Ancient Life at the Extremes: Molecular Fossils and Paleoenvironmental Contexts of Neoproterozoic and Cambrian Hypersaline Settings

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A thesis submitted for the degree of Doctor of Philosophy of
The Australian National University

April 2011

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Candidate’s declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university. To the best of the author’s knowledge, it contains no material previously published or written by another person, except where due reference is made in the text.

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16 April 2011
Abstract

This thesis investigates the molecular remains of microorganisms from Late Precambrian and Early Cambrian hypersaline settings. In particular, it assesses the composition and antiquity of halophilic microbial communities, which survive and flourish under highly saline conditions. While numerous studies have examined modern hypersaline ecosystems, their biological composition in the geologic past, particularly in the Precambrian, is poorly understood. All deposits in this study consisted of evaporites from the Gillen Member of the ~800 Ma Neoproterozoic Bitter Springs Formation and the Early Cambrian Chandler Formation. These evaporites originated from the Amadeus Basin in central Australia and were deposited in inland seas at those times. Due to the shallow nature of these seas and tenuous connections with the contemporaneous oceans, the waters were characterized by elevated salinity levels that resulted in the deposition of dolomite, gypsum and halite.

The biological composition of these ancient hypersaline settings have been assessed by analysing the hydrocarbon remains of lipids, which act as molecular fossils, or biomarkers. However, since all evaporites were collected from a drill core (Mt Charlotte 1) that was contaminated during drilling and storage (e.g. through sampling bags), the syngeneity of the molecules needed to be tested. Such tests were conducted by removing the outer surfaces of the evaporites and quantifying and comparing the amount of hydrocarbons in both the exterior and interior rock portions. This work allowed for the detection of various contaminants, which masked or overprinted indigenous biological signals.

While no indigenous hydrocarbons were detected in the Early Cambrian evaporites, those from the Neoproterozoic yielded a diverse range of syngenetic biomarkers. A detailed analysis of the biomarkers and the enclosing sedimentary textures yielded the oldest current evidence of microorganisms from hypersaline conditions. In particular, the saturate fractions of these samples revealed high ratios of mono- and dimethylalkanes relative to \( n \)-
alkanes. Such a pattern is typical of Precambrian and Cambrian samples and observed in a number of facies settings. An outstanding characteristic of these evaporites are the presence of several pseudohomologous series of both regular (to C\textsubscript{25}) and irregular (to C\textsubscript{40}) acyclic isoprenoids. The relative concentrations of these molecules vary and depend on the mineralogy and textural characteristics of the sedimentary host rock. These isoprenoids are interpreted as the oldest evidence of haloarchaea in the geological record.

Apart from haloarchaea, evidence for cyanobacterial mats was also observed. Molecular evidence for cyanobacteria included elevated concentrations of \textit{n}-heptadecane (\textit{n}-C\textsubscript{17}), and mono- and dimethylalkanes. Such biomarkers were present in anhydrite (altered from gypsum) as well as in layers of dolomite. The carbonate layers can be interpreted as fossil microbial mats based on: 1) evidence for cohesive dolomitized layers that resemble modern mat structures; 2) characteristics of low-temperature dolomite precipitation; 3) concentric frambooidal pyrite inside the dolomite; 4) shape, distribution and association of clay laminae with dolomite crystals and 5) inorganic carbon (δ\textsubscript{13}C) and oxygen (δ\textsubscript{18}O) stable isotope values. These results also indicate that the dolomite was most likely formed through microbial activity, making this the oldest evidence for biologically-induced dolomite precipitation.

Such dolomite-precipitating mats would have also harbored a community of other microorganisms including sulfide-reducers (as evidenced by frambooidal pyrite precipitation) and methanogens, possibly methylotrophic, through the detection of 2,6,11,15-tetramethylhexadecane (crochetane) and 2,6,10,15,19-pentamethyllicosane (PMI). The detection of these two compounds in the Neoproterozoic makes this their oldest occurrence to date and yields insights into the antiquity of methanogens in hypersaline settings.

Through comparisons with published data from modern hypersaline settings, it can be concluded that similar microbial communities as that of today were present on Earth by at least ~800 Ma. Therefore, such results provide new insights into ancient halophilic ecosystems.
Completing a PhD thesis is not an endeavor that can be achieved by one person. It involves the help and assistance of numerous people whose expertise is often required.

Firstly, I would like to thank my supervisor Jochen Brocks, who guided and inspired me through four years of research and writing. Jochen’s passionate and meticulous approach to science has been a real inspiration and a fantastic learning experience. I also appreciated the freedom he has given me in pursuing my own research interests and encouraging me to apply a multidisciplinary approach to my work. His friendly approach and participation in countless lunchtime discussions will be fondly remembered.

Secondly, I thank Janet Hope who served as laboratory manager during my time at the Brocks laboratory. Janet’s helpful, energetic and competent work ethic has been greatly appreciated and admired. I also thank her for inviting me several times to dinner at her house.

Next, I would like to thank the four academics/scientists who agreed to serve on my thesis panel and attended my mid-term review in 2008. In this regard, I thank Lindel Bromham and Robert Burne (Australian National University, ANU), Emmanuelle Grosjean (Geoscience Australia), and Galen Halverson (University of Adelaide). I also like to take this opportunity to thank Robert Burne for inviting me to Shark Bay, Western Australia, in 2007.

Numerous people provided me with technical or administrative assistance. In particular, I thank Junhong Chen, Jamie Lankford, Eddie Resiak, Christian Thun (Geoscience Australia), Maxwell Heckenberg (Northern Territory Geological Survey, Alice Springs), Frank Brink, Joseph Cali, Harry Kokkonen, Josephine Magro, Robyn Petch, Tony Phimphisane, Robert Raap, Hillary Stuart-Williams, and Ulrike Troitzsch (ANU).

I also thank Emmanuelle Grosjean (Geoscience Australia), Michael Moldowan (Stanford University) and Steven Rowland (University of Plymouth) for providing me with samples or standards that were needed for compound identification.

Advice from numerous experts in their fields has been beneficial in advancing ideas that were relevant to my thesis. In particular, I thank Chris Boreham (Geoscience Australia), Judith McKenzie and Chris Vasconcelos (ETH Zurich), Steven Rowland (University of Plymouth), Neil Sherwood (CSIRO, Sydney), and Dioni Cendon Sevilla (Ansto, Sydney).
I greatly appreciated the opportunity to become acquainted with a number of students that were part of the Brocks laboratory over the years that I was there. In particular, I would like to mention Amber Jarrett, Claudia Jones and Carina Lee. I wish them all the best for their futures.

My stay and education in Australia was funded by scholarships from the Research School of Earth Sciences and the ANU. Funding for my research was obtained by Jochen Brocks through the Australian Research Council.

Finally, I would like to thank my parents Andreas and Stana Schinteie for giving me the opportunity to pursue my academic interests and for supporting me over the years that I have been a university student in both New Zealand and Australia. Their love and understanding is greatly appreciated.
# Table of contents

ABSTRACT

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION

1.1 Ancient life at the extremes: investigating Precambrian and Cambrian hypersaline ecosystems

1.2 Molecular proxies and use of lipid biomarkers in the study of ancient biotas

1.3 Definitions of lipids and their biological value

1.4 Preservation of lipids in sediments and their utility as chemical fossils

1.5 Limitations in the applicability of lipid biomarkers to ancient settings

1.6 Thesis outline

1.7 References

CHAPTER 2: GEOLOGIC, MINERALOGICAL AND PETROGRAPHIC CHARACTERISTICS OF ~800 MA NEOPROTEROZOIC AND EARLY CAMBRIAN EVAPORITES: CONTEXT FOR ORGANIC GEOCHEMICAL STUDIES

2.1 Introduction

2.2 Geological setting and previous work
2.2.2 The search for oil and gas in the Amadeus Basin and a current focus on sub-salt hydrocarbons .......................................................... 26
2.2.3 Geological characteristics of the Amadeus Basin evaporites .......... 28
2.2.4 Information on the Mt Charlotte 1 core ........................................ 30
2.2.4.1 The Gillen Member of the Mt Charlotte 1 core ......................... 30
2.2.4.2 The Chandler Formation of the Mt Charlotte 1 core ................. 31
2.3 Materials and Methods ............................................................... 31
2.3.1 Transmitted and reflective light microscopy ............................... 31
2.3.2 X-ray powder diffraction (XRPD) .............................................. 32
2.3.3 Scanning Electron Microscopy (SEM) ......................................... 33
2.3.4 Electron microprobe analysis .................................................... 33
2.3.5 Organic petrography ............................................................... 33
2.3.6 Stable isotope analyses of the dolomite ..................................... 34
2.4 Results ...................................................................................... 34
2.4.1 Overview of sedimentary textures at hand specimen scale ............ 34
2.4.2 X-ray powder diffraction (XRPD) .............................................. 36
2.4.3 Light Microscopy ................................................................. 39
2.4.4 Scanning Electron Microscopy (SEM) ......................................... 41
2.4.5 Elemental Mapping ............................................................... 43
2.4.6 Carbon and oxygen isotopic analyses of the dolomite layers ......... 45
2.4.7 Organic petrography ............................................................... 46
2.5 Discussion ................................................................................ 47
2.5.1 Paleoenvironmental setting ...................................................... 47
2.5.2 Presence of microbial mat textures ........................................... 54
2.5.3 Modern hypersaline mats from coastal sabkhas: potential equivalents of the Gillen Member dolomite laminae? ......................... 56
2.5.4 Precipitation of dolomite ........................................................ 58
2.5.4.1 Abiotic dolomite precipitation and associated problems………………..58
2.5.4.2 Biologically induced dolomite precipitation: a viable alternative……59
2.5.4.3 Comparisons of Gillen Member dolomites with those from modern sabkhas………………………………………………………………………61
2.5.4.4 The co-occurrence of sulfate and dolomite........ .......................63
2.5.5. Presence of frambooidal pyrite.......................... ...63
2.5.6 Clay laminae within dolomite layers…………………………………….65
2.5.7 Carbon and oxygen isotopic analyses of the dolomite layers.............67
2.5.8 Spatial distribution of organic matter.............................................69
2.5.9 Isolated patches of halite within the dolomite layers.......................70
2.5.10 Presence of anhydrite...............................................................71
2.6 Conclusion..................................................................................72
2.7 References..................................................................................72

CHAPTER 3: ANALYTICAL FRAMEWORKS AND METHODOLOGIES FOR EVALUATING HYDROCARBON SYNGENEITY IN ANCIENT ROCKS.......86
3.1 Testing syngeneity of lipid biomarkers from ancient rocks.................86
3.1.1 Susceptibility of ancient biomolecules to contamination...............86
3.1.2 Historical and current ideas on the contamination of Precambrian rocks with emphasis on lipid biomarkers..............................................87
3.2 Techniques employed for the removal, detection and evaluation of hydrocarbon contaminants.................................................................87
3.2.1 The sawing technique for the removal of hydrocarbon contaminants..93
3.2.2 The micro-abrasion technique for the removal of hydrocarbon contaminants.........................................................................................95
3.2.2.1.2 Abrasion procedure..........................................................95
3.2.2.2 Results and discussion......................................................96
3.3 Quantification of bitumens between the exterior and interior rock portions.97
CHAPTER 4: TESTING HYDROCARBON SYNGENEITY OF EVAPORITES FROM NEOPROTEROZOIC AND CAMBRIAN STRATA: A SYSTEMATIC STUDY OF CONTAMINATED DRILL CORE SAMPLES

4.1. Introduction................................................................. 106
4.2. Materials and Methods.................................................... 108
4.2.1 Samples................................................................ 108
4.2.2 Exterior/interior experiment......................................... 109
4.2.3 Processing of samples............................................... 110
4.2.4 Gas chromatography-mass spectroscopy (GC-MS)......... 110
4.3. Results..................................................................... 111
4.3.1 Laboratory sand blanks........................................... 111
4.3.2 Bitumen groups observed in the Cambrian and Neoproterozoic evaporites....................................................... 111
4.3.2.1 Group 1: Bitumens with low concentrations of monomethylalkanes relative to \( n \)-alkanes................................................................. 112
4.3.2.2 Group 2: Bitumens displaying a high concentration of mono- and dimethylalkanes relative to \( n \)-alkanes................................................................. 114
4.3.2.3 Group 3: Bitumens displaying low relative concentrations of mono- and dimethylalkanes relative to \( n \)-alkanes................................................................. 118
4.3.3. Measuring concentration differences of hydrocarbons between exterior and interior rock portions................................................................. 119
4.3.3.1 E/I differences for Group 1 bitumens......................... 119
4.3.3.2 E/I differences for Group 2 and 3 bitumens............... 120
4.3.4. Saturated hydrocarbon composition of a plastic bag from a Mt Charlotte 1 storage box................................................................. 128
4.4 Discussion.................................................................. 129
4.4.1 Biomarker evidence for ancient hypersaline environments............. 130
4.4.1.1 Laboratory sand blanks ......................................................... 130
4.4.1.2 \( n \)-alkane patterns .............................................................. 131
4.4.1.3 Elevated concentrations of heptadecane (\( n \)-C\(_{17} \)) and docosane (\( n \)-C\(_{22} \)) ................................................................. 132
4.4.1.4 Presence of mono- and dimethylalkanes ................................. 135
4.4.1.5 Elevated concentrations of isoprenoids .................................. 135
4.4.1.6 Presence of various hopanes and steranes ............................... 138
4.4.1.7 Presence of various Phanerozoic plant biomarkers .................. 139
4.4.1.8 Presence of aromatic hydrocarbons ...................................... 140
4.4.1.9 Final assessment of bitumen syngeneity from Cambrian and Neoproterozoic evaporites of \( Mt \) Charlotte 1 .................................................. 140
4.4.2 The necessity of using quantitative as opposed to qualitative approaches in testing bitumen syngeneity .................................................. 141
4.5 Conclusions ............................................................................. 144
4.6 References ............................................................................... 145

CHAPTER 5: ASSESSING THE MICROBIAL DIVERSITY OF AN \( \sim \)800 MA NEOPROTEROZOIC HYPERSALINE ENVIRONMENT: EVAPORITES AS ARCHIVES FOR PRECAMBRIAN HALOPHILES ................................................................. 151

5.1 Introduction ............................................................................... 151
5.2 Materials and Methods .............................................................. 153
5.2.1 Samples ............................................................................... 153
5.2.2 Processing of samples ............................................................ 153
5.2.3 Gas chromatography-mass spectroscopy (GC-MS) .................. 154
5.2.4 X-ray powder diffraction (XRPD) .......................................... 155
5.2.5 Total Organic Carbon (TOC), ROCK EVAL, hydrogen and oxygen indices determination ......................................................... 155
5.3 Results ................................................................................... 155
5.4.2.4 Presence of pristane and phytane and their relative concentrations…188
5.4.2.5 Presence of regular, head-to-tail isoprenoids from $C_{21}$ to $C_{25}$……..193
5.4.2.6 2,6,10,15,19-pentamethyllicosane (PMI)……………………………………….194
5.4.2.7 2,6,11,15-tetramethylhexadecane (crocetane)………………………………196
5.4.2.8 Presence of biphytane and its breakdown products………………………197
5.4.2.9 Presence of squalane…………………………………………………………….200
5.4.3 Absence of hopanes and steranes in the Gillen Member samples………202
5.4.3.1 Influence of thermal maturity (catagenesis)……………………………………..203
5.4.3.2 Absence of hopanoid producing members of the Bacteria domain….203
5.4.3.3 Absence of steroid producing members of the Eukarya domain………204
5.4.3.4 Influence of oxic degradation during diagenesis………………………….205
5.4.4 Paleoenvironmental interpretation…………………………………………….207
5.4.4.1 Evidence for photosynthetic primary producers in the Gillen Member………………………………………………………………………208
5.4.4.1.1 Fossil evidence from previous studies……………………………………….209
5.4.4.1.2 Evidence from this study……………………………………………………209
5.4.4.2 Evidence for haloarchaea in the Gillen Member…………………………..211
5.4.4.3 Evidence for methanogens in the Gillen Member…………………………..212
5.4.4.4. Antiquity of halophiles, their ecological communities and associated biogeochemistry…………………………………………………………….214
5.5 Conclusions…………………………………………………………………………216
5.6 References…………………………………………………………………………217

CHAPTER 6: ASSESSING COMPOUND-SPECIFIC CARBON ISOTOPIC SIGNATURES OF BIOMARKERS FROM PRECAMBRIAN EVAPORITES……………………………………………………………………………236
6.1 Introduction……………………………………………………………………….236
6.2 Materials and Methods.................................................................238
6.2.1 Samples.................................................................................238
6.2.2 Interior/exterior experiment.....................................................239
6.2.3 Processing of samples..............................................................239
6.2.4 Gas chromatography-mass spectroscopy (GC-MS).......................239
6.2.5 Silicalite adduction.................................................................240
6.2.6 Isotopic Analyses.................................................................240
6.2.7 Isotope analysis of kerogens...................................................241
6.3 Results.....................................................................................241
6.4 Discussion...............................................................................249
6.4.1 Implications of hydrocarbon contamination on single-compound δ¹³C
values.......................................................................................249
6.4.1.1 Impacts of n-alkane contamination......................................250
6.4.1.2 Impacts of isoprenoid contamination...................................251
6.4.2 δ¹³C comparisons between n-alkanes and kerogen.....................252
6.4.3 δ¹³C variations within Gillen Member samples............................253
6.4.4 δ¹³C variations between Gillen Member samples.........................255
6.4.5 δ¹³C values of crocetane and PMI from the Gillen Member...........255
6.5 Conclusion..............................................................................256
6.6 References...............................................................................257

CHAPTER 7: CONCLUSIONS AND SUGGESTIONS FOR FURTHER
STUDY............................................................................................261
7.1 References...............................................................................268
1.1 Ancient life at the extremes: investigating Precambrian and Cambrian hypersaline ecosystems

Numerous microorganisms survive and flourish in environments that are often considered extreme from an anthropogenic view point. Such environments include hydrothermal vents (e.g. Van Dover 2000; Reysenbach and Shock 2002), hydrocarbon seeps (e.g. Aharon 2000; Campbell 2006), and acid hot springs (e.g. Schinteie et al. 2007). Hypersaline environments are another type of extreme setting where microorganisms, known as halophiles, live under salinity regimes exceeding those of seawater (e.g. Javor 1989; Russell 1992; Oren 2002). Such environments pose special challenges to life and organisms adapted to these settings have evolved specific mechanisms to cope with these difficulties.

In order to understand the antiquity and evolution of hypersaline ecosystems through significant periods of geologic time, the chemical remains of 800 Ma Neoproterozoic and Early (~542 Ma) Cambrian halophiles and their enclosing host rocks will be investigated. Rocks from these time periods are significant, as they were deposited under tremendous environmental and biotic changes (Figure 1.1). The Neoproterozoic (1000-542 Ma), in particular, witnessed the greatest perturbations of the carbon cycle (Bartley and Kah 2004), massive ice ages (so called Snowball Earth events) (e.g. Hoffman et al. 1998), a rise of atmospheric oxygen (e.g. Bekker et al. 2004; Canfield 2005), a shift from dominantly sulfidic or ferrous oceans to the modern state of well-oxygenated clear-waters (Canfield et al. 2007; Butterfield 2009), and the advent of animal life that later culminated in their rampant diversification (called Cambrian Explosion) (e.g. Gould 1989; Narbonne et al. 1998; Xiao et al. 1998; Love et al. 2009).

Currently, the antiquity of halophiles is unknown (Mullen 2002). Furthermore, there is no knowledge on the microbial community composition of Precambrian hypersaline settings or any associated biogeochemical
interactions. While fossil eukaryotes have been used to document biotic changes during the Neoproterozoic (e.g. Knoll et al. 2006; Knoll et al. 2007), including ancient hypersaline settings (Oehler et al. 1979), this method of study is inadequate for their prokaryotic counterparts. Most prokaryotic cells offer few phylogenetic information and mineralogical artifacts can be potentially mistaken for their fossilized counterparts (e.g. Garcia-Ruiz et al. 2003). Therefore, it is not surprising that estimates of ancient microbial diversity are severely incomplete (Conway Morris 1998).

Figure 1.1 Time diagram of Proterozoic and Cambrian events. Note that the Cambrian is in the early Phanerozoic. Oceans: ① = surface waters become increasingly oxygenated, ② = anoxic and possibly sulfidic deep oceans, ③ = possible transition to ferrous oceans and ventilation of the deep oceans with O₂, ④ = predominantly oxygenated deep ocean waters. Oxygen: concentration of O₂ relative to present atmospheric level (PAL). Carbon: carbon isotopic excursions (%) indicating perturbations of the carbon cycle. Quaternary ice ages and origin of modern humans (green circles) placed in time diagram for comparison. Diagram adapted with modifications from Bartley and Kah (2004); Brocks and Banfield (2009).
Rock textures interpreted as the remains of microbial mat structures are often described from Precambrian strata (e.g. Schieber 1986; Hagadorn and Bottjer 1997; Steiner and Reitner 2001). As a result, criteria have been established and classification schemes devised to recognize and understand ancient, lithified mats and their textures (e.g. Eriksson et al. 2007). Such textures are often interpreted through comparisons with modern microbial mat accumulations and their behavior under environmental stressors such as wind, currents and evaporation (e.g. Gerdes et al. 1993, 2000). Modern hypersaline phototrophic mats, in particular, are used for such comparisons. In such extreme settings, the activity of grazing organisms is suppressed, thereby allowing for extensive mats to grow. Similarly extensive growth of microbial mats is believed to have occurred in numerous environmental settings in the Precambrian before the evolution of grazers and burrowers (e.g. Bottjer et al. 2000). Therefore, due to an interest in understanding Precambrian microbial mats, an abundance of published research on the sedimentology, microbiology and organic geochemistry of modern hypersaline mats exists. This abundance of data on hypersaline settings allows for comparisons to be made with ancient equivalents. Thus far, however, only few serious studies (e.g. Oehler et al. 1979; McKirdy and Kantslcer 1980) have been undertaken in understanding Precambrian/Cambrian hypersaline ecosystems. Furthermore, no data exists whereby a detailed molecular and isotopic study of such ecosystems is integrated with petrography and mineralogy. This thesis presents the first of such analyses.

This study has three major aims. The first aim is to investigate whether evaporites, which record hypersaline conditions, can serve as biomarker archives for recording the presence of a halophilic ecosystem in the Precambrian/Cambrian. The second aim is to understand if rock textures in evaporites record the presence of microbial activity and if there are texture-specific biomarkers. The third and final aim intends to evaluate whether Precambrian/Cambrian ecosystems were similar or different to Phanerozoic, including modern, counterparts. Biological evolution has been punctuated
throughout Earth history with numerous series of innovation, diversification and extinction (e.g. Conway Morris 1998). However, the antiquity and evolution of halophilic ecosystems is not well understood. Therefore, investigations of ancient hypersaline environments for the traces of biological molecules could be particularly informative in understanding halophiles and their ecological communities.

The remainder of this chapter will provide the background to a lipid biomarker study of ancient microbial ecosystems. Background information is provided on the nature of these molecular fossils or biomarkers and their applicability to reconstruct ancient ecosystems. The chapter ends with a thesis outline.

1.2 Molecular proxies and use of lipid biomarkers in the study of ancient biotas

Zuckerkandl and Pauling (1965) described living matter as “the one which, in the face of great transformations, preserves inscribed in its organization the largest amount of its own past history”. While DNA (and in some occasions RNA) provides the blueprint for life on Earth, it has become clear that other molecules also retain significant biological information (Schweitzer 2003). To date, a variety of biogenic molecules have been extracted from fossilized remains with the aim of reconstructing past biota: DNA (e.g. Leonard et al. 2002; Barnes et al. 2006); proteins (e.g. Ostrom et al. 2006); and lipids (e.g. Summons et al. 1988a,b; Logan et al. 1995, 1997; Brocks et al. 2005). For a molecule to act as a useful biological marker (biomarker) in geological or palaeoenvironmental studies, it is important for it to exhibit taxonomic specificity and good preservation potential (Brocks and Summons 2004). As a former “blueprint of life”, ancient DNA provides the best source of information on past life. Unfortunately, DNA degrades in a comparatively short period of time. On the basis of empirical and theoretical data, currently accepted maximum ages for DNA survival range from between 100 years to 1 Ma (Pääbo et al. 2004; Hebsgaard et al. 2005). Proteins, although less informative than their DNA
counterparts, can still provide significant biological information. Since protein sequences are directly related to DNA sequences, they provide another insight into the evolution of past ecosystems. Ancient proteins have, for example, been reported from fossils >55 thousand years (Nielsen-Marsh et al. 2002). Although proteins are sturdier than their DNA counterparts, they cannot be used for geologically distant ages such as the Cambrian and Precambrian. Therefore, other molecules are needed that can be preserved in rocks from such ages, yet still retain the biological information necessary to provide an insight into ancient life and past ecosystems. In this regard, lipids have proven to be valuable molecules. These molecules are the principal biological markers (biomarkers) used in this thesis and are introduced below.

1.3 Definitions of lipids and their biological value

The term lipid is often loosely defined as molecules that readily dissolve in organic solvents such as diethyl ether, hexane, benzene, chloroform or methanol (e.g. Christie 1973; Killops and Killops 2005, Peters et al. 2005). However, this definition has been criticized (e.g. Christie 1973; Peters et al. 2005), since other molecules that meet this criterion are clearly not regarded as lipids. A more precise definition can restrict the term to oils, fats, and waxes. However, as noted by Peters et al. (2005), this term excludes such compounds as lipopolysaccharides, lipoproteins, sphingolipids, glycerophospholipids, and glycoglycerolipids. While a precise definition for lipids that is widely agreed on is currently not available, it should be kept in mind that this group of compounds can be structurally diverse and bound to a variety of other molecules including proteins and carbohydrates (Killops and Killops 2005). Examples of a variety of lipids are shown in Figure 1.2.

Lipids play vital roles in the functioning of organisms. In particular, lipids act as: 1) cell membrane fluidity regulators (e.g. Choi et al. 2003); 2) membrane barriers to proton exchange (Haines 2001); 3) long-term energy storage (e.g. Jonsson and Jonsson 2005); 4) pigments (e.g. Volkman et al. 1988); 5)
hormones (e.g. Sommer and Cowley 2001); and 6) vitamins (e.g. Rezamand et al. 2007). Different lipid classes such as steroids, hopanoids, fatty acids and carotenoids have distinct biosynthetic origins. Each structural form of a lipid class has a particular biological function and the most useful compounds for biomarker studies are taxonomically specific (Brocks and Summons 2004; Brocks and Pearson 2005).

Cell membranes provide a key example of the diversity of lipids within the biosphere. Molecular phylogenetic studies have shown that life can be divided into three domains: the Archaea and Bacteria (collectively known as prokaryotes); and the Eucarya (also known as eukaryotes) (Woese and Fox 1977; Woese et al. 1990). Based on data obtained from small-subunit rRNA, a phylogenetic tree was created that encompasses all three of these domains (Figure 1.3).

Close examination of the membrane lipids between these domains shows some fundamental difference in their composition (Figure 1.4). Members of the Bacteria and Eucarya domains possess glyco- and phospholipids that are...
composed of fatty acids connected to a polar head. Members of the Archaea domain, by contrast, are characterized by isoprenoid hydrocarbons bound together by ether linkages. These organisms can possess lipids composed of a single polar head group with isoprenoid side chains ranging from 15 to 25 carbon atoms, or can have two polar head groups joined together by C$_{40}$ biphytanyl isoprenoids. Differences between the domains are also observed in the presence or absence of hopanols and sterols. Hopanols are characteristically present in the Bacteria, whereas virtually all sterols are observed within the Eucarya. A few exceptions exist, where sterols are also observed within the Bacteria. Archaea do not possess such molecules in their membranes. Other lipids, such as chlorophylls can occur in both the Bacteria and the Eukarya, while carotenoids are found in all the domains of life (Brocks and Summons 2004; Brocks and Pearson 2005). Some lipids, in turn, are observed only within certain taxa, such as carbon isotopically depleted 2,6,10,15,19-pentamethylicosane, which is associated with microorganisms involved in the anaerobic oxidation of methane (e.g. Boetius et al. 2000; Valentine 2002).

1.4 Preservation of lipids in sediments and their utility as chemical fossils

The preservation potential of lipids is a key aspect when discussing their capacity as molecular fossils. Once microorganisms die in an aquatic environment, they will slowly drift through the water column. During this journey, numerous biological, chemical and physical processes operate on the organic matter, progressively altering its character or becoming consumed by heterotrophic organisms. Collectively, these processes are branded diagenesis, which refers to the alteration of organic matter in the water column and sediments prior to significant changes caused by heat (Peters et al. 2005). The processes of diagenesis are so intense, that 98% of organic matter is degraded (Wakeham et al. 2002).
The means by which organic matter sinks to the sea or lake floor can vary. While some biomolecules are readily dispersed in the environment when released from a precursor organism, others are influenced by being packaged in resistant biopolymer matrices (e.g. cell walls) or within fecal pellets, sedimentary detritus and colloidal matter (e.g. Logan et al. 1995; Eglinton and Eglinton 2008). The compounds can become adsorbed onto or incorporated covalently into mineral matrices such as clay (e.g. Eglinton and Eglinton 2008). The attachment and incorporation into mineral matrices is particularly frequent when the biomolecules contain polar or reactive functional groups such as amino groups, hydroxyl or carboxylic acid functional groups (e.g. Eglinton and Eglinton 2008).

Figure 1.3 Phylogenetic tree of life, showing the division of life into three domains: Archaea, Bacteria and Eucarya. Adapted with modifications from Prescott et al. (1999).
Figure 1.4 Different types of lipids observed in the three domains of life. Adapted with modifications from Peters et al. (2005).

The accumulation rate of biomarkers is determined by both primary productivity (production) and the extent of degradation during transport through the water column and during burial in the sediment (preservation) (e.g. Sinninghe Damsté et al. 2002). Once buried, lipids are further subjected to both aerobic and anaerobic degradation in the oxic and anoxic zones of the sediment, respectively (e.g. Sinninghe Damsté et al. 2002). The efficiency of organic carbon burial, for example, has been shown to strongly correlate with the length of time accumulating particles are exposed to molecular oxygen in the sedimentary pore waters (Hartnett et al. 1998; Hedges et al. 1999). In addition to altering the amounts of organic matter preservation, oxygen can influence the biomarker fingerprint if oxic degradation of individual lipids takes place at different rates (e.g. Canuel and Martens 1996; Sinninghe Damsté et al. 2002).
Lipid biomarkers may become preserved in sediments and survive the eventual lithification stage into sedimentary rocks. However, through burial and heating of the sedimentary host rocks, another lipid altering process called catagenesis is encountered. This process refers to the thermal alteration of organic matter in rocks that is heated to temperatures of approximately 50 to 150°C (Peters et al. 2005). Changes in sedimentary organic matter with increasing burial and elevated temperatures are generally termed maturation (Killops and Killops 2005). Heating of the host rocks to high temperatures (e.g. >150°C) will result in the destruction of organic matter, essentially removing them from the molecular fossil record (Peters et al. 2005).

While both dia- and catagenetic processes cause structural changes in lipids, the precursors of many lipids can still be identifiable. Due to their preservation potential that is akin to fossilized organisms, such molecules have also been referred to as chemical fossils (Eglinton and Calvin 1967). Such chemical or molecular fossils can provide valuable information about the existence and nature of early life in the absence of recognizable fossils (Eglinton et al. 1964; Brocks and Summons 2004).

Since most organisms in the Precambrian were prokaryotes with simple cell morphologies, it is impossible to ascertain even basic taxonomic status (e.g., domain, kingdom, phylum, and class) to their body fossil record. The identification of numerous Precambrian eukaryotes, which tend to be morphologically more complex than prokaryotes, is also plagued by taxonomic problems. Issues related to the polyphyletic evolution of character traits and the absence of certain trait combinations in stem groups can cause difficulties in the taxonomy of Precambrian eukaryotes (e.g. Knoll et al. 2006; Knoll et al. 2007). Lipid biomarker geochemistry, by contrast, has the potential to determine the composition of Precambrian and Phanerozoic ecosystems at a variety of taxonomic levels. For example, high concentrations of $n$-C$_{17}$, can indicate a cyanobacterial or algal input (e.g. Gelpi et al. 1970), while high concentrations of $n$-C$_{27}$, $n$-C$_{29}$, and $n$-C$_{31}$ is often diagnostic for higher plant input (Eglinton and Hamilton 1967). C$_{20}$ and C$_{25}$ highly branched isoprenoids (HBIs) are
diagnostic for the presence of diatoms (Yon et al. 1982; Kenig et al. 1990; Nichols et al. 1988; Volkman et al. 1994), while the acyclic isoprenoid crocetane (2,6,11,15-tetramethylhexadecane) likely indicates input by methanotrophic Archaea (e.g. Thiel et al. 2001).

Lipid biomarkers also allow for a study of microorganisms in the context of their environments. Since the appearance of life on Earth, biospheres and geospheres have evolved together and their records are chronicled in sedimentary and metasedimentary rocks spanning billions of years (Brocks and Pearson 2005). Since life evolved to cope in different environmental niches, taxonomically diagnostic lipid biomarkers can act as indicators of paleoenvironmental conditions as well. With regards to the reconstruction of paleoenvironments, lipid biomarkers have, for example, been used for elucidating hypersaline conditions from Miocene/Pliocene halite-rich deposits (Grice et al. 1998), a sulfidic ocean in the Proterozoic (Brocks et al. 2005), and an apparent radiation of crenarchaeota associated with Cretaceous oceanic anoxic events (Kuypers et al. 2001). Such examples clearly demonstrate the applicability of lipid biomarker work for elucidating ancient ecosystems and environments. However, there are also important limitations, which need to be taken into account. These limitations are discussed below.

1.5 Limitations in the applicability of lipid biomarkers to ancient settings

While lipid biomarkers are effective tools for paleoecological and paleoenvironmental studies, there are also numerous limitations. Failure to keep these drawbacks in mind may result in serious misinterpretations. For example, not all lipid biomarkers are taxonomically specific. Certain lipids can be representative of particular physiologies or biosynthetic pathways that may have a broad and/or patchy taxonomic distribution (Knoll et al. 2007). In such cases, traditional body fossils may be far more informative, particularly if a combination of distinctive morphological traits are present (e.g. size, segmentation, extracellular ornamentations). Preservation conditions are also
important, in particular since diagenesis results in the loss of functional groups that would otherwise confer important biophysiological and taxonomic information (Knoll et al. 2007).

The most difficult problem faced by biomarker studies of particularly ancient and organically lean rocks is contamination (e.g. Brocks et al. 2008). Since the testing of biomarker syngeneity forms a central theme in this thesis, this topic will be explained in greater detail in chapters 3 and 4.

The study of lipid biomarkers is also restricted to well preserved sedimentary rocks that contain at least a minimum amount of organic matter (kerogen). Rocks that are oxidized, such as red-beds, or have a large sedimentary grain sizes, such as sandstones, rarely contain indigenous biomarkers. The range of sedimentary rocks that may contain biologically informative molecules is also constrained to units that did not suffer high metamorphic temperatures. Moreover, biomarkers are commonly destroyed by secondary replacement of sedimentary minerals by chert or dolomite (e.g. Brocks and Summons 2003; Peters et al. 2005; Knoll et al. 2007). Therefore, not all sedimentary rocks will host indigenous biomarkers and their presence may often be an exception rather than the rule.

1.6 Thesis outline

This thesis is divided into six chapters, which aim to progressively unravel the biotic composition of ancient hypersaline ecosystems. A brief outline is provided below of each of these chapters and is aimed at allowing the reader to gain an appreciation of the content of this thesis:

Chapter 2 provides the context for an organic geochemical study of Neoproterozoic and Cambrian evaporite strata. First, the geological settings as well as previous work on the sedimentary rocks will be outlined. Following that account, a description of the core from which rock samples were collected will be provided. The bulk of this chapter is devoted to the mineralogy, petrography
and stable isotopes of evaporites that have been analyzed for biomarkers. Original work will be presented to show the presence of both biotic and abiotic mineral textures, an interpretation of the depositional environment as well as the presence of organic matter.

**Chapter 3** provides the analytical framework for establishing biomarker syngeneity on Precambrian/Cambrian rocks. As briefly mentioned above, hydrocarbon contamination can severely impair the accurate determination of biomarker signals. The chapter starts with an account on the history of establishing biomarker syngeneity in ancient rocks. Following this account, two techniques are described that help in determining which biomarkers are syngenetic to the rock and which have been introduced at a late stage. Both techniques have been employed in this thesis. Since one of the techniques, the microabrasion method, has never before been described, original experimental results are provided that demonstrate its efficiency in determining hydrocarbon syngeneity.

**Chapter 4** establishes the syngeneity of biomarkers from the Neoproterozoic and Cambrian evaporites. Through the application of the techniques discussed in Chapter 3, it will be shown that the samples were significantly affected by hydrocarbon contamination. However, numerous indigenous biomarkers were obtained and this chapter will discuss how to differentiate between the syngenetic and contaminant hydrocarbons.

**Chapter 5** focuses on the indigenous biomarker signals from the evaporites. All hydrocarbons that yield biomarker data will be individually discussed. The chapter will end with a reconstruction of the ancient hypersaline ecosystems and their evolutionary and paleoenvironmental significance.

**Chapter 6** concentrates on the single-compound carbon isotopic results of the evaporite hydrocarbons. The first part of the chapter will demonstrate that even the isotopic results of individual hydrocarbons can be significantly affected by
contamination. The second part will discuss the isotopic characteristics of the indigenous hydrocarbons.

Chapter 7 will provide a summary of the key findings of this thesis. Furthermore, suggestions will be made on how to advance the study of Precambrian/Cambrian hypersaline ecosystems with additional analytical techniques and rock samples from different localities and/or geologic time intervals.

1.7 References


Chapter 2
Geologic, mineralogical and petrographic characteristics of ~800 Ma Neoproterozoic and Early Cambrian evaporites: context for organic geochemical studies

The aim of this chapter is to investigate the geologic, mineralogical and petrographic contexts of the samples from which hydrocarbons, including a diverse range of biomarkers, were extracted. Through the contextualization of these samples and their associated hydrocarbons, one should be in a better position to understand their paleoenvironmental and paleoecological significance. To achieve this result, a variety of mineralogical, petrographic and isotopic techniques were employed and these are outlined below.

2.1. Introduction
Evaporites are chemical sediments that were originally precipitated from a saturated surface or near-surface brine as a result of solar evaporation (e.g. Nichols 2006; Warren 2006). Under such conditions, minerals precipitate out of solution as ions and become concentrated (e.g. Nichols 2006; Warren 2006). The resulting salts deposit in a well-defined order (Usiglio 1849). Aragonite (CaCO₃), which precipitates out of solution when seawater volume is reduced to ~50%, is the first mineral to form. In cases where the original brine is agitated and/or organisms are present, aragonite precipitation may occur at lower concentrations (e.g. Logan 1987). Other carbonate minerals, particularly dolomite (CaMg(CO₃)₂), are also observed to precipitate at this stage (e.g. Vasconcelos et al. 1995; Bontognali et al. 2010). Gypsum (CaSO₄·2H₂O) precipitates when seawater volume is reduced to ~20% and is the second evaporite mineral to form (e.g. Usiglio 1849; Logan 1987). When the brine volume has been reduced to less than 10% volume, halite (NaCl) begins to precipitate. Residual brines, also known as bitterns, form at less than 5% of original volume and precipitate halite, as well as magnesium and potash salts. See Logan (1987) for a more detailed explanation on the formation of evaporites.
Important relationships have been observed between the occurrence of evaporites and liquid hydrocarbons (Kirkland and Evans 1981). In many cases, evaporites act as impervious barriers, or cap rocks, that trap hydrocarbons and assist in their accumulation. For example, Kirkland and Evans (1981) argued that evaporites overlie or seal an estimated 50% of the world’s known total petroleum reserves. In other cases, a genetic link has been established between the deposition of evaporative carbonates and hydrocarbon formation. Source rocks require anoxic conditions for organic matter to accumulate and preserve in high abundance. Many subaqueous evaporitic settings are localities where organic matter accumulates (Warren 2006). Evaporites require restricted water inflow conditions and are often areas of density stratified waters with a bottom brine body composed of a dense, saline water mass (Warren 2006). Since atmospheric gases do not easily exchange with the bottom brine body, long-term bottom anoxia is maintained (Warren 2006).

In Earth history, it was not until the Mesoproterozoic (1600-1000 Ma) that well developed examples of bedded gypsum/anhydrite appeared. They include the evaporitic sequences of the ~1.7 to 1.6 Ga McArthur Basin, northern Australia (Jackson et al. 1987), the ~1200 Ma Borden Basin, northern Canada (Jackson and Ianelli 1981), and the ~1200 Ma Amundsen Basin, northern Canada (Young and Long 1977). While these basins record sulfate evaporite deposits that are up to tens of meters thick and spread over hundreds of square kilometers (Grotzinger and Kasting 1993), they were eclipsed by ~800 Ma Neoproterozoic equivalents from the Amadeus Basin, central Australia, which, together with other evaporites such as halite, can cover an area of >100,000 km² and be hundreds of meters thick (Stewart 1979; Lindsay 1987).

The Neoproterozoic (1000-542 Ma) ocean has been interpreted as a transition interval, with some sedimentary facies being similar to those of earlier Proterozoic sequences, while others being akin to those of the Phanerozoic (Grotzinger and Kasting 1993). The precipitation of appreciable amounts of the evaporite mineral gypsum is interpreted as one of those transition features that characterize the mid- to latter portion of the Proterozoic. Nevertheless, studies based on the sedimentary sequences of Precambrian evaporites (e.g.章2
Grotzinger 1986; Grotzinger and Kasting 1993) and the isotopic record of sulfate and sulfide (Canfield 2004; Canfield and Farquhar 2009) have demonstrated that sulfate precipitation was still comparatively sparse during most of this period. This phenomenon was due to low sulfate concentrations in these oceans and its conversion into pyrite by microbial sulfate reduction (Canfield and Farquhar 2009).

As one of the oldest known evaporites, the Bitter Springs Formation in central Australia has played a central role in understanding the evolution of sea water chemistry (Holland 1984). This understanding is largely due to the presence of evaporitic sequences that range from carbonates to gypsum ± anhydrite to halite (Holland 1984; Lindsay 1987). Since the order of these precipitates is the same as that observed in modern marine evaporites, it was possible to deduce that the major ion chemistry of sea water was not too different from the present (Holland 1984).

The Cambrian (542-488 Ma), as the first period of the Phanerozoic, witnessed an ocean chemistry akin to that of today. Importantly, it sets itself apart from the Precambrian by the evolution and radiation of metazoans and associated ecological changes such as predation (e.g. Gould 1989; Butterfield 2007). Associated with these biotic changes is the increasing disturbance of sediments by bioturbation, which is known as the “Cambrian substrate revolution” (Bottjer and Hagadorn 1999; Bottjer et al. 2000). Recently, it has been proposed that this biologically-induced disturbance of sediments led to a several fold increase in seawater sulfate concentration (Canfield and Farquhar 2009). Sediment stirring by benthic animals would result in the oxidation of sedimentary sulfides to sulfates. This increase in seawater sulfate is believed to be linked to an increase in the eventual deposition of sulfate evaporite minerals in the Phanerozoic (Canfield and Farquhar 2009). Indeed, the deposition of sulfate evaporite minerals is seen as largely a “Phanerozoic event” (Canfield and Farquhar 2009).

It is in these Neoproterozoic (~800 Ma) and Early Cambrian (>542 Ma) settings where a detailed investigation of a sulfate-rich hypersaline microbial
ecosystem is undertaken (Chapters 4-5). First, however, a study of the geological contexts for these ancient hypersaline settings will be conducted.

### 2.2 Geological setting and previous work

#### 2.2.1 The Amadeus Basin

The Amadeus Basin is one of several Neoproterozoic depocenters in central Australia that are collectively referred to as the Centralian Superbasin (Walter et al. 1995; Skotnicki et al. 2008) (Figure 2.1). These basins were predominantly filled by sedimentary rocks that correlate interbasinally and rest on underlying middle Proterozoic granitic and metamorphic rocks (Lindsay 1987; Walter et al. 1995). Sediments of the Amadeus Basin record shallow marine and non-marine successions that reach a local thickness of up to 15 km and range in age from the early Late Proterozoic (between ~900 and 1076 Ma) to the Devonian (416-359 Ma) (Ambrose 2006).

The Neoproterozoic successions of the Amadeus Basin are divided into four Supersequences that are separated by unconformities (Walter et al. 1995; Skotnicki et al. 2008) (Figure 2.2). Supersequence 1 records the oldest events that include initial basin formation and the subsequent deposition of thick, widespread tidal marine sand sheets of the Heavitree Quartzite followed by shallow, marginal marine carbonates, evaporites and red beds of the Bitter Springs Formation (Lindsay 1999). The Gillen Member, which represents the early, evaporitic part of the Bitter Springs Formation, will be discussed in greater detail below, as it forms the core time interval of this thesis.
Tectonic deformation (Freeman et al. 1991) or salt diapirism (Kennedy 1993) eventually led to the Areyonga Movement, resulting in uplift and erosion of Supersequence 1 and leading to a widespread unconformity (Skotnicki et al. 2008). This unconformity records the beginning of Supersequence 2, which is composed of sandstone, dolomite, and diamictite of the Areyonga Formation and the overlying deeper-water, shale dominated, Aralka Formation (Preiss et al. 1978; Skotnicki et al. 2008). Evidence of glacial deposition is recorded in diamictites of the Areyonga Formation that has been correlated with the Sturtian glacial events described in the Adelaide Rift Complex (Skotnicki et al. 2008).

Deposition of Supersequence 3 was followed by the uplift and erosion during the Souths Range Movement (Lindsay 1989; Shaw 1991). This sequence begins with mudstone, sandstone, conglomerate, and diamictite of the Olympic Formation and its lateral equivalent, the near-shore Pioneer Sandstone (Lindsay 1989; Field 1991; Ambrose 2006). Wells et al. (1970) interpreted the diamictites of the Olympic Formation to represent a second Cryogenian glacial event that is equivalent to the widespread “Marinoan” glacial
event (Preiss et al. 1978; Knoll and Walter 1992; Walter et al. 2000). An angular unconformity has been recognized between the top of the Olympic Formation and the overlying Gaylad Sandstone unit (Freeman et al. 1991). Thick, shale-dominated beds of the overlying Pertatataka Formation grade upward into dolomite and sandstone beds of the Julie Formation (Freeman et al. 1991; Skotnicki et al. 2008). These aforementioned units of Supersequence 3 (Olympic, Pioneer, Gaylad, Pertatataka, and Julie) are suggested to represent a major marine transgression (Freeman et al. 1991). The mainly fluvio-deltaic red Arumbera Sandstone overlies the Julie Formation and spans the Precambrian/Cambrian boundary (Kennard and Lindsay 1991; Walter et al. 1995). This sandstone unit is separated in most places from the Julie Formation by a disconformity, which represents the Petermann orogeny (650-530 Ma) and Supersequence 4 when the superbasin suffered major fragmentation (Kennard and Lindsay 1991; Walter et al. 1995; Ambrose 2006).

The lower and upper Arumbera sandstones are late Neoproterozoic and early Cambrian in age, respectively, and are separated by a disconformity (Ambrose 2006). The Arumbera Sandstone also acts as a reservoir in the Dingo and Orange gas fields and is an important gas target in the northern and southern Amadeus Basin (Ambrose 2006). The Lower Cambrian Chandler Formation overlies the Arumbera Sandstone and is composed of a carbonate and evaporite succession. Like the Gillen Member of the early Neoproterozoic Bitter Springs Formation, this Cambrian formation is also a major focus of this study on halophilic microorganisms. Therefore, this unit is also introduced below. The remaining sequences of the Amadeus Basin include mid- and late-Cambrian to Devonian sedimentary strata. Because these sequences are beyond the time frame of this thesis, they are not discussed in any further detail. However, it should be noted that the prime oil and gas source rocks occur in the Ordovician Horn Valley Siltstone, which have charged the hydrocarbon fields at Mereenie and Palm Valley.
Figure 2.2 Composite stratigraphic section of the Precambrian/Cambrian time interval in the Amadeus Basin, central Australia. Strata of interest for this thesis are highlighted in orange. Adapted with modifications from Skotnicki et al. 2008.

2.2.2 The search for oil and gas in the Amadeus Basin and a current focus on sub-salt hydrocarbons

The Amadeus Basin has been of intermittent focus in the search for oil and gas reserves (Ambrose 2006). Sediment cores from boreholes in the Amadeus Basin have been previously examined for information about the distribution, quality and distinctiveness of source rock intervals (Jackson et al. 1984; Schroder and Gorter 1984; Summons and Powell 1991). These studies concluded that the prime oil and gas source rocks occur in the Ordovician (488-444 Ma) Horn Valley Siltstone, which have charged the hydrocarbon fields at Mereenie and Palm Valley. The Ordovician oils in these fields have been found to be derived from the remains of the alga or cyanobacterium *Gloeocapsomorpha prisca* (Summons and Powell 1991). Organic matter has also been discovered in Proterozoic and Cambrian sediments, although it was
shown that these sediments have poor petroleum source potential (Jackson et al. 1984; Summons and Powell 1991). Kerogen in these sediments was observed to be of low abundance and mostly restricted to shale and beds where TOC rarely exceeded 1% (Summons and Powell 1991).

Low amounts of bitumen were also found in Neoproterozoic and Cambrian carbonates, which differed in composition from those discovered in shale (Summons and Powell 1991). Rock-Eval pyrolysis and biomarker analyses by Summons and Powell (1991) suggest that organic matter in these Cambrian and Proterozoic sediments is mature with respect to petroleum generation. Although initial studies in the search for Cambrian and Proterozoic oil have been less promising, only small intervals have been sampled. Since the early 1990s, petroleum exploration efforts have been directed to the characterization of a Neoproterozoic sub-salt reservoir (Young and Ambrose 2006). Of particular interest has been the southeastern Amadeus Basin, which is largely unexplored. Available data from this area has been derived from 900 km of multi-fold seismic data and the drilling of six wells (Young and Ambrose 2006). A lower Gillen Member-Heavitree Quartzite petroleum system has been identified in that area, which provides an attractive source and seal (Young and Ambrose 2006). Although lower Gillen Member and Heavitree Quartzite sediments extend over the entire Amadeus Basin, target depths of less than 5000 m are generally restricted to the southern part (Young and Ambrose, 2006). The Lower Gillen Member is considered as the primary source rock interval in that area. Magee-1, the only well to penetrate the Lower Gillen Member, contained thin, bitumen-stained, carbonaceous laminae in a 20 m interval of dolostone and siltstone directly overlying the Heavitree Quartzite (Young and Ambrose, 2006). Although this interval was relatively depleted, with TOC ranging from 0.5 to 0.8%, it is regarded as within the oil window due to methylphenanthrene index (MPI)-derived reflectance values of 1.02 (VRe).

2.2.3 Geological characteristics of the Amadeus Basin evaporites

Because of a tenuous connection with the ocean and the broad shallow nature of the Amadeus Basin, the sea water was characterized by elevated salinity
levels. During two time intervals, salinity levels were elevated to such a point that thick evaporite units were deposited. These units have been defined as the Gillen Member of the early Neoproterozoic Bitter Springs Formation (~800 Ma; Lindsay 1987) and the Chandler Formation of the Early Cambrian (~542; Bradshaw 1991). The thickness of the Gillen Member varies as it has been distorted by intraformational salt movements. It appears that the deposition of this evaporite was cyclic and followed patterns identified in other major evaporite basins (e.g. Permian Zechstein evaporites of western Europe): carbonates and sulfates deposited closer to the basin margin, while later stage halite and possibly potassium salts deposited toward the basin center (Lindsay 1987). The conditions that led to the deposition of evaporites occurred after relative sea level reached its maximum in the lower half of the Gillen Member and begun to fall again. A minimum of five smaller depositional cycles, at least two of which contain halite, can also be identified within these sediments (Lindsay 1987). The occurrence of stromatolites and the interpretation of other sedimentary structures suggest that water levels were relatively shallow but deeper and less oxic than during deposition of the underlying Heavitree Quartzite. It was suggested that the apparent sea-level high may relate to basin dynamics, while the cyclicity of evaporite deposition may be a result of eustatic seal level controls that would allow intermittent inflow of sea water (Lindsay 1987). As the Gillen Member graded into the overlying Loves Creek Member, which is also part of the Bitter Springs Formation, cyclic sedimentation became more pronounced. During the reestablishment of a deeper water phase in the Loves Creek Member, depositional cycles involving large stromatolitic reefal structures developed (Southgate 1986).

Oehler et al. (1979) discovered a number of microfossils from the evaporite deposits of the Gillen Member. These fossils were described as being small in size, low in diversity, and of likely prokaryotic origin. Such findings are in direct contrast to the more diverse fossil assemblages observed in the overlying Loves Creek Member of the same formation. The disparity in fossil assemblages is interpreted to be a result of palaeoenvironmental differences between these two units. The Gillen Member fossils are derived from a marine
evaporite sequence formed in a warm, restricted and periodically hypersaline lagoon (Oehler et al. 1979). The fossilized biota of the Loves Creek Member, in turn, appears to have lived in a more open, shallow marine environment. Therefore, it appears that the Gillen Member fossil assemblage may record an environmentally stressful condition that would feature low species diversity, exclude most eukaryotes and is dominated by prokaryotes (Oehler et al. 1979).

The Cambrian evaporite equivalent to the early Neoproterozoic Gillen Member is represented by the Chandler Formation. This formation has been dated stratigraphically as “Lower Cambrian” (~542 Ma) and represents a marine carbonate and evaporite succession (Bradshaw 1991). The carbonate (Chandler carbonate) includes “organic rich”, foetid, mudstone, which is 10 m thick and has source rock potential (Bradshaw 1991; Ambrose 2006). An overlying evaporite section (Chandler salt) is predominantly composed of halite (>95%, with rare occurrences of gypsum, anhydrite and native sulfur) and is between 225 and 470 m thick (Bradshaw 1991). Three depositional facies have been recognized in this formation: 1) desiccation and evaporite precipitation; 2) basin flooding and carbonate deposition; and 3) karstification and evaporite precipitation (Bradshaw 1991; Ambrose 2006). With the exception of small domal stromatolites, the formation has been described as largely devoid of fossils (Bradshaw 1991). Summons and Powell (1991) mentioned the discovery of a restricted assemblage of filamentous and spheroidal microfossils. Summons (1987) and Summons and Powell (1991) analyzed biomarker patterns of the Chandler Formation and observed that these sediments are characterized by abundant low molecular weight alkanes and an absence of acyclic isoprenoids. This feature has been attributed to a dominance of Bacteria (Summons and Powell 1991). Low concentrations of steranes, triterpanes and tricyclic terpanes were also reported.

2.2.4 Information on the Mt Charlotte 1 core

Work presented on Neoproterozoic and Cambrian evaporite sediments in this thesis was based on rock samples derived from the Mt Charlotte 1 core. This core was drilled in the southeastern part of the Amadeus Basin in 1965, at a
latitude of 24°53’41"S and a longitude of 133°59’11”E (Figure 2.1) (McTaggart et al. 1965; Schmerber and Ozimic 1966). The original purpose of this core was to test the stratigraphy and structure of the Mount Charlotte Anticline (137 km south of Alice Springs) to a total depth of 2115 m (Schmerber and Ozimic 1966). Based on the work conducted by McTaggart et al. (1965) and Schmerber and Ozimic (1966), the Mt Charlotte 1 core contains 649 m of the mid-Neoproterozoic Bitter Springs Formation (Gillen and Loves Creek Member), 487 m of the late-Neoproterozoic Pertatataka Formation, 69 m of the Early Cambrian Chandler Limestone, and 249 m of the Mid to Late Cambrian Jay Creek Limestone. Ages of Mt Charlotte 1 samples have been determined through the rubidium/strontium method (McTaggart et al. 1965), while individual strata have been correlated with those from other cores (e.g. Young and Ambrose 2006).

Currently, one half of the core is stored at Geoscience Australia in Canberra, while the other half, with different depth intervals, is stored at the Northern Territory Geological Survey drill core store in Alice Springs. Both drill core repositories where visited for the purpose of this thesis.

2.2.4.1 The Gillen Member of the Mt Charlotte 1 core

Gillen Member strata are derived from 1554 to 2115 m depth. The member in this core is mostly composed of finely laminated dolomite and anhydrite that is interfingered with halite, shale, siltstone and sandstone. In general, anhydrite and dolomite occur as fine rhythmically interlaminated layers. Anhydrite also occurs as enterolithic structures in an anhydrite-dolomite groundmass. Both siltstone and shale occur as thin layers that grade to very fine sandstone. The siltstone has been described by Schmerber and Ozimic (1966) as very hematitic and chloritic and contains scattered, rounded conglomeritic quartz, feldspar, and both igneous and sedimentary rock fragments. The sandstone is interbedded with the siltstone and is composed of well sorted, angular quartz grains with rare potassium feldspar, igneous and sericitized rock fragments (Schmerber and Ozimic 1966). Coarse crystalline halite, which includes suspended pieces of anhydrite (Figure 3.6), occurs as a thick sequence
between 1863 and 1903 m. Additional streaks and fine layers of halite are present at 1925, 1939, 1961, 1996, 1999, 2036, 2048 and 2060 m (Schmerber and Ozimic 1966).

2.2.4.2 The Chandler Formation of the Mt Charlotte 1 core
Chandler Formation evaporites of the Mt Charlotte 1 core are predominantly composed of evaporites with an interspersion of a thin siltstone and dolomite sequence. The Chandler Formation has been recorded to occur between 936 and 711 m with Schmerber and Ozimic (1966) interpreting the paleoenvironment as highly saline with primary halite precipitation and minor deposition of clastics. The halite in this formation is coarse crystalline, light pink in coloration and interbedded with reddish, clayey siltstone and scattered, angular to rounded coarse-grained quartz, potassium feldspar and some green biotite (Schmerber and Ozimic 1966). The dolomite has been labeled as ferruginous, while interspersed mudstone and siltstone has been described as haematitic (Schmerber and Ozimic 1966). Even quartz in this formation exhibits a reddish iron coating and is testament to the highly oxidizing conditions to which these rocks have been exposed. Such oxidizing conditions were not encountered in the Gillen Member.

2.3 Materials and Methods
2.3.1 Transmitted and reflective light microscopy
Evaporite texture and mineralogy was investigated using a Leica DMRX polarizing microscope which can be operated in transmitted or reflected light. The microscope was equipped with a Leica DC500 camera. Polished thin sections were constructed according to standard procedures.

2.3.2 X-ray powder diffraction (XRPD)
Rock samples selected for XRPD analysis were first crushed to powder in agate mortar to a particle size of <0.5 mm. Two grams of this powder were subsequently ground for 10 minutes with ethanol in a McCrone Micronizing Mill (McCrone Research, Chicago, Illinois). Milling of the powder reduced the
particle size further (~20 μm) and ensured a more even particle size distribution for each mineral phase.

X-ray diffraction was carried out with a SIEMENS D5005 Bragg-Brentano diffractometer equipped with a graphite monochromator and scintillation detector, using CoKα radiation. The scan range was 2 to 85° 2-theta, at a step width of 0.02°, and a scan speed of 1 second per step. Samples were loaded in side-packed sample holders. The results were interpreted using the SIEMENS software package Diffracplus Eva (2003).

Clays from an evaporite sample were investigated by a variety of techniques. First, an oriented clay slide was prepared by crushing the sample to powder, dispersing it in water and extracting a subsample of clays that were in suspension. A diffractometer scan was subsequently run from 2 to 20° 2-theta. Afterwards, the clay slide was saturated with ethylene glycol (24 hours at 50°C) and subsequently run on a diffractometer from 2° to 20° 2-theta to search for smectite (which expands to 17 Å with glycol). This glycolation step was followed by heating the slide to 350 and 550°C to allow for the determination of several clay phases. The heating to 350°C allows for smectite and vermiculite to collapse to 10 Å, while chlorite (14.2 Å) does not. Subsequent heating to 550°C results in the disappearance of kaolinite (7 Å), while chlorite will remain unaffected.

2.3.3. Scanning Electron Microscopy (SEM)

Fine-scale evaporite textures and associated mineralogy were examined at the micro- and nanometer scale through SEM. All analyses were conducted with a Zeiss UltraPlus analytical FESEM (Carl Zeiss Co., Germany) fitted with an INCA Energy 450 EDXA system for elemental disperse spectroscopic (EDS) analysis. Samples were investigated at an acceleration voltage of 3, 7 or 15 kV. Prior to analysis, all samples were sputter coated with gold/palladium.

2.3.4 Electron microprobe analysis
Major elemental compositions and their distributions were measured on a polished, carbon-coated thin section of a dolomite and anhydrite-rich evaporite sample. This study was carried out through electron microprobe analysis (EPMA) with a Cameca SX100 at the Australian National University. EPMA analyses were conducted with an accelerating voltage of 15 kV, a beam current of 100 nA, a counting time of 100 milliseconds and a grid spacing of ~ 1 micron. X-ray mapping was carried out using wavelength dispersive spectrometry, measuring K-alpha radiation for carbon, sulfur, magnesium, iron, calcium, aluminum, potassium, and silicon.

2.3.5 Organic petrography

Prior to petrographic analyses, all samples were roughly crushed and set in cold setting polyester resin. They were subsequently polished using P240 followed by P400-P800-P1200 and P2400 grade paper, before final polishing with diamond powder and colloidal silica on a cloth. The examinations were conducted on a Zeiss reflecting light microscope, employing both incident white light and UV/blue light excitation for fluorescence mode. Reflectance measurements were taken on randomly oriented bitumens, in nonpolarised light (cf. 'maximum VR', which is measured in plane polarised light via stage rotation) and an interference filter having a passband peak of 546 nm was used. The photometer was mainly calibrated against a synthetic garnet standard of 0.92% reflectance. Immersion oil having a refractive index of 1.518 at 23 ± 1°C was used on the samples to enhance contrast and resolution.

2.3.6 Stable isotope analyses of the dolomite

Dolomite samples were reacted for 20 minutes with five drops of phosphoric acid at 90°C in a Kiel Carbonate device attached to a Finnigan MAT 251 mass spectrometer. Sample weights ranged from 259 to 817 micrograms depending upon the content of reactive carbonate in the various samples (ranging from 25-100%). The Oxygen analyses were standardized to VPDB and a fractionation factor, calculated from Rosenbaum et al. (1986), was applied. The fractionation factor was applied assuming that the only carbonate in the samples was pure
dolomite. This was confirmed through X-ray powder diffraction analyses of the samples.

2.4 Results

A number of samples were analyzed for both their mineralogical and petrographic characteristic as well as their hydrocarbon contents. In this chapter, only the mineralogical and petrographic characteristics are described. Unless mentioned otherwise, all samples described and discussed below have yielded indigenous hydrocarbons as determined by the techniques described in Chapters 3 and 4. Although halite and red bed sequences are a considerable component of the evaporitic sequences in Mt Charlotte 1, they were devoid of indigenous hydrocarbons and are not further discussed. Furthermore, since no indigenous hydrocarbons were detected in the Cambrian Chandler Formation of Mt Charlotte 1 (discussed further in Chapter 4), evaporite sequences from this time frame are also not described or discussed below.

2.4.1 Overview of sedimentary textures at hand specimen scale

Samples that yielded indigenous hydrocarbons were mostly composed of dolomite or anhydrite or a combination of the two minerals (Figure 2.3). Minor amounts of other minerals were also detected in these samples using analytical instruments such as petrographic microscopy and XRPD and are discussed further below.

The dolomitic samples were either massive in texture or composed of individual layers that are interbedded with anhydrite (Figure 2.3). The individual dolomite layers usually exhibited a very uneven shape and were fragmented, rolled-up, wrinkled folded or experienced thinning (Figure 2.3). Some coherent pieces of dolomite fragments even exhibit displacement. Based on these textural characteristic, it appears that the dolomite layers had a cohesive and elastic property at the time of their deformation.

Several other evaporite samples were predominantly composed of anhydrite (Figure 2.4). However, in many cases small horizons of dolomitic laminae were also observed within the otherwise massive anhydrite (Figure 2.4).
Figure 2.3 Gillen Member evaporite with alternating layers of dolomite (dark grey) and anhydrite (light grey). Dolomite layers show evidence of: A) bending; B) and C) intense folding fragmentation; D) thinning; E) displacement; and F) complete tearing (rust stain in the center). Coin in large picture is 2.5 cm in diameter.

Figure 2.4 Dark Gillen Member anhydrites (A,C) showing fine, interspersed bedding of dolomite in thin section (B,D). Coins in A) and C) are 2.5 cm in diameter.
2.4.2 X-ray powder diffraction (XRPD)

The following samples (and their depths) have been analyzed through XRPD: 07r011 (1566 m); 07r013 (2115 m); 08r006 (1655 m); 08r008 (1654 m); 08r009 (1654.5 m); 08r011 (1651.1 m); 08r022 (1650.8 m); 09r001 (1652 m); 09r004 (2113.6 m); 09r029a (1651.8 m); 09r029b (1651.9 m). The mineralogy of each of these samples is summarized with organic geochemical data in Chapter 5.

XRPD confirmed the presence of dolomite and/or anhydrite as the main mineral phases in the Gillen Member samples where biomarkers have been extracted (Chapter 5). Furthermore, quartz, feldspar, pyrite, illite, muscovite has been detected in most samples. Halite was not detected through XRPD and this may be a result of grinding the samples to powder with ethanol. This alcohol may have resulted in the dissolution of halite, which has been observed in these samples through SEM (see below). All mineral phases are present at various levels of concentrations and Chapter 5 will investigate the sensitivity of extracted biomarkers with regards to varying concentrations of dolomite and anhydrite.

As noted above, several clay minerals were detected in associated with dolomite (Figure 2.5). The highest intensity of the clay phases was observed for the basal 9.97 Å peak (usually reported in the literature at 10 Å), suggesting the presence of muscovite and/or illite. These two clay minerals overlap in their peak positions and the presence of both types of minerals can be ascertained based on differences in their full width at half-maximum (FWHM). The FWHM is a measure of the width of the XRPD peak, at a height half-way between background and the peak maximum (e.g. Jaboyedoff et al. 2001). While illite displays broad FWHM values, muscovite is characterized by much sharper peaks. Since illite progressively transforms into muscovite as temperature increases (e.g. Hunziker et al. 1986; Warr 1996; Frey and Robinson 1999), this transformation will result in a progressively narrower x-ray diffraction peak width for the muscovite as opposed to the illite. In the Gillen Member samples, the 9.97 Å peak exhibits a broad base, followed by a sharp peak (Figure 2.5). Such a pattern suggests the presence of both illite and muscovite. Indeed, FWHM
values of the 9.97 Å peaks in the Gillen Member samples are around 0.159° 2-theta. Such values are relatively broad and are in stark contrast to pure muscovite peaks, which display values of ~ 0.082° 2-theta (U. Troitzsch, personal communication, 2010). Illite tends to be defined as clay with crystal sizes that are <4 μm in size. Muscovite, by contrast, exhibits sizes >4 μm (Srodon and Eberl 1984). Scanning electron microscopy of the Gillen Member evaporites has shown the presence of platy clays of similar sizes with elemental composition of that of illite and muscovite (discussed below).

Other clay minerals were also detected in the Gillen Member evaporites. Kaolinite, was identified by a basal (001) reflection at 7.1 Å (Figure 2.5) and another (002) reflection at 3.53 Å. The collapse of the kaolinite structure to amorphous material takes place on heating to 550°C, which confirms the identification of this mineral. Chlorite was also identified and had a basal reflection at 14.01 Å. Smectite was not detected in any of the samples. Glycolation of the samples did not result in any characteristic shifts of the XRPD diffraction patterns that would indicate the presence of smectite. All clay minerals were predominantly associated with the dolomite phase. The appearance of gypsum in the clay XRPD samples is a result of hydrating the rock powder, which contains anhydrite. No gypsum was detected in unhydrated samples.
2.4.3 Light Microscopy

The use of light microscopy (transmitted and reflected) allowed for a more detailed investigation of evaporite textures and associated mineralogy. The results described herein provided the basis for conducting further studies such as elemental microprobe analysis and more fine-scale observation under the SEM.
Under transmitted light, Gillen Member anhydrite exhibits bedding laminae that are usually between 1 and 3 mm in thickness, but can be smaller in scale (Figure 2.6). Anhydrite crystals are euhedral and form either a polygonal mosaic of equidimensional crystals (Figure 2.6A,B) or are tabular or selenitic in shape (Figure 2.6D,E). The selenitic (i.e. monoclinic) crystal shape is commonly observed in gypsum. The crystals decrease in size (to <10 μm) when in proximity to the dolomite layers, while progressively increasing in size away from it. Framboidal pyrite is observed to intercalate between individual anhydrite crystals.

Transmitted light microscopy of the dolomitic layers revealed a fibrillose texture, consisting of individual strands that are brown in coloration (Figure 2.7A,C). Reflective light microscopy revealed the presence of dolomite crystals that are interbedded with the brown laminae (Figure 2.7B,D) and usually assume their characteristic trigonal rhombohedral shapes. These dolomite crystals are intimately associated with the dark fibrous laminae and only occur in association with each other. Framboidal pyrite grains are intimately associated with the dolomite crystals and either coat the crystal exteriors or are incorporated into the mineral (Figure 2.8). The incorporated pyrite is concentrically distributed inside the dolomite crystals and is in the nm size range.

While most samples exhibit ordered bedding of anhydrite and dolomite, some contain a high detrital input, with subangular quartz, anhydrite and feldspar grains being cemented together by fine, interstitial anhydrite (Figure 2.9). In many cases, a thin (generally <60 μm), brown layer of dolomite would surround such detritus, before becoming cemented together by anhydrite (Figure 2.9D).
Figure 2.6 Transmitted light micrographs of Gillen Member anhydrite crystals (08r022; 1650.8 m). A) and B) mostly polygonal and interlocking anhydrite crystals. Note decreasing crystal sizes in the top and bottom parts of the images. This size decrease corresponds to a close proximity with dolomite (not shown in these images). C) overview showing anhydrite crystals (in color) compared to the dark dolomite layers (labeled “D”). Note the presence of anhydrite within the dolomite layers. D) and E) tabular/selenitic layers of anhydrite crystals.
Figure 2.7 Transmitted (A,C) and reflective (B,D) light micrographs of dolomitic layers (08r008; 1654 m). Dark fibrous layers in all images correspond to clay laminae observed under SEM. These laminae are also interlayer by finely deposited dolomite not visible under the light microscope. Areas labeled “A” refer to anhydrite crystals. Large dolomite crystals are visualized under reflective light and labeled “D”. Areas labeled “B” refer to anhydrite/clay laminae/dolomite mix.

2.4.4 Scanning Electron Microscopy (SEM)

SEM was used for a more detailed investigation of the evaporites and their mineralogy. Furthermore, electron dispersive spectroscopy (EDS), coupled to the SEM, permitted the elemental composition of various minerals to be discerned.

Three principal mineral distributions were observed under SEM: 1) dolomite-rich layers containing variable concentrations of clay layers; 2) isolated patches of halite; 3) layers of anhydrite (Figures 2.10 and 2.11). The description that follows concentrates on the dolomite layers and the halite patches. The anhydrite layers were mostly monotonous and no additional mineralogical information could be discerned under SEM.

Two types of dolomite textures were observed in the Gillen Member samples: 1) dolomite crystals of a largely uniform size with little or no visible interbedding of
the platy minerals (Figure 2.12A); and 2) dolomite crystals of various sizes that are interbedded with platy minerals (Figure 2.12B). EDS spectra of the platy minerals revealed that they are composed of Si, Mg, Fe, Al, and K (Figure 2.11C). In most cases, these platy minerals are between ≥400 nm in size (Figure 2.12B-D). The shape and elemental composition of these plates corresponds to that of illite ((K,H 3O)(Al,Mg,Fe)2(Si,Al)4O10[(OH)2,(H2O)]). A few rare layers exist were the plates are more extensive and cover an area several micrometers in size (Figure 2.12E,F). Based on their shape and elemental composition (similar to that observed for the smaller counterparts, but with less Fe and Mg), these larger plates are also interpreted as clay minerals, probably muscovite (KAl 3Si3O10(OH)2). As was noted above, both illite and muscovite were identified as major clay phases through XRPD. These clays would drape individual dolomite crystals, forming sedimentary “Augen-type” structures that are akin to those described in modern dolomite precipitating hypersaline settings (compare with Figure 2.19C).

Small nanometer sized dolomite crystals are observed to adhere to individual clay layers (Figure 2.13A), and appear to aggregate together while adhering on to the clay (Figure 2.13B). These crystals are interpreted as dolomite, since their EDS spectra display higher concentrations of Ca and Mg compared to the surrounding clay layers. Larger dolomite crystals contain fragments of platy clays within their structure (Figures 2.13C,D). Subsequent growth of these dolomite crystals deformed and fractured the clay minerals, showing that dolomite precipitation occurred in-situ or within the clay laminae and is not detrital (Figure 2.13D). No evidence was found of putative microbial fossils within the dolomite or anhydrite layers.

Halite crystals occur throughout the dolomite layers as isolated patches (Figures 2.10 and 2.14). The crystal exteriors of halite usually appear slightly dissolved, which may be a result of sawing the rocks at the drill core stores with a diamond blade that uses water as a coolant. EDS confirmed the presence of this mineral, exhibiting a spectrum rich in Na and Cl (Figure 2.14).
Chapter 2

Figure 2.8 Reflected light micrographs of Gillen Member dolomite crystals with incorporated light colored grains of framboidal pyrite. Note the concentric layers of pyrite in numerous dolomite crystals. Sample: 09r009 (1654.5 m).

2.4.5 Elemental Mapping

The distribution of numerous elements was investigated on a polished thin section (08r009; 1654.5 m) containing folded layers of dolomite that is interbedded with anhydrite. The principal aim of this analysis was to discern the distribution and extent of the clay layers observed under SEM and to investigate whether they correspond to the brown fibrous laminae observed under the light microscope.

As was determined through the EDS analyses, the clay layers are composed of variable concentrations of Al, Mg, Fe, Si and K. Therefore, the elemental composition of these clays should be distinguishable from that of dolomite (composed of C, Ca, Mg) and anhydrite (identified by the presence of Ca and S).

Figure 2.15A presents the distribution and concentration gradients of S, showing that anhydrite is present as small layers (see white arrows) that fold
around dolomite (dark blue layers). This observation was confirmed under the light microscope prior to elemental analysis. The distribution of Ca is displayed in Figure 2.15B, showing that the dolomite-rich portions contain fine layers that are devoid of this element (white arrows). Therefore, minerals other than dolomite appear to be present there. Carbon distribution is presented in Figure 2.15C, showing that the highest concentrations were detected in the areas where dolomite is present. Although these elemental analyses where conducted on carbon coated thin sections, their absence in the anhydrite portions (compare with Figure 2.15A) shows that the coating is unlikely to have had a major effect on the results. High concentrations of carbon on the fringes of the elemental map are due to the presence of carbon tape in these areas. Note the presence of some isolated carbon-rich patches (white arrows). These areas may point to the presence of accumulated organic matter. The existence of such areas has been confirmed through organic petrography (see below).

Figure 2.16 shows the distribution of Al, Mg, Fe, Si and K. Collectively, these elements have their highest concentrations as fine layers that span the dolomitic portion of that sample. It appears that these layers were originally horizontally deposited and subsequently tilted as a result of folding. These layers were only observed in conjunction with dolomite and not with anhydrite. The distribution of these layers can be best observed in the images that map the distribution of Al and Si. Based on these observations, the fine layers can be confirmed as the clay laminae that were observed under SEM. These layers also have similar distributions as the brown fibrous layers observed under the light microscope (Figure 2.7) and show that they are also likely clay.
Figure 2.9 A) and B) angular to subangular clasts of quartz, feldspar and anhydrite cemented together by pink, microcrystalline anhydrite. C) and D) close up showing cementation of subangular clasts by microcrystalline anhydrite. Note in D) that some clasts on the upper right became enveloped by thin layers of dolomite (red arrow). Sample: 09r010 (1659.7 m).

2.4.6 Carbon and oxygen isotopic analyses of the dolomite layers

Carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) stable isotope values were measured of several dolomite layers from the Gillen Member. The results of these analyses are tabulated in Table 2.1.

The $\delta^{13}C$ values range from -6.2 to 0.8‰ VPDB, with an average of -3.2‰ VPDB, and have an average reproducibility of 0.04‰ (Table 2.1). Most of the $\delta^{13}C$ values are in the negative range. The $\delta^{18}O$ values for the samples range from 1.39‰ to 2.28‰ VPDB, with an average of 1.91‰ VPDB, and have an average reproducibility of 0.04‰ (Table 2.1).
2.4.7 Organic petrography

Three types of bitumen have been observed in the Gillen Member evaporites: 1) bitumen occupying interstitial vugs between dolomite crystals (Figure 2.17A,B); 2) thucholites, which are spherical in shape and are characterized by lower, but variable, reflectance and variable fluorescence, in places forming halos (Figure 2.17C,D,E,F); 3) bitumen balls, similar in morphology to the thucholites but without their diagnostic characteristics and possibly referable to thucholites. Reflectance values of the Gillen Member bitumen range from about 0.5-1.7%. Finely disseminated stringers of organic matter, possibly derived from algal/bacterial sources, have also been observed in the dolomite portions. Framboidal pyrite is often associated with the bitumens.
2.5 Discussion

2.5.1 Paleoenvironmental setting

Rock samples of the Gillen Member from Mt Charlotte 1 are characterized by dolomitized laminae, intraclasts, displacive growth of anhydrite, an input of angular detritus and red beds. These sedimentary features, taken in combination, point to a shallow marine, possibly sabkha-style depositional environment (see Nichols 2006 for discussion on depositional features of sabkhas) (Figure 2.18). A “sabkha” is the Arabic word for a salt enriched flat (Kinsman 1969) and has been defined by Friedman et al. (1992) as surfaces of deflation that were formed by the removal of dry, loose sedimentary particles down to the top of the capillary water zone.
Figure 2.12 SEM micrographs of dolomite layers from the Gillen Member (AS 19; 1532.8 m). A) dolomite crystals of a largely uniform size with little or no visible interbedding of the platy clay minerals. B) dolomite crystals of various sizes that are interbedded with platy minerals. C) and D) close ups of platy clay minerals labeled “C” and dolomite labeled “D”. E) and F) Large platy clay laminae “C” with interbedded dolomite “D”. “A” refers to anhydrite.
Figure 2.13 SEM micrographs showing close ups of the interaction between clay laminae and dolomite (AS 19; 1532.8 m). A) Small, nanometer sized, dolomite crystals (red arrows) adhering to clay laminae. B) Merging of minute dolomite crystals (red arrows) on the clay laminae. Note large dolomite grain in the mid to lower right. C) Dolomite grain between clay laminae. Note that the grain is composed of smaller, nanometer-sized particles and the incorporation of clay laminae (red arrows). D) Cross-section of a large dolomite crystal with incorporated clay laminae (red arrows). Dolomite crystal is surrounded by clay laminae, which exhibit fragmentation in the lower left of the image.

Figure 2.14 SEM micrograph and EDS spectra of an isolated halite patch (“H”) within dolomite (“D”) (08r008; 1654 m).
Figure 2.15 Elemental maps of a Gillen Member sample (08r009; 1654.5) with alternating dolomite and anhydrite layers. A) Sulfur. B) Calcium. C) Carbon.
Figure 2.16 Elemental maps of a Gillen Member sample (08r009; 1654.5) with alternating dolomite and anhydrite layers. A) Aluminum. B) Potassium. C) Silicon. D) Magnesium. E) Iron.

In this setting, evaporation in the supratidal zone results in the updraw of saline groundwater through coastal sediments, resulting in the precipitation of
evaporite minerals within and on the sediment (Nichols 2006). Microbial mats and associated microbialites often form in the intertidal zone of sabkhas (Nichols 2006; Bontognali et al. 2010). A large fraction of samples in this study consist of dolomitized layers that resembles microbial mats (see further discussion below). In modern sabkhas of the Arabian Gulf, authigenic dolomite precipitation occurs within the microbial mats in the intertidal zone (Bontognali et al. 2010). Based on these similarities with modern settings, it appears that the Gillen Member samples were also deposited in a sabkha-style depositional environment, probably in the intertidal zone.

Table 2.1 List of Gillen Member dolomite samples from the Mt Charlotte 1 core and their δ¹³C and δ¹⁸O isotope values

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>δ¹³C</th>
<th>δ¹⁸O</th>
</tr>
</thead>
<tbody>
<tr>
<td>1453</td>
<td>0.8</td>
<td>1.4</td>
</tr>
<tr>
<td>1532.2</td>
<td>-5.5</td>
<td>2.1</td>
</tr>
<tr>
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<td>2.1</td>
</tr>
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<td>-5.1</td>
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<td>-5</td>
<td>1.8</td>
</tr>
<tr>
<td>1537.8</td>
<td>-4.9</td>
<td>1.6</td>
</tr>
<tr>
<td>1539.9</td>
<td>-5.1</td>
<td>1.7</td>
</tr>
<tr>
<td>1651.8</td>
<td>-0.1</td>
<td>1.7</td>
</tr>
<tr>
<td>1652</td>
<td>-0.2</td>
<td>1.9</td>
</tr>
<tr>
<td>1654</td>
<td>0.7</td>
<td>2.1</td>
</tr>
<tr>
<td>1655.9</td>
<td>-4.9</td>
<td>2.1</td>
</tr>
</tbody>
</table>
Figure 2.17 Petrographs of Gillen Member dolomites (08r009; 1654.5 m). A) and B) Bitumen (light grey; ~1.5% reflectance) occupying interstitial vugs between dolomite crystals. Highly reflecting round particles in all white-light images represent framboidal pyrite. C) Thucholite under white light with a reflectance of 0.55%. D) Thucholite as in C) but in fluorescence mode. Note fluorescing halos inside the thucholite. A fluorescing mineral grain is present on the upper right side of the thucholite. E) Thucholite under normal white light. F) Thucholite as in E) but in fluorescence mode.

2.5.2 Presence of microbial mat textures
Numerous Gillen Member evaporites contain layers of dolomite that were folded, exhibited tearing and pinching, and were broken into chips (Figure 2.3). Nevertheless, these perturbed or contorted layers retained a level of cohesiveness and did not experience complete breakup. Furthermore, some textures that show pinching and folding suggest that the layers also had some elastic properties, which allowed a level of flexibility.

![Figure 2.18 Schematic cross-section of a coastal sabkha. Diagram demonstrates how evaporation in the supratidal zone results in the draw-up of saline water through coastal sediments. Adapted with modifications from Nichols 2006.](image)

Similar textures have been previously reported from a variety of ancient sedimentary facies and are often interpreted as the remains of microbial mats that imparted the cohesive and elastic properties (e.g. Schieber 1986; Bouou gri and Porada 2002; Sarkar et al. 2006). This interpretation is based on field observations of modern microbial mats and their behavior as a result of various environmental stressors (e.g. evaporation, gas accumulation) in contemporary coastal or shallow water settings (Gerdes et al. 1993, 2000b). Indeed, modern shallow basin-margin evaporitic systems are characterized by the abundance of thick stratified microbial mats that are dominated by cyanobacteria in the upper layers (e.g. Gerdes et al. 2000a; Javor 2002). Microbial mats are composed of microbial cells, which are embedded in a network of extracellular polymeric
substances (EPS). The EPS is composed of high molecular weight organic compounds (mostly polysaccharides), which, together with the cells, provide significant cohesive strength (Gerdes et al. 2000a; Donlan 2002; Donlan and Costerton 2002). It is this cohesive property of microbial mats that results in the formation of various biogenic structures. In subaerially exposed coastal settings, drying and tearing of microbial mats as well as upcurling of mat margins can give rise to so called “microbial mats chips” (Gerdes et al. 2000a). In subaqueous settings, microbial mats that are loosely attached to the underlying substratum tend to scour and tear, also resulting in folds (Gerdes et al. 2000a).

Upfolding of microbial mats or their lateral expansion can also result due to the precipitation of evaporitic minerals (e.g. gypsum) onto their surfaces (Assereto and Kendall 1977; Park 1977; Gerdes et al. 2000a). Such mineral encrustation of microbial mats is typical of hypersaline settings, where continuous sea water evaporation results in mineral precipitation. Park (1977) noted that such evaporite minerals (e.g. gypsum, halite) can also distort and/or destroy individual lamina. Transmitted light micrographs of the Gillen Member samples (Figure 2.6) show the presence of anhydrite crystals inside individual dolomite laminae which are likely to have distorted the layers, particularly resulting in small-scale folds.

Another process that results in the folding and doming of microbial mats is gas accumulation beneath the mats (Gerdes et al. 2000a). Hypersaline microbial mats that are enriched in reduced sulfur compounds are known for their capacity to produce methane gas (e.g. Kiene et al. 1986; Gerdes et al. 2000a). The accumulated gas elevates the cohesive microbial mat layers, which, in turn retards the escape of gas into the air or water (Gerdes et al. 2000a). In the Gillen Member, biomarker evidence for putative methylotrophic methanogens (Chapter 5) was detected, pointing to the former presence of methane generation in these sediments. Therefore, it is possible that gas accumulation may have also contributed to the perturbed mat textures.

Some dolomite layers also coated individual detrital anhydrite, quartz and feldspar grains. Such layers were quite small in size (generally <60 μm) and only observed under the light microscope. Similar coatings of carbonate on
individual mineral grains (e.g. sulfate minerals) have been previously interpreted as evidence for microbial activity (Gerdes et al. 2000a).

While the above discussion favors a biologic source for the perturbed dolomite textures, abiotic features should not be discounted. Physical current waning, sediment loading and wind-induced shear (e.g. Allen 1985; Dzulynski and Simpson 1966) could also explain some of the textures observed in the Gillen Member. However, as discussed below and in Chapter 5, a number of other mineralogical and biomarker observations support the interpretation of the dolomite layers as fossil microbial mats: characteristics of low-temperature dolomite precipitation; concentric frambooidal pyrite inside the dolomite; shape, distribution and association of clay laminae with dolomite crystals; carbon (δ13C) and oxygen (δ18O) stable isotope values of the dolomite; and dolomite-specific biomarker composition.

2.5.3 Modern hypersaline mats from coastal sabkhas: potential equivalents of the Gillen Member dolomite laminae?

In order to understand what ancient hypersaline microbial mats were like, one can compare the settings of ancient samples with those from modern equivalents. Modern dolomite precipitating mats of coastal hypersaline sabkhas are predominantly composed of cyanobacteria (e.g. Bontognali et al. 2010), which play host to a consortium of various other microorganisms. As shown in Chapter 5, there is biomarker evidence for cyanobacterial mats in the Gillen Member. In modern sabkhas, intertidal/supratidal cyanobacterial mats undergo repeated subaerial exposure and are reported to develop felty cyanobacterial layers in the upper photic zone that are strongly cohesive and feature a leathery surface (Porada et al. 2007). The internal structure of these felty layers has been described as a condensed fibrillar meshwork that is composed of mostly parallel, horizontally stretched ensheathed filament bundles (Gerdes et al. 2000a; Gerdes et al. 2007; Porada et al. 2007). These organisms demonstrate phototactic movement by gliding up and down in a mat to position themselves to optimal light intensities (Gerdes et al. 2000a). Organic matter that is subsequently buried underneath such mats consists of empty abandoned sheaths, immotile filaments and unicells. These features, combined with EPS
provide significant cohesive strength to the mats (Gerdes et al. 2000a). It is this cohesive strength that enables mats to form the various biogenic structures discussed above.

Another common feature of modern shallow water hypersaline conditions are coccoidal, slime-ensheathed cyanobacterial populations (Gerdes et al. 2000a; Gerdes et al. 2007). These cells are reported to be particular abundant during arid conditions, producing copious amounts of EPS, viscous polysaccharide solutions and protective pigmentation to cope with extreme light radiation (Gerdes et al. 2007). While no specific textures were observed in the Gillen Member that would point to coccoidal cell accumulations, their presence during that time cannot be ruled out. Coccoidal cells alternate with filamentous cells during changing environmental conditions (forming the characteristic $L_{h}$/$L_{v}$-laminae outlined in Gerdes et al. 2000a) and their copious slime or EPS production is associated with sites where carbonates and gypsum precipitate (Gerdes et al. 2007). Indeed, Bontognali et al. (2010) reported the precipitation of dolomite on EPS from a microbial mat with coccoidal cyanobacteria (see more detailed discussion below). It is possible that the lack of fossilized filamentous cyanobacteria in the Gillen Member indicates that the Gillen Member dolomites were also precipitated in a microbial mat dominated by coccoidal cyanobacteria. Such fossils, if present, would be difficult to distinguish from other minerals of a similar shape. Below, mineralogical evidence from the Gillen Member is presented that further points to microbial mediation in the formation of dolomite textures.

2.5.4 Precipitation of dolomite
Most evaporite samples of the Gillen Member contain dolomite. This mineral is commonly formed in nature. However, its precise formation mechanism in the geological past and present has eluded researchers for centuries and is known as “The Dolomite Problem” (McKenzie 1991). Below, both abiotic as well as biotic models in dolomite precipitation are discussed. Through the discussion of previous research work on the precipitation of this mineral, it will be shown that
the presence of dolomite in the Gillen Member is most likely the result of biological factors.

2.5.4.1 Abiotic dolomite precipitation and associated problems

The geological record exhibits vast deposits of dolomite with fluctuating but globally decreasing abundance through time. While a link has been suggested between this uneven abundance of dolomite and global environmental changes, it has not yet been established and is still eluding researchers. Compounding this problem is the issue that laboratory synthesis of dolomite under sterile conditions has only been possible at temperatures greater than 100°C (Land 1998; Lippman 1973), or by braking Ostwald's step rule with fluctuating pH conditions (from between pH 5.3 and 6.1 to pHs 8.34 and 8.85; Deelman 1999). While temperatures greater than 100°C are typical of burial conditions in sedimentary basins, they do not explain the formation of primary dolomite in nature under low-temperature conditions (McKenzie 1991). Likewise, fluctuating pH conditions like those described under laboratory conditions by Deelman (1999) are not observed in nature. Currently it is still unclear what exactly inhibits dolomite formation under low-temperature laboratory conditions and what promotes its formation in natural environments.

Since the discovery of Holocene dolomite forming beneath supratidal sabkhas of the Arabian Gulf (Wells 1962) in supratidal crusts in the Bahamas (Shinn et al. 1965) and in alkaline ephemeral lakes of the Coorong region in South Australia (von der Borch 1965), numerous models and hypothesis concerning dolomite formation have been proposed. A number of abiotic models have been suggested that concentrate on the physico-chemical aspects of dolomite formation. In the “sabkha model” (Kinsman 1969; Hsu and Siegenthaler 1969; McKenzie et al. 1980; Mueller et al. 1990; Wood et al. 2002), strong evaporation concomitant with gypsum precipitation in the supratidal zone is held responsible for the formation of dolomite. Because gypsum precipitation decreases the concentration of dissolved Ca\(^{2+}\) and SO\(_4^{2-}\), a supersaturated solution with a high Mg/Ca ratio is left behind. This supersaturated solution is thought to promote dolomite formation. Another well known model is the “Dorag
dolomitization model” or “mixing zone model” (Badiozamani 1973). This purely theoretical model is based on a calculation that mixing of meteoric water with up to 30% seawater produces undersaturation with respect to calcite and thereby increases dolomite saturation. However, mixing zone dolomitization has never been observed in modern environments (Bontognali 2008). Furthermore, a major problem with both the mixing zone and sabkha models is that they do not explain how kinetic barriers of dolomite precipitation under low temperature laboratory conditions can be overcome (Bontognali 2008).

2.5.4.2 Biologically induced dolomite precipitation: a viable alternative

Numerous observations and experiments suggest that biology may play a significant part in the mineralization process of dolomite and that physico-chemical parameters by itself are insufficient in describing the precipitation of this mineral. While the hypothesis of biologically induced dolomite precipitation is not new (Nadson 1928; Neher and Roher 1958), it is only in the past three decades that this concept has been increasingly investigated.

In the early 1980s, Kelts and McKenzie (1982) and Pisciotto and Mahoney (1981) described in situ dolomite formation in organic, carbon-rich deep-sea sediments. The precipitation of this dolomite was found to be in association with microbial sulfate reduction and methanogenesis. In the decades that followed, numerous cases of dolomite formation in organic, carbon-rich sediments have been reported from peritidal, shallow-marine and deep-sea settings (see Mazzullo 2000 and references therein), resulting in the formulation of the “organogenic model” for dolomite precipitation (Compton 1988; Slaughter and Hill 1991). In this model, presumed kinetic barriers for low-temperature dolomite formation are overcome as a result of the metabolic activities of bacterial sulfate reduction and methanogenesis. Alkaline conditions, necessary for the precipitation of carbonates, would be achieved as a result of the metabolic consumption and recycling of buried organic matter. Consequent carbonate saturation together with Ca and Mg dehydration would then lead to dolomite precipitation from fluids with a high Mg/Ca ratio (Compton 1988; Slaughter and Hill 1991).
Further evidence for a biogenic role in dolomite precipitation came in the mid-1990s. Vasconcelos et al. (1995) demonstrated low-temperature dolomite precipitation in the presence of sulfate-reducing bacteria (SRB) that were previously isolated from a modern dolomite precipitating hypersaline lagoon in Brazil. This laboratory experiment, together with the study of the Brazilian hypersaline lagoon, led to the formulation of the “microbial model” for dolomite precipitation (Vasconcelos and McKenzie 1997). This model has been subsequently tested in the laboratory (Warthmann et al. 2000) as well as through further combined laboratory and field studies using SRB (van Lith et al. 2003a; Wright and Wacey 2005), moderately halophilic heterotrophic bacteria (Rivadeneyra et al. 1993) and methanogens (Roberts et al. 2004).

Currently, SRB-influenced dolomite precipitation is the best understood model for the precipitation of this mineral. In most studies, it is postulated that the consumption or excretion of metabolites plays a key role in mineral precipitation (Vasconcelos and McKenzie 1997; Warthmann et al. 2000; van Lith et al. 2003a,b; Wright and Wacey 2005). Concomitant increases in pH and removal of $SO_4^{2-}$, which is considered an inhibitor of dolomite precipitation (Baker and Kastner 1981), provides the condition for dolomite precipitation. A simplified chemical reaction of sulfate reduction can be written as:

$$\text{eq. 2.1: } 4\text{CHO}_2^- + SO_4^{2-} + H^+ \rightarrow 4\text{HCO}_3^- + HS^-$$

The reaction for primary dolomite precipitation is defined as:

$$\text{eq. 2.2: } 4\text{HCO}_3^- + 2\text{Mg}^{2+} + 2\text{Ca}^{2+} \rightarrow 2\text{CaMg(CO}_3\text{)}_2 + 4\text{H}^+$$

In addition to these reactions, the adsorption of $Ca^{2+}$ and $Mg^{2+}$ is proposed as an important factor in microbially-induced dolomite precipitation (van Lith et al. 2003b).

However, while the above mentioned scenarios would work for a living microbial mat, it does not explain present-day dolomite formation in buried 1700 year old microbial mats from the supratidal zone of a coastal sabkha in Abu
Dhabi (Bontognali et al. 2010). Since these mats show no signs of intense microbial activity, this dolomite growth cannot be explained as a result of active metabolic growth. Recently, Bontognali et al. (2010) have observed the growth of dolomite crystals on the exopolymeric substances (EPS) of both living and buried mats from Abu Dhabi. EPS carries a net negative charge with the capacity to bind ions (Bontognali et al. 2010). The role of EPS in templating the precipitation of dolomite only requires a pre-existing mat and no living organisms to sustain it. Nevertheless, as noted by Bontognali et al. (2010), since microbial activity is originally required to produce the EPS, it would be inaccurate to describe this process of dolomite precipitation as “abiotic”.

2.5.4.3 Comparisons of Gillen Member dolomites with those from modern sabkhas

Comparisons of the Gillen Member dolomites with those precipitating in modern microbial mats from hypersaline settings reveal several similarities (Figure 2.19). In the modern samples, dolomite is tightly associated with microbial mat filaments (either cellular or EPS), while in the Gillen Member it is associated with clay laminae (discussed below) only. As noted in section 2.5.6, these clay laminae are interpreted to record the former presence of microbial mats. Therefore, a microbial origin is inferred for the Gillen Member dolomites. SEM observations of the Gillen Member dolomite grains also reveals a striking similarity to those from modern environments (Figure 2.19C,D).

While most Gillen Member dolomites show this intergrowth of clay laminae and dolomite, some parts mostly exhibit dolomite crystals only. This pattern likely indicates that dolomite crystal growth in some parts was considerably intense, resulting in the eventual overgrowth of the dolomite crystals over the clay laminae, while in other parts, the rate of dolomite growth was more moderate.

The incorporation of subrounded to subangular quartz crystals as well as feldspars into the dolomite layers is consistent with modern dolomite precipitating microbial mats (Bontognali et al. 2010). In these modern settings, both quartz and feldspar grains of detrital origin were transported by flood
waters and eventually trapped and bound by the microbial mats (Reid et al. 2000). The angularity of the Gillen Member quartz grains also suggests that the quartz was of detrital origin.

Figure 2.19 Comparisons of microscopic observations between modern-day (A, C) and Neoproterozoic (AS 19; 1532.8 m) (B, D) carbonate precipitation. A) Light microscopic image of carbonate particles (light grey) growing in a modern-day filamentous microbial mat (dark layers). Scale represents 150 μm. B) Reflective light microscopic image of Neoproterozoic dolomite crystals (white crystals) associated with dark laminae of clay minerals. C) SEM image of a growing carbonate particle that is interpreted to have pushed a microbial lamina apart. Scale represents 30 μm. D) SEM image of a Gillen Member dolomite growing between clay laminae. Images of A) and C) are adapted from Gerdes et al. 2000a. Note that these authors referred to these minerals by their general term “carbonate”. Their mineral shape and occurrence suggests that it is dolomite.

2.5.4.4 The co-occurrence of sulfate and dolomite

Sulfate has been regarded as an import inhibitor of dolomite precipitation in seawater. This process is known as the “sulfate inhibition model” (Baker and Kastner 1981) and is based on laboratory precipitation experiments at 200°C, which demonstrates that even small concentrations of sulfate (>2 mM) inhibit calcite transformation to dolomite. In natural environments, sulfate concentrations may be diminished as a result of microbial sulfate-reduction, seawater mixing with meteoric water and gypsum/anhydrite precipitation. These processes may help explain the precipitation of dolomite in sulfate-rich
environments, such as those encountered in the Neoproterozoic Gillen Member. However, it should be pointed out that the sulfate inhibition model has recently also come under attack (Sánchez-Román et al. 2009). Studies conducted on numerous modern hypersaline environments have reported dolomite precipitation from solution with sulfate concentrations that are 2 to 70 times greater than seawater (Folk and Land 1975). Furthermore, as stated above, Baker and Kastner (1981) derived the model from laboratory experiments that were carried out at 200°C, which do not simulate natural, low-temperature conditions in hypersaline settings. It should also be highlighted that low-temperature laboratory experiments have shown no precipitation of dolomite, even in the complete absence of sulfate (Bontognali 2008). These observations make it clear that the presence of sulfate does not necessarily result in the inhibition of dolomite precipitation.

2.5.5. Presence of framboidal pyrite

Framboidal pyrite is composed of densely packed spherical or subspherical clusters of micron to submicron-sized pyrite crystals (Wilkin and Barnes 1997). In the Gillen Member evaporites, framboidal pyrite was mostly associated with the dolomite. This pyrite would either coat the surfaces of dolomite or be concentrically incorporated inside this mineral (Figure 2.8). Framboidal pyrite was also observed in association with organic matter (Figure 2.17) or in the interstitial space between anhydrite crystals.

The presence of framboidal pyrite has long been regarded as evidence for biogenic activity (e.g. Kaplan et al. 1963; Love and Amstutz 1966; Berner 1969; Rickard 1970). While this mineral has also been shown to form abiotically in high temperature hydrothermal systems (e.g. Steinike 1963) and in the laboratory (e.g. Ohfuji and Rickard 2005 and references therein), there is a considerable discrepancy in the precipitation conditions where it forms abiotically compared to where it is commonly observed in the natural environment (Maclean et al. 2008). In the laboratory, inorganic synthesis of framboids has been carried out only at elevated temperatures from between 60 and 85°C (Sweeney and Kaplan 1973) to 150 and 350°C (Graham & Ohmoto
Chapter 2

1994). Such temperatures are in stark contrast to those where frambooids naturally form (~25°C) (Maclean et al. 2008). Assuming that the temperature conditions experienced by the Gillen Member where similar to those of modern sabkha environments of Abu Dhabi (up to ~50°C; Bontognali et al. 2010), it would still be less than those experienced in the laboratory. Further clues to a biotically induced precipitation of frambooids are provided by its precipitation below redox interfaces (Kohn et al. 1998; Popa et al. 2004) and its close spatial relationships with organic matter (e.g. Grimes et al. 2001; Large et al. 2001; Popa et al. 2004). Indeed, macromolecules and polymers have been shown to act as important contributors in regulating size and morphology of such crystals as well as influencing particle aggregation (Mann 1988; Bianconi et al. 1991; Matijevic 1996). Since biogenic organic matter is predominantly composed of macromolecules and polymers, it could explain the close spatial relationship between frambooidal pyrite and sedimentary organic matter.

In the Gillen Member samples, frambooidal pyrite was also closely associated with organic matter (Figure 2.17). A close link between biology and frambooidal pyrite formation can also provide further supporting evidence that the Gillen Member dolomites are biologically induced. As mentioned in section 2.5.4.2, a link was established between sulfate reducers and the precipitation of dolomite. If the frambooidal pyrite is a product of sulfate reduction, then it could explain their presence within the dolomite. A collaborative project has recently been set up with Dr. David A. Fike (Washington University in St. Louis, U.S.A) to measure the sulfur isotope composition of these pyrite grains using NanoSIMS. Such sulfur isotopic compositions may help confirm the biogenic nature of the frambooidal pyrite that was incorporated into the dolomite crystals.

2.5.6 Clay laminae within dolomite layers

In this study, clay laminae where present within the dolomite layers. Moreover, dolomite crystals precipitated within these clay laminae and grew to various sizes (Figures 2.12 and 2.13). When compared to modern dolomite precipitating sabkhas, the clay layers assume a shape and distribution similar to
contemporary microbial mats (Figure 2.19). It is therefore hypothesized that the clay layers represent the presence and former distribution of microbial mats.

Intricate relationships have been previously noted between clay minerals and microbial mats. Studies conducted by Fox et al. (2009) on surface crusts from South African soils observed the presence of silt-sized mica flakes assuming a laminated structure. Some of these laminae were suggested to have formed as a result of biological mediation by microbial films composed of algae, fungi and cyanobacteria (Fox et al. 2009). Microbes have in the past few decades been held responsible for the accumulation of clay minerals by changing the chemistry of the surrounding environment allowing for clay to flocculate (Fox et al. 2009) or nucleate (Ferris et al. 1987; Konhauser et al. 1993). A relatively high pH associated with carbonate precipitation may also have contributed to significant clay flocculation (c.f. Fox et al. 2009).

While authigenic clay mineral formation on microbial mats is a likely scenario, a detrital input cannot be ruled out. Indeed, biological material has been previously suggested to act as a filter to trap clay (e.g. Tiessen and Stewart 1988; Fox et al. 2009). Illite, in particular, has a stable crystal structure, accounting for their abundance as weathering products (Andrews et al. 1996). Therefore, the illite may have had a detrital origin and became eventually trapped in the microbial mats. However, detrital input through weathering of surrounding rocks is usually more common in colder or temperate climates (Andrews et al. 1996). The much warmer climate that would likely have occurred during the deposition of the Gillen Member may not have produced sufficient weathering products.

In the Gillen Member dolomites, illite and mica are the principal clay minerals. A likely reason for the absence or lesser abundance of expandable clays such as smectite is age. While late Tertiary clays are predominantly of the expandable type, they become progressively less common in older rocks (Blatt and Tracy 1996). In lower to middle Paleozoic rocks, for example, expandable clays average less than 10% of all clay minerals. In such rocks, illite, with few or no smectite interlayers becomes more common (Blatt and Tracy 1996). Such
trends have been reported worldwide from numerous stratigraphic sections and result from the following diagenetic alteration:

\[
\text{eq. 2.3:} \text{smectite} + \text{Al(OH)}_3 + \text{K}^+ \rightarrow \text{illite} + \text{H}_4\text{SiO}_4 + \text{Na}^+ + \text{Ca}^{2+} + \text{Fe}^{2+,3+} + \text{Mg}^{2+} + \text{H}_2\text{O}
\]

This diagenetic change can be explained as a result of water loss and heating, resulting in progressive changes in crystal structure from randomly interlayered smectite and illite to an ordered interstratification of discrete illite (Blatt and Tracy 1996). Further water loss causes illite to undergo additional changes in its crystal structure to form muscovite. The conversion of smectite into illite has been reported from temperatures as low as 50°C and is largely completed at 120°C (Blatt and Tracy 1996). Progressively higher temperatures will convert this illite into muscovite. It should be noted that the temperature-clay conversion relationships are far from perfect and depend on variables such as the diagenetic environment, availability of potassium, mobility of aluminum, composition of smectite sheets, subsurface fluid pressure, and the presence of calcite (Blatt and Tracy 1996). The presence of subsurface salt can also affect temperature-clay conversion relationships through a variety of opposing effects. The high thermal conductivity of salt, for example, can increase smectite-illite conversion rates, while sodium sourced from halite can stabilize the smectite (Blatt and Tracy 1996). Potassium derived from posthalite evaporites, in turn, can enlarge the stability field of illite (Blatt and Tracy 1996). Since the Amadeus Basin contains a significant amount of subsurface salt, these effects may have played a significant role on any clay conversions in the samples analyzed for this study.

As shown through SEM (Figure 2.13D), the clay laminae surrounding dolomite grains show evidence of bending and eventual fragmentation. These occurrences demonstrate that the clay laminae were already in place before or during the precipitation of the dolomite crystals. Furthermore, dolomite crystals continued to grow, resulting in the fracturing of the clay.
2.5.7 Carbon and oxygen isotopic analyses of the dolomite layers

Depleted carbon (δ\textsuperscript{13}C) and oxygen (δ\textsuperscript{18}O) stable isotope values were obtained for several Gillen Member dolomite layers (Table 2.1). The values for both elements are similar to those obtained from Pleistocene Sabkha dolomites offshore of Al Jubayl in the Arabia Gulf (Chafetz et al. 1999). Below, δ\textsuperscript{13}C results are discussed first, followed by those from δ\textsuperscript{18}O.

In the Quaternary-age sabkha deposits of Al Jubayl, depleted δ\textsuperscript{13}C values can, in principle, be interpreted as downward percolation of meteoric waters beneath soil zones (Chafetz et al. 1999). In soils, plant matter decay can generating isotopically depleted \textsuperscript{13}C that may infiltrate the top few meters of the underlying dolomite (Chafetz et al. 1999). Such soil horizons could form during the wet pluvials that existed during Pleistocene in the Arabian Peninsula (Chapman 1971; Chafetz et al. 1999). However, as noted by Chafetz et al. (1999), no firm evidence for soil horizons exists in the samples from the Al Jubayl borings and the presence of abundant evaporites does not support the presence of a significantly wetter climate. In the Gillen Member ancient soil horizons have also not been reported and the significant amount of evaporites does not support a wet climate at that time. Therefore, an alternative explanation needs to be found for the depleted carbon isotope values.

In the Al Jubayl deposits, excursions recording particularly low δ\textsuperscript{13}C values correspond to areas that have signs of former reducing condition (Chafetz et al. 1999). This observation led Chafetz et al. (1999) to suggest that dolomitizing microbial mats and their sulfate reducing microenvironment led to the isotopically low δ\textsuperscript{13}C values.

In Abu Dhabi, which acts as a modern analogue for the depositional setting of the Gillen Member evaporites, the tidal zone is colonized by living microbial mats (e.g. Chafetz et al. 1999; Bontognali et al. 2010). These mats can become buried during the progradation of the sabkha sequence and have been traced inland for up to 9 km (Butler 1969; Bontognali et al. 2010). The eventual decay of these buried mats is hypothesized to supply \textsuperscript{13}C-depleted carbon to the surrounding sediments (Behrens and Frishman 1971; Wainright and Fry
1990; Andrews 1991) and produce a zone with predominantly reducing conditions (Chafetz et al. 1999). Common reaction products under such reducing conditions are H₂S, calcite and dolomite (Machel et al. 1995). Indeed, dolomite formation at Abu Dhabi has only been observed under reducing conditions (Patterson and Kinsman 1982), and is in line with laboratory experiments where dolomite forms in the presence of sulfate-reducers (Vasconcelos et al. 1995).

Low δ¹⁸O values, like their δ¹³C counterpart, can also be interpreted as resulting from microbial sulfate-reduction (Chafetz et al. 1999). Indeed, Sass et al. (1991) observed that low δ¹⁸O values of early, diagenetic carbonates in organic-rich sediments can be obtained through organic matter degradation under reducing conditions. Therefore, dolomite samples that display both low δ¹³C and δ¹⁸O values can be interpreted as the result of microbially mediated sulfate reduction processes (Chafetz et al. 1999). The Gillen Member dolomite layers, likewise records reducing conditions, as is evidenced by the presence of organic matter and the precipitation of framoidal pyrite. Furthermore, as mentioned above, multiple signs point to the interpretation of the dolomite layers as evidence of microbial mats. Therefore, the δ¹³C and δ¹⁸O values add further support to this interpretation.

2.5.8 Spatial distribution of organic matter
Organic petrography was conducted on the Gillen Member evaporites to investigate any distribution patterns of organic matter and observe potential associations between different mineralogical phases or textural characteristics. Several types of solid bitumens have been observed in this study. All types were found in the dolomite layers of the evaporites. No organic matter was observed in the anhydrite portion using organic petrography. Since indigenous hydrocarbons have been detected from the anhydrite using gas chromatography-mass spectrometry (see Chapters 4 and 5), this bitumen is likely to be too finely disseminated throughout this mineral phase to be optically resolved. It is also uncertain whether the organic matter detected in the dolomite portions contributed to the hydrocarbons. It may be possible that
additional bitumen exists that is finely disseminated throughout the dolomite and was not detectable using organic petrography.

Solid bitumen is a commonly observed constituent in sedimentary rocks (Jacob and Hiltmann 1985; Robert 1988). A variety of processes can alter oil into solid bitumen. Such processes include cracking of oil due to temperature increases, and biodegradation followed by selective dissolution of organic matter in water (i.e. water washing) (Milner et al. 1977; Taylor et al. 1998). Since indigenous hydrocarbons were detected in the Gillen Member evaporites (see Chapters 4-5), any temperature increases would have to be minimal. High temperatures would have cracked these hydrocarbons.

One type of bitumen in the Gillen Member occupies interstitial vugs between dolomite crystals (Figure 2.17A,B). The restriction of this type of bitumen to such localities indicates that the organic matter must have migrated to some extent. Solid bitumen is generally observed to precipitate on the surfaces of mineral grains or as infill of entire interparticle pore spaces (Taylor et al. 1998). In carbonates, in particular, the shape of solid bitumen is observed to conform to the pore shapes in which they occur (Taylor et al. 1998). If bitumen migration did occur within these evaporites, then it must have been subtle. As noted in Chapter 5, extracted hydrocarbons/biomarkers differed significantly between adjoining dolomite and anhydrite layers. If extensive bitumen migration occurred, this difference between adjoining layers would not be observed.

Numerous thucholites were also observed in the Gillen Member (Figure 2.17C,D). A thucholite is organic matter that is well-known from mineralized zones that contain uranium and thorium (e.g. Prashnowsky and Schidlowski 1967; Taylor et al. 1998). It displays variable reflectance and commonly exhibits fluorescing halos (Figure 2.17D). Previously observed associations of these structures with lamalginitie (microbial remains; e.g. Taylor et al. 1998) are consistent with the thucholites representing alginitie or bitumen that has been variably modified by radioactivity. The presence of thucholites in central Australia is consistent with the occurrence of Proterozoic-age uranium deposits in this area (e.g. Ryan 1979).
The third and final type of organic matter observed through organic petrography were spherical aggregates that resemble the thucholites but show no uraniferous cores or lower reflectance fluorescing halos. They also resemble “bitumen balls” observed from Cambrian playa lake sequences in the Officer Basin, South Australia (McKirdy and Kantsler 1980). Kantsler (1979) suggested that these balls may originate by precipitation of an asphaltene fraction at surface temperature and pressure. If that was the case, the low reflectance of these balls indicates that any than subsequent burial and heating by the geothermal gradient has had only minimal effects on its maturation.

2.5.9 Isolated patches of halite within the dolomite layers

The presence of isolated halite patches within the dolomite layers (Figures 2.10 and 2.14) may be akin to similar textures observed from Late Neoproterozoic to Early Cambrian dolomites of the South Oman Basin (see Schoenherr et al. 2009, their figure 9). In that basin, an influx of a NaCl-supersaturated brine from an overlying halite layer resulted in the deposition of halite cubes within the underlying carbonate mud (Schoenherr et al. 2009). The dissolution, seepage and re-precipitation of halite resulted in what is known as “porosity plugging” within dolomite strata (Schoenherr et al. 2009). In the Gillen Member of Mt Charlotte 1, numerous layers of halite are also present (see section 2.2.4) and it is possible that a similar seepage of NaCl brines into the dolomite layers may have occurred in the geological past.

2.5.10 Presence of anhydrite

In settings that experience sufficiently intense evaporation such as hot, dry sabkhas, gypsum is quickly replaced by anhydrite (e.g. Bush 1973; Shearman 1983) or is deposited as a primary mineral (e.g. Shearman 1966, 1983; Bush 1973). Alternatively, anhydrite may have formed as a result of the dewatering of pre-existing gypsum during burial and subsequent heating by geothermal heat. Examinations of Gillen Member anhydrite under the petrographic microscope show that anhydrite is more likely to be secondary in nature and derived from the replacement of primary gypsum. As noted in section 2.4.3, numerous
anhydrite crystals from Mt Charlotte 1 assume a tabular/selenitic shape. Such shapes are characteristic of gypsum. The absence of gypsum in this core is likely the result of thermal recrystallization, which dehydrated the mineral and converted it to anhydrite. Petrographic work conducted on the drill core Alice Springs No. 3 (also from the Amadeus Basin) by Stewart (1979) showed that gypsum was the primary mineral phase in that setting. Subsequent sedimentary diagenesis resulted in its conversion into anhydrite due to burial. Although the Alice Springs No. 3 core was obtained from a locality ~200 km to the northeast of Mt Charlotte 1, it shows that primary precipitation of gypsum was the most likely form of sulfate deposition during the formation of the Gillen Member.

2.6 Conclusion

This chapter has focused on providing the geological context from which hydrocarbons, including numerous biomarkers, were extracted. It was shown that the Mt Charlotte 1 core from central Australia contains evaporitic strata of both the Neoproterozoic Gillen Member and the Lower Cambrian Chandler Formation. Both time intervals record the presence of “saline giants”, featuring the deposition of exceptionally thick evaporitic strata.

The Neoproterozoic Gillen Member dolomites were shown to possess several textural, petrographic and mineralogical indications of biologically-induced dolomite precipitation. In modern-day hypersaline environments, dolomite is observed to precipitate in association with cyanobacterial mats. In the Gillen Member, a number of isotopic, petrographic and mineralogical observations also support the interpretation of the dolomite layers as fossil microbial mats: 1) evidence for cohesive dolomitized layers that resemble modern mat structures; 2) characteristics of low-temperature dolomite precipitation; 3) concentric framboidal pyrite inside the dolomite; 4) shape, distribution and association of clay laminae with dolomite crystals and 5) carbon ($\delta^{13}$C) and oxygen ($\delta^{18}$O) stable isotope values.

The occurrence of organic matter in the Gillen Member dolomites also demonstrates the presence of former live in these evaporites. The remaining
chapters of this thesis, in particular Chapters 4 to 6, aim to elucidate the characteristics of this life through the analysis of extracted hydrocarbons.

2.7 References


Chapter 2


This chapter outlines the analytical frameworks and methodology for processing and analyzing ancient rocks. A major theme in any study concerned with the remains of ancient biological molecules is their interpretation as original contemporaneous components of a rock sample or as recent additions in the form of contamination. Therefore, a background to the contamination issue is provided in this chapter, with the aim of creating awareness of the problems that have plagued research in this field to this day. An important aim of ancient molecular work is to see through the cover of contamination and make more accurate paleoecological and paleoenvironmental interpretations. For these reasons, a variety of techniques have been introduced and it is a major component of this thesis to utilize them.

3.1 Testing syngeneity of lipid biomarkers from ancient rocks

3.1.1 Susceptibility of ancient biomolecules to contamination

Testing syngeneity is a central issue in any study concerned with ancient molecules from geological or archaeological samples. Contamination of such samples from non-indigenous sources can lead to erroneous information about the nature of the sample being investigated and provide misleading paleoecological and paleoenvironmental data. Problems associated with contamination have been particularly evident in the study of ancient DNA. Large numbers of ancient samples, including dinosaur, Neanderthal and cave bear bones, have been observed to contain exogenous DNA. Prior to the implementation of rigorous criteria to detect and control contamination, erroneous data was obtained from many of these samples. Due to concerns associated with contamination, criteria have been established that need to be addressed before ancient DNA results are authenticated (Pääbo et al. 2004; Hebsgaard et al. 2005). Such criteria include extensive decontamination of samples before DNA extraction, the use of control blanks
for DNA extraction and amplification, the application of biochemical assays such as amino acid racemization and/or assessment of bone histology to test if ancient DNA was likely to be preserved, and the reproduction of results in a second laboratory (Pääbo et al. 2004; Hebsgaard et al. 2005). Concerns over DNA contamination have also led to the establishment of dedicated laboratory facilities that are solely concerned with ancient DNA work and host isolated ventilation systems and nightly UV irradiation of surfaces (Pääbo et al. 2004; Hebsgaard et al. 2005). As with ancient DNA, the extraction and analyses of ancient proteins and microfossils from geological material can also be affected by contamination. In parallel with the stringency applied to ancient DNA analyses, criteria have also been suggested for sample handling, analysis and interpretation of ancient proteins (e.g. Ostrom et al. 2006).

Lipid biomarker analysis has also been recognized to suffer from contamination by various substances ranging from drilling fluids to plastic sampling bags (e.g. Grosjean and Logan 2007; Brocks et al. 2008). In the section below, a historical overview is presented that summarizes key developments in the study of Precambrian biomarkers and the realization that this study is severely plagued by contamination. Following this account, recent work will be presented that states the current position of testing biomarker syngeneity.

3.1.2 Historical and current ideas on the contamination of Precambrian rocks with emphasis on lipid biomarkers

The potential for organic geochemical studies of Precambrian rocks has been perceived in the 1950s (Woodring 1954) with the first biogenic molecules detected in 2.1 Ga Gunflint Chert (Barghoorn 1957). The molecules identified were amino acids that were detected in ppm quantities. This discovery was followed by the identification of more amino acids, as well as fatty acids, porphyrins, \( n \)-alkanes, and acyclic isoprenoids from Archean and Proterozoic rocks (Eglinton et al. 1964; Meinschein et al. 1964; Barghoorn et al. 1965; Belsky et al. 1965; Burlingame et al. 1965;
Meinschein 1965; Oró et al. 1965; Hoering 1966; Johns et al. 1966; Oró and Noon 1967; Prashnowsky and Schidlowski 1967; Schopf and Barghoorn 1967; Han and Calvin 1969; Kvenvolden and Hodgson 1969; Nagy and Nagy 1969). However, the majority of these studies appraised the extracted molecules in terms of biogenic versus abiogenic, underrating or ignoring problems associated with contamination (McKirdy 1974).

By the late 1960s and early 1970s it became evident that many of these “Precambrian” molecules were essentially modern contaminants. For example, it was observed that amino acids obtained from Precambrian cherts were still optically active (possessing the L configuration), even though such molecules should have been fully racemized after only one million years at 25°C (Kvenvolden et al. 1969). The role of contamination was not only confined to amino acids but to a wide range of other molecules, including hydrocarbons. Hoering (1966) pointed out that many Precambrian rocks experienced substantial metamorphism, which is inconsistent with the survival of soluble organic molecules. He noted that most Precambrian kerogen is graphitic in nature with low hydrogen content, indicating that the host rocks have experienced severe thermal histories. The pervasiveness and ease of sample contamination was further demonstrated when Hoering (1966) was able to extract organic matter from Precambrian granite in quantities even greater than those previously reported from other Archean sedimentary rocks. Stable carbon isotopic composition of Precambrian samples also painted a picture of contamination. In a majority of Precambrian samples, kerogen was isotopically lighter than the bulk of the extracted bitumen (Hoering 1966, Hoering 1967). While these stable isotope observations were later argued to represent actual biogeochemical phenomena in the Precambrian ocean (Logan et al. 1995), it was the most widely accepted argument for contamination at that time and paralyzed the search for Precambrian biomarkers until the late 1980s (Brocks 2001).

Further evidence for contamination of Precambrian sedimentary rocks was published in the 1970s. Smith et al. (1970) sequentially extracted Precambrian cherts and demonstrated that saturated hydrocarbons were
concentrated on the outer surfaces or along grain boundaries of the samples. It was also shown that Precambrian kerogen has a tendency to adsorb and retain any hydrocarbon contamination it might come in contact with (Oehler 1977). Adsorption of contaminant hydrocarbons is particularly problematic when pyrolysis is conducted to release apparently bound hydrocarbons from kerogen.

There are many sources of hydrocarbon contamination that can affect the interpretation of Precambrian rocks. Hoering (1966, 1967) and Hayes (1983) listed a variety of potential contaminants including migrated petroleum fluids, soil organic matter from percolating ground waters, anthropogenic petroleum products, airborne substances in a laboratory environment, wrapping paper and aluminum foil used for shipment and storage. It has recently been estimated that more than 70,000 organic compounds have been produced commercially, with an additional 1000 added annually (Simoneit 2005).

Issues associated with contamination virtually stopped research on molecular fossils from Archean and Paleoproterozoic rocks (Brocks 2001). The late 1980s and 1990s saw molecular biomarker studies being concentrated on younger Precambrian rocks, including the mid-Proterozoic sediments of the McArthur Basin in northern Australia (Jackson et al. 1988; Summons et al. 1988a; Taylor et al. 1994) and the Nonesuch Shale from North America (Imbus et al. 1988; Pratt et al. 1991), the late Proterozoic Chuar Group in North America (Summons et al. 1988b) and the Centralian Superbasin in Australia. These rocks revealed abundant hydrocarbons with distinctive Precambrian signatures. However, issues associated with contamination were still problematic. Summons and Powell (1991) mentioned the possibility of oil migration, which would preclude a firm correlation between biomarker composition and microfossil assemblages. They also included material from both core and surface outcrops to preclude contaminants from drilling fluids. However, results of any comparisons were not discussed. Studies on the bitumen composition of Precambrian rocks have often centered on comparisons between other time and/or basin
equivalent rocks (e.g. Logan et al. 1999, 2001). In these studies, biomarker distributions have been described on how they are similar or different from these other rocks. Differences were often regarded as indigenous and of paleoenvironmental significance without involving a discussion of contamination. In other studies (e.g. Logan et al. 1997), authors noted that bitumen reflected the environment of deposition within its source formation. According to these studies, biomarkers indicative of the Precambrian include, for example, elevated concentrations of mono- and dimethylalkanes relative to \( n \)-alkanes (e.g. Jackson et al. 1986; Klomp, 1986; Fowler and Douglas 1987; Summons and Powell 1991; Summons et al. 1988a,b). While most of these studies probably largely reported indigenous Precambrian biomarkers, it is unclear if and how much contamination was also present. Others have relied on the physical appearance of rock sample to determine whether bitumen in the samples is indigenous. Li et al. (2003), for example, regarded the bitumen from Precambrian samples as indigenous because the rocks were compressed in appearance and had no ferric oxide stains. The absence of ferric oxide stains is not an indicator for indigenous bitumen. In addition, microscopic pores and cracks were not taken into account, which would allow contaminants, either natural or anthropogenic, to settle, enter and/or pass through the samples.

Brocks (2011) artificially contaminated Precambrian shale without indigenous bitumen content with crude oil and showed that liquid petroleum products may penetrate compact- and intact-looking shale centimeters deep within days. Although other Precambrian biomarker studies (e.g. Olcott et al. 2005) have made attempts to identify organic contaminants, the methods also fall short in the ability to quantify and distinguish between contaminants and syngenetic bitumen. Olcott et al. (2005) analyzed bitumen from samples that were repeatedly crushed and extracted. Such a method does not take into account microscopic pores that would not fracture after repeated crushing. Such pores could still act as conduits for bitumen to settle or migrate through.

While it would be instructive to have knowledge of the drilling fluid and/or additives used in drilling a particular core (Peters et al. 2005), such
information is commonly not available. Addition of petroleum-based lubricants is often not mentioned in drilling log records (Brocks et al. 2008). In addition, combustion engines from motors of drill rigs can produce hydrocarbon aerosols that may settle on samples and can be difficult to distinguish from indigenous biomarkers (Brocks et al. 2008). Brocks et al. (2008) summarized numerous sources of contamination and their major organic constituents. Drilling fluids, lubricants and plastic bags often contain hydrocarbons such as alkanes and steranes that may mimic indigenous compounds. Moreover, if a particular compound is already present as an indigenous biomarker, an addition from a contamination source may cause quantitative change of biomarker ratios.

Brocks (2011) conducted experiments whereby rock samples were cut into millimeter thick slices, which were individually analyzed for their hydrocarbon content. Through these experiments, information was obtained about the spatial distribution of molecules in their host rocks. In drill core samples, it was observed that saturated and aromatic hydrocarbons of low molecular weight were present in gradually increasing concentrations from the outer surfaces to the center of the rock. By contrast, higher molecular weight hydrocarbons were present in decreasing concentrations with distance from the outer surfaces. Two models were proposed by Brocks (2011) that could explain this gradational distribution of molecules in the rock samples: 1) a contamination model, whereby petroleum-based contaminants would diffuse into the rock; or 2) a live-oil effect, whereby pressure release after drilling would leach indigenous hydrocarbons out of their host rocks. Brocks (2011) tested these models by comparing the hydrocarbon distribution in Archean shales with artificially contaminated rocks and younger mudstones which leached live-oil. Although the live-oil effect model appeared to explain the increase of hydrocarbon concentrations on the surface of shale samples, it still requires untested or unlikely assumptions to explain the pattern of molecular fractionation (Brocks 2011). However, the contamination model successfully replicated the concentration gradients and the chromatographic pattern of the Archean shales. Hydrocarbon contamination can leave chromatographic fingerprints, whereby migrating molecules separate
according to the adsorption energies to mineral surfaces. The most significant chromatographic effects were observed with the aromatics. The concentration even of low molecular weight aromatics such as naphthalenes dropped sharply with increasing penetration depths (Brocks 2011) and is consistent with the stronger adsorption of aromatic hydrocarbons to mineral surfaces (Carlson and Chamberlain 1985). Brocks (2011) compared this chromatographic separation to laboratory fractionation of bitumen on silica or alumina columns (cf. Zhao-An and Philp 1987) and during primary migration in shale under natural, geological conditions (cf. Mackenzie et al. 1983). Further techniques have recently been developed as a result of an increased understanding of hydrocarbon contamination. Brocks et al. (2008) suggested the use of branched alkanes with quaternary carbon (BAQCs) as a convenient marker of whether a rock was infiltrated by petroleum contaminants. Since BAQCs are anthropogenic contaminants derived from polyethylene bags that have similar physical and chemical properties as many hydrocarbons from naturally occurring bitumen, they can be used for estimating the degree to which a rock sample was likely infiltrated by other organic contaminants. A combination of this technique with interior/exterior (see below) and slice extraction experiments (Brocks et al. 2008) would provide a powerful assessment for the syngeneity of bitumen from Precambrian and Cambrian rocks samples.

3.2 Techniques employed for the removal, detection and evaluation of hydrocarbon contaminants

Due to the pervasiveness of hydrocarbon contamination of geological samples, this thesis employed two techniques to remove hydrocarbon contaminants from rock samples: surface removal by sawing and micro-abrasion (Figure 3.1). Either of these two techniques was employed on each drill core sample.
3.2.1 The sawing technique for the removal of hydrocarbon contaminants

This technique relies on cutting off the outer surfaces of rock samples and separately extracting bitumen from the exterior and interior portions (Figures 3.1A, B and 3.2). This procedure is particularly useful when the drill core samples possess an intact outer rounded surface that formed as a result of coring. Since this surface was exposed to drilling fluids, it would be the most contaminated surface and thus provide the most information about these molecules.

Samples chosen for sawing had no visible cracks. This was important, since cracks and pores would act as conduits for contaminants to permeate through. Unfortunately microscopic fissures cannot be adequately identified. Therefore, subsequent analysis of the GC results is required to identify migrated contaminants via the chromatographic effects discussed by Brocks.
Rock samples were cut using an ultra-clean precision saw (Buehler Isomet™ 1000; Illinois, U.S.A) (Figure 3.1A,B). Prior to usage, the blade and sample holder (Figure 3.2B) was cleaned with methanol and dichloromethane (DCM; solvent grade 99.9%, UltimAR®, Mallinckrodt Chemicals). Distilled water was used as the lubricant. Surfaces of quarter cores were cut parallel to the outer rounded surface and perpendicular to the bedding direction to yield cuboids (~2 to 3 cm), representing the interior of the rock (Figure 3.2). For this thesis, between 8 and 37.2 g of exterior rock surface was removed from each sample using the sawing technique. Because the sawing technique and its efficiency in removing hydrocarbon contaminants has been previously described (Brocks et al. 2008; Brocks 2011), it will not be further discussed in this chapter.

Figure 3.2 Schematic illustration of a drill core sample indicating the portions referred to as exterior and interior.

3.2.2 The micro-abrasion technique for the removal of hydrocarbon contaminants

The micro-abrasion technique described herein was first conceived by Jochen Brocks, Australian National University, Canberra (J.J. Brocks, personal communication, 2007). This technique relies on the principle of tumble polishing, whereby the sample surfaces are smoothened by grinding
off the outer or exterior surfaces. Because this technique has not been
previously described, it will be covered in greater detail below.

3.2.2.1 Materials and Methods

3.2.2.1.1 Abrasion equipment

A KG-1 Mini-Sonic tumbler (Diamond Pacific, U.S.A) (Figures 3.1C,D) was
employed to transmit the necessary vibratory energy for micro-abrasion or
tumbling to be carried out. The operation frequency of this machine
is >3,500 vibrations per minute (Diamond Pacific 2002). This tumbler was
supplied with a plastic hopper for holding the samples. Since plastics can be
a leading source of organic contaminants (e.g. Grosjean and Logan 2007),
the hopper was replaced with a steel version designed and constructed at
the mechanical workshop of the Research School of Earth Sciences,
Australian National University. This metal hopper was baked-out at 480°C
for 9 hours, to eliminate hydrocarbon contaminants. The hopper is round at
the edges to ensure continuous movement of rock samples during tumbling.
The widest part of the container is 7.1 cm long, while the opening is 4 x 4 cm
wide. The container has a depth of 6.1 cm. The mechanical workshop also
designed new clips that will hold the metal container in place and ensure that
proper vibratory action is being transmitted.

3.2.2.1.2 Abrasion procedure

This technique is suitable for small rock samples that are between ~5 and 30
mm in diameter. Larger samples will face greater difficulties in moving inside
the metal container and may eventually set the entire tumbling process to a
halt. Smaller samples, on the other hand, may become completely abraded.
Therefore, all samples are crushed to appropriate sizes.

Approximately 40 g of sample (Figure 3.3A), together with a defined mass of
baked-out (450°C/9hrs) silicon carbide grains (2-3 g) (60# grit; Shell-Lap
Supplies Pty Ltd, South Australia), were placed into the metal hopper. These
carbide grains acted as the polishing medium during abrasion. Baked-out (at
450°C/9hrs) ceramic cylinders (6x12 mm cylinders; Shell-Lap Supplies Pty
Ltd, South Australia) were added to enhance the abrasion process. After
emplacement into the container, this mixture was wetted with distilled water
to allow for the adhesion of the silicon-carbide grains to the samples and ceramic cylinders (Figure 3.3B). Samples were tumbled until all the exterior surfaces were visibly smoothened. This process would take between 4 and 20 hours, depending on sample hardness. The smoothened rock material (Figure 3.3C) is here referred to as the interior of the samples, and the abraded material as the exterior. After the tumbling process, the interior was separated from the exterior mud (Figure 3.4) by sieving through a cylindrical steel mash, and ultrasonicating in distilled water. The interior rock chips and abraded rock powder were dried at room temperature. The percentage of abraded material was determined gravimetrically. For this thesis, between 2.2 to 13.75 g of exterior was removed from each sample through abrasion.

3.2.2.2 Results and discussion

The micro-abrasion technique has been successful in testing hydrocarbon syngeneity in samples that are considerably small. Figure 3.5 shows layers and fragments of a muddy anhydrite from the Neoproterozoic Gillen Member that were either underlying an accumulation of halite or became incorporated into it. Prior to the use of the micro-abrasion technique, it would have been difficult, if not impossible, to assess the syngeneity of any extracted bitumen from these samples. However, as shown in Figure 3.5, significant concentration differences were observed between the abraded exterior and the remaining interior of these samples. While the anhydrite exhibited hydrocarbons in the exterior and interior portions, a significantly higher concentration of these molecules (>6x for some n-alkanes) was measured in the exterior. Such concentration differences are indicative of contamination, showing that the interior counterpart became infiltrated by various hydrocarbons. The anhydrite inclusions, in particular, reflect significant concentration differences between the exterior and interior. This result shows that hydrocarbons had not much chance to infiltrate the rock samples, leaving a stark difference in hydrocarbon distribution between rock exterior and interior.

The aromatic bitumen fractions of these anhydrite samples also displayed differences between the rock exteriors and interiors (Figure 3.5). The
exterior exhibited numerous peaks of polychlorinated biphenyls (PCBs), while the interior showed such molecules to be in significantly lower concentrations. PCBs are recognized as being derived from anthropogenic sources (e.g. Helsinki Commission 2001), and the results in Figure 3.5 demonstrates that the micro-abrasion technique has been successful in determining them as contaminants.

3.3 Quantification of bitumens between the exterior and interior rock portions

In this thesis, exterior/interior (E/I) differences in the bitumens are quantified (Figure 3.6). A concentration difference with a 1:1 ratio would indicate that equal amounts of a molecule have been detected in the exterior and the interior of a sample. Deviations from this ratio occurred when higher concentration were observed in the exterior (E/I>1) or in the interior (E/I<1). The former indicates contamination, while the latter results when low molecular weight molecules evaporate from rock surfaces.
Figure 3.3 Photographic images demonstrating the smoothening process of rocks as a result of micro-abrasion. A) Anhydrite-rich rocks collected from the Mt Charlotte 1 drill core. Note the angular surfaces. Image is ~12 cm wide. B) Anhydrite-rich rocks during the process of abrasion in a KG-1 Mini-Sonic tumbler. Opening of the metal container is 4 x 4 cm. C) Anhydrite-rich rocks after the micro-abrasion process. Note the smoothening of the exterior surfaces.
Figure 1.4 Tools for the separation of abraded surfaces mud (i.e. the exterior) and remaining rock samples (i.e. the interior). A) Baked-out glass beaker containing a cylindrical steel mash. Inset showing a close-up of the steel mash, which prevents rock samples larger than a few millimeters from moving into the beaker when washed with water. B) Glass beaker containing water through which the abraded mud settled. The abraded material is visible in the bottom part of the beaker.
Figure 2.5 Bitumens and their total ion chromatograms (TIC) obtained from micro-abraded anhydrite (grey pieces) (07r012; from 1871.3 m depth). Red chromatograms were derived from small anhydrite pieces or inclusions that were intermingled with halite (brown crystals). Green chromatograms were derived from the underlying, horizontal anhydrite layers. Note that the interior portions yield significantly less bitumen than the exterior counterparts. IS = internal standard; PCBs = polychlorinated biphenyls; n-alkanes (n-C_{18} and n-C_{22}) are indicated for reference; signal heights are proportional to concentration. Scale bar represents 20 mm.

<table>
<thead>
<tr>
<th>Exterior/Interior ratio</th>
<th>More hydrocarbons on surface/sample exterior</th>
</tr>
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<tbody>
<tr>
<td>&gt; 1</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Equal concentrations of hydrocarbons in exterior &amp; interior of sample</td>
</tr>
<tr>
<td>&lt; 1</td>
<td>More hydrocarbons in interior of sample</td>
</tr>
</tbody>
</table>

Figure 3.6 Bitumen concentration differences between exterior and interior rock portions (expressed as a ratio).
3.4 References


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Chapter 4

Testing hydrocarbon syngeneity of evaporites from Neoproterozoic and Cambrian strata: a systematic study of contaminated drill core samples

This chapter investigates the syngeneity of hydrocarbons extracted from Neoproterozoic and Cambrian evaporites. As was outlined in Chapter 2, all samples were derived from the Mt Charlotte 1 drill core and mark significant evaporitic events at these times in the Amadeus Basin, central Australia. Through the application of exterior/interior (E/I) rock experiments, this chapter will demonstrate that all evaporites were impacted by a complex series of contamination events. Unraveling this complexity is therefore key in making correct interpretations about the biomarker composition of these ancient hypersaline settings.

4.1. Introduction

Lipid biomarkers serve as important proxies in elucidating palaeoenvironmental and palaeoecological characteristics. They are routinely used in characterizing organic remains from modern and near-recent human history (e.g. Kenig et al. 1995; Jahnke et al. 2008) to the Precambrian when body fossil remains are scarce and often undiagnostic (e.g. Summons et al. 1988a,b; Logan et al. 1997, 1999; Brocks et al. 2005). The petroleum industry heavily relies on such biomarkers for oil-oil and oil-source rock correlations, even when samples of source rocks are not available (e.g. Peters et al. 2005). As a result, considerable effort has been made to identify source- and age-related biomarker parameters that are useful in understanding preserved organic matter.

Over the past decades, some attention has been devoted to the characterization of lipid biomarkers from ancient meso- and hypersaline Phanerozoic environments. Such compounds include elevated concentrations of the C_{21} to C_{25} regular isoprenoids (Grice et al. 1998), and docosane, a C_{22} n-alkane (e.g. ten Haven et al. 1985). Other biomarkers that are prevalent in hypersaline environments include gammacerane and elevated concentrations of pregnanes and homopregnanes (e.g. ten Haven et al. 1988). Indeed, since the
1980s, a genetic link has been established between the deposition of evaporative carbonates and hydrocarbon formation. Many subaqueous evaporitic settings are localities where organic matter accumulates (Sonnenfeld, 1985; Warren 2006). Evaporites require restricted water inflow conditions and are often areas of density-stratified waters with a bottom brine body composed of a dense, saline water mass (Warren 2006). Since atmospheric gases do not easily exchange with the bottom brine body, long-term bottom anoxia is maintained (Warren 2006).

Molecular proxies that are indicative of hypersaline environments can provide important clues to the biotic evolution and community composition of hypersaline settings over time. While body fossil and inorganic isotopic data can provide considerable insights into the biotic composition of ancient hypersaline settings, biomarkers can provide important taxonomic information ranging from the species level to that of the domain of life. Such information is particularly required for the identification of prokaryotes, which constitute a significant part of hypersaline ecosystems. Even if their body fossil remains are preserved, the identification of most prokaryotes is hampered by a lack of taxonomically-relevant morphological features that enable phylogenetic discrimination beyond the broad classification of rods, spheres and filaments. Therefore, the recognition and identification of taxonomically specific biomarkers is of importance when investigating the biotic composition of ancient hypersaline environments.

While biomarker analysis serves as a valuable analytical tool, it has become increasingly clear that such studies are not immune to contamination via migrated oils from nearby geological strata, as well as from drilling fluids and storage bags (e.g. Grosjean and Logan 2007; Brocks et al. 2008; Rasmussen et al. 2008). Although most synthetic contaminants such as UV absorbers and softeners are easily recognized and unlikely to be confused with petrogenic hydrocarbons, a range of other contaminants have proven to be troublesome. Numerous synthetic products, for example, have been shown to contain at least traces of petroleum-based hydrocarbons, which include biomarkers such as hopanes, steranes, \( n \)-alkanes and fatty acids (Brocks et al. 2008). Because hydrocarbon contaminants have the ability to overprint on indigenous biomarkers, it is important to be able to
differentiate between the two hydrocarbon signatures. Therefore, testing the
syngeneity of lipid biomarkers from sedimentary rocks is of importance, especially
in the Precambrian, if one is to prove the first appearances of a range of
microorganisms (e.g. Rasmussen et al. 2008).

This study presents the results of experiments aimed at elucidating the
syngeneity of hydrocarbon biomarkers from Cambrian and Neoproterozoic
evaporites collected from drill cores. Such investigations are important, since drill
cores are frequently used in biomarker analyses from both Phanerozoic and
Precambrian sedimentary sequences. Rocks from such cores are often well
preserved and have not been exposed to the detrimental effect of weathering or
groundwater infiltration (e.g. Sherman et al. 2007). Furthermore, drill cores allow
for the sampling of continuous stretches of stratigraphic horizons, which may not
be available at surficial outcrop exposures.

Since the aim of this chapter is to test hydrocarbon syngeneity, compound
classes are only broadly described. More detailed descriptions of the indigenous
compounds are provided in Chapter 5.

4.2. Materials and Methods

4.2.1 Samples

Rock samples were collected from evaporitic facies of two stratigraphic horizons
from the Amadeus Basin in central Australia: the 800 Ma Gillen Member of the
Neoproterozoic Bitter Springs Formation (Lindsay 1987) and the Cambrian
Chandler Formation (dated stratigraphically as “Lower Cambrian”; Bradshaw
1991). Both sections mark significant evaporative events as a result of a tenuous
connection with the contemporaneous ocean and the broad shallow nature of the
Amadeus Basin. As a result, very thick (100 m to >2000 m) evaporitic units were
deposited at these times (Lindsay 1987).

All samples collected for this study were derived from the Mt Charlotte 1 core
held at Geoscience Australia, Canberra, and the Northern Territory Geological
Survey drill core store at Alice Springs. Rocks were composed of varying quantities
of dolomite, anhydrite, halite, clays, pyrite, detrital quartz and feldspars. A total of
65 samples were collected. Of this amount, a total of 10 samples were collected from the Cambrian Chandler Formation, while the remaining 55 samples came from the Gillen Member of the Neoproterozoic Bitter Springs Formation. Pieces of plastic bags containing rock samples were also collected from the core boxes. Figure 4.1 shows an example of a Mt Charlotte 1 drill core storage box and some of the evaporites that were collected. Some rock samples are kept in thick, translucent plastic bags. Occasionally, plastic bags are also used as space fillers or to cushion fragmented rocks.

![Figure 4.1](image)

**Figure 4.1 Evaporites from the Mt Charlotte 1 drill core.** A) Core material held in a carton box at Geoscience Australia (Canberra). Note rocks wrapped in plastic bags. B) Neoproterozoic halite and underlying anhydrite (07r012; from 1872.3 m depth). Sample is ~10 cm high. C) Neoproterozoic evaporite (08r008; 1654 m) composed of alternating layers of dolomite (dark layers) and anhydrite (light grey layers). D) Dolomite-rich Neoproterozoic sample (08r006; from 1655 m depth) with some light grey anhydrite layers. Note the presence of moisture in the lower rock portion (red arrows) highlighting a horizontal crack in this sample. Coins in C) and D) are 2.5 cm in diameter.

### 4.2.2 Exterior/interior experiment

To assess concentration differences of organic molecules between the exterior surfaces of a rock and the corresponding interior, all rock surfaces were either trimmed by using a clean precision wafering saw (Buehler Isomet 1000; Illinois, U.S.A; blade thickness 340 µm) or abraded by a tumbler (KG-1 Mini-Sonic tumbler,
Diamond Pacific, U.S.A). Between 8 and 37.2 g of exterior rock surface was removed using the sawing technique, while 2.2 to 13.75 g were removed through abrasion. See Chapter 3 for a full description of the sawing and abrasion techniques. The exterior and interior rock portions were separately crushed to powder, extracted and fractionated.

4.2.3 Processing of samples
Rock samples were ground to powder in an alumina ring-mill (Rocklabs, Auckland, NZ). Prior to usage, the mill was cleaned by grinding backed-out (600°C/24 h) quartz-rich sand two to three times for 60s and subsequently washed with methanol and dichloromethane (DCM; solvent grade 99.9%, UltimAR®, Mallinckrodt Chemicals). System blanks consisted of backed-out sand (600°C/24 h). Approximately 5 to 30 g of rock powder was extracted with 100% DCM in a Dionex Automated Solvent Extractor. The extracts were reduced to 100 µl under a stream of purified nitrogen gas and separated into saturated, aromatic and polar fractions using column chromatography over 12 g annealed (450°C/24 h) and dry-packed silica gel (Silica Gel 60; 230-400 mesh; EM Science). Saturated hydrocarbon were eluted with 1.5 dead volumes (DV) n-hexane, aromatic hydrocarbons with 2 DV n-hexane:DCM (1:1 v/v) and polars with 2 DV DCM:methanol (1:1 v/v). An internal standard consisting of 2.3 µg 18-methyleicosanoic acid methyl ester (MEME, Ultrascientific, U.S.A) was added to the saturated and aromatic hydrocarbon fractions. An additional 50 ng of d₄-C₂₉-α,α,α-ethyl-cholestane (D₄; Chiron Laboratories AS) was added to the saturated fraction as an internal standard for metastable reaction monitoring (MRM).

4.2.4 Gas chromatography-mass spectroscopy (GC-MS)
GC-MS analyses of the saturated and aromatic fractions were carried out on a Micromass AutoSpec Premier equipped with a 6890 gas chromatograph (Agilent) and a DB-5 capillary column (60 m × 0.25 mm i.d., 0.25 μm film thickness) using helium as carrier gas. The MS source was operated at 260°C in EI-mode at 70 eV ionization energy and with 8000 V acceleration voltage. Samples were injected in
splitless mode into a PTV injector at a constant temperature of 300°C. For full-scan analyses, the GC oven was programmed at 40°C (2 min), heated to 315°C at 4°C/min, with a final hold time of 17 min. The AutoSpec full-scan duration was 0.7 s plus 0.2 s interscan delay over a mass range of 55-600 Da. For MRM, the GC oven was programmed at 60°C (2 min), heated to 100°C at 8°C/min, further heated to 315°C at 4°C/min and held at the final temperature for 34 min.

4.3. Results

4.3.1 Laboratory sand blanks

Laboratory sand blanks were processed concurrently with the Neoproterozoic and Cambrian evaporite samples. Such blanks allowed for the detection of laboratory-based contaminants that could affect the interpretation of hydrocarbons derived from rock samples.

In the majority of cases, the blanks produced in this study proved to be markedly devoid of any contaminants. No \( n \)-alkanes, isoprenoids, hopanes and steranes were detected. However, in a few cases, a homologous series of \( n \)-alkanes from \( C_9 \) to \( \sim C_{28} \) was observed. Monomethylalkanes as well as pristane and phytane were also detected in these samples. However, the concentration of these molecules relative to standard was minute and cannot account for any of the patterns discussed below. The only molecules that were present at significant concentrations were \( n \)-alkanes from \( C_{12} \) to \( C_{14} \). These molecules were also noted in several Neoproterozoic and Cambrian extracts and are regarded as laboratory contaminants. Hopanes, steranes, and aromatic compounds were not detected in the blanks.

4.3.2 Bitumen groups observed in the Cambrian and Neoproterozoic evaporites

Gas chromatography-mass spectrometry (GC-MS) profiles of saturated bitumens obtained from \textit{Mt Charlotte 1} evaporites clustered into three groups. Below, the bitumen composition of the exterior rock portions is described.
4.3.2.1 Group 1: Bitumens with low concentrations of monomethylalkanes relative to \(n\)-alkanes

The first group of bitumens yielded saturate fractions with relatively low monomethylalkane to \(n\)-alkane ratios (Figure 4.2). The rocks were composed of dolomite, anhydrite or a mixture of these two minerals. The compounds described below were detected in both Cambrian and Neoproterozoic samples. No differences in bitumen composition were observed between evaporites from these different age groups.

A significant hump of unresolved complex (UCM) occurred in most of these samples. The \(n\)-alkane profiles varied substantially; several bitumens displayed a predominance of low carbon number \(n\)-alkanes (\(n\)-C\(_{13}\) to \(n\)-C\(_{20}\)) with a mode at \(n\)-C\(_{17}\) or \(n\)-C\(_{18}\). The \(n\)-alkanes with higher carbon numbers become progressively less abundant in these samples. Other bitumens displayed an opposite trend, with a predominance of higher molecular weight \(n\)-alkanes (especially from \(n\)-C\(_{21}\) to \(n\)-C\(_{37}\)).

A notable feature in many samples is the relatively high concentration of docosane (\(n\)-C\(_{22}\)). The \(R_{22}\) index (\(2 \times C_{22} / (C_{21} + C_{23})\); ten Haven et al. 1988) has been determined for these evaporites, with values ranging from 1.05 to 3.71. A \(R_{22}\) index value >1 has been interpreted as indicative of hypersaline conditions (ten Haven et al. 1988).

Several regular (head-to-tail) acyclic isoprenoids (\(i\)-C\(_{19}\) to \(i\)-C\(_{40}\)) were also detected in most samples (Figure 4.3). Their identification is based on mass spectra and relative elution positions.

Hopanes were detected in all bitumens. Through GC-MS metastable reaction monitoring (MRM), it was possible to detect the complete C\(_{27}\) to C\(_{35}\)-17\(\alpha\)(H),21\(\beta\)(H)-hopane series in M+ →191 transitions (Figure 4.4). MRM M+ →205 mass chromatograms identified the presence of methylhopanes.

Steranes, like hopanes, were also detected in all samples. The major sterane products are the C\(_{21}\) pregnane and C\(_{22}\) homopregnanes (Figure 4.5). In addition, the C\(_{26}\) to C\(_{30}\)-pseudohomologues and diasteranes (Figures 4.5 and 4.6) were also noted. Cholestane (C\(_{27}\)) was the most abundant pseudohomologue comprising, on
average, ~51% of the total C_{27} to C_{29}-regular-steranes, followed by ergostane (C_{28}) comprising ~26% and stigmastane (C_{29}) comprising ~23%. 24-n-Propylcholastane (C_{30}) comprise ~4% of the total C_{27} to C_{30}-regular steranes.

A-ring methylated steranes were also detected (Figure 4.7). A Triassic bitumen (Watchet #5021) was used to identify dinosteranes in these samples.

Various plant biomarkers, were also detected. Most notable was the presence of oleanane and bicadinane in MRM 412→191 and 412→369 transitions, respectively. Taraxastane, possibly derived from terrestrial organic matter (Perkins et al. 1995), was tentatively identified in the 412→191 transition of many samples.

A series of aromatic hydrocarbons and dibenzothiophenes was present in all Group 1 samples (Figure 4.8). A notable feature of all aromatic fractions was the occurrence of several polychlorinated biphenyls (PCBs) and various phenanthrenes. Small relative concentrations of various biphenyls and naphthalenes were also observed.
4.3.2.2 Group 2: Bitumens displaying a high concentration of mono- and dimethylalkanes relative to n-alkanes

The second group of bitumens yielded saturate fractions with high mono- and dimethylalkane relative to n-alkane ratios (Figure 4.8A). A total of ten samples, all from the Gillen Member, displayed GC-MS profiles characteristic of this group. The rocks were composed mostly of dolomite or a mixture of dolomite and anhydrite.

Homologues of n-alkanes with chain length from 9 to 37 carbon atoms were detected under full-scan GC-MS conditions (Figure 4.8A). The n-alkane distribution of these samples is either unimodal and centered on C_{14} or C_{15} or bimodal with centers on C_{14} or C_{15} and C_{22}. Elevated concentrations of n-C_{17} (relative to n-C_{16} and n-C_{18}) and n-C_{22} (relative to n-C_{21} and n-C_{23}) were encountered in most samples. The R_{22} index in these samples ranged from 1 to 1.3.

A UCM hump is a characteristic feature in all samples. It is particularly evident between n-C_{17} and approximately n-C_{28}.

Regular as well as irregular isoprenoids are present in all samples. Isoprenoids were particularly prominent in anhydrite-rich samples. Their identification is discussed in detail in Chapter 5. Regular isoprenoids include pristane (i-C_{19}) and phytane (i-C_{20}), as well as a homologous series of up to C_{36}. In
this series, \( i-C_{24} \) and \( i-C_{25} \) were particularly prominent relative to the other homologues, which differs from that observed in Group 1.

Figure 4.4 MRM distributions of hopanes and other pentacyclic terpanes in the saturated fraction of 08r008 (1654 m). Chromatograms are identified by carbon number and the MRM reaction transition. \( Tm = 17\alpha(H)-22,29,30\)-trinorhopane; \( Ts = 18\alpha(H)-22,29,30\)-trinorneohopane; \( tx = \) taraxastane; \( \gamma = \) gammacerane; \( \alpha\beta, \beta\alpha, \) \( 22S, 22R = \) isomers of a compound; \( DNH = \) dinorhopane.
Figure 4.5 Partial m/z 217 mass chromatogram showing the distribution of diasteranes (D), pregnane (C20), homopregnane (C21) and regular C27, C28, C29 and C30 steranes from Gillen Member sample 09r001 (1652 m).

Figure 4.6 MRM distribution of steranes in 08r008 (1654 m). Chromatograms are identified by carbon number and the MRM reaction transition. βα = 13β(H),17α(H)-diasteranes; ααα = 5α(H),14α(H),17α(H)-steranes; αββ = 5α(H),14β(H),17β(H)-steranes.
Irregular isoprenoids are present as a homologous series of tail-to-tail and head-to-head isoprenoids. The tail-to-tail isoprenoids are 2,6,11,15-tetramethylhexadecane (C_{20}; crocetane), 2,6,10,15,19-pentamethyllicosane (C_{25}; PMI), and squalane (C_{30}). Head-to head isoprenoids are comprised of homologues from C_{32} to C_{40}.

Hopanes, steranes and plant biomarkers were also detected in these samples and featured the same patterns as noted in the Group 1. Therefore, these molecules are not further described here.
The aromatic fraction from Group 2 consisted of a series of aromatic hydrocarbons and dibenzothiophenes and exhibits similar profiles as that observed for Group 1. PCBs and various phenanthrenes were also notable compounds in this group.

4.3.2.3 Group 3: Bitumens displaying low relative concentrations of mono- and dimethylalkanes relative to n-alkanes

This group featured a similar distribution of hydrocarbons as that described in Group 2. However, it differed from the latter by featuring low concentrations of mono- and dimethylalkanes relative to n-alkanes (Figure 4.8C). The UCM was also less prominent than in the above two groups. One sample, from the Gillen Member, belongs to this group and was predominantly composed of anhydrite.
4.3.3. Measuring concentration differences of hydrocarbons between exterior and interior rock portions

While all Mt Charlotte 1 samples contained a variety of molecules, significant concentration differences were observed between the rock exterior and the interior of these three bitumen groups.

4.3.3.1 E/I differences for Group 1 bitumens

Molecules from Group 1 showed the highest E/I concentration differences. The n-alkanes and monomethylalkanes displayed progressively higher E/I ratios with either increasing (Figures 4.9A and 4.10A) or decreasing (Figure 4.9B) molecular weight. E/I differences of up to 30x have been measured for the n-alkanes. Docosane (n-C_{22}) is also observed to feature up to 6x higher E/I values. In the interior of many samples, n-alkanes >C_{22} are not detected.

Regular isoprenoids display progressively higher E/I ratios with increasing molecular weight. E/I ratios of up to 69.5x have been measured for these isoprenoids (Figure 4.11A).

Pronounced E/I concentration differences of hopanes and steranes were also observed. These molecules exist predominantly on the exterior portions (Figures 4.12 and 4.13). The average E/I ratio of hopanes was 6.8, while for steranes it was 5.6. Exterior concentration of steranes progressively increased from C_{27} to C_{30} (Figure 4.14).

The plant biomarkers detected in this bitumen group featured similar E/I patterns where these molecules are predominantly (up to 4x) on the surface.

Aromatic compounds also exhibited significant differences between the rock portions (Figure 4.15). While some of the lighter PCBs and various phenanthrenes were still able to infiltrate the interior or numerous rocks, heavier ones were absent. Average E/I ratios of up to 27x were measured for these compounds. Others, such as the dimethylnaphthalenes, phenanthrene, and pentamethylnaphthalenes exhibited average E/I ratios of 2.1, 2.8, and 6.3, respectively. This indicates higher E/I ratios with increasing molecular weight for these aromatic compounds.
4.3.3.2 E/I differences for Group 2 and 3 bitumens

In Groups 2 and 3, E/I concentration differences were more complex. While some hydrocarbons were predominantly on the exterior, others were present in both rock portions at equal concentrations.

E/I ratios of \( n \)-alkanes would either rise with increasing molecular weight (Figure 4.9C) or to a specific carbon number (e.g. \( n \)-C\textsubscript{25} Figure 4.9D) and then drop. Any smooth increase in E/I \( n \)-alkane ratios can be punctuated by elevated concentrations of individual or groups of homologues. Docosane (\( n \)-C\textsubscript{22}), in particular, was frequently observed to be at significantly higher concentrations on the exterior (up to 3x) (Figure 4.9C). Other \( n \)-alkanes with notably higher concentrations on the exterior, included \( n \)-C\textsubscript{14}, \( n \)-C\textsubscript{15}, \( n \)-C\textsubscript{18}, and a group ranging from \( n \)-C\textsubscript{30} to \( n \)-C\textsubscript{35} (Figure 4.9C,D). E/I concentration differences of \( n \)-alkanes in these bitumen groups were up to 4.8x, although for most samples it was ~2x.

As shown in Figure 4.16, E/I \( n \)-alkane profiles can differ significantly. While relative concentrations of \( n \)-C\textsubscript{22} are particularly high in the exterior, they are much lower in the interior. Other \( n \)-alkanes, particularly from \( n \)-C\textsubscript{30} to \( n \)-C\textsubscript{35}, also feature less prominently in the interior (data not shown).

Monomethylalkanes recorded E/I concentration differences of up to ~1.9x and would mirror the pattern observed for the \( n \)-alkanes (compare Figure 4.9D with 4.10B from the same sample). Dimethylalkanes, by contrast, feature E/I ratios of one.

Isoprenoids exhibited various concentration differences between the rock portions. Most regular isoprenoids, especially pristane and phytane, displayed E/I ratios that are >1. Figure 4.17 shows an example where such molecules exhibit significant E/I differences. Regular isoprenoids ranging from \( i \)-C\textsubscript{21} to \( i \)-C\textsubscript{25} were the only homologues that feature E/I ratios close to one (Figure 4.11B,C). Heavier homologues (\( \geq i \)-C\textsubscript{26}), by contrast, exhibited higher E/I ratios (Figure 4.11B).

E/I concentration differences of crocetane and PMI were more difficult to measure. These molecules co-elute with phytane and regular \( i \)-C\textsubscript{25}, respectively (see Chapter 5). However, rough quantifications indicate no significant E/I
differences. The C_{30} isoprenoid squalane and the head-to-head isoprenoids from (C_{32} to C_{40}) also feature E/I ratios of one.

Aromatic hydrocarbons were present in significant concentrations in both the exterior and the interior portions. While the exterior featured prominent peaks of PCBs, these compounds were either not detected or present in low relative concentrations in the interior (Figure 4.18). The interior also featured prominent series of various benzenes, naphthalenes, phenanthrenes and anthracenes that were not readily observed in the exterior. E/I ratios of the lower molecular weight benzenes and naphthalenes yield values less than one, indicating that many of these compounds evaporated from the surface. The dimethylnaphthalenes, phenanthrene, and pentamethylnaphthalenes exhibited average E/I ratios of 0.8, 1.1, and 1.9, respectively. While this result indicates higher E/I ratios with increasing molecular weight, the values for these compounds are less than those measured for Group 1.
Figure 4.9: Extrapolation concentration differences of n-alkane homologues from (A) 08710.10 (161.3 m); (B) 08710.12
08710.13 (316.5 m); (C) 08710.06 (165.5 m) depth.

122
Figure 4.10 Exterior/interior concentration differences of 2-monomethylalkanes from A) 08r010 (1871.3 m) and B) 07r013 (1613 m).
Figure 4.11 Exterior/interior concentration differences of isoprenoids. A) Regular isoprenoids from 08r010 (1871.3 m). Note that no regular isoprenoids above C_{31} were detected in the interior of these samples. B) Regular isoprenoids from 08r022 (1650.8 m). C) Irregular, head-to-head isoprenoids 08r022 (1650.8 m).
Figure 4.12 Exterior/interior differences in the MRM distribution of hopanes in 08r008 (1654 m). The chromatograms are scaled relative to extract yields and signal heights and can be directly compared.

Figure 4.13 Exterior/interior differences in the MRM distribution of steranes in 08r008 (1654 m). The chromatograms are scaled relative to extract yields and signal heights and can be directly compared.
Figure 4.14 Exterior/interior concentration differences of steranes in 08r022 (1650.8 m).

Figure 4.15 Total ion chromatograms (TIC) showing exterior/interior differences in the aromatic fraction of a Group 2 bitumen (08r010; 1871.3 m). The chromatograms are scaled relative to extract yields and signal heights and can be directly compared. Red = PCBs; green = phenanthrenes; blue = various biphenyls and naphthalenes; IS = internal standard.
Figure 4.16 Partial m/z 85 mass chromatogram showing exterior/interior differences in the n-alkane profiles of 08r008 (1654 m). The chromatograms are scaled relative to extract yields and signal heights and can be directly compared.

Figure 4.17 Total ion chromatograms (TIC) of the pristane (Pr) and phytane (Ph) elution region showing exterior/interior concentration differences. Note E/I differences in relative concentrations between Pr, Ph and C₁₈ monomethylalkanes. The chromatograms are scaled relative to extract yields and signal heights and can be directly compared. Sample: 08r006 (1655 m).
4.3.4. Saturated hydrocarbon composition of a plastic bag from a Mt Charlotte 1 storage box

In order to determine the sources of potential hydrocarbon contaminants in the Mt Charlotte 1 core, a plastic bag sample from a storage box at Geoscience Australia (Canberra) (Figure 4.1A) was obtained and the hydrocarbons extracted. The saturate fraction from this bag featured a homologous series of $n$-alkanes from $C_{16}$ to $C_{30}$ with particularly high relative concentrations of $n-C_{22}$ (Figure 4.19).

Figure 4.18 Total ion chromatograms (TIC) showing exterior/interior differences in the aromatic fraction of a Group 2 bitumen (08r008; 1654 m). The chromatograms are scaled relative to extract yields and signal heights and can be directly compared. Red = PCBs; green = phenanthrenes; blue = benzenes and napthalenes; pink = anthracenes; IS = internal standard.
Chapter 4

4.4 Discussion

This chapter examined bitumen syngeneity of Neoproterozoic and Cambrian evaporites from the Mt Charlotte 1 drill core. A central feature has been the physical removal of the exterior rock surfaces and the quantitative evaluation of E/I hydrocarbon concentration differences. Such experiments help examine if the bitumens are either syngenetic, contaminants, or a mixture of the two. Previous studies (Brocks et al. 2008; Brocks 2011) have shown that hydrocarbon contaminants can be either surficial, or present inside the rock. In the latter case, hydrocarbons will have diffused into fissures and pore spaces. This diffusion leaves concentration gradients whereby lighter molecules (e.g. low molecular \( n \)-alkanes and isoprenoids) will permeate deeper than heavier ones (e.g. hopanes, steranes, and high molecular weight \( n \)-alkanes and isoprenoids).

As was explained in Chapter 3, so-called live-oil effects whereby indigenous hydrocarbons are leached out of host rocks due to pressure release cannot explain the chromatographic separation of molecules. Such effects were investigated by Brocks (2011) who quantitatively analyzed hydrocarbon distributions in Archean shale, artificially contaminated rocks, and mudstones where live-oil effects have been reported.
In this study, all analyzed rock samples from the *Mt Charlotte* core yielded hydrocarbons. However, significantly higher concentrations of numerous compounds have been observed on the rock exteriors of these samples. Other compounds, do not record such differences and display E/I values that are closer to one (i.e. have approximately equal concentrations of hydrocarbon molecules on both rock portions). Below, the results of this study and their implications will be discussed. Importantly, this study will highlight the necessity for a rigorous, quantitative approach in determining hydrocarbon syngeneity in drill core samples.

### 4.4.1 Biomarker evidence for ancient hypersaline environments

All rock samples mentioned in this study have been deposited in a hypersaline environment during the Neoproterozoic and Early Cambrian (see Chapter 2). Such environments tend to host a variety of microorganisms that are adapted to hypersaline conditions and include members from all domains of life (e.g. Oren 2002). Therefore, bitumens obtained from these samples should yield biomarkers indicative of such organisms. This proved to be the case for the *Mt Charlotte* evaporites. These samples yielded biomarkers deemed characteristic of hypersaline environments and include elevated concentrations of docosane, homologous series of both regular as well as irregular isoprenoids, and the detections of numerous hopanes and steranes including pregnanes. Based on these results, it would be very tempting to regard them as indigenous paleoenvironmental signals of ancient hypersaline settings. Below, each of these “hypersaline biomarkers” we be separately evaluated. It will be shown that their origin can be either dubious or real with regards to syngeneity in the *Mt Charlotte* drill core.

#### 4.4.1.1 Laboratory sand blanks

Prior to any investigations on the hydrocarbon syngeneity of rocks, laboratory blanks need to be discussed. Such blanks record the presence of contaminants as a result of laboratory activity (e.g. rock crushing, solvent extraction).
In this study, laboratory contamination is not regarded to have been significant and would have contributed little \( n \)-alkanes, monomethylalkanes, pristane and phytane. The only exception would have been \( n \)-alkanes from C\(_{12}\) to C\(_{14}\), which were present in high concentration in the blanks and were also detected in some of the Neoproterozoic and Cambrian evaporites (Figure 4.2). Therefore, any other contaminants must have been introduced prior to sample analysis (e.g. through contact with drilling fluids and plastic storage bags).

4.4.1.2 \( n \)-alkane patterns
The \( n \)-alkanes usually are the most abundant hydrocarbons in all bitumen groups. However, significant differences were observed between Group 1 bitumens and those from Group 2 and 3. In the first group, E/I concentration differences were significantly higher (up to 30x) than in the other groups (up to 4.8x, but mostly ~2x). These results indicate that Group 1 bitumens are severely affected by \( n \)-alkane contaminants, while those from the other groups are less affected. The observation that \( n \)-alkanes >C\(_{22}\) are often not detected in the interior of Group 1, indicates that all homologues of this compound class are most likely contaminants. Heavier \( n \)-alkane contaminants would not have been able to diffuse through the same rock depths as their lighter counterparts. By contrast, \( n \)-alkanes from the interior of all Group 2 and 3 bitumens ranged up to C\(_{37}\) and no sharp cut-off were observed. As shown in Chapter 6, single-compound carbon isotopic measurements of the exterior and interior \( n \)-alkanes from Group 2 and 3 can yield markedly different \( \delta^{13} \)C values. This observation provides further indication that the bitumens from these groups are a mixture of syngenetic and contaminant \( n \)-alkanes.

Inspection of E/I chromatograms in Figure 4.16 shows that the relative concentrations of mono- and dimethylalkanes to \( n \)-alkanes are more pronounced in the interior than in the exterior rock portion. This observation provides a visual demonstration of the additional \( n \)-alkane contaminants on the exterior, which diluted the relative concentration signals.

E/I ratios of \( n \)-alkanes are not uniform in all samples. Group 1 bitumens can either yield large ratios for the heavier homologues (Figure 4.9A) or their lighter
counterparts (Figure 4.9B). E/I ratios of \(n\)-alkanes in Groups 2 and 3 can also exhibit substantial variations (compare Figures 4.9C and 4.9D). Such differences can be explained when the contaminants were composed of variable \(n\)-alkane profiles. As mentioned in section 4.3.2.1, \(n\)-alkane profiles of Group 1 bitumens can vary substantially and their highest concentrations range from lighter to lighter to heavier homologues. Since these \(n\)-alkanes can be regarded as contaminants, it is expected that they would have also overprinted the Group 2 and 3 bitumens, resulting in varied E/I profiles.

4.4.1.3 Elevated concentrations of heptadecane \((n-C_{17})\) and docosane \((n-C_{22})\)

Elevated concentrations of \(n-C_{17}\) and/or \(n-C_{22}\) were detected in most samples. Below, each of these compounds is separately discussed.

Elevated concentrations of \(n-C_{17}\) were characteristic of Group 2 and 3 bitumens. E/I ratios of this compound did not exhibit the same abrupt concentration differences (relative to nearby homologues) as was observed for \(n-C_{22}\) (Figure 4.9C). Therefore, no proof exists that the elevated concentration of this molecule is due to contamination. Previous studies (e.g. Bühring et al. 2009) have reported high concentrations of this molecule in hypersaline settings and are interpreted to originate from cyanobacteria or algae.

Numerous evaporite samples from this study also exhibited elevated concentrations of \(n-C_{22}\). Such prominent concentrations have been previously reported in a number of studies from ancient Phanerozoic evaporites (e.g. ten Haven et al. 1985, 1988; Gely et al. 1993; Andersen et al. 2001), as well as in modern hypersaline settings (Schreiber et al. 2001). Therefore, a prominent docosane concentration has been assigned as a biomarker for hypersalinity (ten Haven et al. 1985, 1988), resulting in the creation of the \(R_{22}\) index \((2 \times C_{22} / (C_{21} + C_{23}))\) for this setting (e.g. ten Haven et al. 1988; Gely et al. 1993). Since the results of this study are consistent with those conducted in other ancient hypersaline settings, it would appear logical to consider ruling out contamination. Indeed the presence of elevated docosane concentration in evaporites from the
Neoproterozoic would be of particular interest, indicating that the source organisms of \( n\text{-C}_{22} \) occurred in hypersaline environments at \( \sim 800 \text{ Ma} \).

However, the results from the E/I experiments have painted a different picture. Significant concentration differences of docosane were observed between the exterior and interior of rock samples. While the interior of some samples still recorded elevated concentrations of docosane, the concentration of these molecules relative to the exterior would be significantly less. Indeed, E/I concentration differences of up to 6x has been noted for some samples. In other samples, the elevated occurrence of this molecule would be hardly visible in the interior at all. As shown in Figure 4.16, the concentration maxima at \( n\text{-C}_{22} \) disappears in the interior, and reveals a slight odd-over even predominance compared to \( n\text{-C}_{21} \) and \( n\text{-C}_{23} \). This pattern would have been obscured, had the exterior of the sample not been removed.

Based on the above results, elevated \( n\text{-C}_{22} \) concentrations in all Mt Charlotte 1 evaporites are interpreted as contaminants. The hydrocarbon composition of the plastic sample bags of the Mt Charlotte 1 drill core (Figure 4.19) provides a clear source for these elevated concentrations. The discovery that \( n\text{-C}_{22} \) can act as a contaminant, however, is not new. Previous studies by Douglas and Grantham (1973) have identified this molecule as a contaminant in geological samples that were derived from polyethylene vial closures. Lehtonen and Ketola (1993) also reported \( n\text{-C}_{22} \) as an artifact of TLC-plates, while Zhou et al. (2008) identified it as a major component in the foil laminated film of packaging material. This molecule may also occur in anti-foaming agents or other chemicals used in the pulp and paper industry (Aurela et al. 1997).

The leaching of hydrocarbon molecules from plastic sources has long been recognized in the food and pharmaceutical industries. The primary concern for these enterprises has been the transfer of plastic contaminants into food and drug substances (e.g. Jenke et al. 2006). Aurela et al. (1997) conducted tests to determine the permeability barriers of various plastic and non-plastic coated paper plates. Docosane was used as a model substance to test the permeability of these barriers into food simulants. In each of these plates, docosane was shown to have
migrated through the plates, demonstrating the ease with which such a molecule can move.

Organic geochemists have increasingly pointed to the dangers of hydrocarbon contaminants in obscuring or misleading the interpretation of biomarkers in rock samples. The \( n \)-alkanes in particular form a major constituent of products such as polyethylene bags, along with drilling fluids and lubricants (see Brocks et al. 2008 for a list and references).

While the results of this and other studies (e.g. Douglas and Grantham 1973) portray elevated concentrations of \( n\text{-C}_{22} \) as a contaminant, it does not necessarily mean that this is always the case. For example, Schaeffer et al. (1995) reported the presence of elevated docosane concentrations in the sulfur-bound fraction of organic-rich marls deposited during the Messinian salinity crisis. Furthermore, Andersen et al. (2001) measured stable hydrogen and carbon isotopes of the sulfur-bound \( n\text{-C}_{22} \) from this time period and noted a variability that appears to be affected by hydrographic changes caused by extremes of evaporation. Therefore, docosane may occur both naturally and as a plastic-derived contaminant. For this reason, the ability to differentiate between these possible sources is critical.

It should be noted that docosane is not the only plastic-derived molecule that can be mistakenly interpreted as biogenic. Grosjean and Logan (2007), for example, have shown that a range of organic contaminants can emanate from plastic sampling bags. In this regard, branched alkanes with quaternary carbon centers (BAQCs) are an important example of artificially produced organic molecules. They are common constituents of plastic bags and form as polymerization by-products of polyethylene (Takahashi et al. 1980a,b). Nevertheless, the source of these molecules appears to have been largely unknown to the organic geochemical community. Despite the absence of known natural sources, BAQCs reported in publications were interpreted as biogenic (e.g. Kenig et al. 2003; Bai et al. 2006). Only recent work by Grosjean and Logan (2007) and Brocks et al. (2008) have demonstrated that BAQCs are easily transferred from storage bags to rock samples by diffusion into fissures and pores.
4.4.1.4 Presence of mono- and dimethylalkanes

Homologous series of monomethylalkanes were observed in Group 1, 2 and 3 bitumens. However, an additional series of dimethylalkanes was detected only the latter two groups. Like the \( n \)-alkanes, the monomethylalkanes in Group 1 can only be interpreted as contaminants. Measurements demonstrated markedly higher concentrations on the exterior and E/I ratios follow the trends of the \( n \)-alkanes. Furthermore, high molecular weight homologues were more concentrated on the exterior than their lighter counterparts (Figure 4.10A).

Group 2 and 3 monomethylalkanes also exhibited higher concentrations on the exterior (Figure 4.10B). Of note is the observation that E/I plots of monomethylalkanes are similar to those of the \( n \)-alkanes from the same sample (compare Figure 4.9D and 4.10B). Therefore, any effects of hydrocarbon overprinting would be similar as that described for the \( n \)-alkanes. However, E/I differences were not marked (~1.9 x) and no chromatographic differences between light and heavy molecular weight homologues were observed. Therefore, while some contamination overprint of these alkanes did occur, monomethylalkanes are present.

Dimethylalkanes from both Groups 2 and 3 exhibited E/I ratios close to one. Therefore, these molecules are regarded as syngenetic. Based on this observation, any contaminants that affected these rocks did not appear to contain dimethylalkanes at great concentrations.

4.4.1.5 Elevated concentrations of isoprenoids

Acyclic isoprenoids are an important class of compounds when discussing the biomarker composition of hypersaline ecosystems. Although present in all domains of life, isoprenoids are particularly abundant and diverse in members of the Archaea domain, many of which are present in hypersaline environments (e.g. Oren 2002; Patel and Sprott 2006; Jahnke et al. 2008; Orphan et al. 2008). Members of the Archaea differ from those of the Bacteria and Eukarya domains in having a diverse range of isoprenoid chains attached to glycerol by ether links.
rather than fatty acids connected by ester links. Sometimes these isoprenoids link
to glycerol units to form a long tetraether (e.g. Sinninghe Damsté et al. 2002).

For these reasons it would be easy to regard all isoprenoids of Mt Charlotte 1
samples as indigenous. However, results of the E/I rock experiments do not
support such a claim. Below, the syngeneity of isoprenoids from each group of
bitumens is discussed and it will be demonstrated that not all compounds can be
regarded as syngenetic.

Group 1 bitumens contained a homologous series of regular head-to-tail
isoprenoids up to C_{36} (Figure 4.3). However, these isoprenoids are regarded as
contaminants since significantly higher concentrations (up to 69.5x) were detected
on the exterior than the interior portions (Figure 4.11A). Indeed, exterior
concentrations increased at higher molecular weights, exhibiting the
chromatographic effects described by Brocks (2011). Isoprenoids heavier than C_{31}
were not detected in the interior. As with the n-alkanes, this pattern also
demonstrates that shorter chain isoprenoids can infiltrate rock samples more easily
than their larger chain counterparts. The interpretation of these isoprenoids as
contaminants is important, since regular head-to-tail isoprenoids with more than 20
carbon atoms are regarded as diagnostic of hypersaline source-rocks (Peters et al.
2005). Indeed, Li et al. (2003) described a similar distribution of regular isoprenoids
(to C_{36}) in Precambrian carbonates and evaporites and attributed them to
halophiles and other archaea.

Group 2 and 3 bitumens yielded a variety of regular and irregular (head-to-
head and tail-to-tail) isoprenoids. Below, the syngeneity of each type of isoprenoid
is separately discussed.

The regular isoprenoids of this group consisted of pristane and phytane, their
breakdown products, and homologues >C_{20} (Figure 4.8). In anhydrite-rich samples,
C_{21} to C_{25} regular isoprenoids were particularly prominent, although higher
molecular weight homologues (up to C_{36}) were present in smaller quantities. E/I
experiments indicate that some of these regular isoprenoids are contaminants.
Pristane and phytane, in particular, had the propensity to exhibit marked contrasts
between the exterior and interior portions (Figure 4.17). Therefore, contamination
appears to have severely affected these molecules. Carbon isotopic measurements (discussed in Chapter 6) will further demonstrate that the exterior pristane and phytane are contaminants while their interior counterparts are indigenous.

Homologous series of regular isoprenoids that range from $C_{21}$ to $C_{25}$ provide a stark contrast to lighter and heavier homologues of this series. These compounds are particularly prominent in anhydrite-rich samples (discussed more fully in Chapter 5) and show E/I ratios close to one (Figure 4.11B). Such a result indicates that equal amounts of these homologues were detected in both the exterior and interior rock portions. Therefore, this group of compounds is deemed to be syngenetic to the evaporites.

Previous studies support the interpretation of this group of regular isoprenoids as indigenous. Grice et al. (1998), for example, reported regular $C_{25}$ isoprenoids as a predominant component in Miocene/Pliocene anhydrite and halite deposits. Similar results were also observed in other studies of hypersaline lacustrine and marine environments (e.g. McKirdy and Kantsler 1980; ten Haven et al. 1988).

Regular isoprenoids $>C_{26}$ exhibited a different pattern as their lighter counterparts. These molecules, like those in Group 1, exhibited progressive E/I concentration differences with increasing molecular weights. Therefore this homologous series is not regarded as syngenetic to the evaporites.

The $C_{30}$ tail-to-tail isoprenoid squalane was also detected in this group of bitumens. E/I ratios for this isoprenoid were close to one. Therefore this irregular isoprenoid is also interpreted as syngenetic to the host rock. Squalane has been reported from a number of ancient hypersaline settings (e.g. McKirdy and Kantsler 1980; ten Haven et al. 1988; Grice et al. 1998), which provides further support that this molecule is syngenetic to the evaporites.

A homologous series of head-to-head isoprenoids ($C_{32}$ to $C_{40}$) was also identified in this group (Figure 4.8). Their E/I ratios were also close to one (Figure 4.11C), and are therefore interpreted as syngenetic to the evaporites. Recently, caldarchaeol, a likely $C_{40}$ precursor of these head-to-head isoprenoids, was reported from a modern hypersaline environment (Jahnke et al. 2008).
observation provides further evidence for the syngeneity of these irregular isoprenoids in the Gillen Member evaporites.

4.4.1.6 Presence of various hopanes and steranes
Hopanes and steranes were detected in all Neoproterozoic and Cambrian evaporites. However, these molecules were mostly observed on the exterior rock portions. Hopanes and steranes exhibited between 4.9 to 9.4x and 4.5 and 5.5x greater concentrations on the exterior, respectively. Indeed, clear-cut distinctions were observed between E/I rock portions (Figures 4.12 and 4.13). Therefore, these molecules are interpreted as contaminants and are not indigenous biomarkers.

Testing the syngeneity of hopanes and steranes is important as these molecules are biologically informative markers, which can impart significant information on the pro- and eukaryotic diversity in ancient rock samples. Previous biomarker studies on the Gillen Member (e.g. Logan et al. 1997; Summons and Walter 1990) have reported a similar pattern and range of hopanes and steranes as that observed in the exterior samples of Mt Charlotte 1. Some of these occurrences can have far reaching implications in understanding the evolutionary history of numerous microorganisms. Summons and Walter (1990), for example, tentatively suggested that the presence of 4-methyl steranes, including dinosteranes, in the Gillen Member indicate that dinoflagellates probably had ancestors going as far back as the Neoproterozoic. Since no dinoflagellate cysts have been detected in sediments older than the Triassic, this would be an important discovery. However, dinosteranes were only detected in the exterior portion of Mt Charlotte 1 rocks, and cannot be attributed as an indigenous component in these samples. Therefore, this observation raises doubts on the syngeneity of dinosteranes detected in other cores from the Amadeus Basin and whether these molecules originated from the Neoproterozoic rocks.

The major sterane products in the Mt Charlotte 1 samples were the C_{21} pregnane and C_{22} homopregnanes. Elevated concentrations of these molecules could provide further clues on ancient hypersaline biodiversity. Significant concentrations of pregnanes and homopregnanes have been previously detected
in hypersaline paleodepositional environments from numerous parts of the world (e.g. ten Haven et al. 1988; Lu et al. 2009) and are regarded as a biomarker for hypersaline settings (ten Haven et al. 1985). However, these molecules only exhibited high concentrations on the rock exteriors, demonstrating that they are also not indigenous components of the Mt Charlotte 1 evaporitic rocks. Resistance to biodegradation could be a major reason for the elevated concentration of pregnane and homopregnanes in these samples. Indeed, comparable to the diasteranes, these molecules are reported to be significantly recalcitrant (Peters et al. 2005).

Hopanes from the Mt Charlotte 1 core could also provide misleading information. The major compound in the hopane series of this core was 17α(H)-30-norhopane, accompanied by a high relative abundance of 29,30-bisnorhopane (Figure 4.4). Since 30-norhopanes have been identified in several carbonate-derived petroleums (e.g. Seifert et al. 1984; Summons and Powell 1987; Price et al. 1987), the molecular composition of hopanes in Mt Charlotte 1 samples containing dolomite is consistent with what is expected of a carbonate rock. However, as with the steranes, none of these compounds have been detected in the interior. This result shows that despite consistencies between lithologies and expected biomarkers, the latter are not indigenous to the samples.

4.4.1.7 Presence of various Phanerozoic plant biomarkers

Various plant biomarkers such as oleanane and bicadianane were detected in the Neoproterozoic and Cambrian evaporites. Since these biomarkers are largely indicative of flowering plants that evolved much later in the geological record (e.g. van Aarssen et al. 1992; Moldowan et al. 1994), their presence in Neoproterozoic and Cambrian evaporites indicates that they have been exposed to contaminants. The co-occurrence of these molecules with hopanes and steranes of similar molecular weight provides additional support that the latter are contaminants.
4.4.1.8 Presence of aromatic hydrocarbons

Aromatic compounds were detected in all evaporites. However, marked E/I contrasts were observed within both bitumen groups. A notable feature in all samples was the presence of various polychlorinated biphenyls (PCBs). These compounds are industrially produced and have been in commercial use from 1929 to the mid-1980s (e.g. Helsinki Commission 2001). Due to their chemical inertness, heat resistance and non-flammability, PCBs have been used as additives in hydraulic, cutting and lubricating oils (Helsinki Commission 2001). Their presence in the Mt Charlotte 1 samples is therefore a strong indication of contamination that was possibly introduced through the drilling of the core. Since these molecules have been detected in both the exterior and interior portions of many samples, they also display the ability of contaminants to infiltrate rocks.

In Group 1 bitumens, aromatic compounds were predominantly (up to 27x) on the exterior portions (Figure 4.15). Such results were in contrast to those observed for Groups 2 and 3, where most compounds, except PCBs, exhibited E/I ratios of up to 1.9x (Figure 4.18). These results indicate that aromatic compounds from Group 1 are contaminants, while those from Groups 2 and 3 are mostly interpreted as syngenetic.

4.4.1.9 Final assessment of bitumen syngeneity from Cambrian and Neoproterozoic evaporites of Mt Charlotte 1

In this study, hydrocarbons from all Group 1 bitumens exhibit E/I patterns and values that are characteristic of contamination. This group includes all Cambrian as well as most Neoproterozoic samples investigated for this thesis. While bitumens from this group have been interpreted as contaminants, it cannot be ruled out that some syngeneric hydrocarbons are still present. However, their biomarker signals would have been severely overprinted, making any paleoecological or environmental interpretations unreliable. Therefore, these samples are excluded from the paleoecological assessments made in Chapter 5.

Group 2 and 3 bitumens exhibit E/I patterns and values that are characteristic of both indigenous and contaminant hydrocarbons. All hopanes, steranes and plant
biomarkers from this group are contaminants. However, the regular isoprenoids contain a complex mix of contaminants (i.e. those exhibiting E/I ratios >1) and indigenous molecules (i.e. those exhibiting E/I ratios of 1). In this regard, some of the pristane and phytane as well as homologues >C_{25} are regarded as contaminants. However, homologues between C_{21} and C_{25} and some of the pristane and phytane (observed in the interior; see also Chapter 6) are interpreted as syngenetic to the host rocks. All irregular isoprenoids exhibit E/I values of one and are also interpreted as syngenetic. The n-alkanes, like the regular isoprenoids from this group, are made up of a mix of both syngenetic and contaminant contributions. Chapter 6 will provide carbon isotopic evidence for this inference.

While some overprinting from contaminant monomethylalkanes has occurred in Group 2 and 3 bitumens, the effects are minor (E/I ratios of ~1.9x). Indeed, a conspicuous feature of Group 2 bitumens is the elevated concentrations of mono- and dimethylalkanes relative to their n-alkane equivalents. Such a pattern has been observed in a number of Precambrian and Cambrian sedimentary rocks (e.g., Jackson et al. 1986; Klomp 1986; Fowler and Douglas 1987; Summons et al. 1988a,b) and is regarded to be indicative of sample from that time frame. Such hydrocarbon patterns are rarely observed in Phanerozoic samples and provide a strong indication that these hydrocarbons are syngenetic.

4.4.2 The necessity of using quantitative as opposed to qualitative approaches in testing bitumen syngeneity

This study has focused on testing the syngeneity of bitumens from numerous evaporitic samples of the Mt Charlotte 1 drill core. Through the application of quantitative E/I experiments, it has been shown that the syngeneity of numerous compounds (e.g. n-C_{22}) cannot be supported. Data for other compounds (e.g. head-to-head isoprenoids), in turn, appear to support a syngenetic origin. Below we describe the wider implications for this study and why a quantitative approach is necessary in testing the syngeneity of ancient biomarkers.

In the past, non-quantitative have been used to argue for syngeneity and to dispel doubts about a contamination. Schenk (1969), for example, reported prominent concentrations of n-C_{22} in nearly 100 sediment samples ranging in age
from the Permian to the Quaternary. The author excluded field contamination by pointing out that similar results were obtained from samples collected by numerous people at various localities at different times. He also excluded laboratory contamination by indicating that reproducible results were obtained by different operators, utilizing different apparatuses and chemicals.

Over the past decades, numerous authors have provided similar arguments to justify the presence of various hydrocarbon biomarkers from rock extracts as indigenous. An important example is the biomarker result obtained from 2.5 to 2.7 Ga Archean shale collected from the Pilbara craton in Western Australia. These shales yielded hydrocarbons that have been interpreted as evidence for the presence of oxygenic cyanobacteria and ancestral eukaryotes well before the oxygenation of the atmosphere and the appearance of eukaryotic body fossils (Brocks et al. 1999; Summons et al. 1999). The samples for that study came from several cores drilled hundreds of kilometers apart and were independently collected and stored. Based on the thermal maturity levels of the hydrocarbons and their compositional similarity to other Precambrian samples, the biomarkers and other aliphatic hydrocarbons were regarded as “probably syngenic” with their Archean host rocks (Brocks et al. 2003). However, a later study has cast doubts on these results. Rasmussen et al. (2008) compared the carbon isotopic composition of the bitumen with co-occurring pyrobitumen and kerogen. The bitumen was shown to be enriched in $^{13}$C by 10 to 20‰ relative to the pyrobitumen and kerogen. The implication of this isotopic discrepancy meant that the liquid hydrocarbons of the bitumen entered the rock after peak-metamorphism 2.2 Ga by means of migrating petroleum or during drilling and storage (Brocks 2011). Such results demonstrate the dangers in basing biomarker syngeneity solely on their presence in other rock samples from the same age and lithology.

Additional arguments for biomarker syngeneity by various authors include an observed difference in the composition of biomarkers between stratigraphic horizons. However, such arguments by themselves are not adequate for proving an indigenous nature of biomarkers. Differences in the surface properties, as well as the porosity and permeability of different rock types are likely to affect the
pattern of rock contamination (Brocks et al. 2008). Furthermore, drilling operation procedures can vary for a single core. The Mt Charlotte 1 core, for example, was obtained using a mixture of mud, air and mist drilling (McTaggart et al. 1965). Furthermore, the process of air drilling varied, with some sections of the core being obtained with a hammer drill and others without this tool. An additional source of variability is introduced by differences in storage conditions. As shown in Figure 4.1, some samples of the core have been wrapped in plastic bags, while others were only in partial contact with the bags when they were used as space fillers. Other samples, in turn, had no contact with the bags. Such observations are in direct contrast to commonly held assumptions that a single drill core has been subjected to the same handling and storage conditions (cf. Sherman et al. 2007). Therefore, a simple presence or absence scheme of biomarkers between different stratigraphic horizons is not sufficient in determining syngeneity.

Several methods aimed at removing hydrocarbon contaminants from samples are often not sufficient. They lack the quantitative or experimental rigor, which could indicate migration of hydrocarbons into a rock. An example of such a method includes multiple solvent rinses of whole rock broken into increasingly smaller fragments. Chromatographic analysis of each rinse is used to ensure that the extracted bitumen was intimately associated with the rock and not coating it or lining cracks (Peters et al. 2005). However, microscopic pores and cracks are often not taken into account in such arguments, although their presence would allow natural and/or anthropogenic contaminants to settle, enter and/or pass through the samples. Brocks (2011) artificially contaminated Precambrian shale devoid of indigenous bitumen with crude oil and showed that liquid petroleum products may penetrate (centimeters deep) compact- and intact-looking shale within days. Indeed, depending on the porosity and permeability of the rock, hydrocarbons can potentially migrate through to the center of the rock sample.

Another method commonly employed in removing hydrocarbon contaminants is the rinsing and ultrasonication of rocks surfaces with solvents. However, such a method is inadequate in completely removing contaminants. Brocks et al. (2008) have artificially stained rock pieces with petroleum, which were subsequently
subjected to ultrasonication in solvents. Regardless, hydrocarbon contaminants were still present, and have even migrated into rocks. Therefore, the application of such a technique is futile in the complete removal of contaminants.

This study was based on results obtained from E/I experiments, whereby the outer surfaces of core samples were physically removed via cutting or abrading. These processes have been first described by Brocks (2001) and have proved to be far more efficient in hydrocarbon removal than sonication in solvents (see also Sherman et al. 2007). A similar approach has been previously employed on Archean core samples by Sherman et al. (2007) and Waldbauer et al. (2009). However, these studies did not quantify the hydrocarbon concentrations in both the interior and exterior rock portions. Furthermore, they suggested the removal of an arbitrary rock thickness, which was believed to be sufficient in eliminating the impacts of contamination. However, as was shown by Brocks (2011), contaminants can migrate right through a rock. Therefore, E/I quantification of the hydrocarbons is necessary to identify any impacts of migrated contaminants.

4.5 Conclusions

This study investigated the syngeneity of hydrocarbons from Cambrian and Neoproterozoic evaporates. Such a study is vital for properly understanding the paleoecological composition ancient hypersaline ecosystems. The rocks were derived from a drill core that was stored or for several decades in boxes and/or plastic bags. While all samples yielded a diverse range of hydrocarbons, not all of these molecules could be interpreted to be syngeneric with their respective host rocks. This result is particularly intriguing, since many of these contaminants have been previously interpreted as indicative of hypersaline conditions (e.g. elevated concentrations of docosane, isoprenoids and pregnanes). Furthermore, many of these molecules have overprinted samples that yielded syngeneric hydrocarbons.

Results from this study show that the origins of hydrocarbons from a drill core can be particularly complex. Commonly occurring hydrocarbons, especially \( n \)-alkanes, head-to-tail isoprenoids like pristane and phytane, hopanes and steranes should treated with particular caution. Since these molecules are present in most
Phanerozoic oils, the likelihood that they will contaminate Precambrian drill core samples is high. Nevertheless, E/I experiments, whereby the hydrocarbon concentration from a single rock sample is quantitatively determined, provides the means for evaluating their syngeneity. Ultimately, this study provides clearer insights into the paleoecological composition of ancient hypersaline settings.

### 4.6 References


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Chapter 5
Assessing the microbial diversity of an ~800 Ma Neoproterozoic hypersaline environment: evaporites as archives for Precambrian halophiles

This chapter presents a detailed molecular investigation of the indigenous biomarkers from an evaporitic, hypersaline environment in the Neoproterozoic. The aim is to assess the microbial community composition of this environment and to understand the effects of changing salinity levels on this ecosystem.

5.1 Introduction
Numerous microorganisms survive and flourish in environments that exhibit salinity ranges several times higher than that of sea water. Organisms able to survive such hypersaline conditions are referred to as halotolerant or halophilic (Javor 1989). Halotolerant microbes are able to grow in both the presence and absence of salt, while halophiles require the presence of salt (Russell 1992). Phylogenetically, these organisms do not form a coherent group, and in most cases halophiles are closely affiliated with non-halophilic relatives (e.g. Oren 2002). Indeed, microorganisms able to withstand high salinities have been identified in all three domains of life: Archaea, Bacteria and Eukarya (e.g. Oren 2002).

Microorganisms that reside in hypersaline settings are affected by a number of environmental stressors. A drop in oxygen levels or high osmotic pressures, for example, can result in the death or inevitable desiccation of cells that are not adapted to saline conditions (e.g. Oren 2002). In order to survive and grow in these environments, microorganisms evolved special protective mechanism that made them adapt to such conditions. Among these mechanisms, lipids play a vital role. Indeed, the membrane lipid compositions of halophilic microbes display a variety of adaptations and have even been shown to change in response to alterations in salinity (Russell 1992).

Currently, a diverse range of lipids and their diagenetic breakdown products have been identified and labeled as indicative of hypersaline conditions. These
molecules have been utilized in a number of biomarker studies of ancient hypersaline settings in order to understand their microbial composition.

Thus far, most biomarker studies on ancient evaporitic deposits have focused on Phanerozoic sediments. These studies include Miocene/Pliocene halite-rich deposits from the Sdom Formation in Israel (Grice et al. 1998); Paleozoic (Permian), Mesozoic (Triassic, Cretaceous) and Cenozoic (Eocene) saline/hypersaline basins in China (Wang and Fu 1997; Wang 1998); marls of the Eocene-Oligocene Mulhouse Basin in France (e.g. Hofmann et al. 1993); Miocene marls of the northern Apennines in France (ten Haven et al. 1985); and Cambrian evaporite sequences of the Observatory Hill beds, Officer Basin, South Australia (McKirdy and Kantsler 1980).

Molecular studies on Precambrian hypersaline settings are sparse and not detailed. Summons et al. (1988b) suggested that hypersaline conditions prevailed at times during the deposition of the early Neoproterozoic Walcott Member, Chuar Group, North America. Traces of gypsum, quartz and calcite pseudomorphs after gypsum and anhydrite, and solution collapse structures together with dolomite were observed within five vertical meters of two organic rich samples. The authors detected isotopically heavy (+4.5‰ PDB) whole rock carbonate, slightly elevated C_{25} and C_{30} acyclic isoprenoids, and the occurrence of gammacerane. Purported evidence for an even older saline setting was observed in organic rich mid-Proterozoic McArthur basin sediments of the Barney Creek Formation from northern Australia (Summons et al. 1988a). In these rocks, slightly elevated concentrations of acyclic isoprenoids, particularly C_{20} to C_{25} and C_{30}, were detected. Based on these observations it was discerned that the sediments were formed in a hypersaline environment (Summons et al. 1988a).

A feature that is often lacking in these ancient studies is the integration of mineralogical, petrographic and organic geochemical data. Such combined data can provide important information on the environmental conditions encountered at that time and help constrain the settings from which biomarkers were extracted.
Herein, the results of organic geochemical work from the ~800 Ma Gillen Member of the Neoproterozoic Bitter Springs Formation are presented. As was noted in Chapter 2, the Gillen Member of central Australia hosts a variety of evaporites that are indicative of an ancient hypersaline environment and contain evidence of former dolomite-precipitating microbial mats. This chapter shows that these rock samples contain well preserved organic matter, which features an array of biomarkers. Since these result yield insights into the microbiological diversity of a variety of Precambrian hypersaline facies, antiquity constraints as well as changes in microbial community compositions over time can be established.

5.2 Materials and Methods

5.2.1 Samples
For this thesis, 55 samples were collected from the Gillen Member of the Mt Charlotte 1 core. However, as mentioned in Chapter 4, only 11 of these samples yielded indigenous hydrocarbons. Herein, these samples are investigated in greater detail.

The rocks that were analyzed for this chapter consisted of evaporites that were predominantly composed of dolomite and anhydrite. Organic geochemical and lithologic characteristics of these samples are summarized in Table 5.1. The evaporites were deposited in an inland sea that is now central Australia. Due to the shallow nature of that sea and a tenuous connection with the ocean at that time, the water was characterized by elevated salinity levels (Lindsay 1987). Below, the syngenetic hydrocarbons from these rocks are investigated.

5.2.2 Processing of samples
Rock samples were ground to powder in an alumina ring-mill (Rocklabs, NZ). Prior to usage, the mill was cleaned by grinding baked-out (600°C/24 h) quartz-rich sand two to three times for 60 seconds and subsequently washed with methanol and dichloromethane (DCM; solvent grade 99.9%, UltimAR®, Mallinckrodt Chemicals). System blanks consisted of baked-out sand (600°C/24 h). Approximately 5 to 30 g
of rock powder were extracted with 100% DCM in a Dionex Automated Solvent Extractor. The extracts were reduced to 100 µl under a stream of purified nitrogen gas and separated into saturated, aromatic and polar fractions using column chromatography over 12 g annealed (450°C/24 h) and dry-packed silica gel (Silica Gel 60; 230-400 mesh; EM Science). Saturated hydrocarbon were eluted with 1.5 dead volumes (DV) n-hexane, aromatic hydrocarbons with 2 DV n-hexane:DCM (1:1 v/v) and polars with 2 DV DCM:methanol (1:1 v/v). An internal standard consisting of 2.3 µg 18-methyleicosanoic acid methyl ester (MEME; Ultrascientific, U.S.A) was added to the saturated and aromatic hydrocarbon fractions. An additional 50 ng of d₄-C₂₉-α,α,α-ethyl-cholestane (D₄; Chiron Laboratories, UK) was added to the saturated fraction as an internal standard for metastable reaction monitoring (MRM).

5.2.3 Gas chromatography-mass spectroscopy (GC-MS)

GC-MS analyses of the saturated and aromatic fractions were carried out on a Micromass AutoSpec Premier equipped with a 6890 gas chromatograph (Agilent) and a DB-5 MS capillary column (60 m × 0.25 mm i.d., 0.25 µm film thickness) using He as carrier gas. The MS source was operated at 260°C in EI-mode at 70 eV ionization energy and with 8000 V acceleration voltage. Samples were injected in splitless mode into a PTV injector at a constant temperature of 300°C. For full-scan analyses, the GC oven was programmed at 40°C (2 min), heated to 315°C at 4°C/min, with a final hold time of 17 min. The AutoSpec full-scan duration was 0.7 s plus 0.2 s interscan delay over a mass range of 55-600 Da. For MRM, the GC oven was programmed at 60°C (2 min), heated to 100°C at 8°C/min, further heated to 315°C at 4°C/min and held at the final temperature for 34 min. Identification of 2,6,11,15-tetramethylhexadecane (crocetane) and 2,6,10,15,19-pentamethylicosane (PMI) was aided by separation with a 50 m x 0.22 mm x 0.25 µm β cydex column using conditions similar to those reported by Greenwood and Summons (2003) and Barber et al. (2001b).
5.2.4 X-ray powder diffraction (XRPD)

XRPD was carried out with a SIEMENS D5005 Bragg-Brentano diffractometer equipped with a graphite monochromator and scintillation detector, using CoKα radiation. The scan range was 4 to 85° 2-theta, at a step width of 0.02°, and a scan speed of 1 second per step. Samples were loaded in side-packed sample holders. The results were interpreted using the SIEMENS software package Diffracplus Eva (2003) and Siroquant V3.

5.2.5 Total Organic Carbon (TOC), ROCK EVAL, hydrogen and oxygen indices determination

Values for the above measurements were obtained commercially through Geotech, Perth, Western Australia. All samples consisted of crushed rock powder from which bitumens were previously extracted.

5.3 Results

5.3.1 Sample classification based on hydrocarbon signatures

Three major GC patterns from extracts of the Gillen Member were able to be discerned (Figure 5.1). These patterns are broadly described below. The aim here is to provide a simply overview of each type of GC pattern. Subsequent sections will describe the results in increasingly greater detail and provide quantitative data for interpreting their distribution.

One type of GC pattern was characterized by high concentrations of mono- and dimethylalkanes relative to \( n \)-alkanes, and a low relative abundance of most isoprenoids (Figure 5.1A). This type of pattern would be derived from rocks with relatively low concentrations of anhydrite and a greater concentration of dolomite and siliciclastics. Rocks of this type tend to be quite dark in color.

Another type of GC pattern would yield a hydrocarbon signature that also exhibits high concentrations of mono- and dimethylalkanes relative to \( n \)-alkanes (Figure 5.1B). However, this pattern is characterized by significantly high concentrations of regular and irregular (tail-to-tail and head-to-head) isoprenoids.
This type of pattern is derived from samples that have a much larger concentration of anhydrite relative to the first patterns. Nevertheless, significant concentrations of dolomite are still present in the form of flat as well as twisted and deformed laminae. Other minerals include quartz, clays, feldspar, pyrite and halite (Table 5.1; see also Chapter 2).

The third and final pattern is characterized by relatively low concentrations of mono- and dimethylaklanes relative to \( n \)-alkanes (Figure 5.1C). Furthermore, the relative concentration of \( n \)-alkanes relative to the regular isoprenoids is also particularly low. The concentration of extracted bitumen is also relatively low, as is witnessed by the higher concentration of internal standard relative to the \( n \)-alkanes and isoprenoids. This observation is in contrast to the previous two patterns, which exhibited much higher yields of bitumen. The third GC-pattern is derived from samples composed of high concentrations of anhydrite with very little input of dolomite or siliciclastics.

The first two patterns correspond to the Group 2 bitumens described in Chapter 4. The third pattern corresponds to the Group 3 bitumen.

### 5.3.2 Bulk characteristics and saturated compound classes of biomarkers

This section provides a detailed description of the bulk characteristics and saturated compound classes derived from the Gillen Member evaporites. The major focus is on the pattern and distribution of aliphatic (\( n \)-alkanes, mono- and dimethylalkanes acyclic isoprenoids) compounds detected in these samples. The results of full-scan and Metastable Reaction Monitoring (MRM) analyses of hopanes and steranes will also be described.

#### 5.3.2.2 Bulk characteristics

Bulk characteristics are summarized in Table 5.1. The analyzed samples consist of a variety of lithologies and kerogen contents, with total organic carbon (TOC) contents ranging from 0.1 to 0.5%. Total yields of extracted saturated and aromatic hydrocarbons were measured in ppm (\( \mu g/g \) of rock) and approximated by
integration of total GC-MS signals using 18-MEME as the internal standard. Gravimetric analyses were avoided, since solvent removal causes loss of lighter hydrocarbons and residual solvent in bitumen causes large systematic errors in the submilligram range (Brocks et al. 2003a). A weak negative correlation ($R^2 = 0.4$) was noted between increasing amounts of anhydrite and declining total saturate yields (Figure 5.2A), while a corresponding positive correlation ($R^2 = 0.4$) was observed between increasing amounts dolomite and total saturate yields (Figure 5.2B). Sample 08r009 (from 1654.5 m depth) was an exception to this trend, yielding the highest amounts of TOC and total saturate yields (Table 5.1; Figure 5.2C) and did not conform to the correlations observed in Figure 5.2A,B. All samples recorded saturate to aromatic ratios >1.

Figure 5.1 Total ion chromatograms (TIC) of representative saturate fractions from Gillen Member evaporites composed of A) predominantly dolomite (77%) (08r006; 1655 m depth), B) a mixture of anhydrite (76.8%) and dolomite (15%) (08r008; 1654 m depth), C) predominantly anhydrite (90%) (08r022; 1650.8 m depth). Black = n-alkanes; green = monomethylalkanes; red = dimethylalkanes; light blue = regular, head-to-tail isoprenoids; brown = squalane; pink = irregular, head-to-tail isoprenoids. IS= internal standard.
Table 5.1: Bulk maturity and macrofossil characteristics of nominated Glen Member samples.
5.3.2.3 Unresolved complex mixture (UCM)
Most Gillen Member bitumens feature high concentrations of UCMs (Figure 5.1). However, the concentration of these unresolvable mixtures varies and is most prominent in samples with high concentrations of dolomite. Anhydrite-rich samples, in turn, do not feature such high concentrations.

5.3.2.4 n-alkanes
The most abundant compounds in each Gillen Member sample are the $n$-alkanes. Homologues with chain length from 9 to 37 carbon atoms were detected under full-scan GC-MS conditions (Figure 5.1). The $n$-alkane distribution is commonly unimodal and centered on $C_{14}$ or $C_{15}$.

A feature apparent in most samples was the elevated concentration of $n$-C$_{17}$ relative to $n$-C$_{16}$ and $n$-C$_{18}$. Separation of $n$-alkanes from isoprenoids through silicalite adduction (technique explained in section 6.2.5, Chapter 6) indicates that the elevated concentration of $n$-C$_{17}$ is genuine and not a product of co-elution with pristane or other compounds.

5.3.2.5 Cyclohexylalkanes
Alkanes possessing a terminal cyclohexyl ring were detected in low concentrations in all Gillen Member bitumens and feature distributions similar to the $n$-alkane profile. Samples with elevated $n$-C$_{17}$ concentrations also have correspondingly high C$_{17}$ cyclohexylalkanes.
Figure 5.2 Relationship between saturated hydrocarbon yield and increasing amounts of A) anhydrite or B) and C) dolomite (in weight %). Sample 08r009 (1654.5 m depth), which acts as an outlier, is only shown C).
5.3.2.6 *Mono- and Dimethylalkanes*

Monomethylalkanes with the same mass range as that of the *n*-alkanes were detected in all Gillen Member samples (Figure 5.1). The dominating isomers in each monomethyl cluster are the 3-methyl (anteiso-) and 2-methyl- (iso-) alkanes. Centrally branched isomers (especially >C$_5$) closely elute and form elevated peaks.

A marked feature of most Gillen Member samples is the elevated concentration of these monomethylalkanes relative to their *n*-alkane counterparts. This pattern was largely observed in samples that were quite rich in dolomite. In samples predominantly composed of anhydrite, this pattern of elevated relative concentrations of monomethylalkanes was not always observed (Figure 5.1C).

Dimethylalkanes are also observed in these samples. This inference was based on published elution positions (e.g. Kissin 1987; Kenig et al. 1995a) and mass spectra. These molecules are particularly prominent up to C$_{17}$, but sharply decline at higher carbon numbers. Dimethylalkanes were detectable at least up to ~C$_{20}$.

5.3.2.6 *Acyclic Isoprenoids*

Various regular and irregular acyclic isoprenoids were detected in the Gillen Member evaporites. The irregular variants have tail-to-tail and head-to-head structures.

5.3.2.6.1 Regular, head-to-tail isoprenoids

Homologous series of regular isoprenoids were detected at various relative concentrations in all samples. Their identification is based on mass spectra and relative elution positions. These molecules were detected at *m/z* 183 and range from C$_{14}$ to C$_{25}$. The relative concentrations of these molecules, particularly the C$_{25}$ homologue, vary with host rock mineralogy (section 5.3.6). While regular isoprenoids >C$_{26}$ were also detected, they are deemed to be contaminants (see Chapter 4).
Pristane ($C_{19}$) vs. phytane ($C_{20}$) ratios ranged from 1.1 to 3.9 (Table 5.2). Some samples had to be excluded from these measurements since intense co-elution with other molecules resulted in analytical uncertainty. All pristane/phytane ratios were measured on silicalite adducted samples.

![Figure 5.3 Partial m/z 183 mass chromatogram showing the distribution of head-to-tail isoprenoids (with carbon numbers) in 08r022 (1650.8 m depth). Pr = pristane; Ph = phytane. Unlabeled peaks are largely n-alkanes, monomethylalkanes and irregular isoprenoids.](image)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Depth</th>
<th>Pristane/Phytane ratio</th>
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<tbody>
<tr>
<td>07r011</td>
<td>1566 m</td>
<td>1.8</td>
</tr>
<tr>
<td>08r022</td>
<td>1650.8 m</td>
<td>3.9</td>
</tr>
<tr>
<td>08r011</td>
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<td>1.1</td>
</tr>
<tr>
<td>09r001</td>
<td>1652 m</td>
<td>1.4</td>
</tr>
<tr>
<td>08r008</td>
<td>1654 m</td>
<td>2.0</td>
</tr>
<tr>
<td>08r009</td>
<td>1654.5 m</td>
<td>1.9</td>
</tr>
<tr>
<td>07r013</td>
<td>2115 m</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Regular isoprenoids would range to $C_{25}$. Therefore, homologues from $C_{21}$ to $C_{24}$ are probably breakdown products this molecule. In most samples, the $C_{24}$ homologue would have a higher relative concentration than $C_{25}$. To test if the breakdown of $C_{25}$ is related to the process that also result in the putative oxidative/reductive formation of pristane and phytane (e.g. Peters et al. 2005b), the
C$_{24}$/C$_{25}$ isoprenoids are plotted against pristane/phytane in Figure 5.4. The result yields a strong correlation ($R^2 = 0.92$).

5.3.2.6.2 Irregular, tail-to-tail isoprenoids

Close inspection of the phytane elution region of numerous Gillen Member samples have resulted in the identification of the irregular tail-to-tail linked C$_{20}$ isoprenoid crocetane (2,6,11,15-tetramethylhexadecane). This identification was based on comparing the characteristic GC elution and MS spectra of this molecule with synthetic crocetane (provided by Steven Rowland, University of Plymouth) (Figure 5.5 and 5.6) and the Gillen Member. Co-injection experiments were also conducted (Figure 5.7).

Subtraction of the ions at $m/z$ 169-197 in a Gillen Member evaporite resulted in a peak with the same GC elution position as that observed for the pure crocetane at $m/z$ 169 (Figure 5.7). Co-injection of crocetane with a saturate fraction of the Gillen Member resulted in a doubling of the $m/z$ 169-197 peak, confirming the presence of crocetane in the Neoproterozoic.
Figure 5.5 Partial mass chromatograms at \( m/z \) 169 indicating the presence of crocetane in Gillen Member evaporite 09r001 (1652 m depth). A) Elution position of synthetic crocetane (Cr). B) Phytane elution region in the Gillen Member evaporite. C) Position of crocetane (Cr) after co-injection of synthetic crocetane with the Gillen Member sample. Ph = phytane.

Inspection of the regular \( i\)-C\(_{25}\) elution region of numerous Gillen Member samples resulted in the identification of the irregular tail-to-tail linked C\(_{25}\) isoprenoid 2,6,10,15,19-pentamethylicosane (hereafter referred to as PMI). The identification was based on comparing the characteristic GC elution (Figure 5.8) and MS spectra (Figure 5.9) of this molecule with PMI from a Miocene hydrocarbon seep site in New Zealand (Campbell et al. 2008; PMI sample provided by Emmanuelle Grosjean, Geoscience Australia). Co-injection experiments were also conducted (Figure 5.10).
Figure 5.6 Mass spectral comparisons of pure crocetane with crocetane and phytane from Gillen Member evaporite 09r001 (1652 m depth). A) Synthetic crocetane. B) Corresponding elution position in the Gillen Member after background subtraction. C) Phytane from the Gillen Member after background subtraction.
Figure 5.7 Partial mass chromatograms showing a comparison of the elution position of A) crocetane from Gillen Member evaporite 09r001 (1652 m depth) at \( m/z \) 169 with B) the subtraction of ions at \( m/z \) 169-197 in the same Gillen Member sample and C) a co-injected mix of pure crocetane and the same Gillen Member sample as above.

Figure 5.8 Partial mass chromatograms at \( m/z \) 183 indicating the presence of 2,6,10,15,19-pentamethylicosane (PMI) in Gillen Member evaporite 08r008 (1654 m depth). A) PMI from a New Zealand hydrocarbon seep carbonate. B) PMI elution region in the Gillen Member, showing a peak at similar position as in A). Note that the Gillen Member peak co-elutes with regular \( i-C_{25} \).
Figure 5.9 Mass spectral comparisons of pure C_{25} isoprenoids with Gillen Member equivalents from 08r008 (1654 m depth). A) Comparison of regular i-C_{25} standard with B) Gillen Member equivalent after background subtraction. C) Comparison of PMI from a NZ hydrocarbon seep with D) Gillen Member equivalent after background subtraction.
Figure 5.10 Partial mass chromatograms indicating the presence of the regular \( i-C_{25} \) isoprenoid and PMI in Gillen Member evaporite 08r008 (1654 m depth). A) Elution position (blue line) of regular \( i-C_{25} \) standard compared with that from B) the Gillen Member at \( m/z \) 253. C) Elution position (red line) of PMI from the Gillen Member with that from D) the same Gillen Member evaporite that was co-injected with PMI at \( m/z \) 267.

A \( C_{30} \) tail-to-tail isoprenoid, identified as squalane, was also detected in variable concentrations in most Gillen Member samples. Identification of this molecule, at \( m/z \) 183, was based on mass spectra (Figure 5.11) and predicted elution position. The relative concentrations of this molecule would vary with respect to host rock mineralogy and this result is outlined in section 5.3.6.

Visual inspection of GC-MS data from various Gillen Member samples indicates that both squalane and the regular \( i-C_{25} \) isoprenoid tend to co-occur. To investigate this relationship quantitatively, the relative concentrations of these two compounds were plotted together. Figure 5.12 shows a strong positive correlation \((R^2 = 0.90)\) between these two compounds. Note that the concentration of each of these molecules is expressed relative to the nearest two \( n \)-alkanes of lower and higher molecular weight.
Figure 5.11 Mass spectra of the C$_{30}$ tail-to-tail isoprenoid squalane from 08r008 (1654 m depth). Background subtracted.

Figure 5.12 Relationship between the relative concentrations of squalane and regular $i$-$C_{25}$
5.3.2.6.3 Irregular, head-to-head isoprenoids

Head-to-head isoprenoids have been detected in most Gillen Member samples. In order to confirm that these isoprenoids belong to the head-to-head group, the GC elution positions, and MS spectra have been compared with the saturate fraction of a Miocene crude oil from the San Joaquin Basin in California, USA. The saturate fraction from this crude oil was provided by J.M. Moldowan (Stanford University, USA). The head-to-head-isoprenoids in this oil have been previously confirmed by comparing it with an authentic head-to-head \( i-C_{19} - i-C_{19} \) isoprenoid standard (Moldowan and Seifert 1979).

Figure 5.13 compares the GC profiles of the saturate fractions from the Gillen Member and the Californian crude oil. The positions of individual head-to-head isoprenoid peaks assigned in Moldowan and Seifert (1979, their figure 1) match those present in the Gillen Member. Diagnostic MS ion doublets for this type of isoprenoid have been elucidated by Moldowan and Seifert (1979) using authentic standards and are observed at m/z 112-113, 182-183, 252-253, 308-309, 378-379, and 448-449. These ions match those observed in the Gillen Member evaporites (not shown), proving their presence in these samples.

![Figure 5.13 Partial mass chromatogram at m/z 183 showing the distribution of head-to-head isoprenoids in A) Miocene crude oil from California and B) 08r008 (1654 m depth). Unlabeled peaks are \( n \)-alkanes and regular isoprenoids.](image)
To investigate if the head-to-head $i$-C$_{37}$ correlates with the regular $i$-C$_{25}$ and squalane, their relative concentrations were plotted. Figure 5.14 shows positive relationships between the $i$-C$_{37}$ and regular $i$-C$_{25}$ ($R^2 = 0.67$) and squalane ($R^2 = 0.46$).

![Figure 5.14 Relationships between the relative concentrations of head-to-head $i$-C$_{37}$ and A) regular $i$-C$_{25}$ and B) squalane.](image)

**5.3.2.7 Hopanes and steranes**

No indigenous hopanes and steranes were detected in any of the Gillen Member samples. Exterior/interior experiments conducted on the drill core samples have
shown that while these molecules are present (in full-scan and MRM), they are not indigenous to the rocks (see Chapter 4).

### 5.3.3 Diamondoids

Adamantanes ($C_nH_{2n-4}$ series) and diamantanes ($C_nH_{2n-8}$ series) have been detected in all samples. Identification of these compounds was based on the mass spectra and elution profiles presented in Wingert (1992) and Chen et al. (1996). Collectively, these two groups of molecules are known as diamondoids and are the products of rearrangement of polycyclic compounds due to thermal stress under the presence of strong Lewis acids (e.g. Chen et al. 1996; Peters et al. 2005a). While diamondoids have no direct biological precursors, they are of interest here in evaluating the maturity of the rock samples (Chen et al. 1996). The methyl adamantane (MA) index $(1-MA/(1-MA + 2-MA) (%)$) and the methyl diamantane (MD) index $(4-MD/(1-MD + 3-MD + 4-MD) (%)$) were used to evaluate the maturity of the Gillen Member samples. The methyl adamantane indices ranged from 52 to 69%, while the methyl diamantane indices ranged from 33 to 49% (Table 5.1). These results correspond to a vitrinite reflectance value ($R_o$) of 1.1 to 1.6 (Chen et al. 1996), indicating that they are mature to overmature.

### 5.3.4 Aromatic compounds and maturity values deduced from them

A series of aromatic hydrocarbons and dibenzothiophenes have been detected in all Gillen Member samples. However, no aromatic molecules of biomarker value (e.g. aromatic steranes or aryl isoprenoids) were detected. Nevertheless, maturity values were discerned and are discussed below.

#### 5.3.4.1 Aromatic maturity values

Various aromatic compounds were used in this study as maturity indicators. Their application is due to ample evidence that ratios, of benzo- and dibenzothiophenes (Ho et al. 1974; Hughes 1984), phenanthrenes (Radke et al. 1982a; Radke and Welte 1983) and naphthalenes (Alexander et al. 1984, 1985) change in a regular manner with increasing maturity (Radke et al. 1986). Hence, ratios of such
compounds have been frequently used in assessing maturity of source rocks and petroleum (e.g. Radke et al. 1982a; Brocks et al. 2003a). At first, these maturity trends were observed in crude oils, sedimentary rocks and coals within the oil generation window, but were later also found applicable to lower maturity levels (Boreham et al. 1988).

A variety of aromatic maturity indicators have been measured for the Gillen Member evaporites in this study: the methylphenanthrene index (MPI-1); the methylphenanthrene ratio (MPR); the methylphenanthrene distribution factor (MPDF); the dimethylnaphthalene ratio (DNR-1), the methyldibenzothiophene ratio (MDR); and the trimethylnaphtalene ratio (TMR). The equations of these maturity indicators and their results pertaining to the Gillen Member samples are listed in Table 5.1.

Equations 3 and 5 in Boreham et al. (1988) were used to estimate vitrinite reflectance equivalents ($R_c$) from MPDF and MPI-1. When applied to the Gillen Member evaporites, $R_c$ ranges from 0.8 to 1.7%, which correspond to a mature to overmature stage. However, some caution needs applied when interpreting MPI-1 values. These values appear to reach their maximum at a vitrinite reflectance equivalent of ~1.7% and then decrease with higher maturities (Boreham et al. 1988; Radke et al. 1982a). This reversal is related to demethylation of methylphenanthenes to phenanthrene. Therefore, low MPI-1 values can be indicative of immature and highly mature bitumens. However, reversed MPI-1 values can be recognized by high phenanthrene/methylphenanthrene ratios (Phen/MP >1; Brocks et al. 2003a). In this study, phenanthrene/methylphenanthrene ratios range from 0.1 to 0.28, indicating that such a reversal did not occur in the Gillen Member samples. Another factor that can influence MPI-1 and MPDF values is the kinetically controlled methylation of phenanthrene (Alexander et al. 1995). This process occurs at low temperatures when phenanthrene reacts with methyl donors to yield predominantly 9-methylphenanthrene. Therefore, bitumen with high relative concentrations of phenanthrene might also contain high relative concentrations of 9-
methylphenanthrene, resulting in 9-MP/1-MP ratios >1 and consequently lower MPI-1 and MPDF values (Alexander et al. 1995; Brocks et al. 2003a). In the Gillen Member samples, the 9-MP/1-MP ratios ranged from 1.7 to 2.1, indicating that such kinetically controlled methylation of phenanthrene may have occurred. Therefore, MPI-1 and MPDF values may yield inflated maturity values.

An alternative measurement that may be applied is the MPR, which is less affected by methylation reactions (Brocks et al. 2003a). Indeed, MPR might be the most reliable phenanthrene parameter for overmature bitumens (Brocks et al. 2003a). The Gillen Member samples yielded MPR values ranging from 1.2 to 2.25. However, this value also corresponds to a late stage of petroleum generation (see Killops and Killops 2005, their figure 5.47) and therefore is in agreement with the MPI-1 and MPDF values. These results are also in agreement with the DNR-1 and MDR values, which also correspond to a late stage of petroleum generation (Radke 1988; Killops and Killops 2005).

6.3.5 ROCK EVAL, hydrogen and oxygen indices

Gillen Member samples were submitted for ROCK EVAL pyrolysis to investigate maturity (Table 5.1). Previous studies have shown that $T_{\text{max}}$ values (see Table 5.1 for definition) measured during pyrolysis are closely related to the thermal history of organic matter (Tissot and Welte 1984). However, only 08r009 (1654.5 m depth) contained enough organic matter for ROCK EVAL analysis. The $T_{\text{max}}$ value for this sample was 424°C, indicating a maturity close to the entrance of the oil generation window (i.e. early mature; Peters et al. 2005a) if Phanerozoic standards are applied. However, when compared to diamondoid and aromatic maturity indicators (see above) and organic matter from other Precambrian successions (e.g. mid-Proterozoic Barney Creek Formation, northern Australia), the Gillen Member samples may rather fall into the later stages of oil generation. In drill core GR7 of the Barney Creek Formation, $T_{\text{max}}$ values are 436 to 442 °C in the depth range of 39 to 524 m (Powell et al. 1987). In this range, triaromatic steranes and hopanes are preserved to a depth of 427 meters. If the organic matter and ROCK EVAL data of the Gillen Member is comparable to the Barney Creek Formation,
this would indicate a maturity range consistent with the preservation of polycyclic aromatic and saturated biomarkers.

Hydrogen (HI = S2/TOC) and oxygen (OI = S3/TOC) indices record considerably low values (0-50 and 10-50, respectively; Table 5.1), and would plot close to the origin on a pseudo-van Krevelen diagram (e.g. Tissot and Welte 1984; Peters et al. 2005a; Dembicki Jr. 2009). Such low values could be indicative of very high maturity and pose a contradiction to the less mature indicators discussed above. However, it should be noted that the low TOC in these samples is likely giving rise to significantly depressed O and H indices. In sediments with TOC contents of <2%, hydrocarbon retention on mineral grains can result in significantly reduced HI values (e.g. Espitalie et al. 1980; Katz et al. 1983; Dembicki Jr. 2009). Therefore, H and O indices of the Gillen Member samples are likely unreliable.

5.3.6 Quantitative differences in biomarker pattern between dolomite and anhydrite

As mentioned section 5.3.1, significant differences occur in the biomarker distributions between dolomite and anhydrite. These differences were detected by a) selecting samples that are predominantly composed of dolomite or anhydrite (as confirmed by XRPD) (Figure 5.1); and b) plotting concentration differences of biomarkers relative to mineral percentages that were determined through quantitative XRPD (using Siroquant V3).

The combined application of biomarker analyses and quantitative XRPD has been particularly useful in providing insights into the sensitivity of certain biomarkers to increasing saline conditions. In this study, an increase in salinity is defined by a higher concentration (in weight percent) of anhydrite. Lower salinity, in turn, is defined by a higher concentration (in weight percent) of other minerals, especially dolomite. See Chapter 2 for a more detailed discussion on the precipitation conditions of these minerals.

This study investigated the concentration of a number of biomarkers that featured prominently in the mass chromatograms: \( n \)-heptadecane (\( n \)-C\(_{17} \)); the
head-to-tail isoprenoids pristane \((i-C_{19})\) and phytane \((i-C_{20})\) and the regular pseudohomologue with 25 carbon atoms \((i-C_{25})\); the tail-to-tail isoprenoid squalane \((i-C_{30})\); a head-to-head isoprenoid with 37 carbon atoms \((i-C_{37})\); a likely breakdown product of biphytane). The effect of increasing salinity on their concentration is discussed below. Due to the irregular nature of \(n-C_{17}\) concentrations, pristane concentrations were normalized to \(n-C_{18}\). All \(n\)-alkanes where quantified at \(m/z\) 85 and isoprenoids at \(m/z\) 183.

Relative \(n-C_{17}\) concentrations display a weak positive correlation with increasing amounts of anhydrite \((R^2 = 0.29); \text{ Figure } 5.15A\). A similarly weak negative correlation exists for increasing amounts of dolomite \((R^2 = 0.30); \text{ Figure } 5.15B\).

Relative pristane concentrations did not yield any correlations between anhydrite \((R^2 = 0.16)\) or dolomite \((R^2 = 0.19); \text{ Figure } 5.16\). Likewise, no correlations were observed between phytane and anhydrite \((R^2 = 0.16)\) or dolomite \((R^2 = 0.19); \text{ Figure } 5.17\).

A strong positive correlation was observed between the regular \(C_{25}\) isoprenoid and increasing amounts of anhydrite \((R^2 = 0.85); \text{ Figure } 5.18A\). This trend was matched by a negative correlation of this isoprenoid with increasing amounts of dolomite \((R^2 = 0.54); \text{ Figure } 5.18B\).

The irregular \(C_{30}\) isoprenoid squalane shows a similar trend as \(i-C_{25}\). A strong positive correlation was observed between increasing squalane concentrations and anhydrite \((R^2 = 0.79); \text{ Figure } 5.19A\). A weak negative correlation, in turn, was observed with increasing amounts of dolomite \((R^2 = 0.39); \text{ Figure } 5.19B\).

No correlations were observed between the head-to-head \(C_{37}\) isoprenoid and anhydrite \((R^2 = 0.07); \text{ Figure } 5.20A\) or dolomite \((R^2 = 0.005); \text{ Figure } 5.20B\).
5.3.7 Semiquantitative assessment of (crocetane) and 2,6,10,15,19-pentamethyleicosane (PMI)

Differences in the amounts of crocetane and PMI were estimated between samples. Since both of these isoprenoids co-elute with other isoprenoids (see section 5.3.2), only a semiquantitative approach was employed.

The semiquantitative approach applied in this study relied on characteristic differences in mass spectral fragmentations for each compound. Crocetane, for instance, yields particularly strong fragments with $m/z$ 169 (Figure 5.6). While the co-eluting phytane also yields ions at this mass to charge ratio, the probability that such fragments are generated by this molecule is much lower. Therefore,
depending on its concentration, crocetane should contribute a significant proportion of the aforementioned ion. Phytane, by contrast, yields ions at $m/z$ 183, which are not produced by crocetane (Figure 5.6). Based on these observations, relative intensity differences between prominent ions produced by crocetane ($m/z$ 169) and the $m/z$ 183 ions derived from phytane should provide an estimation of relative concentration differences between these two co-eluting molecules.

![Figure 5.16 Relationships between the relative concentrations of pristane ($i$-C$_{19}$) and increasing amounts of A) anhydrite and B) dolomite (in weight %).](image)

Relative concentration differences between Gillen Member samples were also measured for PMI and co-eluting regular $i$-C$_{25}$. PMI yields strong ions at $m/z$ 239 (Figure 5.9). The co-eluting regular $i$-C$_{25}$, in turn, created prominent ions at $m/z$ 253, which is low in PMI (Figure 5.9). Therefore, relative intensity differences between these ions (i.e. 239 for PMI and 253 for regular $i$-C$_{25}$) should also provide
an estimation of relative concentration differences between these co-eluting molecules.

All semiquantitative measurements were conducted by investigating the combined signal height of the co-eluting peaks at $m/z$ 183. Heights of the ions of interest were measured and plotted.

Ratios of intensity differences between ions at $m/z$ 169 over 183 from a variety of Gillen Member samples were measured. However, these measurements demonstrate no correlation between concentration differences of crocetane and the mineralogy (i.e. anhydrite or dolomite) of the host rocks (Figure 5.21).

Similar trends were also observed when plotting intensity differences between ions at $m/z$ 239 over 253 from a variety of Gillen Member samples. The plots also do not suggest that PMI concentrations correlate with host rock mineralogy (Figure 5.22).

In order to investigate if crocetane correlates with PMI and regular $i$-C$_{25}$, their relative concentrations were plotted as shown in Figure 5.23. The results show that crocetane correlates positively with PMI ($R^2 = 0.93$) and negatively ($R^2 = 0.51$) with regular $i$-C$_{25}$.

Correlations of crocetane and PMI with squalane and the irregular, head-to-head $i$-C$_{37}$ were also measured. However the results did not yield any correlations (data not shown).

5.4 Discussion

5.4.1 Organic matter enclosed in Gillen Member evaporites

The amount of bitumen extracted from the Gillen Member anhydrites was lower than in the dolomitic samples (section 5.3.2.2). TOC values for the Gillen Member were also generally lower for anhydrite-rich samples than those predominantly composed of dolomite (Table 5.1). This observation is consistent with data published from other anhydrites, were TOC is consistently lean (Hite and Anders 1991 and references therein). The dewatering of the precursor mineral gypsum into anhydrite has been regarded as a reason for the low organic content in these
deposits (Warren 1986; Hite and Anders 1991). Because gypsum consists of 21% water, it is hypothesized that a considerable amount of water and hydrocarbons could be emitted from these deposits as the critical thickness of overburden and temperature is reached (Warren 1986; Hite and Anders 1991). However, this hydrocarbon expulsion scenario depends on the timing of water loss and the generation of hydrocarbons in this type of deposit (Warren 1986; Hite and Anders 1991). Another explanation for the difference in extract yields/TOC between anhydrite and dolomite is that the former did not enclose a significant biomass. Indeed, as pointed out by Gerdes et al. (2000), a rise in salinity will result in an increase in gypsum deposition that is concomitant with a decline in microbial activity. The lower salinity levels encountered during dolomite precipitation, in turn, would enable a more significant biomass to be established.

![Figure 5.17](image)

**Figure 5.17** Relationships between the relative concentrations of phytane (i-C_{20}) and increasing amounts of A) anhydrite and B) dolomite (in weight %).
Figure 5.18 Relationships between the relative concentrations of regular i-C_{25} and increasing amounts (in weight %) of A) anhydrite and B) dolomite.

5.4.2 Hydrocarbons from Gillen Member evaporites and their biomarker information

5.4.2.1 n-alkanes, especially n-heptadecane (n-C_{17})

The n-alkanes are usually the most abundant hydrocarbons in nonbiodegraded oils and mature bitumens (Brocks and Summons 2004). In the Neoproterozoic, these molecules would likely be derived from various straight-chain molecules such as phospho- and sphingolipids produced from bacteria and algae (Brocks and Summons 2004). The lack of steranes in the Gillen Member (see discussion in section 5.4.3), may indicate that bacteria were the principal source for the n-alkanes.
While most samples from the Gillen Member record high relative concentrations of \( n \)-alkanes over isoprenoids (Figure 5.1A,B), bitumens from the anhydrite can display the opposite trend (Figure 5.1C). While the latter can be explained through biodegradation of \( n \)-alkanes, the bitumen from this anhydrite phase does not yield a high UCM, which is indicative of this destructive process (e.g. Peters et al. 2005b). Therefore, the low concentration of \( n \)-alkanes relative to isoprenoids in the anhydrite may have an ecological explanation; a lesser abundance of microorganisms producing \( n \)-alkanes or their precursors under more saline conditions and/or a greater abundance of isoprenoid-producing organisms.

A prominent feature of most Gillen Member bitumens is the elevated concentration of \( n \)-C\(_{17} \). This straight-chain alkane appears to be slightly more elevated in anhydrite-rich samples than in dolomitic samples (Figure 5.15).

The elevated concentration of \( n \)-C\(_{17} \) in the Gillen Member could have been derived from a precursor fatty acid or \( n \)-alcohol that was present in the organism(s) at that time. In modern environments, fatty acids and \( n \)-alcohols from bacteria and lower plants exhibit an even over odd predominance (e.g. Rezanka and Sigler 2009). Indeed, octadecanoic acid, a potential precursor of \( n \)-C\(_{17} \), is a very common fatty acid in almost all modern environments (e.g. Wood et al. 1965). An alternative precursor could have been elevated concentrations of heptadecanoic acid or heptadecanol. However, with increasing thermal maturation, fatty acids, \( n \)-alcohols and their functionalized diagenetic products are heavily cracked and reshuffled, commonly leaving no trace of the \( n \)-alkane carbon number preference (e.g. Bray and Evans 1961; Peters et al. 2005b). On the other hand, saturated and unfunctionalized \( n \)-alkanes are likely more inert to cracking and rearrangement reactions. Therefore, the most likely precursor for the elevated \( n \)-C\(_{17} \) would have been biologically-produced \( n \)-C\(_{17} \) itself.

Numerous studies detected varying but elevated \( n \)-C\(_{17} \) concentrations in pure cultures of cyanobacteria (Han et al. 1968; Han and Calvin 1969; Gelpi et al. 1970; Paoletti et al. 1976), as well as in cyanobacteria-dominated mats from a variety of different environments (e.g. Grimalt et al. 1992; Thiel et al. 1997; Bühring et al. 1997).
2009). Indeed, an elevated concentration of \( n-C_{17} \) is commonly regarded as a cyanobacterial marker, although some algae have also been shown to produce this alkane in high concentrations (e.g. Tulliez et al. 1975; Řezanka et al. 1982).

![Figure 5.19 Relationships between the relative concentrations of irregular i-C_{30} (squalane) and increasing amounts (in weight %) of A) anhydrite and B) dolomite.](image)

5.4.2.2 Presence of cyclohexylalkanes

Low concentrations of cyclohexylalkanes have been previously reported in bitumens and petroleums from the Proterozoic and Phanerozoic (Brocks et al. 2003b). It is conceivable that cyclohexylalkanes could have a direct biologic source in the uncommon \( \omega \)-cyclohexyl fatty acids isolated from some bacteria (Suzuki et al. 1981). However, the close affinity of the \( n \)-alkane and cyclohexylalkane homologue series in the Gillen Member bitumens points to postdepositional cyclization of straight chain lipids as the likely source (Fowler et al. 1986; Hoffmann...
et al. 1987; Summons et al. 1988a). For that reason, the cyclohexylalkanes probably have the same precursor as the $n$-alkanes.

Figure 5.20 Relationships between the relative concentrations of irregular $i$-$C_{37}$ isoprenoids and increasing amounts (in weight %) of A) anhydrite and B) dolomite.

5.4.2.3 High relative concentrations of monomethyl- and dimethylalkanes

Another outstanding feature of the Gillen Member bitumens is the high monomethyl- and dimethylalkane concentrations relative to their $n$-alkane equivalents. Such a pattern is frequently observed in bitumens extracted from Precambrian and Cambrian samples, such as from the Mesoproterozoic McArthur Basin in northern Australia (Summons et al. 1988), in Neoproterozoic oils from eastern Siberia (Fowler and Douglas 1987), the Neoproterozoic to Cambrian Huqf Formation of Oman (Klomp 1986; Höld et al. 1999) and the early Cambrian
Chandler Formation in central Australia (Summons 1987). However, while often observed in Precambrian/Cambrian rock samples, such patterns are rarely observed in the post-Cambrian. Finding a precise source for these high concentrations of dimethyl- and monomethylalkanes has not yet been achieved, and a number of biogenic precursors as well as abiogenic factors can be considered (e.g. Kissin 1987; Summons 1987; Summons and Walter 1990; Kenig 2000).

![Figure 5.21](image)

**Figure 5.21** Relationships between the relative concentrations of crocetane (m/z 169/183) and increasing amounts (in weight %) of A) anhydrite and B) dolomite.

Currently, the most preferred interpretation is that these molecules originate from cyanobacterial mats. This preference is due to the extraction of a suite of
monomethylalkanes from modern and Holocene-age cyanobacterial mats in hot springs (e.g. Shiea et al. 1990) and hypersaline coastal plains (sabkhas) (e.g. Kenig et al. 1995a). Indeed, cyanobacteria are the only organisms known to produce mid-chain branched monomethylalkanes (e.g. 4- to 8-methylalkanes) and have not been detected in areas where these organisms are absent (Shiea et al. 1990 and references therein).

While it may be tempting to associate the Precambrian/Cambrian monomethyl- and dimethylalkanes with cyanobacteria, contrasts need to be highlighted. Importantly, modern cyanobacterial cultures and mats generally contain monomethylalkanes with a carbon range from C$_{17}$ to C$_{21}$ (e.g. Kenig 2000), which contrast with those obtained from this and other Precambrian/Cambrian studies where a range of homologues from C$_{10}$ to C$_{33}$ was observed (e.g. Jackson et al. 1986; Fowler and Douglas 1987; Summons et al. 1988a,b). This discrepancy led Fowler and Douglas (1987) to suggest that the source organisms for these elevated monomethyls may have become extinct. However, Kenig (2000) obtained a homologous series of monomethylalkanes ranging from C$_{17}$ to C$_{29}$ by pyrolyzing a Holocene-age cyanobacteria-dominated microbial mat from a sabkha environment. Therefore, such molecules are still produced today.

While cyanobacteria are often held as the most likely source for mono- and dimethylalkanes, other sources could potentially provide an important contribution. For example, while the pyrolysis experiments on the sabkha microbial mats conducted by Kenig (2000) may have had a cyanobacterial input, other microbes are likely to have been present. This was acknowledged by Kenig (2000), who recommended further pyrolysis experiments on monospecific cultures of cyanobacteria as well as other microbes commonly noted in such mats. Other microorganisms that could contribute to the monomethylalkane repertoire include the sulfate reducers, which are known to produce a variety of monomethyl fatty acids (e.g. Taylor and Parkes 1983). Sulfate reducing bacteria were likely present in the Gillen Member samples with frambooidal pyrite grains disseminated widely in the dolomitic portions of the evaporites (see Chapter 2). The sulfur isotopic
composition of these pyrite grains is currently being investigated by Dr. David Fike (Washington University in St. Louis). Since sulfate reducers are known to precipitate pyrite, it is quite likely that these bacteria contributed to the monomethylalkane assemblage detected from the Gillen Member. However, it is uncertain if these organisms are the source for all the monomethylalkanes, since the majority of branched fatty acids in such organisms typically have a carbon chain length with relatively lower molecular weight of up to ~C17 (e.g. Taylor and Parkes 1983; Harwood and Russell 1984; Rajendran et al. 1995). Therefore, as with the cyanobacteria, further work needs to be conducted to evaluate the contribution of sulfate reducers to the monomethylalkane repertoire in ancient sediments.

Abiotic processes have also been suggested as potential sources of mono- and dimethylalkanes in ancient sedimentary rocks. For example, Hoering (1981) and Klomp (1986) suggested that monomethylalkanes formed as a result of long-term equilibration of a limited range of isomers. This suggestion is based on the observation that living organisms tend to produce specific isomers of monomethylalkanes, whereas most isomers are present in Precambrian crude oils (Klomp 1986).

Another abiotic process was suggested by Kissin (1987) whereby mono- and dimethylalkanes are produced as a result of acid-catalyzed products of alkenes formed by thermal cracking. Thermolysis experiments of n-alkanes, alkanoic acids and esters in the presence of various minerals resulted in lighter n-alkanes and \( \alpha \)-olefins (Kissin 1987). Kissin (1987) noted that the olefins, in the presence of acidic clays, are converted into mixtures of predominantly monomethyl- and dimethylalkanes. Such results corroborate with recent experimental observations by Alexander et al. (2010), where a range of common sedimentary hydrocarbons (e.g. alkenes and isoprenoid alkanes) were subjected to sealed tube heating experiments on activated carbon between 170 and 340°C. A range of reactions were noted in these experiments and include hydrogen exchange (e.g. double bond isomerization and hydrogenation), heteroatom exchange and carbon
exchange (e.g. dealkylation and transalkylation) (Alexander et al. 2010). Similar reactions may also have given rise to the formation of various mono- and dimethylalkanes.

Abiotic reactions of organic matter are an attractive explanation for the formation of these alkyl-substituted alkanes. It is conceivable that the pyrolysis reactions of cyanobacterial mats by Kenig (2000) resulted in similar reactions as that outlined by Kissin (1987) and Alexander et al. (2010) that changed the makeup of the bitumen. Homologues with carbon numbers above those observed in cyanobacteria could be formed by the joining of various alkyl-substituted alkane chains followed by transalkylation (or reshuffling) reactions that are catalyzed by clay or carbonaceous substances. Since the Precambrian biosphere was believed to have been dominated by cyanobacteria (Knoll et al. 2007), reshuffling processes would have a significant amount of starting material to work on. Subsequent reactions after burial could have resulted in the patterns observed from Precambrian/Cambrian samples. Based on the similarities between the results obtained by Kissin (1987) and the Gillen Member samples, abiotically induced production of alkyl-substituted alkanes is the most favored explanation adopted herein. Further research in this area is likely to shed more light in discerning biotic and/or abiotic factors in the formation of these mono- and dimethylalkanes in ancient rock samples.

5.4.2.4 Presence of pristane and phytane and their relative concentrations
All Gillen Member samples contain the regular, head-to-tail isoprenoids pristane (Pr) and phytane (Ph). These isoprenoids are primarily regarded as a product of chlorophyll-derived phytol degradation and their ratios are commonly used to interpret the redox conditions of ancient depositional environments (e.g. Didyk et al. 1978). This interpretation is based on the observation that reducing or anoxic conditions in sediments promote the conversion of phytol to phytane, while oxic conditions, in turn, promotes the conversion to pristane (see also review by Peters et al. 2005b). Therefore, Pr/Ph ratios <1 are regarded to indicate anoxic conditions,
while ratios >1 are seen as indicative of oxidizing condition. Numerous studies have confirmed these ratios with other redox indicators such as high sulfur content and elevated C\textsubscript{35} homohopane indices (consistent with anoxia; Peters et al. 1995), and relative concentrations of C\textsubscript{27} diasteranes (apparently indicative of oxicity; Moldowan et al. 1986).

While Pr/Ph ratios act as a popular proxy in determining anoxic/oxic conditions, their applicability is complicated by the observation that molecules other than the chlorophyll-derived phytol side chain result in the formation of pristane and phytane. Indeed, as noted by ten Haven et al. (1987), the discovery of these sources greatly weakened the rational for the use of the Pr/Ph ratio. One such source includes tocopherols, a minor constituent of plants and algal membranes, which can result in the formation of pristane (Goossens et al. 1984). Another potential source of pristane are methyltrimethyltridecylchromans (MTTCs) (Li et al. 1995), whose source is currently unknown but believed to be of biological origin (e.g. Sinninghe Damsté et al. 1987; Kenig et al. 1995b). Phytane can also originate from membrane lipids of the Archaea, which are known to contain isoprenoids with a phytanyl moiety (ten Haven et al. 1987). Archaeol (di-O-phytanyl glycerol ether) derived from halophilic Archaea (Orphan et al. 2008) serves as an example. Furthermore, free phytane has been identified as a constituent of one Methanococcus sp. with a variety of dehydrophytenes in other archaeal strains (Tornabene et al. 1979).

In hypersaline environments, phytane is usually attributed to an archaeal source. Jahnke et al. (2008) observed phytane in the surface layer of a modern hypersaline microbial mat in Baja California. However pristane and chlorophyllide a, which are expected breakdown products of oxic chlorophyll degradation, were not detected in that layer. Phytane continued to be detected in the underlying sediment of the mat and was observed to increase with archaeal core lipids. Jahnke et al. (2008) consider these results to indicate that phytane derives from an archaeal source, rather than from phytol degradation.
When phytane concentrations are higher than pristane, low Pr/Ph ratios (<1) will be obtained. Such low ratios are interpreted to be typical of hypersaline environments, where numerous halophiles possess lipids with a phytanyl moiety (ten Haven et al. 1987). Indeed, an inverse relationship has been observed between Pr/Ph ratios and the gammacerane index (Peters et al. 2005b). The gammacerane index has been previously used as an indicator of water column stratification that can result from hypersalinity (Sinninghe Damsté et al. 1995) and higher indices have been shown to result in lower Pr/Ph ratios (Peters et al. 2005b).

While low Pr/Ph ratios appear to be characteristic of hypersaline environments, no such results were obtained in the Gillen Member. All samples from this study had Pr/Ph ratios >1. In a few samples this ratio may have been an artifact of analytical uncertainty due to the co-elution of other compounds with pristane. However, in other cases, no evidence of co-elution was observed. Furthermore, samples with high relative abundances of isoprenoids ≥C_{21}, which are typically regarded as having a halophilic archaeal source (see below), displayed the highest Pr/Ph concentration. Therefore, the contribution of isoprenoids from Archaea was insufficient in causing a Pr/Ph ratio of <1.

It could be argued that the high Pr/Ph ratios of the Gillen Member are a result of high maturity. As noted above, both diamondoid and aromatic maturity indicators suggest that the hydrocarbons from this rock unit have reached a mature to overmature stage. While phytane is often more abundant than pristane in low maturity oils and source-rock extracts (Volkman and Maxwell 1986), Pr/Ph ratios increase in samples with progressively high thermal maturities due to the liberation of pristane or their precursors from kerogen (Connan 1974; Koopmans et al. 1999; Peters et al. 2005b) while Ph/n-C_{18} ratios decrease (ten Haven et al. 1987). However, such Pr/Ph maturity trends are not systematic, with values increasing at first and then declining at higher levels of maturity (e.g. Albrecht et al. 1976; Peters et al. 2005b). A decline in the Pr/Ph maturity trends have been reported at vitrinite reflectance values of between 0.7% (Connan 1984) and 1% R_o (Brooks et al.
In the Gillen Member, diamondoid maturity indices correspond to a vitrinite reflectance value of 1.1 to 1.6% R_0. Such values are well above those where a reversal in Pr/Ph had been observed. Furthermore, different samples yield different Pr/Ph ratios, even if they were derived from similar depths. These results indicate that thermal maturity is unlikely to be a significant cause of the high Pr/Ph values obtained in these samples.

Figure 5.22 Relationships between the relative concentrations of PMI (m/z 239/253) and increasing amounts (in weight %) of A) anhydrite and B) dolomite.

In the Gillen Member samples, the Pr/Ph ratios are largely interpreted to be the result of oxidation of the chlorophyll-derived phytol side chain. This view is supported by a strong positive correlation (R^2 = 0.92) of the regular i-C_{24}/i-C_{25} isoprenoid ratio with Pr/Ph (Figure 5.4). As noted below, the regular i-C_{25}
isoprenoid was most likely derived from either a 2-O-sesterterpanyl-3-O-phytanyl-
\textit{sn}-glycerol (C$_{25}$, C$_{20}$-diether) or a 2,3-di-O-sesterterpanyl-\textit{sn}-glycerol (C$_{25}$, C$_{25}$-
diether) of halophilic archaea. If this was the case, than a similar oxidation of these alcohols to acid could have ensued. A subsequent decarboxylation reaction would have resulted in \(i\)-C$_{24}$. This is the same principle by which pristane would have formed as a result of phytol-side chain oxidation.

Oxic, or at least sub-oxic conditions are required for Pr/Ph ratios >1 (e.g. Peters et al. 2005b). Since anhydrite deposition requires a significant evaporation of shallow water bodies, it is likely that such conditions expose organic matter to greater amounts of oxygen than deposition in a reducing, dolomite precipitating microbial mat (see Chapter 2). The chlorophyll that yielded the phytol side chain could have been derived from the same cyanobacteria that produced the elevated concentrations of \(n\)-C$_{17}$, dimethyl- and monomethylalkanes.
5.4.2.5 Presence of regular, head-to-tail isoprenoids from $C_{21}$ to $C_{25}$

In this study, high relative concentrations of regular isoprenoids from $C_{21}$ to $C_{25}$ have been detected in the Gillen Member. Figure 5.18 shows that a prominence of the $C_{25}$ regular isoprenoid was particularly evident in samples with higher concentration of anhydrite ($R = 0.85$). This would indicate that the precursor of the $C_{25}$ isoprenoid was an important constituent in microorganisms that could withstand higher salt concentrations.

Archaeal lipids are characterized by isoprenoid side-chains, including those with a regular head-to-tail composition (Figure 1.4). Such isoprenoids form the hydrophobic core of cell membranes and impart important physiological functions.
(e.g. Oren 2002; Boucher et al. 2004). The isoprenoids are linked to a polar phosphate head group via ether bonds and impart cellular membrane stability to halophiles exposed to NaCl concentrations of 3-5 M (e.g. Oren 2002; Tenchov et al. 2006). These ether lipids have been estimated to account for 80-95% of archaeal membrane lipids, with the remaining 5-20% consisting of neutral squalenes and other isoprenoids (Sprott 1992). Three types of ether linked isoprenoids have been identified in halophiles: 2,3-di-O-phytanyl-sn-glycerol (C20, C20-diether); 2-O- sesterterpanyl-3-O- phytanyl-sn-glycerol (C25, C20-diether); and 2,3-di-O- sesterterpanyl-sn-glycerol (C25, C25-diether) (e.g. De Rosa et al. 1982, 1983; Kates 1993a; Oren 2002). Some species possess a mixture of these isoprenoids in their membranes. Members of the Halobacteriaceae, for example, have membranes containing 20-carbon and sometimes 25-carbon chains that are bound to glycerol by ether bonds (Oren 2002). The proportion of C20, C20 and C25, C20 core lipids differs among halophilic species (Oren 2002). A regular bilayer of C20,C20 lipids is present in most neutrophilic Archaea (Oren 2002; Tenchov et al. 2006). So-called extreme halophiles (with an optimum growth in 2.5-4.2 mol L⁻¹ NaCl at 35°C) of the Archaea domain are reported to contain significant concentrations of both C20, C25 and C25, C25 regular isoprenoids rather than just the C20 homologue (Patel and Sprott 2006). Such observations are in line with increasing C25 regular isoprenoid concentrations in Gillen Member samples that record higher proportions of anhydrite.

Although C20 regular isoprenoids have also been detected in the Gillen Member samples, it is difficult to estimate the original contribution of this molecule in these deposits. Breakdown products of their C25 counterparts can also result in C20 homologues. Therefore, any discussion on the occurrence of the regular C20 isoprenoids in the Gillen Member needs to be treated with caution.

5.4.2.6 2,6,10,15,19-pentamethylicosane (PMI)

PMI has been positively identified in the evaporitic samples of the Gillen Member. In these evaporites, PMI was detected in both dolomite and anhydrite-rich samples. However, no correlations were obtained (Figure 5.22).
PMI has previously been used as a diagnostic marker for members of the *Archaea* domain and is known to be closely associated with the methane cycle. This compound has been previously detected in microbial mats, sediments and sedimentary rocks associated with hydrocarbon seepage (e.g. Thiel et al. 2001; Birgel et al. 2006, 2008) as well as in gas hydrate settings (Elvert et al. 1999). In these type of environments, PMI exhibits strong isotopic depletion and is associated with the anaerobic oxidation of methane (AOM) that is mediated by consortia of methane-oxidizing archaea and sulfate reducing bacteria (e.g. Elvert et al. 1999; Hinrichs et al. 1999; Boetius et al. 2000; Valentine 2002).

While a vast majority of studies have reported PMI in association with AOM, this compound has also been reported from hypersaline settings. In such environments, PMI is not necessarily derived from methanotrophs, but has been linked with methylotrophic methanogens (Schouten et al. 1997; Summons et al. 1998; Orphan et al. 2008).

The presence of PMI in the Gillen Member evaporates correlates with the occurrence of this molecule in modern hypersaline equivalents. Jahnke et al. (2008) and Orphan et al. (2008) detected an isomeric mixture of PMI (predominantly C$_{25:5}$) in the surface layer of a hypersaline microbial mat in Baja California. Incubation experiments of this mat with methanogenic substrates coupled with combined 16S gene and biomarker analysis links the methylotrophic methanogen *Methanolobus* spp. with the PMI (Orphan et al. 2008). Other studies also link PMI with this methanogen (Schouten et al. 1997; Koga and Morii 2005). These observations led Orphan et al. (2008) to suggest that PMI may be used as an indicator for the presence of methylotrophic methanogenesis in ancient organic-rich sediments.

The discovery of PMI in the Gillen Member provides possible evidence for methanogens in Neoproterozoic hypersaline settings. As is noted in Chapter 6, the PMI in the Gillen Member, like that reported from Baja California (Jahnke et al. 2008; Orphan et al. 2008), does not exhibit a $^{13}$C isotopic depletion characteristic of methane oxidation. Such evidence could further link the Gillen Member PMI to
methylotrophic methanogenesis. Section 5.4.4.3, will examine the case for methylotrophic methanogenesis in the Gillen Member.

Currently, the oldest biomarker evidence for PMI is derived from hydrocarbon seep limestones that dates to the Late Pennsylvanian (300 Ma) (Birgel et al. 2008). The presence of PMI in the Gillen Member extends the oldest date of this molecule by a further 500 Ma.

5.4.2.7 2,6,11,15-tetramethylhexadecane (crocetane)
Crocetane has been identified in a number of the Gillen Member evaporites, although no correlations with host rock lithology were obtained (Figure 5.21). As with PMI, the detection of crocetane in the Gillen Member makes this the oldest reported occurrence of that molecule to date. While crocetane has been previously detected in the 1640 Ma Barney Creek Formation (Greenwood and Summons 2003), this molecule is almost certainly the breakdown product of diaromatic carotenoids (Brocks and Grice 2010). While the Barney Creek Formation contains significant amounts of diaromatic carotenoids (Brocks and Schaeffer 2008), none of these molecules were detected in the aromatic fraction of the Gillen Member evaporites.

Like PMI, crocetane has been an important molecular component in both ancient and modern sites of hydrocarbon seepage (e.g. Thiel et al. 2001; Birgel et al. 2006, 2008) and gas hydrate settings (Elvert et al. 1999). While it is also associated as a diagnostic marker for members of the Archaea domain, the source organism is unknown. Blumenberg et al. (2004) hypothesized that this molecule is associated with methanotrophic ANME-2, which are a phylogenetically distinct group of anaerobic methanotrophs.

Although crocetane has been solely associated with AOM, the molecule has recently been detected in lower parts (100 mm) of modern hypersaline cyanobacterial mats from Guerrero Negro, Baja California (Jahnke et al. 2008; Orphan et al. 2008). Enrichment with trimethylamine (TMA) showed that these parts of the mats produced increased CH₄, while both 16S and functional gene
analyses revealed the presence of diverse archaeal methanogens belonging to the Methanosarcinales (Orphan et al. 2008). In contrast to the crocetane from AOM settings, the hypersaline equivalents did not exhibit any $^{13}$C-depletion and DNA-based phylogenetic analyses did not support a link of this molecule with anaerobic archaeal methanotrophs of ANME-2 (Orphan et al. 2008). As with crocetane from these modern mats, those from the Gillen Member also did not show any $^{13}$C-depletion (see Chapter 6). These findings demonstrate an alternative source for crocetane in hypersaline mat environments. Based on the phylogenetic relatedness between the ANME-2 and the hypersaline methanogens, Orphan et al. (2008) postulated a methanogenic source for the crocetane in their study. Future biomarker studies on modern hypersaline mats, potentially coupled with genome-related investigations, would be needed to resolve the source issue.

5.4.2.8 Presence of biphytane and its breakdown products
An acyclic head-to-head isoprenoid ($i$-C$_{40}$) and various associated breakdown products were detected in most Gillen Member samples. Such compounds are interpreted to have been derived from the C$_{40}$ isoprenoid biphytane, which is considered to be highly specific for the archaea (Peters et al. 2005b). It is typically regarded to originate from $\omega\omega'$-biphytanediol, which is a key component in the di- and tetraether lipids of archaeal membranes (Figure 1.4) (Peters et al. 2005b). These archaeal di- and tetraether lipids are known as biphytanyl glycerol diethers or glycerol dibiphytanyl glycerol tetraethers (GDGTs), respectively (e.g. Sinninghe Damsté et al. 2002b). The breakdown of biphytane typically forms a homologous series of products that consist of a phytane unit ($i$-C$_{20}$) coupled to a lower carbon-number isoprenoid unit in the range of $i$-C$_{11}$ to $i$-C$_{20}$ or a pristane unit ($i$-C$_{19}$) joined to an isoprenoid unit in the range of $i$-C$_{10}$ to $i$-C$_{19}$ (Moldowan and Seifert 1979; Petrov et al. 1990; Stefanova 2000; Peters et al. 2005b).

An alternative to the acyclic variants (also known as caldarchaeol) are those that contain ring(s) in their structure. The rings are composed of either one to eight cyclopentane rings (Hopmans et al. 2000), or, in the case of crenarchaeol, a
cyclohexane ring (Sinninghe Damsté et al. 2002b). Cyclic GDGTs are prevalent in thermophiles (e.g. Kates 1993b) and in planktonic marine types (e.g. DeLong et al. 1998; Sinninghe Damsté et al. 2002a,b). In this study the head-to-head isomers from the Gillen Member were acyclic, while cyclic structures were not detected. Jahnke et al. (2008) made a similar observation in modern hypersaline sediments and interpreted this to indicate that cyclic caldarchaeols may not be essential for Crenarchaeota from such an environment. However, while it may be tempting to link the lack of cyclic biphytanes in the Gillen Member as a product of a hypersaline environment, one should take detection limits of the analytical instruments into consideration.

GDGTs are core membrane lipids that were originally thought to be produced by hyperthermophilic archaea, but have now been found in a variety of low-temperature (<30°C) environments that include open and coastal marine settings (e.g. Schouten et al. 2000; Sinninghe Damsté et al. 2002), lakes (e.g. Blaga et al. 2009), and peat bogs (e.g. Schouten et al. 2000).

A likely source for the acyclic biphytane breakdown products in the Gillen Member samples are GDGTs comprised of two acyclic biphytane chains (GDGT-0). Thus far, GDGT-0 have been linked to planktonic crenarchaeota (DeLong et al. 1998; Sinninghe Damsté et al. 2002a), or to methanogenic archaea (e.g. Weijers et al. 2004). Figure 5.20 shows that no correlation was observed between increasing concentrations of one biphytane breakdown product (\(i\)-C\(_{37}\)) and increasing dolomite or anhydrite contents. This observation indicates that the organisms producing this molecule were present in both the dolomite and anhydrite precipitation stages.

Moderate correlations between the head-to-head \(i\)-C\(_{37}\) and \(i\)-C\(_{25}\) (\(R^2 = 0.67\); Figure 5.14A) and squalane (\(R^2 = 0.46\); Figure 5.14B) indicates that the organisms producing these compounds could have co-occurred in the Gillen Member. However, the lack of any correlations with \(i\)-C\(_{37}\) and crocetane and PMI (data not shown) indicates that such associations with methanogens may have been absent.
Recently, biphytane was observed in a cyanobacterially-dominated microbial mat and underlying sediments from a modern hypersaline lagoon in Baja California (Jahnke et al. 2008). This molecule was obtained after a BBr$_3$/Superhydride treatment of caldarchaeol (dibiphytanyl glyceroltetraether) that was observed in small amounts in most samples. This molecule was particularly abundant in the top 17 mm and coincided with an abundance of 16S rRNA gene clone libraries sharing sequence homologies with uncultured members of the Euryarchaeota group Thermoplasmales (Jahnke et al. 2008). Based on this close association between lipid and clone library abundances, Jahnke et al. (2008) suggested that these Thermoplasmales contributed to the caldarchaeol. If this was the case, then such microorganisms may also have contributed to the biphytane input observed in the Gillen Member evaporites. However, further work would be needed to confirm whether Thermoplasmales really are the genuine contributor of biphytane in this modern hypersaline environment. In such a case, it would be necessary to culture these microorganisms, which is currently difficult since less than 1% of microorganisms are culturable using existing techniques (e.g. Amann et al. 1995; Pace 1997; Schloss and Handelsman 2004). In the study by Jahnke et al. (2008), only the broad phylogenetic grouping could be identified. Nevertheless, the extracted 16S rRNA gene sequences were related to sequences from other hypersaline environments that also showed affinities to the archaeal taxa Thermoplasmales (e.g. Cytryn et al. 2000). This indicates that members from this phylogenetic group may be important contributors of lipids in hypersaline environments, which may have been present in the Neoproterozoic. Nevertheless, it will be important to investigate if Thermoplasmales are the only contributor of biphytane in hypersaline environments, or if there are others that have hitherto not been detected.

Thus far, C$_{40}$ tertraether isoprenoids are rarely documented in ancient hypersaline systems. A notable exception may be the studies by McKirdy and Kantsler (1980) and McKirdy et al. (1984), of Cambrian carbonates of the Officer Basin, South Australia. The carbonates were interpreted to have formed in a
hypersaline setting and chromatograms were published that depict the presence of potential 30+ acyclic isoprenoids with an elution pattern similar to the ones presented in this study of the Gillen Member (e.g. see McKirdy and Kantsler 1980, their figure 11). However, further work needs to be conducted on these samples to confirm if these compounds belong to the same class as those from the Gillen Member. More recently, Wang and Fu (1997) and Wang (1998) detected a series of long-chain acyclic isoprenoids up to C_{40} in Cambrian, Permian and Triassic marine sequences in China. The sequences were composed of carbonates, gypsum and halite and interpreted as “saline/hypersaline”. Based on their mass-spectra, the compounds were interpreted as head-to-head acyclic C_{40} isoprenoids and their diagenetically related <C_{40} homologues.

The detection of biphytane and associated breakdown products in the hypersaline Gillen Member pushes the antiquity of these molecules to at least 800 Ma. Ventura et al. (2007) detected cyclic and acyclic biphytanes, as well as C_{36} to C_{39} breakdown derivatives of these molecules in Late Archean (~2.71-2.68 Ga) metasedimentary rocks. However, the syngeneity of these and other molecules to is doubtful, since the host rocks were a site of hydrothermal gold mineralization and have been further metamorphosed at temperatures between 200 and 300°C (Ventura et al. 2007). Exposures to such high temperatures would most likely have destroyed the bitumen, making the antiquity of biphytane in the Archean questionable (Brocks 2011).

5.4.2.9 Presence of squalane
Squalane, a C_{30} tail-to-tail acyclic isoprenoid, has been detected in numerous evaporitic sections of the Gillen Member. It was particularly prominent in samples containing high concentrations of anhydrite relative to dolomite (Figure 5.19A; R^2 = 0.79), and appears to be a biomarker sensitive to increased salinity.

Squalane has been reported in numerous biomarker studies on sedimentary deposits that record ancient hypersaline conditions. For example, Mello et al. (1993, 1994) observed high relative concentrations of squalane in Brazilian rock sequences deposited under hypersaline conditions (e.g. marls and calcareous
black shales) as opposed to normal (i.e. less saline) marine and lacustrine black shales. Likewise, Grice et al. (1998) detected squalane in the more saline anhydrite deposits of the Miocene/Pliocene Sdom Formation (Israel), but it was not reported from the less saline dolostones of the same formation. Similar results were also reported from Cambrian evaporite sequences of the Observatory Hill beds, Officer Basin, South Australia (McKirdy and Kantsler 1980). Squalane has also been documented in relatively high concentrations in European and North American oils derived from source rocks that are interpreted to be deposited under hypersaline conditions (ten Haven et al. 1988). In all these cases, squalane was inferred to derive from halophilic microorganisms.

Since squalane has been interpreted as a biomarker for hypersaline environments (e.g. ten Haven et al. 1988; Grice et al. 1998) and relatively high concentrations of this molecule have been detected in pure cultures of halophiles (e.g. Kamekura 1993), it is important to determine its source(s). Squalane would be derived from its precursor squalene, which is common to all domains of life and serves as a precursor to polycyclic terpenoids and steroids (Peters et al. 2005b). It is therefore not surprising that squalane has been one of the first biomarkers identified in crude oil (Gardner and Whitehead 1972) and is regarded to occur in most source rocks and oils (Peters et al. 2005b). However, squalene is also a major lipid produced by methanogenic (Tornabene et al. 1979; Brassell et al. 1981), thermoacidophilic (e.g. Tornabene et al. 1979) and halophilic (e.g. Tornabene et al. 1969; Stiehl et al. 2005) archaea and an abundance of squalene is often seen as indicative for the presence of this domain (Peters et al. 2005b). Kamekura (1993) reported that squalenes comprise ~36% of the neutral (nonpolar) lipids in halophiles and that they can be divided into the following components: squalene; dihydrosqualene; tetrahydrosqualene; and dehydrosqualene.

Recently, new source organisms have been proposed for squalene in modern hypersaline environments. Jahnke et al. (2008) detected squalene and several dehydrosqualanes in sedimentary core samples of a modern hypersaline cyanobacterial mat and underlying sediments in Baja California. Jahnke et al.
(2008) and Orphan et al. (2008) regarded methanogens as the source-organisms for this squalene, which had similar depth distributions as PMI. Indeed, an isolate of *Methanohalophilus* was observed to contain abundant squalene together with small amounts of PMI (Jahnke et al. 2008). Trimethylamine (TMA)-amendment of the upper 2 to 22 mm of their mat samples revealed appreciable amounts of squalene at approximately half the concentration of PMI (Orphan et al. 2008).

In addition to squalenes, a novel dehydrosqualane was also detected in the surface 0 to 4 mm zone that spans the oxic-anoxic zone of a modern hypersaline cyanobacterial mat in Baja California (Jahnke et al. 2008; Orphan et al. 2008). Based on thin-layer chromatography, the isoprenoid was associated with glycolipids and mass spectral data indicated a possible glycosidic linkage with this molecule (Jahnke et al. 2008). While the source organism(s) could not be identified for that isoprenoid, it was observed that the compound increased in dark, anaerobic enrichments with addition of TMA or H₂/CO₂, indicating a possible association with methanogens (Orphan et al. 2008). The structure and polarity of the compound appeared to be comparable to those of other membrane lipids (Jahnke et al. 2008). Since isoprenoids characterize archaeal membranes, the compound was regarded as likely to have an archaeal rather than bacterial origin (Jahnke et al. 2008). Based on the abundance of this molecule in the several horizons of this core, Jahnke et al. (2008) suggested that these novel $i$-$C_{30}$ compounds may be the source of squalane in ancient hypersaline environments.

While the Gillen Member squalane cannot be assigned to specific precursor organisms, its strong positive correlation with increasing salinity (Figure 5.19A) indicates that it is a biomarker sensitive to hypersaline conditions. Furthermore, the strong positive correlation with $i$-$C_{25}$ ($R^2 = 0.9$, Figure 5.12) indicates that these molecules may have either been produced from the same group of organism(s) or from different ones that occupied similar ecological niches. A lack of correlation with crocetane and PMI (data not shown) indicates that squalane from the Gillen Member is unlikely to have been derived from methanogenic sources.

### 5.4.3 Absence of hopanes and steranes in the Gillen Member samples
A number of factors could explain the absence of hopanes and steranes in the Gillen Member evaporites. These factors involve the influence of thermal degradation during catagenesis, absence or low abundance of hopanoid and steroid producing microorganisms at that time, or oxic degradation during diagenesis. Below, each of these factors is evaluated.

5.4.3.1 Influence of thermal maturity (catagenesis)

The absence of hopanes and steranes in the Gillen Member could be explained as a result of thermal maturity. Cyclic saturated hydrocarbons are generally observed to have lower thermal stabilities than their acyclic counterparts (Peters et al. 2005a). Therefore, thermal processes associated with burial and exposure to geothermal heat could result in the destruction of these molecules. Indeed, the Gillen Member in Mt Charlotte 1 has been obtained from depths of between 1000 and >2000 m and would likely have experienced heating by the local geothermal gradient.

However, as was noted in section 5.3.5, the thermal maturity of the Gillen Member may be comparable to parts of the mid-Proterozoic Barney Creek Formation in northern Australia where triaromatic steranes and hopanes are abundantly preserved. Therefore, thermal maturity may not provide an adequate explanation for the lack of hopanes and steranes in the Gillen Member.

5.4.3.2 Absence of hopanoid producing members of the Bacteria domain

In this study, no indigenous hopanes were detected from any Gillen Member evaporites. Hopanoids, the precursor of hopanes, are an important class of biomarkers that have widespread occurrence in aerobic (e.g. Farrimond et al. 1998; Brocks and Summons 2003) and anaerobic (e.g. Fischer et al. 2005) members of the Bacteria domain. Hopanoids have been detected in numerous cyanobacterial species, especially the C_{35} bacteriohopanepolyols (e.g. Rohmer et al. 1984; Brocks and Summons 2004). From these molecules, 2-methylhopanoid is often assigned as a marker for cyanobacteria in ancient settings (Summons et al. 1999), although other sources have recently been noted (Rashby et al. 2007).
While an absence of hopanes in the Gillen Member samples can be a result of the destructive processes inherent during diagenesis or catagenesis, it should be noted that not all cyanobacteria produce hopanoids (e.g. Rohmer et al. 1984). As pointed out by Brocks and Summons (2004), cyanobacterial hopanoids from hypersaline environments are poorly studied and future work needs to be directed in this regard. Therefore, an absence of hopanes in the Gillen member samples does not necessarily indicate evidence for the absence of cyanobacteria.

5.4.3.3 Absence of steroid producing members of the Eukarya domain

Previous work on the paleontology of the Gillen Member has not reported any fossil evidence for algae in the Gillen Member (Oehler et al. 1979). This observation is in contrast to the overlying Loves Creek Member, where a more diverse fossil assemblage, including possible evidence for algae, has been reported (Schopf 1968; Schopf and Blacic 1979; Knoll and Golubic 1979). Oehler et al. (1979) regarded this contrast in biotic composition a result of the different environmental conditions inherent between the two members: a restricted, periodically stagnant, hypersaline setting in the Gillen Member; and the more open, shallow marine conditions of the overlying Loves Creek Member. Nevertheless, in modern marine hypersaline settings, algae can form an important biotic constituent. Some of the most widely reported modern halophilic algae include diatoms and Chlorophyta (i.e. green algae) from the genus Dunaliella (e.g. Javor 1989; Oren 2002). While fossil diatoms have not appeared in the geological record until the Mesozoic (especially by the Lower Cretaceous; Sims et al. 2006), the oldest fossils with possible affinity to green algae has been reported at ~750 Ma (Butterfield 2004; Knoll et al. 2006). The presence of green algae at that time indicates that such organisms could have been present when the Gillen Member was deposited.

In this study, no eukaryote-specific biomarkers in the form of indigenous steranes were detected in the Gillen Member. Sterols, the precursor of steranes, occur in all eukaryotes and serve as stabilizers of cell membranes (e.g. Brocks and
as with the hopanes, the absence of steranes could also be a result of diagenesis or catagenesis. However, the results discussed above do not necessarily indicate that. Therefore, eukaryotes may either not have been present in the Gillen Member or did not contribute biomarkers in the locality where samples have been collected.

Another possible eukaryotic marker that has been associated with hypersaline conditions is gammacerane. Although the origin of this pentacyclic triterpenoid is uncertain (Peters et al. 2005b), it may form by the reduction of tertrahymanol (gammacerane-3β-ol) (Venkatesan 1989; ten Haven et al. 1989). Stable carbon isotope values of gammacerane may indicate that it could be derived from bacterivorous ciliates, which is consistent with the observation that such organisms biosynthesize tetrahymanol if their diet when deprived of sterols (Sinninghe Damsté et al. 1995). While this molecule has been noted in a number of hypersaline settings and is regarded as a marker for this type of environment (e.g. ten Haven et al. 1988), it was not detected in the Gillen Member. Provided that diagenesis or catagenesis have not destroyed this or other sterane molecules, it appears that eukaryotic input in the Gillen Member has been absent or not abundant enough to create detectable steranes.

5.4.3.4 Influence of oxic degradation during diagenesis
In modern coastal sabkha environments like those at Abu Dhabi, carbonaceous mats develop in the intertidal zone that is frequently exposed to air (e.g. Bontognali et al. 2010). As a whole, Kenig et al. (1990) described the sedimentary environment of Abu Dhabi as oxidizing, although local accumulation of organic matter in sediments favors sulfate reduction. As noted in Chapter 2, the depositional environment of the Mt Charlotte 1 Gillen Member is interpreted to be similar to that of the Abu Dhabi intertidal setting. Hence, similar oxidizing conditions may have been at play in the Gillen Member, destroying the precursors of hopanes and steranes while preserving more recalcitrant lipids that resulted in molecules such as \( n \)-alkanes and isoprenoids.
Although a range of hopanes and steranes have been noted from various Phanerozoic samples described as “hypersaline” (e.g. ten Haven et al. 1986, 1988), it is unclear what their precise depositional environment had originally been. Hypersaline condition can involve a wide variety of settings and conditions (e.g. sabkhas, lagoons, shallow basins), with sabkha style environments being different from those consistently covered in hypersaline water. Indeed, subaqueous hypersaline facies are localities where organic matter accumulates (Sonnenfeld, 1985; Warren 2006). In such density-stratified waters, a bottom brine body is composed of a dense, saline water mass where atmospheric gases do not easily exchange with the brine and long-term bottom anoxia is maintained (Warren 2006). Such conditions would contrast markedly from the more oxic intertidal sabkha environment.

The energetically most favorable metabolic pathways for bacteria involve oxygen as the electron acceptor (Wakeham and Canuel 2006). Therefore, an increase in oxygen exposure time would result in a concomitant rise in microbial oxidation. Indeed, laboratory-based oxic incubation studies point to the relative instability of sterols under such conditions. For example, Sun and Wakeham (1998) determined that 78% of radiolabelled cholesterol was lost during ~100 days of incubation at the sediment-water interface under oxic conditions.

Field work and laboratory-based studies on modern marine sediments show that different biomarkers are degraded at different rates under extensive oxygenic degradation conditions (e.g. Harvey and Macko 1997; Rieley et al. 1997; Hoefs et al. 2002; Sinninghe Damsté et al. 2002c). Laboratory studies seem to indicate higher resistance of alkenones towards degradation than algal steroids (e.g. Harvey and Macko 1997; Rieley et al. 1997). Based on the biomarker record of Arabian Sea sediments that have been exposed to varying oxygen exposure times, Sinninghe Damsté et al. (2002c) noted that steroids degraded faster under oxic conditions than alkenones, which in turn were more labile than terrestrial \(n\)-alkanes. Such degradation patterns were also apparent from sediment trap studies by Prahl et al. (2000) and Wakeham et al. (2001). Through work conducted on
oxidized turbidites in the Madeira Abyssal Plain (offshore from NW Africa), Hoefs et al. (2002) observed the following general order of resistance to degradation for the free lipid fraction:

\[ n \text{-alkanes} > \text{fatty acids} > n \text{-alcohols} > \text{diols/keto-ols} > \text{alkenones} > \text{steroids} > \text{triterpenoids} \]

For the ester-bound lipid fraction, this general order was observed to be similar, only that the last three orders were:

\[ \text{triterpenoids} > \text{steroids} > \text{alkenones} \]

As a result of these field and laboratory based studies, oxic degradation could be used as valid explanation for the absence of hopanes and steranes in the Gillen Member. These results would also be in agreement with the pristane/phytane ratios discussed in section 5.4.2.4, where oxidation was attributed to the observed results. Therefore, oxic degradation appears to be the most favorable explanation for the absence of hopanes and steranes in the Gillen Member.

5.4.4 Paleoenvironmental interpretation

Contemporary hypersaline environments are of particular interest, since they can host a diverse and highly productive microbial ecosystem (e.g. Jahnke et al. 2008; Orphan et al. 2008; Bühring et al. 2009). Furthermore, the activity of grazing organisms is suppressed in such settings, allowing for extensive mats to grow. This ecological feature is believed to mimic much of the Precambrian when grazing organisms have not yet appeared and extensive microbial mats occurred. Consequently, numerous studies have aimed to better understand modern hypersaline mats and use them as a model in understanding Precambrian microbial mats (e.g. Hoehler et al. 2001; Bühring et al. 2009). However, such interpretations rely on the uniformitarian assumption that ancient hypersaline microbial mats were very similar to those of today. Therefore, the analysis of
molecular proxies indicative of halophilic microorganisms could be useful in discerning the biotic composition ancient of Precambrian hypersaline settings.

Numerous rock samples of geobiologic interest are, unfortunately, devoid of organic matter that could be indicative of the former biological composition. While hypersaline environments play host to specialized communities of halophilic microorganisms, diagenetic and catagenetic process in the sedimentary rock record can render the organic matter unsuitable to molecular analyses (Rouchy and Monty 2000). The presence of indigenous biomarkers in the Gillen Member of Mt Charlotte 1 has provided a rare opportunity to evaluate the uniformitarian assumption outlined above and provide insights into the biological composition of a Precambrian hypersaline setting.

5.4.4.1 Evidence for photosynthetic primary producers in the Gillen Member

Modern hypersaline environments play host to a substantial biomass of primary producers. These organisms create the organic base upon which biogeochemical cycles function in these settings (e.g. Gerdes et al. 2000). Basin-margin evaporite systems are particularly characterized by an abundance of photoautotrophic species that are dominated by cyanobacteria as well as a variety of algae (e.g. Gerdes et al. 2000). In sabkhas and shallow brines, benthic cyanobacteria make the greatest contribution to sediments and associated sedimentary structures (Gerdes et al. 2000). Modern cyanobacteria-dominated mats from the Gavish Sabkha, for example, have been reported to occur in almost all salinity ranges, beginning at the metahaline range (4-7%) and ending close to the level of potash salts (>30%) (Gerdes et al. 2000).

Below, work from previous studies as well as from this study is discussed. This discussion will show that cyanobacteria were most likely the dominant photosynthetic primary producers in the Gillen Member.
5.4.4.1.1 Fossil evidence from previous studies

Fossil evidence for cyanobacteria in the Gillen Member evaporites has been previously reported from a dolomitic sequence containing coarsely crystalline anhydrite (Oehler et al. 1979). The fossils comprise spheroidal to ellipsoidal bundles of densely interwoven tubules (2-10 μm in diameter) that were interpreted as cyanobacterial sheaths. Possible evidence for degraded cellular trichomes was also reported. Schopf (1968), Schopf and Blacic (1979) and Knoll and Golubic (1979) also reported a diverse range of cyanobacterial fossils from the same formation that contains the Gillen Member (i.e. Bitter Springs). However, these samples were reported from the upper member of the formation and may refer to the currently known Loves Creek Member. Rocks from this part are not associated with the underlying, evaporitic Gillen Member.

5.4.4.1.2 Evidence from this study

As was shown and discussed in Chapter 2, the dolomite layers in the Gillen Member were interpreted as fossilized microbial mats. This interpretation was based on 1) evidence for cohesive dolomitized layers that resemble modern mat structures; 2) characteristics of low-temperature dolomite precipitation; 3) concentric framboidal pyrite inside the dolomite; 4) shape, distribution and association of clay laminae with dolomite crystals and 5) carbon (δ\(^{13}\)C) and oxygen (δ\(^{18}\)O) stable isotope values.

In this chapter, biomarkers have indicated the likely presence of cyanobacteria in the Gillen Member. This interpretation is based on the high abundance of mono- and dimethylalkanes relative to \(n\)-alkanes, elevated concentrations of \(n\)-C\(_{17}\) as well as the acyclic isoprenoids pristane and phytane. Indeed, modern evaporitic environments are usually characterized by thick accumulations of cyanobacterial mats, which coat the surface or develop within a gypsum crust (e.g. Oren et al. 1995). Furthermore, cyanobacterial mats have been shown to precipitate dolomite in these sabkha settings (e.g. Gerdes et al. 2000;
Bontognali et al. 2010), providing a further indication of their presence in the Gillen Member.

It is possible that algae were also present in the Gillen Member evaporite. Indeed, elevated concentrations of \( n-C_{17} \) have also been associated with algae (e.g. Tulliez et al. 1975; Řezanka et al. 1982). Furthermore, modern hypersaline settings can harbor eukaryotes such as the unicellular green alga *Dunaliella salina*, which inhabits brine pools (e.g. Oren 2005; Bardavid et al. 2008). However, while fossil evidence from the Gillen Member indicates the former presence of cyanobacteria (Oehler et al. 1979), no evidence currently exists on any algal occurrences (see discussion above). Furthermore, dolomite precipitation has only been observed in cyanobacteria- rather than eukaryote-dominated mats. Therefore, while the existence of algae cannot be ruled out in these samples, it is most likely that cyanobacteria were the predominant photoautotrops in the Mt Charlotte 1 Gillen Member. The absence of detectable steranes in the Gillen Member, as well as breakdown products of carotenoids that are indicative of *Dunaliella salina* may also point to a lack of eukaryotes. However, oxidation may have played a role in removing these molecules.

While cyanobacteria are frequently associated with dolomitizing mats, they are also observed to reside on the surface of as well as within gypsum crystals (e.g. Oren et al. 1995; Gerdes et al. 2000; Canfield et al. 2004). Gerdes et al. (1985, 2000) pointed out that salt crusts, including gypsum, can host a significant biomass through the ideal light-channeling properties of these minerals and act as protective barriers to retain moisture and store nutrients. Microbial mats in modern gypsum deposits are vertically stratified, with individual layers ranging in color from yellow and orange to purple-red and light and dark green (e.g. Gerdes et al. 2000; Canfield et al. 2004). Although numerous microbial taxa are observed in modern gypsum, cyanobacteria form a dominant group in these sulfates. Thin sections of Gillen Member anhydrites demonstrate the presence of thin layers of dolomite between anhydrite crystals (Figure 2.4). The occurrence of this mineral in the anhydrite could also point to the presence of fossil cyanobacterial mats (see
Chapter 2). This would indicate that similar microbial mats as that of today were also present in the Gillen Member sulfates.

In the Gillen Member anhydrites (formed from gypsum; see Chapter 2), biomarkers indicative of cyanobacteria were also detected. Indeed, \( n-C_{17} \), seen as indicative of cyanobacteria, showed a weak positive correlation with increasing anhydrite concentration (\( R^2 = 0.29 \); Figure 5.15A). While elevated concentrations of \( n-C_{17} \) and monomethylalkanes may be associated with cyanobacteria, these molecules do not necessarily derive from the same cyanobacterial sources. As shown in Figure 5.1C, samples composed almost entirely of anhydrite display high elevated concentrations of \( n-C_{17} \), but comparatively low concentrations of monomethylalkanes relative to \( n \)-alkanes. This observation is in contrast to the more dolomitic samples, where higher concentrations of monomethylalkanes have been detected but comparatively lower concentrations of \( n-C_{17} \) has been observed (Figure 5.1A,B). Provided that both of these molecules are of cyanobacterial origin, this result indicates that different cyanobacterial species may have been present in that environment. Indeed, a number of different cyanobacterial species from a variety of genera have been previously observed in modern hypersaline environments (e.g. López-Cortés et al. 2001; Thajuddin and Subramanian 2005). Different cyanobacterial species have also been shown to produce a certain range of monomethylalkanes, with some species not producing any mid-chain branched alkanes (Shiea et al. 1990). Alternatively, differences in growth conditions of a single species between various depositional environments could also explain this difference. Further studies on the lipid diversity between and within different halophilic cyanobacterial species and populations may shed more light on this topic.

5.4.4.2 Evidence for haloarchaea in the Gillen Member

Halophilic archaea are taxonomically diverse and can withstand the extremities of high salinity (between 3-5 M; Falb et al. 2008). DasSarma and DasSarma (2008) proposed that the term haloarchaea be used for halophiles that are members of the
archaeal domain. These microorganisms possess a characteristic lipid membrane that differs significantly from those of the other domains. In particular, halophilic archaeal membranes are characterized by lipids with acyclic isoprenoid side chains that are stable at high salinities. Of these acyclic isoprenoids, those with a regular isoprenoid chain composed of 20 and 25 carbon atoms as well as those with a C\textsubscript{30} chain (i.e. precursors of squalane) are particularly characteristic of haloarchaea (e.g. Grice et al. 1998; Oren 2002; Peters et al. 2005b; Jahnke et al. 2008). Therefore, the Gillen Member harbored haloarchaea that increased in abundance with increasing salinities. Indeed, the strong positive correlations of i-C\textsubscript{25} ($R^2 = 0.85$; Figure 5.18A) and squalane ($R^2 = 0.79$; Figure 5.19A) with increasing anhydrite concentrations indicate that these isoprenoids act as biomarkers for hypersalinity.

5.4.4.3 Evidence for methanogens in the Gillen Member

Methanogens in hypersaline environments are also representatives of the archaea. In most environments, methanogens are facing competition with sulfate-reducing bacteria for the products of fermentation such as hydrogen and acetate (e.g. Lovley et al. 1982; McGenity 2010). Such competition would have been especially intense in the hypersaline environments that are represented by the Gillen Member. Most of the samples analyzed in this study contain significant amounts of anhydrite, a sulfate mineral formed during the dehydration of gypsum. In modern equivalents of such environments, sulfate reduction is the dominant terminal-electron-accepting process as it possesses a higher affinity for these competitive growth substrates (e.g. Lovley et al. 1982). Nevertheless, modern sulfate-rich hypersaline environments play host to methanogens. Indeed, methanogenesis is an important process in the sulfate-depleted zones in deeper sediments (Wilms et al. 2007), in areas with increased hydrogen production (Hoehler et al. 2001; Buckley et al. 2008), and where non-competitive carbon sources such as methanol, dimethylsulfide and methylated amines are available (Oremland et al. 1982; Winfrey and Ward 1983).
In modern hypersaline settings, high levels of methane production coincide with unusually high hydrogen concentrations in subtidal microbial mats at Guerrero Negro, Mexico (Hoehler et al. 2001). Such elevated hydrogen concentrations were associated with cyanobacterial activity in the upper few millimeters, negating competition with sulfate-reducing bacteria (Hoehler et al. 2001). In addition, cyanobacteria may also be leaking the solute glycine betaine, which can give rise to methylamines and thereby resulting methanogenesis (McGenity 2010). Indeed, Smith et al. (2008) observed that methylated amines were the primary route to methane formation in the Guerrero Negro mats and that methylotrophs were the only methanogens detected there. Both laboratory (Smith et al. 2008) and field studies (Jahnke et al. 2008; Orphan et al. 2008) of hypersaline microbial mats have shown that such methylotrophic methanogens tend to be the dominant methanogens in modern hypersaline conditions. Hydrogenotrophic methanogens, by contrast, were shown only to be a small fraction of the Archaeal community (Smith et al. 2008). Methylotrophic methanogens can be an abundant component in hypersaline environments and have been detected in microbial mats at salinities of up to 15 to 20% (Mouné et al. 2003).

Methylotrophic or hydrogenotrophic methanogens may have also been the source of crocetane and PMI in the Gillen Member. However, the precise facies setting with respect to host rock mineralogy is elusive. As noted in section 5.3.7, Gillen Member evaporites displayed no correlation between PMI concentrations and increasing amounts of anhydrite or dolomite (Figure 5.22). Likewise, no correlations were measured between crocetane concentrations and host rock mineralogy (Figure 5.21). A close association between cyanobacterial mats and methylotrophic or hydrogenotrophic methanogenesis could explain the lack of correlations with anhydrite or dolomite concentrations. As was noted in section 5.4.4.1.2, microbial mats in modern hypersaline settings inhabit both gypsum (the precursor of anhydrite) and dolomite precipitating environments. Since these mats could have supported methanogens, methanogenesis would have been encountered at various levels of salinity during the deposition of the Gillen
Member. A negative correlation ($R^2 = 0.51$; Figure 5.23B) between crocetane and regular $i$-C$_{25}$ concentrations indicates that methanogens and various haloarchaea may not have occupied the same ecological niche.

A strong positive correlation of Gillen Member crocetane with PMI ($R^2 = 0.93$; Figure 5.23A) indicate that sources producing these compounds occupied similar niches. While previous work in Guerrero Negro showed that crocetane and PMI were closely associated in modern hypersaline cyanobacterial mats, they were not derived from the same source (Orphan et al. 2008). Therefore, the strong correlation between these compounds in the Gillen Member may indicate a close association between different methanogenic taxa.

5.4.4.4. Antiquity of halophiles, their ecological communities and associated biogeochemistry

In this study, numerous biomarkers were detected that have been previously reported from Phanerozoic and modern hypersaline environment. Some of these biomarkers even show a strong correlation in their abundance relative to salinity increases. Such results point to a conservative nature of lipid function in hypersaline environments. The regular C$_{25}$ isoprenoid, for example, has frequently been assigned as a hypersaline biomarker in Phanerozoic studies and regarded as specific to members of the archaea (e.g. Grice et al. 1998). The presence of this molecule in the Gillen Member shows that the biological precursor of this biomarker has been present in hypersaline settings for the past 800 Ma.

More intriguingly, the presence of a combination of biomarkers (e.g. regular $i$-C$_{25}$, crocetane, PMI, squalane and biphytane) in both the Gillen Member and a modern hypersaline cyanobacterial microbial mat in Baja California (Jahnke et al. 2008; Orphan et al. 2008) points to a similar community composition of prokaryotes and associated biogeochemistry that remained unchanged since at least the Late Precambrian. This similarity is compounded by the interpretation that the dolomitic laminae in the Gillen Member are also likely the remains of phototrophic microbial mats (see Chapter 2). Of course, this interpretation does not indicate that halophiles have not evolved in the intervening time between mid-Neoproterozoic
and today. Recent genomic research, for example, has shown that populations of halophiles can exhibit considerable genetic variation (e.g. Ma et al. 2010 and references therein). However, broad taxonomic groups with specific lipid compositions appear to have been present by mid-Neoproterozoic times. Such similarities between ancient and modern are remarkable, considering the massive environmental perturbations that have existed on Earth since the Neoproterozoic. Indeed, the presence of extensive Neoproterozoic glacial deposits in the Amadeus Basin (e.g. Wells et al. 1970) shows that this area was affected by the severe ice ages of the so-called Snowball Earth. However, halophilic prokaryotes and their ecological communities appear to have survived such worldwide catastrophic events, and are still part of contemporaneous hypersaline settings.

Recent research points to halophiles being able to survive long-term in settings that have experienced environmental perturbations. In hypersaline settings that experienced a drying-out of brines, for example, Baati et al. (2010) showed that halophiles can get entrapped in halite crystal inclusions. Such microorganisms have been shown to retain their viability for long periods after their entrapment (e.g. Norton and Grant 1988). The recent discovery of algal remains entrapped within halite has also led to the speculation that enough organic material was available for the sustenance and survival of entombed heterotrophic halophiles (Ma et al. 2010; Schubert et al. 2010). It is this survival ability within salt that may have allowed halophiles to survive the onslaught of numerous environmental perturbations; reestablishing themselves once more favorable conditions were encountered.

A number of genetic/biochemical adaptations may have also allowed halophilic communities to withstand the consequences of environmental changes. Previous work has shown that hypersaline environments are exceptionally dynamic, offering challenges with respect to light, salinity, temperature, pH and oxygen (DasSarma et al. 2001; Leuko et al. 2010). It is therefore not surprising that halophiles demonstrate significant adaptive versatility in response to different stress-inducing situations (Leuko et al. 2010). The archaeon *Halobacterium* NRC-1, for example, exhibits considerable adaptive plasticity. Leuko et al. (2010)
pointed out that this species can adapt to a variety of environmental parameters that include both high and low saline conditions (Coker et al. 2007; Leuko et al. 2009); ionizing and UV radiation (Baliga et al. 2004; Kottemann et al. 2005; deVeaux et al. 2007; Whitehead et al. 2006); desiccation (Kottemann et al. 2005); different temperature regimes (Coker et al. 2007; Shukla 2006); and stress from transition metals (Kaur et al. 2006). Another halophile, *Haloarcula sp.*, has even been shown to withstand a two week exposure to the space environment (Mancinelli et al. 1998), while *Halococcus dombrowskii* and *Halobacterium sp.* NRC-1 survived a simulated Martian atmosphere for up to six hours (Stan-Lotter et al. 2002). Such adaptations may have rendered halophilic communities immune to environmental perturbations, resulting in at least broad phylogenetic similarities of prokaryotes between late Precambrian and Phanerozoic hypersaline ecosystems.

### 5.5 Conclusions

This study investigated the biomarker composition of numerous evaporites from the Gillen Member of the 800 Ma Neoproterozoic Bitter Springs Formation. The aim of this investigation was to establish the biomarker composition of a Precambrian hypersaline setting and to ascertain any associated ecological and biogeochemical characteristics. Such work allows for a comparison with numerous other biomarker studies that were conducted on hypersaline settings in the Phanerozoic.

Indigenous bitumens extracted from various anhydrites and dolomites of the *Mt Charlotte 1* core, yielded evidence of a Neoproterozoic hypersaline ecosystem. A key feature of this study has been the observation that biomarkers indicative of hypersalinity in the Phanerozoic have also been present at ~800 Ma. Based on the biomarker composition, the Gillen Member ecosystem was characterized by cyanobacteria, methanogens and various haloarchaea. As in modern hypersaline settings, the cyanobacteria would have formed extensive, dolomite-precipitating mats, which encountered oxidizing conditions on the surface and reducing conditions in the interior. Such mats would have harbored sulfide reducers.
(evidenced by the presence of frambooidal pyrite in the dolomite laminae) and methanogens (evidenced by the presence of crocetane and PMI). Indeed, studies of modern environments have shown that cyanobacteria-dominating mats host dense and diverse microbial communities in which biogeochemical cycles occur in close proximity (e.g. Pearl and Pinckney 1996; Visscher and Stolz 2005; Jahnke et al. 2008; Orphan et al. 2008). This study has shown that such communities would have been present at ~800 Ma. A further increase in salinity (as evidenced by the precipitation of sulfates) would have set the conditions for the colonization by haloarchaea. While cyanobacteria would still have been present, the amount of organic matter produced under such conditions would have been more limited.

In conclusion, this study has provided evidence that dolomite and anhydrite can serve as archives in ascertaining the existence and composition of Precambrian hypersaline ecosystems. Indeed, such deposits have yielded the oldest reported biomarker evidence for methanogens and haloarchaea. Furthermore, the biomarkers extracted from such deposits have enabled the most detailed biogeochemical reconstruction of a cyanobacterial mat in the Precambrian. Based on comparisons with reports from modern hypersaline equivalents, such ecosystems would have been quite conserved over at least the past ~800 Ma.

5.6 References


Chapter 5

p. 409–414.


Chapter 5


Chapter 6
Assessing compound-specific carbon isotopic signatures of biomarkers from Precambrian evaporites

This chapter investigates compound-specific carbon isotopes of hydrocarbons extracted from the Neoproterozoic Gillen Member. As noted in the previous chapters, bitumens from these evaporites revealed biomarker signatures characteristic of a Precambrian age and a hypersaline setting. Herein, the aim is to investigate whether the carbon isotopic composition of such hydrocarbons can provide important biogeochemical insights.

6.1 Introduction

Modern hypersaline environments are important ecosystems that play host to both dense and diverse microbial communities in which major biogeochemical cycles occur (e.g. Jahnke et al. 2008; Orphan et al. 2008; Bühring et al. 2009; McGenity 2010). These environments often feature thick, cyanobacteria-dominated mats that have the capacity to develop into stromatolitic structures akin to those reported in Precambrian strata. For these reasons, hypersaline settings have been used as modern analogues in understanding biogeochemical cycling in the Precambrian as well as providing insight into the burial and preservation of organic matter relevant to the interpretation of chemical signatures (e.g. Des Marais et al. 1992a; Des Marais 2003; Schidlowski et al. 1994).

The carbon cycle is a particularly important biogeochemical feature, of which the formation of organic matter is an essential component (e.g. Des Marais 2001). The carbon isotopic composition ($\delta^{13}C$) of organic matter reflects the $\delta^{13}C$ of the utilized carbon source and modifications by biochemical processes and environmental variables involved in its production. Insights into the history of the biogeochemical carbon cycling on Earth have been at least partially afforded through records in the carbon isotopic composition ($\delta^{13}C_{\text{PDB}}$) of carbonate ($\delta_{\text{carb}}$) and reduced carbon ($\delta_{\text{org}}$) in sedimentary and metamorphic rocks (Des Marais 1997, 2001). The isotopic difference between $\delta_{\text{carb}}$ and $\delta_{\text{org}}$ reflects the metabolic pathways of CO$_2$ fixation and carbon metabolism by autotrophic organisms as well
as the pathways and mechanisms of organic carbon transformation and remineralization (Des Marais 1997). Therefore, isotopic characterization of ancient marine-derived organic matter can provide insights into the conditions under which carbon fixation occurred. Such results have the capacity to contribute to the understanding of global marine cycles.

Previous carbon isotopic studies on Precambrian sedimentary rocks have, for example, revealed links between tectonic change and the operation and balance of the global carbon cycle (e.g. Des Marais 1997), as well as associations between biological evolution and ocean-atmosphere-lithosphere chemistry (e.g. Knoll et al. 1986). Indeed throughout Earth history, global as well as local environmental and biological signals have been discerned from the isotopic record of sedimentary inorganic and organic carbon (e.g. Des Marais 2001 and references therein).

While such isotopic work has provided key insights into the Precambrian, they suffer from a lack of physiological or taxonomic specificity on the organisms present at that time. In such studies, carbonaceous residues known as kerogens are studied and can represent accumulations of organic matter from potentially wide variety of organisms. More specific isotope insights have recently been gleaned from ion microprobe measurements of individual Precambrian microfossils (e.g. House et al. 2000). Such studies were not only able to provide taxonomically specific insights of individual microfossils, but also distinguish between different metabolic pathways responsible for isotopic fractionation.

Particularly informative insights on the carbon isotopic records have been gleaned by combining them with molecular signals of hydrocarbons buried in ancient sedimentary rocks. Using a method known as single-compound carbon isotopic analyses, individual lipid-derived hydrocarbons are analyzed for their $\delta^{13}C$ values by isolating the individual molecules and combusting them (Freeman et al. 1990; Hayes et al. 1990). As opposed to bulk carbon isotope measurements, such analysis affords greater resolution of isotope variations at the molecular level and is therefore a valuable tool in reconstructing ancient biogeochemical processes.

The aim of this study is to provide the first single-compound carbon isotopic measurements from biomarkers of a Precambrian hypersaline environment.
Previous compound-specific work aimed to understand the biogeochemistry of modern marine hypersaline settings (e.g. Bühring et al. 2009) as well as older Phanerozoic equivalents such as the Miocene-age Messinian evaporites (e.g. Schouten et al. 2001) and Miocene/Pliocene halite deposits of the Dead Sea Basin (e.g. Grice et al. 1998a). However, to date, no compound-specific isotopic study has been conducted on biomarkers of Precambrian sediments that feature a combination of evaporitic minerals such as carbonates, anhydrite and halite.

This work also differs from other compound-specific studies in that the isotopic signatures are spatially evaluated in each rock sample through exterior/interior experiments discussed in Chapters 3 and 4. Such analyses provide a robust test in evaluating the scale of hydrocarbon contamination on these rock samples and the extent to which they mask biogeochemical signals.

6.2 Materials and Methods

6.2.1 Samples

A total of seven Neoproterozoic evaporites have been analyzed for kerogen and single-compound isotopic analysis of carbon. Their values are shown in Table 6.1 and Figures 6.2 and 6.3. For six out of these seven samples, isotopes were measured on both the exterior and interior rock portions. For the remaining sample, only the interior values were measured. All rocks analyzed in this study were collected from sections of the Mt Charlotte 1 drill core held at Geoscience Australia (Canberra).

The rocks consisted of evaporites (dolomite, anhydrite and halite) that were derived from the Gillen Member of the ~800 Ma Neoproterozoic Bitter Springs Formation. These evaporites were deposited in an inland sea that is now central Australia. Due to the shallow nature of that sea and a tenuous connection with the ocean at that time, the water was characterized by elevated salinity levels (Lindsay 1987). Thick (100 m to >2000 m) evaporite units were deposited (Lindsay 1987) and were noted to harbor both body (Oehler et al. 1979) and molecular fossil
(Chapter 5) evidence of various halophilic microorganisms. It is from rocks of that setting, where hydrocarbons were analyzed for their carbon isotope signatures.

6.2.2 Interior/exterior experiment
To assess concentration differences and measure isotopic differences of organic molecules between the exterior surfaces of a rock and the corresponding interior, all rock surfaces were either trimmed by using a clean precision wafering saw (Buehler Isomet 1000; Illinois, U.S.A; blade thickness 340 µm) or ablated by a tumbler (KG-1 Mini-Sonic tumbler, Diamond Pacific, U.S.A). Between 8 and 33 g of exterior rock surface was removed. The exterior rock surfaces and the interior rock core were separately crushed to powder, extracted and fractionated.

6.2.3 Processing of samples
Rock samples were ground to powder in an alumina ring-mill (Rocklabs, NZ). Prior to usage, the mill was cleaned by grinding baked-out (600°C/24 h) quartz-rich sand two to three times for 60s and subsequently washed with methanol and dichloromethane (DCM). System blanks consisted of baked-out sand (600°C/24 h). Approximately 5 to 30 g of rock powder was extracted with 100% DCM in a Dionex Automated Solvent Extractor. The extracts were reduced to 100 µl under a stream of purified nitrogen gas and separated into saturated, aromatic and polar fractions using column chromatography over 12 g annealed (450°C/24 h) and dry-packed silica gel (Silica Gel 60; 230-400 mesh; EM Science). Saturated hydrocarbon were eluted with 1.5 dead volumes (DV) n-hexane, aromatic hydrocarbons with 2 DV n-hexane:DCM (1:1 v/v) and polars with 2 DV DCM:methanol (1:1 v/v).

6.2.4 Gas chromatography-mass spectroscopy (GC-MS)
Prior to isotopic analysis, GC-MS analyses of the saturated fraction was carried out on a Micromass AutoSpec Premier equipped with a 6890 gas chromatograph (Agilent) and a DB-5 capillary column (60 m × 0.25 mm i.d., 0.25 µm film thickness) using helium as carrier gas. The MS source was operated at 260°C in EI-mode at 70 eV ionization energy and with 8000 V acceleration voltage. Samples were
injected in splitless mode into a PTV injector at a constant temperature of 300°C. For full-scan analyses, the GC oven was programmed at 40°C (2 min), heated to 315°C at 4°C/min, with a final hold time of 17 min. The AutoSpec full-scan duration was 0.7 s plus 0.2 s interscan delay over a mass range of 55-600 Da.

6.2.5 Silicalite adduction
The aliphatic hydrocarbon fraction (ca. 2-3 mg) was adducted with silicalite activated at 120°C for 1 h. The silicalite was tightly packed into a Pasteur pipette (to a height of 4 cm) and washed with dry pentane. The fraction was dissolved in a minimum volume of hexane and applied to the silicalite column and eluted with 4 column volumes of pentane. Pentane, containing the branched/cyclic components was removed by evaporation through a gentle nitrogen stream. The silicalite was dissolved in HF (32%) and the \( n \)-alkanes released. The \( n \)-alkanes were extracted with pentane (4x).

6.2.6 Isotopic Analyses
The carbon isotopic composition of individual hydrocarbons was determined by gas chromatography-isotopic ratio mass spectroscopy (GC-IRMS). The analytical system included a Trace GC connected to a CuO/Ni/Pt combustion unit interfaced via a Finnigan GC III to a Finnigan MAT 252 isotope-ratio mass spectrometer. Samples were manually injected on-column. Helium (2ml/min) was used as the carrier gas (at constant flow) and the GC capillary column was a DB-5 (60 m x 0.32 mm i.d., 0.25 μm film thickness). The GC oven was programmed at 40°C for 10 min, heated to 310°C at 4°C/min and held at the final temperature for up to 10 min. Data was acquired and processed using the software package ISODAT version 7. The system was calibrated and corrected for instrument drift by co-injecting internal standards of perdeuterated \( n \)-alkanes (C\(_{16}\)D\(_{34}\), C\(_{20}\)D\(_{42}\), C\(_{24}\)D\(_{50}\); Chevron) with known isotopic compositions. All \( \delta^{13}C \) values are reported relative to the Pee Dee Belemnite (PDB) standard as follows:

\[
\text{eq. 6.1: } \delta^{13}C = \left[ \frac{(13C/12C)_{\text{sample}}}{(13C/12C)_{\text{PDB}}} - 1 \right] \times 10^3
\]
Isotopic analyses were carried out in duplicates with most reported data achieving an error well within ±1. Maximum deviations of up to ±2‰ were encountered for some compounds with poor baseline separation. Such large deviations were mostly encountered with the isoprenoids, due to difficulties in establishing the baseline.

6.2.7 Isotope analysis of kerogens
Kerogen carbon isotope values were analyzed through a commercial operator at the Research School of Biological Sciences, Australian National University, Canberra. All samples analyzed for kerogen isotopes consisted of crushed rock powder from which bitumens was previously extracted.

6.3 Results
In this study, attention was paid to the possibility of hydrocarbon contaminants overprinting indigenous δ^{13}C values. Therefore, \textit{n}-alkanes and the isoprenoids pristane and phytane were analyzed in both the exterior and interior rock portions. In this chapter, exterior and interior isotope values are plotted in green and pink, respectively (Figure 6.1).

![Figure 6.1 Schematic representation of a drill core sample indicating exterior (green) and interior (pink) rock portions.](image-url)
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*Table 6.1: Sample dataset of given columns exemplified.*
Figure 6.2 $\delta^{13}C$ values of interior $n$-alkanes plotted against chain length. Isotope values for individual samples are presented in Table 6.1.

Figure 6.3 $\delta^{13}C$ values of kerogen (blue), and average $n$-alkanes from the interior (pink) and the exterior (green).
In most cases, marked differences in $\delta^{13}$C values were measured between the exterior and interior of the same evaporite samples. Below, three common exterior/interior $\delta^{13}$C patterns of $n$-alkanes are described.

The first pattern (Figure 6.4) displays a diverging exterior/interior trend. Interior $\delta^{13}$C values become progressively heavier (-24.5 to -27.6‰) with increasing carbon number of the $n$-alkanes ($n$-C$_{15}$ to $n$-C$_{24}$). An exception was observed at $n$-C$_{17}$, which is lighter than $n$-C$_{16}$ and $n$-C$_{18}$. Exterior $\delta^{13}$C values, by contrast, remain more constant and their average range is at 29.1‰.

Like the first pattern in Figure 6.4, the second pattern also displays heavier $\delta^{13}$C values in the interior and lighter ones in the exterior (Figure 6.5). In this second pattern, exterior/interior $\delta^{13}$C values follow a roughly parallel trend, but exhibit considerable differences at varied carbon numbers. In the sample shown in Figure 6.3, marked exterior/interior differences (up to 1.8‰) were noted between $n$-C$_{15}$ and $n$-C$_{23}$. Between $n$-C$_{24}$ and $n$-C$_{31}$, these differences become markedly smaller and are within 1‰.

The third and final pattern (Figure 6.6) displays very little difference in the $\delta^{13}$C composition of interior and exterior $n$-alkanes. While interior isotope values tend to be heavier than their exterior counterparts, differences are slight. Both rock portions record progressively heavier $\delta^{13}$C values with increasing carbon number. In the example shown in Figure 6.6, $\delta^{13}$C values range from -29.3 to -25.9‰ for the interior and -30.4 to -26.8‰ for the exterior. The only exception to this trend was $n$-C$_{17}$, which, as in all the other samples, has a lighter $\delta^{13}$C composition.
Figure 6.4 Representative example of a “first pattern” exterior/interior δ¹³C trend of n-alkanes (08r008; 1654 m). Green data points represent exterior values, while those in pink represent interior values.

Figure 6.5 Representative example of a “second pattern” exterior/interior δ¹³C trend of n-alkanes (08r011; 1651.1 m). Green data points represent exterior values, while those in pink represent interior values.
Figure 6.6 Representative example of a “third pattern” exterior/interior $\delta^{13}$C trend of n-alkanes (09r001; 1652 m). Green data points represent exterior values, while those in pink represent interior values.

Apart from n-alkanes, isoprenoids were a significant component of Gillen Member samples (see Chapter 5). However, single-compound isotopic analysis of these molecules proved to be difficult. Numerous isoprenoids co-eluted with other compounds and could not be chromatographically separated. Such interference potentially masks compound-specific signatures. Furthermore, a hump of unresolved complex mixture (UCM) resulted in many isoprenoids to be situated on slopes where base-line definitions proved to be difficult. Nevertheless, a few insights into their isotopic composition were afforded on samples that displayed high relative concentrations of isoprenoids and a small UCM.

In the samples where isoprenoids could be measured, the regular homologues ($i$-C$_{23}$ to $i$-C$_{25}$) displayed $\delta^{13}$C values between -24.5 and -28.5‰, while the C$_{37}$ to C$_{39}$ head-to-head isoprenoids yielded values ranging from -23.7 to -26.6‰ (Table 6.1). These values are similar to the ones obtained for average n-alkanes of the respective samples (Table 6.1). Since the exterior of these samples exhibited a significant UCM, only the interior $\delta^{13}$C values could be measured for these compounds.
An attempt was made to measure $\delta^{13}C$ values of pristane and phytane in both the exterior and interior rock portions. This was done on one sample (08r008; from 1654 m depth), where significant amounts of these compounds were detected in both rock portions. While pristane and phytane were detected in all samples, significant differences in their relative concentrations were detected between the exterior and interior rock portions (see Chapter 4). In this study, $\delta^{13}C$ differences of up to 6‰ were noted for these molecules between the different rock portions.

It should be noted that the Gillen Member phytane and regular C$_{25}$ isoprenoids usually co-elute with 2,6,11,15-tetramethylhexadecane (crocetane) and 2,6,10,15,19-pentamethylicosane (PMI) (see Chapter 5). While no exterior $\delta^{13}C$ values could be measured for these molecules, the interior values do not deviate significantly from the average $n$-alkane measurements (Table 6.1). Therefore, these molecules are not interpreted to have been contaminated.

The mono- and dimethylalkanes, like most of the isoprenoids, were also strongly co-eluting. Therefore, isotopic measurements were not conducted on these molecules.

In some samples, $n$-C$_{17}$ was isotopically depleted relative to $n$-C$_{16}$ and $n$-C$_{18}$. Indeed, values of up to 29.9‰ were noted in the interior (Table 6.1). This isotopic anomaly is of interest, since elevated concentrations of $n$-C$_{17}$ relative to $n$-C$_{16}$ and $n$-C$_{18}$ have been detected in most samples. In order to examine if there is a potential relationship between these two observations, $\delta^{13}C$ values have been plotted against relative concentrations of $n$-C$_{17}$. As shown in Figure 6.7, a positive correlation ($R^2 = 0.65$) was observed between elevated relative concentrations of $n$-C$_{17}$ and increasingly light carbon isotope values.

Based on the above results, there appears to be at least two different sources for $n$-C$_{17}$ in the Gillen Member; at least one source that contributed to the regular homologous series of $n$-alkanes and another source that contributed to producing the elevated or excess concentrations. Therefore, the $\delta^{13}C$ of $n$-C$_{17}$ is a combination of the $n$-C$_{17}$ that is part of the homologous series and the excess $n$-C$_{17}$. Since the former contribution should exhibit similar isotopic values as the nearby homologues (i.e. $n$-C$_{16}$ and $n$-C$_{18}$), it should be possible to examine the
δ^{13}C values of the excess \( n\)-C\(_{17} \). These results were obtained by determining the excess amounts of \( n\)-C\(_{17} \) molecules relative to the \( n\)-C\(_{16} \) and \( n\)-C\(_{18} \) homologues and using these amounts to calculate the original δ\(^{13}\)C contribution of the excess \( n\)-C\(_{17} \). The results obtained from these mass-balance calculations indicate that excess \( n\)-C\(_{17} \) is depleted in δ\(^{13}\)C by at least 5‰ relative to \( n\)-C\(_{17} \) that is part of the regular homologous series.

![Figure 6.7 Relationship between relative \( n\)-C\(_{17} \) concentrations and their δ\(^{13}\)C values.](image)

Apart from \( n\)-C\(_{17} \), \( n\)-tetraicosane (\( n\)-C\(_{24} \)) also provided relatively low carbon isotopic values in some samples (down to -29.2‰ in the interior; Figure 6.2 and 6.5). However, in contrast to \( n\)-C\(_{17} \), these isotopic values were not associated with elevated concentrations of \( n\)-C\(_{24} \). Indeed, no positive correlation (R\(^2\) = 0.008) was obtained when concentrations of \( n\)-C\(_{24} \) (relative to \( n\)-C\(_{23} \) and \( n\)-C\(_{25} \)) were plotted against δ\(^{13}\)C (data not shown). Furthermore, depleted δ\(^{13}\)C values for \( n\)-C\(_{17} \) are not necessarily accompanied by similar values for \( n\)-C\(_{24} \). However, a positive trend was detected when δ\(^{13}\)C values of \( n\)-C\(_{24} \) were plotted against relative concentrations of \( n\)-C\(_{17} \) (Figure 6.8; R\(^2\) = 0.69). While this correlation may indicate
some relationship between these two molecules, it should be noted that it is largely
carried by one data point (at -29.2‰). Therefore, more values need to be acquired
to evaluate this correlation.

![Figure 6.8 Relationship between relative n-C$_{17}$ concentrations and $\delta^{13}$C values of n-C$_{24}$.](image)

### 6.4 Discussion

This study investigated single compound $\delta^{13}$C values of n-alkanes and some
isoprenoids from Precambrian evaporites that were derived from drill cores in
central Australia. The effects of hydrocarbon contamination were examined and
clearer insights were gained through their removal. Below, the results of these
analyses are discussed, followed by an examination of the $\delta^{13}$C values derived
from the indigenous hydrocarbons.

#### 6.4.1 Implications of hydrocarbon contamination on single-compound $\delta^{13}$C values

Exterior/interior experiments of *Mt Charlotte 1* evaporites from the Gillen Member
revealed that exterior hydrocarbons have lighter $\delta^{13}$C values than their interior
counterparts. Such differences demonstrated the capacity of hydrocarbon
contamination to overprint isotopic signals. As noted in Chapter 4, *Mt Charlotte 1*
core samples became contaminated by a variety of different sources. The above results indicate that the contaminants were isotopically depleted relative to indigenous hydrocarbons.

6.4.1.1 Impacts of n-alkane contamination

As noted above, interior n-alkanes were always heavier than their exterior counterparts. On average, the interior $\delta^{13}C$ values of these molecules ranged from -22.1 to -26.9‰, while those from the exterior ranged from -24.4 to -28.2‰. This result raises the question of why the interior values are heavier than their exterior counterparts.

Since the host rocks record a hypersaline paleoenvironment (see Chapter 2), it could be expected that the indigenous hydrocarbons should record $\delta^{13}C$ values indicative of such an environment. Previous studies on hypersaline settings revealed relatively high $\delta^{13}C_{org}$ values of up to -5‰ (e.g. Schidlowski et al. 1984; Des Marais et al. 1989; Lazar and Erez 1992; Kenig et al. 1994). Such heavy values are often regarded as a proxy for these environments. Hypersalinity itself has been suggested as a key driver in producing these heavy values by causing diffusive inorganic carbon limitation (Schidlowski et al. 1984; Sumner 2001). Additional causes in such settings include: the high biological productivity in microbial mats responsible for decreased fractionation of $^{13}C$ (Des Marais et al. 1989; Schidlowski et al. 1994); an inorganic-carbon-concentrating mechanism (Tchernov et al. 1997), and bicarbonate uptake that tends to suppress isotopic discrimination against $^{13}C$ (e.g. Des Marais et al. 1992b; Hayes 1993).

While heavy $\delta^{13}C$ values are often recorded from functionalized lipids and hydrocarbons of Phanerozoic hypersaline settings, they are not necessarily very heavy (e.g. >-20‰) or restricted to a certain range. Phytane from hypersaline settings, for example, can vary in $\delta^{13}C$ from -25‰ (Grice et al. 1998b) to -33.2‰ (Hollander et al. 1993). Such values are even lighter than those measured in the Gillen Member from this study. As will be further discussed below, environmental factors such as CO$_2$ diffusion limitations could explain such variations.
In contrast to the indigenous hydrocarbons, the $\delta^{13}C$ values of the Mt Charlotte 1 contaminants exhibited markedly lighter values. An examination of the literature (e.g. Peters et al. 2005 and references therein) reveals that $\delta^{13}C$ values of Phanerozoic hydrocarbons tend to average at ~30‰. Such values are similar to those obtained from the exterior rock portions and indicate the isotopic overprinting effect of contaminants on hydrocarbons with different $\delta^{13}C$ values.

While the $n$-alkanes and isoprenoids from the Gillen Member interior can be largely regarded as indigenous (see Chapter 4), it is still possible that contaminants infiltrated the rock interior. Various studies (Brocks et al. 2008; Brocks 2011) have shown chromatographic effects associated with the diffusion of hydrocarbon contaminants into rocks. Such a process leaves concentration gradients whereby lighter molecules (e.g. low molecular $n$-alkanes and isoprenoids) permeate deeper into a rock than heavier ones (e.g. high molecular weight $n$-alkanes and isoprenoids). Therefore, it is possible that at least some $n$-alkanes with a lower carbon number infiltrated the rocks and overprinted any indigenous isotopic signals. Future studies, whereby slice-extraction experiments (see Chapter 3) are coupled with carbon isotope measurements, could potentially provide further insights. Since the slice-extraction technique assesses the spatial distribution of hydrocarbons in a rock at millimeter-scales, one could better track and assess any impacts on isotopic overprints.

### 6.4.1.2 Impacts of isoprenoid contamination

In few limited cases, Gillen Member isoprenoids could be measured for their $\delta^{13}C$ values. These molecules were pristane, phytane/crocetane mix, regular $i$-$C_{25}$/2,6,10,15,19-pentamethylicosane (PMI) mix and their $C_{23}$ and $C_{24}$ homologues, as well as $C_{37}$, $C_{38}$, and $C_{39}$ breakdown products of biphytane. Below, impact of contamination on $^{13}C$ isotope values of Gillen Member isoprenoids is discussed.

Accurate measurements of $\delta^{13}C$ values for pristane and phytane are important and have been used to determine the $^{13}C$ contents in primary products over time. The linking of these molecules with primary production is based on the presence of the phytanyl side chain in chlorophyll. This side chain is often regarded
as the parent molecule of pristane and phytane and the relative ratios of these breakdown products are commonly used to interpret the redox conditions of ancient depositional environments (e.g. Didyk et al. 1978).

Due to the co-elution of pristane and phytane with other compounds in the Gillen Member, their δ\textsubscript{13}C values can only be approximate at present. However, comparisons between their exterior and interior values in one sample showed that a difference of up to 5.3‰ can occur for these molecules (Table 6.1). As noted in Chapter 4, concentrations of both pristane and phytane from the Gillen Member are significantly higher on the exterior portions than in the interior counterparts. Since pristane and phytane are common petroleum constituents, it is very likely that they are present in most contaminants. Therefore, δ\textsubscript{13}C values of these molecules must be treated with caution.

Only the interior δ\textsubscript{13}C value could be measured for isoprenoids other than pristane and phytane. However, these isotope values were similar to the average \textit{n}-alkane isotope composition from the interior. Samples that possessed on average a heavy δ\textsubscript{13}C \textit{n}-alkane composition also have correspondingly heavy values for these isoprenoids.

\textbf{6.4.2 δ\textsubscript{13}C comparisons between \textit{n}-alkanes and kerogen}

In this study, \textit{n}-alkanes from the sample interiors usually possessed δ\textsubscript{13}C values that are heavier than kerogen (Table 6.1; Figure 6.3). This observation is in line with that reported by Logan et al. (1997, their table 3 and figure 7) where the average \textit{n}-alkyls from the Gillen Member were noted to be more depleted in \textsuperscript{13}C compared to the associated kerogen. However, it is unsure by how much previously published values have been affected by contamination. It should be noted that the exterior \textit{n}-alkanes deviated far more from the kerogen in their δ\textsubscript{13}C composition (up to 4.2‰) than the interior counterparts (up to 1.2‰). Furthermore, the interior \textit{n}-alkanes followed a similar trend to kerogen values, while those for the exterior are more erratic (Figure 6.3).
6.4.3 δ\textsuperscript{13}C variations within Gillen Member samples

Individual Gillen Member evaporites recorded variations of more than 3% in the δ\textsuperscript{13}C compositions of \textit{n}-alkane homologues (Table 6.1). The \textit{n}-alkanes likely were derived from various straight-chain molecules such as phospho- and sphingolipids produced by bacteria (Brocks and Summons 2004). Two of the most prominent groups of bacteria in the Gillen Member would have been cyanobacteria and sulfate-reducers (see Chapters 2 and 5). Therefore, some of this variation in δ\textsuperscript{13}C may be attributed to a mixture of at least these two groups.

Currently, little is known about carbon isotope fractionation by sulfate-reducers (Goevert and Conrad 2008). Furthermore, there is indication that different species from this group can fractionate carbon differently (Goevert and Conrad 2008). Therefore, the contribution of sulfide-reducers to δ\textsuperscript{13}C values in the Gillen Member cannot be currently discussed.

Cyanobacterial contributions to δ\textsuperscript{13}C values in the Gillen Member can be further investigated. As noted in Chapter 5, elevated concentrations in heptadecane (\textit{n}-C\textsubscript{17}) have been detected in most Gillen Member samples and attributed to the cyanobacteria. These molecules yielded depleted δ\textsuperscript{13}C values relative to most other \textit{n}-alkanes. This observation is particularly evident in samples that yielded high relative concentrations of \textit{n}-C\textsubscript{17}. The positive correlation between carbon isotopic depletion of \textit{n}-C\textsubscript{17} and its excess concentration (\textit{R}\textsuperscript{2} = 0.65; Figure 6.7), suggests that the organism producing this molecule in high relative abundance would also be responsible for its isotopic depletion. However, these results are in contrast to previous molecular isotopic studies on modern hypersaline environments (e.g. Schouten et al. 2001; Wieland et al. 2008), where \textit{n}-C\textsubscript{17} recorded δ\textsuperscript{13}C values that they are much more enriched than most of the Gillen Member samples.

The correlation between δ\textsuperscript{13}C depletions and elevated \textit{n}-C\textsubscript{17} indicates that at least two different sources contributed to \textit{n}-C\textsubscript{17}: one producing the relatively light excess and the other the more enriched counterpart. Such results may be due to differences in isotopic fractionation between widely different groups of organisms, but also between different cyanobacterial species. Indeed, carbon isotope values
of individual cyanobacterial fossils from the Bitter Springs Formation have been previously investigated (House et al. 2000). The isotopic composition of these microfossils (dated to ~850 Ma) ranged in $\delta^{13}C$ from -21 to -32‰. These values are within the $\delta^{13}C$ range reported herein from the Gillen Member samples.

Previous work on modern microbial mats in hypersaline (e.g. Wieland et al. 2008) and hot spring environments (e.g. Jahnke et al. 2004) may also lend weight to the idea that different cyanobacterial inputs contributed to differences in $\delta^{13}C$. It was shown that the cyanobacterial markers $n$-$C_{17}$, 7-methylheptadecane and 8-methylhexadecane differed significantly in $\delta^{13}C$. Wieland et al. (2008) suggested, among other factors, that differences in cyanobacterial source-inputs could have resulted in the carbon isotopic variation between different lipid biomarkers. As pointed out by Wieland et al. (2008), future studies on modern hypersaline mats should focus on comparative analyses of hydrocarbon synthesis by different mat-building cyanobacterial species. Such studies could clarify whether different carbon isotopic signatures resulted from the use of different biochemical mechanisms or varying environmental conditions.

Environmental influences could also serve as an explanation for these isotopic differences. Cyanobacterial mats in hypersaline settings can develop to a considerable thickness with various biogeochemical processes occurring at different parts of the mat. Wieland et al. (2008) reported a $^{13}C$ depletion for the total lipids with depth in a hypersaline cyanobacterial mat. A difference of up to 6.1‰ was measured between the top and the bottom 10 mm of that mat. While the heavy isotope values were interpreted as the result of limited dissolved inorganic carbon availability in the surface layer of a cyanobacterial mat, the more depleted values were regarded due to remineralization of organic matter due to sulfate reduction and anoxygenic photosynthesis at depths. It would not be surprising if a similar mechanism also occurred in the Gillen Member mats. More CO2 from recycled organic matter

While explanations can be found for depleted $\delta^{13}C$ values of $n$-$C_{17}$, those for $n$-$C_{24}$ prove to be more difficult. Currently, there does not appear to be any information published on the biological origins of the carbon isotope values for this
molecule. Furthermore, unlike \( n\)-C\(_{17}\), \( n\)-C\(_{24}\) does not exhibit associated increases in its relative concentration. In addition, depleted \( \delta^{13}C \) values for \( n\)-C\(_{17}\) are not necessarily accompanied by similar values for \( n\)-C\(_{24}\). While a positive correlation \((R^2 = 0.69)\) between \( n\)-C\(_{17}\) concentrations and the \( \delta^{13}C \) of \( n\)-C\(_{24}\) may indicate some relationship between these two molecules, it should be noted that this correlation relies on limited data.

### 6.4.4 \( \delta^{13}C \) variations between Gillen Member samples

The \( \delta^{13}C \) values of \( n\)-alkanes varied significantly between the samples. For example, \( n\)-C\(_{19}\) exhibited values ranging from -20.8 to -26.8‰ (Figure 6.2). Similar differences were also observed with other \( n\)-alkanes (Table 6.1). The \( \delta^{13}C \) of the kerogen also varied between samples by up to 5‰ (Figure 6.3). Such variation was also observed between samples that exhibited similar petrographic textures, mineralogy and near-identical biomarker compositions.

The most likely explanation of \( \delta^{13}C \) variations between the samples are local or seasonal differences in their carbon availability. Such differences in carbon availability have been previously reported in modern hypersaline settings (e.g. Schidlowski et al. 1994; Pagès et al. 1995). Carbon isotopic heterogeneity among cyanobacteria has also been previously reported from individual carbon isotope measurements of microfossils of the Bitter Springs Formation (dated to ~850 Ma; House et al. 2000). In that study, isotopic compositions of cyanobacterial fossils varied considerably within each taxon. House et al. (2000) regarded these values to be a result of biological or environmental processes occurring at the time and excluded fossilization processes as a cause for the isotopic discrepancy.

### 6.4.5 \( \delta^{13}C \) values of crocetane and PMI from the Gillen Member

Both crocetane and PMI have been detected in the Gillen Member. These acyclic isoprenoids are regarded to be archaea-specific (e.g. Peters et al. 2005). In studies from both modern and ancient methane-rich marine environments, \(^{13}C\)-depleted crocetane and PMI has been used as a proxy for anaerobic oxidation of methane (AOM; e.g. Thiel et al. 1999; Valentine 2002; Birgel et al. 2006). However, a
strong $^{13}$C-depletion that is characteristic of methane oxidation has not been detected in the Gillen Member equivalents. While it must be acknowledged that these compounds co-elute with other isoprenoids (see Chapter 5), it should still be possible to detect some depletion in their $\delta^{13}$C value (possibly to $-40\%$ or less) if they were associated with AOM. However, this was not the case.

Recently, both crocetane and PMI have also been noted in modern hypersaline cyanobacterial mats from Guerrero Negro, Baja California (Jahnke et al. 2008; Orphan et al. 2008). The PMI from that environment was linked to the methylotrophic methanogen *Methanolobus* spp., while the source for crocetane is still unknown in both hypersaline and methane-rich environments. While the isotopic composition of PMI was not discussed, crocetane from Guerrero Negro, like those from the Gillen Member, also did not exhibit any $^{13}$C-depletion. DNA-based phylogenetic analyses on these cyanobacterial mats did not support an association of crocetane with anaerobic archaeal methanotrophs (Orphan et al. 2008). Therefore, these findings demonstrate an alternative source for crocetane in hypersaline mat environments. Based on a phylogenetic relatedness between anaerobic archaeal methanotrophs of the ANME-2 and Guerrero Negro methanogens, Orphan et al. (2008) postulated methanogens as a potential source for the crocetane in their study. As was discussed in Chapter 5, a case for the former presence of methylotrophic methanogenesis may also be made for the Gillen Member.

### 6.5 Conclusion

Single-compound carbon isotopes of biomarkers from the 800 Ma Neoproterozoic Gillen Member of central Australia were investigated. To better understand the carbon isotopic signatures recorded in these biomarkers and to assess the impact of hydrocarbon contamination on these results, exterior-interior experiments on these evaporites were applied for the first time. These experiments quantitatively assessed hydrocarbon concentration differences between the exterior and interior rock portions on which single-compound carbon isotopes have been conducted.
The results revealed that hydrocarbon contaminants overprinted the original Precambrian signature and exerted a significant influence on the carbon isotopic signature of these biomarkers. However, through a better understanding of the impacts of contamination, it was possible to gain insights into the original Precambrian isotopic signatures recorded in these sample. In particular, it was possible to detect δ^{13}C variations within individual and between different Gillen Member evaporites. Such differences have been attributed to biological (i.e. variations in isotope fractionations between different organisms) as well as environmental (i.e. differences in carbon availability) mechanisms.

6.6 References


This thesis aimed to provide a detailed understanding of the biotic composition of Precambrian and Cambrian hypersaline settings. For this work, evaporites from the 800 Ma Neoproterozoic Bitter Springs Formation (Gillen Member) and the Early Cambrian Chandler Formation were selected for biomarker analysis. These rocks were derived from the Amadeus Basin in central Australia, which experienced significant evaporative events as a result of a tenuous connection with the contemporaneous ocean and the broad shallow nature of the local bathymetry. All samples were collected from the Mt Charlotte 1 core, which was drilled in the southwestern part of the basin.

The biotic composition of these evaporites was determined through the analysis of biomarker molecules derived from microorganisms inhabited these hypersaline environments. The indigenous hydrocarbon component was determined through quantitative exterior/interior rock experiments. In order to understand the information that these molecular fossils provide, the sedimentary context of their enclosing host rocks was also investigated. An outline of the key findings of this thesis is listed below:

- All core samples contained either hydrocarbon contaminants or a mixture of both contaminant and indigenous hydrocarbons. The contaminants had the potential to provide misleading information on the former biotic composition (Chapter 4), as well as the capacity to overprint the single-compound isotopic signals (Chapter 6). While no indigenous hydrocarbons were detected in the Early Cambrian Chandler Formation and in many of the Neoproterozoic Bitter Springs Formation evaporites, 11 samples from the latter formation did possess indigenous bitumens (Chapter 4).

- The evaporite host rocks of the indigenous Neoproterozoic hydrocarbons were mostly composed of anhydrite or dolomite or a mixture of these two minerals (Chapter 2). The anhydrite was inferred to originate from
gypsum and represent an increase in hypersaline conditions. The dolomites, on the other hand, suggest less saline conditions and contain evidence for the former presence of microbial mats. The microbial mat inference was based on: 1) evidence for cohesive dolomitized layers that resemble modern mat structures; 2) characteristics of low-temperature dolomite precipitation; 3) concentric framboidal pyrite inside the dolomite; 4) shape, distribution and association of clay laminae with dolomite crystals and 5) carbon ($\delta^{13}$C) and oxygen ($\delta^{18}$O) stable isotope values. A shallow, sabkha-style depositional setting was inferred from these rocks.

- The indigenous hydrocarbon component of the evaporites yielded numerous biomarkers that suggest the former presence of cyanobacteria, putative methylotrophic methanogens and haloarchaea (Chapter 5). The abundance of some of these molecules, especially those derived from haloarchaea, increased with the anhydrite concentration and, thus, rising saline conditions.

- The petrographic and organic geochemical studies of this thesis proved the capacity of evaporites to act as archives for understanding ancient microbial diversity. It also provided the oldest yet reported evidence for haloarchaeal and methanogen-specific molecular biomarkers and textural evidence for biologically-induced dolomite precipitation. If the laminae within the dolomite represent fossilized cyanobacterial mats, this study would provide the most detailed understanding of a microbial mat in the Precambrian.

- The following stages can be proposed in the genetic development of a Mt Charlotte 1 Gillen Member evaporite: 1) Formation of cyanobacterial mats in the intertidal setting of a sabkha (Figure 7.1A). These mats would host sulfide-reducers (evidenced by the presence of framboidal pyrite) and various archaeal taxa that include methanogens. Various lipids such as chlorophylls, phospholipids, crocetane, and mono- and dimethylated alkyl lipids were produced in these mats. Dolomite precipitation was probably induced within the mat. 2) An increase in
salinity resulted in the precipitation of gypsum on top of the cyanobacterial mat (Figure 7.1B). The onset of increasing salinity resulted in a greater abundance of haloarchaea that flourished under these conditions. Nevertheless, cyanobacteria and other microbes still persisted under these increasingly saline conditions. 3) Thin layers of dolomite-producing cyanobacterial mats re-established on top of the precipitated gypsum (Figure 7.1C; see also Figure 2.4). Salinity fluctuations resulted in alternating layers of dolomite and anhydrite. Throughout these stages, dolomite likely continued to precipitate within mats on remaining biological material such as exopolymeric substances (c.f. Bontognali et al. 2010). 4) Diagenesis and catagenesis resulted in the alteration of the preserved lipids into hydrocarbons. Burial and subsequent heating of the evaporites also resulted in the conversion of gypsum into anhydrite, while continuing dolomite precipitation completely lithified the mats (Figure 7.1D).

- Coring of Mt Charlotte 1 and subsequent sample storage introduced a variety of hydrocarbon contaminants, which had the ability to overprint the indigenous biomarker signals of the Gillen Member evaporites.
Chapter 7

Figure 7.1 Schematic reconstructions of the inferred stages in forming a Neoproterozoic Gillen Member evaporite. Such deposits require interactions between microbial biota, mineral precipitation and burial. Possible lipid biomarker precursors and their hydrocarbon products are encircled. A) Cyanobacteria-dominated mat featuring dolomite and framboidal pyrite precipitation indicative of sulfide reducers. B) Gypsum precipitation on top of cyanobacterial mats due to increased evaporation. C) Colonization of gypsum crust by cyanobacteria followed by reestablishment of dolomite-precipitating mat. Eventually, the re-established mat is covered by another phase of gypsum. D) Diagenetic and catagenetic changes altered the lipids into hydrocarbons. Burial would have also caused the conversion of gypsum into anhydrite. Cyanobacterial mats would have become completely dolomitized.
Numerous additional studies could be conducted to further the findings of this thesis and to advance our understanding of hypersaline ecosystems in deep-time. Suggestions for such studies are listed below:

- Conduct single-compound hydrogen isotopic studies on the Gillen Member bitumen. Previous work on hydrogen isotopes on an ancient hypersaline setting from the Miocene has shown that their δD values can be affected by extremes in evaporation (Andersen et al. 2001). Therefore, such analyses could also be applied to investigate changes in single-compound hydrogen isotopes between the dolomite and anhydrite portions of the Gillen Member. Sessions et al. (2004) noted that n-alkane and isoprenoid carbon skeletons undergo extensive exchange of carbon-bound hydrogen in ancient (>340 Ma) rocks. At temperatures between 50 and 100°C, published experimental estimates of such exchanges likely occur on timescales of $10^4$ to $10^8$ years (Sessions et al. 2004). The ~800 Ma Gillen Member evaporites could provide an excellent opportunity to provide further data on such hydrogen-exchanges over a geologically distant period of time and under temperature conditions that would likely have been >100°C.

- Collecting and analysing more evaporite samples from the Gillen Member in the Amadeus Basin or other time-equivalent formations in Australian (e.g. Officer Basin, South Australia) or overseas basins (e.g. Pakistan; Kovalevych et al. 2006). Such studies could provide a better understanding of the regional or global distribution of hypersaline ecosystems in the Precambrian.

- Attempting to extract bitumens from hypersaline deposits of the late Paleoproterozoic (~1.7-1.6 Ga) McArthur Group, McArthur Basin, northern Australia. One of the oldest well developed examples of bedded gypsum/anhydrite have been reported from this basin at that time (Jackson et al. 1987). In addition, previous biomarker work on the McArthur Group has yielded indigenous biomarkers (Jackson et al. 1986;
Summons et al. 1988; Brocks et al. 2005), although a hypersaline environment was either not inferred or only purported. If successful, it would push back the date of biomarkers from hypersaline ecosystems by another ~700 million years, allowing for comparisons to be made from the Paleoproterozoic to the Phanerozoic.

- Continue the search for the oldest textural evidence of biologically-induced dolomite precipitation. Precambrian hypersaline microbial mat structures have been reported in the literature (e.g. Gandin and Wright 2007) and are believed to have been formed by cyanobacteria. Petrographic studies like those conducted for this thesis could potentially extend the oldest textural evidence for biologically-induced precipitation by billions of years.

Some work that was conducted in this thesis could have wider implications in organic geochemistry and might be applied to other, non-hypersaline, depositional environments. Such studies include:

- Further investigate the capacity of contaminant hydrocarbons to overprint indigenous single-compound isotope results. Isoprenoids such as pristane and phytane as well as the $n$-alkanes are commonly used in single-compound isotopic analyses. However, as was noted in Chapters 4 and 6, these compounds are also common contaminants. Therefore, it would be worthwhile to conduct further exterior/interior isotope studies on rocks from various depositional environments to investigate their signal-overprinting effects.

- Apply the illite to muscovite clay transformation property (seen in XRD) to organic geochemistry. This clay transformation is affected by heat from 50 degrees to >200 degrees Celsius (e.g. Blatt and Tracy 1996). Since organic matter appears to be affected by a similar temperature range, it would be interesting to note if there is a correlation between the extend of this clay conversion and the
catagenic alteration of organic matter. Since only the 10 Angstrom XRD peak needs to be investigated, this technique would be a cheap and quick method for screening samples for the potential preservation of bitumen. This technique would be particular suitable to shale, which is rich in illite (e.g. Blatt and Tracy 1996).

7.1 References


