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)

David Hamilton Case

ORCID: 0000-0002-1023-0040

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

ACKNOWLEGEMENTS

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 accomlishment, 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, JonMichael Rasmus, Bill Bloomenkranz, 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 chemosynthetic 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 microorgansisms 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.

Mason*, O.U., Case*, D.H., Naehr, T.H., Lee, R.W., Thomas, R.B., Bailey, J.V., Orphan, V.J., 2015. Comparison of Archaeal and Bacterial Diversity in Methane Seep Carbonate Nodules and Host Sediments, Eel River Basin and Hydrate Ridge, USA. Microbial Ecology 70, 766-784. doi:10.1007/s00248-015-0615-6. *indicates shared first authorship.

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.

Case, D.H., Pasulka, A.L., Marlow, J.J., Grupe, B.M., Levin, L.A., Orphan, V.J., 2015. Methane Seep Carbonates Host Distinct, Diverse, and Dynamics Microbial Assemblages. mBio 6, e01348-15. doi:10.1128/mBio.01348-15.

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.

Case, D.H., Yoshinaga, M.Y., Pasulka, A.L., Dawson, K.S., Greve, A.J., Hinrichs, K.U., Orphan, V.J., in preparation. Extent and Rate of Carbonate-Associated Microbial Biomarker Turnover During *in situ* Simulated Methane Seep Quiescence.

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.

Case, D.H., Pasulka, A.L., Trembath-Reichert, E., Connon, S.A., Dawson, K.S., Skennerton, C.T., Orphan, V.J. Development of an In-House Preparation, Processing, and Analysis Workflow for Next Generation Sequencing Data. Freely available by request from the authors.

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 geochemical 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.

Case, D.H., Morono, Y., Ijiri, A., Tavormina, P., Orphan, V.J., Inagaki, F., in preparation. *In Situ* Deployable Reactor Enables Examination of Microbial Communities from High Pressure Environments.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

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

CHAPTER ONE

Tables

Figures

CHAPTER TWO

Tables

Figures

CHAPTER THREE
Tables

Figures

CHAPTER FOUR
Tables

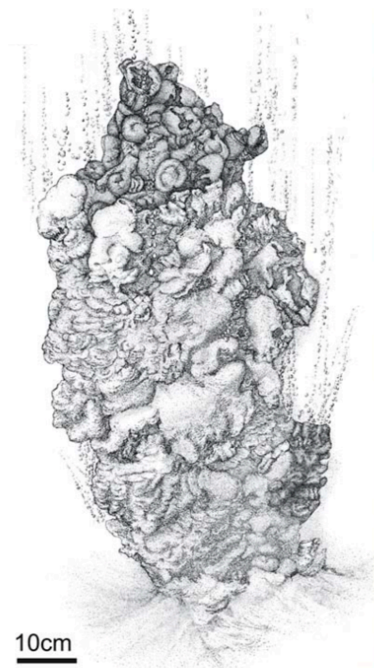
Figures

APPENDIX ONE
Tables

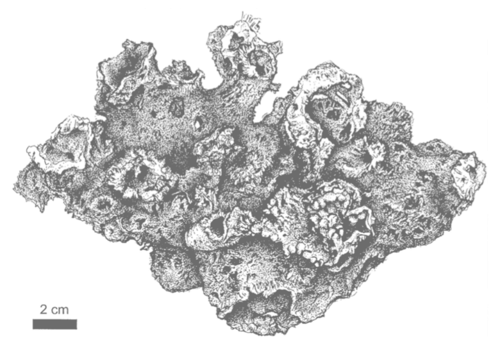
Figures

LIST OF ACRONYMS & ABBREVIATIONS

AEG	acyletherglycerol
Alk	alkalinity
ANME	anaerobic methane-oxidizing archaea
ANOSIM	analysis of similarity
AOM	anaerobic oxidation of methane
AR	archaeol
cmbsf	centimeters below seafloor
DAPI	4',6-diamidino-2-phenylindole
DEG	dietherglycerol
DAG	diacylglycerol
DHVEG	deep sea hydrothermal vent group
DIC	dissolved inorganic carbon
DSV	deep submergence vehicle
EMP	Earth Microbiome Project
ERB	Eel River Basin
HP	high pressure
HR	Hydrate Ridge
GDGT	glyceroldibipytanylglyceroltetraether
IPL	intact polar lipid
iTAG	massively parallel high-depth DNA sequencing (also, "iTag")
MBGB	marine benthic group B
MBGD	marine benthic group D
mbsf	meters below seafloor
mbsl	meters below sea level
NGS	next generation sequencing (see also, "iTAG")
NMDS	nonmetric multidimensional scaling
OH-AR	hydroxyarchaeol
OTU	operational taxonomic unit
PC	phosphatidylcholine (in the context of organic geochemistry)
PC	push core (in the context of seafloor sampling)
PCR	polymerase chain reaction
PG	phosphatidylglycerol
PI	phosphatidylinositol
PS	phosphatidylserine
QIIME	Quantitative Insights Into Microbial Ecology
RFLP	restriction fragment length polymorphism
ROV	remotely operated vehicle
RV	research vessel (also, "R/V")
SIMPER	similarity percentage
SMTZ	sulfate-methane transition zone
SRA	Sequence Read Archive
SRB	sulfate-reducing bacteria
TRFLP	terminal restriction fragment length polymorphism
XRD	X-ray diffraction



Sketch of a carbonate precipitate in the Black Sea from 230 meters below sea level in anoxic waters (Reitner et al., 2005. *Facies* 51: 66-79).



Sketch of a carbonate precipitate in the Black Sea from 188 meters below sea level in anoxic waters (Peckmann et al., 2001. *Marine Geology* 177: 129-150).

“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