New Constraints on Methane Fluxes and Rates of Anaerobic Methane Oxidation in a Gulf of Mexico Brine Pool via In Situ Mass Spectrometry

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New constraints on methane fluxes and rates of anaerobic methane oxidation in a Gulf of Mexico brine pool via *in situ* mass spectrometry

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46 **Abstract**

47 Deep sea biogeochemical cycles are, in general, poorly understood due to the
48 difficulties of making measurements *in situ*, recovering samples with minimal
49 perturbation and, in many cases, coping with high spatial and temporal heterogeneity. In
50 particular, biogeochemical fluxes of volatiles such as methane remain largely
51 unconstrained due to the difficulties of accurate quantification *in situ* and the patchiness
52 of point sources such as seeps and brine pools. To better constrain biogeochemical fluxes
53 and cycling, we have developed a deep sea *in situ* mass spectrometer (ISMS) to enable
54 high-resolution quantification of volatiles *in situ*. Here we report direct measurements of
55 methane concentrations made in a Gulf of Mexico brine pool located at a depth of over
56 2300m. Concentrations of up to 33mM methane were observed within the brine pool,
57 while concentrations in the water directly above were three orders of magnitude lower.
58 These direct measurements enable the first accurate estimates of the diffusive flux from a
59 brine pool, calculated to be $1.1 \pm 0.2 \text{ mol m}^{-2} \text{ yr}^{-1}$. Integrated rate measurements of
60 aerobic methane oxidation in the water column overlying the brine pool were $\sim 320 \text{ } \mu\text{mol}$
61 $\text{m}^{-2} \text{ yr}^{-1}$, accounting at most for just 0.03% of the diffusive methane flux from the brine
62 pool. Calculated rates of anaerobic methane oxidation were 600 to 1200 $\mu\text{M yr}^{-1}$, one to
63 two orders of magnitude higher than previously published values of AOM in anoxic
64 fluids. These findings suggest that brine pools are enormous point sources of methane in
65 the deep sea, and may, in aggregate, have a pronounced impact on the global marine
66 methane cycle.

67

67 **Introduction**

68 **1.1. *Global importance of methane***

69 The marine methane cycle has been the subject of much investigation in recent
70 years, in large part due to burgeoning interest and concern over deep ocean methane
71 hydrates. The deep ocean methane reservoir represents an enormous and dynamic pool
72 of carbon likely exceeding reserves of conventional oil and gas (Collett and Kuuskraa,
73 1998). In deep ocean regions, characterized by low temperatures, high pressure and
74 sufficient methane concentration, methane exists largely in the solid form of a gas
75 hydrate (Kvenvolden, 1993). Methane seeps and associated gas hydrates have been
76 identified along many passive and active continental margins (Kvenvolden and Lorensen,
77 2008). Because the destabilization of hydrates is sensitive to increases in temperature or
78 decreases in pressure, it has been postulated that increases in mean global temperatures
79 might trigger a release of methane into the ocean and atmosphere. A significant release
80 of methane into the atmosphere could ultimately lead to a catastrophic greenhouse effect;
81 this mechanism has been invoked as an explanation for past deglaciation events (Dickens,
82 2003; Sloan *et al.*, 1992; Zachos *et al.*, 2001).

83 Despite recent, numerous studies of methane hydrates, modern fluxes of methane
84 from the deep sea into surface waters and ultimately the atmosphere are very poorly
85 constrained. Estimates of methane flux have been aided, to some degree, by recent
86 advances in our understanding of marine microbiological influences on the global
87 methane cycle. Aspects of the marine methane cycle remain largely unconstrained due to
88 limitations in methods and technologies that enable accurate assessment of methane

concentration and flux – as well as rates of biological methanogenesis or methanotrophy. Pressure and temperature have a pronounced effect on methane solubility. As such, upon retrieval of methane-saturated waters or hydrate-rich sediments from the deep ocean, methane rapidly outgasses to the atmosphere. Thus, it has been challenging to constrain flux and microbial activity *in situ*, under environmentally relevant conditions. Because previous data have shown that methane oxidation, both aerobic and anaerobic, are the largest methane sinks in marine environments (Reeburgh, 2007), understanding what controls methane oxidation -including concentration and abiotic flux- are paramount to understanding global methane dynamics.

To better constrain the methane flux in chemically reducing environments – and ultimately to quantify the influence of biotic and abiotic processes on the methane cycle – we employed a newly developed *in situ* mass spectrometer (ISMS) to conduct direct measurements of methane concentration which - in concert with shipboard microbiological measurements - were used to generate more robust estimates of diffusive flux and net methane oxidation rates in a newly discovered brine pool in the Gulf of Mexico.

2. Geologic Setting

Along the continental shelf in the Gulf of Mexico, massive reservoirs of liquid and gaseous hydrocarbons lie buried beneath kilometers of sediment accumulated from the Mississippi River drainage basin. Due to compression and dewatering of the overlying sediments, underlying evaporite deposits have undergone plastic deformation resulting in salt-diapir driven tectonic activity (Kennicutt *et al.*, 1988). The resulting system of fractures and faults provides conduits for the emission of hydrocarbons to the

seafloor via seepage (Roberts and Carney, 1997). Hydrocarbon seeps are often characterized by abundant chemosynthetic based macro- and microfaunal communities including tubeworms, mussel beds, and bacterial mats which thrive on the reduced organic compounds emanating from below (Fisher *et al.*, 2007; MacDonald *et al.*, 1990; MacDonald *et al.*, 2003). In addition, hypersaline brine fluids seep from the seafloor in many locations (MacDonald *et al.* 1990; Joye *et al.* 2005, 2009). Previous studies have provided insight into the geochemical composition of these brine pools, though to date volatile flux and net rates of methane oxidation remain poorly constrained due to the challenges in quantification resulting from off-gassing (at *in situ* pressures and temperatures relevant here, methane saturation is $\sim 174 \text{ mmol kg}^{-1}$ (Duan and Mao, 2006)). Accurate sampling of fluids with high gas content using conventional methods (e.g., Niskin Bottles) has thus proven impractical for volatiles.

The brine pool characterized in this study (AC601) is located in the Alaminos Canyon lease block 601 (26° 23.53 N; 94° 30.85 W; Roberts *et al.* this issue; (Roberts *et al.*, 2007). The brine pool is estimated to be $\sim 250\text{m}$ in diameter and approximately 2334 meters below sea surface. This brine pool was visited during expeditions on board the *RV Ronald H. Brown* using the *DSV Jason II* during expeditions from May 6 through June 4, 2006 and June 3 through July 6, 2007 (see Roberts *et al.*, this issue), for further description of the expeditions and site locations). Discrete geochemical measurements of this brine pool were made during the 2006 and 2007 expedition, while deployment of the ISMS was carried out during the 2007 expedition.

3. Methods

3.1. *Fundamentals of Membrane Inlet Mass Spectrometry/ISMS*

Over the past five decades, the use of membrane inlet mass spectrometry (MIMS) has proven to be a powerful tool for measuring complex mixtures of dissolved gases in both industrial and laboratory settings (Johnson *et al.*, 2000; Ketola *et al.*, 2002). MIMS represents an optimal technique for mixed environmental gas analysis, having a high degree of sensitivity and precision, with minimal sample perturbation (Kana *et al.*, 1994). MIMS has been used over the past several decades to measure and monitor a wide range of dissolved gases in aquatic and terrestrial environments, including studies of bacterial mats, peat bogs, estuarine sediments, forest soils, and tree canopies to name a few (Hemond, 1991; Kana *et al.*, 1998; Lloyd *et al.*, 1986; Lloyd *et al.*, 1998). MIMS has also been used to study metabolite flux during shipboard high-pressure experiments (e.g., (Girguis *et al.*, 2002; Girguis *et al.*, 2000). MIMS has also emerged as an important tool for analyzing dissolved gases in seawater, (e.g. dissolved gases in surface waters analyzed continuously shipboard;(Kaiser *et al.*, 2005; Tortell, 2005a; Tortell, 2005b) and more recently for *in situ* marine surface waters (Bell *et al.*, 2007; Camilli and Hemond, 2004; Kaiser *et al.*, 2005; Tortell, 2005b, c).

The recent adaptation of MIMS to *in situ* environmental analyses demonstrates the utility of such an instrument operating while underway at sea – allowing the continual monitoring of many gas species in real time. This approach allows highly accurate monitoring of spatially explicit biogeochemical changes. For example, changes in O₂/Ar indicate changes in net community production in ocean surface waters in different regions of the eastern equatorial Pacific (Kaiser *et al.*, 2005). Additionally, N₂/Ar has

been used to identify areas of active denitrification (e.g., N₂ production) in seasonally oxygen-depleted bottom waters via MIMS in Saanich Inlet (Tortell, 2005b). Underway shipboard trace gas analysis has also shed light on dynamics of dimethylsulfide (Tortell, 2005a).

Given the apparent utility of real-time quantification by MIMS, we aimed to develop a MIMS that would achieve comparable performance in waters deeper than 1000 meters. Currently, investigation of deep water samples still generally requires the collection of individual samples and shipboard analysis (e.g., Tortell, 2005b), risking contamination and/or degassing. Furthermore, individual sample collection and analysis can often result in delays between sampling and data interpretation. Our understanding of deep-sea biogeochemistry would greatly benefit from real-time, *in situ* dissolved gas analysis. Here we present results from a real-time *in situ* membrane inlet mass spectrometer designed to A) operate at depths up to 450 atmospheres of pressure, B) provide real-time data to the user (when used on human occupied submersibles or remotely operated vehicles), and C) enable sampling with high spatial and temporal resolution using an *in situ* pumping system.

3.2. ISMS Design and Calibration

This ISMS consisted of three primary sub-systems: 1) a high-pressure membrane inlet (Fig. 1a) with a small volume seawater pumping system;; 2) a quadrupole mass spectrometer (Fig. 1e, f) and oil-less vacuum pumping system (Fig. 1 d, g); and 3) an underwater housing (either a 2000 meter-rated aluminum 3300 alloy housing, or a 4500 meter rated 6AL-4V titanium housing, both of which are approximately 120 cm in length and 24 cm in diameter). The membrane inlet assembly consisted of a circular sheet

(0.625 in. diameter) of polydimethylsiloxane (PDMS) membrane structurally backed by an integrated woven fiber (Franatech, Germany). This pliable membrane material was supported by a sintered stainless steel frit (5 μ m pore size, Applied Porous Materials, Tariffville, CT), which was in turn supported by the titanium body of the inlet housing. Sample water was pumped through the inlet housing assembly (2ml internal volume) at a flow rate of ~3ml/min using a small solenoid pump (The Lee Company, Westbrook, CT), which was controlled by an adjustable timing circuit located inside of the pressure housing.

The membrane assembly was connected to a Stanford Research Systems Residual Gas Analyzer (SRS RGA100) via standard vacuum flanges. Within the vacuum system, a pressure of $\sim 10e^{-5}$ Torr was maintained by a turbo-molecular pump (model: ATH 31+; Alcatel, France) backed by a diaphragm roughing pump (model: ANDC83.4; KNF-Neuberger, Trenton, NJ). Open source electron impact ionization was carried out with a thoriated iridium wire filament. The mass spectrometer and pumps were protected from membrane failure by a high-pressure / high-vacuum solenoid valve (Circle Seal, Inc., Corona, CA), which is actuated upon intrusion of water. The entire mass spectrometer assembly was housed in one of the aforementioned housings. 24 VDC power and two independent RS-232 channels (for serial communications with the turbo pump control board and continuous feedback from the RGA analyzer) were supplied via a wet-connect underwater cable (SubConn, Inc., North Pembroke, MA). In this configuration, real time monitoring of fluid chemistry is achievable during submersible or ROV operations, which allows for informed site selection for fluid measurements as well as adaptive sampling of biological specimens.

To conduct the benchtop high-pressure calibrations (Fig. 2), model 110A HPLC pumps (Beckman-Coulter, Fullerton, CA) were used to deliver calibrated solutions past the membrane surface at various flow rates and pressures. Hydrostatic pressure against the membrane inlet was manually controlled with a backpressure valve (StraVal Valve, Garfield, NJ) and monitored with high pressure gauges. Various calibration solutions were used including air-sparged DI water or seawater, degassed distilled water and/or seawater equilibrated with gas mixtures of interest (e.g., CH₄), including the use of a high pressure equilibration system for generating very high CH₄ concentrations (Fig. 2). A gas chromatograph (HP 5890 Series II plus with a TCD) outfitted with a custom gas extractor (Childress *et al.*, 1984) designed for quantification of rapidly degassed seawater samples was used to obtain independent analyses of dissolved methane concentrations, while previously described equations of state were used to calculate dissolved methane concentrations at very high pressures (Duan and Mao, 2006) (Fig. 2b). During lab experiments, relative changes in signal intensity were proportional to changes in the permeation of gas through the membrane (either due to changes in permeate concentration or changes in the permeability coefficient). We and others have observed that changes in hydrostatic pressure can have an influence on permeation of gases through membrane materials, in particular PDMS, interpreted to be caused by compression of the membrane pore space through which analyte gas passes (Bell *et al.*, 2007). A change in the relationship between dissolved gas concentration and signal intensity was observed during large changes in hydrostatic pressure (Fig. 2a). To account for this response, we conducted calibrations using methane dissolved in seawater over a range of *in situ* pressures and used these results to develop an empirical correction as

previously described (Bell et al., 2007). While this approach corrects for implicit changes in membrane behavior, it should be noted that the ISMS dataset presented here is comprised entirely of fluids sampled at a relatively constant depth ($\sim 2330\text{m} \pm 2$ (bar)) and temperature and as such effects due to differential pressure or temperature among the samples collected were negligible. Based on benchtop calibrations, the accuracy of the ISMS methane concentrations in the configuration described here was $\pm 11\%$, primarily due to the correction required for the pressure effects on the PDMS membrane (accuracy is improved through the use of alternate membrane material (e.g. Teflon AF)). Notably, however, the precision of the ISMS measurements is much better than this and, based on benchtop calibrations, is within $\pm 1\%$.

3.3. *Water Column Methane and Oxygen Concentration*

To determine brine pool and seawater column methane concentrations, a CTD rosette was lowered into the brine pool, using sonar to identify the brine pool as the rosette approached the bottom. Niskin bottles were tripped during descent to prevent contamination of bottles as gas came out of solution during the rosette's ascent. Two bottles were tripped in the brine pool itself, while two were tripped 1 meter above the brine-seawater interface (the interface was confirmed by the real-time conductivity signal). After securing the rosette on deck, water samples were immediately transferred to 1-liter PET-G bottles using gas-impermeable tubing. Bottles that were tripped in the brine were substantially over-pressured and not suitable for gas quantification (though samples were transferred to the PET-G bottles for rate measurements). A second sample was transferred to a 250 mL BOD bottle for determination of dissolved oxygen using a high sensitivity Orion[®] oxygen electrode. Methane was extracted using an adaptation of

the sonication/vacuum extraction technique (Suess *et al.*, 1999) followed by gas chromatography for quantification. Prior to dissolved gas extraction, samples were stored at bottom water temperature (4 °C). Two individual samples were analyzed from each rosette bottle.

3.4. ISMS Deployments and Determining Brine Pool Methane Concentrations

Upon reaching the brine pool, the submersible took care not to disturb the brine-seawater interface. Using the ROV manipulator, the ISMS sample inlet was positioned and held in place until the ISMS response reached steady state from which *in situ* concentrations of CH₄ were calculated. A total of five independent sets of measurements were made, beginning with two just above the brine fluid and three at depths of approximately 5, 20 and 80 cm into the fluid (Figs. 3 and 4).

3.5. Methane Oxidation Rates

Aerobic methane oxidation occurs according to the following stoichiometry:

Accordingly, aerobic methane oxidation rates were determined by incubating samples with C³H₄ and tracking the production of ³H₂O (Carini *et al.*, 2005; Valentine *et al.*, 2001). Typically, triplicate live and dead (Hg killed; i.e. samples were amended with HgCl₂ to arrest all biological activity) samples from each depth were incubated for 36 hours at *in situ* temperatures. Unreacted C³H₄ tracer was removed by purging samples with water-saturated CH₄ and the oxidation product, ³H₂O, was quantified by liquid scintillation counting (Carini *et al.*, 2005).

In general, marine anaerobic methane oxidation in hydrocarbon seeps and brine pools is coupled to sulfate reduction, with the net reaction:

Anaerobic methane oxidation rates were also determined by incubating samples with $^{14}\text{CH}_4$ and tracking the production of $^{14}\text{CO}_2$ (as in Joye *et al.*, 1999; Valentine *et al.*, 2001). Triplicate live and dead (Hg killed) samples from the surface (~20 cm) and sub-surface (~100 cm) brine were incubated for 48 hours at *in situ* temperatures. Unreacted $^{14}\text{CH}_4$ tracer was removed by purging with water-saturated CH_4 and the $^{14}\text{CO}_2$ oxidation product was quantified following acid extraction and trapping on a phethylamine wick, followed by liquid scintillation counting (Carini *et al.*, 2005).

3.6. *Sulfate Reduction Rates*

Samples for sulfate reduction were collected into gas tight glass tubes, amended with radiotracer ($^{35}\text{SO}_4^{2-}$) and incubated for 24 hours (as in Joye *et al.*, 2004; Orcutt *et al.*, 2005). For each depth, triplicate samples were incubated along side controls (killed at time zero). After incubation, samples were transferred from the tubes to 50 ml centrifuge tubes and mixed with 20% zinc acetate. Samples were processed and rates calculated as presented in Orcutt *et al.* (2005).

3.7. *Major Ion chemistry*

Concentrations of major ions (SO_4^{2-} , Cl^- , dissolved inorganic carbon (DIC), dissolved organic matter (DOC and DON) and dissolved inorganic nitrogen (NH_4^+ , NO_2^- and NO_3^-) were determined using previously reported methods (see (Joye *et al.*, 2004) and Joye *et al.* this volume and references therein).

4. Results

4.1. General geochemical composition of brine pool AC601

Geochemical data on the waters collected from the brine pool are given in Table 1. The waters of the brine pool were anoxic ($O_2 < 2\mu M$) with a pH of ~ 6.3 . Salinities were substantially elevated above seawater at 82 and 92 for the 20 and 100 cm depths, respectively, with chloride measuring 1366 and 1533 mM at each depth. Water from both depths was highly enriched in dissolved inorganic carbon (DIC; 11.2 mM at 20 cm, 12.8 mM at 100 cm). Sulfate concentrations were lower than seawater, decreasing with depth into the pool (Fig 4). Dissolved inorganic nitrogen was dominated by very high NH_4^+ (1750 and 2195 μM , 20 and 100 cm, respectively), with NO_3^- disappearing sharply in the top meter of the brine. The dissolved organic matter content was also high with a low C:N of ~ 4.6 suggesting the importance of autochthonous production within the brine waters.

4.2. Water Column and Brine Pool CH_4 Oxidation and SO_4^{2-} Reduction Rates

Aerobic methane oxidation rates measured in the water column above the brine pool from depths of 300m to 2313m (or heights above the brine pool from 0 to 2013m) ranged from 0.00 to $6.33 \pm 0.9 \text{ pmol L}^{-1} \text{ d}^{-1}$ (hereafter pM d^{-1}) (Fig 4). The aerobic methane oxidation rate in the sample taken from directly above ($\sim 3\text{m}$) the brine pool (2328m) was significantly higher, $129.6 \pm 18.2 \text{ pM d}^{-1}$, than rates at any other depth. Using the methane concentrations determined via gas chromatography and the empirically derived oxidation rates, we calculate an integrated methane oxidation rate in the water column above the brine pool of $320 \mu\text{mol m}^{-2} \text{ yr}^{-1}$.

Within the brine pool, two samples (20cm and 100cm) were retrieved and used for sulfate reduction and anaerobic oxidation of methane (AOM) rate measurements (Table 2). Methane concentrations measured in the bottles used for the rate measurements were 454 and 1320 μM , respectively (Table 1), giving rates at these depths of 78.8 ± 7.6 and $62.1 \pm 13.1 \text{ nmol L}^{-1} \text{ d}^{-1}$, respectively. Sulfate concentrations in the brine were depleted relative to seawater with concentrations of 20 and 16mM at 20cm and 100cm, respectively. Sulfate reduction rates were 107 and 50 nmoles per liter per day (hereafter nM d^{-1}) at 20cm and 100cm, respectively, and were comparable to the rates of AOM on a per mole basis. As mentioned above, these oxidation rates were measured using water taken from CTD rosette bottles, which, when sampling gas-charged waters, are subject to outgassing and gas phase exchange during recovery. Thus, these rates are considered to be conservative estimates of anaerobic methane oxidation. *In situ* methane oxidation rates are expected to be higher as methane concentrations increase (i.e., on a first order basis up to k_{max}) and are calculated below.

4.3. *Water Column and Brine Pool Methane Concentrations*

Methane concentrations measured in the water column (Fig 4) directly above the brine pool ranged from 30nM to 70nM at depths between 2000 and 300m, representing concentrations that were 15 to 32 times that of atmospheric equilibrium and underscoring the transport of methane from below. At depths below 2000m, closer to the brine pool, concentrations increased sharply and ranged from 111 nM to 24 μM . Methane concentrations, as measured by the ISMS approximately 5 cm above the brine fluid/seawater interface near the shore of the pool and 1 cm above the brine fluid/seawater interface in the center of the brine pool were 180 and 590 μM ,

respectively. Approximately 1m above the brine surface, the ISMS-measured methane concentration was approximately $\sim 35 \mu\text{M}$, which is in general agreement with the methane concentrations measured in the CTD rosette at this depth ($24 \mu\text{M}$, well below the saturation of methane at one atmosphere, so these particular Niskin measurements are not compromised by off gassing, see Fig. 4). Concentrations at depths of 5, 20 and 80cm into the brine fluid near the center of the pool were orders of magnitude higher (Fig 4) with values of 14.3, 20.3 and 33.3 mM, respectively ($\pm 2\%$), and more than an order of magnitude in excess of concentrations measured with Niskin sampling (see Table 1).

5. Discussion

5.1. *In situ rates of brine pool anaerobic methane oxidation*

The *in situ* rates of AOM reported here exceed values in other anoxic waters by at least one to two orders of magnitude. Our measured rates of AOM from two sampling depths allowed calculation of first order rate constants of 0.063 and 0.017 yr^{-1} from depths of 20cm and 80cm, respectively. Coupling the *in situ* methane concentration measurements from the ISMS to the aforementioned rate constants yields estimates of the actual rates of AOM of 1285 ± 125 and $572 \pm 121 \mu\text{M yr}^{-1}$ at the depths of 20 and 80cm in the brine pool, respectively. To the best of our knowledge, these AOM rates are by far the highest documented in an anoxic water body. Whereas there has been some evidence that AOM is inhibited by high chloride concentrations (e.g., (Joye *et al.*, 2009; Oren, 2002)), our data (Tables 1 and 2) suggest that moderately high salinities may in fact not be inhibitory to AOM and that coupled sulfate reduction and AOM may yet play an important role in many Gulf of Mexico hydrocarbon/brine environments.

358 Many studies have measured AOM in deep sea environments with high
359 concentrations of methane, and the highest rates are generally found within sediments,
360 particularly hydrate-influenced sediments (e.g., (Devol, 1983; Girguis *et al.*, 2003; Joye
361 *et al.*, 2004; Reeburgh, 1980)). Indeed, far fewer studies have measured AOM occurring
362 in anoxic water columns, and the rates reported in these studies are generally much lower
363 than sediment rates (as is the case with most biogeochemical processes primarily due to
364 microbial density being substantially higher in sediments). Rates of AOM measured in
365 the anoxic waters of the Cariaco Basin ($\sim 1.5 \mu\text{M yr}^{-1}$; Ward *et al.*, 1987), Saanich Inlet
366 ($7.3 \mu\text{M yr}^{-1}$; Ward *et al.*, 1989) and the Black Sea ($0.6 \mu\text{M yr}^{-1}$; Reeburgh *et al.*, 1991)
367 were all orders of magnitude lower than those observed in this study. Joye *et al.* (1999)
368 measured rates as high as $17.5 \mu\text{M yr}^{-1}$ in the bottom waters of alkaline, saline Mono
369 Lake. Notably, these rates were measured during a period when the lake waters were
370 well mixed. More recent data collected during a period of extended meromixis in Mono
371 Lake exhibit substantially higher rates of AOM (up to $365 \mu\text{M yr}^{-1}$) (Joye *et al.*,
372 submitted).

373 The rates of AOM presented here are also consistent with the extremely high *in*
374 *situ* concentrations occurring at these depths. Turnover times of methane in the anaerobic
375 brines were on the order of 16 to 58 years, more than long enough to maintain supply of
376 SO_4^{2-} via diffusion. Such long turnover times also might suggest that supply to the
377 overlying water via diffusion is likely to be a substantial methane sink relative to removal
378 by AOM (or aerobic oxidation at the brine-seawater interface) in similar brine pool
379 environments. Indeed, given that the rate constants were lower than many other
380 comparable environments, the rates presented represent a conservative estimate and, as

previously mentioned, the rate constants would likely increase at the higher methane concentrations found *in situ*.

5.2. Estimates of CH₄ flux from the brine pool

Research on deep-sea fluxes and transformations of biological compounds is constantly challenged by the need to sample at extreme temperatures, depths and pressures. Measurement of these compounds *in situ* provides more rigorous constraints on their fluxes and transformation rates. In the context of the current study, the *in situ* mass spectrometer allowed direct measurement of methane concentrations in a gas-charged brine pool. These concentration measurements were used to calculate a diffusive flux of methane from the brine pool into the overlying water column of $1.1 \pm 0.2 \text{ mol m}^{-2} \text{ yr}^{-1}$, illustrating the magnitude of methane flux from benthic environs into the overlying mixed layer (discussed in detail below).

Specifically, discrete *in situ* measurements taken over a depth profile across the seawater-brine interface provide a context for calculating diffusive geochemical fluxes of methane into the overlying water column. This approach has been used in numerous studies for estimating the mass transfer (e.g., fluxes) of solutes from one region into another. Brine pools such as AC601 are generally very quiescent in nature with fluid advection playing a small role in controlling fluxes (Joye *et al.*, 2005; Joye *et al.*, 2009). In these locations, where diffusion is the dominant mode of mass transfer, Fick's first law is used to make first order estimates of the diffusive flux based on the measured concentration gradients. The range of possible flux values is estimated based on error in the spatial resolution of the gradient (i.e., since high precision positioning of the sampling wand was not possible, we estimate the potential vertical position $\pm 10\text{cm}$). Moreover,

we adopted a value of $1.38\text{e}^{-5} \text{ cm}^2 \text{ s}^{-1}$ for the diffusion coefficient of methane in seawater adjusted for the average viscosity of the brine using the Stokes-Einstein relationship (Mao and Duan, 2008; Sahores and Witherspoon, 1970).

Even with our lower-bound estimate of diffusive methane flux ($1.1 \text{ mol m}^{-2} \text{ yr}^{-1}$), water column integrated methane oxidation rates (Fig. 4) measured directly above the brine pool ($\sim 320 \text{ } \mu\text{mol m}^{-2} \text{ yr}^{-1}$) indicate that only a very small fraction of methane escaping the brine pool is biologically consumed in the overlying water column (0.02 to 0.03%). While it is likely that lateral advection plays a large role in the disconnect between brine pool flux and water column methane oxidation rates above the brine, upper water column concentrations are nonetheless >10 times that of methane in equilibrium with the atmosphere, confirming the transport of methane from depths $>2000\text{m}$ into the mixed layer which easily escapes, un-oxidized, into the atmosphere.

Our estimates of diffusive methane flux should be taken as a lower bound on flux from environments such as Gulf of Mexico brine pools. They also underscore the value of *in situ* measurement for constraining methane fluxes. For example, other studies (Lapham *et al.*, 2008; Schmidt *et al.*, 2003) have modeled diffusive and/or advective fluxes from brine seep environments by comparing profiles of a non-conservative solute (e.g., methane) to conservative solutes (e.g., chloride, temperature). Using a 1-D reaction transport model together with chloride and methane profiles, advective methane fluxes of up to $2 \text{ mol m}^{-2} \text{ yr}^{-1}$ were estimated from brine-influenced sediments characterized by a strong advective flow (Lapham *et al.*, 2008). However, these calculated fluxes were based on methane concentrations from sediment cores that had degassed upon collection, and were thus substantially lower than methane concentrations *in situ*. In another study,

Solomon et al. (2008) employed osmotic samplers demonstrating that net seafloor methane fluxes range from $0.89 \text{ mol m}^{-2} \text{ yr}^{-1}$ from a mussel bed environment up to $29 \text{ mol m}^{-2} \text{ yr}^{-1}$ from a bacterial mat environment. Hereto, because methane concentrations could not be reliably determined at *in situ* pressure and temperature, fluxes were calculated assuming that porewater was in equilibrium with methane hydrate under *in situ* conditions. However, others have shown that methane in sediments around hydrate can be far from saturated (Lapham *et al.*, pers. comm.), which would result in much lower flux estimates. Future studies should aim to couple *in situ* methane measurements with direct fluid flow measurements to better constrain the contribution of advective flux to water column methane flux.

6. Summary and Conclusions

Our calculated *in situ* AOM rates, using empirically derived rate constants, are higher than those previously published by one to two orders of magnitude. The diffusive flux was estimated to range as high $1.8 \text{ mol m}^{-2} \text{ yr}^{-1}$ from the brine pool, while integrated oxidation rates in the overlying 2000m water column could only account for $0.32 \text{ } \mu\text{mol m}^{-2} \text{ yr}^{-1}$. These data suggest that a very large component of the diffusive brine pool methane flux escapes both aerobic and anaerobic oxidation in the water column above the brine pool and may be released into the atmosphere (or at least subject to dispersion via lateral advection). These first *in situ* measurements of methane concentration from a brine pool using the ISMS enabled robust quantification of methane concentrations at *in situ* conditions in these gas-charged brines and reflect the strong influence of the surrounding hydrocarbon seep environments. Such integrated approaches – wherein geochemical determinations are coupled with microbiological activity measurements –

450 are the best means of providing a rigorous constraint on methane diffusive fluxes and
451 transformation rates. This will improve our understanding of the role that hydrocarbon
452 seeps may play in the delivery of methane into the ocean and ultimately the atmosphere.
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Figure captions

Figure 1: Schematic of the in situ mass spectrometer. a) membrane inlet housing, b) front end plate of titanium pressure housing, c) high pressure solenoid for isolation of vacuum chamber, d) Alcatel ATH-31+ Turbo Pump, e) vacuum flight tube housing the SRS Quadrupole RGA-200 including ion source, quadrupoles and detectors f) electronics head for controlling and reading spectrometer signals g) KNF Neuberger roughing pump model ANDC-84.3. Sample gas is continuously extracted across the membrane located in (a) into the high vacuum system (d, g), ionized in (e) and analyzed by the detector and electronics control unit housed in (f). The instrument is approximately 1m in length.

Figure 2: a) Normalized response at m/z 15 over a range of hydrostatic pressure for three example fluid temperatures and concentrations, 10°C 1160 μ M CH₄ (grey squares), 2°C 800 μ M CH₄ (black triangles) and 14°C 180 μ M CH₄ (grey triangles). Responses to pressure were experimentally fit under a wide range of temperatures and concentrations (as in Bell et al 2007) with values of b' ranging between 0.02 to 0.24 and values of k ranging between 0.84 to 0.94. b) The response of m/z 15 (corrected for pressure effects – see Fig 2a) was linearly proportional to methane concentrations as measured independently by gas chromatography (grey triangles) and as calculated after Duan et al 2006 during high pressure calibration measurements (black circles).

Figure 3: Photo from the Pilot Cam of ROV Jason showing the starboard manipulator reaching through the seawater/brine pool interface and sampling at a depth of approximately 80cm.

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489 **Figure 4:** a) Depth profile of methane concentration and methane oxidation rates in the
490 water column above brine pool AC601. Note log scale. Open circles are concentration
491 measurements made from Niskin bottle samples, while black circles are those made *in*
492 *situ* using the ISMS. b) Close-up of seawater/brine pool interface and profile into the
493 brine fluid. Note the linear scale in contrast to panel a. Measured rates of anaerobic
494 methane oxidation (AOM) at two depths within the brine pool are shown. Note that
495 these, when corrected for in situ CH₄ concentrations, these rates are 30-45 times higher.
496 Sulfate concentrations are depleted in the brine, consistent with its role in AOM.

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Table 1: Major chemical components of brine pool AC601

Depth cm	pH	salinity	oxygen	Concentration mM						Concentration mM						
				DIC	H ₂ S	SO ₄ ⁻²	Cl ⁻	CH ₄ ^a	CH ₄ ^b	NH ₄ ⁺	NO _x	DIN	TDN	DON	DOC	DOC:DON
5									14.35							
20	6.29	82	<2 μM	11.2	0.00	20	1366	0.454	20.29	1750	3.4	1753.4	1843.5	90.1	423.5	4.7
80									33.29							
100	6.25	92	<2 μM	12.8	0.25	16	1533	1.320	38.40*	2195	0.3	2195.3	2280.5	85.2	380.0	4.5

^aConcentrations measured via gas chromatography on samples retrieved with a CTD rosette and Niskin bottles

^bConcentrations measured via *in situ* mass spectrometer

* estimated by regression of the three ISMS data points collected above

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Table 2: Rates of sulfate reduction and anaerobic methane oxidation in Gulf of Mexico brine pool AC601

Depth cm	Rate nM/d			
	Sulfate Reduction	Anaerobic Methane Oxidation*	Anaerobic Methane Oxidation^	
20	107.1 ± 14.6	78.8 ± 7.6	3502.0 ± 340	
100	49.8 ± 8.4	62.1 ± 13.1	1807.4 ± 330	

* measured using water samples collected via CTD rosette and Niskin bottles
^ estimated rates corrected for measured concentrations *in situ* and using rate constants measured from shipboard incubations of brine pool water collected via Niskin bottles

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Wankel Figure 1

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Wankel Figure 2

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Wankel Figure 3

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Wankel Figure 4