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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 methanotrophy (microbial methane oxidation) as a control on methane release, we measured water column methane profiles (concentration and $\delta^{13}\text{C}$) 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 $^3\text{H}\text{-CH}_4$.

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 $5.2 \text{ mmol CH}_4 \text{ m}^{-2} \text{ yr}^{-1}$, whereas the upper portion of the water column averaged only $0.14 \text{ mmol CH}_4 \text{ m}^{-2} \text{ yr}^{-1}$; the depth-integrated oxidation rate for deep water in the 25-km^2 area encompassing the venting field averaged $2 \times 10^6 \text{ g CH}_4 \text{ yr}^{-1}$. Stable isotope values ($\delta^{13}\text{C}\text{-CH}_4$) 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 ($\delta^{13}\text{C}\text{-CH}_4$ about -57 to -69‰), further supporting our observations of extensive methane oxidation in this environment. Copyright © 2001 Elsevier Science Ltd

1. INTRODUCTION

Methane (CH_4) is a radiatively important trace gas in the atmosphere, and its concentration has increased dramatically because of anthropogenic activity. Past changes in CH_4 concentration from glacial to interglacials have contributed to the idea that CH_4 may play an important role in modulating climate (Cicerone and Oremland, 1988). The oceans constitute a vast CH_4 reservoir, mainly in the form of CH_4 hydrates, although they are thought to play only a small role in the modern CH_4 cycle (Cicerone and Oremland, 1988). In addition, little is known about the magnitude and fate of CH_4 emissions from continuous venting systems (Hovland et al., 1993). However, evidence suggests that massive releases of oceanic CH_4 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 CH_4 in future climate change.

Methane clathrate hydrates (hydrates) are solid, nonstoichiometric compounds formed from CH_4 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 CH_4

hydrate reservoir is estimated at up to $5 \times 10^{18} \text{ g}$ (Collett and Kuuskraa, 1998), some of which may become destabilized in the event of ocean warming (MacDonald, 1990). The atmospheric CH_4 budget includes hydrates as a source of CH_4 to the atmosphere ($5\text{--}10 \text{ Tg yr}^{-1}$), 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 CH_4 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 CH_4 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, CH_4 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 CH_4 to the atmosphere.

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Fig. 1. Map of the study region.

Anaerobic CH_4 oxidation (also referred to sulfate-dependent CH_4 oxidation) is an important CH_4 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; Valentine et al., unpublished data), in advective seeps (Boetius et al., 2000), and in active vents (Hinrichs et al., 1999). Anaerobic CH_4 oxidation modulates the flux of CH_4 from sediment to the water column, quantitatively consuming CH_4 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 CH_4 oxidation in the water column overlying decomposing CH_4 hydrates. This article reports CH_4 oxidation above decomposing CH_4 hydrates in the Eel River Basin, off the coast of northern California. A combination of techniques were utilized, including direct oxidation rate measurements with tritium-labeled methane ($^3\text{H}-\text{CH}_4$), as well as depth profiles of CH_4 concentration and ^{13}C isotope composition. A new method to synthesize tritium-labeled CH_4 is also described.

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

Fig. 2. Temperature ($\square$), salinity ($\circ$), and oxygen (Δ) measured at station 1, typical of conditions at all stations occupied.

expedition run jointly between Scripps Institution of Oceanography and the Monterey Bay Aquarium Research Institute. Sampling focused on areas of active CH_4 venting identified with the ROV *Ventana*. Active CH_4 venting occurs in the region at the P-T phase boundary of CH_4 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 CH_4 concentration analyses. The third bottle was set aside for shore-based CH_4 concentration analyses and the fourth bottle for $\delta^{13}\text{C}-\text{CH}_4$ analyses. All samples for shore-based analyses were treated with 0.1 mL saturated HgCl_2 . 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 CH_4 were performed at sea by introducing a 10-mL headspace into the 125-mL sample bottle by displacement with ultrahigh-purity N_2 , vigorously shaking, equilibrating for 12 h, and analyzing the headspace in two 5-mL aliquots. Samples for shore-based CH_4 concentration analyses and for stable isotope analyses of $\delta^{13}\text{C}-\text{CH}_4$ 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 $\delta^{13}\text{C}-\text{CH}_4$ 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, IOA-1000) and an ultraviolet detector (SPD-6AV, Shimadzu Corp.) set at 210 nm. The mobile phase consisted of 0.5 mM H_2SO_4 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. CytoScint 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 ³H-H₂O product. Methane oxidation in the water column is a microbially mediated process in which oxygen acts as the oxidant (Eqn. 1).

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 (³H-H₂O) 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_{ox}*) 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 ([CH₄]), 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.

$$r_{ox} = k[CH_4] \quad (2)$$

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 time point at *t* = 0. The use of a blank was supported by control experiments performed in the laboratory that demonstrated no label incorporation into the aqueous phase when the incubation was terminated within a few minutes after injecting the label.

Methane turnover time was calculated as the reciprocal of the fractional turnover rate (1/*k*), which is also equal to the concentration divided by the oxidation rate ([CH₄]/*r_{ox}*). The range of fractional turnover rates measured in this study give turnover times of 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 methylotrophic 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).

Methanosaeta thermophila (DSMZ 3870) is an obligately aceticlastic, 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 ZnCl₂, 0.4 mg AlCl₃ · 6H₂O, 0.3 mg NaWO₄ · 2H₂O, 0.2 mg CuCl₂ · 2H₂O, 0.2 mg NiSO₄ · 6H₂O, 0.1 mg H₂SeO₃, 0.1 mg H₃BO₃, 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 mCi at 15 Ci mmol⁻¹, 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 out of the original 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 seated into the stopper of the evacuated serum vial. The needle of the syringe 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 ³H-H₂O 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 heated for 30 min with a heat gun (Master Appliance Corp.) after combustion to ensure that the ³H-H₂O was transferred to the traps. More than 99% of the recovered label was located in the first trap, and controls showed that little residual ³H-H₂O remained in the system

after 30 min. The concentration of $^3\text{H-CH}_4$ was determined by diluting 100 μL of $^3\text{H-CH}_4$ 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 $^3\text{H-CH}_4$ (77% 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 $^3\text{H-CH}_4$

The use of *M. thermophila* for $^3\text{H-CH}_4$ synthesis is preferable to the use of other aceticlastic methanogens, and this strain has also been used to synthesize high-purity $^{14}\text{C-CH}_4$ (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 Anguish, 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 $^{14}\text{C-CH}_4$ in areas with high CH_4 concentrations (Reeburgh et al., 1991; de Angelis et al., 1993; Ward et al., 1987). However, $^3\text{H-CH}_4$ is preferable to $^{14}\text{C-CH}_4$ for CH_4 oxidation rate studies in low- CH_4 waters. The specific activity of $^3\text{H-CH}_4$ can be almost 2000-fold higher than $^{14}\text{C-CH}_4$, which allows tracer-level measurements of CH_4 oxidation rates at water column concentrations. If the same activity of $^{14}\text{C-CH}_4$ 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 $^3\text{H-CH}_4$ added to the incubation vials (9.5 μCi , 2.92 Ci mmol^{-1}) increased the CH_4 concentration by <25 nM. Another advantage of $^3\text{H-CH}_4$ is that the oxidation product is $^3\text{H}_2\text{O}$, not CO_2 gas. By use of $^3\text{H-CH}_4$, the aqueous sample can be directly counted after unreacted CH_4 has been stripped from the sample.

The synthesis and use of $^3\text{H-CH}_4$ also has disadvantages compared with $^{14}\text{C-CH}_4$. 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 $^3\text{H-CH}_4$. 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 $^3\text{H-CH}_4$ and ambient water is unlikely (Whiticar et al., 1986). Any $^3\text{H-CH}_4$ 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_2O because isotope exchange is not observed even on geologic time scales (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

Table 1. Results from control experiments.

Station	Depth (m)	CH_4 Ox rate (nM yr^{-1}) ^a	Control Ox rate (nM yr^{-1}) ^b	Comments ^c
1	522	27	67	Duplicate
2	519	45	46	Duplicate
	519	45	1.2	+ HgCl_2
	487	98	102	Duplicate
	462	21	0	+ HgCl_2
3	555	273	193	Duplicate
	555	273	55	+ HgCl_2
	437	26	0	+ HgCl_2
	55	1.3	0.2	Duplicate
8	539	22	45	Duplicate
	539	22	19	48-h incubation
	539	22	2.8	+ HgCl_2
	453	0.7	0	Duplicate
	453	0.7	0.4	48-h incubation
	453	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 $^3\text{H-CH}_4$, 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 $^3\text{H-CH}_4$ addition (as a check on $^3\text{H-CH}_4$ purity).

factor of ~ 2 . Two measurements performed with 48-h incubation times also yield similar rates to the equivalent 24-h rate measurements.

Depth profiles of CH_4 concentration, CH_4 oxidation rate, and $\delta^{13}\text{C-CH}_4$ 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 $\delta^{13}\text{C-CH}_4$ in the upper portion of the water column shows a strong atmospheric influence (atmospheric $\delta^{13}\text{C-CH}_4 = -47\text{‰}$). 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 $\delta^{13}\text{C-CH}_4$ minimum in the same depth range as the first six stations. Station 12 is located farther

Fig. 3. Water column depth profiles of CH₄ concentration ($\square$), CH₄ oxidation rate ($\circ$), and $\delta^{13}\text{C}$ -CH₄ (Δ) 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 ($\times$), each representing the duplicate of a sample from the same depth.

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 $\delta^{13}\text{C}$ -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 ^{13}C 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

Table 2. Depth-integrated CH₄ concentration, oxidation rate, and turnover time for each station.

Station	Depth range (m)	Latitude	Longitude	CH ₄ oxidation rate (mmol m ⁻² yr ⁻¹)	CH ₄ burden (mmol m ⁻²)	CH ₄ turnover time (yr)
1	0–370	40°47.07'N	124°35.78'W	0.3	2.1	7
	370–530			2.7	3.8	1.4
2	0–370	40°47.08'N	124°35.64'W	0.06	1.9	32
	370–524			4.0	5.4	1.4
3	0–370	40°47.08'N	124°35.34'W	0.15	2.2	15
	370–555			6.9	7.9	1.1
5	0–370	40°47.01'N	124°36.06'W	NA	NA	NA
	370–561			1.7	4.0	2.4
6	0–370	40°47.53'N	124°35.93'W	NA	NA	NA
	370–570			5.7	10.8	1.9
8	0–370	40°48.82'N	124°36.94'W	0.1	2.9	29
	370–568			8.6	7.9	0.9
11	0–370	40°50.30'N	124°37.21'W	0.1	1.1	11
	370–815			7.0	13.3	1.9
12	0–575	40°46.70'N	124°49.10'W	0.5	2.9	5.8
	575–912			5.3	9.9	1.9
Average ^a	0–370			0.14	2.0	14
	370–			5.2	7.6	1.5

^a Excludes station 12.

hydrate, or pore fluids that are undersaturated in CH₄ relative to hydrate formation. The observations made at station 12 indicate that the observed distribution of CH₄ oxidation is not dependent on active hydrate dissociation and may be a more general phenomenon along the continental margins.

Integrated water column CH₄ oxidation rates and burdens are shown in Table 2. The total burden of CH₄ in the water column is heavily influenced by CH₄ 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 CH₄ burden is 7.6 mmol CH₄ m⁻², whereas the average integrated oxidation rate is 5.2 mmol CH₄ 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. CH₄ Oxidation

The goal of this study was to quantify the role of water column methanotrophy as a control on CH₄ 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 CH₄ oxidized adjacent the area of active CH₄ venting; the turnover time of CH₄ with respect to oxidation; and the extent of CH₄ 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 methanotro-

phic activity in the Eel River Basin. These observations may also be representative of other similar settings. Assuming a constant CH₄ 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 CH₄ oxidation for the 25-km² area encompassing the venting field can be estimated at 2×10^6 g CH₄ yr⁻¹. This value may eventually be compared to estimates of CH₄ sources in the region to further elucidate the relationship between CH₄ venting and water column CH₄ oxidation.

4.3. Turnover Times

Calculation of CH₄ 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 CH₄ plume mixes with low-CH₄ 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 CH₄ is relatively constant (~1.5 yr) in deep samples with high CH₄ concentrations, but that the turnover time is much longer (decades) in shallow samples with low CH₄ concentrations. The difference in turnover time between these two types of samples clearly indicates that methanotrophic bacteria are active in areas that contain high CH₄ and further indicates that active populations have not developed in areas characterized by low CH₄ concentrations. Although the deep waters may mix with low-CH₄ waters before most of the CH₄ 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 CH₄ turnover time in coastal areas with active CH₄ venting (~1.5 yr) is much shorter than turnover times (~50 yr) re-

Fig. 4. Aggregate plot of methane concentration vs. turnover time for seven similar stations (1, 2, 3, 5, 6, 8, and 11). Samples are differentiated as deep ($\circ$, depths >370 m) and shallow ($\square$, depths <370 m). Turnover time is defined as the CH₄ concentration divided by the oxidation rate. The following three deep samples are off the scale of the graph: 1) CH₄ = 243 nM, turnover time = 0.9 yr; 2) CH₄ = 243 nM, turnover time = 1.3 yr; and 3) CH₄ = 192 nM, turnover time = 0.9 yr. The maximum turnover time of 40 yr represents the lower detection limit for these studies, and all samples with radioactivity levels indistinguishable from background were assumed to have a turnover time of 40 yr.

ported for low-CH₄ waters (Scranton and Brewer, 1978; Rehder et al., 1999).

4.4. $\delta^{13}\text{C}\text{-CH}_4$

Changes in the $\delta^{13}\text{C}\text{-CH}_4$ are an unambiguous indicator of the occurrence and extent of CH₄ oxidation, provided that environmental conditions and isotopic fractionation factors are well known. The CH₄ emitted from sediments in the Eel River Basin has a $\delta^{13}\text{C}$ of about -50 to -60‰ (unpublished data). Hydrates in the region have previously been found with $\delta^{13}\text{C}\text{-CH}_4$ values of -57.6 to -69.1‰ (Brooks et al., 1991). The isotopic fractionation (α) factor for aerobic methane oxidation as determined for a variety of terrestrial systems is ~ 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 $\delta^{13}\text{C}\text{-CH}_4$ of -55‰ and an isotope fractionation (α) factor of 1.025 requires an oxidation of $\sim 45\%$ of CH₄ to produce a sample with the observed $\delta^{13}\text{C}\text{-CH}_4$ of -40‰ .

The $\delta^{13}\text{C}\text{-CH}_4$ can also be considered in conjunction with the concentration and rate data to describe patterns of CH₄ release and oxidation around venting fields. Results shown in Figure 3 demonstrate a clear CH₄ maximum and $\delta^{13}\text{C}\text{-CH}_4$ 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 CH₄ is being injected into the water column from vents and seeps around this depth. The recently injected CH₄ is isotopically light relative to meth-

ane elsewhere in the water column and is inferred to have undergone little oxidation. Conversely, CH₄ 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 CH₄ release from the sediment.

4.5. CH₄ Transport

The extent of CH₄ 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 CH₄ as it mixes away from the source region. Because no CH₄ bubbles appear to reach the atmosphere from the sediments of the Eel River Basin, the eventual fate of CH₄ is either oxidation or ventilation (controlled by mixing). The turnover time of CH₄ with respect to oxidation is ~ 1.5 yr, but mixing will act to dilute CH₄ 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 CH₄. Determining such a threshold is the key to understanding the eventual fate of dissolved CH₄ that undergoes significant mixing and is not rapidly ventilated to the atmosphere. In pure cultures of methanotrophs, CH₄ thresholds tend to be high (≥ 10 nM CH₄), but CH₄ oxidation in nature is known to occur at much lower levels (≥ 0.6 nM CH₄) (Hanson and Hanson, 1996). It is unclear whether the active methanotrophic populations that accompany high CH₄ will continue to consume CH₄ once the CH₄ (and the microbial population) have mixed with low-CH₄ waters. However, evidence from the deep (low CH₄) waters of the North Sea and the Atlantic Ocean indicates that CH₄ is consumed even at low concentration, with a turnover time of ~ 50 yr (Scranton and Brewer, 1978; Rehder et al., 1999).

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