CARBONATE-ASSOCIATED MICROBIAL ECOLOGY AT METHANE SEEPS

ASSEMBLAGE COMPOSITION, RESPONSE TO CHANGING ENVIRONMENTAL CONDITIONS, AND IMPLICATIONS FOR BIOMARKER LONGEVITY

Thesis by

David Hamilton Case

In Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

Caltech

CALIFORNIA INSTITUTE OF TECHNOLOGY

Pasadena, California

2016

(Defended May 12, 2016)
DEDICATION

Dedicated with love to,

C. Randy Case & Beth Ann Hamilton,
Charles, Monica, & Madeleine Case,
Peter, Aneta, Michael, & Alexander Case,
V. Edward & D. Jane Hamilton
Charles & Ilyeene Case
ACKNOWLEDGEMENTS

I am indebted to my thesis advisor, Victoria Orphan, for providing an open and supportive environment in which to explore science. Perhaps Victoria’s greatest strength as a mentor is the smile she imparts on every student as they walk into her office – without exception, I never once left a meeting with her feeling anything but better about my science and myself as a scientist. Such support is a great gift from Victoria to her students – thank you!

In addition, many other thoughtful, helpful, and friendly scientists have rotated through the Orphan lab and provided mentorship and friendship to me during the past six years. In thanking them all, I would like to especially acknowledge Derek Smith, Ally Pasulka, Connor Skennerton, Stephanie Connon, Elizabeth Trembath-Reichert, Roh Bhartia, Hank Yu, Jeff Marlow, Kat Dawson, Patty Tavormina, Roland Hatzenpichler, Silvan Scheller, Anne Dekas, Greg Wanger, and Sean Mullin for playing important parts in my graduate career.

Other mentors and peers at Caltech have also indelibly influenced my experience and shaped the scholar I have become. I have received invaluable advice from my committee members: Woody Fischer, Lisa Levin, Jess Adkins, and Jared Leadbetter. Members of my original graduate class – officemates in the “Pit” – have made the last six years more meaningful than merely an academic experience: Jeff Prancevic, Jena Johnson, Stephen Cox, Sarah Slotznick, and Elizabeth Trembath-Reichert. Paul Asimow, Cassandra Horii, and Daniel Thomas shaped my extracurricular interest in high-quality teaching, which I explored extensively while at Caltech.

My completion of this thesis would not have been possible without the support and influence of countless others. It is impossible to list the names of every person who has touched my life, leading to this accomplishment, and I cannot put into words how grateful I am to the cast of family, friends, colleagues, and peers who have shaped my journey. Acknowledging that a mere list does not sufficiently connote the importance of the following people, and that many important names are not given here, I nonetheless want to note the following people who mean so much to me: Brian Kohan, Chris Marotta, the Kahn family, Joel and Elaine Ziskind, Sophie Hines, Greg Adamson, Kevin Okura, Jason Lopez, Lee Coleman, Susan Chadwick & family, Divina Bautista, Alice Sogomonian, Charles Niesen, Linnea Larson, Ben Willis & the DWA team, Dan Giammar, Keith Troche, Steve Cory, Jaina Krueger, Michelle Pai, Kate Wilson, Gordon Johnston, Andrew Harris, Jon Michael Rasmus, Bill Bloomenkrantz, Sarah Scallon, Greg Emmerich, Bennett Javenkoski, Brian Stoveken, Jessica Pellegrino, Sharon Stahl, Andrew Harris, Julie Jensen, Joe Spolar, Larry Henderson, and Mary Schmidt.

To everyone, named here and otherwise – Thank You
ABSTRACT

Methane seeps are globally distributed geologic features in which reduced fluid from below the seafloor is advected upward and meets the oxidized bottom waters of Earth’s oceans. This redox gradient fuels chemoautotrophic communities anchored by the microbially-mediated anaerobic oxidation of methane (AOM). Both today and in Earth’s past, methane seeps have supported diverse biological communities extending from microorganisms to macrofauna and adding to the diversity of life on Earth. Simultaneously, the carbon cycling associated with methane seeps may have played a significant role in modulating ancient Earth’s climate, particularly by acting as a control on methane emissions.

The AOM metabolism generates alkalinity and dissolved inorganic carbon (DIC) and at a 2:1 ratio, promoting the abiogenic, or authigenic, precipitation of carbonate minerals. Over time, these precipitates can grow into pavements covering hundreds of square meters on the seafloor and dominating the volumetric habitat space available in seep ecosystems. Importantly, carbonates are incorporated into the geologic record and therefore preserve an inorganic (i.e., $\delta^{13}C$) and organic (i.e., lipid biomarker) history of methane seepage. However, the extent to which preserved biomarkers represent a snapshot of microorganisms present at the time of primary precipitation, a time-integrated history of microbial assemblages across the life cycle of a methane seep, or a view of the final microorganisms inhabiting a carbonate prior to incorporation in the sedimentary record is unresolved.

This thesis addresses the ecology of carbonate-associated seep microorganisms. Chapters One and Two contextualize the extant microbial diversity on seep carbonates versus within seep sediments, as determined through 16S rRNA gene biomarkers. Small, protolithic carbonate “nodules” recovered from within seep sediments are observed to be capable of capturing surrounding sediment-hosted microbial diversity, but in some cases also diverge from sediments. Meanwhile, lithified carbonate blocks recovered from the seafloor host microbial assemblages demonstrably distinct from seep sediments (and seep nodules). Microbial 16S rRNA gene diversity within carbonate samples is well-differentiated by the extent of contemporary seepage. In situ seafloor transplantation experiments further demonstrated the microbial assemblages associated with seep carbonates to be sensitive to seep quiescence and activation on short (13-month) timescales. This was particularly true for organisms whose 16S rRNA genes imply physiologies dependent on methane or sulfur oxidation. With an improved understanding of the modern ecology of carbonate-associated microorganisms, Chapter Three applies intact polar lipid (IPL) and core lipid analyses to begin describing whether, and to what extent, geologically relevant biomarkers mimic short-term dynamics observed in 16S rRNA gene profiles versus archive a record of historic microbial diversity. Biomarker longevity is determined to increase from 16S rRNA genes to IPLs to core lipids, with IPLs preserving microbial diversity history on timescales more similar to 16S rRNA genes than core lipids. Ultimately, individual IPL biomarkers are identified which may be robust proxies for determining whether the biomarker profile recorded in a seep carbonate represents vestiges of active seepage processes, or the profile of a microbial community persisting after seep quiescence.
PUBLISHED CONTENT AND CONTRIBUTIONS

The material presented in Chapter One is published in Mason & Case et al. (2015). In the publication, D.H.C. and O.U.M. share co-first authorship. D.H.C. performed iTag processing and analysis, beta diversity analyses, and was the principal and coordinating author of the manuscript. O.U.M. processed the samples and optimized DNA extraction, as well as terminal restriction fragment length polymorphism and clone library analyses. V.J.O. conceived of the study and collected samples at sea. T.H.N. provided X-ray diffraction data, R.W.L. performed the isotopic composition analyses, J.V.B. provided thin section images, and R.B.T. performed the pore water geochemical measurements. D.H.C., O.U.M., and V.J.O. principally wrote the manuscript.


The material presented in Chapter Two is published in Case et al. (2015). D.H.C. performed the lab work, prepared the samples for sequencing, analyzed and interpreted all data, and was the principal and coordinating author of the manuscript. V.J.O. and L.A.L. led field sampling and coordinated the seafloor experiments. All authors, including A.L.P., J.J.M., and B.M.G., provided intellectual and writing contributions.


The material presented in Chapter Three is in preparation for publication by the listed authors. D.H.C. is the principal and coordinating author of the manuscript, acquired all iTag and geochemical data, performed all computational analyses, and generated interpretations of the data. M.Y.Y. and A.J.G. performed bench-top lipid extraction and quantification. V.J.O. led field sampling and coordinated the seafloor experiments. All authors, including A.L.P., K.S.D., and K.U.H., provided intellectual and writing contributions.


The material presented in Chapter Four is available as a laboratory resource and will be openly provided to any researchers interested in the results. To facilitate in dissemination of this data, the authors are preparing to make the chapter and data publically available online, including as part of this thesis through thesis.library.caltech.edu. D.H.C. and S.A.C. coordinated bench-top lab work, D.H.C. performed data analyses and generated interpretations. All authors, including A.L.P., E.T.-R., K.S.D., C.T.S., and V.J.O., provided intellectual and writing contributions.


The material presented in Appendix One is in preparation for publication by the listed authors. D.H.C. is the principal and coordinating author of the manuscript, performed all microbiological bench-top laboratory work, and generated interpretations of the data. A.I. provided geochemoal data. P.T. helped with clone library assays. D.H.C., Y.M., A.I., and F.I. conceived of the study. All authors, including V.J.O., provided intellectual and writing contributions.

# TABLE OF CONTENTS

Dedication .....................................................................................................................v  
Acknowledgements ......................................................................................................vi  
Abstract .........................................................................................................................vii  
Published Content and Contributions ..........................................................................viii  
Table of Contents ...........................................................................................................ix  
List of Figures and Tables ..............................................................................................xi  
List of Acronyms & Abbreviations ..............................................................................xiv  

Introduction ..................................................................................................................1  

Chapter One: Comparison of Archaeal and Bacterial Diversity in  
Methane Seep Carbonate Nodules and Host Sediments, Eel River Basin  
and Hydrate Ridge, USA ............................................................................................19  
1.0 Abstract ..................................................................................................................20  
1.1 Introduction .............................................................................................................21  
1.2 Methods ..................................................................................................................24  
1.3 Results .....................................................................................................................33  
1.4 Discussion ...............................................................................................................41  
1.5 Conclusions ............................................................................................................48  
1.6 Acknowledgements .................................................................................................50  
1.7 Tables ......................................................................................................................51  
1.8 Figures .....................................................................................................................53  
1.9 Supplemental Text ...................................................................................................58  
1.10 Supplemental Tables ..............................................................................................60  
1.11 Supplemental Figures ............................................................................................65  
1.12 References ............................................................................................................67  

Chapter Two: Methane Seep Carbonates Host Distinct, Diverse, and  
Dynamic Microbial Assemblages ..............................................................................75  
2.0 Abstract ..................................................................................................................76  
2.1 Importance .............................................................................................................77  
2.2. Introduction ..........................................................................................................78  
2.3 Materials and Methods ..........................................................................................80  
2.4 Results and Discussion ..........................................................................................84  
2.5 Conclusions ............................................................................................................98  
2.6 Acknowledgements ................................................................................................99  
2.7 Figures .....................................................................................................................100  
2.8 Supplemental Text ................................................................................................105  
2.9 Supplemental Tables .............................................................................................113  
2.10 Supplemental Figures ..........................................................................................117  
2.11 References ............................................................................................................123  

Chapter Three: Observed and Modeled Turnover of Carbonate-  
Associated Microbial Biomarkers During in situ Simulated Methane Seep  
Quiescence ..................................................................................................................129  
3.0 Abstract ..................................................................................................................130  
3.1. Introduction ..........................................................................................................131  
3.2 Materials and Methods .........................................................................................135
LIST OF FIGURES AND TABLES

Abbreviated as (Chapter Number)-(Figure/Table Number)

CHAPTER ONE

Tables

1-1: Geochemical Observations .................................................. 51
1-2: Microbiological Observations ............................................. 52
1-S1: Archaeal iTAG sequence data ........................................ 60
1-S2: Bacterial iTAG sequence data .......................................... 61-64

Figures

1-1: $\delta^{13}$C variation at Hydrate Ridge and Eel River Basin ........ 53
1-2: Nonmetric Multidimensional Scaling Ordinations ................. 54
1-3: Petrographic thin sections of nodules ................................ 55
1-4: Phylogenetic analysis of archaeal clones ............................. 56
1-5: Phylogenetic analysis of bacterial clones ............................. 57
1-S1: Procrustes comparison between iTAG and TRFLP datasets ...... 65
1-S2: XRD spectra from nodules .............................................. 66

CHAPTER TWO

Tables

2-S1: All samples listed with their accompanying metadata .......... 113-114
2-S2: List of ANOISM test results ............................................. 115
2-S3: Relative abundance of OTUs presented in Figure 3, 4, and 5 .. 116

Figures

2-1: Non-metric multidimensional scaling plot of microbial assemblages .... 100
2-2: Collector’s curves of estimated Chao1 OTU$_{97}$ richness ........ 101
2-3: Boxplot of OTU relative abundances from the 82 native samples .. 102
2-4: Comparison of OTU$_{97}$ overlap among samples and treatments ... 103
2-5: Boxplot of carbonate-associated data of key OTUs ............... 104
2-S1: Overview map of sampling locations ............................... 117
2-S2: Additional alpha diversity metrics .................................. 118
2-S3: Additional non-metric multidimensional scaling analyses .... 119
2-S5: Mineralogical analysis ............................................... 120
2-S6: Distance-decay plot of native carbonates .......................... 121
2-S7: OTU overlap between samples from station HR-9 .............. 122
CHAPTER THREE
Tables
3-1: Samples and their accompanying metadata.................................162
3-2: Timepoints used to define terms in Equation 1..........................163
3-3: Timepoints used to define the model of specific biomarker turnover ......164
3-S1: Standards for correcting IPL concentrations................................183
3-S2: Raw lipid data for the IPL and ASL datasets..............................184
3-S3: Possible sources of archaeal polar lipids....................................185
3-S4: Pearson R correlations between 16S rRNA gene OTUs and lipids........186
3-S5: Biomarker data used to generate Venn diagrams in Figs. 1 and S1........187

Figures
3-1: Lipid and geochemical data from authigenic carbonates....................165
3-2: Specific IPL ratios of native carbonates......................................166
3-3: Ternary diagrams of lipid biomarkers associated with mineralogy.........167
3-4: Non-metric multidimensional scaling ordinations............................168
3-5: Venn diagrams of lipid richness for parallel transplant experiments .........169
3-6: Model of biomarker richness over time......................................170
3-7: Box plots of specific biomarker abundances................................171
3-8: Model of specific biomarker shifts over time................................172
3-9: Calculated cell concentrations of ANME-1 and ANME-2...................173
3-S1: Venn diagrams of biomarkers by mineralogy...............................188
3-S2: Non-metric multidimensional scaling of IPLs................................189
3-S3: Cross-plot of $\delta^{13}C_{\text{org}}$ vs concentration of various archaeal IPLs ........190

CHAPTER FOUR
Tables
4-1: Relative abundances of taxa in the plasmid mock communities..........231
4-2: Relative abundances of taxa in the genomic mock communities...........232
4-3: Synthetic relative abundance data of species from five samples..........233
4-4: Bray-Curtis similarity of five synthetic samples in Table 3...............233
4-5: Ranked similarities of five synthetic samples in Table 3..................233

Figures
4-1: Flow chart of procedures for processing iTag samples......................234
4-2: Examination of loss of sequences at each step of data processing ........235
4-3: Relative abundance of taxa in negative controls............................236
4-4: Relative abundance of OTUs in the plasmid mock communities...........237
4-5: Reproducibility (precision) of iTag sequencing of mock communities.....238
4-6: Accuracy of iTag sequencing of plasmid mock communities...............239
4-7: Comparison of single vs two-step PCR results..............................240
APPENDIX ONE

 Tables

A1-S1: Relative abundance of all 16S rRNA gene iTag data ............................286

 Figures

A1-1: Contextualization of study site .......................................................277
A1-2: Schematic of samples and analyses .............................................278
A1-3: Log of HP-Core temperature and pressure during incubation ..........279
A1-4: Time-resolved record of HP-Core incubation geochemical data .......280
A1-5: Heat map of major OTUs in the iTAG16S rRNA gene dataset ..........281
A1-6: Nonmetric multidimensional scaling plot of 16S rRNA gene data ...282
A1-7: Maximum likelihood tree of pmoA sequences ..............................283
A1-8: Ambient methane oxidation rates in this and previous studies .......284
# LIST OF ACRONYMS & ABBREVIATIONS

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AEG</td>
<td>acyletherglycerol</td>
</tr>
<tr>
<td>Alk</td>
<td>alkalinity</td>
</tr>
<tr>
<td>ANME</td>
<td>anaerobic methane-oxidizing archaea</td>
</tr>
<tr>
<td>ANOSIM</td>
<td>analysis of similarity</td>
</tr>
<tr>
<td>AOM</td>
<td>anaerobic oxidation of methane</td>
</tr>
<tr>
<td>AR</td>
<td>archaeol</td>
</tr>
<tr>
<td>cmbsf</td>
<td>centimeters below seafloor</td>
</tr>
<tr>
<td>DAPI</td>
<td>4',6-diamidino-2-phenylindole</td>
</tr>
<tr>
<td>DEG</td>
<td>dietherglycerol</td>
</tr>
<tr>
<td>DAG</td>
<td>diacylglycerol</td>
</tr>
<tr>
<td>DHVEG</td>
<td>deep sea hydrothermal vent group</td>
</tr>
<tr>
<td>DIC</td>
<td>dissolved inorganic carbon</td>
</tr>
<tr>
<td>DSV</td>
<td>deep submergence vehicle</td>
</tr>
<tr>
<td>EMP</td>
<td>Earth Microbiome Project</td>
</tr>
<tr>
<td>ERB</td>
<td>Eel River Basin</td>
</tr>
<tr>
<td>HP</td>
<td>high pressure</td>
</tr>
<tr>
<td>HR</td>
<td>Hydrate Ridge</td>
</tr>
<tr>
<td>GDGT</td>
<td>glyceroldibiphytylglyceroltetraether</td>
</tr>
<tr>
<td>IPL</td>
<td>intact polar lipid</td>
</tr>
<tr>
<td>iTAG</td>
<td>massively parallel high-depth DNA sequencing (also, “iTag”)</td>
</tr>
<tr>
<td>MBGB</td>
<td>marine benthic group B</td>
</tr>
<tr>
<td>MBGD</td>
<td>marine benthic group D</td>
</tr>
<tr>
<td>mbsf</td>
<td>meters below seafloor</td>
</tr>
<tr>
<td>mbsl</td>
<td>meters below sea level</td>
</tr>
<tr>
<td>NGS</td>
<td>next generation sequencing (see also, “iTAG”)</td>
</tr>
<tr>
<td>NMDS</td>
<td>nonmetric multidimensional scaling</td>
</tr>
<tr>
<td>OH-AR</td>
<td>hydroxyarchaeol</td>
</tr>
<tr>
<td>OTU</td>
<td>operational taxonomic unit</td>
</tr>
<tr>
<td>PC</td>
<td>phosphatidylycholine (in the context of organic geochemistry)</td>
</tr>
<tr>
<td>PC</td>
<td>push core (in the context of seafloor sampling)</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PG</td>
<td>phosphatidylglycerol</td>
</tr>
<tr>
<td>PI</td>
<td>phosphatidylinositol</td>
</tr>
<tr>
<td>PS</td>
<td>phosphatidylserine</td>
</tr>
<tr>
<td>QIIME</td>
<td>Quantitative Insights Into Microbial Ecology</td>
</tr>
<tr>
<td>RFLP</td>
<td>restriction fragment length polymorphism</td>
</tr>
<tr>
<td>ROV</td>
<td>remotely operated vehicle</td>
</tr>
<tr>
<td>RV</td>
<td>research vessel (also, “R/V”)</td>
</tr>
<tr>
<td>SIMPER</td>
<td>similarity percentage</td>
</tr>
<tr>
<td>SMTZ</td>
<td>sulfate-methane transition zone</td>
</tr>
<tr>
<td>SRA</td>
<td>Sequence Read Archive</td>
</tr>
<tr>
<td>SRB</td>
<td>sulfate-reducing bacteria</td>
</tr>
<tr>
<td>TRFLP</td>
<td>terminal restriction fragment length polymorphism</td>
</tr>
<tr>
<td>XRD</td>
<td>X-ray diffraction</td>
</tr>
</tbody>
</table>
“It is not intuitively obvious why a square yard of meadow, say, should not be exactly like the next square yard in species-composition, yet it must have been noted many times, and from the earliest times, that it rarely or never is so.”

— F.W. Preston, *Time and Space and the Variation of Species*, 1960