier (20 mL min⁻¹). The length of tubing between the combustion tube and the Harvey traps was held for 30 min with a heat gun (Master Appliance Corp.) after combustion to ensure that the ³H₂O was transferred to the traps. More than 99% of the recovered label was located in the first trap, and controls showed that little residual ³H₂O remained in the system.
after 30 min. The concentration of $^{13}$CH$_4$ was determined by diluting 100 μL of $^{13}$CH$_4$ gas into 3.0 mL of ultrahigh-purity N$_2$ gas, measuring the resulting mixing ratio of CH$_4$ by gas chromatography (with a flame ionization detector), and back-calculating the original CH$_4$ concentration.

The synthesis utilized 2.6 mCi of tritium-labeled acetate with a specific activity of 15 Ci mmol$^{-1}$. The synthesis yielded 2 mCi of $^3$H-$\text{CH}_4$ (77.5% conversion) with a specific activity of 2.92 Ci mmol$^{-1}$. These yields are similar to those of Sandbeck and Reeburgh (1989), and a similar dilution in specific activity was observed in both cases. The cause of the dilution in specific activity is not clear.

2.3.2. Use of $^3$H-$\text{CH}_4$

The use of $M$. thermophila for $^3$H-$\text{CH}_4$ synthesis is preferable to the use of other aceticlastic methanogens, and this strain has also been used to synthesize high-purity $^{13}$C-$\text{CH}_4$ (Miller et al., 1999). $M$. thermophila has a rapid growth rate (12-h doubling time), which allows synthesis to proceed more rapidly than isotope exchange. The culture also produces little hydrogen (H$_2$) as a metabolic byproduct (Zinder and Anuguish, 1992), avoiding any problems with H$_2$ oxidation confusing the CH$_4$ oxidation rate measurements. $M$. thermophila is also capable of utilizing acetate more completely (10–20 μM threshold) than other aceticlastic methanogens (Min and Zinder, 1989), which allows the synthesis to be performed with small quantities of radioactivity, but at high specific activity. This synthesis technique does not require the use of an anaerobic chamber.

Previous studies of CH$_4$ oxidation in the water column have successfully utilized $^{13}$CH$_4$ in areas with high CH$_4$ concentrations (Reeburgh et al., 1991; de Angelis et al., 1993; Ward et al., 1987). However, $^3$H-$\text{CH}_4$ is preferable to $^{13}$CH$_4$ for CH$_4$ oxidation rate studies in low-CH$_4$ waters. The specific activity of $^3$H-$\text{CH}_4$ can be almost 2000-fold higher than $^{13}$C-$\text{CH}_4$, which allows tracer-level measurements of CH$_4$ oxidation rates at water column concentrations. If the same activity of $^{13}$C-$\text{CH}_4$ was used in these studies (9.5 μCi, 60 mCi mmol$^{-1}$), the CH$_4$ concentration in the incubation bottles would increase by 1200 nM, overwhelming the ambient CH$_4$ pool and introducing a major potential artifact. The $^3$H-$\text{CH}_4$ added to the incubation vials (9.5 μCi, 2.92 Ci mmol$^{-1}$) increased the CH$_4$ concentration by <25 nM. Another advantage of $^3$H-$\text{CH}_4$ is that the oxidation product is $^3$H-H$_2$O, not CO$_2$ gas. By use of $^3$H-$\text{CH}_4$, the aqueous sample can be directly counted after unreacted CH$_4$ has been stripped from the sample.

The synthesis and use of $^3$H-$\text{CH}_4$ also has disadvantages compared with $^{13}$C-$\text{CH}_4$. The reactant, tritium-labeled acetate, readily undergoes isotope exchange in water, and the synthesis must be performed within a few days of forming the aqueous solution. We have also had problems with long-term storage of the $^3$H-$\text{CH}_4$. We have noticed an increased aqueous background in stock solutions stored for >1 yr. This is not surprising, as radioactive decays in multiply-labeled methane have the potential to create other chemical forms. Isotope exchange between $^3$H-$\text{CH}_4$ and ambient water is unlikely (Whiticar et al., 1986). Any $^3$H-$\text{CH}_4$ stored for more than a few months should be transferred through a desiccant into a new storage vial before use.

3. RESULTS

Results of several controlled-rate measurements are shown in Table 1, along with their equivalent rate measurement. Six rate measurements performed in bottles amended with 0.1 mL of saturated mercuric chloride (killed controls) show lower rates than the equivalent live samples. One of the killed controls from station 3 did show significant conversion of tritium-labeled CH$_4$ to the aqueous phase. This conversion is unlikely to be caused by isotope exchange between CH$_4$ and H$_2$O because isotope exchange is not observed even on geologic timescales (Whiticar et al., 1986). It is more feasible that this conversion may be due to enzymatic or microbial activity not inhibited by the mercuric chloride. Seven rate measurements performed as duplicates yield similar rates to the equivalent rate measurement, although two of the duplicates differ by a factor of ~2. Two measurements performed with 48-h incubation times also yield similar rates to the equivalent 24-h rate measurements.

| Station | Depth (m) | CH$_4$ Ox rate (nm yr$^{-1}$) | Control Ox rate (nm yr$^{-1}$) | Comments |
|---------|-----------|------------------------------|------------------------------|----------|
| 1       | 522       | 27                           | 67                           | Duplicate |
| 2       | 519       | 45                           | 46                           | Duplicate |
| 3       | 452       | 21                           | 0                            | + HgCl$_2$ |
| 4       | 437       | 26                           | 0                            | + HgCl$_2$ |
| 5       | 555       | 273                          | 193                          | Duplicate |
| 6       | 555       | 273                          | 55                           | + HgCl$_2$ |
| 7       | 437       | 26                           | 0                            | + HgCl$_2$ |
| 8       | 539       | 22                           | 45                           | Duplicate |
| 9       | 539       | 22                           | 19                           | 48-h incubation |
| 10      | 437       | 0.7                          | 0.3                          | + HgCl$_2$ |
| 11      | 555       | 273                          | 193                          | Duplicate |
| 12      | 539       | 22                           | 2.8                          | + HgCl$_2$ |
| 13      | 437       | 0.7                          | 0.4                          | 48-h incubation |
| 14      | 539       | 0.7                          | 0.3                          | + HgCl$_2$ |

$^a$ CH$_4$ Ox Rate signifies the experimentally determined oxidation rate of methane.

$^b$ Control Ox rate gives the oxidation rate determined from a control experiment.

$^c$ Four types of control experiments were employed: 1) duplicate incubations in which two samples were treated identically, 2) killed controls in which two samples were treated identically except that mercuric chloride (HgCl$_2$) was added to one before addition of the $^3$H-$\text{CH}_4$, 3) extended incubations in which two samples were treated identically except that the incubation period for one was increased to 48 h, and 4) zero time measurements conducted in the laboratory before and after the cruise in which the incubation was stopped immediately after $^3$H-$\text{CH}_4$ addition (as a check on $^3$H-$\text{CH}_4$ purity).

Depth profiles of CH$_4$ concentration, CH$_4$ oxidation rate, and $^{13}$C-$\text{CH}_4$ are shown in Figure 3 for each of eight stations occupied. Methane concentrations are generally low in the upper 370 m of the water column, though an expected subsurface maximum is frequently observed (Burke et al., 1983; Cynar and Yayanos, 1992; Ward, 1992). The $^{13}$C-$\text{CH}_4$ in the upper portion of the water column shows a strong atmospheric influence (atmospheric $^{13}$C-$\text{CH}_4 = -47‰). Maximum CH$_4$ concentrations are observed in samples around 500 m depth; this depth is coincident with both the upper limit of hydrate stability for the region, and with the CH$_4$ vents observed by the ROV Ventana.

Measured oxidation rates correlate well with CH$_4$ concentrations in the deep samples and are very low in the upper portion of the water column. The data show two types of samples: shallow samples (<370 m), with typically low methane concentrations and oxidation rates, and deep samples (>370 m), with higher CH$_4$ concentrations and elevated rates of methane oxidation.

The first six stations are located near vents and show similar distributions of CH$_4$ concentration and oxidation rate. Large spikes in CH$_4$ concentration are observed at depth; smooth CH$_4$ distributions are not expected at these sites because of the direct venting of CH$_4$ bubbles into the water column. Station 11 is located in a canyon nearby to one of the observed vents and displays a CH$_4$ maximum and $^{13}$C-$\text{CH}_4$ minimum in the same depth range as the first six stations. Station 12 is located farther
off the shelf (900-m water depth) and was chosen as a control station because it is outside of the venting field. However, this station shows similar depth distributions of CH₄ concentration, oxidation rate, and δ¹³C-CH₄ to the other stations, although the depth is shifted down by ~200 m.

Results from station 12 indicate a benthic CH₄ source at depths within the range of hydrate stability. The CH₄ concentration and δ¹³C profiles for this station appear similar to the other stations, and the observed methane maximum at 700 m corresponds to the observed isotopic minimum. The methane oxidation rates also appear similar to the other stations, supporting the presumption that methane oxidation in the water column occurs independently of the nature of the CH₄ source. The CH₄ observed at station 12 may originate upslope and advect laterally to station 12. The source of the CH₄ may be advecting pore fluids that are in equilibrium with methane.

Fig. 3. Water column depth profiles of CH₄ concentration (□), CH₄ oxidation rate (○), and δ¹³C-CH₄ (△) for each of eight stations occupied. The number of each station is given in the upper left corner of each panel. Oxidation rate measurements were also performed with several killed controls (×), each representing the duplicate of a sample from the same depth.
hydrate, or pore fluids that are undersaturated in CH$_4$ relative to hydrate formation. The observations made at station 12 indicate that the observed distribution of CH$_4$ oxidation is not dependent on active hydrate dissociation and may be a more general phenomenon along the continental margins.

Integrated water column CH$_4$ oxidation rates and burdens are shown in Table 2. The total burden of CH$_4$ in the water column is heavily influenced by CH$_4$ residing in the surface waters, whereas the depth-integrated oxidation rate is dominated by oxidation in deeper water. Considering only the deep water (depth >370 m) at all the stations excluding station 12, the average CH$_4$ burden is 7.6 mmol CH$_4$ m$^{-2}$, whereas the average integrated oxidation rate is 5.2 mmol CH$_4$ m$^{-2}$ yr$^{-1}$. This leads to a turnover time estimate of 1.5 yr for deep water in the area.

4. DISCUSSION

4.1. CH$_4$ Oxidation

The goal of this study was to quantify the role of water column methanotrophy as a control on CH$_4$ release to the atmosphere. However, the source strength of methane seeps and vents is not known for this region. In addition, the rate, concentration, and isotope measurements made during this study are all instantaneous measurements made in a dynamic area. However, these data allow us to estimate several important factors directly relating to this goal including: the quantity of CH$_4$ oxidized adjacent the area of active CH$_4$ venting; the turnover time of CH$_4$ with respect to oxidation; and the extent of CH$_4$ oxidation for individual samples.

4.2. Integrated Oxidation Rates

The integration of oxidation rate measurements with depth allows us to consider this process at a regional level. The integrated oxidation rates for each station are of similar magnitude (Table 2) and are indicative of substantial methanotrophic activity in the Eel River Basin. These observations may also be representative of other similar settings. Assuming a constant CH$_4$ supply, the integrated oxidation rates at each station should remain relatively constant over time. By averaging the integrated oxidation rates (Table 2), the overall amount of deep-water CH$_4$ oxidation for the 25-km$^2$ area encompassing the venting field can be estimated at 2 $\times$ 10$^5$ g CH$_4$ yr$^{-1}$. This value may eventually be compared to estimates of CH$_4$ sources in the region to further elucidate the relationship between CH$_4$ venting and water column CH$_4$ oxidation.

4.3. Turnover Times

Calculation of CH$_4$ turnover time is a useful tool to describe methanotrophic activity in marine waters. However, there are caveats associated with these calculations. One critical assumption is that oxidation will continue with first-order kinetics as the CH$_4$ plume mixes with low-CH$_4$ waters, an assumption neither supported nor refuted by our data. More accurately, these calculations yield “instantaneous turnover times,” and potential changes in rate should be considered carefully when interpreting the results.

The results shown in Table 2 and Figure 4 demonstrate that the turnover time of CH$_4$ is relatively constant (~1.5 yr) in deep samples with high CH$_4$ concentrations, but that the turnover time is much longer (decades) in shallow samples with low CH$_4$ concentrations. The difference in turnover time between these two types of samples clearly indicates that methanotrophic bacteria are active in areas that contain high CH$_4$ and further indicates that active populations have not developed in areas characterized by low CH$_4$ concentrations. Although the deep waters may mix with low-CH$_4$ waters before most of the CH$_4$ is oxidized, the turnover time may be useful in conjunction with the hydrography to determine the extent of methane oxidation. One important observation made in this study is that the CH$_4$ turnover time in coastal areas with active CH$_4$ venting (~1.5 yr) is much shorter than turnover times (~50 yr) re-
CH$_4$ oxidation over decomposing hydrates

The CH$_4$ emitted from sediments in the Eel River Basin has a minimum around the upper limit of the hydrate stability zone at 370 m. Hydrates in the region have previously been found with 13C-CH$_4$ of about $-50$ to $-60\%$ (unpublished data). Hydrates in the region have previously been found with 13C-CH$_4$ of $-57.6$ to $-69.1\%$ (Brooks et al., 1991). The isotopic fractionation ($\alpha$) factor for aerobic methane oxidation as determined for a variety of terrestrial systems is $\sim 1.025$ (Reeburgh, 1996; Reeburgh et al., 1997; Liptay et al., 1998; Snover and Quay, 2000). A closed-system calculation that used a sediment source 13C-CH$_4$ of $-55\%$ and an isotope fractionation ($\alpha$) factor of 1.025 requires an oxidation of $\sim 45\%$ of CH$_4$ to produce a sample with the observed 13C-CH$_4$ of $-40\%$.

The 13C-CH$_4$ can also be considered in conjunction with the concentration and rate data to describe patterns of CH$_4$ release and oxidation around venting fields. Results shown in Figure 3 demonstrate a clear CH$_4$ maximum and 13C-CH$_4$ minimum around the upper limit of the hydrate stability zone at six of the stations occupied. One implication of this observation is that isotopically light, hydrate-derived CH$_4$ is being injected into the water column from vents and seeps around this depth. The recently injected CH$_4$ is isotopically light relative to methane elsewhere in the water column and is inferred to have undergone little oxidation. Conversely, CH$_4$ located above and below this depth appears to have undergone substantially more oxidation. Methane oxidation rates in the “new plumes” are relatively high, providing evidence that methanotrophic activity occurs soon after CH$_4$ release from the sediment.

4.5. CH$_4$ Transport

The extent of CH$_4$ oxidation adjacent to areas of hydrate decomposition is controlled by both physical and biological processes. The hydrography of such regions is complex and is characterized by extensive mixing and advection. In this study, only vertical profiles are considered. This approach constitutes a snapshot of the system because it considers only a limited area and does not consider the fate of CH$_4$ as it mixes away from the source region. Because no CH$_4$ bubbles appear to reach the atmosphere from the sediments of the Eel River Basin, the eventual fate of CH$_4$ is either oxidation or ventilation (controlled by mixing). The turnover time of CH$_4$ with respect to oxidation is $\sim 1.5$ yr, but mixing will act to dilute CH$_4$ and may also increase the turnover time. Without more knowledge of the methane source distribution, the hydrography, or both, it is not currently feasible to estimate the fraction that survives oxidation and is transported to the atmosphere away from the source region.

One important factor that is not addressed in this study is the threshold concentration below which methanotrophs are unable to consume CH$_4$. Determining such a threshold is the key to understanding the eventual fate of dissolved CH$_4$ that undergoes significant mixing and is not rapidly ventilated to the atmosphere. In pure cultures of methanotrophs, CH$_4$ thresholds tend to be high ($\geq 10$ nM CH$_4$), but CH$_4$ oxidation in nature is known to occur at much lower levels ($\geq 0.6$ nM CH$_4$) (Hanson and Hanson, 1996). It is unclear whether the active methanotrophic populations that accompany high CH$_4$ will continue to consume CH$_4$ once the CH$_4$ (and the microbial population) have mixed with low-CH$_4$ waters. However, evidence from the deep (low CH$_4$) waters of the North Sea and the Atlantic Ocean indicates that CH$_4$ is consumed even at low concentration, with a turnover time of $\sim 50$ yr (Scranton and Brewer, 1978; Rehder et al., 1999).

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