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Progress Report (Period ending 9/30/2014)

Assessing the response of methane hydrates to environmental change at the Svalbard continental margin
Project Period (11/1/2013 to 10/31/2015)

Submitted by:
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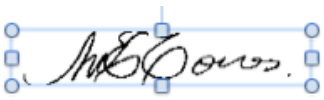

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EXECUTIVE SUMMARY

In November 2013, Oregon State University initiated the project entitled: **Assessing the response of methane hydrates to environmental change at the Svalbard continental margin**. In this project, we will take advantage of a unique opportunity to collect samples from the Svalbard continental margin. The overall objective of this research is to constrain the biogeochemical response of the gas hydrate system on the Svalbard margin to environmental change. The locations sampled shall provide key datasets that allow examination of the system with respect to sediment temperature fluctuations driven by thermal changes in the overlying water column and by hydrothermal circulation in the sediments. Because of a delay in the planned expedition, we reconfigured the program based on discussions with NETL program managers and submitted a revised SOPO. In the new plan, we will collect samples in two expeditions, the first of which happens Oct 7-21, 2014. During this period we re-vamped the project design, planned for the new expedition, continue testing instrumentation, numerical models and microbial methods in support of this project.

PROGRESS, RESULTS, AND DISCUSSION

1. New project planned. Based on conference calls with NETL project managers we modified the field program as follows:
    a. We have secured participation it the upcoming cruise to Vestnesa Ridge on the Norwegian RV Helmer Hanssen cruise; scheduled to sail from Svalbard on the 7th and return on the 18th of October. We will be able to conduct the geochemistry and microbiology tasks of the DOE funded project on that region, i.e., sampling across a transect with different levels of methane flux. WeiLi Hong (currently funded on the DOE grant) will sail on this cruise and collect the pore water and sediment chemistry. Norwegian scientist Friederike Gründger will collect samples for microbiology. The Arctic University of Norway (CAGE Center) will provide some shipboard supplies (e.g. reagents, liquid nitrogen, core handing equipment, etc.). Additional supplies needed for the expedition were sent from Bremen, or hand-carried by WeiLi Hong from OSU. This expedition is currently underway. Details of the OSU component are listed in the Appendix
    b. We have secured participation on the German RV Heincke for next year. This expedition is scheduled from 30 July to 25 September and we will revisit Vestnesa to collect samples aided by a remotely operated vehicle (ROV), as well as conduct the water column aspects of the project. The RV Heinke's original task for this expedition is to deploy and test a new AUV system under development. However Prof. Bohrmann has agreed to allocate 35% of the ship-time for me to conduct the DOE-related tasks.
2. Instrument update-
    a. We continue testing and calibrating the green house analyzer (results attached). We conducted preliminary field testing on a one day expedition offshore Germany (Erkenforde Bay) on July 2nd. The instrument performed adequately but we encountered problems with dilution protocols needed to match the sample volumes required by the instrument. New procedures have been developed to stream-line the operation
    b. We received delivery of the second instrument (Range 3 isotope analyzer). Preliminary testing revealed significant scatter of the isotope data, beyond what is

acceptable. We conducted several conference calls with the technical support (Bob Provenzal, Los Gatos Research). He identified a software issue with the laser temperature control and we continue working remotely to fix this problem

c. Additional Field testing- To test the long-term performance of the instruments and obtain enough samples to cross-calibrate with other instruments/laboratories we are conducting a time-series of methane analyses using samples collected from a Peat Bog in Bremen, Germany.

3. Microbiology. We continue developing methods for microbial analyses. To date we have:
    a. Amplifiable (16s gene) DNA from practice (sandstone) sediments was extracted with MoBio kits
    b. Have extracted DNA on practice samples using hot alkali/phenol:chloroform method that may give more accurate representation of microbial community (Morono et al 2014)
    c. Plan to test out DNA extraction method that separates extracellular and intracellular DNA (Alawi et al 2014)
    d. Simultaneous DNA/RNA extraction for microbial activity measurements will use MoBio powersoil RNA kit with additional DNA cleanup kit
    e. Qubit fluorometer used to accurately measure [DNA], will also be used for [RNA]
    f. Taking advantage of a DOE-funded cruise to the Cascadia margin (October 2014), we will collect sediment samples at CH_4 seepage and non-seep sites above/below/within SMTZ, and use these samples to optimize DNA/RNA extractions, sequence 16s genes for analysis of communities, and compare biases of extraction methods
4. Modeling- We began an effort to review the methane hydrate stability and saturation expressions derived from several authors. These expressions will be cross-validated by results from lab experiments. Results of this exercise are shown in the appendix.

PROBLEMS OR DELAYS

We encountered a significant set-back when the planned expedition in the R/V M.S. Merian got cancelled due to massive engine failure of the vessel. We immediately notified the program management and set up a set of conference calls to decide the best options to move forward. This was resolved by participating on two expeditions (October 2014, July-September 2015)

PRODUCTS

- Revised PMP submitted to NETL detailing the new program plan
- Plan for R/V H. Hanssen cruise
- Report on Numerical Simulations
- Continuation Presentation (ppt)

MILESTONE STATUS

We are well within our planned progress regarding Milestone 1 (revised PMP):
Title: Complete preparations for expedition-
Planned Date: Currently underway

RV Helmer Hanssen
07. 10. 2014 – 18. 10. 2014

Methane venting and gas hydrates off west coast of Svalbard

Scientific Rationale

The cruise primarily is aiming to collect field data in the area of the West-Spitsbergen continental margin (**Figure 1**) that will allow investigating the gas hydrate dynamics. Our objectives are:

- To evaluate stability conditions of the gas hydrates based on the analysis of the geochemical compositions and temperature lance measurements.
- To obtain a better understanding of stratigraphic development and sedimentation rate on the Vestnesa Ridge.
- To obtain baseline information on fluid expulsion processes on the Vestnesa Ridge which allow an optimized design of a planned seafloor observatory to be deployed in this area.

Geochemistry plan

Goals:

Prepare for and collect water and sediment core samples from a high latitude setting (the Svalbard Margin) across gradients where methane hydrates show vulnerability to environmental change. Samples will be used for chemical and microbiological analyses to assess changes in chemistry and microbiology across vertical gradients (i.e., within a core) and horizontal gradients (i.e., across the putative upper edge of gas hydrate stability) that constrain the biogeochemical response at locations where methane hydrates are sensitive to environmental change.

Sampling procedure:

Things which will be prepared before coring

- Fill up 5ml 1M NaOH into 20ml glass serum vial, close it with stopper (no crimp)
- Label glass vials: content, analysis, date, cruise part,
- Production of 50ml 4% and 500ml 2% PFA buffer for cell fixation
- Agilent Vials containing 10µl $HgCl_2$
- Drill holes into the empty liner, cover holes with tape

CORING

1. Clean & label the core:
   large arrow pointing to the top of the core
   label core no. & cm (section no.) on each 30cm core intervals
   mark TOP & BOTTOM on each core section
2. Fill out the core log for the core
3. Cut core into 30-cm section
4. Collect the sed. from the bottom end of the fresh cut core section
5.

GAS ANALYSIS (every 30cm from bottom to top)

6. use 5-ml cut-off syringes
7. take the sed. through the pre-drilled hole in the liner- NO, this is from the fresh cut section of the core above
8. extrude 2 x 3ml sed. into 20-ml glass serum vial containing 5ml 1M NaOH
9. close with stopper and crimp-top
10. label with core no. & cm
11. store upside-down at 4C

RHIZONE SAMPLING (every 30cm from bottom to top)

12. Carry out the rhizone sampling within 4 hours of pulling up the cores (note hours)
13. Put Rhizone through the pre-drilled hole in the liner into the core sed.
14. Use *acid-washed* 20-mL syringes for shallower sediments (more water) and *acid-washed* 10-mL syringes for deeper sediments
15. Use woody sticks to keep vacuum in the syringes
16. Make a note of depth from the top of each core section (core no./section no.) for each rhizon sample

17. After porewater sampling leave Rhizones in the core sediment
18. Filter pore water through Accrudisk during subsampling

SO_4 precipitation test

19. add ~250µl porewater in clear PCR tube
20. add ~20µl $BaCl_2$
21. see if milky-white precipitate/turbidity forms → first sample where sulfate is abscent → SMTZ

POROSITY SAMPLING (every 30cm from bottom to top)

22. use 2-ml cut-off syringes
23. put **3ml sed**. into a ***pre-weighed*** **glass vial**
24. record exact volume taken
25. label with core no. & cm
26. store at ??? NOTHING SPECIAL, ROOM TEMPERATURE IS FINE

TOTAL SEDIMENT SAMPLING (BULK) (every 30cm from bottom to top)

27. take 1-2 scoops (~20ml) sed. via spoon or cut-off syringes
28. put into a Whirlpak bag
29. if calcium carbonate deposits are identified, take extra samples.

PORE WATER SUBSAMPLING

SALINITY TEST by Refractometer

30. Put a drop of porewater on the refractometer
31. record results
32. cleaned off between each sample using DI water and Kimwipes
33. calibrate refractometer periodically against sample of known salinity (IAPSO?)

$\delta^{13}C$

34. put 1ml pore water in an Agilent Vial containing 10µl $HgCl_2$
35. If H_2S is present, a brown precipitate will form

CHLORIDE

36. Put 2ml (min. 1ml) porewater in an empty Wheaton glass vial

SO_4

37. Add 1.5 ml porewater to a Wheaton glass vial OR 2-ml Eppendorf tubes) containing 0.1ml 10% ZnAc solution

NUTRIENTS

38. Put 3ml porewater in a 15-mL Falcon tube
39. Freeze at -20C

REMAINDER

40. Put remainder in acid-washed Nalgene bottles
41. Make sure bottles are tightly closed
42. put all Nalgene bottle samples from one core in a Ziploc freezer bag
43. Note how much porewater was added to each bottle
44. Acidify with ultra pure HNO_3^-

Microbiology program

Goals:

Analyze the samples to assess changes in chemistry and microbiology across vertical gradients (i.e., within a core) and horizontal gradients (i.e., across the putative upper edge of gas hydrate stability) that constrain the biogeochemical response at locations where methane hydrates are sensitive to environmental change. In particular, examine how microbial communities influence carbon, iron, manganese and sulfur cycling.

Sample request:

Samples for microbiological analysis will be obtained from the cores in close physical proximity to samples obtained for geochemical and physical parameters of the sediments. Ideally we would like to obtain samples for DNA and RNA every 25cm within the SMTZ, at 1 and 2 meters above and below the SMTZ, and every 5 meters along the core from the seabed to the bottom of the core. Additional "samples of opportunity" will be taken if sediment features such as fractures indicate active fluid movement in the samples, or biofilms are observed in the cores.

Optimally we need 50 g sediment for each DNA and RNA (roughly 40 cc's sediment per falcon tube). The samples can be collected just by pushing a 50 ml falcon tube down into the core until the tube is ~40 ccs full.

DNA extraction samples should be put in liquid nitrogen as soon as possible. The RNA samples should also either be frozen in liquid nitrogen immediately or preserved with RNAlater to prevent RNA from breaking down. At the end of the expedition frozen samples will be shipped to OSU in dry shippers (MVE Biomedical Inc., Washington, PA) for analysis.

Analyses:

Molecular ecology analysis of the samples will be conducted with sample selection for intensive molecular characterization being guided by the results of the geochemical porewater analyses that occur shipboard or shortly thereafter.

We will extract DNA from cells using a method optimized for marine sediment communities (Luna et al. 2006) and with which we have had success (Colwell et al. 2011; Briggs et al. 2011; Briggs et al. 2012).

We will determine the presence and numbers of genes for methyl coM reductase subunit A (mcrA), dissimilatory bisulfite reductase (dsrAB), fermentation (hydA), and particulate methane monooxygenase (pmoA) which are indicative of methanogens/anaerobic methane oxidiz-

ers, sulfate reducers, fermenters, and aerobic methanotrophs, respectively, all involved in methane and organic carbon cycling in the sediments. To enumerate these genes we will use quantitative polymerase chain reaction (qPCR) as we have previously (Colwell et al. 2008; Nunoura et al. 2008) and with recent improvements of primers and methods to distinguish between ANME-I and methanogens, both of which possess mcrA (Lever, 2008; Joye et al. 2009; Lever 2013; M. Lever, personal communication).

To complement the qPCR studies we will determine the diversity of Bacteria and Archaea in selected samples using high-throughput, nextgeneration Illumina sequencing.

On selected sediment cores we will examine the relative activity of microbes that play a key role in methane carbon cycling. This will be approached using either 16SrRNA:rDNA ratios for microbial communities (Muttray and Mohn 2000) or an approach that targets the mRNA (messenger RNA) characteristic of the ANME cells as identified in the aforementioned studies (Chen et al. 2007; Freitag et al. 2010). These methods provide specific data on the activity of selected microbes based on the relative amounts of specific rRNAs or mRNAs.

Microbiological data obtained through qPCR to enumerate key functional genes will be compared using multivariate statistics (PC-ORD ver. 5.0; MjM Software, Inc.; McCune and Mefford, 2006) and QIIME (Caporaso et al. 2010) to determine the degree of similarity of the communities. Non-metric multidimensional scaling overprinted with biplots highlighting the values of abiotic parameters measured in the sediments will be used to evaluate how the microbial community patterns are aligned with key environmental parameters (Colwell et al. 2011; Huber et al. 2010; Briggs et al. 2012).

We will explore the possibility of using transcriptomics (RNA) to determine classes of genes microbes are transcribing, to hopefully gain insights into additional biogeochemical cycles that involve microbial activity.

MICROBIOLOGY:

DNA/RNA (CAGE & OSU)

OSU: every 30cm within a 2-m range of the SMTZ
~1m (0.5m if SMTZ is shallow) above and ~1m below the SMTZ range
from the bottom of each core

CAGE: every 30cm from bottom to top

4. use 50-ml cut-off syringes sterilized
5. extrude **50ml sed. into 50-ml Falcon tube** (2x)
6. label with core no. & cm
7. put tube into liquid N_2
8. store at -80C

SEDIMENT FOR CULTIVATION (CAGE) (every 30cm from bottom to top)

9. use 50-ml cut-off syringes
10. extrude **2 x 50ml sed. into 100-ml Schott bottle**
11. close bottle under nitrogen flush with black rubber and red screw cap
12. label with core no. & cm
13. store at 4C

FISH (CAGE) (every 30cm from bottom to top)

14. use 2-ml cut-off syringes
15. from 2ml sed. extrude **0.5ml sed. into each 2-ml Eppendorf tube** (2x2ml tubes)

16. label with core no. & cm (use Tough-Tags)
17. store at 4C until preservation

FISH (OSU)

every 50 cm within the 2 m SMTZ range
~1m (0.5m if SMTZ is shallow) above and ~1m below the SMTZ range for all cores
from the bottom of each core

18. use the same 2-ml cut-off syringes (as for FISH (CAGE))
19. extrude **5ml sed. into 50-ml Falcon tube**
20. label with core no. & cm
21. store at 4C until preservation

Preservation technique (CAGE):

add 1ml 4% Formaldehyde/PBS and mix
preserve at 4C for max. 12h
centrifugation at 13000rpm, 4°C, 10min and discard the supernatant
wash twice with 1ml 1xPBS, centrifugation, discard the supernatant
add 0.5ml PBS and 0.5ml cold EtOH (98%, sterile filtered)
store at -20C.

Preservation technique (OSU):

Add 15mL 2% PFA to 50-ml Falcon tube
preserve at 4C for 12 hours.
Add 15mL 1xPBS and 15ml EtOH (98%)
store at -80C

Numerical simulation: Stability and saturation of methane hydrate in marine sediments

Motivation

To review the methane hydrate stability and saturation expressions derived from several authors. These expressions will be cross-validated by results from lab experiments.

Setting the stage

We will focus this study on the four sites drilled during the 2010 UBGH2 expedition in Ulleung Basin, East Sea. These four sites were chosen as their pore water Cl profiles fall into two different categories: pore water freshening in one site and shallow Cl enrichment in the other three sites. As pressure and temperature are the most fundamental and important parameters to calculate methane hydrate stability and saturation, the information including water depth, BSR depth, and geothermal gradient for these four sites was compiled and provided in Table 1. The pressure and temperature ranges we are interested in are therefore 273-300 K and 9to 25 MPa. Cl content of these sites ranges from ~0 to 1438 mM or 0 to 82.4 PSU (practical salinity unit).

We will focus on the stability and saturation of sI hydrate with methane as the only gas component. We will therefore use methane hydrate, instead of gas hydrate, hereafter to specify the single component sI hydrate. Three phase equilibrium (aqueous-hydrate-vapor) will be computed to define the base of hydrate stability zone (HSZ). However, as our model takes into account only aqueous and hydrate phase (i.e., no multicomponent transport), the equilibrium of these two phases is more important. We consider NaCl as the only thermodynamic inhibitor. Other electrolytes such as KCl or $CaCl_2$ also serve as inhibitors (ref); however, due the an-order-of-magnitude higher content of NaCl comparing to other inhibitors and to simplify problem, we will primarily concern the effect from NaCl.

There are different ways to express the content of NaCl in the water. Units such as wt%, mole fraction, molarity (M=mol of NaCl per liter water), and molality (m=mol of NaCl per kg water) are most common in the literatures. In some literatures, salinity, which is defined as the total dissolved salt in the water (ref), instead of NaCl content is used in the literatures. Common units are PSU, wt%, or permil (‰). Conversion between salinity and NaCl content requires additional as-

sumption. In this work, we assume the salinity of seawater is 35 PSU and the concentration of major salts equals to the composition listed in Table 1 (DOE report by Dickson and Goyet, 1994). Calculating from this composition, the concentration of Cl is 0.56675 m or 0.55027 M assuming seawater density is 1.030 kg/L. This is therefore equals to 33.12 g/kg for NaCl content. We will follow this calculation to unify the different units from the literatures so that they are comparable.

Source of references

Laboratory measurements

A lot of the data used in this study is compiled in Sloan and Koh, (2008). The detail citation will be specified in the following paragraphs. Dickens and Quinby-Hunt (1994) measured hydrate stability in pure water and seawater for 3-11 MPa. Maekawa et al. (1995) conducted experiments with different NaCl concentrations under pressure as high as 18 MPa. These lab measurements are valuable in terms of validating different theoretical calculations.

Field observations

Depths of BSR and down-hole temperature measurements are also valuable information to evaluate theoretical calculations. These observations include all possible complexities from natural. A success theoretical calculation should be able to describe all these complexities. We used information from ten boreholes drilled during UBGH2 expedition in 2010 (ref). Depths of BSR and temperature measurements were reported elsewhere (ref). Different pore water composition at these sites (data from ref) is considered in this study when calculating methane hydrate stability.

Theoretical approaches

Stability and saturation expression from three different sources will be discusses here. Davie et al. (2004) presented expressions for methane solubility at three-phase boundary (water-hydrate-vapor or the hydrate stability zone, HSZ) based on the theoretical work done by Zatsepina and Buffett (1997). They extended the solubility below HSZ (i.e., into the free gas zone) by simple parametric models. Their expressions were applied to four different locations where the depths of three-phase equilibrium were well constrained. Their calculation is applicable from ~273 to 295

K, 10 to 30 MPa, and 0 to 1 molality of salinity (Zatsepina and Buffett, 1998). The second expression is from Tishchenko et al. (2005) where thermodynamic equations were established using the method of Pitzer (1991). These thermodynamic equations were approximated by empirical algorithms which are easily applicable to a wide range of condition (273-293 K, 0-50 MPa, and 0-70 salinity). The last source is from Sloan and Koh (2008) and the software CSMGem in the book. This is by far the most comprehensive expression which covers the equilibrium of different phases (aqueous, vapor, ice, sI hydrate, sII hydrate, sH hydrate, and salt precipitates) and can account for up to 30 different kinds of hydrocarbon gases and 4 non-hydrocarbon gases in the hydrate cage. Six different thermodynamic inhibitors are as well considered. The results of CSMGem have been cross-validated with lab data as well as other commercially-available software which does the similar calculation.

Only few lab measurements are available to validate these models.

Methane hydrate stability

T-P relationship at the three-phase-equilibrium without thermodynamic inhibitors

Before comparing the saturation prediction from different models, it is fundamentally important to discuss the relationship between temperature and pressure at the three-phase-equilibrium (i.e., water-hydrate-vapor). Such relationship can be calculated empirically from temperature measurements in the field and the depth where bottom simulator reflector is observed from seismic profiles. Alternatively, lab experiments were setup to obtain this relationship in a control environment. Theoretical calculation based on the statistical thermodynamic approach was developed (Sloan and Koh, 2008) and can be used to derive such T-P relationship. Here we provide reviews of relevant literatures.

Based on the work during ODP Leg141 at Chile Triple Junction, Brown and Bang (1995) derived temperature and equivalent water depth:

$$T(z) = m_3 + m_1 \times \log(\frac{z}{1000}) - m_2 \times (\log(\frac{z}{1000}))^2 \quad (1)$$

where $T(z)$ is temperature in ºC and as a function of depth (z) in meter. m_1, m_2, and m_3 are fitting parameters with 20.334, 2.296, and 12.949, respectively. Tishchenko et al. (2005) derived thermodynamic equations for methane hydrate stability and fitted the equations with an empirical algorithm. Detailed equation is not included here but the results were plotted in Figure 1a. A more recent work by Sloan and Koh (2008) developed the software CSMGem, based on the models proposed by Barrer and Stuart (1957), Waals and Platteeuw (1959) and Ballard (2002), to calculate methane hydrate stability by the statistical thermodynamic approach. The detail consideration and assumption can be found from their book. We calculated a series of temperature and pressure at the three-phase equilibrium with the software.

Predictions from three different works and measurements from lab experiments (Roberts et al., 1940; Deaton and Frost, 1946; Kobayashi and Katz, 1949; McLeod and Campbell, 1961; Marshall et al., 1964; Jhaveri and Robinson, 1965; Galloway et al., 1970; Verma, 1974; de Roo et al., 1983; Thakore and Holder, 1987; Adisasmito et al., 1991; Dickens and Quinby-Hunt, 1994; Dyadin and Aladko, 1996; Nakano et al., 1999; Nakamura et al, 2003) compiled by Sloan and Koh (2008) were included in Figure 1a. Excellent agreement among all the measurements and model predictions can be seen within the temperature and pressure range of interest suggesting the success of all models.

T-P relationship at the three-phase-equilibrium with thermodynamic inhibitors

Dickens and Quinby-Hunt (1994) determined the T-P condition at three-phase equilibrium from lab experiments with pure water and seawater (33.5 ‰ salinity). The seawater data were later fitted as:

$$\frac{1}{T} = 3.79\times10^{-3} - 2.84\times10^{-4}(\log P) \quad (2)$$

where T and P are temperature and pressure in K and MPa. Brown and Bang fitted Eq. (1) with the data from Dickens and Quinby-Hunt (1994) and concluded m_1, m_2, and m_3 are 20.5, 2.2, and 11.66, respectively, for seawater salinity (33.5 ‰). Calculations of methane hydrate stability were done with the algorithm proposed by Tishchenko et al. (2005) with seawater composition

and CSMGem with 0.55 M of dissolved NaCl concentration (0.55 mole + 57.2 mole of water assuming water density is 1030 kg/m^3). These calculated results along with the lab measurements from Dickens and Quinby-Hunt were plotted in Figure 1b.

First of all, one can notice the very sparse lab measurements suggesting more experiments, especially for pressure over 10 MPa, are necessary. Except for the predictions by Dickens and Quinby-Hunt (1994), which should only be applicable within the T and P ranges of their measurements, the rest three models predict very similar values within the range of interest. The range of temperature predicted by the three models for a certain pressure is ~0.5 degree. Temperature and pressure at 10 sites drilled during UBGH2 were also plotted to verify the relationships under high pressure (10-24 MPa) from different works.

Depths of BSR were identified from seismic profiles (ref). Temperature sat each sites were calculated from seafloor temperature and geothermal gradient as detailed by ref and listed in Table 2.Salinty of these sites ranges from 18.2 to 38.3 PSU (ref). With the knowledge of pressure and salinity, we estimated temperature at three phase equilibrium with different methods. P-T relationships enclose this salinity range were calculated with three different methods and presented in Figure 1c. In sum, both algorithms proposed by Tishchenko et al. (2005) and Xu (????) provide accurate estimations. We estimated the equilibrium temperature with the knowledge of pressure and pore water composition (Cl, K, and Ca content in pore water) with CSMGem. The estimation is 0.1 to 0.7 degree different from what was measured at all sites. The temperature at UBGH2-7 is the only site that its temperature can not be accurately estimated by all methods. We therefore suggest that the base of HSZ at UBGH2-7 is over-heated by flow from beneath.

For methane hydrate stability at even higher salinity (>35 PSU), the expressions derived by Brown and Bang (1995) and Dickens and Quinby-Hunt (1994) are no longer applicable. We validated the expressions from Xu (????), Tishchenko et al. (2005), and CSMGem with lab measurements from Kobayashi et al. (1951), de Roo et al. (1983), and Maekawa et al. (1995). Maekawa et al. (1995) fitted their lab measurements of hydrate stability under different salinity conditions with the following equation:

$$Ln(\frac{P}{P_0}) = -926.815 + \frac{31979.3}{T} + 144.909Ln(T) + 5847.92x + 322.026x^2 + 5840.5Ln(1-x)$$

where P and P_0 are pressure in MPa at different depth and atmospheric pressure (0.101 MPa). T is temperature in K. x is the mole fraction of NaCl in the aqueous phase. Only the estimation by CSMGem can provide estimation that agrees with these lab measurements (Figure 1d).

In conclusion, most of the theoretical estimation based on thermodynamic calculation can provide excellent estimation of methane hydrate stability under the condition of no thermodynamic inhibitor (e.g., NaCl). Less estimation is satisfactory under sea water condition; even less satisfactor when the pressure is over 10 MPa. Field observations from the 10 sites during UBGH2 expedition can be well reproduced by the calculation by Xu (????), Tishchenko et al. (2005), and CSMGem (Sloan and Koh, 2008). For methane hydrate stability higher under higher salinity (> 35PSU), only CSMGem can provide good estimation when comparing with lab measurements.

Methane hydrate saturation

Parametric model from Davie et al., (2004)

The expression developed by Davie et al. (2004) is basically a fitting algorithm to the theoretical calculation done by Zatsepina and Buffett (1997). This expression has two essential equations:

$$C_3(T,P,S) = (1-\beta S)\left[C_3(T_0,P_0,0) + \frac{\partial C_3(T,P,0)}{\partial T}(T-T_0) + \frac{\partial C_3(T,P,0)}{\partial P}(P-P_0)\right] \quad (3)$$

$$C_{eq}(T) = C_3(T,P,S)\exp(\frac{T-T_3}{\alpha}) \quad (4)$$

$C_3(T,P,S)$ is the solubility of methane hydrate (i.e., methane concentration) under at three phase equilibrium. This solubility can be uniquely defined with the knowledge of salinity (S) and either temperature (T) or pressure (P) following the approaches provided in next paragrpah. T_0 and P_0 in Eq. (3) are the temperature and pressure of some reference state. β is a parameter determined from the theoretical calculation of Zatsepina and Buffett (1997). C_{eq} in Eq. (4) is the methane hy-

drate saturation within HSZ (i.e., within the condition where only two phases are present). a in Eq. (4) is obtained in by fitting the parametric model of Davie et al. (2004) with the theoretical calculation in Zatsepina and Buffett (1997).

The relationship between T and P at the three phase equilibrium is experimentally determined by Dickens and Quinby-Hunt (1994) for pure water and seawater (S=33.5 ‰).

The parameters required in Eqs. (3) and (4) are provided in Davie et al. (2004) and listed in Table 3.

Table 1 Basin parameter of the four study sites in Ulleung Basin

	UBGH2-2_2	UBGH2-3	UBGH2-7	UBGH2-11
Water depth (m)	2093	898	2145	2082
P at seafloor (MPa)[a]	21.13	9.06	21.65	21.02
Seafloor temperature (K)[b]	273.35	273.45	273.55	274.35
BSR depth (mbsf)	180.5	131.6	124	159
P at BSR (MPa) [a]	22.95	10.39	22.90	22.62
BSR temperature (K)[c]	292.7	286	294.8	292.2
First hydrate appear-ance depth (mbsf)[d]	67.9	6.2	7	7
P at 1st GH (MPa) [a]	21.81	9.13	21.71	21.09
1st GH temperature (K)[c]	273.4	274	274.6	275.1
Geothermal gradient (°C/m)[e]	0.107	0.095	0.171	0.120
Salinity at BSR (kg/kg)	1.44E-2	2.94E-2	2.50E-2	1.73E-2

[a] Pressure was calculated assuming 1030 kg/m^3 for seawater density and 9.8 m^2/sec for gravitational acceleration.

[b] seafloor temperature was measured at each of the drilling site (Lee et al., 2013).

[c] temperature is estimated from seafloor temperature and geothermal gradient

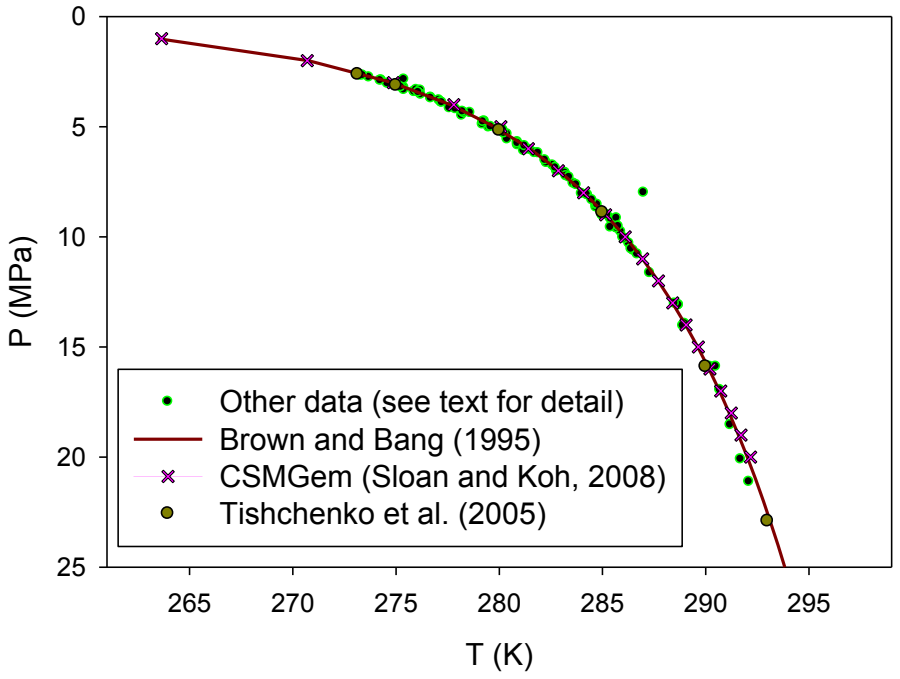
[d] The depth of hydrate first appearance was determined by either visual observations of hydrate of pore water anomalies.

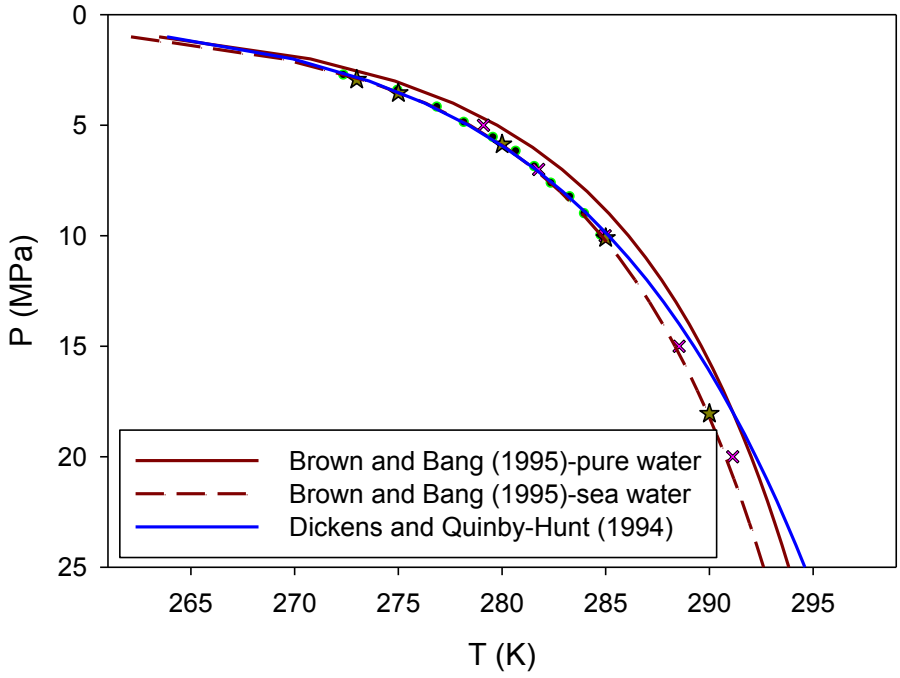
[e] geothermal gradient determined from linear regression of downhole temperature measurements at all UBGH2 drill-sites (Riedel et al., 2013).

Table 2 Parameters required in Eqs. (3) and (4) to calculate methane hydrate stability and saturation following the Davie et al. (2004) approach.

Parameters	T_0	P_0	α	β	$C_3(T_0,P_0,S)$	$\frac{\partial C_3(T,P,0)}{\partial T}$	$\frac{\partial C_3(T,P,0)}{\partial P}$
Values suggested by Davie et al. (2004)	292 K	20 MPa	14.4 ºC	0.1 mol^{-1}	153.36 mM	6.34 mM/K	1.11 mM/MPa

Figure 1


0
5
10
15
20
25
P (MPa)
265
270
275
280
285
290
295
T (K)
Other data (see text for detail)
Brown and Bang (1995)
CSMGem (Sloan and Koh, 2008)
Tishchenko et al. (2005)


0
5
10
15
20
25
P (MPa)
265
270
275
280
285
290
295
T (K)
Brown and Bang (1995)-pure water
Brown and Bang (1995)-sea water
Dickens and Quinby-Hunt (1994)

:

ATTACHMENT 2

STATEMENT of PROJECT OBJECTIVES

Assessing the Response of Methane Hydrates to Environmental Change at the Svalbard Continental Margin

A. Objectives

The overall objective of this research is to constrain the biogeochemical response of the gas hydrate system on the Svalbard margin to environmental change. The locations sampled shall provide key datasets that allow examination of the system with respect to sediment temperature fluctuations driven by thermal changes in the overlying water column and by hydrothermal circulation in the sediments. The specific project objectives are:

1) Prepare for and collect water and sediment core samples from a high latitude setting (the Svalbard Margin) across gradients where methane hydrates show vulnerability to environmental change.

2) Analyze the samples to assess changes in chemistry and microbiology across vertical gradients (i.e., within a core) and horizontal gradients (i.e., across the putative upper edge of gas hydrate stability) that constrain the biogeochemical response at locations where methane hydrates are sensitive to environmental change.

3) Use a kinetic modeling approach to synthesize the expected response of carbon cycling pathways around the sensitive sulfate methane transition zone (SMTZ) to environmental change, and combine the results from this research with previous results from Cascadia and Ulleung Basin settings.

B. Scope of Project

The scope of work involves collecting samples, sample analysis, and numerical modeling of data derived and integrated from the analyses. During Phase 1 of the project, the Recipient shall prepare for and then participate in the sampling of the Svalbard Margin. The main goal is to investigate the active interplay of fluid and gas flow processes from the sediments to the water column and the gas hydrate dynamics in relation to seawater temperature changes. To this end, the Recipient shall collect field data during Phase 1 to determine the geochemical and microbiological properties of pore water, sediment, and the water column. The Recipient shall also begin some of the sample analyses (e.g., using the CRDS and Deep Water Analyzer) and modeling during Phase 1.

Phase 2 involves analysis of the sediments, pore water, and hydrate lattice water for geochemical and microbiological parameters from Phase 1, plus participation on a second expedition. During Phase 3, we will complete the geochemical and microbiological

analyses and the data from both expeditions shall be integrated into the numerical model with the aim to constrain methane-carbon cycling in the SMTZ and determine the environmental consequences due to temperature perturbations. During this phase the Recipient shall prepare the data for release to the scientific community and to the public.

C. Tasks and Subtasks to Be Performed

PHASE 1/BUDGET PERIOD 1

Task 1.0 – Project management and planning

The Recipient shall work together with the DOE project officer upon award to develop a project management plan (PMP). The PMP shall be submitted within 30 days of the award. The DOE project officer shall have 20 calendar days from receipt of the PMP to review and provide comments to the Recipient. Within 15 calendar days after receipt of the DOE's comments, the Recipient shall submit a final PMP to the DOE project officer for review and approval.

The Recipient shall review, update, and amend the PMP (as requested by the DOE project officer) at key points in the project, notably upon schedule variances of more than 3 months and cost variances of more than 10%, which require amendments to the agreement and constitutes a re-base lining of the project. The PMP shall define the approach to management of the project and include information relative to project risk, timelines, milestones, funding and cost plans, and decision-point success criteria.

The Recipient shall execute the project in accordance with the approved PMP covering the entire project period. The Recipient shall manage and control project activities in accordance with their established processes and procedures to ensure subtasks and tasks are completed within schedule and budget constraints defined by the PMP. This includes tracking and reporting progress and project risks to DOE and other stakeholders.

Task 2.0 – Pre-expedition preparation and preliminary analysis

> *Subtask 2.1 - equipment purchase and testing:* The Recipient shall purchase and calibrate two cavity ring-down spectrometer CRDS for in situ measurements of methane, and methane isotopes. The systems shall be calibrated and tested the Recipient shall report to DOE system readiness (see Section D. Deliverables).
>
> *Subtask 2.2 - preliminary microbial analyses:* The Recipient shall conduct preliminary microbiological analyses to optimize both the extraction of DNA from cells in sediments and the use of primers and reaction conditions for qPCR, and also to determine the levels of RNA and DNA that are expected in the samples from

the Svalbard Margin based on studies with model sediments and literature values for related systems.

Subtask 2.3 - model adaptation: The Recipient shall adapt the kinetic-transport-reaction model for integration of data from the Svalbard Margin systems. Specifically, the Recipient shall develop code to 1) test the AOM efficacy to stop methane leaking to water column under a big gas hydrate dissociation event; and 2) allow for 2-D modeling in the sediments along the planned cored transect.

Task 3.0 – Expedition #1 participation

Subtask 3.1 - mobilization: This task involves procurement of the required materials and supplies necessary for shipboard sample collection and analyses. The Recipient shall confirm the transit and loading of all equipment, supplies etc. and proper setup in the chemical laboratories.

Subtask 3.2 - core sampling and onboard analysis: Gravity cores obtained during the expedition by scientists shall be sampled onboard by the Recipient to collect sediment, and pore water for subsequent geochemistry and microbiological studies.

Pore water samples and analyses shall be conducted using established techniques. Pore water samples shall be obtained by rhizons prior to core splitting for analysis of ammonium, DIC, phosphate, chloride, sulfate, and dissolved cations (Ca, Mg, Sr, K, Ba, S, Mn, Si, B, Li). The Recipient shall have access to these data to support subsequent modeling efforts in Task 5.0. Additional aliquots of the pore water shall be taken to determine the concentration of sulfate and $\delta^{18}O$ of pore water analysis at the Recipients labs.

Samples for microbiological analysis shall be obtained from the cores in close physical proximity to samples obtained for geochemical and physical parameters of the sediments. For each of the split cores microbiological samples shall be collected approximately every 25-60 cm through the core depth. Additional “samples of opportunity” shall be taken if sediment features such as fractures indicate active fluid movement in the samples. Microbiological samples (100-200 g) shall be collected from the interior of the cores and then placed into sterile bags resistant to -80oC. Samples for DNA analyses shall be frozen immediately in liquid N2 and samples for RNA analysis shall be preserved with RNALater or in a similar solution to retard the activity of RNases, and then refrigerated or frozen at -20°C. At the end of the expedition frozen samples shall be shipped to the Recipient’s labs in dry shippers for analysis.

PHASE 2/BUDGET PERIOD 2

Task 4.0 – Post-expedition analysis

Subtask 4.1 - geochemistry analyses: The geochemical shore-based program includes analyses of pore water for isotopic composition of the water and the dissolved inorganic carbon. These analyses shall be conducted by the Recipient at the Oregon State University CEOAS isotope ratio mass spectrometer (IRMS) facility.

Subtask 4.2 - microbiology analysis: Molecular ecology analysis of the samples shall be conducted post-expedition at the Recipient's microbiology lab with sample selection for intensive molecular characterization guided by the results of the geochemical porewater analyses that occur shipboard or shortly thereafter. The Recipient shall extract DNA from cells using a method optimized for marine sediment communities. The Recipient shall determine the presence and numbers of genes for methyl coM reductase subunit A (mcrA), dissimilatory bisulfite reductase (dsrAB), fermentation (hydA), and particulate methane monooxygenase (pmoA) which are indicative of methanogens/anaerobic methane oxidizers, sulfate reducers, fermenters, and aerobic methanotrophs, respectively, all involved in methane and organic carbon cycling in the sediments. To enumerate these genes the Recipient shall use quantitative polymerase chain reaction (qPCR) utilizing recent improvements of primers and methods to distinguish between ANME-I and methanogens, both of which possess mcrA. To complement the qPCR studies, the Recipient shall determine the diversity of Bacteria and Archaea in selected samples using high-throughput, next generation techniques such as Illumina sequencing.

On selected sediment cores determined to be in high methane flux locations by geochemical and physical measurements and from cores deemed to be useful control sediments where methane flux is low, the Recipient shall examine the relative activity of microbes that play a key role in methane carbon cycling. The Recipient shall utilize an approach that provides specific data on the activity of selected microbes based on the relative amounts of specific rRNAs or mRNAs.

Microbiological data obtained through qPCR to enumerate key functional genes shall be compared using multivariate statistics (PC-ORD ver. 5.0; MjM Software, Inc.) and QIIME to determine the degree of similarity of the communities. Non-metric multidimensional scaling overprinted with biplots highlighting the values of abiotic parameters measured in the sediments shall be used to evaluate how the microbial community patterns are aligned with key environmental parameters.

Task 5. Expedition 2

Subtask 5.1 - mobilization: This task involves procurement of the required materials and supplies necessary for shipboard sample collection and analyses. The Recipient shall confirm the transit and loading of all equipment, supplies etc. and proper setup in the chemical laboratories.

Subtask 5.2 - water column surveys/sampling: To further constrain the distribution of methane in the water column in relation to potential locations of methane release from sediment sites the Recipient will conduct a series of hydrographic stations using a CTD/rosette. Sampling intervals shall be determined in real time while onboard to best capture observed acoustic signals with higher resolution sampling within an active plume and lower resolution outside the plume to determine background methane concentrations onboard. Additional water column samples obtained during the expedition by scientists shall be sampled onboard by the Recipient for subsequent chemistry analyses.

Subtask 5.3: - -core sampling: Sediment samples collected during the cruise will be subsampled with rhizones for pore water analyses and sediment will be collected for microbiological studies. The sampling stations will be guided by remote-operated vehicle (ROV) surveys

Subtask 5.4 - gas hydrate composition and abundance: The Recipient shall support onboard gas sampling activities. Gas samples shall be obtained from sampling of gravity cores, and if recovered, controlled dissolution of hydrate pieces. Gas samples from gravity cores shall be analyzed onboard headspace techniques followed by analyses by the DOE-purchased CRDS. Intact gas hydrate pieces shall be dissociated at ambient temperature onboard, and the released gas phase analyzed by CRDS.

PHASE 3/BUDGET PERIOD 3

Task 6.0 – Expedition 2 Analyses

Subtask 6.1 - geochemistry and microbiology analyses: Sediment samples recovered from Expedition 2 will be analyzed for geochemistry and microbiology as detailed in Task 4

Task 7.0 Numerical modeling

Data from the microbiological and geochemistry analysis tasks shall be integrated as they become available into the kinetic-transport-reaction model. This model, developed by the Recipient using the FORTRAN-based CrunchFlow, shall be used to constrain and quantify reaction network around the SMTZ and changes to the carbon cycling pathways resulting from variations in methane flux.

Task 8.0 – Data synthesis and integration

Chemistry, microbiology, and numerical modeling studies shall be completed and fully integrated. Data shall be synthesized and prepared for presentation at science meetings and for inclusion in draft manuscripts for submission to peer review journals for publication. Draft manuscripts shall be provided to the DOE Project Officer in accordance with

the Reporting Requirements. The Recipient shall also provide a draft article of integrated findings for the Methane Hydrates Fire-in-the-Ice (FITI) newsletter.

D. Deliverables

The periodic, topical, and final reports shall be submitted in accordance with the attached "Federal Assistance Reporting Checklist" and the instructions accompanying the checklist.
In addition to the deliverables identified on the "Federal Assistance Reporting Checklist," the Recipient shall provide the following deliverables:

Phase 1

1. Task 1 – Project Management Plan, due to DOE 30 days after start of project

2. Task 2 – Report (ca. five pages) to DOE Project Officer on status of expedition preparations. Report shall include a section that details receipt of the CRDS analyzer. In addition, the report shall provide for preliminary sampling and onboard analysis plan for the expedition. Due 30 September 2014

3. Task 3 – Expedition report to DOE Project Officer detailing the daily and cumulative samples logged and those made available to Task 4. The report shall include a summary of preliminary findings of shipboard analyses. Due 30 days post-expedition.

Phase 2

4. Task 4 – Report to the DOE Project Officer summarizing progress related to microbiology and geochemical analyses including preliminary findings and initial results of the numerical modeling. To be submitted within six months of completing the expedition.

5. Task 5 - Report (ca. five pages) to DOE Project Officer on status of expedition #2 preparations. The report shall provide for preliminary sampling and onboard analysis plan for the expedition. Due 15 June 2015.

Phase 3

6. Task 6 -Expedition report to DOE Project Officer detailing the daily and cumulative samples logged. The report shall include a summary of preliminary findings of shipboard analyses. Due 30 days post-expedition.

7. Task 6- Report to the DOE Project Officer summarizing progress related to microbiology and chemical analyses including preliminary findings and initial results of the numerical modeling. To be submitted within six months of completing the expedition #2.

8. Task 7 – Peer-review paper submissions draft manuscript and draft FITI article. Due by project end-date;

E. BRIEFINGS/TECHNICAL PRESENTATIONS (If applicable)

The Recipient shall prepare detailed briefings for presentation to the Project Officer at the Project Officer's facility located in Pittsburgh, PA or Morgantown, WV. These presentation briefings may be conducted via Webex at the Program Officer's discretion. Briefings shall be given by the Recipient to explain the plans, progress, and results of the technical effort. The Recipient shall present project briefings as follows:

- Project Kick-off – within 60 days of award
- Continuation Application – within 60 days of Recipient's request to proceed to next project Budget Period
- Final Project Presentation – within 30 days of project end date

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