Introduction

The supporting information provides a derivation of the isotopic fractionation factor once methane has reached equilibrium between a liquid and gas phase. This expression was used in the main text to calculate the isotopic ratios of methane dissolved in a seawater sample. We also provide a derivation for the Instantaneous Product Rayleigh model used in the main text to relate isotopic ratios of dissolved methane to the fraction of methane that has dissolved since bubble emission at the seafloor. Finally, the supporting information also includes several sampling diagrams for the GISR G08 cruise onboard the E/V Nautilus. The goal was to obtain samples from different areas within the gas bubble flare in order to assess whether any spatial distributions existed in regards to methane concentrations and stable isotopic ratios. The gas bubble flare was visualized using acoustic instrumentation and in most situations the flare took the form of a teardrop shape. Sampling locations were chosen in order to sample along the length and width of the bubble flare. Samples were collected within the flare where bubbles were abundant, along the edge of the flare, and sometimes outside the flare where bubbles were no longer present. The circles in the diagrams represent the location where samples were collected, and the number within the circles correspond to the Sample ID.
found in the submitted data set presenting methane concentration and isotopic ratio data. The distance between each sample is presented in meters, although the lines connecting the circles are not to scale. Samples were collected in numerical order.
Text S1.

Definition of Terms

$L = \text{Subscript used to denote the light isotope of methane or } ^{12}\text{CH}_4$

$H = \text{Subscript used to denote the heavy isotope of methane or } ^{13}\text{CH}_4$

$X_L = \text{The mass of the light methane isotope of the dissolution product}$

$X_H = \text{The mass of the heavy methane isotope of the dissolution product}$

$R_L = \text{The mass of the light methane isotope of the reactant}$

$R_H = \text{The mass of the heavy methane isotope of the reactant}$

$k_L = \text{The first order reaction rate constant of the light methane isotope}$

$k_H = \text{The first order reaction rate constant of the heavy methane isotope}$

$t = \text{time that has passed since methane bubble emission at the seafloor}$

$R_X = \text{The ratio of the heavy to light isotopes of the dissolution product}$

$R_R = \text{The ratio of the heavy to light isotopes of the reactant}$

$R_0 = \text{The initial isotopic ratio of methane within a bubble at the time of bubble emission at the seafloor}$

$R_{std} = \text{The isotopic ratio of the Pee Dee Belemnite (PDB) standard}$

$\alpha_d = \text{The fractionation factor defined as the ratio of the kinetic reaction constant between}$

$\text{the light and heavy isotopes of methane}$

$f = \text{The fraction of methane that has been dissolved since bubble emission at the seafloor}$

$\delta X = \text{The isotopic ratio of the dissolution product converted to delta notation}$

$\delta R_0 = \text{The initial isotopic ratio of methane within a bubble converted to delta notation}$
**Isotope Exchange Reaction = Isotope Equilibrium Reaction**

An isotope exchange (also known as isotope equilibrium) reaction is characterized by zero net chemical reaction between the bulk species, however, the distributions of the isotopes change between the individual chemicals or phases. In addition, it is assumed that the redistribution of the isotopes has fully occurred and reached a new equilibrium.

In the case of CH₄ isotopes equilibrating in a sample vial headspace:

\[ ^{12}CH_4(d) + ^{13}CH_4(d) \leftrightarrow ^{12}CH_4(h) + ^{13}CH_4(h) \]

Where \((d)\) and \((h)\) denote the isotopes of CH₄ dissolved or in the headspace, respectively.

Generalized, this reaction becomes:

\[ A_L + B_H \xrightleftharpoons[{k_L}{k_H}]{k_H}{k_L} A_H + B_L \]

where the subscripts denote the light, \(L\), and heavy, \(H\), isotopes, and \(k_L\) and \(k_H\) are the rate constants for the forward and reverse reactions, respectively. Since the fractionation factor for any kinetic process is defined in its most fundamental sense as the ratio of the rate constants \((k_L / k_H)\), it is often the most useful parameter as it characterizes the extent of the isotope fractionation effect. For isotope exchange reactions, the fractionation factor \((\alpha)\) is equivalent to:

\[ \alpha_{A-B} = R_A / R_B \quad \quad \alpha_d = (^{13}CH_4/^{12}CH_4)_d / (^{13}CH_4/^{12}CH_4)_h \quad \text{(Eq. 2)} \]

This equivalency can be witnessed when viewing the rate law with the assumption that the isotope exchange process has occurred and a new equilibrium has been reached.

\[ \frac{dA_L}{dt} = -k_LA_LB_H + k_HA_HB_L = 0 \]

\[ k_LA_LB_H = k_HA_HB_L \]
Mass spectroscopy analyses produce values of \( \delta_A \) and \( \delta_B \), and Eq. 1 can be manipulated to give \( R_A \) and \( R_B \) which can be used to calculate the fractionation factor.

\[
R_A = \left( \frac{\delta_A}{1000} + 1 \right) R_{\text{Std}}
\]

(Eq. 3)

\[
\alpha = \frac{R_A}{R_B} = \left( \frac{\delta_A}{1000} + 1 \right) R_{\text{Std}} = \frac{\delta_A + 1000}{\delta_B + 1000}
\]

\[
\alpha = \frac{k_L}{k_H} = \frac{R_A}{R_B} = \frac{\delta_A + 1000}{\delta_B + 1000}
\]

\[
\alpha_d = \frac{\delta d + 1000}{\delta h + 1000}
\]

(Eq. 4)

**Instantaneous Product Rayleigh Model Derivation**

The Rayleigh model assumes that only one process, in this case dissolution, controls the mass of \( \text{CH}_4 \) and its isotopic ratio inside a gas bubble. We assume that dissolution is similar to a first order reaction where the dissolution rate depends on a reaction rate constant and on the mass of \( \text{CH}_4 \) inside the bubble. The reactant, \( R \), is the \( \text{CH}_4 \) inside the bubble in gas form while the product, \( X \), is \( \text{CH}_4 \) dissolved in the water column. Since dissolution has been shown to fractionate isotopes we treat each isotope individually, and assign different reaction rate constants. Equations 5 and 6 are the
differential equations that govern the production of the heavy and light isotopes of dissolved CH$_4$ over time.

\[
\frac{dX_H}{dt} = k_H * R_H \quad \text{(Eq. 5)}
\]

\[
\frac{dX_L}{dt} = k_H * R_L \quad \text{(Eq. 6)}
\]

\[
\int_0^{X_H} dX_H = k_H * R_H * \int_0^t dt
\]

\[
\int_0^{X_L} dX_L = k_L * R_L * \int_0^t dt
\]

\[
X_H = k_H * R_H * t
\]

\[
X_L = k_L * R_L * t
\]

Combining the expressions for $X_H$ and $X_L$:

\[
\frac{X_H}{X_L} = \frac{k_H * R_H * t}{k_L * R_L * t}
\]

The isotopic ratio is defined as the heavy over the light isotope, and the fractionation factor is defined as the ratio of reaction rate constants of the light over the heavy isotope:

\[
R_X = \frac{1}{\alpha_d} * R_R \quad \text{(Eq. 7)}
\]

In (Leonte et al., 2017) the isotopic ratio of the reactant was related to the starting isotopic ratio of CH$_4$, $R_0$, and the fraction removed since $t = 0$:

\[
R_R = R_0 * (1 - f) \left( \frac{1}{\alpha_d} - 1 \right) \quad \text{(Eq. 8)}
\]

By substituting the product for the reactant:

\[
R_X = \frac{R_0}{\alpha_d} * (1 - f) \left( \frac{1}{\alpha_d} - 1 \right)
\]

The isotopic ratio can be converted to $\delta$ notation using the Pee Dee Belemnite (PDB) standard, $R_{\text{std}}$:
\[ R = \left[ \frac{\delta R}{1000} + 1 \right] \times R_{std} \]

\[ \left[ \frac{\delta X}{1000} + 1 \right] \times R_{std} = \frac{\delta R_{0} + 1000}{\alpha_d} \times R_{std} \times (1 - f)^{\frac{1}{\alpha_d} - 1} \]

\[ \delta X = \frac{(\delta R_{0} + 1000)}{\alpha_d} \times (1 - f)^{\frac{1}{\alpha_d} - 1} - 1000 \]  

(Eq. 9)
Figures S1 to S5.

Dive: H1402
Altitude: 95 m
Depth: 793 m

Figure S1. Sampling diagram for dive H1402.
Figure S2. Sampling diagram for dive H1404.
Figure S3. Sampling diagram for dive H1406.
**Figure S4.** Sampling diagram for dive H1407, for samples collected 202 m above the seafloor.
Figure S5. Sampling diagram for dive H1407, for samples collected 302 m above the seafloor.