PhD Thesis in Paleobiogeochemistry

RECONSTRUCTING EARTH’S ALIEN ANCIENT ECOLOGY – A MULTIPROXY STUDY OF THE 1.64 BILLION-YEAR-OLD BARNEY CREEK FORMATION, NORTHERN AUSTRALIA

Modern stromatolites at Shark Bay, Western Australia may resemble Proterozoic shallow water habitats.

Benjamin J. Nettersheim
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RECONSTRUCTING EARTH’S ALIEN ANCIENT ECOLOGY – A MULTIPROXY STUDY OF THE 1.64 BILLION-YEAR-OLD BARNEY CREEK FORMATION, NORTHERN AUSTRALIA

A thesis submitted March 2017 by Benjamin Jakob Nettersheim (né Bruisten) for the degree of Doctor of Philosophy of The Australian National University under the supervision of J.J. Brocks at the Research School of Earth Sciences.

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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.

Date, Signature:

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The vast majority of analyses and interpretations were conducted by the author, assisted by others as outlined on the following page.

This thesis contains an estimated ~80 000 words in the main text.
Contributions to this work

The following people assisted in analytical measurements and interpretations:

Jochen Brocks was involved in all aspects of the project, from acquiring original funding, over sampling and experimental design to extensive scientific discussions and editorial assistance. I hope we managed to smooth-out the worst in the limited amount of time available for such an extensive project. Jochen also analysed some BCF samples that were used in this project. These samples include GR7: 38.7, 41.8, 45.35, 50.3, 77.2, 101.6, 116.0, 126.4, 151.1, 180.0, 238.8, 252.9, 426.7, 638.54 and 870 m (used in chapters 9 – 11), as well as some samples from other drill cores that were used for comparison but are not included in this thesis. He also conducted the slice extraction experiment (45.35 m, GR7) that formed the basis for my syngeneity assessments in chapter 7. Janet Hope taught me most that I know about laboratory analyses and assisted in performing some of the pyrolysis and GC-MS co-injection experiments and generally helped with analytical issues. Romain Guilbaud conducted parts of the Fe-speciation analyses, and he and Simon Poulton hosted me in Leeds and taught me the iron speciation analytical procedures. They assisted with proxy interpretations, and their colleague Rob Newton conducted δ34S measurements of silver sulphides obtained during Fe-speciation analyses (chapter 5). Linda McMorrow assisted with ICP-MS analyses while setting-up the Fe-speciation procedure at ANU. Ulrike Troitzsch conducted the XRPD & Geoscience Australia & Labwest the elemental analyses, and Stewart Fallon and Rachel Woods performed the organic carbon isotopes measurements (mostly chapter 5). Pierre Adam and Philippe Schaeffer performed RuO4 oxidation experiments, hosted me in Strasbourg, supplied a dammarane- and monoaromatic lanostane-standard, and assisted with mass spectral interpretations (mostly chapters 9 & 10). Junhong Chen provided a lanostane (geological) standard (chapter 9) and Julien Langley conducted additional pyrolysis experiments with sulphur addition (chapter 10). Jim Duggan from Australian Petrographic prepared ultra-thin thin-sections, and Sam Spinks (CSIRO) provided XRF scans of these (chapter 4 & 6). I also want to thank the Northern Territory Geological survey for access to drill core material. I discussed my interpretations with several friends and colleagues, most notably CSIRO cluster members, my supervisory panel, Chris Hallmann and my colleagues in Canberra and Bremen. Thanks a lot everyone!
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The discrepancy of these statements stems from the great diversity and complexity reflected in a modern PhD program. The PhD adventure is not a simple lifestyle choice, but forms an integral part of the student’s life, dominating it in good and bad times alike. Throughout the years there are many highs and lows, a lot of leisure but also a lot of pressure. In short, I would not have been able to survive the PhD adventure without the love and support of my caring wife, Julia, who followed me to the ‘boring bush capital’ of Australia, friends, colleagues and fellow PhD students, my supervisors, my parents and family. Without the love and encouragement of my parents, I would never have been able to even enter a PhD program in the first place, let alone completing it. I am most grateful to them and everyone else who supported me and enabled this unique experience of PhD studies.

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The essence of my PhD experience might be summed up in the following quote by George Bernard Shaw: “Science never solves a problem without creating ten more”. Indeed, it felt like that for any problem I solved at least ten more were created and Jochen’s encouragement that this would be typical for good science would offer little comfort. The myriads of potential explanations when dealing with such ancient systems greatly complicated my scientific interpretations and I would never have imagined how difficult it would be and how long it would take to finish writing the thesis. Thus, my thanks also extend to Chris Hallmann and the Agouron Institute who let me start my postdoc while still wrapping up my thesis, and to my supportive new colleagues. I am most grateful to all of you.
Abstract

This thesis aims at reconstructing the paleo-ecology of the 1.64 billion year (Ga) old Barney Creek Formation (BCF) in the McArthur Basin, northern Australia through multi-proxy analyses. The first focus is the reconstruction of paleo-redox conditions and basin ecology. Iron speciation analyses indicate dominantly ferruginous conditions across the southern McArthur Basin. Our analyses revealed unique correlations between mineralogy, paleo-redox conditions and microbial communities that are best explained by a two-end member mixing model, in which BCF waters fluctuated frequently, potentially seasonally, between ferruginous and sulphidic conditions, accompanied by intermittent oxidation events. Shifts in redox conditions were likely driven by variations in terrestrial sediment and nutrient influx, in turn controlled by fluctuations in precipitation and evaporation. Episodic nutrient influx fuelled primary production, leading to euxinia. Mixed layer depth varied significantly. Shallow stratification characterised ferruginous periods. The chemocline was deeper during euxinic periods, which is best explained by variations in wind activity or atmospheric cooling. Chemocline variations resulted in a community shift from purple to green sulphur bacteria. Abundant purple sulphur bacterial biomarkers during ferruginous periods provide the first evidence for photoferrotrophy in the geological record. The inferred redox fluctuations may even have controlled the formation of giant stratiform base metal sulphide deposits such as HYC, causing the fine lamination of the ore.

The oldest indigenous, clearly eukaryotic steranes emerge in the biomarker record at ~0.8 Ga. Despite microfossil evidence for eukaryotic emergence by >1.6 Ga, no demonstrably indigenous saturated steranes have been detected in mid-Proterozoic sediments, including the BCF. However, the BCF hosts dominantly 4-methylated tri-aromatic steroids. The existence of these aromatic steroids, but apparent absence of saturated steranes, was one of the great puzzles of Precambrian biomarker geochemistry. The problem was solved in this work by the discovery of the saturated C₃₀ sterane lanostane and a new ‘protosterane’ structure and a variety of mono- and di-aromatic lanosteroid derivatives. Intriguingly, the entire BCF steroid inventory with dozens of structures may be derived from just cycloartenol and/or lanosterol, the products of the first cyclisation step in sterol biosynthesis. The last common ancestor of
eukaryotes was already able to produce most modern sterols, and since BCF steroids are products of a strongly abbreviated sterol biosynthetic pathway, any eukaryotic source organisms must have belonged to the stem group of the domain. The abundance of the steroids suggests that these organisms were ecologically important. This discovery leads to the controversial proposition that most mid-Proterozoic eukaryotic fossils are stem group representatives, also explaining why they lack crown group eukaryotic features.

We also detected dammaranes, a variety of tri- and tetra-aromatic triterpenoids and typical aromatic arborane-derivatives that are likely derived from biomolecules such as isoarborinol. In addition, the BCF triterpenoid inventory encompasses hopanes, cheilanthanes, gammacerane and aromatic derivatives. As a plant origin can be excluded, the triterpenoids are all of microbial origin. Based on the most plausible scenario for the evolution of polycyclic triterpenoid cyclases, dammaranoid cyclases are evolutionary precursors of sterol cyclases. The dammarane in the BCF thus provides evidence for yet deeper branching ancestors.
Table of contents

Preface
Contributions to this work III
Acknowledgements IV
Abstract VI
Thesis outline XVI

Chapter 1. Introduction, aims and concepts
1.1 Introduction 1
1.2 Aims of thesis 5
1.3 Overview of important scientific concepts 6
   1.3.1 Molecular fossils as biomarkers 6
   1.3.2 The thermal maturity concept 7
   1.3.3 The Fe-speciation concept 8
1.4 References 10

Chapter 2. Methodology and samples
2.1 Samples 13
2.2 Iron speciation 16
2.3 S-isotopes 19
2.4 Total organic carbon, kerogen δ13C and source rock analyses 19
2.5 Mixing simulation 20
2.6 Biomarker analyses 20
   2.6.1 Previous biomarker analyses by Jochen Brocks 20
   2.6.2 New biomarker analyses 20
   2.6.3 Bitumen extraction 21
   2.6.4 Gas chromatography–mass spectrometry 21
   2.6.5 Pyrolysis experiments and hydrogenation experiments 22
   2.6.6 Cyclopropane-ring opening experiments with HCl gas 22
2.7 Mineralogical and elemental analyses 23
2.8 References 25

Chapter 3. Geology
3.1 Introduction 26
Chapter 5. Reconstructing a “mixed-up” record: a multi-proxy interpretation of the BCF ecology

5.1 Introduction 83
5.2 Methodology and samples 87
5.3 Results and discussion 88

5.3.1 Iron speciation analyses 88
5.3.1.1 FeHR/FeT 88
5.3.1.2 FePy/FeHR 92

5.3.2 Mineralogy 94

5.3.3 Adding additional proxies to reveal ecological dynamics 96
5.3.3.1 TOC 99
5.3.3.2 Ti/Ca 99
5.3.3.3 Organic carbon isotopes 100
5.3.3.4 Total iron (FeT) 102
5.3.3.5 FePy/FeHR 103
5.3.3.6 FeHR/FeT 104
5.3.3.7 Chromium 104
5.3.3.8 Pyrite sulphur isotopes (δ34S) 104
5.3.3.9 G/(G+P) 105
5.3.3.10 Benthic communities as potential arylisoprenoid sources 109
5.3.3.11 Planktonic communities as potential arylisoprenoid sources 111
5.3.3.12 AIR-G and AIR-P 113
5.3.3.13 Pr/Ph 115

5.3.4 Environmental models based on proxy data 117
5.3.4.1 Gradual environmental model 117
5.3.4.2 Two endmember mixing models 118
      5.3.4.2.1 Results and discussion of mixing simulations 119
      5.3.4.2.2 Tests for two-endmember mixing 120
      5.3.4.2.3 Determination of endmembers 124
      5.3.4.2.3.1 ‘Green’ endmember ecology 125
      5.3.4.2.3.2 ‘Purple’ endmember ecology 125
7.3.7 Arborane/fernane derivatives 174
7.3.8 Aromatic 8,14-seco hopanoids 175
7.3.9 Picenes 175
7.4 Conclusions 177
7.5 References 178

Chapter 8. Triterpenoids of the Barney Creek Formation

8.1 Introduction 179
8.2 Samples and methodology 185
8.3 Results and discussion 186
8.3.1 Saturated triterpanes 186
  8.3.1.1 Regular hopanes and tricyclic terpanes (cheilanthanes) 186
  8.3.1.2 Dammaranes 187
  8.3.1.3 8,14-Secohopanes 191
  8.3.1.4 Gammacerane 192
8.3.2 Aromatic triterpenoid derivatives of the BCF 193
  8.3.2.1 Tetra-aromatic triterpenoids (TeAT) 193
  8.3.2.2 Tri-aromatic triterpenoids (TrATs) 198
  8.3.2.3 Benzohopanes and 8,14-secohopanoids with fluorene moiety 202
  8.3.2.4 8,14-secohopanoids withacenaphthene moiety 204
  8.3.2.5 Regular monoaromatic 8,14-secohopanoids 206
  8.3.2.6 Arborane/fernane-type aromatics 212
  8.3.2.7 Picenes 217
8.4 Conclusions 221
8.5 References 222

Chapter 9. Paleoproterozoic Protosteroids

9.1 Introduction: Sedimentary steroids and eukaryotic evolution 225
  9.1.1 Phanerozoic steroid record 227
  9.1.2 The published Precambrian sterane record 229
9.1.3. Previous studies on BCF steroids  230
9.1.4. The ‘cleaned up’ Precambrian sterane record  231
9.1.5. The Bloch Hypothesis and sterol evolution  232
9.1.6. Eukaryotic body fossil evolution 235
9.1.7. Potential for BCF eukaryotes  236

9.2. Methodology and samples  238
9.3. Results and discussion of new BCF sterane discoveries  239
9.3.1. Arcane BCF triterpanes with sterane characteristics  239
  9.3.1.1. Syngeneity of the triterpanes  241
  9.3.1.2. Structure elucidation  242
  9.3.1.2.1. Structural inferences from GC-MS bitumen analysis  243
  9.3.1.2.2. The nature of the 5th double bond equivalent  249
  9.3.1.2.3. Pyrolysis, acidified PtO2 hydrogenation and HCl gas treatment  250
  9.3.1.2.4. Potential structure of unknown BCF triterpanes 254
  9.3.1.2.4.1. Formation of a cyclobutane ring by cleavage of the cyclopropyl-ring  254
  9.3.1.2.4.2. Bridge formation  254
  9.3.1.2.4.3. Potential ring-opening products 258
  9.3.1.3. Summary of new insights into unknown BCF triterpanes 262
9.3.2. BCF steranes and artificial maturation experiments  262
  9.3.2.1. Lanostanes  262
  9.3.2.2. Pyrolysis experiments  266

9.4. Conclusions and outlook  270
9.5. References  273

Chapter 10. BCF aromatic steroids

10.1. Introduction  277
10.2. Samples and methodology  279
10.3. Results and Discussion  280
  10.3.1. Comparison of different catalysts in pyrolysis experiments  280
10.3.1. Lime catalysis 280
10.3.1.2. Clay catalysis 281
10.3.1.3. Active carbon catalysis 282

10.3.2. BCF aromatic steroids and comparison to pyrolysis experiments 285

10.3.2.1. Triaromatic steroids (TAS) 285
   10.3.2.1.1. M/z 231 triaromatic cholesterol (C₃₀) 286
   10.3.2.1.2. M/z 245 4-methyl-triaromatic cholesterol (C₁₉ to C₃₀) 287
   10.3.2.1.3. M/z 259 triaromatic steroids (C₃₀) 289

10.3.2.2. Diaromatic steroids (DAS) 291
   10.3.2.2.1. C₃₆ diaromatic steroids of the m/z 376 series 291
   10.3.2.2.2. C₃₈ diaromatic lanosteroids 294
   10.3.2.2.3. C₄₀ DAS of the m/z 404 series 297
   10.3.2.2.4. C₄₂ DAS of the m/z 362 series 299

10.3.2.3. Monoaromatic steroids (MAS) 302
   10.3.2.3.1. C₃₈ monoaromatic steroids with m/z 379 base ion 302
   10.3.2.3.2. C₃₆ monoaromatic steroids with m/z 365 base ion 306

10.3.2.4. Pentacyclic monoaromatic triterpenoids in a biodegraded oil 306

10.4. Conclusions and outlook 311
10.5. References 313

Chapter 11. Biogenic precursors, diagenetic pathways and ecological implications of BCF triterpenoids

11.1. Introduction 315
11.2. Methods and samples 317
11.3. Results and discussion – the BCF triterpenoid inventory and plausible biogenic precursors 319
   11.3.1. Triterpenoid quantification 319
   11.3.2. Comparison of total triterpenoid concentrations 322
   11.3.3. Comparison of TeAT concentrations 324
   11.3.4. Comparison of aromatic steroid concentrations 327
   11.3.5. Biogenic precursors and diagenetic products of BCF triterpenoids 328

XIV
11.3.5.1. Cheilanthanes 328
11.3.5.2. Gammacerane and picenes 329
11.3.5.3. Aromatic arborane/ferbane derivatives, TrATs & TeATs 332
11.3.5.4. Hopanes, seco-hopanes and aromatic derivatives 338
11.3.5.5. Dammaranes 342
11.3.5.6. Protosteroid derivatives 344
11.3.6. Summary of biogenic precursors and diagenetic summary scheme 349

11.4. Discussion of source organisms and ecological implications 352
11.4.1. BCF steroids in light of the geological rock record 353
11.4.2. Molecular oxygen and BCF steroids 360
11.4.3. BCF triterpenoids in light of the evolution of triterpenoid cyclases 362

11.5. Synthesis 367

11.6. References 370

Chapter 12. Highlights, outlook and future work

12.1 Research highlights 376
12.1.1 Proxy correlations and two-endmember mixing 376
12.1.2 Large scale proxy variations and orbital climate cycles 378
12.1.3 Implications for base metal mineralisation 378
12.1.4 Aromatic triterpenoids 380
12.1.5 Arborane/ferbane-derivatives 381
12.1.6 Steroids 381
12.1.7 Dammaranes 382
12.1.8 The combined BCF triterpenoid record 382

12.2 Future work and implications for other studies 384
12.3 References 388

Appendix 389
Thesis outline

Due to the complexity of the subjects, the thesis is divided into the following twelve chapters:

**Chapter 1: Introduction**

This chapter introduces the wider context of the thesis, the main aims and analytical concepts. It gives a general overview over the biomarker, iron speciation and thermal maturity concept, and elucidates the thermal maturity of BCF sediments in GR7, information relevant for meaningful interpretations of biomarker proxies.

**Chapter 2: Methodology**

This chapter provides an overview over samples and methodologies employed in this thesis.

Appendix 2 gives an overview over elements and detection limits from our Labwest analyses.

**Chapter 3: Geology**

Discusses the geology of the BCF, with a focus on geologic information relevant for the discussion of the subsequent ecological reconstructions.

**Chapter 4: Depositional environment**

Discusses the depositional environment of the BCF based on a literature review of previous studies and new petrographic observations, elemental and mineralogical analyses in order to assist ecological interpretations in the following chapters.

**Chapter 5: Reconstructing a mixed-up record**

In this chapter, we present a detailed multi-proxy reconstruction of the BCF ecology. Results of a detailed Fe-speciation study of BCF sediments across the McArthur Basin are followed by the incorporation of biomarker and other proxies that allow detailed palaeoecological reconstructions. Four different ecological models are presented that can explain the combined proxy data: a conventional model of gradual change, and three
different two-endmember mixing models. I discuss two geographic models, each with one pelagic and one littoral endmember, and a temporal mixing model. Published criteria for the recognition of two endmember mixing in geological samples are put to the test of the BCF data, that are also compared to results of a basic two-endmember mixing simulation. Long term trends in the BCF proxy data are discussed separately, followed by a conclusion about the general implications of our model for Proterozoic ecology and climate.

This chapter has two appendices, Appendix 5.1 contains results of all the iron speciation analyses and Appendix 5.2 all cross plots of mixing simulations for all proxies discussed in the main text.

Chapter 6: Zn/Pb mineralisation

This chapter reviews important aspects of the stratiform base metal deposits in the McArthur basin. A correlation of Zn and Fe-speciation ratios is followed by a discussion of the implications of the ecological models on base metal mineralisation and the potential of Fe-speciation analyses to assist future exploration.

Chapter 7: Syngeneity

This short chapter establishes the syngeneity of the new biomarkers presented in the subsequent chapters.

Chapter 8: BCF triterpenoids

This chapter presents the triterpenoid inventory of the BCF sediments with a focus on newly discovered aromatic triterpenoids. Discussions are structured according to compound class: (i) saturated triterpanes including dammaranes, (ii) tetra-aromatic and (iii) tri-aromatic triterpenoids (iv) benzo-hopanes and 8,14-secohopenoids with fluorene and (v) 8,14-secohopenoids with acenaphthene moieties, (vi) regular monoaromatic 9,14-secohopenoids (v) fernane/arborane-type aromatics, (vi) methyl-picenes.

This chapter has two appendices, Appendix 8.1 gives an overview over peak and structure assignments used in chapters 7 to 11. Appendix 8.2 shows the mass spectra of the inferred tetra aromatic BCF triterpenoids (TeATs).
Chapter 9: BCF steranes

presents newly discovered BCF steranes in the context of a discussion of the Proterozoic sterane record. The introduction discusses the topics of Precambrian steroids and eukaryotic evolution by explaining the conventional and ‘cleaned-up’ Precambrian sterane record, previous studies on BCF steroids, the Bloch hypothesis of sterol evolution, and how the hypothesis might now be tested in the rock record, elucidating the Proterozoic eukaryotic microfossil record, and discussing the potential for eukaryotes in the BCF.

This is followed by a section on unknown BCF triterpenoids, that are here identified as protosteranes. All the new information regarding these enigmatic compounds is presented, including several different laboratory experiments, culminating in the proposition of the most plausible structures. The discovery of new BCF steranes (lanostanes) is discussed with respect to pyrolysis and a sulphuric acid dehydration experiment.

Chapter 10: BCF aromatic steroids

In this chapter, I present the results of artificial maturation experiments of primitive sterols in relation to the aromatic steroid inventory of the BCF sediments. Steroids are discussed according to the compound classes (i) triaromatic, (ii) diaromatic and (iii) monoaromatic steroids. The aromatic fraction of a severely biodegraded oil, which yielded high concentrations of protosteranes in the saturate fraction, is discussed separately.

Appendix 10 contains the mass spectra of additional di- and monoaromatic lanosteroids produced during pyrolysis experiments.

Chapter 11: Diagenetic scheme and ecological implications

In this chapter, I discuss plausible biogenic precursors and diagenetic pathways leading to the BCF triterpenoid and steroid inventory. The ecological implications of the new BCF biomarkers are discussed in light of the wider Proterozoic microfossil and contamination-free biomarker record. Plausible source organisms are discussed in the light of early triterpenoid evolution, considering insights from previous analyses of XVIII
biological costs and enzyme divergence, the Phanerozoic and Precambrian steroid record, the Proterozoic steroid record and molecular clock estimates for eukaryotic evolution. The discussion of the potential implications of the new BCF findings for the evolution of triterpenoid cyclases and corresponding organisms focuses on the ecological role of early eukaryotes. The ‘grand diagenetic scheme’ (Figure 11-16 & 11-17 in section 11.3.6) summarises potential biogenic precursor compounds and diagenetic pathways towards the diverse BCF triterpenoids, and also contains inferred fragmentation pathways for most compounds. This scheme is duplicated as the last two pages of the Appendix and can be cut out or copied by the reader to better follow the discussions. Having figures 11-16 and 11-17 at hand, may greatly assist in following the discussions of potential structures, precursors and diagenetic processes in chapters 8 to 11.

Appendix 11 contains ion response factors calculated for each major BCF triterpenoid class in order to improve quantifications and particularly comparability of different compound classes. It also shows concentrations of individual triterpenoid classes for 9 BCF samples.

Chapter 12: Conclusions and outlook:

This chapter synthesises the most important discoveries, conclusions and research highlights of this PhD project. The chapter also reiterates the most significant open questions and discusses meaningful approaches of future research.
1 Introduction, aims and concepts

1.1 Introduction

This thesis investigates the molecular remains of microorganisms that inhabited our planet long before complex, multicellular life evolved. In the Paleoproterozoic, 2.5 to 1.6 billion years ago (Ga), the oldest interval of Earth’s Middle Age, our planet still had an alien appearance. It would take almost another billion years until primitive animals would colonise the oceans and even longer still until plants grew on land. The Proterozoic was thus the realm of microorganisms. The absence of grazing organisms favoured the formation of microbial mats in many settings. Rocky stromatolites grew in shallow, light-filled waters, often forming complex reef systems, while flat phototrophic microbial mats may have covered other parts of the sun-lit seafloor. Even the sediments of deep waters may often have been covered by non-phototrophic microbial mats that lived off infalling organic matter and local redox gradients, or fixed inorganic carbon chemoautotrophically. Bacteria and archaea were presumably the dominant forms of life, while the ecological role and phylogenetic state of eukaryotes in the Paleoproterozoic is still controversial.

Atmospheric oxygen concentrations were low, possibly lower than 1% of present levels (e.g. Planavsky et al., 2014), and only the upper mixed layer of the oceans can be expected to have been mildly oxygenated. The deeper waters were likely largely oxygen-free and rich in dissolved iron, with marine euxinia being more widespread than on the modern Earth (Poulton and Canfield, 2011). Euxinic waters, rich in toxic hydrogen sulphide have been inferred in particular for the McArthur Basin, a shallow sea that covered parts of what is now northern Australia. Based on molecular fossil evidence, green and purple sulphur bacteria inhabited the Paleoproterozoic seas. Even a Proterozoic Purple Ocean was hypothesised, which would have been tinted by the abundant purple pigments of sulphide oxidising Chromaticeae (Brocks et al., 2005). Based on comparisons with their modern relatives that live in the euxinic waters of many stratified lakes, these bacteria may have oxidised hydrogen sulphide using the energy of the sun light captured with the help of their characteristic colourful accessory pigments (Brocks and Schaeffer, 2008).
This study revisits the Barney Creek Formation (BCF) sediments that were deposited ~1640 million years ago in the McArthur Basin. Over the last decades, advances in paleo-redox proxy research changed our understanding of Proterozoic redox conditions. Earlier interpretations of fully oxygenated oceans were challenged by the ‘Canfield Ocean’ hypothesis that predicted sulphidic deep marine waters (Canfield, 1998). The BCF molecular fossils of green and purple sulphur bacteria discussed above provided important support for this widely accepted hypothesis. However, the refinement of the Fe-speciation proxy led to another paradigm change in palaeo-redox research as it was revealed in recent years that iron-rich (ferruginous) instead of hydrogen sulphide-rich (euxinic) waters dominated the Proterozoic oceans (Poulton and Canfield, 2011, Planavsky et al., 2011). Locally, ferruginous conditions were also inferred for the BCF sediments by iron speciation analyses (Planavsky et al., 2011), seemingly contradicting previous biomarker interpretations of photic zone euxinia. Thus, the detailed temporal and spatial redox conditions of the BCF waters remain unresolved. (Brocks et al., 2005).

It is now known that not only purple- but also green sulphur bacteria are able to thrive in anoxic iron-rich and not necessarily euxinic waters (Crowe et al., 2008). Was the McArthur Basin ferruginous and the phototrophic sulphur bacteria represent the first evidence of phototrophic iron oxidation in the rock record or did ferruginous and euxinic conditions both occur in the McArthur Basin waters separated in space and/or time? If there was a spatial or temporal variability in local redox conditions, how would this have impacted the microbial communities? The BCF hosts giant stratiform and potentially syngenetic Zn/Pb deposits like HYC McArthur River. Understanding the basinal paleo-redox conditions during ore formation may also help to understand the formation of these deposits, possibly assisting future metal exploration.

However, not only is our picture of the Proterozoic redox landscape constantly evolving, but the same can be said about our understanding of early life and eukaryotic evolution. In case of the Precambrian biomarker record, interpretation is not only impeded by the antiquity of the sediments that experienced several geologic events that may have overprinted original signatures, but also by anthropogenic contamination. Contamination of rock samples with petroleum products particularly during drilling, but also during sampling, handling and storage greatly obfuscate the original biomarker signatures (Brocks et al., 2008, Jarrett et al., 2013, Schinteie and Brocks, 2014).
Precambrian biomarkers are thus in the centre of heated scientific debates and previous reports of Archaean biomarkers have been refuted (French et al., 2015, Brocks, 2011). Similar problems exist for the post-Archaean sterane record as most Pre-Ediacaran steranes reported so far resemble Phanerozoic assemblages and a probable contamination-source should be considered until the syngeneity is unambiguously proven (Pawlowska et al., 2013). This leads to a puzzling gap in the geological record of microfossils and molecular fossils (biomarkers) of eukaryotes. Steranes are characteristic biomarkers of eukaryotes, but the oldest clearly indigenous steranes do not occur until ~820 Ma (Brocks et al., 2017), while the oldest probably eukaryotic microfossils date to ca. 1.8-1.6 Ga (Knoll, 2014). The apparent discrepancy in molecular- and microfossil evidence for eukaryotes and the inferred low activity of eukaryotes in most recorded environments is commonly attributed to taphonomic biases (Pawlowska et al., 2013) and the restriction of early eukaryotes to oxygenated niches or nutrient-rich near-shore environments (Javaux et al., 2001), nutrient limitations (Anbar and Knoll, 2002), or hydrogen sulphide poisoning in largely anoxic oceans (Poulton et al., 2010, Brocks et al., 2005). Even more puzzling is the occurrence of a unique assemblage of dominantly 4-methylated triaromatic steroids in BCF sediments that is currently attributed to a possible bacterial source (Brocks et al., 2005), but the apparent absence of saturated steroid equivalents from the same samples has to our knowledge not been observed in any other formation. Non-methylated triaromatic steroids that occur in low relative concentrations could be diagenetic degradation products of methylated steroids or be derived form cholestane-type steroids of crown group eukaryotes like red algae. One of the biggest questions regarding the BCF biomarker record is thus why there appear to be no saturated equivalents of the characteristic triaromatic steroids. Will it be possible to tie the unusual triaromatic steroids to more specific biogenic precursors?

This PhD thesis revisits the BCF biomarker inventory and provides one of the most detailed combined biomarker and iron speciation analyses conducted to date, allowing unprecedented insights into the dynamicity and potential environmental controls of Proterozoic redox conditions. The most detailed analysis of BCF triterpenoids to date further allows unprecedented insights into the early evolution of triterpenoids. The
detailed analyses of unusual steroid derivatives may provide the first molecular glimpse of our early eukaryotic ancestors.
1.2 Aims of thesis

This thesis aims at reconstructing the paleo-ecology of the McArthur Basin 1.64 Ga ago through a multi-proxy approach encompassing organic and inorganic geochemistry, petrographic and mineralogical analyses. The first main aim is to reconstruct the paleo-redox conditions as primarily elucidated by iron speciation analyses and complemented by trace metal, isotope and biomarker analyses. The main aim is to identify microbial communities and metabolisms of the Paleoproterozoic ecosystem through biomarker analysis. In this regard, the triterpenoid and steroid records are of particular importance. One of the major aims is to find out if any unusual triterpenoids were overlooked in previous studies and if the biogenic precursors of the unusual triaromatic BCF steroids can be inferred. Another aim is to compare redox reconstructions to the paleo-ecology as reconstructed from biomarker analyses. Is there a link between prevailing redox-conditions and microbial communities and if so, which environmental factors controlled these Paleoproterozoic ecosystems? Another aim is to test if new paleo-ecological reconstructions can help in understanding stratiform base metal mineralisation and if Fe-speciation or organic analyses may assist future base metal exploration.
1.3 Overview of important scientific concepts

1.3.1 Molecular fossils as biomarkers

*Molecular biological markers, or biomarkers, are natural products that can be assigned to a particular biosynthetic origin* (Brocks and Summons, 2003).

Most of the organic matter produced by living organisms is recycled in trophic processes, but a small fraction can get incorporated into sedimentary rocks. Biomolecules like the lipids of cell membranes are subject to diagenetic and geologic/thermal alterations when deposited in sediments. The functionalised biolipids are reduced to hydrocarbon skeletons that can retain much of the biological information and can contain enough diagnosticity to be considered molecular fossils or biomarkers (e.g. Brocks and Pearson, 2005 and references therein). The biomarker concept goes back to Alfred Treibs who thus became ‘the father of organic geochemistry’. In the 1930s he recognised that plant-derived chlorophyll-derivatives can be found in petroleum, confirming the biological origin of petroleum (Treibs, 1934).

Steroids, like cholesterol, are mostly produced by eukaryotic organism. If eukaryotic organisms like red algae get buried in marine sediments, the functionalised biomolecules that are not eaten by microorganisms lose their functional groups upon diagenetic/geologic alteration (e.g. Brassell and Eglinton, 1981). Cholesterol, for example would lose the hydroxyl-group and double bond to form the sterane hydrocarbon cholestane (Figure 1-1) (Mackenzie et al., 1982). Steranes, the hydrocarbon skeletons of steroids can thus be regarded as a biomarker for eukaryotes. Importantly, hydrocarbon biomarkers can be preserved for billions of years if enclosed in intact sedimentary rocks with a mild thermal history (Brocks and Pearson, 2005). Deposited organic matter can be buried underneath thousands of meters of sediments that are turned into sedimentary rocks under the influence of elevated pressure and temperature. Cholestane and other stable hydrocarbon skeletons derived from the source organisms can survive these harsh conditions. Besides microfossils encompassing fossilised, i.e. silicified, remains of microorganism, and isotopic signatures, molecular fossils or biomarkers are among most powerful means of studying life in the Proterozoic and are therefore employed in this study of BCF ecology.
1.3.2 The thermal maturity concept

Functionalised biomolecules and even saturated hydrocarbons are sensitive to heat. This is one of the challenges, but also one of the major applications of biomarker research. During geological heating, hydrocarbons are converted into thermally more stable derivatives such as progressively more aromatised biomarkers (e.g. Mackenzie et al., 1981) and higher molecular weight hydrocarbons get cracked to lower molecular weight compounds, losing all or most of their biological specificity (e.g. Seifert and Michael Moldowan, 1978). Beyond the so called ‘oil window’ most solvent extractable hydrocarbons have been cracked and expelled leaving only insoluble kerogen (see e.g. Peters et al., 2005 and references therein). It is thus crucial for biomarker preservation that the sedimentary rocks were never heated significantly above the oil window or otherwise the preservation of diagnostic molecular remains cannot be expected. A crucial part of syngeneity assessment is thus to confirm that the bitumen maturity is consistent with the thermal maturity of the host rock – otherwise most or all of the bitumen probably entered the rock at a later stage (allochthonous origin), usually by migration of younger oils through the strata or by sample contamination with anthropogenic hydrocarbon products during drilling, sawing, storage, sampling or handling.

Originally identical sediments that have been heated to varying degrees would exhibit different biomarker signatures. It is therefore important to know the thermal maturity of sedimentary rocks in order to estimate which biomarker signatures are related to ecology and which are induced by thermal maturation. At the same time, different molecular ratios such as those of sterane or hopane isomers, aromatic steroids,
phenanthrenes or diamondoids can be used to assess the thermal maturity of crude oils or sedimentary rocks (e.g. Mackenzie et al., 1981, Mackenzie et al., 1980, Seifert and Michael Moldovan, 1978, Tissot and Welte, 1984, Radke, 1988, Chen et al., 1996). This information is crucial for thermal maturity reconstructions employed in the exploration for hydrocarbon deposits as source rocks need to reach the oil window for significant petroleum accumulation, and the thermal maturity of a sedimentary rock informs petroleum geologists if oil or gas discoveries can be expected. The organic geochemical studies are thus also of interests for petroleum exploration that is currently active in the McArthur Basin.

There are different approaches to assess the thermal maturity of sedimentary rocks and oils. Most important for this study are biomarker and source rock analyses. In the later, rock powder is heated in an inert atmosphere and it is measured how much carbon compounds are released at different temperatures (free HCs, bound HCs, CO2) (e.g. Peters, 1986). Most important as a maturity indicator is the temperature at which the largest amount of hydrocarbons is released from the rock (Tmax) (Espitalié, 1986). Since different hydrocarbons have different thermal stabilities, the ratios of thermally more stable to less stable compounds are often used to estimate the degree of thermal stress a rock has been subjected to. Important for Proterozoic studies are phenanthrene based proxies like the methylphenanthrene index (MPI-1 = 1.5*(2MP + 3 MP)/(P + 1MP + 9MP)) (Radke, 1988), triterpane-ratios like the norhopane Ts/Tm ratio (Ts/Tm = 18α(H) 22, 29, 30 trisnorhopane/17α(H) 22, 29, 30 trisnorhopane) (Seifert and Michael Moldowan, 1978) and the diamondoid ratios methyl-adamantane index (MAI = 1MA/(1MA + 2MA) and dimethyl-adamantane index (DMI = 4MD/(1MD + 3MD + 4MD) (Chen et al., 1996).

1.3.3 The Fe-speciation concept

Iron Speciation analyses can be used to evaluate palaeodepositional redox conditions. To do this end, iron is extracted from different pools of a sedimentary rock: pyrite (FePy), magnetite (FeMag), ferric oxides (FeOx) and carbonate minerals (FeCarb). The highly reactive iron (FeHR) can react to form iron sulphides and eventually pyrite (FeS2) either in the water column or during early diagenesis. FeHR includes FePy, FeMag, FeOx and FeCarb (Poulton et al., 2004). Pyrite is measured gravimetrically by releasing H2S
from FeS₂ in a boiling chromous chloride solution and re-precipitation as AgS in silver nitrate. The AgS can also be used to measure the sulphur isotopic composition of bulk pyrite. FeCarb, FeOx and FeMag are released sequentially by shaking sediment powder first in a sodium acetate (at 50°C for 48 hours), then a sodium dithionite (2 hours at room temperature) and finally an ammonium oxalate solution (6 hours at room temperature). Extracted iron concentrations are then measured by ICP Atomic Emission Spectroscopy (AES) or Atomic Absorption Spectroscopy (AAS). The total iron content of the sample (FeT, comprising FeHR as well as Fe associated with silicate minerals) is measured separately, either by elemental analysis like X-ray Fluorescence (XRF) or a hot HCl digestion procedure.

Figure 1-2 illustrates the use of Fe-speciation analyses to distinguish oxic, ferruginous and euxinic depositional conditions. The ratio of FeHR/FeT can be used to distinguish between oxic and anoxic depositional conditions (Poulton et al., 2004). FeHR/FeT of sediments deposited beneath anoxic bottom waters usually exceeds 0.38 (Raiswell and Canfield, 1998, Poulton and Raiswell, 2002). If anoxic conditions prevailed (FeHR/FeT > 0.38), the ratio of FePy/FeHR can further be used to distinguish sulphidic (H₂S-rich) from ferruginous (Fe(II)-rich) conditions. This is because H₂S is highly reactive towards Fe(II) forming pyrite, effectively titrating iron from the water column. A ratio of FePy/FeHR ≥ 0.7 is usually indicative of euxinic depositional conditions ([H₂S]>[Fe(II)]), FePy/FeHR < 0.7 usually indicates ferruginous conditions (März et al., 2008).

**Figure 1-2.** Fe-speciation as a tool to distinguish oxic, ferruginous and euxinic depositional conditions (after Poulton and Canfield, 2005, März et al., 2008). FeHR = Highly Reactive Iron (carbonates, Fe-III oxides, magnetite, pyrite), FeT = Total Iron (all iron, including siliciclastic), FePy = Pyrite Iron.
1.4 References


BROCKS, J. J. & SCHAEFFER, P. 2008. Okenane, a biomarker for purple sulfur bacteria (Chromatiaceae), and other new carotenoid derivatives from the 1640 Ma Barney Creek Formation. Geochimica et Cosmochimica Acta, 72, 1396-1414.


2. Methodology and samples

2.1. Samples

This study focuses on samples from the ~1.64 Ga BCF in the McArthur Basin, although a few adjacent formations were also analysed. The BCF consists of carbonaceous, dolomitic siltstones and shales with variable carbonate content and was probably deposited below wave base in an intracratonic marine basin, representing the deep-water-facies of a Paleoproterozoic carbonate platform. A detailed description of the BCF sedimentology was provided by Bull (1998).

Our samples come from cores drilled across the southern McArthur Basin. Cores were stored, sometimes for decades, in the Northern Territory Geological Survey drill core store in Darwin, NT. Samples are usually quarter cores or pieces thereof. Most of the samples were collected by Benjamin Nettersheim and Jochen Brocks, and cut with a regular, non-combusted rock saw in Darwin. Samples were then crushed at ANU with a stainless-steel puck mill to a fine powder. Where possible, aliquots of the same non-extracted powder were used for biomarker and inorganic analyses. If not possible, I tried to obtain representative material from as close as possible to those parts of the samples employed for the other analyses.

Drill core locations are shown in Figure 2-1. The most important samples employed in this study come from drill core GR7, where the following samples were used for biomarker and inorganic analyses. Biomarker analyses by Jochen Brocks: 38.7, 41.8, 45.35, 50.3, 77.2, 101.6, 116.0, 126.4, 151.1, 180.0, 238.8, 252.9, 426.7, 638.54 and 870 m. Biomarker analyses by Benjamin Nettersheim: 47.55, 67.14, 71.65, 82.95, 90.3, 106.28, 162.85, 199.08, 218.1 and 328.8 m. Biomarker analyses included in this study were also conducted for drill core MY4 (12Z083, 103.3 m depth), LV09001 (12B117, 382.2 m) (chapter 8). Interior-exterior extracts of samples B03200 (GR7, 683 m), B03224 (GR7, 870 m), B04016 (HYC deposit) and B03132 (McA5) prepared by Jochen Brocks are also considered in chapter 8. In addition to B03162, samples from 683 and 870 m (GR7), B03016 (HYC), B03288 (LY1), B03132 (McA5) extracted by Jochen Brocks were also considered in chapter 7. Sample 12Z083 from 103.3 m depth (MY4), comprising solid bitumen filling an open vug in the Coxco dolomite directly underlying the BCF, is also
discussed in more detail in chapter 9. For comparison, extracts from drill cores GR5, GR11, MY4, MY5, McA5, LY1, BB5, WM6, LV09001 and HYC analysed by Benjamin Nettersheim and Jochen Brocks were also considered in chapter 9.
Figure 2-1. Geologic formations and structural elements of the southern McArthur Basin, locations of drill cores included in this study and thermal maturity of Barney Creek Formation sediments in selected drill cores (modified from Crick, Boreham et al. 1988).
A total of 190 samples were subjected to Fe-speciation analysis, without replicates, a total of 161 samples was analysed, including 110 from the BCF proper. FeHR/FeT was determined for 158 and FePy/FeHR for 155 samples. For the BCF proper, FeHR/FeT was determined for 109 samples and FePy/FeHR for 107 samples. These samples span different stratigraphic horizons and come from ten drill cores (Warramara 6: 25 samples, Myrtle 4: 23, McArthur 5: 19, LV09001: 26, Glyde River 7: 37, Glyde River 10: 2, Glyde River 5: 1, Cow Lagoon: 1 and Bing Bong 5: 18 samples), forming a north-south transect along the Emu Fault zone (Figure 2-1). More details are provided in chapter 5 and Appendix 5.1.

2.2. Iron speciation

As part of this project, an iron speciation set-up was installed at ANU. However, most of the iron speciation data presented in this study were measured by the author and Romain Guilbaud in Simon Poulton’s iron speciation laboratory at Leeds University (UK).

Iron speciation analysis consists of three parts, analysis of total iron content (FeT), carbonate (FeCarb), oxide (FeOx) and magnetite (FeMag) iron, and determination of pyrite iron (FePy). In this study, FeT of sedimentary rock samples was measured as part of a more comprehensive elemental analysis. Rock samples were crushed at ANU with a stainless-steel puck mill (Rocklabs Ltd, New Zealand) in order to be able to use aliquots of the same powders employed for biomarker analyses. Considering the high Fe content of most BCF samples, potential trace iron contamination from mill abrasion was considered negligible. The elemental data were measured by Geosciences Australia according to their standard procedures. Most samples were oxidised with LiNO₃ and taken up to temperature slowly to retain S. Fused beads were prepared by heating the rock powered with a lithium borate flux to 1050°C in Pt crucibles, and beads were then analysed on a Philips PW2404 4kW sequential wavelength dispersive spectrometer fitted with a rhodium X-ray tube. Results were verified using certified reference materials that were analysed with each batch of samples. Replicate analyses of three different BCF
samples gave a relative standard deviation (RSD) of 1.9% for Fe₂O₃. For some samples, multi-element assay was performed at Labwest Geoservices in Australia using a microwave-assisted multi-acid digestion with HF (MMA-04) followed by ICP-OES/MS analysis for 60 elements (see Appendix 2 for list of elements and detection limits) including many trace metals. Certified reference materials were used for quality control. Duplicate analyses of three different BCF samples yielded FeT RSD = 4.2%. Comparability of FeT analyses conducted by Geoscience Australia (GA) was confirmed by analysing one sample in duplicate at GA (RSD = 1.1%) and once at Labwest (for the three analyses average FeT = 2.2%, total RSD = 0.75%).

Iron bound in three different pools is extracted sequentially in an incubator shaker (Poulton and Canfield, 2005). First, the iron bound in carbonates (Fe₉⁸) is extracted for 48h with a sodium acetate solution buffered at pH 4.5 at 50°C. Then, the Fe(III)oxides are extracted for 2h employing a sodium dithionite solution buffered to pH 4.8. Finally, the magnetite bound iron is extracted with an ammonium oxalate solution buffered at pH 3.2 for 6h (Poulton and Canfield, 2005). Iron concentrations are then measured for each extract using atomic absorption spectroscopy (AAS) at Leeds University. Standard deviation for 5 ppm Fe reference solutions was 2.2%. Fe-speciation in the Poulton laboratory usually has a relative standard deviation RSD <4% for each step, leading to <8% for calculated FeHR, which is comparable to the precision obtained by other laboratories (Guilbaud et al., 2015). Reproducibility was comparable in our study with non sulphidised fraction of FeHR (Fe₉⁸ + Fe₀ + Fe₉⁷) yielding a RSD of 6.3% for duplicate analyses of seven different samples.

The amount of iron bound in pyrite is measured indirectly by dissolving the pyrite in a hot chromous chloride solution and subsequently precipitating the released sulphur as silver sulphide in a silver nitrate solution (Canfield et al., 1986). The silver sulphide is filtered, dried and weighed and pyrite iron (FePy) calculated using the stoichiometry FeS₂. A distillation with 8mL 6N boiling HCl prior to the Cr(II)Cl₂ oxidation ruled out the potential presence of acid volatile sulphides. We used ca. 1g of rock powder for carbonate-poor and up to 6g of rock powder for very carbonate-rich samples. Three replicate analyses for pyrite extractions yielded an average RSD of 8.4%, while two replicate analyses for the total FeHR determination gave an average RSD of 6.6% for
FePy/FeHR ratios. For the replicates of these two samples, FeHR/FeT values gave RSD = 1.3%, although FeT was only measured once, so actual RSD be slightly higher if uncertainties in FeT determination are taken into account.

Iron speciation has been calibrated based on modern environments and geological samples for which redox conditions have been well constrained by other proxies, and according to other authors can be extrapolated to the Proterozoic (see e.g. Poulton and Canfield, 2011). The paleo-redox conditions are reconstructed using two iron ratios: The ratio of highly reactive to total iron (FeHR/FeT) and the ratio of pyrite to highly reactive iron (FePy/FeHR) (see e.g. Poulton and Canfield, 2011). Highly reactive iron is all the iron that can react to form pyrite in biological and diagenetic processes (FeCarb + FeOx + FeMag) as well as iron bound in pyrite (FePy).

The ratio of FeHR/FeT can be used to distinguish between oxic and anoxic depositional conditions (Poulton et al., 2004). FeHR/FeT of sediments deposited beneath anoxic bottom waters usually exceeds 0.38, while oxic conditions commonly lead to FeHR/FeT < 0.2 (Raiswell and Canfield, 1998, Poulton and Raiswell, 2002). If anoxic conditions prevailed (FeHR/FeT > 0.38), the ratio of FePy/FeHR can be used to further distinguish sulphidic (H₂S-rich) from ferruginous (Fe²⁺-rich) conditions. This is because H₂S is highly reactive towards Fe(II). The formation of Fe-sulphides from freely available Fe(II) and H₂S effectively titrates iron from the water column. Observations from the Black sea suggest that FePy/FeHR = 0.8 constrains the upper limit of ferruginous deposition (Anderson and Raiswell, 2004) and is an often used threshold for euxinia ([H₂S]>[Fe(II)]). However, the extraction scheme used in earlier studies does not fully quantify FeCarb or FeMag, and the application of the refined iron speciation analysis to Phanerozoic sediments suggests that an FePy/FeHR threshold of 0.7 is more to indicative of euxinia (März et al., 2008). According to a recent calibration for carbonate samples, FeHR/FeT ratios are only reliable for samples with FeT > 0.5 wt% (Clarkson et al., 2014), which is the vast majority of BCF samples.
2.3. S-isotopes

Pyrite S isotope compositions were determined (via EA-IRMS at Leeds University) on Ag₂S precipitates from the chromous chloride extractions. All data are reported relative to the Vienna Canyon Diablo Troilite standard.

2.4. Total organic carbon, kerogen δ¹³C and source rock analyses

Powdered rock samples were decarbonised with hydrochloric acid (HCl) in several steps starting with 2mL 50vol% conc. HCl. After no more bubbles were visible, samples were centrifuged, decanted and 2ml conc. HCl added. This was repeated twice. Then 8mL conc. HCl were added and samples were left for 2 days to make sure all carbonate dissolved, before samples were washed ten times with distilled water. Samples were dried at 60°C overnight and weighted to correct TOC for carbonate dissolution, then ~10-50 mg of powder (to yield ~300μg carbon) was weighed into small tin cups. Vanadium oxide was added to boost combustion. ¹²C and ¹³C abundances were measured at ANU with a Sercon 20-22 isotope ratio mass spectrometer (IRMS) connected to an ANCA-GSL Elemental Analyzer operating in continuous flow. This allowed determination of δ¹³C_(org) as well as the Total Organic Carbon (TOC) content. Samples were normalized to the Vienna Pee Dee Belemnite (V-PDB). The RSD of δ¹³C_(org) is 1.0% (standard deviation 0.25‰) based on an alanine standard (n = 11) and 0.3% for duplicate analyses of two BCF samples. The relative standard deviation of TOC analysis is 7.1% for the carbon-rich alanine standard (40.5% TOC) and is 6.8% for duplicate analysis of two BCF samples with 2% and 3.6% TOC.

Source rock analyses, the equivalent to Rock Eval analyses, were conducted commercially according to standard procedures at Adelaide University using a Source Rock Analyser (SRA TPH Workstation, Weatherford Laboratories Instrument Division). Samples are purged in helium before being raised into desorption furnace at 300°C for 3 minutes, resulting in the release of free hydrocarbons (S1). The sample is then ramp to 600°C at 25°C/min to release bound hydrocarbons (S2). Hydrocarbons are detected by FID and quantifications are calibrated against a certified reference material of known S1 and S2 response.
2.5. Mixing simulation

To test if simple two endmember mixing can explain the data distributions observed in the upper BCF, a two-endmember mixing was simulated in Microsoft Excel. Plausible endmember configurations were iteratively tested by adjusting the concentrations of individual variables to fit the BCF data distributions with the aim of simultaneously fitting the mixing lines as closely as possible to 60 different mixing plots (all shown in Appendix 5.2). Components from both endmembers were mixed according to a mixing ratio. Proxy ratios such as Pr/Ph or Ti/Ca were calculated after mixing of the individual components (e.g. Pr, Ph, Ti and Ca). The biomarker concentrations are dimensionless and only ratios considered due to post-mixing effects as discussed in chapter 5. All mixing plots, compositions of endmember and simulated mixed samples and an example of how values were calculated in the simulations are given in Appendix 5.2.

2.6. Biomarker analyses

2.6.1. Previous biomarker analyses by Jochen Brocks

Included in this study were biomarker analyses previously conducted by Jochen Brocks. In addition, some interior-exterior and a slice extraction experiment (GR7, 45.35 m) were conducted by Jochen Brocks (Brocks et al., 2008). Included in this study were the following previously analysed samples from GR7: 38.7 m, 41.8 m, 45.35 m, 50.3 m, 77.2 m, 101.6 m, 116 m, 126.4 m, 151.1 m, 180 m, 238.8 m, 252.9 m, 426.7 m, 683.54 m and 870 m.

2.6.2. New biomarker analyses

Biomarker syngeneity was tested by quantifying biomarkers in extracts of the slice extraction experiment described above and confirmed by their absence of exterior fractions of mature to overmature BCF samples subjected to interior-exterior experiments as described above.
2.6.3. Bitumen extraction

Rock samples were crushed with a stainless-steel puck mill (Rocklabs Ltd, New Zealand). Between samples, the mill was cleaned with solvent grade dichloromethane (DCM), methanol (MeOH) and hexane (HEX) and combusted (600°C, 9h) quartz sand. Annealed quartz sand was also used as a procedural blank with each batch of biomarker analysis. Bitumen was extracted with 100% DCM from the rock powders by using a Dionex Accelerated Solvent Extractor (ASE 200, USA). Total lipid extracts were reduced under a stream of pure nitrogen gas and fractionated on a micro-column of annealed (300°C, 12h) and dry packed silica gel (Silica Gel 60; 230-600 mesh; EM Science). Saturated hydrocarbons were eluted with 1.5 dead volumes (DV) of n-hexane, aromatics with 2 DV n-hexane:DCM (4:1 v:v) and the polar fraction with 3 DV DCM:methanol (1:1 DV). 18-methyl-eicosanoic acid methyl-ester (18-ME ME; Chiron Laboratories AS) was added to the saturated and aromatic fraction, while d4-C29ααα-ethylcholestane (D4; Chiron Laboratories AS) was added to the saturates only.

2.6.4. Gas chromatography–mass spectrometry

Gas chromatography–mass spectrometry (GC-MS) analyses were carried out on an Agilent 6890 gas chromatograph coupled to a Micromass Autospec Premier double sector mass spectrometer (Waters Corporation, Milford, MA, USA). The GC was equipped with a 60 m DB-5 MS capillary column (0.25 mm i.d., 0.25 mm film thickness; Agilent JW Scientific, Agilent Technologies, Santa Clara, CA, USA), and helium was used as the carrier gas at a constant flow of 1 mL min⁻¹. Samples were injected in splitless mode into a Gerstel PTV injector at 60 °C (held for 0.1 min) and heated at 260 °C min⁻¹ to 300 °C. For full-scan, MRM, and SIR analyses, the GC oven was programmed from 60 °C (held for 4 min) to 315 °C at 4 °C min⁻¹, with total run time of 90 to 120 min (see also (2013)Jarrett et al. (2013)). All samples were injected in n-hexane to avoid deterioration of chromatographic signals by FeCl₂ build up in the MS ion source through use of halogenated solvents (Brocks and Hope, 2014). Mass spectra shown in the thesis are generally background subtracted (i.e. may contain subtraction artefacts).
2.6.5. Pyrolysis experiments and hydrogenation experiments

Similar to the procedure described by Alexander et al. (2011) and Berwick et al. (2011), ca. 10 mg of activated carbon (heated at 340°C in air for at least two hours to remove water) is inserted into a glass tube with ~3 mm i.d. About 1 mg of organic substrate is added and the tube sealed under vacuum. For pyrolysis experiments, the tubes are placed in an oven set between 180 and 340°C for 1 to 89 h depending on the experiment. After cooling to room temperatures, tubes are broken open, pyrolysis products extracted with DCM, and the charcoal filtered through a Pasteur pipette with glass wool plug with ~4 mL hexane and DCM. The pyrolysate is blown down under a stream if nitrogen and transferred into 2 mL vials for GC-MS analysis in hexane, without prior fractionation. Pyrolysates are analysed by full scan or MRM GC-MS analysis. For mineral catalysis, Montmorillonite K10 clay (courtesy of P. Adam and P. Schaefer) and common lime were used.

Hydrogenation was performed in 4 mL vials filled with hexane with a small hydrogen stream bubbling through. Either Pt/C or PtO₂ are used as a catalysed and hexane or DCM with acetic acid as solvents depending on the experiment. A stirring bar is used to agitate the solution. For longer experiments the hydrogen stream is stopped after 2-4 hours and vials are closed with a lid (and Teflon tape) and continue stirring until the end of the experiment (e.g. after 70 h). After hydrogenation, the catalyst is washed like the activated carbon with at least ten rinses of DCM and hexane (ca. 2 mL total) and sonicated for better recovery. The sample is blown down under a stream of nitrogen and transferred in hexane for subsequent GC-MS analysis. In between samples, the capillary is washed in a DCM and hexane solution, before H₂ is bubbled through a hexane bank solution for at least 30 min. to avoid cross-contamination. Blanks are stored for future analyses if potential cross contamination is suspected. For acidic hydrogenation experiments, PtO₂: hydrogenation was conducted in ~2 mL DCM to which 5 drops of acetic acid were added with a Pasteur pipette.

2.6.6. Cyclopropane-ring opening experiments with HCl gas

A small-scale adaption of the HCl gas production method of Arnaiz was used to react HCl gas with cycloartenol, cycloartane and a biodegraded oil from the McArthur Basin. To generate HCl gas, conc. HCl was dripped onto CaCl₂ and the generated HCl gas...
bubbled into a 4 mL vial filled with the organic substrate dissolved in ca. 2 mL DCM as schematically illustrated in Figure 2-2 (see also Arnáiz, 1995). The 4-mL vial was connected with a GC column to another 4-mL neutralising vial filled with water and lime powder. After being flushed several times with HCl gas, the reactant vial was capped and stirred with a magnetic stirring bar overnight. DCM was washed with water (4 times) and dried over an NaSO₄ column, transferred into a 2-mL vial and replaced with hexane prior to GC-MS analysis.

![Figure 2-2. Schematic of HCl ring opening experiment.](image)

### 2.7. Mineralogical and elemental analyses

X-ray powder diffraction (XRPD) analysis was carried out on ground bulk-rock samples with a SIEMENS D501 Bragg-Brentano diffractometer equipped with a graphite monochromator and scintillation detector, using CuK radiation. The scan range was 2 to 70° 2-theta, at a step width of 0.02°, and a scan speed of 1° per minute. The results were interpreted using the SIEMENS software package DiffracplusEva 10 (2003) for identification, and Siroquant V3 for quantification (Bruker AXS, 2003). A clay separation procedure was applied to four samples to determine the clay content more accurately. Since the clays were similar in all samples, we used these clays for quantification of all other samples from the core. Amorphous content was determined by comparison to an Aluminium standard.

Elemental analysis was conducted by Liz Taylor at Geoscience Australia according to standard XRF procedures, and by Labwest employing the microwave-assisted total
digestion technique MMA-04 for 60 elements (see Iron speciation section for details). Relative standard deviations for XRF duplicate analyses of three different BCF samples yielded relative standard deviations of 0.48% for SiO₂, 0.64% for TiO₂, 0.57% for Al₂O₃, 0.67% for Fe₂O₃, 0.88% for MnO, 0.44% for MgO, 0.46% for CaO, 0.55% for K₂O, 2.64% for SO₃ and 8.5% for Zn (average 44 ppm). Labwest duplicate analyses of three different BCF samples yielded relative standard deviation of 2.3% for Al, 4.5% for Ca, and 65% for Zn. Mg yielded very high deviations with RSD = 32%. However, two samples had Mg contents < 0.5wt%, and duplicate analyses of the sample with significant Mg content (3.2%), which is more representative of most BCF samples, yielded a RSD of 0.44%.
2.8. References


3 Geology

3.1 Introduction

The BCF is located within the Proterozoic McArthur Basin on the north Australian Craton. The craton is host to well-preserved late Paleoproterozoic to early Mesoproterozoic sedimentary sequences. These sequences are of significant economic and scientific interest as they contain some of the world’s largest sedimentary Pb-Zn deposits (reproduced from Leach et al., 2010, Bull, 1998), show significant petroleum potential (Crick et al., 1988) with ongoing hydrocarbon exploration, contain the oldest clearly syngenic biomarkers on Earth (Pawlowska et al., 2013), and have been of great importance for elucidating Paleoproterozoic redox conditions (Brocks et al., 2005, Planavsky et al., 2011, Shen et al., 2003). A variety of models have been proposed for the plate tectonic framework. One of the most recent models proposes that the intracratonic north Australian sedimentary basins developed in a wide region of intermittently extending continental crust in the overriding plate at a complex convergent plate boundary that developed synchronously along the southern margin of the North Australian craton (Giles et al., 2002). According to Giles and colleagues (2012), the tectonic setting can thus essentially be considered as a far-field continental backarc setting.

The McArthur Basin is located near the eastern edge of the North Australian Craton. It contains mainly late Paleoproterozoic to early Mesoproterozoic sedimentary rocks that form a ~5-15 km thick platform cover sequence comprising a mixed carbonate-siliciclastic succession with minor volcanics near the base. Basin exposures cover ~180,000 km² in a roughly north-northwest trend along the west coast of the Gulf of Carpentaria. The McArthur Basin is surrounded by older Paleoproterozoic basement rocks of the Arnhem Inlier in the north, the Pine Creek Inlier in the northwest and the Murphy Inlier in the southeast (Rawlings, 2004) (Figure 3-1).
The McArthur Basin is thought to have been part of the Mt Isa Superbasin that also comprises the Mt Isa Basin. Both basins contain giant stratiform lead zinc sulphide deposits that are among the biggest in the world (Figure 3-1). The Mt Isa Superbasin hosts about a quarter of the world’s known sediment hosted Pb-Zn reserves (see Fig. 13 in Leach et al., 2005). The largest deposit discovered so far in the McArthur Basin is HYC McArthur River hosted in Barney Creek Formation sediments in the southern McArthur Basin close to the Emu Fault (Figure 3-1, Figure 3-2). There are two main genetic models for HYC, a diagenetic replacement model (e.g. Williams, 1978, Eldridge et al., 1993) and a syngeneric exhalation model, potentially of the Red Sea brine pool-type, which is favoured in recent work (e.g. Large et al., 1998, Ireland et al., 2004b, Ireland et al., 2004a). There remains significant exploration potential, and exploration as well as the understanding of ore genesis at HYC may be aided by a better understanding of the paleo-redox conditions in the McArthur Basin.

The McArthur Basin contains one of the best-preserved carbonate platform sequences of the late Paleoproterozoic. The unmetamorphosed Proterozoic rocks are gently folded
and faulted. The sediments appear to have been deposited in mostly shallow environments in an intracratonic basin that was temporarily dominated by a prominent north-trending fault zone (Jackson et al., 1987).

3.2 Regional geology of the southern McArthur Basin

The focus of this study is on the southern McArthur Basin. As described by Jackson et al. (1987) and shortly summarised below, the sequence is divided by regional unconformities into four stratigraphic groups: The Tawallah, McArthur, Nathan and Roper Group. The stratigraphy of the southern McArthur Basin is summarised in Figure 3-3, a regional geological map of the southern McArthur Basin is shown in Figure 3-2.

Figure 3-2. Regional geological map of the Southern McArthur Basin including position of deep seismic reflection lines (red) (Fig. 2 in Rawlings, 2004).
Figure 3-3. Stratigraphic table for the Southern McArthur Basin (from Rawlings, 2004). Focus of this study highlighted in red.

The ~ 4.5 km thick Tawallah Group unconformably overlies the ~ 1.8 Ga crystalline basement. It mostly comprises weathering resistant quartz sandstone that alternates
with much thinner formations of deeply weathered basic volcanics and fine-grained clastics. Arkosic and conglomeratic sediment of mainly continental origin at the base are succeeded by monotonous orthoquartzites of marine and aeolian origin. Stromatolitic dolostone units similar to those higher in the stratigraphy also occur sporadically. The uppermost rock units comprise potassium-rich felsic igneous rocks (Jackson et al., 1987).

The McArthur and Nathan Groups unconformably overlie the Tawallah Group and have a combined thickness of ~ 5.5 km. They are dominated by evaporitic and stromatolitic formations of cherty dolostones interbedded with dolomitic fine-grained siliciclastics (Jackson et al., 1987). According to Jackson and colleagues (1987), the formations are of shallow water origin and contain evidence of exposure and desiccation. The main depositional environments are considered peritidal, lagoonal, lacustrine and possibly fluvial (Jackson et al., 1987).

The stratigraphy of the McArthur Group in the southern McArthur basin is summarised in Figure 3-3. Potassium-rich tuffs occur at intervals throughout the McArthur Group sequence and over most of the McArthur Basin (Brown et al., 1969). The Nathan Group overlies the McArthur Group and almost all older units with regional disconformity or unconformity. The middle Nathan Group has been dated to 1589±3 Ma. The Nathan Group consists of dolomitic rocks similar to those of the McArthur Group and is thought to have been deposited in a shallow marine or continental sabkha setting. Basal fluvial chert-clast sandstone is overlain by shallow water stromatolitic and ooidal dolostone and minor siliciclastic sandstone (Rawlings, 2004 and references therein).

The ~1429±31 Ma Roper Group rests unconformably on the Nathan Group and various older units. It is in turn overlain by Neoproterozoic to Mesozoic cover. The Roper Group is a widespread cyclic succession of fine- and coarse-grained siliciclastic rocks deposited in a variety of shallow marine, near shore to shelf environments, potentially representing five progradational coarsening upward cycles (Rawlings, 2004 and references therein).
3.3 Geology of the McArthur Group

The McArthur Group is divided by a local unconformity into two parts of similar thickness, the lower Umbolooga and upper Batten Subgroup. Most sedimentary units were deposited in a restricted intracontinental lacustrine to marine setting with variable influence of tidal and supratidal processes (Rawlings, 2004). The stratigraphic subdivision of the McArthur Group is summarised in Figure 3-3. The lithostratigraphic units of the middle McArthur Group, Leila Sandstone, Myrtle Shale, Emmerugga Dolomite, Teena Dolomite, Barney Creek Formation, Reward Dolomite, Lynott Formation and Yalco Formation belong to the River Supersequence, one of nine sequence stratigraphic supersequences of the Mt Isa Superbasin (Southgate et al., 2000). The River Supersequence is a dominantly shallow marine carbonate platform sequence that is subdivided by McGoldrick and colleagues into three higher order sequences. The oldest is the Emmerugga Depositional Sequence, that is followed by the Barney Creek Depositional Sequence which is in turn followed by the Lynott Depositional Sequence (McGoldrick et al., 2010).

According to McGoldrick et al. (2010) each of the three depositional sequence boundaries is marked by distinctive lithofacies-shallowing or local unconformity and has a particular pattern of accommodation and resulting deposition. The Teena Sequence boundary separates the Emmerugga from the Barney Creek Depositional Sequence and is recognised by a sharp transition from deep-subtidal stromatolitic dolostone to shallow-water lithofacies of coarse-grained carbonate grainstone or flat-pebble conglomerate. The lithofacies shallows gradually in the uppermost Barney Creek Depositional Sequence which is commonly referred to as Reward Dolomite (McGoldrick et al., 2010). McGoldrick and colleagues interpret the existing lithostratigraphic units as likely diachronous and time transgressive, arguing against a simple layer-cake succession in the Emmerugga-Barney Creek-Reward depositional sequence. Characteristic facies associations are genetically related and coeval (McGoldrick et al., 2010). The McGoldrick model does however not consider the McArthur Basin seismic survey (Rawlings, 2004) that suggests relatively flat lying and more continuous formations in the southern McArthur Basin that are only locally controlled by the Fault zones (see Figure 3-4).
As in McGoldrick et al. (2010), deposition of the McArthur Group is traditionally regarded as having been fault-controlled and restricted to the Batten Fault Zone (‘Batten Trough’) in the southern McArthur Basin. The Batten Subgroup was thought to be the most restricted stratigraphic group in its distribution due to intensified marginal fault movements (Rawlings, 2004). However, a seismic survey across the southern McArthur Basin did not provide any evidence for either the Batten ‘Trough’ or asymmetric half grabens (Rawlings, 2004). Rawlings (2004) finds that the sedimentary succession appears to continue away from the implied boundary of the ‘Trough’ towards the east and west. He proposes a gently east-dipping carbonate ramp at McArthour Group times as the most plausible geologic model, with third-order subbasins generated along the Emu Fault at specific time intervals (Rawlings, 2004). The interpretation of the seismic data as shown in Rawlings (2004) is reproduced in Figure 3-4. The seismic data support the concept of a steep and complex strike-slip geometry of the Emu Fault zone with sedimentary growth taking place within negative flower structures along the Emu Fault during deposition of the middle McArthur Group (Rawlings, 2004). Rawlings (2004) infers that rapid local deposition and growth were facilitated by differential subsidence of the fault along transtensional releasing bends. This resulted in the formation of local sub-basins that were inverted during post-Roper deformation when these areas were transformed to transpressional restraining bends. Contrary to most literature, the seismic survey further suggests that the McArthur Group continues to the east of the Emu Fault with significant thickness and almost parallel reflections, indicating that the fault did not have first order control on deposition or post-deformational sediment distribution (Rawlings, 2004).
McGoldrick et al. (2010) distinguish 26 individual lithofacies in the McArthur Group that are defined by sedimentary structures and fabrics, component grains and/or microbialite type and morphology. The lithofacies can be grouped into seven genetically related facies associations of unique depositional settings inferred from geometry and interrelationships of associated lithofacies and their dynamics. These are (i) continental, (ii) peritidal, (iii) shallow-subtidal, (iv) deep subtidal, (v) platform margin, (vi) slope, and (vii) basinal facies as described in Table 1 of McGoldrick et al. (2010).

The sequence of sediment types near the Emu Fault is interpreted by Brown and colleagues (1969) to have resulted from a major transgression followed by regression. The change from predominantly subaerial siltstone (Myrtle Shale Member), through mainly intertidal dolomite (Mara Dolomite Member) and shallow marine carbonates (Mitchell Yard and Coxco Dolomite Members) to deeper water shaly carbonates, tuffaceous mudstones, shales with sulphides and locally graded arenites and breccias (Barney Creek Formation) is attributed by Brown et al. to transgression. A return to predominantly carbonate sediments (Reward Dolomite) and local development of
unconformities is interpreted to be due to regression (Brown et al., 1969). The physiography of depositional environments represented by this sequence as inferred by Brown et al. (1969) is shown in Figure 3-5.

Figure 3-5. Schematic physiographic plan of depositional environments (after Fig.3 in Brown et al., 1969)
3.3.1 Barney Creek Depositional Sequence

According to McGoldrick et al. (2010), the lowermost Teena dolomite constitutes a carbonate platform-facies that is succeeded in areas of local subsidence by the transitional red green dolomitic siltstone lithofacies of the W-fold shale Member of the BCF. This is followed by deeper water sedimentation of basinal siltstone to carbonaceous shales of the Barney Creek formation in local structural controlled subbasins according to McGoldrick (2010), and probably across the southern McArthur Basin following Rawlings (2004) interpretation of the seismic survey. In the local depocenters, a sulphide-rich bottom interval of the BCF is often referred to as the HYC pyritic shale member. The BCF is followed by the Reward Dolomite that is considered by Bull (1998) to be at least partly time-equivalent to the BCF.

McGoldrick and colleagues (2010) suggest that variations in lateral thickness and facies throughout the Barney Creek Depositional Sequence delineate a mosaic of fault-bounded depocenters that record tectonically accommodated development along and between traces of the Tawallah and Emu Faults. They infer that a fundamental change from generally stable shallow-marine platform conditions of the lower McArthur Group sedimentation to the complex and highly variable facies mosaic of the Barney Creek Depositional Sequence required foundering and fragmentation of the platform, resulting in additional accommodation space (McGoldrick et al., 2010). According to this interpretation, basin infill in the Batten area comprises a structurally controlled mosaic of facies associations that represent coeval sedimentation in a variety of environments from evaporitic peritidal, shallow marine carbonate platform, slope, to sub-storm wave-base deep marine settings. Shallow platforms are interpreted as carbonate sources that provided fine detritus for the deeper water deposits, whereas platform margin failure and tectonic uplift provided detritus for slope and coarse-grained deeper water deposits (McGoldrick et al., 2010). It is unclear, how the McGoldrick model can be reconciled with the seismic survey that suggests a gently east-dipping carbonate platform (Rawlings, 2004). It would appear that the middle McArthur Group formations are less diachronous than assumed by McGoldrick and that the main effect of the fault activity around Barney Creek Formation times was the formation of local accommodation space resulting in faster and more turbidic infill and generally deeper water deposition, potentially
accompanied by a deepening of the entire carbonate platform. Following Rawlings (2004) interpretation of the seismic survey, deep water sedimentation should not be restricted to local subbasins, but extend to the east beyond the Emu Fault system. The sub-basins are thus likely local and steep subaqueous depressions in an extensive marine carbonate platform, constituting foci for sedimentary material delivered into the basin from the land to the west and shallow areas of the carbonate platform. In these depressions, settling of suspended material in a low energy sub wave base environment would frequently be interrupted by mass flow events of various scales that redeposit sediments from the surrounding slopes and shallower areas of the basin Figure 3-6.

Figure 3-6. Schematic model of BCF depositional environment (not to scale). Shallow water stromatolitic carbonates grade into deeper water carbonatic siltstones. Surrounding sediment is transported into local subbasins like Glyde River.

### 3.3.1.1 The W-fold Shale Member

The W-fold shale Member is thought to reflect a transition from shallow water environments of the Coxco Dolomite to deep water conditions characteristic of BCF deposition (Large et al., 1998). It conformably overlies the Coxco Dolomite Member of the ca. 1641 (±6) Ma old Teena Dolomite (Page et al., 2000). According to Brown et al. (1969), the W-fold Shale is the most laterally-persistent member of the BCF and between 5 and 150 m thick. It contains variable amounts of dolomite. Thinner sections consists dominantly of shaly laminated dolomite, while thicker sections are characterised by dolomitic shale (Brown et al., 1969). Distinctive manganese enrichments and interlayered (Fe²⁺-bearing) green bands deposited in anoxic waters, and (Fe³⁺-bearing)
pink bands supposedly deposited in oxic waters are interpreted by Large and colleagues (1998) to reflect mixing of anoxic deep waters with oxic shallow waters. Mn-enrichment may have formed where the chemocline impinged on the shelf, although the Mn-enrichment in the dolomites was likely related to diagenetic processes in the first few meters below the sediment water interface (Large et al., 1998). By comparing the W-fold Shale Mn-distribution with that from Middle Ordovician Boyds Creek carbonate sediments in Eastern Tennessee (USA), Large et al. (1998) speculate that the water depth may have exceeded several hundred meters during sedimentation of the upper W-fold Shale Mmb close to the HYC ore deposit.

3.3.1.2 Barney Creek Formation

The 1639 ±2 Ma (Page and Sweet, 1998) Barney Creek Formation (BCF) is a regionally extensive but poorly outcropping dolomitic siltstone-dominated unit. It consists of mainly dolomitic, carbonaceous and pyritic siltstone and shale with locally abundant tuff beds, breccias and graded units (Jackson et al., 1987). Undivided BCF outcropping in the Top Crossing area is described by Jackson et al. (1987) as interbedded fine doloarenites and ferruginous silty dololutite exhibiting red-weathering in the lower part, overlain by finely laminated dolomitic siltstone and silty dolostone. 2-10 cm thick K-rich mudstone beds are common. Jackson et al. (1987) also report the occurrence of acicular crystals after anhydrite or gypsum in one area. In many subbasins, particularly at HYC, thick breccias occur towards the base of the formation.

Walker and colleagues (1978) distinguish three types of breccias: (i) type I breccia beds are chaotic, ungraded and unsorted with up to 10 m big clasts that are derived from all the underlying McArthur Group formations, (ii) type II breccia beds are common in the higher parts of the HYC pyritic shale member with clasts derived from the Emmeruga and Teena Dolomites, (iii) type III breccia beds are confined to the uppermost sections of the HYC member and are chaotic, ungraded and unsorted with clasts derived from underlying parts of the McArthur Group and Gold Creek Volcanics ranging from a few mm to several tens of meters (Jackson et al., 1987, Walker et al., 1977).
The HYC pyritic shale that follows the W-fold member in many areas of the McArthur Basin is described as a carbonaceous, dolomitic, pyritic siltstone (McGoldrick et al., 2010). According to McGoldrick et al. (2010), the massive carbonaceous (± pyritic) shale lithofacies typically consists of faintly bedded to massive carbonaceous shale, occasionally with irregular bands of fine-grained pyrite and pyrite nodules. Commonly interbedded are fine-grained dolomitic sandstone and siltstone units. Also evident are bedding-parallel carbonaceous stringers (McGoldrick et al., 2010).

The unmineralised higher parts of the BCF are generally very fine grained. K-rich mudstones with tuff constituents are also common. Most sediments are finely laminated, but graded beds, scour structures, ripple marks, flame structures and soft-sediment slumping are also evident in places (Jackson et al., 1987). According to Brown et al. (1969), the BCF contains much more non-carbonate than the underlying dolomites. The principal non-carbonate components are reported as microcrystalline potassium feldspar, terrigenous silt and clay and locally abundant sulphides and carbonaceous and bituminous material (Brown et al., 1969).

One of the most detailed sedimentological BCF studies was conducted by Bull (1998) on drill core DDH BMR McArthur2 where he distinguishes three BCF facies as detailed in the following:

1. Thinly bedded dolomitic sandstone/siltstone/mudstone facies (facies 1)

The dominant facies in McA2 (ca. 88% by length) is characterised by pale grey dolomitic siltstone to fine-grained sandstone beds that vary in thickness from < 1 cm to ~20 cm, but mostly between < 1 to 6 cm. Beds are characterised by sharp bases, often exhibiting scours with relief of up to 2 cm as well as load cast and flame structures. Normal grading characterised by an upward decrease in grainsize and increase in the proportion of carbonaceous mudstone is frequently observed by Bull (1998). Other sedimentary structures described by Bull are planar and ripple cross-lamination. Bull finds that all these features are consistent with deposition from small-volume, turbulent mass flows. Ambiguous features described by Bull are rare pebble breccia horizons with platty intraclasts oriented roughly parallel to bedding. The clasts are jigsaw-fit and according to Bull have clearly been formed by relatively minor disruption of several thin but competent
dolomitic siltstone laminae. Bull interprets these features as having formed by the disruption of early diagenetic crusts and disputes a specific paleoenvironmental diagnosticity. The thinly bedded dolomitic mass-flow deposits characteristic of facies 1 can be seen in thin section to consist of an interlocking framework of silt- to fine-grained sand-sized fragments of monocrystalline quartz and dolomite. Proportions of both components vary and quartz- as well as dolomite-dominated endmembers are present. Dolomite-rich areas appear crystalline and to have a similar grain size as associated quartz fragments, but in detail they are amorphous under crossed-polars and are thus interpreted by Bull to represent a recrystallised dolomitic siltstone/mudstone matrix. Increasing proportions of mudstone are reported to be present towards bed tops. Often, they first occur as discrete, bedding-parallel stringers of black carbonaceous material within the grey recrystallised amorphous matrix and then grade into massive grey to black mudstone. These units consist of a fine-grained interlocking mosaic of black carbonaceous material and dolomite with varying proportions of dispersed silt-sized and finer quartz fragments as observed by Bull in thin section. The mudstone is interpreted by Bull to represent ambient depositional conditions where fine-grained material accumulated from hemipelagic suspension in a quiet, sub-wave base environment (Bull, 1998).

2. Massive carbonaceous (± pyritic) mudstone \((\text{facies 2})\)

In the lower half of McA2, seven discrete mudstone intervals are considered by Bull (1998) as discrete facies. The mudstone-dominated intervals range in thickness from 0.5 to 6.5 m and have gradational contacts with adjacent \(\text{facies 1}\) units. They are described by Bull as faintly bedded to massive, black (carbonaceous) mudstone that occasionally contain diffuse bands of fine-grained pyrite. In thin section, a foliation delineated by bedding-parallel carbonaceous stringers similar to those in the upper parts of \(\text{facies 1}\) sandstone beds is observed by Bull in some samples. Dolomitic siltstone units are a minor component and generally < 1 cm thick. Bull interprets \(\text{facies 2}\) in the same manner as mudstone intercalated within \(\text{facies 1}\). The massive nature and lower proportion of dolomite is however taken as evidence that hemipelagic sedimentation was largely uninterrupted by periodic dolomitic mass-flows and considered by Bull as relatively ‘starved’ in terms of clastic sediment input (Bull, 1998).
3. Intraclastic dolomitic sandstone (*facies 3*)

*Facies 3* of Bull (1998) comprises thicker (up to 0.5 m) bedded and coarser grained (up to very coarse-grained sand-size) dolomitic sandstone beds scattered throughout the BCF in McA2. Bull describes typical mass-flow features like sharp, scoured bases, sometimes with load and flame structures. Normal grading is common and highlighted by the presence of basal mudstone intraclasts. The medium to very coarse-grained fragments can be seen in thin section to consist of intraclasts of massive dolomitic mudstone and occasionally dolomitic quartz sandstone. Grain fabrics range from close packed in a dolomitic mudstone matrix to dispersed in a fine-grained dolomitic siltstone matrix similar to *facies 1*. Bull interprets the intraclasts to be derived by high energy erosion of older competent dolomitic mudstone and sandstone units – either older lithified sedimentary units structurally elevated during BCF times, or shallow surficial early diagenetic crusts (Bull, 1998).

3.3.1.3 Reward Dolomite

The Reward dolomite mostly consists of carbonate and subordinate dolomitic shale, dolomitic coarse sandstone and breccia and potash-rich mudstone. Most of the dolomite is dololutite, pelletal or intraclastic doloarenite, often with abundant chert nodules (Brown et al., 1969). According to Brown and colleagues (1969), characteristic features of the Reward are marked lateral changes in thickness and rock type. These generally mirror the facies of the underlying BCF in that thinner sections consist mainly of dolomite and overlie thin sections of dolomitic BCF, while thick shaly sections overlie thick shale sections of BCF. The Reward is interpreted by Bull (1998) as the partially time-equivalent shallow water facies of the BCF. Brown et al. (1969) report abundant columnar stromatolites and some Conophyton stromatolites in some of the thinner sequences. Locally, the stromatolitic dolomites are interbedded with or overlain by coarse to very coarse sandy intraclastic doloarenite or coarse to very coarse dolomitic sandstone, often with pebbles of stromatolitic dolomite or chert. Only vague traces of flat bedding are found in the coarse dolomitic sandstone and doloarenites, but sets of large-scale cross-beds occur in places (Brown et al., 1969). Other sequences reported by Brown and colleagues (1969) contain a variety of rock types and sedimentary structures.
Laminated and thin-bedded grey and brown dololutite with abundant chert in small nodules and thin bands were reported to contain variable potash content, grading into potash-rich mudstone. Fine sandy and pelletal doloarenite in isolated ripple lenses and thin beds with current ripple laminations are also common. In places, arenites and rudites, generally with abundant coarse to very coarse quartz are interbedded with the fine-grained sediments (Brown et al., 1969).

A variety of depositional environments are inferred by Brown and colleagues (1969) for the Reward Dolomite. Columnar stromatolitic and Conophyton dolomites are interpreted to represent a shallow subtidal facies. Restricted pools such as lagoons are suggested by acicular gypsum needles, while the associated cross-bedded arenites may represent tidal channels. An intermediate facies of oncolithic and thrombolitic dolomites may reflect more open-marine conditions, but in waters less than ~60 m deep that allowed penetration of sunlight to phototrophs living at the bottom. Dolutite and potash-rich mudstone facies may represent greater water depths in the order of tens rather than hundreds of meters. Weak traction currents or turbidity currents may have transported the current-rippled fine doloarenites. Interbeds of coarser arenites and breccias are interpreted as turbidites (Brown et al., 1969). Small scale overfolds in finer sediments are considered by Brown et al. (1969) as indicative of appreciable depositional slopes. According to Brown et al. (1969), the thin shallow water sequences around the margins of the basin that contain land-derived grains were reworked to provide the thicker sequences of muddy sediments and interbedded turbidites in the deeper water areas. These remained below sea level throughout deposition of both the BCF and Reward Dolomite (Brown et al., 1969). According to Jackson et al. (1987), the available evidence indicates subaqueous deposition, sometimes alternating with possible emergent phases in areas of sporadic tectonic activity that resulted in tilting, slumping and concomitant erosion. Deep-water depositing is however nowhere indicated (Jackson et al., 1987). ‘Oncolites’ and ‘thrombolites’ described by Brown et al. (1969) are interpreted by Jackson et al. (1987) as alteration textures of post depositional emergence and vadose weathering. Overall, Jackson et al. (1987) favour very shallow-water to emergent conditions for deposition of the Reward Dolomite in small bodies of standing water like lakes or lagoons at or near the regional groundwater table.
3.3.1.4 The BCF in the Glyde River area and drill core GR7

Of particular interest for this thesis is the BCF in drill core Glyde River 7 (GR7) from the Glyde River area because it contains “the most intriguing assemblage of biomarkers reported for the Proterozoic” (Lyons et al., 2012) and the excellent biomarker preservation allows detailed palaeoecological reconstructions (Brocks et al., 2005). GR7 is located in the northern part of the Glyde River ‘subbasin’. The basin lies ~110 km south of Barroloola Township and 80 km south of HYC (Figure 2-1).

According to Davidson and Dashlooty (1993), the Glyde River area represents a fault-bounded depocenter adjacent to the margin of the Batten Fault zone. The geometry is consistent with an origin in a releasing bend of the Emu Fault during oblique right lateral extension. This allowed the deposition of a very thick sequence of below wave base carbonaceous siltstones of the BCF (Davidson and Dashlooty, 1993). The BCF is thickest in drill core GR7 (~900 m). The Glyde River subbasin is separated by a non-depositional horst into a northern and southern depression. Internal syn-sedimentary normal faulting lead to the formation of seven major fault blocks (Davidson and Dashlooty, 1993). According to Davidson and Dashlooty (1993), the horst was submerged and greater water circulation established during deposition of undifferentiated BCF. However, the horst continued to be an east-west barrier to clastic and volcanoclastic gravity flows. Rhyolitic volcanisms in the Glyde area commenced in the W-fold Shale Mmb and became common in the overlying BCF (Davidson and Dashlooty, 1993). Volcanic constituents increase from 4.4 to 17.5% over 18 km, which lead Davidson and Dashlooty (1993) to propose a southern rhyolitic source within 6-30 km. Drill core GR7 is located in the northern part of the basin close to the Emu Fault to the east.

The general geology of the BCF in the Glyde River areas is described in Dashlooty (1982) and summarised in the following. The W-fold shale Mmb is characterised by thinly bedded wavey laminated green and red shale with pink and grey dolomite. Dashlooty reports evaporite pseudomorphs in the dolomite from the middle of the unit downwards. The W-fold shale grades into the overlying HYC pyritic shale member in a transition zone. The HYC pyritic shale Mmb mostly consists of black pyritic carbonaceous, sometimes dolomitic shales. Thickness of the HYC Mmb ranges from 20 m in drill core GR5 to 50 m in GR7. The HYC Member can be distinguished from the
overlying rock units by an abrupt increase in carbonaceous content. The upper rock units in the Glyde River area consist of interbedded pyritic dolutite, dolomitic siltstone and dolarenite with vitric tuff and intercalated graded dolomite breccia. In the northern part of the subbasin where GR7 was drilled, the thickness and number of the graded dolarenite-dolorudite interbeds increases gradually from top to bottom within the upper third of the unit and then decreases gradually towards the base. Thickness of the upper rock units varies from 40 m in drill core GR4 to 805 m in GR7. The thickness variation is attributed by Dashlooty to erosion in the upper section of the unit (e.g. GR4 and GR11) and non-deposition of the lower portions of the unit (e.g. GR1, 3, 5 and 9) (Dashlooty, 1982) although syn-depositional subsidence could also have led to locally enhanced sedimentation rates (e.g. due to turbidites).

A lithographic column for GR7 is shown in Figure 3-7. A small interval of brecciated tuffaceous dolomite at the bottom of the core is attributed by Dashlooty (1982) to the W-fold shale member and is followed by about ten meters of highly shared black & tuffaceous material interpreted as a transition zone. This is followed by about 50 m of dolomitic, carbonaceous, pyritic black shale of the HYC Pyritic Shale Member. Most of the upper rock units are logged by Dashlooty as carbonaceous dolomitic shale grading cyclically to silty shale. Between ca. 200 and 220 meters Dashlooty observed numerous dolomitic graded slum breccias and a facies change to dolomitic shale and siltstone that continues to about 30 m depth where an unconformity separates the BCF from the Cambrian Bukalara Sandstone (Dashlooty, 1982) (Figure 3-7).
Figure 3-7. Lithographic column of drill core GR7 (after Fig. 7 in Dashlooty, 1982) and approximate position of biomarker samples in the upper 350 m of the core (blue circles).
3.4 Thermal maturity of BCF sediments

Thermal maturity data (mainly Rock Eval) is available for most drill cores investigated in this thesis. All data presented in this chapter are from Crick et al. (1988), unless noted otherwise. Figure 2-1 shows a thermal maturity map of the drill cores studied. The least mature BCF sediments occur in the Glyde River (GR) region with thermal maturities ranging from marginally mature to mature. The highest thermal maturities are found in the central and northern part close to the Emu Fault zone (Figure 2-1). There, BCF sediments are generally in the mature to overmature stages of oil generation. Fair hydrocarbon (HC) yields are encountered in a few of the shallow samples, but they diminish rapidly with depth and are virtually nil below 200 m depth. Away from the major fault zones the thermal maturity is somewhat lower however and most BCF samples fall in the mature zone. Locally, maturation levels can also vary rapidly and may be associated with hydrothermal fluid movement along fault zones (Crick et al., 1988). All Proterozoic McArthur Basin samples analysed in the study of Crick and colleagues (RE and/or TOC analysis has been conducted on a total of 1130 samples) were at least marginally mature (Crick et al., 1988). According to Crick et al. (1988), burial history suggests that hydrocarbon generation occurred during deposition of Mesoproterozoic strata.

According to Crick et al. (1988), Tmax measurements of BCF sediments show the same relationship to hydrocarbon generation as Phanerozoic sediments and can thus be employed for maturity assessment. Tmax generally increases gradually downcore, but there are also significant variations of Tmax values for a single depth interval. Crick et al. (1988) found Tmax variations of 2-3°C and sometimes even as much as 5°C for adjacent samples in drill core Glyde River 7 (GR7) and attribute this to variations in organic matter concentrations and chemistry of rock matrix and OM. Therefore, the Tmax values of single samples are not sufficient for reliable maturity estimates, instead a suite of samples should be analysed. Based on a plot of extractable hydrocarbon (HC) yields versus Tmax values Crick et al. (1988) define the oil window in the McArthur Basin between ca. 435°C and ca. 470°C Tmax. These temperatures are the same as for much younger sediments. In a similar plot of HC yields versus mean maximum reflectance (Crick et al., 1988), the oil window seems to fall between ca. 0.15% (fluorescent
lamalginite) and ca. 1.4% reflectance (bitumen and non-fluorescent lamalginite). Adjusted to equivalent vitrinite reflectance values, the oil window falls between ca. 0.55-0.65% and 1.4% Vr. The main zone of hydrocarbon (HC) generation is also expressed in a downhole increase in PI values from < 0.1 to > 0.2 around 440°C Tmax in GR7. A marked increase in the PI is observed in GR7 and GR10 at vitrinite reflectance values (calculated from the MPI-1) of 0.65-0.7% which is in accordance with the usual onset of the peak oil generation window (e.g. 0.65%, p. 612 in Peters and Moldowan, 1993). Crick et al. (1988) define the thermal maturity based on Tmax values as follows: < 440°C: immature to marginally mature, 440-470°C: mature, > 470°C: overmature.

It must be kept in mind that correlations between different maturation parameters are only approximate, because each indicator reflects a different chemical process that might proceed at a different rate as the process for another indicator (Crick et al., 1988). Crick et al. (1988) found vitrinite reflectance values calculated from the Methyl Phenanthrene Index to be higher than those measured on actual lamalginite and solid bitumen as might be expected of hydrogen-rich organic matter. Nevertheless, systematic changes in reflectance have been observed with depth by Crick et al. (1988) and can be used with calibration to assess thermal maturity of BCF sediments. Different maturity parameters can thus be applied to BCF sediments but correlations might only be approximate.

3.4.1 Thermal maturity assessment for drill core Glyde River 7

Important palaeobiogeochemical findings (Brocks et al., 2005) have been possible in the Glyde River region due to the pristine nature of organic matter that has never experienced geological heating above the oil window. Therefore, biomarker analyses in this study are focused on drill core Glyde River 7 (GR7), which might allow a detailed reconstruction of the environmental and depositional conditions of the McArthur basin during BCF times.

Maturity trends are very similar for different drill cores from the Glyde River area, but GR7 contains some of the least mature organic matter and encompasses almost 1 km of BCF sediments (Crick et al., 1988). Thermal maturity is discussed in more detail for this core only. Usually Rock Eval (RE) analysis is considered reliable only for samples with
TOC exceeding 0.5% (R. Littke, personal communication) so that in the following RE data (all from Crick at al. 1988) is shown only for such samples.

In the Glyde River subbasin, sedimentary organic matter shows a clear trend of increasing thermal maturation with increasing burial depth. This is evident from Figure 3-8 that plots the Rock Eval maturity parameter Tmax with depositional depth for drill core GR7. Tmax values indicate that the shallow BCF sediments are in the early mature and below ca. 200 m in the mature stage of petroleum generation.

![Graph showing Tmax versus depth for GR7 core](image)

**Figure 3-8.** Tmax versus depth for drill core Glyde River 7 (data from Crick et al., 1988).

The hydrogen index (HI) is another Rock Eval parameter that can be used for maturity assessments. HI also shows a strong dependence on burial depth for GR7 (Figure 3-9) HI is probably mainly influenced by thermal maturity in GR7, but a change in kerogen-type might also occur at ca. 300 m. The change in the HI-depth trend could also mark the onset of intense hydrocarbon generation from the kerogen with oil expulsion resulting in lower hydrocarbon yields. Between 350 and ca. 580 m there seems to be an OM-lean interval however and most samples have TOC < 1%. A mineral matrix effect is therefore the most likely explanation for suppressed HI values in this interval.
The production index (PI) is another Rock Eval parameter influenced by thermal maturation. There is a strong increase of PI with increasing depth in GR7 (Figure 3-10) which represents release of hydrocarbons from the kerogen. Below 600 m the depth trend is less apparent and some lower PI values occur again, which might indicate that the maximum of oil generation is reached around 600 m. Some of the samples show high PI however and suppressed PI might indicate that hydrocarbons have been expelled from the formation. Below ca. 300 m there is a marked increase in PI from values around 0.1 to higher values which might indicate onset of main oil generation and could also explain the offset in the HI-depth trend at this depth.
3.4.2 Thermal maturity conclusions

Regional trends in burial maturation are relatively well known from previous studies. The least mature BCF sediments occur in the Glyde River (GR) region with thermal maturities ranging from marginally mature to mature. This is one of the reasons we selected drill core GR7 as a main target of the PhD project as these sediments seem ideally suited to reconstruct the originally composition of the BCF organic matter and gain as much palaeo-ecological information as possible.
3.5 Base metal sulphide deposits

The BCF in the McArthur basin hosts major Zn-Pb sulphide deposits and has a great potential for further discoveries. Depositional and paleo-redox conditions are presumably important for the formation of stratiform base metal deposits and insights gained from multi-proxy reconstructions may help to better understand the formation of the mineral deposits and potentially assist future exploration. The two biggest currently known deposit are HYC McArthur River (now ‘MRM’) in the HYC subbasin and Myrtle in the Myrtle subbasin, both of which are located along the Emu Fault that probably acted as a conduit for mineralising fluids.

The HYC McArthur River Zn-Pb-Ag deposit is the largest known example of a stratiform, sediment-hosted Zn-Pb-Ag deposit (Large et al., 1998). It is situated about 70 km southwest of Borroloola, close to the Emu Fault (Figure 3-1). The location of the major base metal deposits in the McArthur and Isa Basin is shown in Figure 3-1. Half of the world’s 10 Super Giant base metal deposits are found in the McArthur (HYC) and Isa Basin (George Fisher, Hilton, Mt Isa, Sullivan, Century) containing 102 Mt Zn and lead metal (Mulholland, 2011). In the Mt Isa Basin, greenschist grades of metamorphism and later fluid flow events obscured original textures. HYC is similar to many of the Mt Isa Basin deposits, but primary textures are well preserved at HYC and biomarker hydrocarbons are well preserved in the unmetamorphosed host sediments. HYC and the distal BCF sediments are therefore the ideal targets for biogeochemical studies of Palaeoproterozoic sediment-hosted stratiform Pb-Zn deposits (Logan et al., 2001). Despite decades of research the formation of the stratiform HYC deposit is still debated with two principal mineralisation models of predominantly exhalative processes leading to mineralisation at the sediment surface (e.g. Croxford et al., 1975, Large et al., 1998, Ireland et al., 2004a) and predominantly early diagenetic processes at shallow burial depths (e.g. Eldridge et al., 1993, Logan et al., 2001, Chen et al., 2003).

As the mineralisation models are still debated and the prospectivity for further deposits is considered high (Northern Territory Government, 2013), there is a need for new analytical approaches like organic and Fe-speciation analyses of the host sediments that may assist in understanding the prevailing environmental conditions that favoured formation of the exceptional base metal deposits.
3.5.1 The Myrtle Pb-Zn deposit and further prospectivity

According to the NTGS CORE lead, zinc, silver factsheet, there is a high prospectivity for further deposits in the McArthur Basin. This is highlighted by the recent discovery of significant resources at Myrtle. The Myrtle prospect is located only 20 km south of the HYC and according to (Mulholland, 2011) is already ranked ~40 in the world (top 1/3 of SEDEX deposits) despite yet incomplete exploration. A cross section of the Myrtle deposit is shown in Figure 3-11. The Myrtle deposit has a similar stratigraphy to HYC and also consists of a series of stacked ore lenses.

Other deposits have also been encountered in the McArthur Basin (Table 3-1) and it is astonishing that a major deposit such as Myrtle in close proximity to one of the world’s biggest Zn-Pb mines has only been recognised in 2005 (Mulholland, 2011), 60 years after discovery of HYC. The existing mine infrastructure at McArthur River and the experience available from exploiting the HYC deposit enhance the economic feasibility of exploiting other deposits in the area. The seismic survey conducted by (Rawlings, 2004) further enhances the prospectivity of the basin since it is now known that the BCF extends well to the east of the Emu Fault with more potential in this largely unexplored region. It thus seems that the exploration potential of the McArthur Basin is far from exhausted and a deeper understanding of BCF depositional and environmental setting could enhance future base metal exploration.


<table>
<thead>
<tr>
<th>Deposit</th>
<th>Resources (Mt)</th>
<th>Zn (%)</th>
<th>Pb (%)</th>
<th>Ag (g/t)</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>McArthur River (HYC)</td>
<td>171.3</td>
<td>11.14</td>
<td>4.74</td>
<td>48.5</td>
<td>Xstrata PLC</td>
</tr>
<tr>
<td>Myrtle</td>
<td>43.6</td>
<td>4.1</td>
<td>0.9</td>
<td>Na</td>
<td>Rox Resources Ltd</td>
</tr>
<tr>
<td>Bulman</td>
<td>1.2</td>
<td>11</td>
<td>6.5</td>
<td>Na</td>
<td>Admiralty Resources NL</td>
</tr>
<tr>
<td>Coxco</td>
<td>7.8</td>
<td>4.2</td>
<td>1</td>
<td>Na</td>
<td>Xstrata PLC</td>
</tr>
<tr>
<td>Teena</td>
<td>11.3</td>
<td>10.9</td>
<td></td>
<td>14</td>
<td>Rox Resources Ltd</td>
</tr>
</tbody>
</table>
3.5.2 HYC McArthur River deposit

The HYC deposit is mined by McArthur River Mining Pty Ltd (MRM) a subsidiary of GlencoreXstrata (MRM, 2013). The total resources are reported as 171.3 Mt with 11.1% Zn, 4.7% Pb and 49 g/t Ag (Table 3-1) and the production corresponds to ~3% of the world’s total zinc resources used by all types of smelters each year (MRM, 2013). The fine-grained nature of the ore hampers beneficiation, and underground mining thus only commenced in 1996 (MIM Exploration) – about 50 years after the discovery. Mining has since been converted to open cut (Logan et al., 2001).

The stratiform mineralisation occurs as eight stacked separate ore lenses at the base of the HYC Pyritic Shale Member (Large et al., 1998). The ore bodies are separated by base metal-poor sediments rich in dolomite (Lambert and Scott, 1973), described by Large et al. (1998) as sedimentary breccias and related dolomitic sediments. There is a general decrease in the sizes of the orebodies from bottom to top, and the portions with higher metal content exhibit an eastward shift. The inter-ore layers are mainly composed of shaley and silty dolomite towards the bottom of the deposit and of rapidly deposited dolomitic arenites and graded breccias higher up in the sequence. The sulphide minerals
in the ore layers are mainly very fine-grained, often not exceeding a few micrometres in diameter, and occur as thin conformable bands and disseminated crystals. Coarser grained accumulations are less common. The main sulphide minerals are (in order of decreasing abundance) pyrite, sphalerite (with variable iron content of up to ca. 6 wt%) and galena. Minor constituents are chalcopyrite, arsenopyrite, marcasite, chalcocite and covellite. The main constituents of the enclosing shales are quartz, potassium feldspar, illite, chlorite, dolomite and bituminous matter (Lambert and Scott, 1973).

Despite the excellent preservation, the mode of ore formation at HYC is still strongly debated. The two principal mineralisation models consist of predominantly exhalative processes leading to mineralisation at the sediment surface (e.g. Croxford et al., 1975, Large et al., 1998, Ireland et al., 2004a) and predominantly early diagenetic processes at shallow burial depths (e.g. Eldridge et al., 1993, Logan et al., 2001, Chen et al., 2003). The proposed environmental models are a shallow water lacustrine or sabkha depositional environment (e.g. Walker et al., 1977), or a deeper water setting (e.g. Bull, 1998). The shallow water interpretation is largely based on the presence of presumably evaporitic nodular dolomite within and around the ore body, but the nodular carbonate nodules were later found differ from shallow-water nodules and re-interpreted to have formed from diagenetic or hydrothermal processes (Large et al., 1998). The deep water interpretation is largely based on sedimentological and geochemical interpretations (e.g. Bull, 1998, Large et al., 1998) and supported by thick and abundant breccia beds.

In the exhalative, brine pool mineralisation model as envisaged by Ireland et al. (2004), dense metal-bearing fluids migrate downslope towards the centre of the sub-basin forming a stratified bottom hugging brine pool from which base metal precipitation would occur via bacterial sulphate reduction (BSR) in sediments and thermochemical sulphate reduction (TSR) in the hotter bottom waters of the brine pool (see also chapter 6). Background precipitation of early pyrite (Py1) would occur in the anoxic water column overlying the brine pool (Ireland et al., 2004b). Geochemical halos around the deposit are in favour of an exhalative model (Large et al., 2001).

In diagenetic mineralisation models (Figure 3-12), mineralising fluids flow roughly parallel to bedding through the sediments at shallow burial depth, temperatures of mineralising fluids are much higher and fluids may even contain abundant H2S. For hot
fluids, a much smaller fluid volume would be required to form the deposits. In such a model, BSR would be responsible for the production of biogenic pyrite, while metal sulphides would be the products of the reaction of metal-rich hydrothermal brines with sulphate and organic matter in the sub-surface. The process may be thermochemical in hotter zones of the brine flow path, but Logan et al. (2001) envisage it to be mostly mediated by BSR where fluids cool. The bicarbonate created by the oxidation of organic matter is precipitated downstream as nodular carbonate and carbonate crusts at the sediment-water interface above the ore formation zone where silica gel would also be deposited from brine seeps (Logan et al., 2001, Chen et al., 2003).

![Image]

**Figure 3-12.** Diagenetic mineralisation model at HYC (Logan et al., 2001).

Abundant polycyclic aromatic hydrocarbons (PAH) in HYC bitumen resemble hydrothermal petroleum generated at high temperatures (Chen et al., 2003), indicative of high temperatures of the ore forming fluid. High mineralisation temperatures are indicated by strongly elevated Tmax and reflectance values in the mineralised zones (Logan et al., 2001). The elevated thermal maturity of the mineralised zones suggests higher
temperatures than consistent with brine pool mineralisation. It would however be possible that a lower maturity signal imposed by syngenetic brine-pool mineralisation was later overprinted during (shallow) burial by hydrothermal fluid migration. In this scenario, a large proportion of mineralization would have occurred syngenetically from exhaled metal-rich brines, while on their way to the surface, these same fluids would also have migrated (sub) horizontally through the previously deposited sediments, heating them up and leading to additional early diagenetic mineralisation and recrystallisation. The locally elevated thermal maturity of the mineralised zone, strongly argues against a purely exhalative mineralisation model. Due to the high thermal maturity of the HYC sediments, any biomarker signatures (Logan et al., 2001, Holman et al., 2014) are prone to contamination overprint and we caution to give too much weight to ecological interpretations without quantifications of biomarker abundances on interior and exterior rock portions. Therefore, constraints from biomarker studies are not further considered here.

An isotopic study of HYC bitumen by Williford et al. (2011) hypothesises that the PAH formed in the underlying Wollogorang Formation and hydrogen equilibration during ore genesis with a fluid from an evaporitic basin. This is consistent with a possible source of the base metals from leached volcanics in the Tawallah Group (Cooke et al., 1998). Cooke et al. (1998) found that volcanic and intrusive rocks of the Middle Tawallah Group have been affected by hematite-chlorite and hematite-orthoclase alteration assemblages in the Mallapunyah Dome, ca. 50 km southwest of HYC. More than 80% loss of Zn and Pb is indicated in these rocks that yield a secondary palaeomagnetic pole similar to HYC. Cooke et al. (1998) thus interpret the alteration of Tawallah Group volcanics and formation of the HYC deposit as coeval. In light of these data, Williford et al. (2011) favour a mineralisation model in which fluids originate in an evaporitic deposit lower in the basin, interact with metals and organic matter in the Tawallah Group at temperatures exceeding 250 °C and about 6 km depth and then ascend along a flower structure associated with the Emu Fault cooling to 200 ± 20 °C before they reach the BCF sediments (Williford et al., 2011).
3.6 References


4 Depositional environment of the Barney Creek Formation

4.1 Introduction

Geological and sedimentological analyses of the BCF have been ongoing for decades, but the depositional environment is still poorly resolved, with shallow and deep-water models. The latter is favoured in most recent publications (e.g. Large et al., 1998). This information is scattered in journals of various disciplines, from sedimentology to economic geology and organic and inorganic geochemistry and often hidden in government reports. Important information, such as the results from the Southern McArthur Basin Seismic Survey (Rawlings, 2004) is thus often neglected and conflicting interpretations are provided by different authors. This chapter aims at summarising the information from previous studies that is most important for the following ecological interpretations, supplemented by new geological data and observations, resulting in a more detailed reconstruction of the depositional environment than possible in the geology chapter.

New mineralogical analysis presented here are restricted to drill core GR7, supplemented by some photographs from drill core LV09001. Data presented in this chapter encompass elemental maps of thin sections (courtesy of Sam Spinks, CSIRO) and carbonate content as determined by XRD, and elemental analyses as well as Ca/Ti ratios from elemental analyses. More details of the methodology can be found in the methodology chapter.
4.2 Results and discussion of BCF sedimentological context

4.2.1 Biogeochemistry of the BCF

For our palaeoecological reconstructions, we need to be familiar with the most important previous findings about the BCF ecology. BCF biomarker analyses were mainly conducted by Roger Summons and colleagues from the late 1980s (e.g. Summons et al., 1988, Logan et al., 2001, Chen et al., 2003) and later by Jochen Brocks and co-workers (Brocks et al., 2005, Brocks et al., 2008, Brocks and Schaeffer, 2008, Brocks et al., 2009, Lee and Brocks, 2011). In the earlier studies, it was recognised that the overall hydrocarbon patterns are similar for most BCF samples.

BCF extracts are generally similar to other Precambrian bitumens (Pawlowska et al., 2013) with a relative high and broad UCM, high relative concentrations of mono- and dimethyl alkanes and often either very high or very low relative concentrations of isoprenoids like pristane and phytane. Cyclic compounds like cyclohexanes are also abundant in BCF extracts. Originally, trace steranes and a dominance of hopanes was interpreted to indicated dominantly prokaryotic source organisms and minor eukaryotic input (Summons et al., 1988).

Brocks et al. (2005) reported that regular steranes were generally below detection limits, but that unusual triaromatic steroids, dominantly methylated at C-4, are ubiquitous in the thermally best preserved BCF sediments. These steroids may be derived from methylotrophic bacteria (Brocks et al., 2005). Many BCF sediments contain hopanes that are produced by a variety of bacteria. Also reported were 2α –methylhopanes of potential cyanobacterial origin (Brocks et al., 2005). While 2-methylhopanoids can also be produced by other bacteria (e.g. Welander et al., 2010) and probably originated in alphaproteobacteria (Ricci et al., 2015), cyanobacteria likely significantly contributed to the BCF organic matter and may be the principal biogenic source of abundant β- and γ-carotane (Brocks et al., 2005). 3β-methylhopanes are present in high relative concentrations and may indicate a high activity of type I-methanotrophic bacteria (Brocks et al., 2005). Thermally unstable intact carotenoids were detected in the shallow BCF sediments of the Glyde River area, including okenane, chlorobactane, isorenieratane, renieratane and renierapurpurane. These arylisoprenoids are biomarkers
for green and purple sulphur bacteria that painted the picture of a stratified water column with sulfidic conditions extending into the photic zone (Brocks et al., 2005).

Brocks and colleagues (2008) found that in contrast to regular bacterial hopanes, eukaryotic C27 to C29 steranes show a typical contamination pattern with strong enrichment on core surfaces. These biomarkers are thus not indigenous to the Paleoproterozoic sediments and cannot be used for paleo-ecological reconstructions. Most samples only contain trace steranes, indicative of generally small contaminant signatures. The absence of clearly indigenous sterane still has an important impact for palaeobiological interpretations as they were the only clear evidence of eukaryotic activity in the BCF waters.

Clearly indigenous steranes could to date not be detected in the BCF (Pawlowska et al., 2013), despite the high abundance of, dominantly 4-methylated, tri-aromatic steroids (Brocks et al., 2005). Surprisingly, no indigenous saturated equivalents of these compounds could yet be detected, which is a unique and enigmatic character of the BCF biomarker signature. Saturated extracts with high isoprenoid concentrations were suggested by Pawlowska et al. (2013) to be representative of typical Proterozoic facies 2 biomarker pattern of phototrophic microbial mats where a planktonic (eukaryotic) signature was taphonomically excluded by a hypothesised ‘mat seal-effect’, but this remains a speculative hypothesis.

4.2.2 Marine versus lacustrine environments and sulphur systematics

For ecological interpretations, it is important to know if the sediments were deposited under marine or lacustrine conditions. It is very difficult to clearly distinguish between marine and lacustrine conditions for sediments as old as the BCF, but the scientific consensus lies on marine conditions. Evidence for a marine setting of McArthur Group deposition comes from so called “Coxco needles” of the Coxco Dolomite Member underlying the BCF. The radiating fans of acicular crystal casts, up to 10 cm in length, are pervasively pseudomorphosed by sparry dolomite cement and thought to represent aragonite precipitation from ambient seawater directly onto the seafloor (Winefield, 2000). Previously, the morphological similarity of these crystals to trona was used as an
argument for a lacustrine setting (Jackson et al., 1987). O/C isotopic patterns of BCF carbonates also fall on a presumed ‘marine’ Umbolooga Group trend and some are even relatively enriched in $^{18}$O indicative of more hypersaline conditions (Veizer et al., 1992).

Brown and Large found clusters of acicular gypsum pseudomorphs cutting across stromatolitic laminae in the Reward dolomite (Brown et al., 1969) and Jackson et al. (1987) report similar from the upper BCF in one area – potentially in the transition to the Reward. Deposition of evaporitic minerals points towards a restricted (shallow) marine environment. However, the paragenetic relationship to stromatolitic laminae reported by Brown et al. (1969) would also be consistent with a late diagenetic/hydrothermal origin of the sulphate minerals and sulphate-rich basinal fluids may be derived from adjacent evaporitic formations.

Strong evidence for a marine origin came from the biomarker 24-n-propyl-cholestane thought to be indicative of marine chrysophyte algae (e.g. Logan et al., 2001). Latter analyses showed that these sterane markers constitute younger contamination (Brocks et al., 2008), so this evidence is not valid anymore.

The strongest evidence for a restricted marine origin of the BCF waters comes from strongly depleted sulphur isotopes of the pyrite minerals (Johnston et al., 2008). $\delta^{34}$S$_{pyr}t$e between 18.2‰ and 23.4‰ have been reported for the lower Reward Dolomite, similar to inferred seawater sulphate values between ~20‰ and 24‰ (Shen et al., 2002). Johnston et al. (2008) report $\delta^{34}$S$_{pyr}$ between 18.8‰ and 27‰ for the Reward Formation in drill core McA2 and 19.7 to 35.2‰ for the BCF in drill core GR10 from the Glyde River area (Johnston et al., 2008). Such values are difficult to reconcile with a freshwater sulphur source that is isotopically depleted relative to marine sulphate (Bottrell and Newton, 2006). In the Phanerozoic, freshwater $\delta^{34}$S$_{SO4}$ usually falls between 0‰ and 10‰ (Paytan et al., 2012) with the meteoric standard $\delta^{34}$S value being 0‰, so the heavy BCF isotopes would be very difficult to achieve through near-quantitative depletion and Rayleigh-enrichment of freshwater-derived sulphate.

According to Johnston (2008), a quantitative reduction of seawater sulphate is the simplest explanation for the observed $\delta^{34}$S composition. Variations in $\delta^{34}$S$_{pyr}$ may reflect secular changes. The basin could have experienced different degrees of closed system
behaviour with Rayleigh fractionation leading to isotopically enriched basin water sulphate and subsequently sulphide. A stratigraphic trend towards lighter values could record recovery from a more restricted setting towards a higher sulphate influx. This could be achieved by communication with a larger, possibly global, sulphate reservoir. This could be understood in terms of a two endmember mixing model, where basin sulphate represents an enriched endmember (maybe ~+35‰) and a less-enriched endmember (possibly ~+20‰) could be the ocean (Johnston et al., 2008). Although freshwater is usually not sulphate-rich, lighter values could also be achieved by increased influx of isotopically light fresh-water sulphate. At low basinal sulphate concentrations as indicated by the likely (near) quantitative reduction of seawater sulphate in Reward sediments (Johnston et al., 2008), a relatively small increase in sulphate influx could have a relatively strong effect on basinal sulphate concentrations. For example, freshwater influx would likely have been significantly lighter than basinal sulphate (e.g. ~0‰ freshwater versus ~35‰ basinal sulphate pool) and importantly, if (even slightly) increased sulphate availability resulted in a non-quantitative depletion of sulphate (in sedimentary pore waters during diagenesis), the bacterial sulphur fractionation would be more fully expressed, also resulting in lower δ34S_{pyrite} signatures. This dual effect of a higher abundance and lower isotopic signature of basinal sulphate through freshwater influx may potentially also have a significant effect on δ34S_{pyrite} in low sulphate environments.

Generally enriched pyrite throughout the sampled stratigraphy in the range of inferred seawater sulphate values, strongly support a dominant seawater sulphate source for McArthur Basin pyrite sulphur. In balance, the available evidence points to a restricted marine depositional system for the BCF.

4.2.3 Water depth

Water depth has a strong control on which types of ecosystems can be expected, particularly if phototrophic microbial mats may have been an important biomarker source. Both, a shallow- and a deeper, subwave-base, depositional environment has been advanced for the BCF. Brown and colleagues (1969) proposed that deeper water shaley carbonates were deposited at the peak of a sea-level transgression. A different
depositional model of shallow water marginal marine or sabkha deposition was first proposed in an abstract by Williams and Logan (1981) and extended by Muir (1983) as a purely lacustrine/sabkha depositional model for the BCF (Muir, 1983, Williams and Logan, 1981). A shallow water or sabkha setting was also favoured by Jackson et al. (1987). Nodular dolomite textures interpreted as pseudomorphs after shallow-water evaporites were important evidence for the sabkha model. However, the nodules were re-interpreted by Large et al. (1998) as related to diagenetic or hydrothermal processes in potentially deep waters as they are distinct from typical evaporitic nodules and formed by displasive growth and cementation. More recent studies return to a deep water depositional setting for the BCF (Bull, 1998, Large et al., 1998).

The BCF strongly differs sedimentologically from the underlying formations and the higher silt- and clay content, together with the lack of unambiguously shallow water sedimentary features, are most plausibly explained by a deeper water environment during deposition of the BCF compared to adjacent formations. The co-occurrence of a mineralogical shift and basin subsidence as indicated by variable lateral thickness of BCF sediments, talus breccia’s and abundant mass flow deposits of various scales, strongly suggests that an increase in water depth was a first-order control on the higher siliciclastic content of the BCF compared to the directly under- and overlying formations. The BCF lithology of silt- to mudstones is typical for quiet, deep water (below wave base) settings. In contrast to many other McArthur Group formations, the BCF lacks any unambiguous evidence of shallow water deposition like ripple marks, desiccation cracks and post-depositional current-reworking. In contrast to the lack of evidence of shallow water deposition, the abundance of mass-flow deposits and evidence for hemipelagic deposition indicate a subaqueous, sub-wave base depositional environment (Bull, 1998). Generally, wave base depth varies significantly for different depositional environments.

The expected depth of wave penetration is for example 100 m on the Atlantic side of Florida, and only 50 m on the Gulf side (Peters and Loss, 2012) and absolute Proterozoic water depth estimates are thus speculative. The absence of wave-influenced features from the intracratonic BCF suggest however considerable water depth, probably exceeding several tens of meters. Thick breccia beds (e.g. Walker et al., 1977) also indicate significant water depth at least in parts of the McArthur Basin during BCF times.
Based on the fine grain size and persistent laminations, Brown et al. (1969) interpreted the W-fold shale and HYC pyritic shale member of the BCF to be “deposited below wave base, in water deep enough to inhibit growth of algae and precipitation of abundant carbonate”. They further conclude that water depth must have been too great for sunlight to penetrate, so that waters were probably deeper than 50 m. A maximum water depth of 550 m at HYC is contrived from the vertical thickness of talus slope breccia (Coooley Dolomite Member), but the talus breccia was probably deposited slowly during progressive subsidence, and at any time the true water depth was interpreted to be less than total breccia thickness (Brown et al., 1969). Large and colleagues (1998) inferred water depth of several hundred meters for the W-fold shale based on the comparison of Mn-distributions to that of a Phanerozoic example, implying that the deeper waters of the BCF exceeded several hundred meters at least in the Glyde River area and other local depressions. In balance, the evidence clearly points to sub-wave base depositional environment with water depth exceeding tens to hundreds of meters. According to the lateral extension of BCF sediments interpreted from the seismic survey by Rawlings (2004), deep water deposition should have extended across large parts of the basin, so the BCF siltstones are unlikely to just reflect localised low-water energy environments such as lagoons. If there were significant benthic microbial mats in the BCF, they would thus rather have been heterotrophic.

4.2.4 Redox conditions

Water column redox chemistry is one of the key drivers of microbial ecosystems. Most eukaryotes thrive in oxygenated conditions and are poisoned by hydrogen sulphide which may also precipitate bio-essential trace metals out of the water column. Anoxyogenic phototrophs in contrast employ reduced hydrogen sulphide or ferrous iron as terminal reductant, the electron donor during photosynthesis. Palaeo-redox conditions are thus of great importance for informed palaeo ecological reconstructions as attempted in this project. The reconstruction of Proterozoic marine redox conditions is also generally important for tracing the oxygenation of Earth’s atmosphere and oceans that paved the way for complex life to evolve. Importantly, water column redox
conditions may also have been crucial for the formation of giant stratiform base metal deposits that are hosted in the BCF and elsewhere in the Mount Isa Superbasin.

Carbonaceous material and sulphides were taken by Brown et al. (1969) as evidence for reducing conditions although this is a poor indicator of paleo-redox conditions. A study of the Reward Dolomite in core McArthur River 2 by Shen and colleagues (2002) reports high degrees of pyritisation (DOP), at the time interpreted to indicate euxinic conditions. The samples consisted of parallel laminated siltstones and shales that were partly dolomitised. Large and colleagues (1998) report so called degree of pyritisation (DOP) values for the W-fold shale, HYC ore horizon and overlying HYC pyritic shale at the HYC deposit. Low values indicating oxic conditions for the W-fold shale are succeeded by strongly fluctuation degrees of pyritisation in the HYC ore zone and pyritic shale. The values fall within the oxic, dysoxic and anoxic zones and are interpreted by Large et al. (1998) to indicate deposition of oxic and anoxic sediments. They observe the presence of at least three end-member microlayers in the HYC ore lenses (i) pelagic muds (ii) quartz-carbonate layers interpreted as turbidites and (iii) zinc-lead-rich bands. The high organic matter content of pelagic muds is interpreted to indicate background deposition from an anoxic water column consistent with high degrees of pyritisation. Low DOP values are attributed to quartz-carbonate turbidites that according to Large and colleagues also have low levels of organic carbon and are interpreted to be derived from an oxic water column. They consider it unlikely that the basin conditions oscillated from reduced to oxidised millions of times during BCF sedimentation and thus infer repeated incursions of oxygenated mass flows derived from a marginal shallow-water oxic environment into the anoxic deep waters of the basin floor (Large et al., 1998).

It must be noted that the DOP used in the earlier studies of Shen et al. and Large et al. (1998) cannot distinguish between anoxic waters that are rich in dissolved iron (ferruginous) and hydrogen sulphide (euxinic). It was later found that ferruginous conditions dominated Proterozoic seas (e.g. Canfield et al., 2008, Planavsky et al., 2011) and these conditions can be revealed by the refined iron speciation procedure developed by Poulton and colleagues (Poulton and Canfield, 2005). The refined iron speciation analyses are also better suited to distinguish between oxic and anoxic conditions as boiling HCl extractions remove a variety of Fe phases, including (oxyhydroxides) and
sheet silicates (Poulton and Canfield, 2005). A recent iron speciation study by Planavsky et al. (2011) included BCF samples from drill core MY3 and a handful of samples from drill core MY4, and revealed dominantly ferruginous conditions (Planavsky et al., 2011). Planavsky’s analyses were however restricted to the Myrtle subbasin that also hosts a large stratiform lead zinc deposit. As for the HYC sediments analysed by Large et al. (1998), it is currently unclear if the proxy signatures may have been overprinted by local hydrothermal activity or if similar redox conditions were characteristic for BCF waters across the McArthur Basins.

In contrast to Fe-speciation evidence for ferruginous Myrtle waters, biomarker evidence of green and purple sulphur bacteria in the Glyde River area, and in a few samples from cores across the basin, were interpreted reflect indicate photic zone euxinia (Brocks et al., 2005).

4.2.5 Carbonate versus siliciclastic sedimentation

To interpret ecological trends across the basin, it would be important to know what controlled the deposition of carbonate-rich, potentially shallow water, and siliciclastic-rich potentially deep water, sediments. So, what does the carbonate distribution look like in the BCF?

The Ti/Ca ratio and carbonate content as determined in this study by XRF and calculated from elemental data is shown for the upper 350 m of BCF in GR7 in Figure 4-1. Carbonate calculated from Ca and Mg concentrations is slightly higher than determined by XRD (in Figure 4-1B) which can be at least partly attributed to Ca and Mg from non-carbonate phases like certain silicate minerals. Ti/Ca shows less variability than the carbonate curves, but around 90 m depth, a carbonate-poor interval is apparent in all curves.
The carbonate-distribution in thin sections of individual BCF samples employed in this study from the upper 300 m of drill core GR7 is illustrated in Figure 4-2. These XRF calcium maps show a relatively even distribution of carbonates throughout the samples. Some sand-sized grains are present, but most grains are silt-sized. Particularly in carbonate-rich samples (e.g. Figure 4-2A), calcium is finely and evenly distributed throughout the sample matrix. Calcium is more restricted to carbonate-grains in samples with a higher proportion of non-carbonate minerals. In carbonate-poor samples (Figure 4-2C, G), most carbonate occurs as silt to sand-sized grains that are evenly distributed across the samples. Samples with more intermediate carbonate-content (Figure 4-2B, E, F) contain many silt to fine-sand size and a few larger carbonate grains, but also have calcium disseminated throughout the rock matrix. In GR7, most carbonate is thus either finely disseminated throughout the rock matrix or occurs in dominantly silt- to fine sand-sized grains and to a lower proportion in larger carbonate grains.
In the upper BCF, we find that carbonate-poor intervals can be optically identified in drill core. They are often darker and the rock is crumbled. In places, the core is highly oxidised with white epsomite (MgSO₄·7H₂O) growing on core surfaces (Figure 4-3D).
Epsomite growth is attributed to a higher pyrite content (and surface weathering in storage), while crumbling may be facilitated by a lack of carbonate. At 90.3 m, carbonate content was calculated to be ~9% based on Ca and Mg concentrations and ~1% by XRD analysis, while pyrite iron was calculated as ~1.9 wt%. In contrast, carbonate-rich adjacent core intervals are often lighter, more intact and lack significant epsomite growth (Figure 4-3A). At 67.14 m, carbonate content was ~26% based on Ca and Mg concentrations and ~18% by XRD, while pyrite iron was ~0.7 wt%. We think that these carbonate-poor, pyrite-rich intervals are present in most drill cores across the McArthur basin, such as GR10 and McA5 (Figure 4-3). These intervals are also recognised by the Hylogger due to epsomite growth as a result of pyrite-weathering (Figure 4-3D). Hylogger results confirm that the BCF interval shown in Figure 4-3C is carbonate-poor and it seems to contain quartz, white mica and feldspar (Belinda Smith, personal communication 2016). Similarly, there is low to no Hylogger response in carbonate wave lengths in the epsomite-rich intervals in McArthur 5 (see data in Smith, 2014). It is thus possible that a carbonate-poor interval extends across the McArthur Basin, which could indicate periods of low level or enhanced terrestrial sediment influx.

The carbonate-poor interval in the upper BCF in GR7 likely correlates to interval 4 in core McArthur 2 shown in Figure 4-5A which Bull (1998) attributes to a second period of increased water depth and the upper maximum flooding surface inferred by Lindsay and Brasier (2000) in core Bing Bong 2 (Figure 4-5). Thus, there are at least two carbonate-poor, siliciclastic-rich intervals present in the BCF across the southern McArthur Basin. Examples of laminated BCF samples are shown in Figure 4-6 and of unlaminated BCF sediments in Figure 4-4.
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Figure 4-3. Hylogger pictures of the BCF (NTGS, Australia) A) Carbonate-rich interval in drill core GR7, B) highly crumbled carbonate-poor interval with epsomite growth in drill core GR7 C) similar, presumably carbonate-poor interval in drill core GR10, D) Hylogger data for drill core McA5 highlighting epsomite distribution (Smith, 2014).
Carbonate and/or clay content in the BCF can be interpreted in a sequence stratigraphic framework (Lindsay and Brasier, 2000) and may reflect fluctuations in sea-level and distance to the shore line. A comparison of the GR7 gamma ray log (Figure 4-5C) to that of Bing Bong 2 (Figure 4-5B) reveals that a similar, but less expressed, sequence of retrogradation at the bottom, progradation in the middle BCF and retrogradation in the upper formation may be recorded in the BCF sediments of the Glyde River area with a potential correlation of maximum flooding surfaces indicated by dotted lines. A similar approach was taken by Bull (1998) in the interpretation of BCF and Reward sediments in drill core McArthur 2 where he subdivides the stratigraphic succession into five intervals that reflect subtle paleoenvironmental variations, mostly in water depth. According to his interpretation, the BCF contains two stratigraphic intervals that represent periods of water depth maxima, with the lower correlating to the HYC mineralised interval. The inferred water depth curve of Bull (1998) is shown in Figure 4-5A. While this record does not directly inform about causes of these trends, it seems quite possible that carbonate-poor intervals can be correlated across the McArthur Basin. It is currently not clear however, if siliciclastic or carbonate delivery controlled carbonate content and if it was controlled by shore-line distance during deposition of the BCF or for example by riverine influx.
4.2.6 Microbial mats versus pelagic ecological signatures

Microbial mats may have strongly altered ecological proxies and it would thus be important to recognise pervasive, particularly phototrophic, microbial mats, but currently there is no firm indicator for microbial mat deposits in siliciclastic environments. The BCF contains siltstones and shales with continuous thin organic- and pyrite-rich laminae. It is unclear if these are pelagic or of biofilm origin, their origin may greatly affect paleo-ecological interpretations (chapter 5).
Many slumped sediments contain organic matter-rich streaks (e.g. Figure 4-7). These could reflect shallow ripped up shallow water microbial mats that were transported over a long distance or pelagic laminae or in-situ mats that were transported for a short distance only. As these flakes may potentially have a large impact on ecological proxies, particularly biomarkers, their origin is of great importance for paleo-ecological reconstructions.

In the well-preserved drill core LV09001, laminated sediments (Figure 4-7 bottom) are sometimes overlain by a grey matrix with streaks (Figure 4-7 top). Also contained within the grey matrix are several pieces of laminated sediment. The association of intact laminated sediments, ripped-up cm-scale pieces and small streaks strongly suggest that the streaks originate by disruption and short distance transport of laminated sediments.
Figure 4-7. Laminated sediments in drill core LV09001 that may be pelagic sediments or microbial mats. Streaks and clasts in grey matrix above could reflect microbial mat fragments.
The laminae could represent phototrophic or heterotrophic microbial mats or pelagic sedimentation. Importantly, Crick et al. (1988) and Crick (1992) showed that BCF contain lots of elongated, generally bedding parallel filaments ranging in length from <2 μm to several hundred microns. In places the filaments appear to group together and form thicker bodies or stands that are generally <500 μm long by 50 μm thick, sometimes forming apparently continuous mats (Crick, 1992). According to Crick (1992), these organic filaments are petrographically similar to a type described from a number of Phanerozoic oil shales, which was originally called alginate and is now referred to as lamalginite. “Streaks” or “flakes” reported from the BCF (Bull, 1998, Lyons et al., 2012) and observed in many samples included in our study (e.g. Figure 4-7) thus seem to be organic in nature. The lamalginite is fluorescent in the thermally well preserved upper stratigraphy in some cores, and non-fluorescent in the thermally more mature (>0.4% bitumen reflectance) stratigraphy. In GR7, the switch from fluorescent to non-fluorescent lamalginite occurs around 600 m (Crick et al., 1988).

In the Phanerozoic, small unicellular or thin-walled colonial benthic or planktonic algae are the source organisms for lamalginite (Hutton, 1987). According to Crick (1992), lamalginite in the McArthur Basin probably originated from a variety of microorganisms that inhabited the basin. The comparison of Phanerozoic lamalginite derived from colonial green algae (Pediastrum) to fluorescence BCF laminae in Figure 4-8 clearly shows that the BCF organic streaks can be derived from (phyto)planktonic sources.
Several sedimentological features of BCF sediments could potentially be attributed to microbial mat features. Most notable are organic matter streaks that could reflect ripped-up shallow microbial mats washed into the deeper basin and pyrite-rich laminae. Currently, flat microbial mats in siliciclastic sediments cannot be convincingly identified and a microbial mat origin of BCF sediments remains speculative. In drill core LV09001, it is obvious that the organic matter streaks can be produced by disruption and short-distance transport of laminated sediments. These fragments of organic-rich laminae are thus unlikely to be transported over a long distance to the deeper basin. Laminated sediments themselves could reflect flat microbial mats. Considering the inferred depth of BCF waters, such mats would most likely be heterotrophic, although low light adapted anoxicogenic GSB may also have lived there. Similar laminations are however also produced by pelagic process, most notably fluctuations in clastic influx and carbonate deposition and planktonic blooms that result in the formation of organic matter and pyrite-rich laminae. As common pelagic process can explain the BCF features and there is no clear evidence for pervasive microbial mats. Most importantly, the BCF ‘mats’ and streaks strongly resemble Phanerozoic lamalginite deposits and a pelagic interpretation is thus favoured here.
4.2.7 Depositional time scales

Estimates of depositional time scale can assist the interpretation of which kind of environmental processes may have controlled long term trends in BCF proxy data like that exhibited by the carbonate curve in Figure 4-1. The zircon age data is indistinguishable for the three samples and 127 analyses give a pooled age of 1639±2 million years (Page and Sweet, 1998). Four million years (Ma) can thus be considered a reasonable maximum estimate for the time of deposition of ~150 m BCF. Actual deposition can be expected to have been much faster however. Simo (1989) compiled linear accumulation rates of 16 Cenozoic and Mesozoic carbonate platforms. The sedimentation rates range from 20 to 600 mm/ka (m/Ma) with an average of ~150 m/Ma (see Simo, 1989 and references therein). Assuming 100 m BCF thickness at LV09001 (without the bottom breccia), this would result in a time range of ~180 000 to 5 000 000 years with an average estimate of 670 000 years for deposition of the BCF.
4.3 Conclusions

The BCF provides one of the most detailed windows into Paleoproterozoic ecology. At BCF times, the McArthur Basin was probably a restricted, relatively deep (in places probably a few hundred meters deep) marine basin with a generally evaporitic climate. The shallow water facies of the BCF is most likely represented by the Reward Dolomite that may encompass a variety of depositional environments from lagoons to shallow shelf dominated by carbonate deposition. The water was deepest along active faults, particularly the Emu Fault, along which local depocenters formed due to tectonic subsidence. These depocenters were probably filled relatively quickly by sediments supplied by mass flow events of different scales from the surrounding areas. Although sedimentation rates were very variable and are difficult to estimate, deposition of the BCF took less than a few million years and likely happened within a few hundred thousand years. Ecological variations like the carbonate trend observed in Figure 4-1 thus likely happened in the time scale of orbital (climate) cycles. It is currently unclear if the BCF carbonate content is controlled by sea level and/or shore line distance or rather by siliciclastic influx controlled for example by riverine delivery. It appears that two major periods of low carbonate deposition can likely be traced across the southern McArthur Basin. Although very important for ecological reconstructions, significant microbial mat remains can currently not be confidently recognised in siliciclastic sediments. In fact, it seems more likely that organic matter streaks and mat-like layers represent pelagic organic matter analogous to Phanerozoic lamalginite. The fine lamination seen in some BCF samples could thus be caused by similar process as those inferred for the Zechstein sea where riverine influx had a strong control on carbonate deposition.
4.4 References


5 Reconstructing a “mixed-up” record: a multi-proxy interpretation of the BCF ecology

5.1 Introduction

The temporal evolution of the redox state of the oceans and atmosphere is intricately linked to the evolution of life on Earth and therefore of major interest in the Earth and Palaeobiological Sciences. Present atmospheric oxygen concentrations around 21% support large animals and complex oxygen-dependent ecosystems. However, for most of Earth’s ~4.5 billion-year (Ga) history, oxygen concentrations remained much lower. The first major rise in atmospheric oxygen concentrations, termed the “Great Oxidation Event” (GOE) can be recognised in the rock record around 2.3 Ga by the appearance of oxidised detrital iron minerals and the disappearance of a mass independence fractionation signal in rare sulphur isotopes of syngenic pyrite in shales (Bekker et al., 2004, Farquhar et al., 2000). A second rise in atmospheric oxygen concentrations is suspected to have occurred in the Neoproterozoic around 800 to 600 Ma ago, and has been tentatively linked to the evolution of complex multicellular life (e.g. Planavsky et al., 2014). The inferred evolution of Earth’s atmospheric oxygen content through time is summarised in Figure 5-1.

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Figure 5-1. Evolution of Earth’s atmospheric oxygen content through time (Figure 1 in Lyons et al., 2014).

The redox state during the almost one-billion-year time interval of Earth’s middle age, the mid Proterozoic (~1.8–0.8 Ga), has been enigmatic for a long time. However, great advances have been made in the last decade, largely through the development of a refined iron speciation analysis technique (Poulton and Canfield, 2005) and the application of iron speciation (e.g. Poulton and Canfield, 2011, Planavsky et al., 2011) and trace metal studies to the Proterozoic rock record (e.g. Planavsky et al., 2014, Scott
et al., 2008). In 1998, Canfield hypothesised that the global ocean was euxinic for most of the Mid-Proterozoic (Canfield, 1998). This model was supported by large abundances of biomarkers of anoxygenic phototrophic sulphur bacteria recovered by Brocks et al. (2005) from the 1.64 Ga Barney Creek Formation that were interpreted to indicate euxinic conditions up to the photic zone. Yet subsequent iron speciation studies suggested that anoxic iron-rich (ferruginous) conditions prevailed (Poulton and Canfield, 2011, Planavsky et al., 2011) with euxinia being restricted to productive ocean margins (Poulton et al., 2010). Overall, an increasingly resolved and quantitative picture of Proterozoic redox evolution is emerging with a statistically higher proportion of basinal euxinia in Mesoproterozoic basins (Sperling et al., 2015). According to Sperling’s (2015) statistical analyses of global iron speciation data, sediment and water column sulphide production reached a minimum in the Neoproterozoic oceans. The Proterozoic redox landscapes appear to have been highly heterogeneous with for example marine sediments deposited around 1.5 Ga in different localities showing evidence of oxic, ferruginous and euxinic basin conditions (Shen et al., 2003, Planavsky et al., 2011, Sperling et al., 2014). Still, much remains to be learned about the dynamics of the mid-Proterozoic redox landscape and the complex relationships between life and environments at the time, calling for more detailed analyses and a better integration of organic and inorganic proxies.

The mid-Proterozoic is also the prime interval for sediment hosted base metal deposits (Leach et al., 2010). The redox state of the palaeo-water column and seafloor sediments are presumably crucial factors for synsedimentary base metal mineralisation. The BCF hosts one of the world’s largest stratiform Zn/Pb sulphide deposits at the current locale of the HYC (Here’s your chance) mine along the McArthur River. The Mt Isa Superbasin, which contains both the time equivalent McArthur and Mt Isa basins, contains one quarter of the global zinc reserves (Leach et al., 2005). Euxinic conditions in the lower water column may be a prerequisite for exhalative mineralisation (see e.g. Ireland et al., 2004). The microbial ecology, in turn, is also greatly influenced by the redox state of the water column and base metal precipitation might be directly or indirectly induced by microorganisms (see e.g. Labrenz et al., 2000). Biological sulphate reduction (BSR) and concurrent oxidation of organic matter may for example be responsible for the precipitation of base metal sulphides. It is therefore important to elucidate the redox-
state of the palaeo-water column and sediments, as well as possible connections between redox-state and microbial communities. Lateral variations in the redox conditions of marine basins could have controlled where exactly mineralisation occurred. If the mineralisation process was predominantly exhalative as proposed e.g. by Large et al. (1998) and Ireland et al. (2004), then the occurrence of a locally euxinic water column in an otherwise ferruginous or oxic basin might be used as a guide towards new deposits and so will not only aid palaeobiological studies but also mineral exploration.

The redox structure of BCF waters is also of major importance for elucidating connections between the early oxygenation of oceans and atmosphere and the evolution of microbial ecosystems and complex eukaryotic life. Probable eukaryotic microfossils are known from at least 1.8 Ga, indicating that eukaryotes existed at BCF times (Knoll, 2014), yet the BCF and other Proterozoic Formations seem to be devoid of indigenous biomarkers diagnostic for eukaryotes (Brocks et al., 2008, Pawlowska et al., 2013). Bio-essential trace metal drawdown (Anbar and Knoll, 2002) and H:2S poisoning under euxinic conditions may have greatly affected early eukaryotic communities.

The BCF has been in the centre of debate about the evolution of ocean redox conditions in the Paleoproterozoic (e.g. Brocks et al., 2005, Planavsky et al., 2011). Biomarkers from the Glyde River area of the McArthur Basin (Figure 2-1) appear to indicate euxinic conditions extending up into the photic zone of the water column, only overlain by a very shallow oxygenated upper layer (Brocks and Schaeffer, 2008, Brocks et al., 2005). Planavsky et al. (2011) on the other hand conducted an iron speciation study on two drill cores of the Myrtle subbasin (Figure 2-1) that indicated prevailing ferruginous conditions, similar to other Proterozoic settings around the world (Planavsky et al., 2011). So, were the prevailing redox conditions in deeper BCF waters sulphidic or ferruginous? Were the BCF sediments analysed by Planavsky et al. (2011) maybe influenced by local hydrothermal fluids that are responsible for formation of the nearby Myrtle Pb/Zn deposit (Mulholland, 2011), while euxinic conditions prevailed in most of the McArthur Basin as proposed by Brocks et al. (2005)? Or are ferruginous conditions indeed dominant for the BCF and euxinia was restricted spatially and/or temporally to the Glyde River and other local subbasins? Or was the redox structure even a more complex interplay between ferruginous, euxinic and oxic waters?
It is the aim of the Fe-speciation and biomarker analyses in this study to find answers to the above questions and to reconstruct the paleo-redox conditions across the McArthur Basin. Fe-speciation analysis is used to establish the redox state of BCF sediments across different locations within the McArthur Basin and across different stratigraphic levels. This is the first detailed study directly linking Fe-speciation to biomarker and additional geochemical proxies.
5.2 Methodology and samples

To reconstruct the paleo-redox conditions during deposition of the 1.64 Ga BCF, a total of 190 Fe-speciation analyses were conducted across the southern McArthur Basin. Without replicates, a total of 161 samples was analysed, including 110 from the BCF proper. Across the basin, FeHR/FeT was determined for 158 and FePy/FeHR for 155 samples. For the BCF proper, FeHR/FeT was determined for 109 samples and FePy/FeHR for 107 samples. The samples span different stratigraphic horizons and come from ten drill cores (Warramara 6: 25 samples, Myrtle 5: 9, Myrtle 4: 23, McArthur 5: 19, LV09001: 26, Glyde River 7: 37, Glyde River 10: 2, Glyde River 5: 1, Cow Lagoon: 1 and Bing Bong 5: 18 samples), forming a north-south transect along the Emu Fault zone (Figure 2-1). In addition to the BCF proper (n = 110), 13 samples are from the Reward Dolomite, 11 were logged as Surprise Creek and it is unclear what this is supposed to mean as Surprise Creek as a Mt Isa Basin formation (likely BCF), and 7 came from the W-fold shale/transition zone of the BCF depositional sequence. Additionally, 10 samples are from the overlying Lynott Formation (Caranbirini Mmb = 6) and 4 samples from the underlying Teena and 2 samples each from the even older Emmeruga and Mara Dolomites.

Due to the excellent thermal preservation, the thickness of preserved BCF sediments and the wealth of information from preceding studies, drill core GR7 from the Glyde River area was chosen as an exemplary location for a more detailed multi-proxy analysis. In GR7, Fe-speciation and δ³⁴S pyrite analyses were conducted on 37 samples and the uppermost 22 of these samples were further subjected to biomarker, TOC, δ¹³Corg, XRD, and XRF analyses for a multi-proxy comparison. 12 of the BCF samples were previously extracted by Jochen Brocks and Romain Guilbaud (University of Leeds) assisted in conducting the Fe-speciation analyses. Elemental maps of double polished thin sections of 7 samples were kindly provided by Sam Spinks (CSIRO, Perth). A more detailed description of the methodology can be found in chapter 2.

Ecological interpretations of course greatly benefited from discussions with colleagues, with main contributions from Jochen Brocks, Romain Guilbaud and Simon Poulton (University of Leeds).
5.3 Results and discussion

5.3.1 Iron speciation analyses

The concept of iron speciation analysis is explained in chapter 1. A technical description of iron speciation procedures is given in chapter 2, and all iron speciation data is compiled in Appendix 5-1. A stratigraphic trend including sulphur isotopes and a lithological column are shown for drill core GR7 in Figure 5-2.

![Figure 5-2. Iron speciation analysis results for drill core GR7.](image)

5.3.1.1 FeHR/FeT

Figure 5-3 shows a cross plot of FeHR/FeT versus FePy/FeHR for all samples across the McArthur Basin where both values were determined (n = 154). A few samples have been divided into sub samples (Appendix 5-1), but these are reported here as average values. The vast majority of these samples are from the undifferentiated BCF (n=109), but a few samples from directly over and underlying units (Reward Dolomite = 11, W-fold shale = 7, Surprise Creek = 11) and older and younger formations within the McArthur Group (Lynott Formation = 8, Emmeruga Dolomite = 2, Teena Dolomite = 4, Mara Dolomite = 2) are also included. Only 5 of the 154 samples (~3%) yield FeHR/FeT ≤ 0.22 indicative of
oxic depositional conditions. 9 samples (~6%) are in the borderline range between 0.22 and 0.38 were oxic depositional conditions may potentially have prevailed. Thus, the vast majority of McArthur samples was deposited under clearly anoxic conditions. FeHR/FeT averages ~0.66 across the McArthur Basin for samples from all formations and ~0.64 for BCF samples.

The crossplot of FeHR/FeT vs FePy/FeHR in Figure 5-4A only includes those samples that were logged as (undifferentiated) BCF in drill core reports and samples are colour coded by drill core. Also included are 14 MY3 and 2 MY4 BCF samples analysed by Planavsky et al. (2011). For the Myrtle area, our results are broadly comparable to those obtained by Planavsky et al. (2011). Samples from the northernmost drill core WM6 generally displays the lowest FeHR/FeT values and while there is a larger spread, samples from nearby drill core BB5 also plot more towards the left (see Figure 2-1 for drill core locations). Drill core GR7 from the southern end of the McArthur Basin also displays relatively low FeHR/FeT values. Samples from drill cores from the central part of the McArthur Basin, particularly from LV09001 and MY3 and MY4, in contrast plot more towards the right of the diagram.
Another way of highlighting differences in FeHR content between the cores is to plot FeT versus FeHR contents (Figure 5-5A). The vast majority of central basin samples, but hardly any northern or southern core samples plot above, while almost all central but very few northern or southern samples plot below the dotted blue line (FeHR/FeT = 0.8). A histogram of FeHR/FeT values for samples from central and the northern & southern parts of the basin also illustrated the significant difference in FeHR/FeT contents (Figure 5-5B). Samples from GR7 and WM6 show a normal distribution with a FeHR/FeT average of 0.53. The distribution of FeHR/FeT values of samples from MY3, MY4, MY5 and LV09001 in contrast is strongly skewed, with most FeHR/FeT values > 0.8 resulting in an average of 0.86. Except for one LV sample with an unrealistically high measured FeT value of 8.32 and therefore excluded from the correlations, all other central basin samples (MY3, 4, 5 and LV) show a strong linear correlation between FeHR and FeT (R² = 0.86, n = 49, p < 0.01), and cluster around the red regression line in Figure 5-5A. The correlation suggests that deposition of FeT in the central basin was mostly controlled by the deposition of FeHR.
It thus seems like there was a higher FeHR influx relative to FeT in the central part of the southern McArthur Basin. The cause for this distribution is difficult to ascertain as no
information is available for differences in the proximity to land or connectivity to the ocean between central and southern parts of the basin and central regions are not necessarily deeper. However, the major known base metal deposits of the basin are located in the central region, and the elevated FeHR flux may be related to proximal exhalative activity. Around the HYC deposit, the lower BCF is known as the HYC pyritic shale member and the high pyrite content is suggestive of significant iron influx even after cessation of base metal deposition. A lithgeochemical halo of carbon and oxygen isotopic anomalies in carbonates was previously shown to extend at least 15 km to the southwest of the HYC deposit (Large et al., 2001). Interestingly, carbonate iron also seems to be elevated in this “Zn-Pb-Tl-ferroan dolomite” halo with Fe/Mg molar ratios in carbonate > 0.1. In light of these observations, it is possible that a significant FeHR enrichment is part of a much wider halo that extends from HYC and Myrtle to the Leviathan area. The relationship between FeHR enrichment and distance to base metal deposits should be tested in more detail in future studies.

5.3.1.2 FePy/FeHR

For anoxic samples, the ratio of FePy/FeHR can be used to distinguish between iron-rich (ferruginous: FePy/FeHR < 0.7) and sulphide-rich (euxinic – FePy/FeHR > 0.7-0.8) paleo water column conditions (März et al., 2008). In earlier studies, a FePy/FeHR threshold of 0.8 (Anderson and Raiswell, 2004) was used which is reached by only 2 samples (~1%). The FePy/FeHR threshold of 0.7 (März et al., 2008) was crossed by 14 (~9%) samples. A potential caveat is posed by the weathering of pyrite during storage to form oxidised iron species, which would alter FePy/FeHR to lower values. On drill core samples, some pyrite weathering is apparent by the formation of sulphate minerals on core surfaces due to the humid conditions in the Northern Territory where the cores are stored. This was even observed in the freshest drill core material available and can thus not be fully avoided. Therefore, the FePy/FeHR threshold of 0.7 appears to be better suited to assess euxinia in the BCF. To test how strongly pyrite weathering could potentially have altered Fe-speciation data in a worst-case scenario, all FeOx could be assumed to be derived from the secondary weathering of pyrite. In this extreme scenario, still only about a quarter of all samples would cross the threshold for euxinia. It can thus be concluded
that beneath an oxygenated mixed layer, the waters of the McArthur Basin were mostly
derruginous. It is however noteworthy, that most of the samples yield intermediate
FePy/FeHR values between 0.4 and 0.7. A similar distribution was reported for the 1.45
Ga Newland Formation and 1.2 Ga Borden Basin, but not from the 1.7 Ga Chuanlinggou
Fm (Planavsky et al., 2011) or most other Neoproterozoic Formations (Guilbaud et al.,
2015).

Our FePy/FeHR values are comparable to those reported by Planavsky et al. (2011) for
samples from the two Myrtle drill cores MY3 and MY4, supporting the validity of our
data. We can show for the first time that dominantly ferruginous conditions were not
restricted to the Myrtle area but extended across the McArthur Basin. Particularly,
samples from the Glyde River area where exceptional biomarker preservation allows
detailed environmental reconstructions, also yield a dominantly ferruginous signal. Iron
speciation analyses, considered in isolation thus indicate that ferruginous conditions
dominated the McArthur Basin redox landscape during deposition of the Barney Creek
Formation.

While FeHR or FeHR/FeT show a geographical dependence in our data set, such a
relationship is not observed for FePy/FeHR values. In contrast to FeHR/FeT (Figure 5-
5B), our FePy/FeHR values show similar distributions for samples of the central and
northern and southern part of the basin (Figure 5-5C). FePy/FeHR values are only
slightly shifted towards higher values in the central basin samples. The majority of
FePy/FeHR of all samples plot between 0.4 and 0.7 and central and southern end
northern basin samples show almost identical averages of 0.57 and 0.55 respectively.

The relationship of FePy/FeHR and FeHR/FeT in northern and southern versus central
basin samples thus suggest an enhanced influx of highly reactive iron into the central
basin that is not buffered by enhanced iron sulphide precipitation in a euxinic water
column – another indication of generally ferruginous conditions. Thus, the flux highly
reactive iron into the central basin is not accompanied by a flux of reduced sulphur.
5.3.2 Mineralogy

Table 5-1 summarises results of X-ray powder diffraction (XRPD) mineral quantification (see Appendix 5-2 for more details). The BCF sediments in the upper 330 m of drill core GR7 consist of variable contents of carbonate and silicate minerals. Maximum carbonate content is 40 wt%. Most samples have a significant proportion of carbonates, with 77% (17 of 22) of samples yielding more than 15 wt% carbonate. In contrast, 14% (n = 3) have less than 5% carbonate. Average carbonate content is 22 wt%. The other main mineral constituents are quartz (average: 22.8 wt%), clays (average: 26 wt%) and feldspars (average: 9.2%), with silicate minerals on average comprising 62 wt%. Amorphous material comprises on average 12.8 wt%. Amorphous content shows the strongest negative correlation with quartz content (R² = 0.52). The amorphous content might be partly an artefact of sample preparation due to crushing the rocks in a stainless-steel ring mill in order to use the same powder that has been employed for the biomarker analyses, instead of using the more homogenous micronizing mill usually preferred for XRPD analysis at ANU. The negative correlation may potentially be explained by a higher quartz content decreasing the crushing efficiency due to the mineral’s hardness, thus preventing material from being crushed too fine for recognition by XRPD. Trace constituents are pyrite and possiblyapatite, gypsum or similar sulphates (probably mostly weathering products of pyrite and commonly in the form of the magnesium sulphate epsomite) and potentially even some marcasite in some samples. Due to the low abundances, there is a larger error associated with both identification and quantification of minor constituents.

Table 5-1. XRPD quantification in GR7 (amo: amorphous material, qtz: quartz, fsp: feldspar (K-feldspar & plagioclase), carb: carbonate (calcite & dolomite), py: pyrite, gyp: gypsum, marc: marcasite, ap: apatite, clays: mica or illite & illite/chlorite interlayer & chlorite, silicates: qtz+fsp+clays, avg: average).

<table>
<thead>
<tr>
<th>depth (m)</th>
<th>amo</th>
<th>qtz</th>
<th>fsp</th>
<th>carb</th>
<th>gyp</th>
<th>py</th>
<th>ma</th>
<th>rc</th>
<th>ap</th>
<th>clays</th>
<th>silicates</th>
</tr>
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<tbody>
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</tr>
<tr>
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<td>8.0</td>
<td>32.8</td>
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<td>0.0</td>
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<td>27.5</td>
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<td>1.6</td>
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<td>9.9</td>
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<td>1.0</td>
<td>33.8</td>
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</table>
The carbonate content derived from XRPD quantification correlates very well with carbonate content calculated from Mg and Ca concentrations obtained by X-ray fluorescence (XRF) analyses (Figure 5-6). There is however, an offset from the 1:1-line towards higher carbonate contents obtained from XRF. At least part of this off-set can be attributed to a fraction of the Mg and Ca being bound in silicate minerals, slightly overestimating the carbonate content calculated from elemental abundances. On the other hand, if some of the carbonate was part of the amorphous phase in XRPD, this would result in a slight underestimation of carbonate content by this method. Generally, the good correlation suggests that both methods give a good estimation of carbonate content.

**Figure 5-6.** Comparison between carbonate content (sum calcite and dolomite) as quantified by XRD and calculated from elemental Mg and Ca concentrations (assuming 100% Ca/MgCO₃).
5.3.3 Adding additional proxies to reveal ecological dynamics

Organic proxies can provide invaluable information about ancient ecosystems and depositional conditions (see e.g. Brocks and Summons, 2005, Peters and Moldovan, 1993). However, with deeper burial and increasing thermal maturity, catagenetic processes lead to the conversion, thermal cleavage and expulsion of hydrocarbon compounds, hampering ecological interpretations. Therefore, only few BCF sections can be employed for detailed ecological reconstructions based on biomarkers. The best-suited drill core for our study was GR7, whose biomarkers are exceptionally well preserved. At the time of study, it was the only available core with detailed biomarker information. Therefore, drill core GR7 was chosen for further investigation of environmental proxies. Biomarker analysis is restricted to the upper 330 m of sediments and only samples to 200 m depth are included in correlations because to avoid thermal maturity artefacts (Lee and Brocks, 2011).

Table 5-2 summarises the results of different organic and inorganic proxy analyses for the thermally well-preserved upper 350 m section of the BCF in drill core GR7. Figure 5-7 provides depth trends for a variety of proxies. Most proxies show two excursions in the intervals around 80 m and 220 m depth. Figure 5-7K shows the first principal component (CP1) of the proxies shown in Figure 5-7A-J, explaining >50% of all data variability. The different parameters are explained in the following discussion.

Table 5-2. Different ecological parameters in the thermally immature sediments of the upper BCF in drill core GR7.

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<th>depth (m)</th>
<th>AIR-G</th>
<th>AIR-P</th>
<th>G/(G+P)</th>
<th>Pr/Ph</th>
<th>δ¹³S (‰)</th>
<th>δ¹³Corg (‰)</th>
<th>TOC (%)</th>
<th>carb. (%)</th>
<th>FeH</th>
<th>R/Fe</th>
<th>FePy/F</th>
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<td>0.49</td>
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<td>25.08</td>
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<td>31.21</td>
<td>0.64</td>
<td>0.61</td>
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</table>
Figure 5-7. Depth trends of BCF environmental proxies in the upper 330 m of GR7.
5.3.3.1 TOC

TOC ranges from 0.4 to 3.9% in the upper 350 m of GR7, with an average of 1.8%. Although the depth trend of TOC appears somewhat similar to other proxies below about 150 m depth (Figure 5-7G), overall there is no correlation between TOC and carbonate content (Figure 5-8) or other any other proxy in the upper 200 m of the core.

![Figure 5-8. TOC versus carbonate content in the upper 350 m of GR7.](image)

5.3.3.2 Ti/Ca

The Ti/Ca ratio reflects the relative abundance of titanium versus calcium as determined by elemental analysis. The Ti/Ca ratios for the upper 350 m of BCF sediments in drill core GR7 are shown in chapter 4. Ti/Ca shows large variations, ranging from 0.01 to 0.26 with an average of 0.06. Ti/Ca is only significantly elevated in an interval around 90 m depth. Titanium is usually part of the siliciclastic fraction and Ti/Ca can be used instead of carbonate content (Figure 5-7H) for determining the relative importance of carbonate versus siliciclastic input. Ti/Ca shows a strong correlation with FePy/FeHR in the upper 200 m (Figure 5-9D). While there is a quasi-linear correlation ($R^2 = 0.64$, $n = 18$), a logarithmic regression line gives a better fit ($R^2 = 0.78$, $n = 18$) and the actual correlation line seems to be of more hyperbolic-form as indicated by the red line in Figure 5-9D that reflects a two endmember mixing simulation (see section ‘Two endmember mixing models’). Independent of the mathematical form of the correlation, there is a surprisingly close relationship between siliciclastic content and the degree of pyritisation.
5.3.3.3 Organic carbon isotopes

In GR7, organic carbon isotopes ($\delta^{13}C_{org}$) range from -30.9 to -34‰ with an average of -31.21‰ (Table 5-2). $\delta^{13}C_{org}$ does not show a significant correlation with any of the proxies shown in Figure 5-7. Changes in kerogen isotopes could reflect changes in the carbon isotopic composition of the dissolved inorganic carbon pool. Carbonate isotope data is not yet available.

For the upper ~250 m of BCF in GR7, $\delta^{13}C_{org}$ shows a linear decrease towards the top of the formation ($R^2 = 0.69, n = 19$) (Figure 5-10). This could be related to a shallowing of the basin towards the overlying Reward Dolomite, with more intense carbon recycling and heavier organic carbon values in deeper waters. If that was the case, then water depth fluctuations are unlikely to explain variations in carbonate content in the upper 250 m of GR7 as carbonate content does not follow a linear depth trend (Figure 5-7E).
A similar, near-linear trend in organic carbon isotopes is seen in the deep water Newland Formation and overlying lower Greyson Formation that may indicate the return to shallow water conditions at the Deep Creek locality closest to the basin main branch in the Mesoproterozoic Belt Basin (Stüeken, 2013). According to Stüeken’s description of the trend, δ\(^{13}\)C\(_{org}\) averages around -20.1 ±0.8‰ throughout the first 1.2 km (n = 10) but then steadily declines to -29‰ at the end of the section another 1.3 km above. Stüeken (2013) finds that in the Belt basin, carbon isotopes are generally more strongly fractionated in onshore environments and relatively shallow water. δ\(^{13}\)C\(_{carb}\) does not follow a linear depth trend and varies only slightly, ca. ± 1‰ around +1.5‰ over the Newland to Greyson section. Similarly, small variations of ca. ± 1‰ seem to be recorded in the BCF record as reported from drill core Bing Bong 2, although the BCF average somewhere around -1‰ is slightly lower than in the Newland. Formation Stüecken interprets the absence of small Δ\(^{13}\)C values as a probable reflection of a shallower water column with a relatively larger oxygenated surface layer and possibly less efficient entrainment of anoxic deep waters from the main basin (Stüeken, 2013). In view of the Belt data, interpretation of the organic carbon isotopic trend in GR7 as reflecting the gradational shallowing of the water column towards deposition of the Reward dolomite is a plausible possibility. Following Stüecken’s (2013) interpretations of the Belt isotopes, ecologically the BCF trend may reflect a gradational trend in cyanobacteria fixing CO\(_2\) under different environmental conditions or nutrient supplies, or onshore-offshore gradients in microbial community structure, possibly related to the differential activity of methanotrophs. As thermal maturity also shows a linear depth trend, a maturity influence, i.e. related to preferential cracking of lighter carbon moieties from the kerogen further downcore, cannot fully be excluded. A notable feature of the depth trend is a deviation from the linear depth trend between ~106 m and 77 m, an interval that is characterised by elevated FePy/FeHR values and perturbations in most ecological proxies (upper grey area in Figure 5-7). In contrast to most other proxies, the return of δ\(^{13}\)C\(_{org}\) towards ‘non-excursion’ values begins earlier (deeper in the stratigraphy). Thus, δ\(^{13}\)C\(_{org}\) may respond to ecological perturbations in this interval, but the potential meaning of these trends remains speculative. Considering the other ecological proxies, it is possible that organic matter degradation is enhanced for example through higher rates of bacterial sulphate reduction (related to higher FePy/FeHR in the grey intervals) or
intermittent oxidation events (related to higher AIR), or that isotopically heavy biomass of GSB that use the reverse TCA cycle contributes strongly to the OM pool (related to higher G/(G+P) in the grey intervals). However, it is also consistent with an excursion to deeper waters.

![Graph showing δ¹³Corg (%) vs depth (m)](image)

**Figure 5.10.** Organic carbon isotopes for the upper 350 m in drill core GR7. The sample from 328.8 m (red) does not follow the trend and is excluded from the regression to illustrate a potential depth trend in the upper ~250 m of BCF that may vanish in deeper parts of the formation.

### 5.3.3.4 Total iron (FeT)

In the upper 330 m of GR7, iron concentrations range from 1.44 to 3.92% with an average of 2.24%. FeT exhibits a similar depth trend as most other proxies (Figure 5-7) and shows a strong positive correlation with FePy (R² = 0.89, n = 18, p < 0.01) and poorly reactive iron (FePR; R² = 0.80, n = 18, p < 0.01) in the upper 200 m of GR7 (Figure 5-7) which suggests that the degree of iron enrichment depends on the rate of pyrite formation and the influx of detrital iron. A similar correlation with siliciclastic content (calculated here as (100% -wt% carbonate) (Figure 5-11) shows that both detrital Fe-influx and pyrite deposition are enhanced during periods of enhanced siliciclastic influx. The non-sulphidic highly reactive iron content (FeHR-FePy) does not correlate with the other variables (Figure 5-11). In the upper 200 m of GR7, non-sulphidic FeHR with an average of 0.54 wt% is dominated by carbonate iron (FeCarb) with an average of 0.37 wt% (68% of FeHR-FePy), 0.15 wt% oxide iron (FeOx) and traces of magnetite iron (FeMag). FeOx shows a strong positive correlation with FeCarb (R² = 0.78, n = 18, p < 0.01). A slight positive correlation of FeCarb with carbonate content is not statistically significant (R² = 0.102).
0.31, n = 18, p = 0.21) and essentially equalised by a slight negative correlation of FeOx with carbonate content. The upper BCF in GR7 thus experienced a constant rate of non sulphidic FeHR deposition under dominantly ferruginous conditions and FeT content is fully controlled by influx of detrital iron minerals and the rate of pyrite deposition. These relationships indicate that pyrite deposition was controlled by bacterial sulphate reduction that was enhanced during periods of higher siliciclastic influx. As the rate of BSR is controlled by sulphate and susceptible organic matter (OM) availability, one or both components should have been enhanced during periods of enhanced siliciclastic influx.

**Figure 5.11.** FeT versus non-sulphidic highly reactive iron (FeHR-FePy), poorly reactive iron (FeHR), pyrite Fe (FePy) and, on the secondary Y-axis, silicates (computed as 100% minus wt% carbonates) for samples from the upper 200 m in GR7 (n = 22).

### 5.3.3.5 FePy/FeHR

In the upper 350 m of GR7, FePy/FeHR ranges from 0.45 to 0.86 with an average of 0.61. Thus, most samples show intermediate FePy/FeHR values, but only three out of the 22 samples cross the euxinia threshold of März et al. (2008) (FePy/FeHR = 0.7). The GR7 data thus indicate dominantly ferruginous conditions, similar to the wider McArthur Basin. Nevertheless, an unusually high number of samples has intermediate to high FePy/FeHR values generated under seemingly ferruginous conditions. As FeHR-FePy is constant, rates of iron-oxide and iron-carbonate formation, and thus dissolved iron concentrations, do not seem to play a role in pyrite formation. Instead, rates of hydrogen
sulphide formation may have been greatly variable may rarely have been high enough to titrate out all of the dissolved iron from the water column.

5.3.3.6 FeHR/FeT

In the upper 350 m of GR7, FeHR/FeT reaches from 0.35 to 0.78 with an average of 0.61 (n = 22). With the exception of a single sample falling into the borderline range between 0.22 and 0.38, all samples were clearly deposited under dominantly anoxic depositional conditions. FeHR/FeT ratios are lower than those of central basin cores, but comparable to those from WM6 from the north of the southern McArthur Basin and therefore seem to reflect background values are not significantly influenced by exhalative activity in the central basin. Since FeT is strongly controlled by pyrite formation and detrital iron influx, FeHR by pyrite formation and detrital iron influx and pyrite formation show a strong correlation, FEHR/FeT does not co-vary with the other ecological proxies over most of the stratigraphy Figure 5-9.

5.3.3.7 Chromium

Chromium concentrations range from 17 to 75 ppm with an average of 41.3 ppm (Figure 5-7I). They show a similar trend to the other proxies with a slight enrichment in intervals of high pyrite content (Figure 5-7). Chromium enrichment in carbonate-poor intervals could reflect a carbonate-dilution effect with chromium-incorporation being largely restricted to siliciclastic minerals as marine sediments can experience a significant detrital input of Cr (Reinhard et al., 2013). However, chromium is generally also incorporated in carbonate minerals and is a redox sensitive element (Frei et al., 2011). The slight enrichment in pyrite-rich intervals may thus be redox controlled with preferential accumulation during euxinic periods.

5.3.3.8 Pyrite sulphur isotopes (δ34S_{pyrite})

The sulphur isotopic composition of BCF sulphides in the upper 350 m of drill core GR7 is shown in Table 5-2 and plotted against depth in Figure 5-2 and Figure 5-7. The pyrites in GR7 show a heavy isotopic signature similar to that observed by Johnston et al. (2010)
for nearby drill core GR10 as discussed in chapter 4. This indicates sulphate depletion during pyrite formation, most likely due to (near)quantitative sulphate reduction. The heaviest values may even indicate Rayleigh fractionation which may be due to restricted conditions of the McArthur basin (see Johnston et al., 2008 for a more detailed discussion). The $\delta^{34}S_{\text{pyrite}}$ values are slightly lower in the pyrite-rich intervals, consistent with higher sulphate availability. There are at least two plausible explanations for this phenomenon. The first encompasses higher sulphate availability in the basin waters during these periods. This would imply that sulphate availability generally limited the establishment of euxinia in the BCF waters. The second explanation encompasses a higher proportion of pyrite formation in the water column where sulphate supply is less restricted, as compared to pyrite formation in the sediments where sulphate replenishment is limited by diffusion. A higher proportion of water-column pyrite formation is consistent with water column euxinia. Even under euxinic conditions however, a proportion of pyrite would form in the sediments, so that it is difficult to distinguish between the two possibilities that are not mutually exclusive.

5.3.3.9  $G/(G+P)$

$G/(G+P)$ measures the abundance of supposedly green sulphur bacterial-derived $C_{13-37}$ 2,3,6- arylisoprenoids relative to the sum of 2,3,6- and supposedly purple sulphur bacterial-derived 2,3,4-trimethylarylisprenoids. Intact $C_{40}$ arylisoprenoids have been detected in many of the well-preserved BCF samples. The intact aromatic carotenoids are dominated by okenane, a 2,3,4-trimethylarylisprenoid (AI) probably derived from biological okenone, and by chlorobactane, a 2,3,6-AI likely derived from biological chlorobactene (Brocks and Schaeffer, 2008) (Figure 5-12). Also present, albeit in much lower concentrations than okenane and chlorobactane, are purpurane, renierapurpurane, renieratane, β-renieratane and isorenieratane (Brocks et al., 2005, Brocks and Schaeffer, 2008). In the BCF, okenane, renierapurpurane, renieratane and β-renieratane are interpreted as biomarkers for purple sulphur bacteria (PSB, Chromatiaceae). Chlorobactane is probably derived from green pigmented and isorenieratane from brown pigmented green sulphur bacteria (GSB, Chlorobiaceae) (Brocks and Schaeffer, 2008).
2,3,4- and 2,3,6-trimethylarylisprenoids with 13 to 37 carbon atoms have been reported to be the dominant biomarkers in the aromatic fraction where they constitute ~0.1% of the total mass (Brocks and Schaeffer, 2008). These molecules are the typical breakdown products of aromatic carotenoids (Brocks and Summons, 2003). The relationship between intact carotenoids and arylisoprenoid degradation products is illustrated in Figure 5-13A that shows a complete homologous series from C_{40} to C_{13} arylisoprenoids in the BCF. Renieratane is the only arylisoprenoid yielding both, 2,3,4- and 2,3,6-methylation, but only occurs in insignificant amounts in the BCF (see Fig. 1c in Brocks et al., 2005). A contribution to the 2,3,6-AI signal from the degradation of other carotenoids (Koopmans et al., 1996, Koopmans et al., 1997) cannot currently be ruled out, but considering the presence of intact GSB C_{40} carotenoids and the stratified water column conditions indicated by iron speciation and Pr/Ph data, we propose to use the sum of 2,3,6-arylisprenoids as a relative measure of the GSB-, and the sum of 2,3,4-arylisprenoids as a relative measure of PSB-contribution to the sediments. For the intact biological pigments okenone and isorenieratane, it was shown in modern Lake Cadagno (Switzerland) that their relative abundance corresponds to that of PSB and GSB as
determined from 16S rDNA (Wirth et al., 2013). Therefore, the $G/(G+P)$ ratio is here proposed as a proxy for the relative ecological importance of green versus purple sulphur bacteria. The ratio of $G/(G+P)$ measures the abundance of $C_{13,37}^{2,3,6}$ relative to the sum of $2,3,6$- and $2,3,4$-trimethylarylisoprenoids using respective peak areas in $m/z$ 134 full scan or selected iron monitoring (SIM) mass chromatograms.

$G/(G+P)$ values in the upper BCF in GR7 follow a somewhat cyclic depth trend with higher values around ca. 67 to 102 m depth and below ca. 200 m depth (Figure 5-7). In GR7, the $G/(G+P)$ ratio ranges from 0.26 to 0.73 with an average of 0.49 (Table 5-2). The arylisoprenoid distribution of a typical BCF samples with $2,3,4$-AI dominance is shown in Figure 5-13A and with $2,3,6$-AI dominance Figure 5-13B.

These values are broadly comparable to $G/(G+P)$ ratios that we estimated from okenone and isorenieratene abundances reported from the modern stratified Lake Cadagno by Wirth et al. (2013). $G/(G+P)$ values estimated from the data presented in Wirth et al. (2013) generally fall between ca. 0.1 and 0.8 based on both rDNA and okenone/isorenieratane data (although two samples with GSB rDNA did not yield any isorenieratane, resulting in $G/(G+P)_{rDNA} = 0$). While $G/(G+P)$ are not reported by Wirth et al. (2013) and data are tabulated making estimates difficult, visually there seem to be on average only slightly higher abundances of GSB- versus PSB-DNA and -pigments, thus average $G/(G+P)$ ratios are likely slightly higher than 0.5 (see Fig. 6c & d in Wirth et al., 2013). By measuring the width of the rectangles representing okenone and isorenieratane abundance for ten sample intervals in Fig. 7 of Wirth et al. (2013), we calculated $G/G(G+P)$ ratios between 0.38 and 0.88 with an average of 0.64, not far from the BCF results. Flood and mass-movement events resulting in turbidity increase, and/or nutrient availability fuelling primary production and resulting in shading by oxygenic phototrophs are interpreted to favour GSB over PSB communities in Lake Cadagno (Wirth et al., 2013).
Figure 5-13. M/z 134 partial ion chromatograms of aromatic fractions of typical BCF samples with A) 2,3,4-trimethyl arylisoprenoid (A1) dominance in sample B03163 (GR7, 47.55 m) and B) 2,3,6-trimethyl arylisoprenoid dominance in sample B03169 (GR7, 90.3 m). Ok: okenane, Cl: Chlorobactane. green squares: 2,3,6-, purple triangles: 2,3,4-trimethyl-arylisoprenoids, numbers indicate carbon atoms. The inset highlights the degradation of okenane that is hampered at methylation-sites resulting in the characteristic arylisoprenoid distribution pattern with low or missing pseudo-homologs at C_{17}, C_{23}, C_{28} and C_{33}. 
The comparison of absolute values from modern settings to those of the BCF is not straightforward, as GSB and PSB can produce different amounts of pigments, and PSB also produce non-aromatic carotenoids like lycopene (Brocks and Schaeffer, 2008). We therefore consider relative changes in the $G/(G+P)$ ratio to be more significant for ecological variations. Nevertheless, the consistent trends in deposited pigment concentrations and bacterial abundance in Lake Cadagno and the similarity to the BCF ratios allow for the possibility of similar relative bacterial abundances in the Paleoproterozoic McArthur Basin as observed in modern Lake Cadagno.

### 5.3.3.10 Benthic communities as potential arylisoprenoid sources

PSB have greater light requirements and a higher oxygen tolerance than GSB, and therefore usually live at shallower depths, both in planktonic and benthic environments. PSB require around four times more light than green pigmented GSB in order to grow at comparable rates (Biebl and Pfennig, 1978), with GSB being able to grow at only ~0.0005% surface light intensity (Overmann et al., 1992). Chromatiaceae typically thrive beneath the surface of photosynthetic microbial mats or below the chemocline in stratified lacustrine to hypersaline waters. The accessory pigment okenone appears to be ideally suited to capture light in planktonic environments with an absorption maximum in the yellow-green spectrum around a wavelength of 520 nm (Brocks and Schaeffer, 2008). Accordingly, it is the most common carotenoid of planktonic Chromatiaceae, observed in about 63% of cases (Van Gemerden and Mas, 1995). Okenone-producing PSB only thrive in modern environments were the chemocline is not deeper than ~12–20 m (Brocks and Schaeffer, 2008). In contrast, all known benthic species produce carotenoids with a lycopene skeleton and okenone has not been detected in microbial mats (Brocks and Schaeffer, 2008). Based on uniformitarian considerations, okenone is thus best interpreted as a biomarker for planktonic purple sulphur bacteria.

Several proxies such Pr/Ph < 1 and FeHR/FeT > 0.38 point to anoxic waters during deposition of the BCF. In such an aquatic setting, one would expect an oxygenated upper mixed layer above a chemocline inhabited by dense microbial populations that shade the underlying water column and sediments. If benthic PSB were the source of okenone in the BCF, the chemocline would need to be extremely oligotrophic, in order for enough
light to penetrate through the water column, the shading by oxygenic primary producers, microbial populations at the chemocline and lastly the upper mat layers, to sustain the anoxic phototrophic purple sulphur bacteria in deeper mat layers. Due to the light limitations of okenone-producing PSB discussed above, it thus seems most unlikely that *in-situ* microbial mats are the primary source of okenone in the BCF.

An alternative explanation for PSB and GSB biomarkers in deep-water BCF sediments is transportation of shallow water microbial mats that may be represented by organic streaks (Lyons et al., 2012). In drill core LV09001 where laminated sediments are representative of ripped-up, transported and re-deposited material (Figure 4-7) streaks and underlying intact laminae optically and chemically seem to be made of similar material. Together with the presence of bigger fragments of laminated sediments within the transported sediment layer, *in-situ* disruption and short distance transport is indicated. These ‘streaks’ thus seem to have been locally generated in deepwater environments. Additionally, Fe-speciation analyses yielded an anoxic signature throughout the BCF in LV09001 (see Appendix 5.1), including subsamples of laminated sediments and overlying transported material. So even if the overlying material had been transported from distal shallow water environments, these environments should still have been anoxic and favouring planktonic over benthic sulphur bacterial communities. Since extant benthic PSB employ pigments other than okenone, a benthic source for the BCF PSB biomarkers is unlikely.

One mechanism that could account for the laminated to streaky appearance of many PSB biomarker-rich sediments could be buoyant rafting associated with seasonal die-off of planktonic sulphur bacterial communities. Association with minerals may help transport the buoyant organic matter below salinity gradients. In meromictic Mahoney Lake, rafted biomass from planktonic PSB communities is also washed into littoral environments from where it may be transported downslope together with shallow water sediments that integrate indigenous shoreline microbial communities (Bovee and Pearson, 2014). Under the shallow chemocline conditions required for planktonic sulphur bacterial growth, a large proportion of the littoral zone would be anoxic, allowing accumulation of rafted material under reducing conditions. Subsequently, well-preserved planktonic signatures could be associated with shallow water sediments.
as an indirect (but not the only) route for influx of planktonic biomass to deeper parts of the basin.

5.3.3.11 Planktonic communities as potential arylisoprenoid sources

Figure 5-14 illustrates the distribution and ecology of planktonic PSB and GSB under varying chemocline depth-conditions, and the main carotenoids expected in the water column and sediments. In microbial mats and in very shallow stratified water bodies, PSB usually live at the oxic-anoxic boundary above green pigmented GSB, who are succeeded by low-light adopted brown pigmented GSB. Brocks and Schaefer (2008) reviewed planktonic sulphur bacterial occurrences. Okenone-producing PSB blooms were observed between ~1.5 and 24 m depths, with 75% at less than 12 m and an average depth of 8.4 m (n =33). Chlorobactene-producing green pigmented GSB blooms were observed between ~2 and 16 m depth, with 75% not deeper than 6 m and an average of 5.7 m (n = 28). Isorenieratene-producing brown pigmented GSB were observed between 2 and 80 m depth with 7% not deeper than 17 m (n = 19) (Brocks and Schaeffer, 2008), but they can even thrive >100 m chemocline depth as observed in the euxinic Black Sea (Repeta et al., 1989) and the ferruginous waters of Lake Matano in Indonesia (Crowe et al., 2008).

Light availability can thus be considered to be one of the main controls on the relative importance of planktonic GSB versus PSB. This is for example observed in Lake Cadagno, where a shift from the usual PSB ecosystem (e.g. years 1994–2001) to a GSB dominated ecosystem was observed in 2002 and 2003. The population shift was accompanied by a reduction in transmitted light reaching the chemocline from mostly >10 μE m-2 s-1 before the year 2000 to <10 μE m-2 s-1 thereafter (Tonolla et al., 2005). PSB communities are thus extremely sensitive to changes in light availability. Brown-pigmented GSB in contrast still thrive in low lightning environments.

Light availability can be controlled for example by the depth of the chemocline, or turbidity of the overlying water column related to suspended sediments or shading by oxygenic primary producers. For example, the ecosystem drawn in Figure 5-14 (1) can be expected to be dominated by PSB with minor green pigmented GSB as is usually the
case in Lake Cadagno with its modern shallow stratification (Tonolla et al., 2005). Okenane and minor chlorobactane and the respective 2,3,4- and minor 2,3,6-AI diagenetic breakdown-products would be expected to be the most dominant biomarkers from such sediments. However, if the chemocline would extend to a depth below 50 m, then the ecosystem would be expected to be dominated by brown pigmented Chlorobiaceae, producing isorenieratene (and beta-isorenieratene) and leaving mostly isorenieratane and 2,3,6-arylisoprenoid breakdown products as biomarkers in the sediments.

Figure 5-14. Schematic illustration of the habitat of purple and green sulphur bacteria and some of the carotenoids expected in water column and sediments. 1. PSB (purple) commonly thrive close to the chemocline, above green pigmented (green) and brown pigmented GSB (brown). 2. Okenone-producing PSB are usually not found deeper than 12-15 m. 3. Green-pigmented GSB can be found at chemocline depth of up to 16 m and mostly produce chlorobactene pigments (Brocks and Schaeffer, 2008). 4. Brown pigmented GSB can thrive below 110-120 m and produce isorenieratene (Crowe et al., 2008). In the sediments, arylisoprenoids are degraded by diagenetic processes. 6. More intact and longer-chain AI are preserved under strongly reducing conditions. Intermittent oxidation results in sedimentation of shorter degradation products (Schwark and Frimmel, 2004).
All GSB produce 2,3,6-arylisprenoids, so variations within the GSB are less likely to significantly affect the G/(G+P) ratio than variations within PSB communities. A switch in carotenoid production within PSB communities, e.g. from okenone to lycopene would however significantly affect G/(G+P) ratios. Intra- and intercommunity variations may be triggered by fluctuations in nutrient availability, temperature or other physicochemical factors such as redox potential, oxygen or sulphide concentrations (Tonolla et al., 2003). Differences in arylisoprenoid production related for example to varying pigment densities within the bacterial populations or variations in reproduction rates between different species would also influence G/(G+P) ratios. Planktonic sulphur bacterial remains could also be rafted and deposited in shallow water environments, potentially subjecting them to oxic degradation and heterotrophic reworking and then redeposited in deep water settings as observed in Lake Mahoney (Bovee and Pearson, 2014). Delivery of planktonic biomarkers to basinal sediments via the littoral environment may affect arylisoprenoid preservation (i.e. AIRs), but is unlikely to affect relative abundances of green versus sulphur bacterial markers (i.e. G/(G+P)). Since G/(G+P) and AIR-G and AIR-P co-vary in GR7, the littoral route is unlikely to control the Paleoproterozoic GSB and PSB biomarker ratios.

In summary, G/(G+P) is considered to reflect the relative ecological importance of green and purple sulphur bacteria. Light intensity is expected to be one of the main controls and can in turn be controlled by chemocline depth, water turbidity or shading by oxygenic primary producers possibly related to nutrient availability.

### 5.3.3.12 AIR-G and AIR-P

The arylisoprenoid ratio (AIR) measures the relative abundance of short- versus intermediate chain-length 2,3,6-trimethylarylisprenoids. This proxy was introduced by Schwark and Frimmel (2004) as a measure of the oxic degradation of aromatic carotenoids produced by green sulphur bacteria. Here, we define AIR-G = \( \sum(C_{13-17})/\sum(C_{18-22}) \) 2,3,6-trimethyl arylisoprenoids and AIR-P = \( \sum(C_{13-17})/\sum(C_{18-22}) \) 2,3,4-arylisoprenoids. AIR-G and AIR-P are considered to reflect the degradation of green and purple sulphur bacterial carotenoids respectively.
In the upper 330 m of BCF sediments in drill core GR7, AIR-G ranges from 0.12 to 3.16 with an average of 1.49, while AIR-P ranges from 0.07 to 2.86 with an average of 1.15 (Table 5-2). Both parameters show a good linear correlation, particularly in the upper 200 m (Figure 5-15). The deepest sample considered in this study from 328 m depth is a stark outlier in the diagram. This is likely caused by co-elution with other compounds in more mature samples. AIR-G is higher than AIR-P from the same samples, causing a deviation of the regression form the 1:1-line (Figure 5-15).

![Figure 5-15](image)

**Figure 5-15.** AIR-G vs AIR-P for BCF above 200m depth (blue dots + regression line), and between 200 and 330 m (red dots).

AIR-G and AIR-P also follow a similar depth trend that is also comparable to the G/(G+P) ratio (Figure 5-7). AIR-G and AIR-P yield linear correlations with G/(G+P), particularly in the upper 200 m (AIR-P: R² =0.61, n = 18, p < 0.01; Figure 5-9A), AIR-G: R² = 0.50, n = 18, p < 0.01). These correlations indicate that arylisoprenoid degradation is greater when GSB are more abundant. This relationship is also illustrated in the BCF chromatograms shown in Figure 5-13. Samples with low G/(G+P) ratios yield an extended series of arylisoprenoids often preserving intact carotenoids such as okenane and chlorobactane (Figure 5-13A), while extracts with high G/(G+P) ratios only yield short- to medium-chain arylisoprenoids (Figure 5-13B).

Aromatic carotenoids are highly susceptible to oxic degradation and AIR is thought to reflect the degree of oxygen exposure (Schwark and Frimmel, 2004). As Chlorobiaceae
and Chromatiaceae live below the oxic-anoxic interphase, their carotenoids are produced in the anoxic part of the water column. Any oxidation must therefore reflect oxygen influx into the usually anoxic part of the water column (Schwark and Frimmel, 2004). Thus, low AIR values indicate largely stable and persistent photic zone anoxia, whereas elevated values indicate an intermittently overturning water column or circulation of oxygenated waters to the sediment-water interface.

5.3.3.13 Pr/Ph

The ratio of the regular isoprenoids pristane and phytane (Pr/Ph) shows only minor variations between 0.44 and 0.83 in the upper 330 m of BCF in drill core GR7, with an average of 0.65 (Table 5-2). In deeper section (i.e. below ~200 m), there is likely an increasing influence of thermal maturity on Pr/Ph ratios and ratios become less reliable as redox indicators.

Generally, there are several potential sources for Pr and Ph, but in our low maturity BCF sediments low in elemental and organic sulphur that were deposited before the emergence of land plants and zooplankton, the most likely sources are chlorophylls from (oxygenic) phototrophs and potentially additional phytane input from archaeal ether lipids (e.g. King et al., 1998). Pristane is an oxidation product, and phytane a reduction product, of the phytol side chain of chlorophyll (Figure 5-16) and the ratios is thus a frequently applied molecular redox indicator (Peters and Moldowan, 1993). It is currently not possible to assess a potential contribution of archaea to the phytane pool, but considering the abundance of chlorophylls in photosynthetic organisms and the abundance of other phototrophic biomarkers like carotenoids, redox conditions are considered the most like primary control on Pr/Ph in GR7. Pr/Ph values < 0.8 have been empirically determined as indicative of anoxic deposition (Peters and Moldowan, 1993). Reducing conditions as indicated by Pr/Ph are supported by the abundance of arylisoprenoids and preservation of intact carotenoids in many samples, as well as high FeHR/FeT values, supporting the hypothesis that redox conditions are the main control on Pr/Ph ratios.
In contrast to aromatic carotenoids that are produced in the anoxic part of the water column, phytol-containing (bacterio)chlorophylls are expected to be dominantly produced by cyanobacteria in the oxygenated surface waters. Exposure to oxygen and oxic degradation of phytol to pristane would thus commence during the passage through the upper mixed layer (Schwark and Frimmel, 2004). In stratified settings like the BCF, Pr/Ph ratios may thus largely depend on the exposure time to oxygen while sinking through the upper water column and the exposure time may thus be controlled by the mixed layer depth (MLD). Under the low atmospheric oxygen conditions of the Paleoproterozoic, the MLD would likely have approximately coincided with the depth of the chemocline.

Pr/Ph shows a similar depth trend patterned to AIR-G and AIR-P (Figure 5-7) and particularly to G/(G+P) ($R^2 = 0.77$, $n = 18$, $p < 0.01$, Figure 5-9B). As discussed previously, one of the main controls on green and purple sulphur bacterial communities may be the lighting conditions, which are commonly controlled by the depth of the chemocline, with PSB usually thriving above 12 m chemocline depth and GSB even below 110 m chemocline depth. The strong correlation between Pr/Ph and G/(G+P) then suggests that both parameters are likely controlled by chemocline depth. A secondary control on Pr/Ph would be expected from intermittent oxidation events such as storm or mass-flow induced injections of oxygenated surface waters or seasonal overturn of basin waters. The AIR is a proxy for intermittent oxidation of anoxic waters, and the correlation between AIR and Pr/Ph suggests that both proxies may have been affected by such oxygenation events.
5.3.4 Environmental models based on proxy data

As shown in Figure 5-7, a variety of ecological proxies follow a similar depth trend in the BCF. Many of these proxies even show linear correlations as exemplified in Figure 5-9. Some of the strongest positive linear correlations are displayed by the iron speciation proxy FePy/FeHR, which distinguishes between ferruginous and euxinic conditions, and Ti/Ca or, inversely, carbonate content (Figure 5-9D). Strong correlations are further observed between FePy/FeHR versus AIRs (Figure 5-9C), AIRs versus G/(G+P) (Figure 5-9A), and G/(G+P) versus Pr/Ph (Figure 5-9B). To a lesser extent, Cr, FeT and δ^{34}S_{pyrite} also follow similar depth trends and/or exhibit linear correlations with the other proxies (Figure 5-7). Three principally different environmental models are discussed in the following to explain these correlations in the framework of the BCF geology and depositional environment (see chapter 3 and 4).

5.3.4.1 Gradual environmental model

The correlations between environmental, ecological and sedimentological proxies may reflect a true causal link between the variables, implying that these parameters are linked linearly to each other or respond in a linear fashion to a common parameter such as sea level change, or a shift in the evaporation/precipitation regime. To maintain quasi linear relationships between such diverse proxies over geological timescales seems rather unlikely however. For example, a synchronous, gradual linear change is difficult to invoke over extended timescales for processes controlling G/(G+P) and Pr/Ph. Pr/Ph variations can largely be explained by variations in MLD, which in turn should control chemocline depth. Gradual trends in Pr/Ph can be explained by gradual deepening of the mixed layer, such that e.g. a doubling of the mixed layer depth would cause a doubling of oxygen exposure time of phytol and thus a linear increase of Pr/Ph with extension of the mixed layer. A linear correlation between G(G+P) and Pr/Ph would then require that GSB relative to PSB abundance increases in a linear response to chemocline depth and the same proportional variations would then have to be maintained for ten to hundred thousands of years. A gradual increase in G/(G+P) would probably be maintained only over a limited range of environmental conditions and cannot be
expected over geological timescales. For example, at a chemocline depth >20 m, no PSB can be expected to be present anymore \((G/(G+P) = 1)\). However, such values are never observed even for somewhat elevated Pr/Ph and in inference greater MLD. \((G/(G+P))\) should also be influenced by a variety of other factors like shading by oxygenic phototrophs, turbidity and nutrient availability. Over geological time scales it is again very unlikely that all these factors show little variation, while chemocline depth changes gradually. So, a simple linear response of \((G/(G+P))\) to mixed layer depth is implausible, making it difficult to explain the \((G/(G+P))\) versus Pr/Ph correlation in a gradual environmental model.

High FePy/FeHR values further indicate that eukinic conditions were established during some periods. The switch from ferruginous to eukinic conditions would mark a major change in basin ecology. It is very unlikely that the linear correlations with organic proxies are maintained across an ecological divide. Overall, it thus appears very unlikely that the BCF observations can be explained by a gradual ecological model.

5.3.4.2 Two endmember mixing models

Instead of reflecting a gradually changing ecological system, the proxy correlations can be explained by mixing varying proportions of two ecological endmember signatures. Many natural aquatic systems fluctuate between two or more ecological states that may be controlled by seasonal variations. Examples are dry and wet seasons, seasonal over-turn of lakes, mixed layer depth variations and seasonal ecological patterns such as planktonic spring blooms. Additionally, many systems exhibit distinct ecological variations in space. For example, microbial mats possess a distinct microbial ecology compared to the overlying water column. Near shore waters may harbour planktonic communities different from those in the open sea. As a local depocenter, the deep part of the Glyde River subbasin at GR7 provides an ideal location were sediments of different geographic origin could have been mixed. For example, in situ pelagic sedimentation could have been mixed with material transported from shallow waters.
5.3.4.2.1 Results and discussion of mixing simulations

To test if two endmember mixing can explain the BCF data distribution, we performed a mixing simulation (see methodology chapter and Appendix 5.2). In the simulation, endmember compositions were iteratively adjusted to obtain the best overall fit with BCF correlations. 60 x-y plots with combinations of 12 different individual variables and ratios were considered. The different variables and the number assigned to each graph are shown in Table 5-3. All plots and data are shown in Appendix 5.2. Selected graphs are shown in the following.

Table 5-3. Overview of mixing simulations and number of graph in Appendix 5.2. Due to the similarity to Ca, not all carbonate plots are shown (X).

<table>
<thead>
<tr>
<th>FePy/FeH</th>
<th>Ti/Ca</th>
<th>Ti</th>
<th>Ca</th>
<th>FePy</th>
<th>Si</th>
<th>FeT</th>
<th>AIR-G</th>
<th>AIR-P</th>
<th>Pr/Ph</th>
<th>G/(G+P)</th>
<th>carbonate</th>
</tr>
</thead>
<tbody>
<tr>
<td>FePy/FeH</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>7</td>
<td>11</td>
<td>16</td>
<td>22</td>
<td>29</td>
<td>37</td>
<td>46</td>
</tr>
<tr>
<td>Ti/Ca</td>
<td>-</td>
<td>3</td>
<td>5</td>
<td>8</td>
<td>12</td>
<td>17</td>
<td>23</td>
<td>30</td>
<td>38</td>
<td>47</td>
<td>X</td>
</tr>
<tr>
<td>Ti</td>
<td>-</td>
<td>6</td>
<td>9</td>
<td>13</td>
<td>18</td>
<td>24</td>
<td>31</td>
<td>39</td>
<td>48</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>-</td>
<td>10</td>
<td>14</td>
<td>19</td>
<td>25</td>
<td>32</td>
<td>40</td>
<td>49</td>
<td>49</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>FePy</td>
<td>-</td>
<td>15</td>
<td>20</td>
<td>26</td>
<td>33</td>
<td>41</td>
<td>50</td>
<td>57</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Si</td>
<td>-</td>
<td>21</td>
<td>27</td>
<td>34</td>
<td>42</td>
<td>51</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FeT</td>
<td>-</td>
<td>28</td>
<td>35</td>
<td>43</td>
<td>52</td>
<td>58</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIR-G</td>
<td>-</td>
<td>36</td>
<td>44</td>
<td>53</td>
<td>59</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIR-P</td>
<td>-</td>
<td>45</td>
<td>54</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pr/Ph</td>
<td>-</td>
<td>55</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/(G+P)</td>
<td>-</td>
<td>60</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>carbonate</td>
<td>-</td>
<td></td>
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</table>

G/(G+P) versus AIR-P and Pr/Ph, FePy/FeHR versus AIR-P and Ti/Ca, the simulated mixing curves are plotted in Figure 5-9. Visually, the mixing curves exhibit a similar or better fit to the BCF data than linear correlations, suggesting that the correlations may be explained by two-endmember mixing. Figure 5-17 shows a selection of additional mixing curves to illustrate that meaningful mixing curves can be obtained for all relevant BCF proxies.

The mixing curves illustrate that correlations in two-endmember mixing plots are linear only under certain circumstances. Hyperbolic mixing curves are instead exhibited by most ratio plots. The hyperbolic shape of many data distributions is typical for ratios in two endmember mixing processes, because individual components are mixed. The shape of mixing lines can reach from a strong hyperbolic curvature to quasi linear (Langmuir et al., 1978). The mixing simulation reflects the distribution of diverse geochemical BCF data.
According to Langmuir (1978), there are three tests for mixing if the data appear to be consistent with a two-endmember mixing model:

1. All plots of appropriate elements should conform to mixing curves
2. The individual samples should maintain the same relative distance to each other on all plots
3. The data should plot as straight lines on so-called companion plots

As ecosystems are never fully homogenous over time, ideal mixing scenarios cannot be expected for ecological proxies, but any proxies that show distinctive differences
between two endmembers and are subjected to two endmember mixing should broadly conform to above requirements.

Proxies that conform to two endmember-mixing should thus follow mixing lines on all possible mixing plots. This is generally the case as can be seen from the different mixing plots shown in Figure 5-17, Figure 5-18, and in Appendix 5-2. Due to different intra-endmember variability and different degrees of post-mixing alteration, significant deviations from mixing lines may be expected, particularly from organic proxies. Overall, however, the observed BCF distribution is remarkably close to theoretical mixing lines in virtually all plots, in our view passing test 1 for two endmember mixing, supporting the hypothesis that the BCF in the upper 200 m of drill core GR7 records mixing of sediments with two distinct endmember ecologies.

Ecological intra-endmember-variability and post mixing-processes change to some degree the order of samples on the mixing lines. A comparison of the relative position of samples on the mixing lines in four different mixing plots is shown in Figure 5-9 and Figure 5-18. It can be seen that samples that plot close to each other in one plot, usually also plot close to each other in other plots, although some variability is also apparent. Considering the type of data however, in our view the BCF dataset also passes test 2 for mixing.
Figure 5-18. Comparison of elemental ratio plots and companion plots for (A) Ti/Ca vs FePy/FeHR (left), and Ca/FeHR vs FePy/FeHR (right), B) Si vs Ti/Ca (left), and Si vs Ca/Si (right) (C) G/(G+P) vs AIR-P (left), and G/(G+P) vs C18-C22 Al-P///(G+P) for C13-C37 (green) and C13-C22 Al (blue), simulation for C13-C22 Al only (right), (D) G/(G+P) vs AIR-G (left), and G/(G+P) vs C18-C22 Al-G///(G+P) for C13-C37
According to Langmuir (1978), so-called companion plots, which plot one of the original ratios versus the ratios of the denominators of the original ratios, can be constructed as another test for mixing. The overall curvature of a mixing curve depends on the ratios of the denominators of the endmembers. Therefore, all intermediate points should have the ratios of their denominators proportionally intermediate to the values of the two endmembers. On the companion plot, the data will plot as straight line, if the mixing curve on the original plot applies to all data (Langmuir et al., 1978).

Figure 5-18 shows different ratio- and corresponding companion-plots for BCF data. The inorganic closely follows a proposed hyperbolic mixing and shows strong linear correlations in the companion plots (Figure 5-18A, B) as expected for mixing of two almost homogenous endmembers. The BCF inorganic proxies thus also pass test 3 for endmember mixing, and additionally suggest a surprisingly low intra-endmember variability. The case is not as clear for organic proxies (Figure 5-18C to E). As expected for complex ecological parameters, G/(G+P) versus AIR-P (Figure 5-18C) and AIR-G (Figure 5-18D) shows much more scatter from any potential mixing lines than the inorganic data. Nevertheless, the data (green triangles) show a linear trend in the companion plots and deviations from the regression line are surprisingly small for most samples with larger deviations only exhibited by ~3 samples in each plot. Surprisingly, companion plots of 2,3,4- and 2,3,6-trimethyl arylisoprenoids (AI) are different when only C13 to C22 instead of C13 to C37-AI are considered (blue dots). The linear correlation in the companion plot of G/(G+P) vs AIR-P is even stronger. In contrast, no correlation is apparent anymore in the companion plot of G/(G+P) vs AIR-G. It is currently unclear, why this is the case, but one explanation may be that the C18-C22 range of 2,3,6-AI is particularly susceptible to post-mixing alteration. For example, co-eluting compounds could lead to a non-systematic overestimation of the denominator (C18-C22) AI-G. Similarly, β-carotene degradation could lead variable, non-endmember related addition of particularly short-chain 2,3,6-AIs, altering the abundances of short-chain AI more than total AI. Similarly, no clear linear trend can be observed anymore in organic
versus inorganic companion plots (Figure 5-18E). This can be explained by significant post-mixing alteration of biomarker abundances. Hydrocarbons migrate out of the rocks during thermal maturation and are cracked, leading to additional post-mixing alteration of absolute concentrations, but not necessarily biomarker ratios. Absolute elemental concentrations would not be affected by such processes. If post-mixing processes affect absolute biomarker abundances, but not biomarker ratios, the data can still largely conform to mixing lines in ratio plots, but lose a linear-relationship in companion plots as seen in Figure 5-18E. Considering intra-endmember variability and post-mixing alteration expected for, particularly organic, ecological proxies, we think that the companion plots are also consistent with two-endmember mixing in the upper BCF in GR7.

Ecological endmembers and particularly organic data are expected to be more complex and show higher deviations from mixing curves than e.g. magma mixing. Therefore, relatively large deviations from ideal mixing scenarios can be expected. Deviations are surprisingly small for many inorganic proxies. Considering the type of data set, all three tests for two-endmember mixing are, in our view, satisfactory, making two-endmember mixing a plausible explanation for the BCF correlations.

5.3.4.2.3 Determination of endmembers

For the ecological interpretations, it is important to note that FePy/FeHR closely follows the ideal mixing line in Ti/Ca (Figure 5-9D) and AIR-P plots (Figure 5-9C). This close association may provide important insights into the mechanisms that control the redox conditions in the McArthur Basin. The companion plots can be used to adjust the mixing lines by applying a least square fit to the linear data (Langmuir et al., 1978). In Figure 5-18, linears of BCF data are shown in blue, and linears of the mixing simulation in red. There is a very good fit between the two lines in Figure 5-18A to C, indicating that a reasonable endmember estimate was used for these proxies in the mixing simulation.

According to Langmuir (1978), the asymptotes and intercepts on the hyperbolic plots or the intercepts on companion plots may be used to constrain endmembers after fitting the shape-optimised mixing lines to the data. As discussed above, meaningful companion
plots cannot be applied to all BCF data plots, for example due to post-mixing alteration and a more thorough statistical treatment was not permitted by the time-frame of the thesis. Therefore, a manual iterative fitting was conducted in this thesis to extrapolate endmember ecologies, but an automated least square fitting approach may result in slightly improved endmember constraints in the future.

5.3.4.2.3.1 ‘Green’ endmember ecology

The proxy correlations and mixing simulation allow us to estimate the ecological signals of the two endmembers. The values estimated for the most important ecological variables are summarised in Table 5-4. One endmember has a high proportion of GSB relative to PSB biomarkers (G/(G+P) ~0.71). In this ‘green’ endmember state, carbonate content is low (~5%) and silicium content high (~31.3%), as is the iron content (~3.7%). Pr/Ph is relatively elevated (~0.78), but still indicative of generally reducing conditions (Peters and Moldowan, 1993), while elevated AIR-P and AIR-G (~3) indicate intermittent oxygen exposure of organic matter (Schwark and Frimmel, 2004). Importantly FePy/FeHR (~0.74) indicates euxinic conditions (März et al., 2008).

Table 5-4. Assumed composition of the two endmembers as employed in the mixing simulation.

<table>
<thead>
<tr>
<th>AIR-P</th>
<th>AIR-G</th>
<th>G/(G+P)</th>
<th>Pr/Ph</th>
<th>carbonate (wt%)</th>
<th>FePy/FeHR</th>
<th>Ti/Ca</th>
<th>FeT (wt%)</th>
<th>Si (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.00</td>
<td>3.21</td>
<td>0.71</td>
<td>0.78</td>
<td>5.00</td>
<td>0.74</td>
<td>0.26</td>
<td>3.70</td>
<td>31.00</td>
</tr>
<tr>
<td>0.13</td>
<td>0.09</td>
<td>0.26</td>
<td>0.50</td>
<td>51.00</td>
<td>0.37</td>
<td>0.01</td>
<td>1.10</td>
<td>16.00</td>
</tr>
</tbody>
</table>

5.3.4.2.3.2 ‘Purple’ endmember ecology

The second endmember has a high proportion of PSB relative to GSB biomarkers (G/(G+P) ~0.26). In this ‘purple’ endmember state, carbonate content is relatively high (~51%) and silicium content relatively low (~16%). Pr/Ph (~0.5) and AIR (~0.1) indicate strongly and persistently reducing conditions (Peters and Moldowan, 1993) and photic zone anoxia (Schwark and Frimmel, 2004). Iron content is relatively low (~1.1%), but high enough for robust interpretation of the iron speciation proxies (Clarkson et al., 2014), with low FePy/FeHR values (~0.37) indicating ferruginous conditions (Poulton
and Canfield, 2011). FeHR/FeT values do not show a significant difference between the two endmembers, indicating anoxic conditions.

5.3.4.2.4 Two endmember mixing models

Drill core GR7 is located in a deep depression with a vast surrounding carbonate platform and distal land to the west. Deposition of pelagic sediments, carbonates, and abundant mass flows provide several ways of mixing sediments from different sources, like for example distant mudflats and proximal carbonate microbialites. Resedimentation of previously deposited material that is transported down-slope into the local depocenter would further intensity the degree of mixing, resulting in more ‘averaging’ of sediment signatures. Even within the same depositional environment, two distinct ecological signatures could for example be present as microbial mats versus pelagic deposition of sediments and planktonic organic matter, or the ecosystem could temporarily fluctuate between two distinct ecological states like for example observed during low- and high-tides in tidal flats. We find it most unlikely, that the ecological signatures of two different types of phototrophic microbial mats were recorded, without recording planktonic phototrophic signatures, so if phototrophic microbial mat signatures are reflected by one endmember, the other endmember should reflect a planktonic signature. Considering the BCF depositional setting, local geology, general Paleoproterozoic ecology and potential modern ecological analogues and proxy interpretations, we identified three plausible two-endmember mixing models that are discussed in the following.

5.3.4.2.5 Spatial mixing Model I: PSB mats

One way to create correlations in a spatial endmember model is to mix the background sedimentation of a deep-water pelagic endmember with transported shallow-water sediments (Figure 5-19). In the spatial model (I), PSB and minor green- and brown-pigmented GSB (G/(G+P) ~ 0.26) thrived in the anoxic layers of microbial mats located on the platform fringes underneath oxygenated waters. A deep chemocline, most of the time located between >16 and 120 m depth, would often only allow brown-pigmented GSB to thrive in the anoxic photic zone of open basinal waters (G/(G+P) ~ 0.71).
Carbonate deposition was largely restricted to the shallow water environments, and particularly to microbial mats, where microbial activity induces carbonate precipitation (~51 wt% carbonate). Siliciclastics were deposited throughout the basin with fine particles accumulating preferentially in the deep subbasin (~5 wt% carbonate, Ti/Ca ~0.26). The biomarkers in the mats were shielded from oxic waters above through a protective coating of exopolymeric substances (Pawlowska et al., 2013), while quick depletion of oxidants through microbial activity resulted in low Pr/Ph (~0.5) and low AIR (~0.1). In this model, redox proxies of the purple endmember would reflect redox conditions prevailing in the anoxic part of benthic phototrophic mats.

![Figure 5-19. Schematic of sulphidic basin with PSB mats model (not to scale).](image)

Conceptionally, the model is similar to that proposed by Lyons et al. (2012). They further argue that euxinic waters sustained at very shallow depth (12–20 m) required for planktonic PSB are hard to reconcile with normal surface-ocean mixing processes and therefore prefer a microbial mat origin of the purple sulphur bacterial biomarkers. They propose that the PSB biomarkers in the carbonate-rich layers are sourced from fragments of shallow bacterial mats that are transported into the deeper basin by frequent mass flow events (Lyons et al., 2012). The model is based on Bull’s (1998) observation of variable lateral transport by debris flows of carbonate mud supposedly sourced from the shallow shelf. Lyons et al. (2012) report that PSB markers occur dominantly in transported shallow water carbonate facies. The source PSB and GSB would have lived
in microbial mats on the surface of very shallow-water sediments in likely oxic, saline and carbonate-rich settings. These sediments were episodically eroded and transported by turbidity currents into the deep waters. They also suggest that GSB, but not PSB, inhabited the water column of the Glyde area.

The PSB mat model fits best with Bull’s (1998) interpretation of the sedimentology of the BCF in core McA2, and is consistent with potentially mat-derived OM streaks that can also be observed in many carbonate-rich GR7 samples (see chapter 4). Lyons et al. (2012) do not present any evidence for the inference that PSB biomarkers are mostly restricted to transported shallow water carbonate facies. We also observed dark streaks in several GR7 samples with elevated PSB biomarkers like B03162 (45.35 m depth, G/(G+P) = 0.29), which would be consistent with this idea. Theoretically, these streaks could be derived from shallow water phototrophic mats and introduced the PSB biomarkers into the Glyde River subbasin.

However, there are also several arguments against Model I. Most importantly, the organic matter streaks in the BCF strongly resemble Phanerozoic lamalginite and are therefore likely sourced from planktonic organisms. As previously discussed, it is rather implausible that benthic PSB communities thrived underneath a shading chemocline, so this model would require an oxic depositional environment for the purple endmember, which is inconsistent with the observed FeHR/FeT > 0.38 indicative of anoxia. Hence, this model requires that Fe-speciation proxies in their current form cannot be applied to such settings. Potentially, the elevated highly reactive iron content could be explained by the high organic carbon content of microbial mat layers and the trapping of riverine Fe-flux in the anoxic layers of microbial mats in oxygenated proximal shallow water settings as proposed by Schieber for pyritic shales of the Belt Basin (Schieber, 1990). The model further requires a non-actualistic behaviour of benthic PSB in that they would have produced okenone rather than lycopene (or other carotenoids that appear to be better suited for benthic photosynthesis and are produced by PSB in modern settings) in the Paleoproterozoic McArthur basin.

Assuming that waters were in fact oxic and allowing for sufficient light penetration for PSB to grow in microbial mats, the mat material would have been transported over significant distances through oxic waters in order to be eventually deposited in the local
depocenter downslope. At the same time however, the occurrence of mm to sub-mm sized organic matter flakes shows that these mats, if originally present, were often torn apart (Figure 4-4). Therefore, one may expect that in contrast to the GSB biomarkers produced in the anoxic part of the water column, the biomarkers produced in the shallow water mats would be subjected to intense oxic degradation. However, the BCF data point to the opposite scenario of high oxic degradation in the green- (e.g. AIR-G \(-3.2\)) and low oxic degradation in the purple-endmember (e.g. AIR-G \(-0.1\)). A long-distance transport of organic matter streaks also stands in contrast to the apparent in-situ disruption and short-distance transportation of laminated BCF sediments in drill core LV09001 (Figure 4-7), which suggest that organic streaks are more likely to derive from proximal sediments than from distant shallow-water deposits. Additionally, the green endmember AIRs indicate intermittent deep-water oxidation, and one would expect the transported organic matter of the purple endmember to be oxidised during these events. Importantly, organic matter streaks in the BCF, even when potentially forming continuous mats, strongly resembles lamalginite formed from planktonic biomass in many Phanerozoic formations (see Fig. 4-8 and discussion in chapter 4). Therefore, it seems more likely that the organic matter streaks in the BCF reflect disrupted lamalginite rather than benthic microbial mat pieces.

Model I is consistent with several sedimentological observations, including the occurrence of inferred organic matter streaks in several samples with purple endmember ecological signals. It requires redox proxies in the purple endmembers to reflect redox conditions prevailing in the anoxic layers of phototrophic microbial mats, but as far as we are aware, such a strong mat effect on redox proxies has never been observed in proxy-calibration studies. Model I further requires a non-actualistic behaviour of okenone-producing PSB, which we consider very unlikely due to the physical properties of okenone that make it an ideal carotenoid for planktonic and not benthic PSB. Model I is further difficult to reconcile with the observation of oxidised arylisoprenoids a planktonic green, but well preserved arylisoprenoids in a transported purple ecological endmember. Considered in balance, model I is not our preferred ecological model to explain the proxy correlations in GR7.
In model II, the carbonate-rich sediments of the purple ecological endmember are a planktonic/pelagic component, whereas silicate-rich sediments of the green endmember reflect benthic microbial mat ecosystems. Following the interpretations of Schieber (1990) for striped shales of the Proterozoic Belt Basin, the silicate-rich siltstones of the BCF may record silty facies of flat microbial mats inhabiting oxygenated shallow water mud flats that could in theory have covered large areas of the McArthur Basin, or local depressions in a carbonate platform. The iron is then sourced from proximal rivers and trapped in the anoxic mat layers largely as iron sulphides (Schieber, 1990). A riverine source of the iron would be consistent with the correlation between iron proxies and siliciclastic content (e.g. Figure 5-9D) if both elements were washed into the basin near-synchronously.

Spatial mixing model II is schematically illustrated in Figure 5-20. Washed-in silicate minerals and iron oxides are trapped on the sticky surfaces of microbial mats in lagoons or even at the basin margins, with carbonate facies occupying the rest of the basin. It has been proposed that, only based on light intensity (~2500 lux), cyanobacterial could grow at depth of up to 100 m in the clearest ocean water, less than 50 m in average ocean water and due to generally much greater turbidity even shallower in lakes. A 3 mm-layer mat layer of living cyanobacteria attenuates full normal summer sunlight of ~70 000 lux by 96-97% to ~2100-2800 at the limit of cyanobacterial growth (Brock, 1976 and references therein). While it seems as if cyanobacterial mats would only thrive in very shallow environments, under certain conditions such as encountered in Lake Huron sinkholes were anoxic groundwater flows downslope and decreases turbidity, cyanobacterial mats may thrive at up to ~30 m depth (Biddanda et al., 2009). As green sulphur bacteria thrive underneath the cyanobacterial layer in many phototrophic microbial mats, below the light limit of cyanobacteria with as little as ~0.0005% surface light intensity (Overmann et al., 1992), based on light availability, benthic green sulphur bacteria could thrive below 50-100 m water depth in many marine systems. Thus, a large proportion of the relatively shallow McArthur basin may have been covered by microbial mats if sedimentation rates were low enough to sustain mat growth. In other parts of the basin, particularly in slightly deeper waters as well as areas of higher water energy or higher sedimentation
rates related to carbonate precipitation, the shallow-water dolomite facies of the BCF (i.e. the Reward dolomite) hosts different microbial communities such as a variety of stromatolites (Brown et al., 1969) and probably other carbonate binding organisms.

Flat microbial mats would probably be restricted to flat, shallow areas like mudflats, estuaries and lagoons with increased siliciclastic influx. The washed-in iron oxides trapped on mat surfaces are sulphurised in the deeper anoxic layers of the microbial mats, where sulphate reducing bacteria produce hydrogen sulphide from sulphate and organic detritus derived from the water column and upper mat layers. Depending on light availability, cyanobacteria may have inhabited mat surfaces, while sulfidic mat layers below were dominated by green- and minor okenone producing purple sulphur bacteria, followed by sulphate reducing bacteria and other heterotrophs in the deeper mat layers. Overlying waters may have been oxic most of the time or exhibited a very oligotrophic chemocline to allow for enough light to reach the anoxic mat layers for phototrophic sulphur bacteria to thrive. Due to the strong redox gradients and periodic burst in oxygen concentrations as a consequence of increased cyanobacterial activity during the day, the organic matter produced by the benthic sulphur bacteria would also be stronger degraded than that of planktonic material deposited in areas not covered by pervasive microbial mats where due to low atmospheric oxygen concentrations even the mixed layer may have exhibited low oxygen concentrations. Oxygen concentrations in shallow microbial mats can almost reach supersaturated levels during the day. Even in modern hypersaline lagoons, oxygen concentrations in upper mat layers can be four times higher than in the overlying water column (Gingras et al., 2011). In the Paleoproterozoic with atmospheric oxygen concentrations potentially as low as 0.02% (Planavsky et al., 2014), the difference in oxygen concentration between the water column and phototrophic mats was likely even greater, providing a plausible explanation for a higher degree of biomarker oxidation in the green endmember. In such a scenario, one would however expect 2,3,6-AI to be better preserved than 2,3,4-AIs as green sulphur bacteria are obligate anaerobes, but the opposite trend is observed in the BCF. One explanation could be that well preserved planktonic detritus dominated by 2,3,4-AI would also have been incorporated into the mats. Although originally coming from the purple endmember, their biomarker signature may have been overprinted in
the microbial mats, but may have been slightly shielded absorbed to clay surfaces or within bigger organic matter aggregates rafts. In this respect, it is interesting to note that in Lake Huron anoxic sinkholes, are sinks for carbon fixed by phytoplankton and not for carbon fixed by cyanobacterial mats. By upward migration of cyanobacterial filaments towards sunlight, cyanobacterial filaments bury pelagic carbon deposited on their surface into underlying anoxic sediments, which may facilitate pelagic carbon burial and preservation. In Lake Huron, the cyanobacterial mat production seems to be consumed on site or lost to the surrounding lake ecosystem (Biddanda et al., 2009). In this way, some planktonic PSB could be incorporated into microbial mats. Alternatively, green sulphur bacterial mats growing in anoxic waters could have been periodically poisoned by oxygen influx during intermittent water column overturn.

The purple ecological signature would stem from basinal environments distal the flat microbial mat facies, where deeper waters were anoxic and ferruginous below the chemocline. Due to the anoxic basin waters, planktonic biomarkers show little degradation as they were produced in and transported through anoxic waters when sinking to the bottom. The shallow chemocline was dominated by purple and minor green sulphur bacteria.

To obtain mixing curves in this model, varying proportions of sediments from different geographical locations would have to be deposited in the local Glyde River depocenter where our samples were collected. The purple endmember signatures represent transported material from upslope anoxic mat-free environments, while the green endmember signature had to be transported by long distance mass flow events into the local depocenter at GR7 (Figure 5-20).
Although extensive mudflat or estuarine facies have not been recognised in the BCF, the GSB-mat model is consistent with the general sedimentology at BCF times with the shallow-water Reward Dolomite facies showing a number of depositional environments encompassing emergent to subaqueous conditions and sedimentation from standing waters of bodies like ponds or lagoons (Jackson et al., 1987). It can also explain all the organic proxy data. Within the carbonate facies, high sedimentation rates and a dense planktonic community at the chemocline could have hampered the establishment of phototrophic microbial mats, leading to the preservation of the purple-endmember biomarker signal. The low degradation of the biomarkers indicates persistently anoxic conditions into the photic zone (low AIR-P and AIR-G, low G/(G+P) & low Pr/Ph). The abundance of PSB further suggests extremely shallow stratification with a chemocline probably not deeper than 12–20 m (Brocks et al., 2005). GSB mat material would likely not only be deposited under oxic conditions, but would further have to be transported through oxygenated waters on its way to the depocenter.

Re-sedimentation of different proportions of purple- and green-endmember sediments in the local Glyde River depocenter would have resulted in a ‘mixed’ bulk signature. Higher sea-level may result in a more extensive flooding area, leading to more deposition of green endmember sediments from mudflats, whereas under lower sea-level carbonate depositional environments may have been more dominant, resulting in deposition of more purple endmember sediments. Re-deposition of more siliciclastic mudflat material under higher sea-level may reconcile this model with the
sedimentological interpretation of BCF mudstone facies to reflect deep water deposition, whereas most recognised shallow water environments are carbonate-rich (Bull, 1998). The model does also not account for stromatolite facies in the Reward, but it is possible that this is a third ecological endmember that is minor and not resolved, but may add to the noise in depocenter sediments.

Another problem of the GSB-mat model is inconsistency with the iron speciation proxy. If microbial mats in shale facies were widespread and lead to significant iron enrichments in certain environments and, most importantly, lead to a near quantitative sulphurisation of the highly reactive iron pool as proposed by Schieber (1990), it would mean that the iron speciation redox proxies cannot be readily applied to Proterozoic sediments where mats were prevalent. However, this contradicts the common interpretation of the well-calibrated iron speciation proxy. Iron speciation analyses on microbial mat facies may shed light on the applicability of the iron proxies to these ecologies. Lithified and unlithified stromatolite samples yielded FePy below and close to the detection limit respectively and low FeT values (Clarkson et al., 2014), but flat microbial mats may yield a different Fe-speciation signal. Thus, it is possible that the Fe-speciation proxy fails in flat microbial mats in environments with a high-enough riverine iron influx. This would have wide-ranging implications for Proterozoic redox interpretations that needs to be tested in the future.

Since model II can theoretically account for all other proxy observations, it builds upon sedimentological observations of the Belt Basin striped shale facies, and detailed iron speciation analyses of microbial mat deposits are currently lacking, benthic mats should be considered as a source for the BCF pyrite-rich siltstone facies, as an alternative to traditional proxy-interpretations. However, it is also difficult to reconcile with the apparent absence of shallow water siliciclastic-rich facies in the BCF depositional sequence and the current interpretation of the BCF mudstone facies as pelagic (Bull, 1998) and not re-deposited shallow water material. Unless planktonic PSB material was incorporated into the microbial mats and altered, but to a lesser degree than the original mat material e.g. due to a predominance of larger rafted organic matter accumulations, there would have to be 2,3,4-AI producing PSB in the mats, that were slightly less
affected by oxic degradation than the obligate anaerobic GSB. Due to these difficulties, model II is not our preferred model, but provides an interesting alternative explanation.

5.3.4.2.5.2 Model III: Temporal mixing

Instead of reflecting mixing of sediments from two geographic locations with distinct ecologies, the proxy correlations can also be explained by a temporal two endmember mixing model. In this scenario, the ecosystem fluctuates over time between two dominant environmental states, such as dry and a wet season. Such patterns are observed in many natural aquatic systems, where the switch between two distinct ecologies is often controlled by seasonal weather patterns or climatic variability. Non-climatic processes resulting in two distinct ecological states are for example observed in tide influenced ecosystems such as the Wadden Sea, where coastal mudflats are submerged and exposed twice a day. In addition to dry and wet seasons (e.g. El Nino versus La Nina events), other examples where climatic processes can result in two dominant ecological states in aquatic ecosystems are hurricane seasons versus calm periods, summer-winter cycles, the seasonal overturn of temperate lakes, ice-covered and ice-free systems, mixed layer depth variations, and seasonal wind and precipitation. Climatic variability that could result in distinct ecological states are for example the Arctic and Antarctic oscillations of northern and southern annular mode wind patterns, and glacial versus interglacial periods. Climate cyclicity is often orbitally controlled, mostly by Earth’s precession (~20ka), obliquity (~40ka) and eccentricity (~100 and 400ka). For the Paleoproterozoic (at 14 Ga), obliquity cycles of 21-30 ka, and precession cycles of 14-17 ka were recently calculated, while Earth’s long orbital eccentricity of 405 ky was considered unchanged over geological time (Zhang et al., 2015).

BCF samples analysed in this study are several centimetres thick and, depending on sedimentation rates, may reflect tens to thousands of years of deposition. Average varve counts in three drill cores yielded for example average sedimentation rates of ~10-30 cm/1000a in the Black Sea (Hay, 1988), so deposition of ~3cm of BCF may have encompassed ~100-300a if sedimentation rates were somewhat comparable. While the BCF sediments are compacted, the basin was not as deep and sedimentation rate probably somewhat higher. Thus, it is expected that BCF samples integrate sediments
deposited over a similar magnitude of time (i.e. hundreds to thousands of years time scale). The two endmember-mixing correlations suggest that frequent mixing occurred on a subsample scale, which in a temporal model points towards at least decadal to centennial fluctuations between ecological states. Low deviations from mixing lines as displayed for example by FePy/FeHR vs Ti/Ca (Figure 5-18A), may point towards a high mixing frequency that may have averaged out potential intra-endmember variabilities, so ecological fluctuations may have occurred on an even smaller, seasonal or even daily, scale. Frequent weather or climatic events like summer-winter cycles, dry-wet seasons, monsoon seasons, or even day-night cycles may thus be potential drivers for the ecological variations.

The temporal mixing model is schematically illustrated in Figure 5-21. Major ecological changes within anoxygenic phototrophic communities from PSB to GSB dominance, as indicated by variations in G/(G+P) from ~0.26 in the purple and ~0.71 in the green ecological state, can be explained by variations in the chemocline depth from less to more than ~20 m and such chemocline variations can explain the strong correlation between G/(G+P) and Pr/Ph (Figure 5-9B) that varies between ~0.5 in the purple and ~0.78 in the green ecological state. Iron speciation analyses provide further ecological information in our temporal two-endmember model. FeHR/FeT values do not correlate with other proxies and values >0.38 indicate anoxic conditions in both ecological states. The FePy/FeHR ratio however switches from ~0.37 in the purple to ~0.74 in the green ecological state, indicating a transition from iron-rich (ferruginous) waters to free hydrogen-sulphide in the water column (euxinic). GSB were more prevalent during periods of increased H2S availability, while PSB were dominant during ferruginous conditions. The growth of PSB and GSB in ferruginous waters is unexpected because extant strains commonly use reduced sulphur species as electron acceptors for photosynthesis. While these organisms can thrive on traces of sulphide even in ferruginous waters, phototrophic growth under such conditions is slow (Crowe et al., 2014). Considering the high concentrations of anoxygenic phototrophic pigment remnants in BCF sediments (Brocks et al., 2005), PSB and GSB must have exploited a more abundant electron source, namely direct oxidation of ferrous iron as observed in some modern strains (Posth et al., 2013). This provides the first hint towards phototrophic iron oxidation in the geological record, confirming the antiquity of this
metabolism and supporting an important role of such organisms throughout much of Earth’s ferruginous history (Kappler et al., 2005).

Figure 5-21. Temporal mixing model for BCF ecology. O2: oxygen (oxic water), Fe(II) dissolved iron (ferruginous waters), PSB: purple sulphur bacteria, GSB: green sulphur bacteria, H2S: hydrogen sulphide (euxinic waters), BSR: bacterial sulphate reduction, dotted line: chemocline. A) Prevailing purple state. B) System fluctuates to green state in likely seasonal to centennial intervals.

A third redox parameter that helps to determine the causes of covariation between iron speciation chemistry and chemocline depth are the arylisoprenoid ratios AIR-G and AIR-P that vary between ~0.1 in the purple and ~3 in the green ecological state and gauge the degree of oxygen-induced cleavage of carotenoid pigments of GSB and PSB respectively. As discussed, AIRs increase when the pigments produced below the chemocline become exposed to oxygen between cell death and permanent burial, thus recording injection of oxygenated waters beneath the chemocline by turbulence, currents or an overturning water column. Together, the correlations suggest that GSB were the dominant anoxygenic phototrophs during episodes of temporary injection of oxygen into deep waters, e.g. by through increased water energy or salinity or temperature related density increase of surface waters, a deeper chemocline, e.g. caused by increased wind activity, and development of sulfidic conditions. Conversely, during periods of persistent stratification, PSB thrived in ferruginous waters beneath a shallow mixed zone.

To find causes for the two environmental states of the McArthur Basin, we explored seasonal weather cycles in a modern analogue basin. The summer in the Black Sea area is relatively calm and dry (Romanou et al., 2010), permitting a shallow mixed layer (mostly < 25 m) across the basin (Kara et al., 2009). The winter, however, brings storms
(Romanou et al., 2010) that cause wind mixing of surface waters and extension of the mixed layer to depths > 50 m and in places 110 meters (Kara et al., 2009). Atmospheric cooling further facilitates the mixing process, and deep currents re-distribute oxygenated cold waters within the basin to a depth of 170 meters (Grégoire and Lacroix, 2001). Similar processes may have operated in the McArthur Basin. During the sulphidic state, storms and falling temperatures may explain the deepening of the mixed layer and the injection of oxygenated waters into the deeper basin.

Winter storms and precipitation in modern basins also increase the flux of clastics (Kwiecien et al., 2009). Thus, if weather cycles were responsible for the redox fluctuations in the BCF, then the turbulent sulphidic state should correlate with an elevated sedimentary content of clastics, while sediments deposited beneath calm ferruginous waters should represent drier periods with increased evaporation and carbonate precipitation. Generally, clastic influx during periods of pelagic carbonate sedimentation can be quantified using the Ti/Ca ratio (Grégoire and Lacroix, 2001). In the BCF, a hyperbolic two end-member mixing curve between Ti/Ca and FePy/FeHR (Figure 5-9D) suggests that increased continental run-off was the main driver for the establishment of sulphidic conditions. Runoff caused elevated influx of nutrients, promoting primary productivity and sulphide production through bacterial sulphate reduction (BSR). During storm periods, BSR was probably further stimulated by the reintroduction of nutrients into the photic zone through turbulent mixing and current-driven upwelling. For example, an increase in primary production was observed after the usually stratified tropical Lake Nkugute was violently stirred (Beadle, 1966).

Turbulence caused by stormy seas is also considered an important mechanism for mixing marine nutrients upwards (Palter, 2015). A recent study showed a tenfold increase in surface nutrients during a storm, followed by two short-lived nutrient bursts afterwards. Chlorophyll concentrations rose by ~50% between the last day of the storm and several days later (Rumyantseva et al., 2015). Thus, storms can provide upward nutrient flow even after they cease. Wind blowing over the sea during a storm event sets the surface layer in motion, creating a sharp change in ocean-current velocity at the interface between the surface and interior waters. This process leads to instabilities in the circulation and turbulence that can mix nutrient into the sunlit region (Palter, 2015).
Such turbulence may be responsible for the injection of oxygenated waters beneath the chemocline and increased AIR during the green environmental state. Intertidal oscillation can lead to additional nutrient spikes after cessation of the storm (Palter, 2015). Similarly, the injection of oxygenated surfaces waters into the deeper McArthur Basin may have replenished the bottom water sulphate pool, increasing the rates of BSR. Enhanced organic matter availability and in turn facilitate the quick removal of oxygen from below the physical mixed zone leading to subsequent euxinia.

A similar ecological scenario was invoked for sediments from the northerly part of the proto-North Atlantic where high trade wind intensity triggered coastal upwelling, primary production (as evidenced by high TOC), deposition of redox sensitive-element Mo and development of euxinia as evidenced by biomarkers of green sulphur bacteria. Intermittent oxidation is however indicated by interlayered green shales that are TOC poor and show evidence for bioturbation, providing strong evidence for alternations between euxinic and oxic conditions (Zhang et al., 2015 and references therein) similar to our BCF interpretations.

The second ecological endmember exhibits much higher carbonate content relatively to siliciclastics (low Ti/Ca), which can be explained by more evaporitic conditions leading to enhance carbonate precipitation, and less rainfall and run-off decreasing the siliciclastic influx. The purple ecological state is characterised by calm, evaporitic conditions dominated by carbonate deposition, and stable and shallow stratification with ferruginous waters being dominated by purple sulphur bacteria in the photic zone.

If the temporal mixing model is correct, then small-scale laminations, especially (insipient) pyrite laminae observed in some green endmember samples (Figure 4-6) should reflect pelagic sedimentation and not microbial mat deposits. This is consistent with the lamination observed in many Phanerozoic anoxic sediments like Zechstein facies described by Turner and Magaritz (1986). The temporal model proposed here for the BCF compares well to the Zechstein environmental model, where organic matter-rich laminae are due to seasonal phytoplankton blooms resulting from increased riverine siliciclastic and nutrient influx. Intriguingly, the organic matter-rich Zechstein intervals are rich in total iron, which dominantly occurs as pyrite, corroborating the assumption that pyrite-rich laminae in the BCF may correspond to periods of enhanced primary
production. Zechstein dolostones on the other hand were interpreted to be deposited in periods of low riverine clastic influx (Turner and Magaritz, 1986). The Zechstein depositional model of Turner and Magaritz (1986) can thus explain the variations in carbonate versus siliciclastics influx, degree of pyritisation and FeT observed in the BCF.

Poulton et al. (2010) observed that euxinic conditions extended over 100 km from the palaeo-shoreline in the 1.88–1.83 Ga Animikie Group from the Superior region, north America. A sulphidic wedge was proposed along continental margins as a results of an increased organic carbon flux and a decreased flux of ferrous iron from the ferruginous deep waters (Poulton et al., 2010), and sulphidic wedges are now widely considered to be characteristic of mid-Proterozoic ocean margins. The BCF temporal model may be interpreted as the waxing and waning of a sulphidic wedge (Figure 5-22), but in the BCF, the proxy correlations allow to better constrain the controlling processes and resulting ecological perturbations. The BCF data suggest that the influx of nutrient-rich terrestrial material, as well as nutrient re-distribution by mixing processes, played an important role in the establishment of euxinia, and thus potentially the waxing and waning of a euxinic wedge. Such a nutrient-control is similar to the upwelling related organic productivity-increase hypothesised by Poulton et al. (2010) for development of euxinic wedges in the Animikie group. This is the first study that can link such as a redox variability to environmental processes as inferred from other proxies.
Lyons et al. (2012) claim that PSB markers dominantly occur in transported shallow-water carbonate facies and that shallow stratification (12-20 m) required for planktonic PSB “is hard to reconcile with normal surface-ocean mixing processes beneath an oxygenated atmosphere.” In contrast, the depth of the mixed layer in modern marine systems suggests that such shallow stratification is not unlikely. The mixed layer depth of many marine basins like the Black Sea (Kara et al., 2009) and even certain areas of the oceans, like the Antarctic Ocean (70°S, 100°W) (Kara et al., 2003) and Eastern Equatorial Pacific Ocean (Lorbacher et al., 2006), is at least temporally above the depth requirements of planktonic PSB. In the Black Sea for example, the mixed layer depth is <25 m for most of the year. The reason why GSB are currently only found below 100 m is thus not the
depth of physical mixing as such. Instead, a dense layer of suboxic waters is found between the mixed layer and chemocline (Oguz, 2002). There remain a lot of open questions about this intermediate layer (see e.g. Oguz, 2002). It is likely that the intermediate layer was not always present. Further, it was established that the thickness of the intermediate layer is variable on a time scale of decades (Konovalov and Murray, 2001). Even before anthropogenic disturbance, a chemocline rise of at least 40–50 m was inferred around ≥ 250–300 years ago (Lyons et al., 1993). With atmospheric oxygen concentrations as low as 0.02% (Planavsky et al., 2014), it seems very unlikely that such an intermediate layer could have been sustained in Paleoproterozoic basins especially with significant OM export as indicated by significant TOC values of BCF sediments (average 1.9% in GR7, n = 20). With probably the MLD roughly coinciding with chemocline depth, planktonic PSB communities could thus have well been present in Proterozoic marine basins and even parts of the open ocean if surface mixing processes were similar to those affecting the present oceans. The argument of surface-ocean mixing processes can thus not be used as an argument against planktonic PSB. On the contrary, seasonal variations in MLD observed in a variety of modern marine settings support the temporal mixing model.

5.3.4.2.7 Reasons for data deviations from theoretical mixing lines

Not all data distributions follow the mixing lines to the same degree. Some proxies generally follow the same trends and thus seem to be part of the endmember mixing process, but nevertheless show significant deviations from the ideal mixing lines. For example, in the Pr/Ph versus G/(G+P) plot the distribution is very similar to that expected in an ideal mixing model (Figure 5-9B). However, both biomarker ratios show a lot of scatter when compared to other ratios (e.g. Figure 5-23).
Deviations from the endmember lines can have the following reasons:

- Part of the signal does not belong to one of the two endmembers (e.g. a third endmember, or there is a variety of different additional sources)
- Intra-endmember variability
- Late diagenetic & catagenetic effects creating systematic offsets or noise
- Early diagenetic processes after mixing creating systematic offsets or noise
- Analytical noise

Mixing line trends can only be expected for variables that show statistically significant differences in both endmembers due e.g. due to different ecological processes and that are relatively constant in each endmember. For most deviations, the best explanation is intra-endmember variability. This envisages that the composition of the endmember variables is not constant but fluctuates around an average value. Variables or ratios that show little deviation from the mixing lines exhibit little, while others such as Pr/Ph and G/(G+P) large intra-endmember variability.

Another explanation for deviations from ideal mixing lines are post-mixing alteration processes like cracking and expulsion of biomarkers or weathering of pyrite. Such processes may explain why biomarker correlations become weaker in deeper intervals of drill core GR7. Any alteration would alter Pr/Ph different ratios differently.

Another important factor for some deviations from the mixing lines are early diagenetic processes. These may e.g. affect FePy/FeHR through pyrite weathering, but are most likely to influence biomarker parameters. For example, oxygenation events may affect the previously deposited sediment layers potentially changing organic redox proxies.
post-mixing. This may explain why AIR-P and AIR-G show a very good linear correlation, because 2,3,4- and 2,3,6-trimethyl carotenoids may have partially been oxidised together after mixing. O₂ influx affecting the upper sediments post-mixing could particularly explain the absence of intact C₄₀-carotenoids from many samples with a significant green endmember signature. Sedimentary oxygen exposure may also affect absolute biomarker concentrations through temporarily enhanced heterotrophic reworking.

5.3.4.2.8 General implications of two-endmember mixing for paleo-ecological reconstructions and data-interpretation

A very important aspect illustrated by the mixing simulation is that the original endmember compositions can be estimated even if pure endmember samples are not preserved. The hyperbolic shape of two endmember mixing lines and the three tests for endmember mixing can be used to infer two-endmember mixing from complex ecological datasets. In the Precambrian, this approach may allow the recognition of terrestrial or shallow water ecologies that are not commonly preserved in the original environments, but may have been washed into and preserved in anoxic deep waters. It may also help to differentiate between planktonic and benthic signals and thus refine paleo-ecological interpretations.

The influence of two endmember mixing on the interpretation of ecological proxies is illustrated in Figure 5-24. The blue dots show the measured BCF data (< 200 m depth in drill core GR7), while the red dots show the simulated values at 10% mixing increments, while the green and purple triangles indicate the derived endmember compositions. FePy/FeHR of 0.7 is often considered as the threshold for euxinic deposition (e.g. März et al., 2008, Guilbaud et al., 2015). For our BCF model, this value roughly corresponds to a 20% purple- to 80% green-endmember mixing ratio. Essentially all samples would have contained some proportion of sediments deposited under euxinic conditions. Assuming a temporal mixing model, the BCF data thus indicates that excursions to euxinia were frequent, while Fe-speciation data in isolation indicates essentially persistently ferruginous conditions and misses this important environmental aspect. Averaged bulk data may entirely eclipse euxinia. However, by inducing sulphide
poisoning and trace-metal drawdown, euxinic excursions may have exerted important controls on Proterozoic ecosystems.

![Diagram](image)

**Figure 5-24.** Influence of mixing on GR7 (< 200 m depth) proxy interpretations illustrated by 10% mixing increments (red triangles).

5.3.5 Discussion of long term trends in proxy data

The temporal endmember mixing model is based on frequent fluctuations between two ecological states on a subsample scale, resulting in quasi-linear to hyperbolic mixing lines in ratio plots. There are however also long-term trends exhibited by the proxy data in Figure 5-7, with rather gradual changes over ~300 m of stratigraphy. Long term trends are summarised by the first principal component (CP1) that explains >50% of all data variability (Figure 5-7K).

The grey shaded areas in Figure 5-7 highlight two periods of elevated clastic influx and sulphide precipitation and surface mixing with great mixed layer depth and intermittent injection of oxygenated surface waters beneath the chemocline. According to the mixing simulation, 'green state' ecological conditions may have been almost persistent at the maximum of the proxy excursions, as plausible endmember values to create the diverse mixing lines coincides with BCF extreme values (Figure 5-24). One explanation for the long-term trend is that endmember states are controlled by prevailing weather patterns and that more gradual long-term trends in climatic conditions control the frequency and
duration of ‘green state’ sulphidic excursions. For example, the grey shaded areas in Figure 5-7 may represent stages of a high-energy climate, where storm seasons were longer or more intense, cooler climatic periods were winter cooling lead to periodic overturn of the water column and nutrient redistribution in the basin and/or wetter climatic periods when influx of siliciclastics and nutrients was, on average, greatly enhanced. Based on estimates of BCF depositional time scales (chapter 4), CP1 shows rudiments of cyclical patterns with a periodicity in the order of ten to hundred thousand years, broadly consistent with orbitally forced climate cycles.

The most important drivers for synchronous changes in a variety of ecological parameters are climatic processes. For example, orbital forcing was proposed to control Cretaceous river discharge in tropical Africa. Based on geochemical data and simulations, it was concluded that alternating periods of arid and humid African climate were driven by orbital precession, with ocean anoxia and black shale sedimentation appearing to be directly caused by high river discharge and occurring specifically when the northern equinox coincided with the perihelion. A 400 ky periodicity was interpreted as the expression of long-a term drying/cooling cycle (Beckmann et al., 2005). Beckmann et al. (2005) proposed that in a warm climate the oceans off continental margins in the tropics respond rapidly and sensitively to even minor changes in river discharge. In the African sediments studied by Beckmann et al. (2005), black shale formation is thought to have been triggered by discharge of freshwater and nutrients into a semi-enclosed marine basin, resulting in circulation reversal and development of anoxia/euxinia, and even periods of photic zone euxinia. According to their simulations, black shale formation requires maximum insolation to occur during wet seasons, fostering seasonal contrasts and massive freshwater discharge in spring and a freshwater cap of ~ 1 to 0.7 m comparable to the modern Black Sea (0.7 m), but 2-3 times higher than for the modern Arctic ocean (0.36 m) (Beckmann et al., 2005 and references therein).

The driving forces for marine euxinia in Cretaceous anoxic events seem to be consistent with the BCF proxy data. Orbitally-controlled river discharge may have introduced siliciclastics and nutrients into the McArthur Basin, while seasonal contrasts, specifically winter cooling like observed in the modern Black Sea, may have led to temporal overturning of the water column, despite a potentially thicker freshwater cap. The
inferred BCF climate cycles may thus be Paleoproterozoic counterparts of the Cretaceous orbital-controlled river-discharge cycles responsible for oceanic anoxic events.

Similar orbitally-driven climate variations were recently invoked to explain the cyclicity in sediment geochemical dynamics in the 1.4 Ga Xiamaling Formation China. Sediment geochemical fluctuations were interpreted to reflect orbitally controlled changes in wind patterns and ocean circulation that influenced organic matter flux, trace metal accumulation and detrital particle source (Zhang et al., 2015). These independent observations from another mid-Proterozoic marine basin thus support our BCF interpretations.

It is an exciting notion that paleoclimate, marine chemistry and even microbial ecology may have been driven by such processes 1.6 billion years ago. If true, orbital climate cycles may have exerted an important control on the development of euxinia along continental margins and particularly in marine basins of limited extent.
5.4 Conclusions

Precambrian iron speciation and biomarker distributions are interpreted for the first time in the framework of a two-endmember mixing model, with important implications for ecological interpretations. For example, mixing of ferruginous and sulphidic sediments would mostly result in a ferruginous bulk signature. Frequent euxinic excursions with a great impact on the prevailing ecology can thus be easily overlooked. The recognition of mixing lines between different proxies allows to account for frequent fluctuations that occurred on a sub-sample scale, and to estimate the composition of the original endmember ecologies.

Instead of persistently ferruginous conditions, as inferred from traditional Fe-speciation interpretations (Planavsky et al., 2011), our analyses reveal the highly dynamic nature of the BCF redox landscape. The previously unintelligible full range of FePy/FeHR values in the BCF, can now be understood as the temporal proportion of sedimentation under ferruginous and sulphidic conditions, greatly improving ecological interpretations. The recognition of sulphidic cycles in an otherwise ferruginous basin may also explain the paucity of typical eukaryotic biomarkers in the BCF and other apparently ferruginous basins throughout the mid-Proterozoic (Poulton and Canfield, 2011), as modern eukaryotes are sensitive to H2S poisoning and sulphide-induced depletion of bio-essential trace metals (Anbar and Knoll, 2002).

Commonly, the mid-Proterozoic is perceived as a period of geochemical stability. In contrast, our data indicate fast redox fluctuations in a relatively large basin, confirming weak redox buffering at generally low sulphate levels. Rapid redox fluctuations may be a common characteristic of the Proterozoic, but will often remain eclipsed by proxy measurements on bulk samples. The relationships between microbial and environmental proxies presented here offer a new approach to recognise such hidden ecological oscillations. During the prevailing ferruginous conditions, the high abundance of PSB biomarkers provides the first geological evidence for phototrophic iron oxidation, confirming the antiquity of this nowadays rather unusual metabolism that may have played an important role throughout much of Earth’s ferruginous history. Our findings concurrently strengthen hypotheses about the potential involvement of phototrophic iron oxidisers in the deposition of ancient banded iron formations (Konhauser et al.,
Redox fluctuations in the BCF may also have important implications for the formation of some of the world’s largest syn-sedimentary metal sulphide deposits as discussed in chapter 6.

In addition to ferruginous/sulphidic fluctuations, organic proxies further allow to recognise intermittent oxidation events, likely caused by storms or water column overturn associated with atmospheric cooling, that affected the usually anoxic bottom waters and sediments and give us a more vivid picture of a marine environment during Earth’s middle ages. The interpretation of the unique correlations of diverse ecological proxies allow for the first linkage between environmental processes and the composition of planktonic communities. A strong correlation between Pr/Ph and G/(G+P) indicates that the composition of anoxygenic phototrophic communities was controlled by the extent of the mixed layer, which is commonly controlled by physical mixing processes related to the prevailing weather patterns.

All BCF proxy observations can be plausibly explained by modern climatic processes, like wind and precipitation patterns, suggesting that climatic variability may have played a crucial role in controlling Proterozoic ecosystems. The long-term trends of the proxy data are evocative of orbital climate cycles that control Phanerozoic ecosystems. This opens the intriguing possibility of an orbital control on Earth’s climate and ecosystems already 1.6 billion years ago—an assumption that is supported by recent analyses of other mid-Proterozoic settings (Zhang et al., 2015). In a low oxygen world (Planavsky et al., 2014) with shallow redox stratification (Brocks et al., 2005) periods of a high energy climate with more frequent and more intense storm events may have controlled much of the ecology of marine basins, from redox conditions to microbial communities. While the prevailing ecosystems, dominated by prokaryotes and anoxic ferruginous and at times sulphidic seas may appear alien to us, the underlying processes that controlled these ancient ecosystems may have been similar to those operating on the modern Earth.
5.5 References


BROCKS, J. J. & SCHAFFER, P. 2008. Okenane, a biomarker for purple sulfur bacteria (Chromatiaceae), and other new carotenoid derivatives from the 1640 Ma Barney Creek Formation. Geochimica et Cosmochimica Acta, 72, 1396-1414.


6 Zn/Pb-mineralisation

6.1 Introduction

The BCF is host to giant stratiform base metal accumulations such as the HYC McArthur River deposit and the Myrtle deposit (chapter 3). An overview over the geology and mineralisation models at HYC is provided in chapter 3. A largely syngenetic origin is favoured in the latest mineralisation models. In these models, water column-redox conditions may have been crucial (e.g. Ireland et al., 2004, Large et al., 1998). As illustrated in Figure 6-1, base metal precipitation from a hydrothermal brine may have required a continuous supply of reduced sulphur from a sulphidic water column (Ireland et al., 2004). Redox fluctuations in the BCF may therefore offer new insights into the formation of some of the world’s largest syn-sedimentary metal sulphide deposits.

![Figure 6-1. Schematic brine pool mineralisation model (not to scale) at HYC (modified from Ireland et al., 2004). Py = pyrite, sp = sphalerite (1 = early, 2 = later formed), C$_{org}$ = organic carbon. White arrows indicate brine flow. Bottom hugging brine flows into local depression, but some hydrothermal fluids potentially also flowed laterally through the sediment pile. Brine accumulates in depression and interacts with sediment and overlying water column (modified brine).](image)

At the HYC deposit, hosted in the BCF some 80 km north of GR7, the mineralised horizons essentially yield two sedimentological endmembers. ZnS/PbS ore occurs in sub-mm laminae that are interbedded with fine non-mineralised clastic horizons. These clastic event beds are currently interpreted as turbidites triggered by fault movement during fluid discharge with thousands of vault-valve release events causing the fine lamination (Large et al., 1998). This model requires local sulfidic conditions for the ore
to precipitate. Water column redox fluctuations have previously been discounted as a control on mineralisation mainly based on the high event frequency required (Large et al., 1998). The observation of two distinct ecological states in the BCF at GR7 that seem to be characterised by ferruginous and sulphidic conditions respectively, provide an alternative mechanism to explain the observed lamination at HYC. Instead of reflecting brine pulses, mineralised horizons could reflect periods of enhanced water column hydrogen sulphide replenishment resulting from the development of euxinia in the basin waters.
6.2 Results of Fe and Zn distributions

A large proportion of carbonates and siliciclastics in GR7 was transported there from up-slope locations via mass flow events of different scales, and primary sedimentary textures reflecting endmember phases are rarely preserved. However, pyrite and carbonate distributions in several samples are consistent with the preservation of primary textures caused by fluctuations between carbonate-poor/pyrite-rich and a carbonate-rich/pyrite-poor depositional phases. For example, a sample (218.1 m; GR7) with a euxinic signature (FePy/FeHR = 0.78) shows a low number of pyrite-poor laminae (Figure 6-2). In particular, a prominent band, poor in pyrite and rich in carbonate, is apparent in the centre of the picture. The carbonate-rich layer also shows Zn enrichment, particularly towards the top and the carbonate layer is further enriched in phosphorous.

![Figure 6-2. XRF image of a thin section of a dominantly euxinic BCF sample taken 50 km south of HYC at GR7 (218.1 m). (A) Carbonate map reveals a carbonate-rich layer. (B) Phosphate map reveals enrichment in carbonate layer. (C) Zn map reveals enrichment towards top of carbonate layer. (D) Sulphur map reveals pyrite lamination and probably ferruginous conditions in carbonate layer. (E) Composite map. Bruker XRF mapper images kindly supplied by Sam Spinks, CSIRO.]

To test whether there is a link between paleo-redox conditions and Zn enrichment in the mineralised horizons, we combined Fe-speciation with elemental analyses in drill core MY5, close to the Myrtle base metal deposit (Figure 6-3). In MY5, a Zn enrichment of ~0.4 wt% at ~325 m depth corresponds to a euxinic excursion (albeit based on only one sample). Although this relationship needs to be confirmed with a higher sampling density, it hints at a connection between water column redox conditions and base metal deposition in the BCF.
Elevated FeHR/FeT ratios in central basin drill cores (chapter 5) may reflect enhanced Fe-influx into the central basin due to the geographic position or enhanced exhalative activity in the wider area. There is however also the possibility of a more local extent of FeHR/FeT enrichment, forming a more extensive alteration halo, in which case this ratio could be used as another vector towards base metal deposits. Since exhalation of Fe-rich fluids seems to continue even after cessation of base metal exhalation (Large et al., 2000), FeHR enrichments may be useful as vectors for mineralisation.
6.3 Discussion of redox implications for mineralisation

Pyrite distributions observed in several elemental maps of thin sections are generally consistent with our temporal mixing model, where deposition fluctuated between euxinic and ferruginous conditions. In our temporal mixing model, the pyrite distribution in Figure 6-2 would largely correspond to green ecological endmembers with very few and short ferruginous periods. Based on the proxy correlations, one would expect that ferruginous periods of the purple endmember would not only be low in pyrite, but also result in a high carbonate content. As can be seen from the calcium map in Figure 6-2A, this is indeed the case for the pyrite-poor central horizon and at least two additional pyrite-poor bands that, in our model, reflect temporal switches to ferruginous conditions and carbonate precipitation. The major ferruginous band is accompanied by a distinctive phosphorus enrichment (Figure 6-2B). Similar phosphorus enrichment was observed for temporal ferruginous excursions in otherwise euxinic sediment of the Cretaceous Oceanic Anoxic Event 3 and can be ascribed to water column phosphorous scavenging during precipitation of iron (oxyhydr)oxides in the upper water column under ferruginous conditions (März et al., 2008). During re-establishment of sulphidic bottom water conditions, März et al. (2008) observed ZnS peaks, as the formation of ZnS is faster and occurs prior to pyrite formation (Morse and Luther Iii, 1999). The same phenomenon is observed in the BCF (Fig. 3C). Zn accumulation in the basin waters may be related to temporal exhalative activity somewhere in the basin, so Zn enrichment is not expected to occur in all ferruginous-sulphidic transitions.

The elemental distributions allow for the possibility that frequent fluctuations between ferruginous and sulphidic conditions occurred in the BCF waters and that the frequency and ‘severity’ or longevity of sulphidic events varied through time. The occurrence of pyrite-rich siliciclastic intervals in various drill cores (Figure 4-5), indicates that these long-term sulphidic excursions occurred across the McArthur Basin and would thus likely also have influenced the water column chemistry at local sites of exhalation of base metal-rich hydrothermal fluids such as HYC and Myrtle. The formation of the giant stratiform base metal deposits may thus have required the co-occurrence of local hydrothermal exhalation and climate-induced sulphidic excursions of the McArthur Basin waters. On the one hand, long periods of climatic conditions favouring the
formation of euxinic basin waters may be required in order to form giant base metal deposits, while on the other hand, short-scale weather conditions such as strong seasonal contrast may be needed to initiate or maintain euxinia, leading to small-scale lamination of the giant base metal deposits.

According to the latest mineralisation models (Ireland et al., 2004, Large et al., 1998), oxidised base metal-rich brines would accumulate in local depressions adjacent to exhalation sites at deeply rooted faults like the Emu fault, and base metal precipitation would require diffusion of water column sulphide into the brine pool and delivery of pelagic organic matter to fuel BSR in the brine pool and sediments. During ferruginous periods with low rates of organic productivity and BSR, mineralisation rates may thus be slow and proportions of the base metal may eventually escape the brine pool, disperse into the wider basin and eventually escape into the ocean. During sulphidic periods however, exhaled base metals could directly react with free hydrogen sulphide in the water column and precipitate locally, while any base metals accumulated in the basin waters during ferruginous periods would also precipitate. This could explain the Zn enrichment observed in the GR7 sample (Figure 6-2) distal to all known mineralisation. In this scenario, one would further expect to find Zn enrichment during euxinic periods in prospective horizons close to major base metal deposits, but absence during ferruginous intervals, which can explain the restriction of Zn to the sulphidic interval in MY 5 (Figure 6-3) proximal to the Myrtle deposit.
6.4 Lessons from the Myrtle discovery

Stratiform base metal deposits in the Mt Isa Superbasin in northern Australia commonly exhibit geochemical halos that can be used as vectors towards new deposits. Vectors developed for the Mt Isa Basin generally also seem to work in the McArthur Basin and Large et al. (2000) showed that a geochemical halo may extend as far as 15 to 23 km away from HYC. This extensive halo is in contrast to the similarly zoned halo around the Lady Loretta deposit which only extends for about 2 km. Considering the much smaller mineralisation of Lady Loretta (~8 Mt compared to ~237 Mt at HYC) (Large et al., 2000), the smaller halo could be due to less exhalative activity during formation of the deposit. The extent of the halo may thus scale with deposit size.

The large extent of the HYC Zn-Pb-Tl ferroandolomite halo around HYC is called into question by the recent discovery of the Myrtle deposit some 17 km to the south of HYC (Large et al., 2000) and the occurrence of several other mineral prospects like the Teena deposit in a ~20 km radius around HYC. Drill cores MY5, MY4 and MY3 approximately fall into a line towards the Myrtle deposit with MY3 being located ~1.5 km, MY4 ~ 3.5 km and MY5 5.5 km away from the mineralised zone. MY3 shows few favourable SEDEX vectors in the stratigraphic horizon hosting the base metal mineralisation. Slightly elevated values of MnOd and Sedex Al occur higher up in the BCF stratigraphy. In MY4, most vectors are close to or just above background values, with a slight Zn enrichment and MnOd. In the lower BCF, a small spike in Zn concentrations and Tl concentrations may hint towards a favourable horizon, but overall vector response is very subtle. In contrast, MY5 shows a clear spike in most vectors in the upper W-fold shale and lower BCF, with Zn concentrations of more than 0.4%, Tl > 10 ppm Al3 > 30 and MnOd of almost 1.5 wt%. Originally, this vector pattern was interpreted to either reflect the distal HYC signature (with MY5 being slightly closer to HYC) or, more likely, a local Myrtle basin source closer to MY5 than to the other cores. The discovery of the Myrtle deposit ~1.5 km away from MY5 confirmed the latter interpretation. Currently, Myrtle is considered to have ~44 Mt Zn+Pb, but is not fully explored yet and targeted at +100 Mt, so it may be anywhere between ~1/5 to ½ the size of the HYC deposit, and is probably at least five times larger than Lady Loretta. Despite the significant size of the Myrtle deposit, the alteration halo is very subtle just a few kilometres away from the deposit.
Myrtle vectors thus seem to indicate distal mineralisation ~15 to 23 km away, while in reality the deposit is ten times closer. Despite the much larger deposit size, the Myrtle halo thus seems to be more comparable to that of Lady Loretta than HYC.

The two most plausible explanations for the apparent halo differences at HYC and myrtle are that either the halo size relates to deposit size, with the HYC halo extending for ~15 km and the Myrtle halo for only ~ 5 km, or that the HYC halo is overestimated, and elevated values at Barney 3 relate to different exhalative activity proximal to the drill core, making the area prospective for base metal exploration. Irrespective of the extent of the HYC, the experience from Lady Loretta and Myrtle shows that even giant base metal deposits in the McArthur basin can have a very limited spatial extent of their vector halos, greatly enhancing the prospectivity of areas with elevated vector signals. I thus propose, that simultaneous elevation of several base metal vectors, in the range of ‘distal’ Mt Isa Basin signatures may also indicate proximal deposits of smaller, but still significant size.

If smaller halos could be confirmed by future analyses around smaller deposits, this would have very important implications for base metal exploration in the McArthur Basin. Based on the recent experience from the giant Myrtle base metal deposit and a similarly zoned alteration around the Lady Loretta deposit I thus propose that the areal extent of the most significant alteration halos around base metal deposits in the McArthur basin is commonly much smaller than generally assumed. Supposedly “distal signatures” may indicate distal giant deposits comparable in size to HYC, but are more likely to reflect proximal (within 1-6 km) smaller, but potentially still significant deposits, comparable in size to e.g. Lady Loretta or Myrtle. Based on probability considerations one may expect to find for each deposit the size of HYC several smaller deposits, and indeed there are many smaller deposits (like Teena) or prospects recognised in the vicinity of HYC, arguing for multiple local exhalation sites within a 20 km radius around HYC.
6.5 Conclusions

Fluctuations between ferruginous and euxinic periods may explain the distinctive base metal distribution at HYC with small-scale lamination of mineralised and unmineralised horizons. The ‘fault-valve’ model, which involves thousands of earthquake triggered small-scale turbidites, was largely based on the assumption that it would be unlikely to have such a high number of redox fluctuations (Large et al., 1998). A sub-sample frequency of redox fluctuations is however predicted in our temporal mixing model and consistent with elemental distributions (Figure 6-2). We thus propose that rather than seismic turbidites, the clastic event beds constitute tempestites deposited during high-energy weather cycles, and the sulphide laminae were precipitated during the ensuing sulphidic conditions. Generally, trends towards euxinia, as observed in Figure 6-3, may serve as vectors towards stratiform sulphide deposits in such settings.

While FePy/FeHR ratios may point towards long term-euxinic periods that may be required to form giant deposits when base metal-rich brines are exhaled locally, FeHR/FeT ratios could also have potential as distal vectors towards exhalative activity. A switch form base-metal to iron-rich fluids during basin evolution means that FeHR enrichment could even be a useful vector much higher up in the stratigraphy where sediments may be exposed or more accessible for drilling. The extent of FeHR/FeT enrichments around base metal deposits should be investigated in more detail in future studies. ‘Traditional’ Mt Isa Superbasin vectors may also be more prospective than generally assumed (Large et al., 2000). Smaller halos around smaller, but still significant deposits like Lady Loretta and Myrtle suggest that ‘distal’ signatures may be more likely to reflect proximal deposits, of potential still significant size, in my view greatly enhancing the prospectivity of the McArthor basin for future exploration.
6.6 References


7 Syngeneity of BCF steroids and triterpenoids

7.1 Introduction

Contamination with petroleum (products) is one of the major obstacles in Precambrian biomarker research. Therefore, the discussion of the ecological significance of Precambrian biomarkers must be preceded by the confirmation of their syngeneity. Due to a lack of awareness of the full scope of the contamination problem, most older Precambrian biomarker studies are fraught with contamination and even today many biomarker studies inadequately address biomarker syngeneity. The problem is most pressing in the sterane record and the resulting reconstruction of eukaryotic evolution. The possibly oldest clearly indigenous biomarkers known on Earth are now those from the 1.64 Ga BCF (Brocks et al., 2005, Pawlowska et al., 2013) that did not yield any indigenous steranes despite relatively high indigenous hopane concentrations (Brocks et al., 2008). It is not until the Neoproterozoic that clearly indigenous, yet unusual, sterane distributions are observed in the rock record (Summons et al., 1988a, Brocks et al., 2015).

Hydrocarbon contamination of geological sample material is often caused by diesel oil or petroleum products like plastics or lubricants. Thus, many contaminants introduce common constituents of crude oil like normal and methylated alkanes, steranes and hopanes (Schinteie and Brocks, 2014, Jarrett et al., 2013, Grosjean and Logan, 2007, Brocks et al., 2008). New saturated and aromatic steroids and aromatic triterpenoid derivatives discovered in this study are rare in many oils, pointing against a contaminant origin. However, the fact that a contamination source appears unlikely based on distinct biomarker patterns has repeatedly deceived geochemist in past studies (e.g. Brocks et al., 1999). Additional measures are necessary to prove that the biomarkers of interest are syngenetetic to the host rock and are not of allochthonous origin. The most robust analytical techniques to assess biomarker syngeneity are interior-exterior experiments that compare biomarker concentrations on exterior and interior portions of rock samples. Contaminant hydrocarbons are typically enriched on the exterior surfaces. These techniques comprise exterior removal or preparation of slices across a rock sample by ultra clean saws like described by Brocks et al. (2008) or the tumbling of fissile material
like described by Jarrett et al. (2013). Bitumen extracts of the original slice extraction experiment conducted by Jochen Brocks that confirmed the syngeneity of most BCF biomarkers including hopanes and showed the allochthonous nature of regular steranes (Brocks et al., 2008) are used in the following to test the syngeneity of the new BCF biomarkers described in the consecutive chapters. Since the syngeneity needs to be confirmed before the use of biomarkers for palaeobiological reconstructions, the syngeneity of compound classes whose discovery in the BCF is first described in the following chapters is assessed here prior to their detailed description and discussion.
7.2 Methodology and samples

In order to analyse millimetre-scale biomarker concentration gradients, cm-scale block of a biomarker-rich BCF siltstone sample (B03162, GR7 45.35 m) that included the rounded exterior surface and centre of the core, was cut by Jochen Brocks from diamond drill core and subsequently cut into 1-mm slices with a clean precision saw parallel to the outer rounded surface and perpendicular to the bedding direction. Details of the methodology, including fractionation and GC-MS conditions are described in Brocks et al. (2008). Biomarkers were analysed separately for each slice and the chromatograms acquired by Jochen Brocks were employed in this study to calculate concentrations of BCF triterpenoids and assess their syngeneity based on the concentration profiles.

To determine the syngeneity of picenes that were not detected in the slice extraction experiment, we further examined the interior exterior experiments of GR7 samples from 683 and 870 m, B03016 from HYC, B03288 from LY1, B03132 from McA5 and GR7 regular extracts B03065, 073, 068, 67, 69, 70, 72, 63, 74, 64 and 75, all extracted by Jochen Brocks as detailed in Brocks et al. (2008).
7.3 Results and discussion of syngeneity assessment

7.3.1 Regular steranes

Brocks et al. (2008) illustrated the allochthonous nature of regular steranes in BCF extracts in a slice extraction experiment conducted on thermally well-preserved sample B03162 from the upper BCF in GR7. A contaminant source for the steranes is indicated by the restriction of significant yields to the exterior slice A that represents the rounded outer surface of the sediment core (see core diagram in Figure 7-1E), and a strong concentration gradient from the outside to the inside (Figure 7-1D). Together with other interior-exterior experiments conducted by Jochen Brocks on BCF sediments from different drill cores and stratigraphies (Brocks et al., 2008), it can be concluded that previous reports of C27 to C30 steranes (e.g. Summons et al., 1988b) that were used to infer the presence of evolved eukaryotes and constituted importance evidence for marine depositional conditions (Logan et al., 2001), are most likely also due to contamination overprint. Erring on the side of caution, the presence of sterane-producing organisms during BCF times should not be inferred until sterane syngeneity can be unambiguously proven by interior-exterior experiments.

In contrast to the regular steranes, the hopanes show a relatively even distribution across all slices, with only a minor enrichment on slice A (Figure 7-1C). The hopane distribution pattern greatly differs from that of the steranes, despite a similar chemical structure and resulting similar chromatographic behaviour of both compound classes. Significant hopane concentrations are found in the sample interior, indicating a dominantly syngenetic signature. The hopane and sterane distributions in the slice extraction experiment can thus be used as a reference to distinguish between indigenous versus allochthonous hydrocarbons to which the concentrations of other BCF biomarkers can be compared.

A slight enrichment on core surfaces like exhibited by the hopanes (Figure 7-1C) can be due to a minor contamination overprint on a largely indigenous signature, but can also be caused by a life-oil effect related to hydrocarbon expulsion upon pressure release after drilling (see Brocks, 2011). The absence of allochthonous steranes from the interior further indicates that this sample is well sealed against contamination, so bulky
contaminants like polycyclic compounds are not expected to penetrate the sample interior significantly. Since aromatics show a strong retention behaviour (see Brocks, 2011), aromatic hydrocarbons are even less likely to penetrate the sample interior. Thus, a near even concentration profile across the different slices similar to the hopane distributions indicates that the biomarkers are (predominantly) syngentic to the host rock.

**Figure 7-1.** Schematic of the slice extraction experiment conducted by Jochen Brocks illustrating biomarker concentrations with increasing distance from the rounded drill core surface to core centre. C) summed concentration of C_{27} to C_{35} hopanes (ng/g rock), D) summed concentrations of C_{27} to C_{29} steranes, E) diagram of sectioned drill core (Figure from Brocks et al., 2008).
7.3.2 New BCF steranes discovered in this study

The concentration profiles of saturated hydrocarbons with a cholestane-like side chains, lanostanes (peaks 94 – 97), dammarane (5) and protosteranes (82 & 83) and inferred protosterane ring-opening products (1 & 2, see chapters 9 & 10), are shown in Figure 7-2 together with the concentrations of the C30 α,β-hopane (81) as determined in this study from m/z 191 partial ion chromatograms. In this sample, the concentration of lanostanes is an order of magnitude lower than that of the C30 α,β-hopane, but the relative distribution is almost identical. Concentrations are relatively even across the slices and variations are insignificant. Again, there is very little variation across the slices, in particular there is no significant enrichment on the exterior slices like observed for the C27 to C29 steranes. The syngeneity of the new BCF steranes and dammarane (5) is thus confirmed by the slice extraction experiment.

![Figure 7-2](image)

**Figure 7-2.** Syngeneity assessment of BCF triterpanes (peak numbers in parentheses). A) protosteranes (82 + 83, m/z 274), sum of lanostanes (94 to 97, m/z 259) and 8b(H),9a(H) lanostane (Chen et al., 1989), and C30 α,β hopane (81, m/z 191). B) dammarane (5, m/z 301) and potential
proto-spirosteranes (1 & 2, m/z 301). Uncorrected partial ion chromatogram quantification relative to D4 C27 ααα (20R)-cholestane standard (m/z 221).

7.3.3 Hopanes

Brocks et al. (2008) could show with the slice extraction experiments that regular hopanes are dominantly indigenous, with only a minor enrichment on the outermost slice (Figure 7-1). The A-ring methylated equivalent of the sum of C31-C35 homohopanes is the sum of C31-C36 3β-hopanes, and both show a very similar distribution to the total hopanes (C27 to C35) plotted by Brocks et al. (2008). The 3β-hopane profile is even slightly flatter, indicating negligible contamination overprint even on the outermost surface. The 2α-hopanes in contrast show a stark enrichment on the outermost surface, indicating significant contamination overprint on the rock surface. The C31-2α-methyl hopane index defined as the abundance of the C31 2α-methyl hopane relative to the C30 desmethyl hopane, shows a similar distribution. Both, the absolute concentrations and 2α-MHI are ~50% higher on slice A compared to the other slices, indicating a significant alteration through contamination overprint. Absolute concentrations on slice A are slightly lower than those of regular steranes (Figure 7-1). Hopanes and steranes have very similar adsorption and diffusion properties (Carlson and Chamberlain, 1986), yet in contrast to the steranes, 2α-hopane concentrations remain significant and show a relatively even distribution across the other slices. This shows that while 2α-hopane concentrations are low and strongly affected by contamination overprint even in the hopane-rich sample, there are also indigenous 2α-methylhopanes in these 1.64 Ga sediments. We can thus confirm the antiquity of both of the unusual methylation pathways. Since 2α-methylhopanes are easily adulterated by contamination however, one has to be careful with paleo-ecological interpretations of 2α-methylhopanes distributions.
The concentration profile of the main C30 8,14-secohopane isomer (6) in sample B03162 is shown in Figure 7-4. The profile confirms that the 8,14-secohopanes in the BCF are dominantly indigenous.

**Figure 7-4.** Concentration profile of main (first eluting) C30 8,14-secohopane isomer (ng/g rock). Uncorrected partial ion m/z 414 chromatogram quantification relative to D4 (m/z 221).
7.3.5 Gammacerane

The concentration profile of gammacerane in sample B03162 is shown in Figure 7-5. The profile confirms that gammacerane in the BCF is dominantly indigenous.

Figure 7-5. Concentration profile of gammacerane (ng/g rock, uncorrected MRM (412→191) quantification relative to D4 (404→221).

7.3.6 Aromatic steroids

Concentrations profiles of the most important aromatic steroids are shown Figure 7-4. The C_{28} and C_{29} diaromatic steroids, C_{26} to C_{28} triaromatic steroids and C_{29} B-ring monoaromatic lanosteroids (G) all show a very similar concentration-profile with significant concentrations in the innermost slices and only a slight enrichment on the outermost slice A. Considering that a variety of unusual triterpenoids that are unlikely to be present in significant quantities in the contamination, the most plausible explanation for the slight enrichment on the core surface observed in almost all plots is the live-oil effect discussed above. The concentration gradient is orders of magnitude lower than that of the steranes and aromatic steroids should have an even lower ability to migrate to the sample interior during contamination. Thus, the slice extraction experiment confirms the syngeneity of the aromatic steroids.
Figure 7-6. Concentration profiles of A) triaromatic steroids (C_{26}: m/z 231, C_{27}: m/z 245, C_{28} m/z 250), B) diaromatic steroids (C_{28}: m/z 376, C_{29}: m/z 390), C) B-ring monoaromatic lanosteroid (m/z 379) and D) aromatic arborane/fernane-derivatives (DPAH 2: m/z 388, DAPH 1, m/z 374, MAPH: m/z 377, C_{23}-des E: m/z 231) quantification relative to D14 p-terphenyl (m/z 244).

7.3.7 Arborane/fernane derivatives

The concentration profile of typical arborane/fernane derivatives DAPH 2 (66), DAPH 1 (67), MAPH (68) and a C_{23} des-E aromatic triterpenoid are shown in Figure 7-6D. The relatively even concentration profile with only a minor enrichment on slice A confirms the syngeneity of these triterpenoid derivatives.

Aromatic triterpenoids

Some of the most abundant peaks of each class of aromatic triterpenoid derivatives were selected for syngeneity assessment. The concentration profiles shown in Figure 7-7A & B. The aromatic triterpenoids show a concentration-profile very similar to the aromatic steroids with only minor concentration gradient across the slices and minor enrichment on slice A. The slice extraction experiment thus confirms the syngeneity of the aromatic triterpenoids.
7.3.8 Aromatic 8,14-seco hopanoids

The concentration profiles of different types of aromatic 8,14-secohopanoids are shown in Figure 7-7C & D. Compounds with fluorene- (44) and acenaphthene-moiety (45), as well as 21-methylated regular monoaromatic hopanoids (46 to 49) and 21-nor monoaromatic 8,14-secohopanoids (60 & 61) show a relatively even concentration profile with only minor enrichment on slice A. The slice extraction experiment thus confirms the syngeneity of the aromatic 8,14-seco hopanoids.

7.3.9 Picenes

The only type of newly discovered BCF triterpenoids that were not encountered in the slice experiment extracts are the tentatively identified methylated picenes. These are only found in many, but not all, of the aromatic fractions recovered by Jochen Brocks
with a slightly more polar solvent mixture than used for new extracts obtained in this PhD project. We therefore suspect that they often elute in the polar fractions. Due to the absent in the slice extraction experiment, their syngeneity can currently not be confirmed. However, they are absenting in all extracts (including exteriors) of highly mature (biomarker-lean) samples prepared by Jochen Brocks (683 and 870 m GR7, B03016 HYC, B03288 LY1, B03132 McA5) and the laboratory blank, while present in most of his extracts of thermally well-preserved samples (B03065, 073, 068, 067, 69, 70, 72, 63, 74, 64 and in low concentration in B03075). These compounds are not known as contaminants and the BCF sterane profiles show that contamination overprint is generally low, while the picenes are very abundant. We thus consider the BCF picenes to probably be indigenous, but their syngeneity needs to be confirmed with additional interior-exterior experiments in the future.
7.4 Conclusions

In this chapter, we investigated the syngeneity of diverse BCF triterpenoids discussed in the following chapters. For each class of newly discovered triterpenoids at least one representative peak (usually the highest) was selected for syngeneity assessment. In contrast to the regular steranes that are restricted to the exterior slices and the 2α-methylhopane that are strongly enriched on slice A, all other triterpenoids show a relatively even concentration profile in the slice extraction experiment. The biomarker distributions are similar to that of the dominantly indigenous hopanes as described by Brocks et al. (2008). It can therefore be concluded that all the unusual aromatic BCF steroids and triterpenoids, including tri- and tetra-aromatic- and typical arborane/fernane derivatives, as well as the newly discovered protosteranes, lanostanes and dammaranes are indigenous to the BCF and can be employed for palaeoecological reconstructions in the following chapters.
References


8. Triterpenoids of the Barney Creek Formation

8.1 Introduction

Triterpenoids are lipids that are derived from three terpane units which are in turn each made up of two isoprene units consisting of five carbon atoms (Figure 8-1). Some triterpenoids do not strictly follow the isoprene rule due to loss of methyl groups, like for example the C27 sterol cholesterol (see e.g. Peters et al., 2005b). Triterpenoids are widely distributed in bacteria (mainly hopanoids) and eukaryotes (mainly sterols) and ubiquitous in many sediments and petroleum of all geological eras (Brocks and Summons, 2003). The aromatic (chapter 10) and saturated steroids (chapter 9) are discussed separately. This chapter focuses on the other saturated and aromatic polycyclic triterpenoids that were detected in BCF sediments in this study.

The recognition of hopanoids as abundant chemical fossils led to their discovery in living organisms. Hopanoids are often referred to as “the most abundant natural products on Earth” (Ourisson and Albrecht, 1992). Most hopanoids are produced by bacteria (e.g. Rohmer et al., 1984). Hopanoids were found in about half of some 100 bacterial strains belonging to diverse taxonomic groups like cyanobacteria, methylo-trophs and several gram-negative and -positive chemoheterotrophs, but not in purple sulphur bacteria or archaea (Rohmer et al., 1984). Originally thought to be diagnostic of aerobic bacteria, they are now also known from many anaerobic bacteria including photosynthetic purple non-sulphur bacteria, fermentative Zymonas mobilis, specific planctomycetes and some sulphate reducing bacteria (see Coolen et al., 2008 and references therein). Over 100 individual hopane derivatives have been isolated from sediments (Ourisson et al., 1979).

The basic hopanoid structure consists of a C30 pentacyclic carbon skeleton derived from the cyclisation of squalene (Figure 8-1) (see e.g. Rohmer et al., 1979). The simplest prokaryotic hopanoids are diplopterol and diploptene (Figure 8-1) that are present in all hopanoid-containing prokaryotes (Ourisson et al., 1987). The amphiphilic bacteriohopanepolyols (BHP) found in bacteria are the functional precursors of C30-C35 hopanes (Figure 8-1). In these lipids, a five-carbon sugar-derived moiety is attached to the C-30 carbon skeleton and may have additional sugar, amino acid or other polar groups (Brocks and Summons, 2003). The saturated diagenetic products, C30-C35
hopanes, (Figure 8-1) are among the most abundant biomarkers in many types of sediments. It is hypothesised that BHP are the bacterial equivalents of sterols that optimise the fluid lipid membranes of eukaryotic cells (Ourisson et al., 1979).

**Figure 8-1.** Overview of important (building blocks of) triterpenoid structures. For 2-methylhopanoids $R_1 = \text{CH}_3$ and for 3-methylhopanoids $R_2 = \text{CH}_3$ for C35 aminobacteriohopanepentols $Z = \text{N}$, $X \& Y = \text{OH}$, tetrahymanol has a gammacerane structure hydroxylated at C-3, hexaprene a hexaprenol structure missing the oxygen.
Specific hopane-product relationships are poorly constrained. This is mainly due to the huge variety of potential source organisms and the relatively low number of cultured bacteria. Without additional information, the hopanoid content of a particular sample cannot be readily attributed to any specific biological source (Brocks and Summons, 2003). Of particular interest for palaeoecological reconstructions are hopanes methylated at position-2 thought to be specific to cyanobacteria (Summons et al., 1999) and position-3 as they may be specific to methanotrophic and acetic acid bacteria (e.g. Zundel and Rohmer, 1985). However, the biosynthetic history of 2-methylhopanoids was recently reconstructed through a phylogenetic analysis of C-2 hopanoid methylase and it was found that it originated in a subset of the alphaproteobacteria and was likely transferred via horizontal gene transfer to cyanobacteria after their major divergence. The original function of 2-methylhopanoid production was possibly related to stress resistance in ancient alphaproteobacteria (Ricci et al., 2015). 2-methylhopanes help to pack lipids more tightly together, resulting in more rigid membranes (Wu et al., 2015). 2-methylhopanes seem to promote fitness in stressful environments like those supporting plant-microbe interactions or oceanic anoxic events (Ricci et al., 2015) which in the Phanerozoic have been correlated with peaks in 2-methylhopanes (Knoll et al., 2007).

Further clues about biological sources of geological hopanoids can be drawn from polar side-chains with different numbers and types of substituents. The functionalisation in turn affects subsequent diagenesis and the types of hopanes and other products recorded in the sediments (Brocks and Summons, 2003). For example, type-I methanotrophs not only produce diagnostic 3β-methyl substituents but also hexafuctionalised side-chains (Zundel and Rohmer, 1985). For example, hexafuctionalised C₆₅ aminopentol (Freeman and Hayes, 1990) (see Figure 8-1) is the most abundant hopanoid in type-I methanotrophs Methylomonas sp. and Methylococcus capsulatus (Zundel and Rohmer, 1985, Neunlist and Rohmer, 1985) and the most likely degradation products are 30-norhopanes (Freeman and Hayes, 1990). Burhan et al. (2002) reported a C₂₇ and C₂₉ hopane predominance that may be partly due to the diagenetic transformation of lipids functionalised at C-29 or C-30 such as aminobacteriohopanepentol. Strong ¹³C depletions indicate that the hopanes were derived from aerobic methanotrophic bacteria (Burhan et al., 2002). This is consistent with previous observations of strongly ¹³C-depleted C₂₇
and C₂₉ hopane signatures coinciding with relatively high concentrations of these hopanoids in extracts from the Messel shale (Freeman and Hayes, 1990). High relative concentrations of C₂₉ or C₂₇ hopanes may thus be indicative of methanotrophic bacteria if accompanied by additional evidence like carbon isotopic depletions.

Most triterpenoids with non-hopane skeletons like oleanane are mostly restricted to land plants and can thus not be expected in Proterozoic sediments. An exception to this is tetra-cyclic dammarane (Figure 8-1). Although rarely reported in the literature, a detailed investigation of IODP drill cores by Meunier-Christmann et al. (1991) revealed that dammarenes and dammaranes occur in numerous Pleistocene to Jurassic sedimentary settings, mostly from continental margins. The occurrence of dammarenes in many marine sediments with variable and often minor terrigenous constituents points to an as yet unrecognised microbial source (Meunier-Christmann et al., 1991). Meunier-Christmann et al. (1991) speculated that a primitive cyclase may exist that catalyses squalene to dammara-13(17),24-diene. Fischer and Pearson (2007) proposed that a compound with a dammarane skeleton may have been cyclised by a proto-triterpenoid cyclase from which all other triterpenoid cyclases may have subsequently evolved (Fischer and Pearson, 2007).

According to Fischer and Pearson (2007), the very first triterpenoid cyclase may have led to a favourable tricyclic triterpenoid with a malabaricane or maybe even cheilanthane skeleton (Figure 8-1), if instead of hexaprenol a hexaprene-substrate (Figure 8-1) was used, followed by the development of a cyclase producing compounds with dammarane skeletons. From there, triterpenoid cyclase evolution may have diverged with the emergence of hopane- and subsequently gammacerane-cyclases on the one side, and sterane- and subsequently arborane-cyclases on the other side (Fig. 4 in Fischer and Pearson, 2007) (see Figure 8-1 for structures). Other scenarios regarding the evolution of the major classes of polycyclic triterpenoid cyclases are also possible. According to Fisher and Pearson (2007), it is nevertheless likely that all cyclases diverged from a common ancestor with a tricyclic triterpenoid-cyclase diverging first, most likely followed by divergence of dammaranoid cyclases.

Other common triterpenoids are those with a fernane, arborane or gammacerane skeleton. Triterpenoids with a fernane- or arborane-skeleton are enantiomers (Figure 8-
and cannot be readily distinguished from each other in the ancient rock record. They are produced by functionalised precursors like fernene/ferneol or isoarborinol (Figure 8-1) and often preserved as aromatic derivatives (Hauke et al., 1992b). In addition to land plants, it was proposed that bacteria or algae produced isoarborinol found in the Messel shale (Hauke et al., 1995), similar to the prediction by Ourisson and colleagues that an as yet unknown aerobic (inferred from the 3-OH group) bacterium may produce isoarborinol that is found in many ancient and modern sediments (Ourisson et al., 1982). It was recently discovered that the marine heterotrophic bacterium Eudoraea adriatica produces two isoarborinol-like lipids. Phylogenetic analyses revealed that the oxidosqualene synthase is homologous to bacterial lanosterol synthases and distinct from plant triterpenoid cyclases. Metagenomic analyses further suggest that there may be additional arborinol producers in marine and lacustrine environments (Banta et al., 2016). According to the potential evolutionary relationships proposed in Fischer and Pearson (2007), compounds with an arborane-skeleton in very ancient sediments could also have been produced by (primitive) eukaryotes. Gammacerane is the diagenetic product of tetrahymanol produced by certain ciliates, but can also have a bacterial source and occurs in trace amounts in almost all oils and bitumens (Brocks and Summons, 2003).

Other triterpenoids commonly encountered in ancient sediments are tricyclic triterpenoids (cheilanthanes). Although extended series up to C_{54} have been reported, shorter homologues up to C_{30} are more frequently encountered. Due to the isoprenoid side chain, C_{17}, C_{22} and C_{27} homologues occur in low relative concentrations. Regular poly-isoprenols such as C_{30} hexaprenol found in bacterial membranes and malabaricatrienes produced by algae or bacteria could be intermediates in the biosynthetic pathway of cheilanthanes (Peters et al., 2005a and references therein). It was inferred from the molecular properties of tricyclohexaprenol and the widespread occurrence of tricyclic terpanes that they originate from the cell membranes of prokaryotes and that tricyclic terpanes with 30 or fewer carbon atoms are derived from tricyclohexaprenol (Ourisson et al., 1982).

The triterpenoid inventory of the BCF was partly discussed in previous studies. Summons et al. (1988) showed the typical hopane MRM transitions for the least mature BCF sample of drill core GR7 (38.7 m). They tentatively identified 29,30-bisnorhopane,
and the C27 hopanes Ts and Tm, 28,30-bisnorhopane, C29 to C35 homohopanes and methyl-hopanes. Hopane distributions were dominated by the C30 pseudohomologue and in most samples an increase of the ratio of TS/Tm was observed with increasing maturity both down core and laterally across several drill cores. Furthermore, the occurrence of a homologous series of putative re-arranged C29-C34 (neo)hopanes at about 10% of the concentration of the normal series was observed in BCF samples (Summons et al., 1988). They were later shown to be 17α-diahopanes and not neo-hopanes (Moldowan et al., 1991). Brocks et al. (2005) described high relative concentrations of 3β-methylhopanes attributed to type Ⅰ methanotrophs or other microaerophilic proteobacteria. High 3β-methylhopane indices of 5.7% on average in drill core GR7 (n = 9) were interpreted as evidence for a high abundance of type Ⅰ methanotrophs. In contrast, very low concentrations of 2α-methylhopanes were reported, with C31-2α-methylhopane indices <1% (Brocks et al., 2005). BCF sediments thus exhibit a wide array of hopanoids ranging from extended series of 2α- and 3β-methylhopanes, homohopanes, to nor- and neo-hopanes.

In this chapter, the triterpenoid inventory of the BCF is re-visited with a focus of unusual structures and potential products of primitive oxidosqualene cyclases. In particular, we will look at the potential aromatic triterpenoid derivatives. Can we find additional, as yet unrecognised, triterpenoids in the BCF?
8.2 Samples and methodology

Samples analysed in this study include: drill cores MY4 (12Z083, 103.3 m depth), LV09001 (12B117, 382.2 m) and GR7 (B03171, 106.28 m; B03169, 90.30 m; B03178, 199.08 m; B03163, 47.55 m; B03166, 67.14 m; B03072, 151.1 m; B03162, 45.35 m and B03062, 180 m). Other BCF samples, particularly from GR7, were used to support the observations made on the above samples but are not further discussed. Interior-exterior extracts of samples B03162 (GR7, 45.35 m), B03200 (GR7, 683 m), B03224 (GR7, 870 m), B04016 (HYC deposit) and B03132 (McA5) prepared by Jochen Brocks were further used to check for picenes. Syngeneity of the other compound types was established in chapter 6 using the slice extraction experiment (Brocks et al., 2008) of GR7 sample B03162.

The methodology of biomarker extraction and analysis is detailed in chapter 2. Briefly, bitumen was extracted from powdered rock samples with dichloromethane and separated by column chromatography into saturated, aromatic and polar fractions. Saturate and aromatic fractions were then analysed by GC-MS.
8.3 Results and discussion

8.3.1 Saturated triterpanes

8.3.1.1 Regular hopanes and tricyclic terpanes (cheilanthanes)

All thermally well preserved BCF samples show homologous series of hopanes and tricyclic terpanes of the cheilanthane-type. In GR7 there two endmember hopane and cheilanthane signatures roughly coincide with the purple and green ecological signatures discussed in chapter 5. Most BCF samples yield higher concentrations of hopanes than tricyclic triterpanes (cheilanthanes) as shown in Figure 8-2A for the sample from 106.28 m depth in GR7. This sample exhibits the typical hopane signature of thermally well preserved BCF samples. The m/z 191 partial ion chromatograms are dominated by the C₃₀ α,β-hopane and yield a homologues series of homohopanes usually extending to C₃₄ or C₃₅. Diahopanes are also abundant and reported to occur usually at ~10% of the concentration of the normal series (Summons et al., 1988). At 106.28 m, the C₃₀ diahopane has ~30% of the concentration of the C₃₀ α,β-hopane (Figure 8-2) despite a rather moderate clay content of ~21% as determined by XRPD (Appendix). Cheilanthanes are present in lower concentrations, as is a C₂₄ tetracyclic terpane. The homologous series of cheilanthanes extends to at least C₂₅, but probably C₂₆ (e.g. 199.08 m depth in GR7). It is currently unclear if the 30-norhopane dominance could partly be a contamination artefact, which needs to be investigated in more detailed future studies.

A few other samples are in contrast are dominated by cheilanthanes with the C₂₃ homologue being most abundant (Figure 8-2B). The sample from 90.30 m shown in Figure 8-2B also shows high concentrations of an unidentified C₁₉ tricyclic with intense m/z 123 ion, but this compound is not prominent in other cheilanthane-dominated samples.
8.3.1.2 Dammaranes

Several BCF samples contain compounds that yield intense $m/z$ 414, 301 and 191 mass fragments typical of dammaranes. To test if dammaranes are present, a standard mixture was kindly provided by P. Adam and P. Schaefer (Université Louis Pasteur, Strasbourg). The standard was prepared by Meunier-Christmann and colleagues by conversion of (20S)-20-hydroxydammar-24-en-3-one isolated from *Dipterocarpus*-resins into a mixture of two pure dammar-13(17)-enes, one of which was separated and hydrogenated (see Meunier-Christmann et al., 1991 for details).

Figure 8-3 shows the $m/z$ 301 partial ion chromatogram of the dammarane standard mixture (A), a Jurassic bituminous black shale from Albania where dammaranes were present (B) and co-elution of the dammarane mixture and Albanian extract (C) as reported by Meunier-Christmann et al. (1991). The MRM $m/z$ 414 → 301 transition for the same dammarane standard mixture under our GC-MS conditions is shown in Figure 8-3E. Figure 8-3D shows the MRM $m/z$ 414 to 301 transition of a BCF sample (B03178, GR7,
199.08 m depth) in black and the co-injection experiment of the same BCF extract with the dammarane standard mixture in red. Based on the co-injection experiment and mass spectra, compounds d2 and d4 from the Jurassic sample are also present in the BCF (peaks 2 and 5). Although peak 4 occurred in a similar elution position as d3, the spectra are different with 4 yielding a much smaller 191 fragment. Comparison of the mass spectra of 3 and 5 with the dammaranes d2 and d4 from the standard mixture (Figure 8-4) confirms that the two BCF compounds are indeed dammaranes. Although the M⁺ 414 fragment is not apparent in the background subtracted mass spectrum of 5, the peaks in the MRM m/z 414 to 301 and 191 transitions confirm 414 Dalton as the molecular mass.

**Figure 8-3.** A-C m/z 301 fragmentograms from Meunier-Christmann et al. (1991) of A) Dammarane mixture obtained after hydrogenation of one of the dammar-13(17)-ene standards, B) non-aromatic hydrocarbon fraction from an Albanian sample, C) co-injection experiment of A and B, D-E m/z 414 to 301 MRM transitions of D) BCF sample B03178 (199.08 m, GR7) (black) and co-elution with dammarane standard (red), E) same dammarane standard as in A in our analyses (courtesy of P. Adam and P. Schaeffer, Strasbourg). Compounds identified by Meunier-Christmann et al. (1991) by NMR analysis and molecular mechanics calculations as d1: (20S)-13α,17β(H)-dammarane, d2: (20S)-13β,17α(H)-dammarane, d3: (20R)-13α,17β(H)-dammarane, d4: (20R)-13β,17α(H)-dammarane (Stereochemistry at positions C-20 and at positions C-13 and C-17 of d2 and d4 is tentative).
In most BCF samples, the identified dammaranes are not the highest peaks in the m/z 301 partial ion chromatograms or MRM m/z 414 → 301 traces. Instead, these chromatograms are dominated by two slightly earlier eluting peaks 1 and 2 (Figure 8-3D). At the elution position of 2, there is also a very small peak in the m/z 301 partial ion chromatogram of the dammarane standard mixture. Due to the very low concentration of this compound, it cannot be distinguished in the co-elution experiment. The mass spectra of 1 and 2 are shown for the biodegraded oil sample 12Z083 MY4 in Figure 8-5, 103.3 m, since 1 and 2 co-elute in most samples with secohopanes 8 and 9.
dammarane mixture yields a mass spectrum similar to that of 2, but small differences are also apparent (Figure 8-5). For example, there are m/z 151 fragments in 1 and 2, but they lack m/z 177 and 231 fragments. 2 does thus most likely not correspond to the small peak in the dammarane standard mixture. The earlier eluting BCF compounds 1 and 2 could still be as yet unrecognised dammarane isomers.

Figure 8-5. Mass spectra of peaks in m/z 301 partial ion chromatograms of sample 12Z083 (MY4, 103.3 m) peaks 1 (A) and 2 (B), dammarane standard mixture at elution position of peak 2.
8.3.1.3 8,14-Secohopanes

The $m/z$ 414 traces of some BCF saturate fractions also yielded a series of four peaks, 5 to 9, eluting around the same time as C$_{27}$-hopanes (Figure 8-6). The mass spectra for these compounds are shown in Figure 8-7. Note that 7 co-elutes with Tm under our chromatographic conditions (Figure 8-6), resulting in subtraction artefacts in the mass spectrum. These BCF compounds have mass spectra and elution positions that are very similar to 8,14-secohopanes. Schmitter (1982) reported four 8,14-secohopane isomers from a Nigerian crude oil with the first eluting slightly before and the last eluting slightly after Tm, similar to BCF peaks 5 to 9. Comparison of the mass spectrum of the first eluting 8,14-secohopane reported by Schmitter (1982) (Figure 8-7A) with 6 (Figure 8-7E) supports the assumption that the unknown BCF triterpenoids are 8,14-secohopanes. These compounds are also present in the biodegraded oil 12Z083 (MY4, 103.3 m), where 6 can be distinguished in TIC, indicating a relatively high biodegradation resistivity of the 8,14-secohopanes. 8,14-secohopanes are present in most oil and mature source rocks, often exhibiting a complete C$_{27}$ and C$_{29}$-C$_{35}$ series (e.g. Fazeelat et al., 1995, Fazeelat et al., 1994).

![Figure 8-6](image_url)

**Figure 8-6.** Partial ion chromatograms of A) $m/z$ 414 and B) $m/z$ 191 traces of BCF sample 12B117 (LV09001, 382.2 m)
Figure 8-7. Mass spectra of (A) first eluting 8,14 seco-hopanoid in a Nigerian crude oil (Schmitter et al., 1982), BCF peaks B) 9, C) 8, D) 7, E) 6 in sample 12B117 (LV09001, 382.2 m). Peak 7 yields some substraction artefacts due to co-elution with Tm.

8.3.1.4 Gammacerane

Gammacerane occurs in low concentrations in several BCF samples. Due to low concentrations, it cannot be used to make ecological inferences and may have a bacterial source.
8.3.2 Aromatic triterpenoid derivatives of the BCF

The aromatic fraction of the BCF has yielded a wealth of biologically informative molecules such as arylisoprenoids produced by green and purple sulphur bacteria and unusual 4-methylated aromatic steroids that may be attributed to methanotrophic bacteria (Brocks et al., 2005) The aromatic fractions contain a wide variety of yet unidentified compounds that may reveal important paleo-ecological insights. Aromatic triterpenoids not previously described from the BCF are examined in the following. The aromatic triterpenoids are tentatively identified based on their mass fragmentation patterns and comparison to published spectra and elution patterns. Structures proposed for BCF aromatic triterpenoids together with some of the inferred fragmentation mechanisms are summarised in Figure 11-16 and 11-17. Appendix 8-1 contains a list of peak and structure numbers. Appendix 8-2 summarises the mass spectra of peaks interpreted to represent tetraaromatic triterpenoids. The syngeneity of all major compound classes reported here was established by slice extraction experiments presented in the preceding syngeneity chapter (chapter 7). The inferred diagenetic and biological origins of the saturated and aromatic triterpenoids are discussed in chapter (11).

8.3.2.1 Tetra-aromatic triterpenoids (TeAT)

Figure 8-8 shows the TIC and m/z 281, 295 and 309 partial ion chromatograms of the high boiling range of a representative thermally well preserved (i.e. marginally mature) BCF sample of drill core GR7. These fragment masses are consistent with pentacyclic units with four aromatic rings and methyl, di-methyl or tri-methyl substitution. Mass spectra further suggest variable substitution of the pentacyclic core fragment with methyl-, ethyl- and propyl-groups respectively. The most abundant compounds of each type are summarised in Table 8-1. The mass spectra of all numbered peaks are shown in Appendix I. The tentative identification of these compounds as tetra-aromatic (chrysene) hopanoid derivatives is based on the mass spectrum reported by Spyckerelle (1975) for compound Ib. Structures are labelled here according to cyclic core (I reflecting mono-, II di- and III- trimethylated chrysene structures) and side chain alkylation (a = methyl-, b = ethyl-, c= isopropyl-substitution at the five membered E-ring) (Figure 8-8, Table 8-1). As can be seen in Figure 8-9, the mass spectrum of the 4-methylated chrysenoid with
ethyl-side chain is very similar to that of BCF peak 11. Greiner et al. (1976) synthesised the compound discovered by Spykerelle, confirmed the structure by NMR, and reported the relative intensities of the most prominent mass fragments. The relative intensities of the fragments of Ib (Greiner et al., 1976) match that of BCF peak 11 (Figure 8-9C), indicating that these are the same compounds. In sample B03166 (GR7, 67.14 m) Ib (~2.7 μg/g of rock) is one of the most abundant aromatic compounds and in similar abundance as the 4-methyl triaromatic cholesteroids (~4.1 μg/g) described by Brocks et al. (2005) (Figure 8-9), highlighting the abundance of the newly discovered aromatic triterpenoids in the BCF.

**Figure 8-8.** Tetraaromatic triterpenoids in high boiling range of aromatic fraction of GR7 sample B03166 (67.14 m). Partial mass chromatograms of A) m/z 309 B) m/z 295, C) m/z 281, D) total ion chromatogram (TIC). Colour code: alkyl-substitution at E-ring.
Table 8-1. Overview of tetraaromatic hopanoids inferred from BCF sample B03166 (GR7, 67.14m) (see Figure 8-8).

<table>
<thead>
<tr>
<th>Peak #</th>
<th>structure #</th>
<th>core fragment (m/z)</th>
<th>M+</th>
<th>side chain</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Ia</td>
<td>281</td>
<td>296</td>
<td>Methyl</td>
</tr>
<tr>
<td>11</td>
<td>Ib</td>
<td>281</td>
<td>310</td>
<td>Ethyl</td>
</tr>
<tr>
<td>12</td>
<td>Ib</td>
<td>281</td>
<td>310</td>
<td>Ethyl</td>
</tr>
<tr>
<td>13</td>
<td>Ic</td>
<td>281</td>
<td>324</td>
<td>Isopropyl</td>
</tr>
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<td>IIa</td>
<td>295</td>
<td>310</td>
<td>Methyl</td>
</tr>
<tr>
<td>15</td>
<td>IIb</td>
<td>295</td>
<td>324</td>
<td>Ethyl</td>
</tr>
<tr>
<td>16</td>
<td>IIb</td>
<td>295</td>
<td>324</td>
<td>Ethyl</td>
</tr>
<tr>
<td>17</td>
<td>IIc</td>
<td>295</td>
<td>338</td>
<td>Isopropyl</td>
</tr>
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<td>26</td>
<td>IIIc</td>
<td>309</td>
<td>352</td>
<td>Isopropyl</td>
</tr>
</tbody>
</table>

Peak 12 yields the same molecular mass and base ion and can thus be interpreted as a different positional isomer of Ib. Most triterpenoids retain one of the C-4 methyl groups during aromatisation (cf. e.g. Greiner et al., 1977, Greiner et al., 1976, Wolff et al., 1989) and it is therefore considered likely that all TeATs are C-4 methylated, while the position of additional methyl groups currently cannot be further constrained. The other compounds with significant m/z 281 fragments (Figure 8-8) show similar fragmentation patterns (Appendix 8.2), but different molecular ions, indicating different types of ring E substitution. Structure Ic of a 4-methylchrysene aromatic hopanoid with isopropyl side chain was assigned by Greiner et al. (1977) to the M+ 324 compounds with m/z 281 base ion in the Messel shale (Greiner et al., 1977). In this manner, peak 10 is preliminarily identified as Ia with a methyl-substituent at ring E (Figure 8-10A), and peak 13 as Ic with an isopropyl-side chain, forming a series of 4-methyl tetraaromatic hopanoids (I). Greiner et al. (1977) also report the detection of higher homologues of I with butyl- (M+ 338) and pentyl-side chains (M+ 352). However, such compounds were not detected in the BCF.
Figure B-9. Mass spectrum of A) BCF peak 11 in sample B03163 (GR7 47.5 m, see Figure 8-8), B) Comparison of fragments of compound reported in Greiner et al. (1976) and BCF peak 11 and C) mass spectrum and structure of this 4-methylated tetraaromatic hopanoid with ethyl side-chain (from Spyckerelle, 1975).
The compounds in the m/z 295 and m/z 309 partial mass chromatograms show similar fragmentation patterns as the m/z 281 series (Appendix I). Again, there are mass differences of 15, 29 and 43 Dalton between the mass of the core fragment and the molecular mass, indicating methyl-, ethyl- and propyl-substitution at ring E (Table 8-1). In series II, the core fragment is 15 and series III 30 Dalton heavier than that of series I, indicating additional methylation or di-methylation of the aromatic core respectively. Therefore, peaks 14 (Figure 8-10B) to 19 (Figure 8-10C) are interpreted to represent dimethylated chrysene derivatives with methyl- (14 and 18), ethyl- (15 & 16) and propyl-substitution (17 and 19) at ring E, constituting isomers of compounds IIa, b and c respectively (Table 8-1, Figure 8-8). It is likely that the additional methyl group is also attached to the A-ring since most potential triterpenoid precursors are trimethylated at ring A. However, B-, D- or E-ring methylation would also be possible. In the same
manner, compounds with \( m/z \) 309 base ions (20 - 26) are interpreted as tri-methylated chrysene derivatives with methyl- (20, 23, 24, 25), ethyl- (21 and 22) and propyl-substitution (26) at ring E (Table 8-1, Figure 8-8, Figure 8-10D).

Potential hopanoid- or arboranoid/fernenoid-precursors allow for additional methylation in different positions. For example, the aromatisation of a regular hopane could result in a methyl-shift from C-4 to C-3, C-8 to C-7, C-10 to C-1, C-14 to C-15 or C-18 to C-19. Similarly, an arborane/fernane-type precursor could result in a shift from C-4 to C-3, C-10 to C-1, C-13 to C-12, C-14 to C-15. Thus, if a C-7, C-19, or C-12 methylation could be determined in the future, it may be diagnostic of hopane- or arborane/fernane-type precursors respectively. The position of the additional methyl groups can thus currently not be further constrained and a number of different positional isomers are possible. The different members of series I, II and III show an irregular distribution (Figure 8-8). In the \( m/z \) 309 series there are four isomers with \( R = \) methyl, only two with \( R = \) ethyl and one with \( R = \) isopropyl.

8.3.2.2. Tri-aromatic triterpenoids (TrATs)

Similar to the series of methyl-to isopropyl-substituted tetraaromatic triterpenoids, the BCF also contains three series of compounds with 4 Dalton lighter core fragments, pointing towards methyl-, ethyl- and isopropyl-substituted triaromatic triterpenoids Figure 8-11. Although it is difficult to obtain clean mass spectra for the TraTs due to generally low concentrations and frequent coelution, peak 35 shows a spectrum very similar to a methyl-substituted B,C-D-aromatised hopane-derivative (VII, Figure 8-12) which was first reported by Spykerelle (1975) and subsequently confirmed with synthesised reference standards (Greiner et al., 1976).

However, this compounds is apparent only in the \( m/z \) 299 traces of a few BCF samples (Figure 8-11B), the \( m/z \) 299 chromatograms of most other samples (Figure 8-11C) are dominated by peaks with significant \( m/z \) 243 fragments and lacking the \( m/z \) 142 fragment characteristic of 35 (Figure 8-13C, D). Due to co-elution problems, it is difficult to determine all fragments, particularly of the \( m/z \) 313 compounds with e.g. 38 coeluting with TeAT 22, but most peaks seem to yield \( m/z \) 229, \( m/z \) 243, but also \( m/z \) 257 fragments.
The $m/z$ 243 fragment seems to stem from a two-ring cleavage and may be analogous to the two-ring cleavage fragments produced by B-ring monoaromatic fernane/arborane derivatives reported by Hauke et al. (1992a) (Figure 8-13D, E). We therefore propose a A,C,D-aromatic structure with methyl- (IV), dimethyl- (V) or trimethyl- (VI) substitution of the cyclic core and methyl- (a), ethyl- (b) or isopropyl-substitution at ring E (c) (Figure 8-11). They could be derived from either hopane- or fernane/arborane-derivatives having lost their D-ring methylation, possibly as a consequence of interrupted D-ring aromatisation in the pathway leading to TeATs, or possess a methylation at C-17 or C-18 instead of C-4. Due to the different fragmentation patterns, they are unlikely to be B,C,D-ring aromatised hopane-derivatives, although this possibility cannot be excluded with the available information. It is however noteworthy, that the BCF contains high relative concentrations of isopropyl-substituted TeATs and TrATs, whereas ethyl-substituted derivatives seem to more common geological samples (Greiner et al., 1976, Spyckerelle, 1975). This also argues for a source other than hopanoids for at least a proportion of the BCF TrATs and TeATs. A,B,C-aromatic TrATs could potentially also represent side-chain cyclised, 21-nor-triaromatic steroids, but such a reaction-chain with loss of the 21-methyl group, cyclisation but not aromatisation of the remaining side-chain and aromatisation of three but not four rings is considered very unlikely. The similarity to the TeATs suggests that the TrATs are also likely to possess a (hydro)chrysene-type structure and not five six-rings, and that they are likely intermediates in the aromatisation pathway towards TeATs. The abundance of isopropyl-substituted derivatives in the BCF argues against dominant C-30 functionalised hopanoid precursors as inferred for the more common ethyl-substituted TrATs and TeATs (Greiner et al., 1976). A-ring instead of side-chain or E-ring functionalisation would not only explain the preservation of the isopropyl-side chain, but also the occurrence of A,B,C-ring aromatisation reflecting progressive aromatisation starting from ring A, analogous to other examples where aromatisation was induced by the loss of the usual 3-hydroxylation of pentacyclic triterpenes (Greiner et al., 1976).

In conclusion, ferenol, isoarborinol or other unusual 3-hydroxylated triterpenoids with a five-membered E-ring are considered the most likely sources for at least a proportion of the BCF TrATs and related TeATs. There could however be several types of
compounds with different precursors in the TrAT and TeAT series, which would explain the wide spread in elution positions. For example, B,C,D-ring aromatic hopanoids are also present in at least some of the BCF samples.

Figure 8-11. Partial ion chromatograms of the boiling point range of GR7 samples (A, C, D, E: B03178, 199.08 m. B: B03072, 151.1 m) A) m/z 313, B) m/z 299 inset showing main peaks at 151.1 m (brown star indicates VII, B,C,D-aromatic with ethyl-substitution), C) m/z 299, D) m/z 285, E) total ion chromatogram (TIC).
### Table 8.2. Overview of BCF triaromatic triterpenoids (from Figure 8-11).

<table>
<thead>
<tr>
<th>Peak #</th>
<th>Structure</th>
<th>Core (m/z)</th>
<th>M+</th>
<th>Side Chain</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td>VIc</td>
<td>313</td>
<td>356</td>
<td>Isopropyl</td>
</tr>
<tr>
<td>37</td>
<td>VIc</td>
<td>313</td>
<td>356</td>
<td>Isopropyl</td>
</tr>
<tr>
<td>36</td>
<td>Vc</td>
<td>299</td>
<td>342</td>
<td>Isopropyl</td>
</tr>
<tr>
<td>35</td>
<td>VII</td>
<td>299</td>
<td>328</td>
<td>Ethyl</td>
</tr>
<tr>
<td>34</td>
<td>Vc</td>
<td>299</td>
<td>342</td>
<td>Isopropyl</td>
</tr>
<tr>
<td>33</td>
<td>Va</td>
<td>299</td>
<td>314</td>
<td>Methyl</td>
</tr>
<tr>
<td>32</td>
<td>Va</td>
<td>299</td>
<td>314</td>
<td>Methyl</td>
</tr>
<tr>
<td>31</td>
<td>Va</td>
<td>299</td>
<td>314</td>
<td>Methyl</td>
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<tr>
<td>30</td>
<td>Va</td>
<td>299</td>
<td>314</td>
<td>Methyl</td>
</tr>
<tr>
<td>29</td>
<td>IVc</td>
<td>285</td>
<td>328</td>
<td>Isopropyl</td>
</tr>
<tr>
<td>28</td>
<td>IVb?</td>
<td>285</td>
<td>314</td>
<td>Ethyl?</td>
</tr>
<tr>
<td>27</td>
<td>IVc?</td>
<td>285</td>
<td>328</td>
<td>Isopropyl?</td>
</tr>
</tbody>
</table>

### Figure 8.12. A) Mass spectrum of 35 in sample B03072 (GR7, 151.1 m) compared to B) B,C,D-aromatised hopanoid.
8.3.2.3 Benzohopanes and 8,14-secohopanoids with fluorene moiety

Benzohopanes (39-42) are hopanoid derivatives with side-chain cyclisation and aromatisation that retain the m/z 191 base ion (VIIIa, Figure 8-16). These compounds could be detected in some BCF samples and were tentatively identified based on their elution position relative to compounds in the AGSO standard (Figure 8-14) and their mass spectral characteristics (Figure 8-16) (Hussler et al., 1984a). Their concentrations are very low in the BCF (Figure 8-14C, Figure 8-17A). Instead, a late eluting peak with M+ 414 is noticeable in the aromatic fraction of many BCF samples (43, Figure 8-14). A comparison to published spectra (Sinninghe Damsté et al., 1998) led to the tentative identification of 43 as a C31 8,14-seco hopanoid with a fluorene moiety (VIII, Figure 8-15). The compound isolated by Sinninghe Damsté et al. (1998) from a very immature sample had the 8α(H),9α(H) stereochemistry. Considering that the BCF is thermally more mature, the compound occurring in the BCF may have a different stereochemistry.
then the one isolated by Sinninghe Damsté et al. (1998). It is currently unclear why the C31 isomer seems to be predominant (Sinninghe Damsté et al., 1998), possibly loss of C3 unit from hopanopolyols with particularly side-chain functionalisation induces side-chain cyclisation. Carillo- Hernández et al. (2001) propose that 43 is not formed by maturity-driven processes, but rather originates from a specific intermediate or precursor, consistent with the occurrence in very immature samples and an equatorial methyl at C-8. Damste et al. (1998) suggested that the distribution in Kimmeridge Clays sediments may favour the formation of 43, but it is also possible that the source organisms only thrive under certain environmental conditions. Both the BCF and, at times, the Kimmeridge Clay have experienced anoxia and photic zone euxinia, so 43 could potentially be related to such conditions.

![Figure 8-14. A) M/z 191 chromatogram of AGSO standard with benzohopanes with assigned carbon numbers compared to BCF sample B03163 (GR7, 47.5 m) showing B) m/z 414, C) m/z 191 and D) total ion chromatograms. 43 corresponds to a monoaromatic hopanoid with fluorene moiety (VIII, Figure 8-15).](image)
8.3.2.4 8,14-secohopanoids with acenaphthene moiety

Also present in some samples are small peaks yielding molecular ions M+ 402 (45) and M+ 416 (44) that elute before and after the C₃₂ benzohopane (39) (Figure 8-17). Concentrations of these compounds generally are very low in the BCF, in the range of
Triterpenoids

Chapter 8

the benzohopanes (Figure 8-17). Based on the mass spectral similarity, they probably correspond to and 8,14-seco-hopanoids with an acenaphthene moiety (Carrillo-Hernández et al., 2001). These are reported to have roughly equal abundances of M+ and the mass fragment of the C\textsubscript{18}H\textsubscript{16} acenaphthene-moiety (m/z 195, plus 14 Dalton for each higher homologue) like displayed by the BCF peaks. These compounds should have a few other mass fragments in low abundance (<20%) and their absence in the BCF spectra is probably due to the low concentrations and subtraction artefacts. The C\textsubscript{32} to C\textsubscript{34} homologues reported by Carillo-Hernández et al. (2001) were not observed in the BCF. Carillo- Hernández et al. (2001) propose that these compounds are generated during thermal maturation from benzohopanes that are converted into more stable derivatives through cleavage of the 8,14-bond and formation of a fused aromatic ring by hydrogen transfer reactions.

Figure 8-17. Comparison of A) m/z 191, B) m/z 416 and C) m/z 402 mass chromatograms for the aromatic fraction of B03178 (GR7, 199.08 m).
8.3.2.5 Regular monoaromatic 8,14-secopanoids

Many BCF samples yield appreciable quantities of two compounds with intense \( m/z \) 365 fragments (Figure 8-19A). In some samples, the molecular ions \( M^+ 394 \) and \( M^+ 408 \) can also be distinguished, indicating \( C_{29} \) and \( C_{30} \) compounds with 7 double bond equivalents. This is consistent with monoaromatic steroid or seco-hopanoid structures. In the \( M^+ 408 \) compound, the difference of 43 Dalton to the base ion and the absence of significant \( (M^+ -15) \) or \( (M^-29) \) fragments indicate an isopropyl sidechain, characteristic of \( C_{30} \) hopanoids. Similarly, an ethyl-side chain is indicated for the \( C_{29} \) compound. Based on the similarity of elution patterns (Figure 8-19) and mass spectra (Figure 8-20) compounds are tentatively interpreted as \( C_{29} \) and \( C_{30} \) monoaromatic 8,14-secopanoids X (Figure 8-20A) that were isolated from crude oils and structure elucidated by NMR (Hussler et al., 1984b). The elution patterns strongly suggest that the same compounds are present in the BCF. In addition to the corresponding molecular ions \( M^+ 394, 408, 422 \), the BCF peaks 46, 47 and 48 also show characteristic \( m/z \) 187, 201 and 215 fragments respectively, corresponding to cleavage of the carbon bridge between the two ring systems (between C-11 and C12) and the same fragments with additional side-chain-loss \( (m/z 187) \). All
compounds of the series are dominated by the side-chain cleavage fragment \( m/z \) 365 (Figure 8-20). The BCF likely contains, the entire homologous series to \( \text{C}_{35} \), and maybe \( \text{C}_{27} \) (Figure 8-19), analogous to saturated hopanes. However, the distribution pattern of the monoaromatic 8,14-secohopanoids with the strong dominance of \( \text{C}_{29} \) and \( \text{C}_{30} \) compounds is different to that of the hopanes usually showing a (less pronounced) \( \text{C}_{30} \) dominance, indicating source-differences. For example, the ratio of \( \text{C}_{30}/\text{C}_{31} \) hopanes is \( \sim 1.1 \) in the most low-maturity GR7 samples like B03162. In contrast, the ratio of \( \text{C}_{30}/\text{C}_{31} \) monoaromatic 8,14-secohopanoids in B03162 is \( \sim 5.2 \). It thus seems that \( \text{C}_{30} \) hopanoids more readily formed the monoaromatic secohopanoids than higher homologous. In \( \text{C}_{30} \) hopanes like diplopterol or diploptene the functionalisation is closer to the ring system than in hopanepolyols (Figure 8-1). This may have facilitated double bond migration into the ring system, potentially initiating aromatisation. Diagenetic re-arrangement of arborane/fernane-type precursors may also result in the formation of \( \text{C}_{30} \) monoaromatic 8,14-secohopanoids (Vliex et al., 1994). This would require migration of the methyl group at C-13, but such a behaviour was observed from fernenes under acidic conditions (Ageta et al., 1987), while a shift of the C-17 methyl group during aromatisation of the D-ring in fernane/arborane-type derivatives would lead to the C-21 methylation in the seco-aromatic derivatives.
Figure 8-19. Comparison of A) m/z 365 ion chromatogram of the aromatic fraction of BCF sample B03162 (GR7, 43.35 m) to B) m/z 365 (top) and m/z 159 (bottom) ion chromatograms from Hussler et al. (1984b).
Figure 8-20. A) Structure of monoaromatic 8,14-secohopanoid provided by Hussler et al. (1984b). R = H (fragmentation shown); CH₃ to C₆H₁₂. Comparison of mass spectra of B) C₂₉ monoaromatic 8,14-secohopanoid provided by Hussler et al. (1984b), C) peak 46, D) 47, E) 48 of BCF sample B03162 (GR7, 45.35 m).
In the m/z 351 and m/z 145 partial ion chromatograms of the aromatic fractions of some BCF samples, at least three double peaks can further be observed in low concentrations (Figure 8-21). Although signal to noise ratios are low, the main ions and elution positions indicate that these compounds correspond to monoaromatic C_{28}-C_{30} 28-nor-secohopanoids (XI, Figure 8-21F) or demethylated seco-hopanoids (DSH) reported by Killops (1991). Killops proposed that these compounds share the same basic structures as the regular monoaromatic 8,14-secohopanoids (Killops, 1991). In the absence of detailed structure elucidation by NMR, it is also possible that these compounds lack C-27 instead of C-28. It is more likely however, that C-28 was lost, rather than undergoing an 1,3-migration like required for formation of X, during aromatisation of the D-ring (Killops, 1991). In contrast to the regular monoaromatic 8,14-secohopananes, the DSH occur as isomeric pairs for C_{30}-C_{34} homologous, with two of the four C_{30} isomers co-eluting, resulting in three peaks with a relative abundance of 1:2:1 (Nytoft et al., 2000). Indeed, the C_{30} member of the BCF series consist of a peak-triplet (62, 63 and 64) and area of 63 correspond to the combined area of (62 + 64) (see Figure 8-21B). We hypothesise that associated with the C-28-loss, C-21 isomerises, potentially due to the migration of a double bond from the side-chain to ring D during aromatisation. We therefore propose that the DSH exhibit 21α and β(H), as well as the common 22-R and -S configurations for higher homologous. The DSH show different homologue distributions than both the regular hopanes and monoaromatic 8,14-secohopananes, with high relative concentrations of not only C_{28} and C_{29}, but also C_{30}, equivalent to C_{29}-C_{31} in non-demethylated hopane derivatives. This indicates that the DSH originate from different biological precursors and/or different diagenetic processes. The distinction between a C_{30} dominance with C-21 methylation in 46, 47 on the one hand, and the high abundance of butyl-substituted homologous in the 28-demethylated derivatives supports the hypothesis of a potential arborane/fernane-source of the former and a hopane-type source for the later, where methylation at C-18 hampers methyl-shift to C-21, preferentially resulting in formation of 28-demethylated derivatives.
Figure 8-21. Comparison of selected ion chromatograms of A) m/z 145, B) m/z 351 and C) m/z 365 of BCF sample B03163 (GR7, 47.55 m), and C) m/z 351 and D) m/z 365 from Killops (1991). F) Structure and fragmentation proposed by Killops (1991) for 60, 61.
Many BCF samples yield significant quantities of compounds with molecular ions M+ 374 and M+ 388. The molecular masses indicate C_{28} and C_{29} compounds with 10 double bond equivalents, consistent with a diaromatic pentacyclic structure. A peak yielding a molecular ion of M+ 392 is also present, consistent with a C_{29} compound with 8 double bond equivalents such as a monoaromatic pentacyclic structure. One of the samples with the highest abundances of such compounds is B03162 (GR7, 45.35 m). As shown in Figure 8-22, one of the peaks in each of the m/z 374, 388 and 392 partial ion chromatograms shows the same relative elution positions as aromatic compounds reported by Auras et al. (2006).

The mass spectrum of the late eluting peak in the m/z 374 ion chromatogram (67) is very similar to that of an arborane-derived aromatic compound isolated from two Eocene and one Permian shale (Hauke et al., 1992b). Hauke et al. (1992) identified the structure by NMR and reported the mass fragmentation pattern. The similarity of the BCF spectrum and the diaromatic compound 5 of Hauke et al. (1992) is shown in Figure 8-23. Similarly, the mass spectrum of the BCF compounds is almost identical to the same compound termed DAPH 1 (diaromatic pentacyclic hydrocarbon) by Auras et al. (2006) (Figure 8-24C, D). Elution position and mass spectrum thus indicate that the BCF sediments contain DAPH 1, a 24,25-dinorarbora-13,5,7,9-pentaene (XIII) or the corresponding fernane-enantiomer (24,25-dinorferna-13,5,7,9-pentaene) that exhibits an identical mass spectrum and elution position.

The late eluting peak in the m/z 388 ion chromatogram (66) yields a mass spectrum that is almost identical to that reported by Auras et al. (2006) for a compound called DAPH 2 (iso-25-dinorarbora(ferna)-1,3,5,7,9-pentaene) (Figure 8-24E, F). This compound (XIV) has the same principal structure as DAPH 1, but an additional methylation at C-3 (Auras et al., 2006). The same relative elution position together with the similarity in the mass spectra indicates that DAPH 2 is also present in the BCF extracts.
Figure 8.22. Partial ion chromatograms A) m/z 402, B) m/z 388, C) m/z 374, D) m/z 392 of the aromatic fraction of BCF sample B03162 (GR7, 45.35 m), and E) comparison to chromatogram published in Auras et al. (2006) showing the elution positions and structures of aromatic fernane/arborane-derivatives (Auras et al., 2006) MAPH (XII), DAPH 1 (XIII) and DAPH 2 (XIV) and the hypothetical structure of DAPH 3 (XV).
The M* 392 compound in the BCF (68) yields a mass spectrum that is almost identical to that reported by Auras et al. (2006) for the C29 monoaromatic hydrocarbon MAPH (Figure 8-24A, B) a 25-norarboran(ferna)-5,7,9-triene. MAPH was isolated from a French Jurassic limestone and an Italian Triassic black shale and the structure was resolved by NMR (Hauke et al., 1992a). The same relative retention time, together with the similarity in the mass spectrum indicates that MAPH is also present in the BCF. The three BCF compounds from Figure 8-22 can thus be confidently interpreted as DAPH 1, 2 and MAPH.

A small peak also occurs in the m/z 402 partial ion chromatogram of sample B03162 in the expected elution position of a C30 DAPH (Figure 8-22A). It would thus seem that potentially a “DAPH 3” compound, the tri-methylated analogue of DAPH 2 may also be present. A good mass spectrum cannot be obtained due to the low concentration and co-elution problem, but the expected main fragments m/z 402, 235 and 245 are present in the background-subtracted mass spectrum (Figure 8-24G). The inferred DAPH 3 may correspond to the diaromatic counterpart of the tri-methylated tetraaromatic hopanoid IIIc. Artificial maturation experiments with arborane/fernane type compounds may establish if such a compound can indeed form from the potential precursors.
In addition to the pentacyclic arborane-type aromatisation products, most BCF samples also yield (relatively low) concentrations of one $M^+$ 310 and one $M^+$ 324 compound with
intense m/z 213 ions (Figure 8-25). This is consistent with C_{23} and C_{24} compounds with seven double bond equivalents as in a monoaromatic tetracyclic structure. The mass spectrum of 69 is very similar to that of a des-E arboritane-type monoaromatic (XVI) reported by Hauke et al. (1993) (Figure 8-25A, C). This compound was isolated from an Italian Triassic black shale and identified by MS and NMR spectroscopy (Hauke et al., 1993). This compound is interpreted to be derived from an arborane/fernane-type precursor (Borrego et al., 1997), although diagenetic formation from functionalised des-E hopane-precursors is also plausible (Hauke et al., 1993). It is difficult to obtain a clean mass spectrum of 70 due to the low concentration and co-elution problems, but the the similarity of the background subtracted mass spectrum (Figure 8-25B), to that of 69 (Figure 8-25C) suggests a similar structure with additional methylation. Since additional methylation at ring A shifts the base ion to m/z 227 as observed for compound 2 in Hauke et al. (1993), 70 probably exhibits additional methylation of the D-ring.
Many BCF aromatic fractions contain high abundances of compounds with molecular masses indicative of penta-aromatic hydrocarbons (Figure 8-26, Figure 8-27). Sample
B03063 has some of the highest relative concentrations of the compounds, with 75 being the highest peak in the high boiling point range of the aromatic fraction (Figure 8-26). Molecular masses reach from M⁺ 292 to at least 348, consistent with mono- to penta-methyl substitution. The dominance of the molecular ion in the mass spectra indicates that probably only methyl groups are attached to the aromatic core. The mass spectra of 72 to 74 are compared to that of the published 2,9-dimethyl picene (Chaffee and Fookes, 1988) in Figure 8-28. The similarity of the mass spectra suggests that the BCF compounds are also dimethylated picene derivatives. None of the BCF M⁺ 334 compounds yields an intense m/z 319 ion like the 2-isopropyl-9-methylpicene reported by Chaffee and Fookes (1988), arguing against an isopropyl-substitution. The BCF picenes thus seem to be mono- (71) to penta-methylated (77).

Due to their absence from any exterior fractions and laboratory blanks, but presence in most aromatic fractions eluted with relatively polar solvents, and their high concentrations in the BCF which contrasts with the very low (regular) sterane contamination signature, we consider the BCF picenes as probably indigenous, but syngeneity needs to be confirmed with additional interior-exterior experiments in the future (see chapter 7).

![Figure 8-26. High molecular weight compounds in BCF sample B03063 (GR7, 180 m). A) m/z 348 ion chromatograms, B) m/z 334, C) m/z 320, D) m/z 306, E) m/z 292, F) TIC.](image)
Figure 8-27. Mass spectra of peaks 71 to 77 in BCF sample B03063 (GR7, 180 m).
Figure 8-28. Mass spectra of M· 306 BCF compounds A) 74, B) 73, C) 72 compared to D) dimethylated picene (Chaffee and Fookes, 1988).
8.4 Conclusions

This study provided new insights into the triterpenoid inventory of the BCF. In addition to previously reported homohopanes and their 2- and 3-methylated counterparts, we detected 8,14-secohopanes. Identified for the first time in the Precambrian were dammaranes and their identity was confirmed by co-injection experiments with an authigenic standard.

Additionally, several unusual aromatic hopanoids were tentatively identified. Benzohopanes, the typical hopane aromatisation products are in low relative concentrations, but the BCF yields a number of aromatic 8,14-secohopanoids, including derivatives with fluorene- and acenaphthene-moiety. It was found that aromatic triterpenoids are important constituents of the aromatic fractions and are among the most abundant biomarkers in many samples. In contrast to the hopanes that show extended series up to C35, abundant tetra-aromatic triterpenoids are dominated by isopropyl or ethyl-substituted derivatives and thus appear no to be derived from homohopanes, but from C30 or C29 hopanes or C30 triterpenoids like isoarborinol. For the first time in the Precambrian, typical aromatic arborane/fernane-derivatives were also detected. Some samples further contain high concentrations of (poly)methylated picenes. The only pentacyclic triterpene with 5 six-rings known from the BCF is gammacerane, making tetrahymanol a plausible biogenic precursor of the picenes.

Potential biological precursor compounds and diagenetic schemes of the aromatic triterpenoids, and the ecological implications of the new discoveries are discussed in more detail in chapter 11.
8.5 References


9. Paleoproterozoic Protosteroids

9.1. Introduction: Sedimentary steroids and eukaryotic evolution

Eukaryotes, including animals, fungi, plants, algae, and a large variety of protists, are characterised by cell nuclei and a generally high degree of cellular complexity. Instead of hopanoids produced by many bacteria, most eukaryotes employ steroids as membrane lipids. Sterols have an important structural role in many eukaryotes (Pendse et al., 2011). They regulate rigidity, permeability and fluidity of cell membranes and are involved in the anchoring of membrane proteins and the formation of membrane rafts (Brocks et al., 2015). The geologic products of the \( C_{27}-C_{29} \) sterols, the saturated steranes cholestane, ergostane and stigmastane, sometimes called ‘sterane trifecta’, are the most common steranes in the Phanerozoic rock record. In contrast to cholestane, ergostane and stigmastane show a side chain alkylation at C-24, with a methyl- and ethyl-group attached respectively. Cholesterol (C\(_{27}\)) (XVIII in Figure 9-1) is one of the most important sterols in humans and other vertebrates. It circulates in the plasma and is a metabolic precursor of bile acids, hormones and fat-soluble vitamins (Pendse et al., 2011). Fungi mostly produce ergosterol (C\(_{28}\)) (XIX) and plants produce photosterols like sitosterol (C\(_{28}\)) (XX). C\(_{30}\) 24-\( n \)-propyl cholesterol steroids are produced by some pelagophyte marine algae and foraminifera (XXI). C\(_{30}\) sterols with 4-methylation, the 4,23,24-trimethyl cholesterol steroids that are also known as dinosteroids (XXII), are produced in significant concentrations by dinoflagellate algae. Steroids are almost exclusively produced by eukaryotes and can thus point to paleo-eukaryotic activity and may even indicate the paleo-activity of specific groups of eukaryotes, although a few bacteria can also synthesise sterols (see e.g. Volkman, 2005 and references therein, Wei et al., 2016). The molecular fossils of biological steroids, saturated steranes and aromatic steroids are thus the molecular complement of eukaryotic microfossils studied by palaeontologists, and can aid in reconstructing past eukaryotic ecologies.
Detailed reviews about sterols in microorganisms were conducted for example by John Volkmann who described the sterols in microorganisms (Volkman, 2003), their role as markers for marine and terrigenous organic matter (Volkman, 1986) and the source specificity of biosynthetic sterol and triterpenoid pathways (Volkman, 2005). The sterol numbering system and some important structures regarding BCF steroids and potential precursors are shown in Figure 9-2.

When deposited, biogenic sterols (e.g. XVIII to XXII and XXIX to XXXI) are converted by diagenetic and catagenetic processes to saturated steranes and aromatic steroids. This is illustrated for the C₂₉ 4,24-dimethylcholestene (XXIII), one of the 4-methyl sterols typical for dinoflagellates (Amo et al., 2010). Common geologic products are 4,24-dimethylcholestan (XXIV) and the C₂₈ 4,17,24-trimethyl triaromatic steroid (XXV). During aromatisation of the A-ring, the C-19 methyl group is usually lost, whereas the C-18 methyl group is transferred from C-13 to C-17. C-ring monoaromatic steroids are also common in geological samples. A-ring monoaromatic and A,B-diaromatic steroids are rare (Peters and Moldowan, 1993). The 4-demethylated 17, 24-dimethyl triaromatic steroid is a potential geologic product of ergosterol (XXVI), whereas the 17-monomethyl aromatic steroid (cholesteroid, XVII) is a typical product of cholesterol (XVIII). The triaromatic steroids in the BCF are dominated by 4-methyl cholesterol. These could be theoretically derived from 4-methylcholestanol, another one of the main dinoflagellate lipids (Amo et al., 2010), but also from 4-dimethylated sterols such as cycloartenol (XXIX), lanosterol (XXX) or cucurbitadienol (XXI).
9.1.1 Phanerozoic steroid record

The diagenetic and catagenetic products of biological steroids, the aromatic steroids and saturated steranes, are ubiquitous in thermally well preserved Phanerozoic sediments. The ratio of regular steranes to 17α-hopanes is often used as a proxy for the relative input of eukaryotes versus bacteria. Steranes are usually slightly more abundant than hopanes in Phanerozoic marine sediments (sterane/hopane ≥ 1) (Peters and Moldowan, 1993). In the Phanerozoic, sterane assemblages reflect a history of marine primary producers that, to a first approximation, parallels that inferred from the microfossil record and molecular clock estimates (Knoll, 2014). The relative abundance of C_{27}, C_{28} and C_{29} steranes was shown to vary through geologic time in accordance with the evolution and ecological dominance of certain (groups of) organisms. For example, in marine sediments C_{28} and C_{29} steranes are indicators for the presence of green algae and C_{27} steranes for red algae respectively. Therefore, the relative abundances of steranes allows the investigation of algal diversification and evolution (Schwark and Empt, 2006). Schwark et al. (2006) found that the C_{28}/C_{29} sterane ratio reflects changes in the algal assemblage during extinction events and evolutionary progress in the Lower
Carboniferous. They conclude that the C28/C29-sterane ratio complements the paleontological record of algal evolution by providing insights into the ecological history of non-fossilising green algae (Schwark and Empt, 2006). The C28/C29-sterane ratios compiled for the Phanerozoic by Grantham and Wakefield (1988) and Schwark et al. (2006) are shown in Figure 9-3. Another example of the imprint of algal evolution on the geological record comes from dinoflagellates that together with coccolithophores and diatoms are the most important marine primary producers today. The microfossils record reveals a limited number of dinocysts in later Triassic deposits, followed by a major radiation recorded in the Early Jurassic (Falkowski et al., 2004). This is reflected in varying dinosteroid abundance in Lower to Upper Triassic marine sediments and their ubiquity in Upper Triassic and younger marine strata (Fensome et al., 1996).

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Figure 9-3. Schematic illustration of ecological importance of different groups of organisms and C28/C29 sterane ratios of 500 rock samples (filled circles) averaged over stages and 400 oil samples (squares) analysed by (Grantham and Wakefield, 1988). Figure from Schwark et al. (2006).
9.1.2 The published Precambrian sterane record

Considering the evolution of the Phanerozoic sterane record as illustrated in Figure 9-3, and the major differences in the Phanerozoic versus pre-Ediacaran eukaryotic body fossil record, one would expect the Precambrian sterane record to be distinctively different from that of the Phanerozoic, potentially reflecting the evolution of algae, or at least changes in dominant primary producers similar to those of the Phanerozoic. In contrast, most reported Precambrian sterane distributions are remarkably similar to Phanerozoic (e.g. Jurassic) sterane assemblages. In fact, the sterane trifecta and dinosteranes have been reported from sediments predating the Great Oxidation event (e.g. Brocks et al., 1999). Together with apparent cyanobacterial hopanoids, the discovery of eukaryotic biomarkers that are produced in an oxygen-intensive pathway, was taken as strong evidence that oxygenic photosynthesis had evolved at least 2.7 billion years ago. The Archaean studies followed rigorous state of the art protocols at the time, but Brocks and colleagues later realised the allochthonous nature of the Archaean biomarkers (Rasmussen et al., 2008). Novel procedures for the recognition of trace contaminants were developed by the Paleobiogeochemistry Group at ANU (Brocks et al., 2008, Brocks, 2011, Jarrett et al., 2013, Schinteie and Brocks, 2014). These studies revealed that the Archean biomarkers such as steranes and hopanes are strongly enriched on rock surfaces, indicating an allochthonous origin. The recognition of Archaean, but also many other pre-Ediacaran, biomarker occurrences as later contamination was for a long time not accepted by the wider geochemical community. Most studies still employ conventional techniques or hybrid-techniques that provide poor-contamination control, like removing surfaces without quantifying biomarker concentrations, or solvent washing samples, which may facilitate contaminant infiltration to sample interiors. Therefore, conventional steranes continue to be frequently reported from diverse Precambrian settings.

To resolve the debate about the syngeneity of Archaean biomarkers, a very elaborate, cost and resources intensive contamination-free drilling project was led by the Agouron Institute. Using only water as lubricant and employing the strictest possible contamination controls during storage and handling, biomarker analyses by independent laboratories finally confirmed that biomarkers were indeed not preserved
in the Archaean sediments (French et al., 2015). The Agouron campaign also illustrated the effectiveness of the interior-exterior experiments developed by the Brocks group for syngeneity assessment. These findings appear now to be more widely accepted and other groups also routinely conduct interior-exterior experiments that confirm the absence of evolved steranes from most Pre-Ediacaran samples analysed to date (for example Flannery and George, 2014, Blumenberg et al., 2012).

9.1.3 Previous studies on BCF steroids

Most biomarker studies of BCF sediments describe the sterane trifecta of cholestane, ergostane and stigmastane (e.g. Summons et al., 1988). Of particular importance for the depositional environment are 24-\(n\)-propyl steroids (C\(_{30}\)) that constituted some of the strongest evidence for the marine nature of the BCF as they were interpreted as biomarkers for marine pelagophyte algae (Logan et al., 2001). Brocks et al. (2005) reported sterane concentrations to be mostly below detection limit in thermally well-preserved samples. Abundant were however triaromatic steroids of which generally >90% are methylated at C-4 (Brocks et al., 2005). At the time, C\(_{26}\), C\(_{27}\) and C\(_{28}\) triaromatic steroids were detected in the BCF.

Some biological steroids yield an additional methylation at position four. This is observed in lanosterol, cycloartenol (see Figure 9-4) and dinosterols (V in Figure 9-1). Dinosterols are produced by dinoflagellate algae, and their Proterozoic ancestors could have contributed 4-methylated steroids to the BCF sediments. However, dinosteranes were not detected by Brocks et al. (2005) and the aromatic steroids were instead attributed to methylotrophic bacteria. These are some of the few non-eukaryotic organisms capable of de-novo steroid synthesis and also produce 4-methylated steroids (Brocks et al., 2005). Astonishingly, the saturated counterparts of these aromatic hydrocarbons have never been detected. It has been argued that the sulphidic nature of BCF waters was suppressing eukaryotic activity (Anbar and Knoll, 2002), which could explain the paucity of steroids in the BCF (Brocks et al., 2005). In 2008, it was shown that conventional (trace) BCF steranes are allochthonous and previous reports of steranes (not the aromatic steroids) can be explained by contamination (Brocks et al., 2008).
9.1.4 The ‘cleaned up’ Precambrian sterane record

All pre-Ediacaran biomarker assemblages that were analysed following rigorous protocols did not yield evolved steroids (Pawlowska et al., 2013). Currently it is not possible to test all previous sterane-reports, but erring on the side of caution, a contaminant origin should be considered for all pre-Ediacaran sterane occurrences until the syngeneity can be proven by detailed interior-exterior experiments or ultra-clean drilling efforts. Thus, the emerging Precambrian sterane record is distinctively different than previously reported, and greatly differs from that of the Phanerozoic. Clearly indigenous pre-Ediacaran steroids are dominantly 4-methylated triaromatic steroids from the BCF (Brocks et al., 2005), Tonian cholestanes, 2- and 3- methyl cholestanes, and a new tentatively identified 26-methyl-cholestan (see Brocks et al., 2015). Currently, trifecta steranes, as well as 24-n-propyl and 24-isopropyl cholestanes first appear just below the Ediacaran boundary (Love et al., 2009) and low concentrations of syngenetic cholestan were detected in ~820 Ma sediments (Brocks et al., 2017). There is thus a ~820 Ma gap between the BCF triaromatic steroids and the first clearly indigenous steranes in the geologic record. Ediacaran sterane assemblages commonly show an outstanding stigastane predominance attributed to chlorophyte algae that may have been the major marine primary producers (Knoll et al., 2007).

The absence of syngenetic steranes from most pre-Ediacaran biomarker assemblages is surprising not only because it contrasts with dozens of reports in the scientific literature, which can be explained by contamination with Phanerozoic petroleum products, but also considering the occurrence of indigenous hopanes in most thermally well-preserved samples. Due to the strong structural similarity, hopanes should have a similar thermal stability as steranes, so that sterane-absence cannot simply be attributed to poor thermal preservation. One explanation for sterane absence but hopane presence is a taphonomic bias. For example, hopane producing bacteria may have thrived in most anoxic settings where biomarkers were preserved, whereas eukaryotes may have been restricted to oxic settings with poor biomarker preservation. Similarly, a mat-seal-effect may have hindered the incorporation of planktonic organic matter into the sedimentary record (Pawlowska et al., 2013), hampering the preservation of eukaryotic biomarker signatures.
Taphonomic rules that govern biomarker conservation differ from factors that determine body fossil preservation. Membrane lipids of soft organisms will be preserved almost as well as those of organisms with thick cell walls or skeletons. Biomarkers thus offer a more quantitative view of major taxonomic groups (Brocks et al., 2015). Biomarkers are also likely to reflect more than just the direct depositional environment and are often transported over significant distances. Thus, even deeper anoxic depositional settings are likely to receive some influx of shallow water material. For example, terrestrial biomarkers are often abundant in near-shore environments and can overwhelm those derived from marine sources even in marine basins like the Black Sea (Simoneit, 1977). Thus, even if Proterozoic eukaryotes where largely restricted to oxygenated shallow water settings, one would expect some contribution to the deeper water biomarker pool, as most preserved Proterozoic sediments are relatively proximal to ancient shore lines. Thus, eukaryotic biomarkers could be expected to be low relative to bacterial markers, but should not be (near) quantitatively absent as indicated by interior/exterior experiments where interior extracts are often completely devoid of steranes.

The idea that Precambrian sterane-paucity represents an ecological rather than a taphonomic signal is supported by the occurrence of certain steranes in some Ediacaran settings. These unique sterane assemblages suggests that pre-Ediacaran eukaryotic ecosystems where qualitatively and quantitatively still fundamentally different (Brocks et al., 2015). The latest research shows that the first crown eukaryotic biomarkers (cholestanes) emerge around 820 Ma ago, and that a distinctive shift occurred in sterane assemblages from the Neoproterozoic through the Ediacaran/Early Cambrian to the Phanerozoic with modern-like algae rising to ecological dominance ~645 Ma ago (Brocks et al., 2017).

9.1.5 The Bloch Hypothesis and sterol evolution

Konrad Bloch, a winner of the 1964 Nobel Prize in Physiology or Medicine, hypothesised that the sterol biosynthetic pathway parallels sterol evolution (Bloch, 1983). This idea became known as the Bloch Hypothesis. According to this hypothesis, natural selection over a long timescale led to stepwise improvements of sterol properties with regards to
membrane function, ultimately resulting in cholesterol as the optimised end-product (Bloch, 1994). The sterol synthesis in different eukaryotic groups is summarised in Figure 9-4. The acyclic \( \text{C}_{30} \) isoprenoid oxidosqualene is cyclised to either lanosterol or cycloartenol, the enigmatic bifurcation in sterol biosynthesis that has no apparent influence on the structure of the functional steroid at the end of the pathway (Nes, 2011)

From these most primitive ‘true steroids’ (Nes, 2011), all other steroids are biosynthesised by enzymatic modifications like oxidative demethylations and desaturations that result in the terminal sterol product. Most extant eukaryotes employ either the lanosterol (e.g. animals and fungi) or cycloartenol (e.g. plants) route of sterol synthesis, but with varying degrees of modifications of the initial cycloartenol or lanosterol products (see e.g. Summons et al., 2006). According to Bloch, the different sterol modifications would have subsequently evolved in eukaryotic evolution.

Evolutionary modification of the hydrocarbon skeletons may be recorded by hydrocarbon biomarkers in ancient sediments, so in theory the Bloch hypothesis may be tested in the rock record. Following Bloch’s hypothesis, early eukaryotes may only have produced the initial sterol products lanosterol or cycloartenol. Subsequent evolutionary events would then have led to a step-wise improvement of the steroids, and in theory this step-wise sterol-evolution should be recorded in the ancient rock record.
Figure 9-4. Generalised pathway of sterol biosynthesis (reproduced with small modifications from Summons et al. (2006)).
9.1.6 Eukaryotic body fossil evolution

The fossil evidence of early eukaryotes is highly controversial. A number of microfossils of unknown affinity, so called acritarchs, are interpreted by some authors as eukaryotic. The oldest potentially eukaryotic stem group fossils are large (30-300 μm) spheroidal vesicles from 3.2 Ga shales (Javaux et al., 2010). Mid Proterozoic rocks generally contain abundant fossils of probably eukaryotic origin of only modest diversity. Large vesicles in up to 1.8 Ga old rocks may well be eukaryotic, but mostly lack diagnostic features. A higher degree of diagnosticity is exhibited by microfossils in up to 1.6 Ga old shales that combine large size (>100 μm) with complex ultrastructure, complex ornamented or tessellated walls and variably formed surface processes (Knoll, 2014 and references therein). Important evidence for Mesoproterozoic eukaryotes comes from the Roper Group sediments that were deposited in the McArthur Basin ~1.4 Ga ago. The eukaryotic Roper Group microfossils show a varying species distribution among physical habitats with an onshore-offshore pattern of decreasing abundance, declining diversity and changing dominance. The most distinctive Roper Group fossils are _Tappania plana_ acritarchs with up to 160 μm wide irregularly spheroidal organic vesicles with bulbous protrusions and up to twenty hollow, cylindrical processes (Javaux et al., 2001).

In a paleontological sense, the last common ancestor of all extant eukaryotes (LECA) and all its descendants are considered as crown group eukaryotes, while older lineages are considered as stem groups. Stem group eukaryotes are compatible with the Paleo- to Mesoproterozoic eukaryotic microfossil record (e.g. Knoll, 2014). The first plausible evidence for crown group eukaryotes comes from _Bangiomorpha_ microfossils attributed to bangiacean red algae (Butterfield, 2000). This fossil indicates that crown group eukaryotes were likely present by ~1.1 Ga. However, while a late Mesoproterozoic age is most likely, the age of the host rocks is absolutely constrained by two dyke systems that bracket the fossil-bearing interval between ca. 723 and 1270 Ma (Butterfield, 2014). Thus, it is theoretically possible that this inferred red algal fossil is much younger than commonly assumed. Older proposed evidence for crown group eukaryotes is highly controversial like simple spheroids with unclear affinities (Knoll, 2014) from ca. 1.8 Ga old rocks that are interpreted by some authors as green algae (Moczydlowska et al., 2011). The first half of recorded eukaryotic history reflects only moderately increasing
fossil diversity until about 800 Ma ago. Pronounced diversification within major eukaryotic clades is indicated around this time by microfossils and molecular clock estimates. Unprecedented taxonomic richness is reflected in vegetative cells and resting cysts with complex morphologies and an increased diversity of coenocytic and simple multicellular eukaryotic populations. Cladophoralean green algae can also be confidently interpreted (Knoll, 2014 and references therein). Further, simple foraminifera and vase-shaped microfossils comparable to tests of modern testate amoebas that were completely unrecorded before ~800 Ma (Knoll, 2014) are abundant in mid-Neoproterozoic rocks form around the world (Porter and Knoll, 2000).

According to Knoll (2014), the geologic microfossil record represents a sliding scale of uncertainty from confident identification of protists in 1.4 to 1.6 Ga old rocks to more ambiguous evidence at 1.8 Ga to even more debated morphologies in older sediments (Knoll, 2014). The microfossil record thus indicates that eukaryotes evolved before BCF times but Paleoproterozoic microfossils cannot be confidently attributed to eukaryotic crown groups and the relatively low complexity would be consistent with stem group eukaryotes (e.g. Knoll, 2014).

9.1.7 Potential for BCF eukaryotes

The BCF hosts the oldest clearly syngenetic biomarkers and thus provides the only detailed molecular insights into Paleoproterozoic ecosystems. The body fossil record shows that eukaryotes evolved by BCF times, but the oldest microfossils that can be confidently attributed to eukaryotes are only marginally older (between 1.6 and 1.8 Ga) than the BCF. The BCF thus provides the unique opportunity to assess the relative importance of these early eukaryotes in marine ecosystems. The recognition of two endmember ecologies in this study provides insights into two fundamentally different ecosystems. One endmember ecology probably reflects ferruginous and the other as sulfidic ecosystems with shallower and deeper extents of the mixed layer. Alternatively, the endmembers could record phototrophic microbial mat versus planktonic, or shallow versus deep water ecosystems (see chapter 5). The preservation of abundant phototrophic biomarkers suggests that any significant eukaryotic community that inhabited the BCF waters should be recorded in at least one of the two distinct biomarker
signatures. The only indigenous steranes known to date are however the dominantly 4-methylated triaromatic steroids attributed to a potential bacterial source (Brocks et al., 2005). Does this mean that no eukaryotes inhabited the BCF waters? The absence of saturated counterparts of the BCF triaromatic steroids is one of the greatest puzzles of Precambrian biomarker research over the last decade and the subject of investigation in this chapter. A particular focus is on unusual steroidal structures that may have been overlooked in previous studies. The thermally exceptionally well preserved BCF sediments may be of particular importance in establishing if the absence of trifecta steranes is due to a taphonomic bias, an insignificant ecological role of eukaryotes, or if it may instead reflect limited sterol-synthesising capabilities of early eukaryotes.
9.2 Methodology and samples

The focus of this and the subsequent chapter is on thermally well preserved BCF samples from the upper ~300 m of drill core GR7 from the Glyde River region. Also analysed was strongly biodegraded bitumen from drill core MY4 of the Myrtle area (12Z083). At 103.3 m depth, the solid bitumen filling an open vug in the Coxco dolomite directly underlying the BCF (Figure 9-5) is sourced either from the BCF or even older formations. Regular biomarker analysis of drill core GR7 is supported by interior-exterior experiments on selected samples for syngeneity assessment (see chapters 2 and 6). For comparison, samples from cores GR5, GR11, MY4, MY5, McA5, LY1, BB5, WM6, LV09001 and HYC were also analysed but are not fundamental for this chapter.

In an attempt to produce some of the BCF steroids from biological precursors, artificial maturation experiments were conducted on lanosterol, cucurbitadienol and cycloartenol by heating in evacuated glass tubes with a clay, lime or in most experiments active carbon catalyst with subsequent hydrogenation under a stream of H2 gas. HCl gas was used for a propyl-ring cleavage experiment on cycloartane and the biodegraded oil. Commercially available cycloartane was purchased (CAS# 511-64-8 BOC Sciences, NY, USA) and analysed by GC-MS. Further details of the methodology can be found in chapter 2.

Figure 9-5. Bitumen-infill of vugs in the Coxco dolomite underlying the BCF may reflect one of the oldest liquid oil accumulations preserved on Earth. Although a BCF source is likely, the oil could potentially even stem from older formations. Top vug (black) was DCM extracted as sample 12Z083.
9.3 Results and discussion of new BCF sterane discoveries

9.3.1. Arcane BCF triterpanes with sterane characteristics

In 1988, Summons and colleagues reported that a “distinctive, but unidentified, doublet of peaks for a pentacyclic C₃₀ compound eluting between the C₂₇ and C₂₉ hopanes appeared in the M⁺412 → m/z 205 chromatograms of most samples from the BCF” (Peaks P, Figure 9-6). They further noted that it elutes much earlier than the C₃₀-methyl hopane commonly found in other sediments. The compounds were even visible in full scan GC-MS from several samples. Summons and colleagues reported ions at m/z 412 (M⁺ 70% RI), 397 (M⁺-CH₃, 35), 299 (66), 274 (100) and 205 (70) (Summons et al., 1988). In the current study, the unknown compound could be identified in a sample coming from the same depth in drill core GR7 as the original sample that was used by Summons et al. (1988). The comparison of Summons original data to the recent biomarker analysis is shown in Figure 9-6. Since the two samples come from the same depth and the double peak shows the same relative elution position and similar mass spectra, the compound can be positively identified as the one described by Summons et al. (1988). The mass spectra for the doublet of compounds P of Summons et al. (1988) as measured in the current study are shown in Figure 9-7.

Like reported by Summons and colleagues, we could also detect the unknown triterpenoids 81 and 82 in m/z 274 partial ion chromatograms of most BCF samples (see chapter 2 for analysed samples). They have a similar depth distribution as hopanes in GR7, pointing to a similar degree of thermal stability. For example, they are found in all thermally well-preserved samples from the upper BCF in drill core GR7, but like hopanes could not be discovered in samples from 683 and 870 m depth. The compounds were also detected in different drill cores from different parts of the McArthur Basin: GR5, GR7, MY4 and LV09001. The triterpanes were not found in cores BB5, GR11, MY5, WM6, McA5, and LY1, although only between one and four samples have been analysed per core, so the triterpanes could be preserved in other sections of the cores. Absence from these cores is generally attributed to a higher degree of thermal maturation as these cores have a higher thermal maturity than GR7 (Crick et al., 1988). Preservation of the unknown triterpanes is thus only expected for thermally well-preserved samples.
Figure 9-6. A) BCF triterpenoid MRM transitions reported by Summons et al. (1988). Partial ion chromatograms for a sample from the same depth in drill core GR7 (38.7 m, B03066) B) m/z 191, C) 205, D) m/z 412. Compounds as marked in Summons et al. (1988): A Ts, B Tm, P: doublet of unknown C30 triterpenoid, F): C29 αβ-hopane, H: C30 αβ-hopane corresponding to peaks 78 to 83 in our analyses.

Figure 9-7. Mass spectra for double peak 82, 83 of unknown triterpanes (compounds P1 and P2 in Summons et al. 1988).
9.3.1.1. Syngeneity of the triterpanes

82 and 83 are not detected in any exterior fraction when not present in the interior. They are absent from both the interior and exterior fraction from the McA5 sample (B03132), from HYC (B04016), LY1(B03200) and the two deeper GR7 samples (683 and 870 m depth), arguing against a contamination source. The unknown triterpanes have not been described from common oils and are only present in trace concentrations in the AGSO standard oil. They are thus very unlikely to be found in most common contaminants. Syngeneity is confirmed by a relatively even distribution across different slices in the GR7 slice extraction experiment as illustrated in Figure 9-8. The triterpanes are thus indigenous and a unique characteristic of thermally well preserved BCF samples where they are found across the basin.

![Graph](image)

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**Figure 9-8.** Syngeneity assessment of unknown BCF triterpanes 82, 83 in sample B03162 (GR7, 45.35 m). A) concentration of 82, 83 and C_{30} \alpha,\beta-hopane as measured in this study, and for the same
9.3.1.2. Structure elucidation

In drill core MY4, we discovered solid bitumen filling an open vug at 103.3 m depth in the Coxco dolomite directly underlying the BCF (Figure 9-5). This may be one of the oldest occurrences of reservoired oil in the world, sourced either from the BCF or even older strata in which case it would also contain the oldest biomarkers in the world. Figure 9-9 shows the total ion chromatogram (TIC) of the saturate fraction of the oil. Apart from the internal standard (IS) the chromatogram mainly consists of an unresolved complex mixture (UCM). The oil is strongly biodegraded. N-alkanes, pristane and phytane are degraded. There are only traces of hopanes.

The aromatic fraction is also dominated by a UCM with most cyclic biomarkers being below detection limit (see chapter 10). Triaromatic steroids are among the most biodegradation-resistant compounds known (Peters and Moldowan, 1993) and are abundant in most thermally well preserved BCF samples. The absence of these compounds from the aromatic fraction of the oil indicates that it may be placed in category 10, the highest ranking of the biodegradation scale of Peters and Moldowan (1993). Nevertheless, two chromatographic peaks, 82 and 83, with a molecular mass of 412 Dalton are well resolved (see also inset in Figure 9-9). They yield almost identical mass spectra (Figure 9-7) and correspond to the unknown triterpenoids P₁ and P₂ of Summons et al. (1988), offering the possibility for a more detailed characterisation of this arcane BCF biomarkers.

In theory, they could be products of biodegradation rather than selective enrichment, which would make them the by far oldest biodegradation biomarkers of probably Proterozoic age. The widespread occurrence of the unknown triterpanes throughout the BCF indicates however that they are enriched, and not produced, during biodegradation. Their enrichment in the severely biodegraded oil thus indicates an unusual structure that greatly increases the resistivity towards biodegradation. With an unprecedented enrichment in a sample that may be categorised in category 10 on Peter and Moldowan’s biodegradation scale, they are among the most biodegradation resistant biomarkers known to date.
Based on mass spectra and chromatographic behaviour, some first structural information can be inferred about 82 and 83. The molecular mass of 412 Dalton points to C₃₀ a compound with 5 double bond equivalents. That means it should contain 5 rings or double bonds. Non-aromatic double bonds are highly unstable and commonly do not survive in mature sediments over geologic times. Since the compound elutes in the saturate fraction, it should also not contain an aromatic ring. Thus, the unknown compound is probably a C₃₀ pentacyclic saturated hydrocarbon as suggested by Summons et al. (1988).

Due to the unusual enrichment of the two unknown isomers of the triterpene in the biodegraded oil, it is possible to obtain clean mass spectra even for low concentrated cleavage products. Figure 9-10 shows selected mass chromatograms of an inferred homologous series corresponding to the side-chain degradation products of the unknown compounds. 82 and 83 are the unknown C₃₀ compounds (m/z 412). 84 to 92 are
compounds corresponding to the subsequent loss of one CH₃ unit. Eight CH₃ units are lost at the end of the series at M⁺ 300 (92). Two members of the series, at m/z 384 and 314 are beneath detection limits (‘x’), pointing to branching positions. In total, the pseudo homologous series points to a 6-methylhept-2-yl side chain, the type of side chain also observed in cholestane. Moreover, all pseudohomologues from 82 to 89 show two isomers with nearly identical mass spectra, while 90 to 92 are single peaks. This likely indicates that the doublets are caused by a chiral centre in the side-chain marked with a star (*) in Figure 9-10.

Figure 9-11 shows the mass spectra of compounds 82 to 92. The arrows follow particular fragments, whereby constant mass throughout the pseudo-homologous series (red arrows) indicates that the fragment belongs to the left-hand side (part of polycyclic core without side-chain), whereas a shift of 14 mass units, or 28 where members are missing at branching positions, indicates that fragments belong to the right-hand side (side-chain-including fragment). The main side-chain-including fragments of the inferred parent compound 82 (M⁺ 412) are m/z 397, 288, 274, 259 and 205. In contrast, the m/z 299 fragment is constant in 82 to 91 and is represented by m/z 300 in the last member of the series (92). The gain of one mass unit in the last member indicates that no further methyl group is lost, i.e. substitution with hydrogen instead of alkyl-group. This confirms that the m/z 299 fragment corresponds to the polycyclic core, reflecting loss of the complete side chain.
Figure 9-10. Pseudo-homologous series of lower pseudo-homologs of 82 & 83. 84 to 92 inferred breakdown series formed through subsequent loss of fragments. In G) and B) x denotes missing members of the series. Structures inferred from this series and corresponding mass fragments are also shown, symbol (*) denotes inferred chiral centre.
Assuming that the series shown in Figure 9-11 represents saturated hydrocarbons, 82 must be a pentacyclic C₃₀ compound with a cholestane-like side chain (see Figure 9-10I). To our knowledge, the only biogenic carbon skeleton with a C₂₂ pentacyclic core and C₈ cholestane-type side chain is cycloartane XXXII (Figure 9-12C). A comparison of the spectra of 82 with the NIST database gave a good agreement (63% probability) with cycloartane (Figure 9-12A & B). 82 contains very similar mass fragments, although masses are slightly shifted and relative intensities vary.
To test whether the unknown compounds may be geological cycloartane isomers, commercially available cycloartane was purchased (CAS# 511-64-8 BOC Sciences, NY, USA) and analysed by GC-MS. As can be seen in Figure 9-13B, the commercial cycloartane comprises one main peak eluting about two minutes after the unknown BCF compounds, and a smaller peak eluting about 1 minute earlier. Both cycloartane isomers yield similar mass spectra to that of the NIST database. Commercial cycloartane was synthesised from cycloartenone and has the same stereochemistry as biological cycloartenol. If 82 and 83 are derived from cycloartenol, then they would have to be
different isomers. This would be expected, as all geological isomers of steranes in mature bitumens have a stereochemistry that differs from the biological configurations. Cycloartane has however never been observed in geologic samples and it is unclear if the highly strained propyl-ring can be preserved in mature sediments.

**Figure 9-13.** A) Unknown BCF compounds 82 and 83 versus B) commercially available cycloartane (MRM 412→274 transition).

An argument for the presence of the cyclopropyl structure in 82 is the abundance of m/z 288 (and m/z 274) corresponding to right hand-side C-ring cleavage fragments, whereas in lanostane XXXIII m/z 274 corresponds to a left hand-side fragment. This fragment is a characteristic feature of 9β-19-cyclosteroids and triterpanes. It is not observed in the mass spectra of related compounds without a cyclopropane ring (Zaikin and Mikaya, 1984). Zaikin et al. (1984) also observe that the mass number of this fragment is independent of ring-A and B substitutions giving evidence that the side chain is retained in the ion, as also seems to be the case in the breakdown series of the unknown triterpanes. The suggested fragmentation mechanism involves initial ionisation of the 9-10 bond that relieves the strain imposed on ring-B, leading to fission of the activated 5-6 bond followed by ‘McLafferty-type’ rearrangement of one of the activated C-11 hydrogens to the core fragment (Aplin and Hornby, 1966). In cycloartane, the resulting fragment has a mass of 288 Dalton. Subsequent loss of a methyl group results in m/z 273 fragments, while side chain loss leads to m/z 175 fragments. Boehme et al. (1997) also present key ions of cycloartane derivatives (alcohols and ketones) and inferred fragmentation patterns. Based on these patterns, the main fragmentation of cycloartane is shown in Figure 9-12C. Fragments resulting from the scission of the cyclopropyl ring
and show a mass of 288 and 273. The unknown triterpane 82 yields \( m/z \) 274 instead of a \( m/z \) 273 fragment, and the \( m/z \) 274 intensity is relatively higher compared to the NIST cycloartane. This might indicate that 82 has a different stereo configuration relating to the B-ring and cyclopropyl-ring, with a slightly different strain distribution favouring loss of an additional CH\(_2\) unit. The breakdown series of 82 also indicates that the \( m/z \) 274 and 288 fragments originate from cleavage through ring-B. Thus, the unknown compound shows a very similar fragmentation pattern, but the slight differences in mass spectral fragmentation may indicate that the 5\(^{th}\) double bond equivalent is at a different position than in cycloartane and the most important differences may be fragments \( m/z \) 123, 205, 218, 274 in compound 82 versus \( m/z \) 121, 203, 220, 273 in cycloartane (see Figure 9-12A & B) that may reflect a structural differences in ring-B or C. A lanostane skeleton with a bridge across ring-A seems theoretically possible but fails to explain the prominent ring-B cleavage products.

9.3.1.2.2. The nature of the 5th double bond equivalent

One explanation for a 5\(^{th}\) double bond equivalent in 82 and 83 would be a free double bond. The degradation series indicates that there is no double bond in the side chain. Instead, the unsaturation would be located in the ring system to lead to the prominent \( m/z \) 299 left hand side fragment. To test for a potential double bond, the biodegraded oil sample was subjected to prolonged hydrogenation. The 19h hydrogenation on Pt/C catalysts is sufficient to even hydrogenate many aromatic hydrocarbons. However, the M\(^+\) 412 double peak did not disappear, strongly arguing against the presence of a free double bond. The only possibility would then be a sterically hindered double bond within the ring system. The absence of even a sterically strongly hindered double bond was excluded through a RuO\(_4\) oxidation experiment conducted by Pierre Adam and Philippe Schaefer (Strasbourg University). In contrast to lanostene that was quantitatively converted under the same conditions, the unknown triterpanes did not react, strongly indicating the absence of a double bond. This means, that the unknown compounds are pentacyclic.
The cyclopropane ring in cycloartenol is a strained structure and it is unlikely that it could be preserved in thermally mature sediments. Still, the unknown compounds must an unusual structural feature that hinders biodegradation and allows for the cycloartane-like mass spectral fragmentation. The only known biogenic C₃₀ carbon skeletons with a cholestane-like C₈ side-chain are lanostane, dammarane, cucurbitane and cycloartane. Of these, cycloartane is the only one with 5 double bond equivalents (M⁺ 412), and also yields the only similar mass spectrum. Thus, a propyl-ring must be considered despite the predicted instability. To test for the presence of a propyl-ring, the biodegraded oil was subjected to strong heating, acidified PtO₂ hydrogenation and treatment with HCl gas.

Pyrolysis of cycloartenol resulted in quantitative ring cleavage, indicating that the strained cyclopropyl-ring has a very low thermal stability. To test if the unknown compounds 82 and 83 are affected under the same conditions, ~1/50 of the saturate extract of the biodegraded oil was heated to 300°C for 18h in an evacuated silica tube. However, 82 and 83 were not affected by elevated temperatures, arguing against the presence of a highly strained propyl-ring.

Figure 9-14 shows the comparison of the full scan mass chromatograms of untreated commercially available cycloartenol (Figure 9-14B) and cycloartenol subjected to 70 h PtO₂ hydrogenation upon acidification with 5 drops concentrated acetic acid (Figure 9-14A). According to the mass spectra that reveal a molecular mass of the product of 430 Dalton, cycloartenol was quantitatively converted to lanostanol. This shows that the propyl-ring is cleaved during acidic hydrogenation. In contrast to the cycloartenol experiment, when the experiment was repeated with an aliquot of the biodegraded oil, the M⁺ 412 double peak in the biodegraded oil did not change. The double peaks still yielded the same mass spectra, and no additional M⁺ 412 or 414 compounds were observed. This was even the case after another subsequent 4 h PtO₂ hydrogenation, again strongly arguing against the presence of a double bond or a cyclopropyl-ring.
A small-scale adaption of the HCl gas production method of Arnaiz (see chapter 2) was used to react HCl gas with cycloartenol, cycloartane and the biodegraded oil. The reaction of cycloartenol with HCl gas lead to the formation of two compounds reflected in a double peak. Based on mass spectra, the two compounds are probably lanostenone isomers with molecular ions of M+ 416. This was confirmed by hydrogenation, which resulted in almost quantitative conversion to lanostanone (and minor lanosterol). This experiment shows how easily and quantitatively the propyl ring is cleaved in the presence of HCl gas.

The experiment was repeated using commercially available cycloartane (Figure 9-15A), resulting in three peaks (Figure 9-14B). The three products a2, a3 & a4 possess M+ 412 fragments, but are strongly dominated by m/z 397 fragments (Figure 9-15B to D). Although other fragments are of much lower intensity, the compounds show the typical fragmentation pattern of lanostenes (Figure 9-15A). It thus seems that cycloartane was quantitatively converted into three different lanostane isomers, again confirming the strong effect of HCl on the propyl-ring. The mass spectrum of a2 is more similar to a1 then the other products, so it is possible that minor quantities of cycloartane still co-elute. The high intensity of the m/z 299 fragment in cycloartane (a1) and the very small intensity in a2 (and similarly m/z 288/299 ratios) strongly argue against cycloartane-coelution however. If there was any cycloartane left, it would have to be traces that cannot explain the elevated intensity of e.g. m/z 288 in a2 compared to a3 and a4. Instead, it is envisaged...
that the double bond is located in a different position in \( a_2 \), inducing a more cycloartane-like fragmentation through the \( B \)-ring. This shows that a \( B \)-ring cleavage similar to that envisaged for \( 82 \) and \( 83 \) can be induced not only by a propyl-ring, but also by other structural features added to a lanostane-type skeleton. Finally, an aliquot of the biodegraded oil was subjected to the same procedure, but no change was observed. The fact that the \( M^+ 412 \) compounds again did not react, is additional evidence that the unknown compounds do not contain a cyclopropyl-group.

**Figure 9-15.** Cycloartane HCl gas treatment experiment. A) commercial cycloartane, B) commercial cycloartane after HCl treatment.
Figure 9-16. A) Mass spectrum of lanost-8-ene from the NIST database, B) to D) mass spectra of peaks a4 to a2 from cycloartane HCl gas experiment, E) mass spectrum of commercial cycloartane.
9.3.1.2.4. Potential structure of unknown BCF triterpanes

9.3.1.2.4.1. Formation of a cyclobutane ring by cleavage of the cyclopropyl-ring

Since the presence of cyclopropyl-ring or double bond can be precluded for the M⁺ 412 compounds 82 and 83, they probably constitute unusual pentacyclic C₃₆ steranes with a carbon-bridge and a cholestanol-like C₈ side chain. One re-arrangement product of lanostane-isomers is known that contains an additional ring system across ring B. 11-oxolanostanol is photo-isomerised in high yield to form the 11α-hydroxy,11β,19-cyclobutane (Imhof et al., 1969) with a cyclobutyl-ring extending between C-10 and C-11 (Figure 9-17). However, a cyclo-butyl ring is not significantly more stable than a propyl-ring and entropically less favourable. It is thus unlikely to form diagenetically or biologically and probably would not have survived the thermal maturation of the BCF sediments.

![Figure 9-17. M⁺ 412 compound with cyclobutyl-ring (11α-hydroxy,11β,19-cyclobutane).](image)

9.3.1.2.4.2. Bridge formation

Diagenetic cleavage of the cyclopropyl-ring of a cycloartane-like precursor compound may lead to the formation of carbon bridges that form thermally more stable five or six rings. There are several theoretical possibilities to create such compounds with a 5ᵗʰ double bond equivalent in the form of a carbon-bridge from a known biogenic C₃₀ compound (Figure 9-18). The depicted structures are considered most likely since they are thermally stable and potential diagenetic products of known biological compounds. Compounds XXXIV and XXXV require a dammarane-type precursor like dammarene, XXXVI could form from a cucurbitane- or cycloartane-type precursor such as
cucurbitadienol or cycloartenol, while XXXVII and XXXIII could form from a
cycloartane- or lanostane-type precursor such as cycloartenol or lanosterol.

**Figure 9-18.** Different possibilities of M⁺412 bridge structures with 30 carbons and cholestane-like
side chain and plausible biogenic precursor compounds of 82 & 83: A) dammarenetype precursor,
B) cycloartenol-precursor, C) cycloartenol or lanosterol-precursor.

Hexacyclic oleanane derivatives (OI30/6) with methylene bridge between C-10 and C-14
were recently discovered in a number of oils, isolated and characterised by NMR (Nytoft
et al., 2016), confirming that there are, as yet enigmatic, pathways for bridge-formation
in triterpenoids. Like proposed for the protosteranes, the methylene bridge stabilised the
C-ring in the oleanane derivatives and the intense C-ring cleavage fragment m/z 191
typical for oleananes does not form in GC-MS analysis. In contrast to the protosteranes,
there is however also no prominent B-ring cleavage in the oleanane derivatives where
both C- and B-rings are bridged. This observation confirms the assumption that
prominent B-ring fragments in the protosteranes are due to stabilisation of the C-ring,
rather than a bridge in the B-ring. The very different fragmentation pattern strongly
indicates that the carbon-bridge of the protosteranes is in a different position.
There are two biological precursor compounds providing a likely mechanism for formation of a bridge. In cycloartane-type compounds, propyl-ring cleavage may result in the formation of a radical precursor that may proceed to form XXXVI or XXXVII. Similarly, functionalisation (or loss thereof) at C-11, like commonly encountered in alisols such as alisol B (XXXIX), could be responsible for the formation of a bridge between C-11 and C-14, resulting in XXXV. Formation of XXXIV and XXXVIII seems unlikely as we cannot envisage any plausible reaction mechanism. Compounds XXXVI and XXXVII also yield an additional quaternary carbon compared to XXXIV, XXXV and XXXVIII, which may increase the thermal stability of the bridge and diminish sites of attack during biodegradation.

The mass spectra of the 82 and 83 are similar to cycloartane, suggesting structural similarity. Some of the most significant mass spectral differences in the spectra of 82 and 83 versus cycloartane are a shift form m/z 121 (cycloartane) to 123, m/z 135 to 137 and m/z 203 to 205. The shift from m/z 123 to 121 indicates that there is no double bond equivalent across or attached to ring A. Most interesting is the shift from major m/z 288 and minor 273 in cycloartane to major m/z 274 and minor 274 in the protosteranes. Following the mass fragmentation of 9,19-cyclocompounds depicted in Boehme (1997) (see Figure 9-12C), the m/z 288 fragment in cycloartane results from simultaneous cleavage of the 10,19- and 6,7-bond in cycloartane and the m/z 273 fragment results form a subsequent methyl-loss of the right-hand fragment. M/z 288 and 274 fragments in protosteranes indicate that B-ring cleavage still occurs, but not with a similar methyl-loss, arguing against a bridge across the B-ring. Another major difference between the mass spectra of 82, 83 and cycloartane is the prominent m/z 205 fragment in the protosteranes. According to the pseudohomologous series, this should represent a right-hand fragment (Figure 9-
11) and the fragment mass is indicative of a C\textsubscript{15} unit with 3 double bond equivalents. Production of such a fragment from XXXIV is not plausible, arguing against this structure. Shift of the methyl group from C-13 to C-17 as would be required in bridge formation from cycloartane-type precursor in XXXVI, also occurs in the formation of C-ring aromatic steroids. It is envisaged that a bridge across the C-ring would stabilise this ring and favour B-ring cleavage, similar to cycloartane where this is favoured by the unstable propyl-ring. To a lesser extent, C ring-cleavage, accompanied by D and A,B-ring cleavage as in lanostanes can still occur. The combination of lanostane and cycloartane-type fragmentation thus results in the high number of both left- and right-hand side fragments. Figure 9-20 illustrated how such a fragmentation may look like for structure XXXVII illustrating that it is plausible to form all protosterane fragments from such a C-ring-bridged structure.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig920}
\caption{Proposed fragmentation of XXXVII. Red numbers: left-hand, blue numbers: right-hand cleavage indicated from homologous series. purple arrows: typical cycloartane-, orange arrows: typical lanostane fragmentation.}
\end{figure}
9.3.1.2.4.3. Potential ring-opening products

The saturated fraction of the biodegraded oil is strongly dominated by the protosteranes and their side-chain degradation products. In close proximity to the protosterane double peak the saturate fraction of the biodegraded oil yields an additional double peak (1 and 2) in \textit{m/z} 414, 301 (Figure 9-21A) and 191 chromatograms. As C\textsubscript{30} compounds with four rings, these are plausible ring-opening products of the protosteranes.

![Figure 9-21. Biodegraded Paleoproterozoic oil from MY4 (12Z083, 103.3 m). A) \textit{m/z} 301 partial ion chromatogram, B) total ion chromatogram.](image)

The mass spectra of 1 and 2 are shown in Fig. Figure 9-22. They are different from those of the 8,14-secohopanes, but similar (with minor differences) to the dammaranes and were introduced as potential new dammarane isomers in chapter 8. A comparison of their mass spectra to those of dammaranes was shown in Fig. 5 and their elution position relative to the dammaranes in Fig. 3 of chapter 8. Despite the close similarity of the mass spectra, 1 and 2 were not found in the authigenic dammarane standard. Some of the notable spectral differences compared to the dammaranes are a higher relative intensity of \textit{m/z} 123 and lower intensity of \textit{m/z} 137 and occurrence of a \textit{m/z} 207 in addition to the dammarane \textit{m/z} 205 fragment.
The close similarity to the dammarane mass spectra would be most easily explained if 1 and 2 would be configurational dammarane isomers. Protostanes (XLI) are dammarane stereoisomers with characteristic stereo structures in positions 8α-CH$_3$, 9β-H, 13α-H, and 14β-CH$_3$ (Figure 9-23). They are produced by certain plants (Alisma) in the form of highly functionalised alisols (XL), but formation of a protostane-cation is also an intermediate step the biosynthesis of (phyto)sterols, so that they are considered the ‘prototype’ of steroids (Zhao et al., 2013). It is thus possible, that 1 and 2 are protostanes, formed directly from a common protosterols precursor or as bridge-cleavage products of the protostananes. The regular BCF dammarane peaks are not recognisable in the biodegraded oil sample (Figure 9-21) and were likely also biodegraded. Thus, if peaks 1 and 2 were dammaranes/protostanes, then they likely formed after biodegradation as ring-opening products of the biodegradation resistant protostananes.
All mass spectral differences between dammaranes and 1 and 2 could be explained by a different relative stability of the C-ring. This can plausibly be explained by ring-rupture of XXXVII, with cleavage of the 8,14-bond, analogous to 8,14-secohopane derivatives that are abundant in the BCF, resulting in the former bridge becoming part of the new C-ring in a highly unusual di-spiro configuration (XLIV). The new structure of the C-ring would be expected to result in a different relative stability. Formation of C-ring cleavage fragment ions the both sides can explain the co-occurrence of m/z 205 and m/z 207 and, in association with the prominent side-chain loss, can also explain a higher relative abundance of m/z 123 fragments. All other mass fragments can also plausibly form from such proto-spirosteranes as shown in Figure 9-22.

In the biodegraded oil, there is indeed another, earlier eluting, peak (93) in the m/z 414 and m/z 301 chromatograms with slightly more than twice the concentration of the potential spiro-steranes (Figure 9-21). Most compounds that can be recognised in sample 12Z083 seem to be related to the protosteranes, so 93 may also represent a protosterane ring-opening product. The mass spectrum of 93 shown in Figure 9-24, is again similar
to that of dammaranes and potential proto-spirosteranes or protostanes, but lacks their characteristic \( m/z \) 191 C-ring-fragment, pointing towards a tetracyclic C\( _{30} \) triterpane with uncommon methylation. Notably, \( m/z \) 207 seems to be part of the spectrum and is indicative of a C\( _{15} \) two ring fragment which is difficult to account for in XLII and XLIII, the potential ring opening products of XXXV. Thus, either 93 is not related to the protosterane, or the protosterane does not have structure XLV. Since 93 occurs as a single and not a double peak as expected for a protosterane derivative, it may in fact not be related at all, but it is also possible that the expected R and S isomers co-elute under our chromatographic conditions, which would explain why 93 has a peak area slightly bigger than that of 1 and 2 combined. As shown in Figure 9-24, the mass spectrum appears to be consistent with ‘cucurlanorane’ XLV, that yields a methylation at C-9, C-10 and C-14, but not at C-13 and thus combines characteristics features of the cucurbitane-, lanostane- and dammarane-skeleton.

**Figure 9-24.** Mass spectrum of peak 93 in the biodegraded oil sample 12Z083 (MY4, 103.3 m).

1, 2 and 93 are consistent with ring opening products of XXXVII, while 1 and 2 could also be ring opening products of XLV. Cycloartenol is the only simple triterpenoid for which a plausible bridge-formation mechanism via the cyclopropane-ring cleavage can
be envisioned. 11-hydroxylation as found in plant triterpenoids with a protostane skeleton would also provide a potential bridge-formation mechanism, but there are no known microbial sources and such a functionalisation is highly unusual in nature.

9.3.1.3. Summary of new insights into unknown BCF triterpanes

Severely biodegraded oil sourced from the BCF or surrounding formations provided the unique opportunity to elucidate the structure of the unknown pentacyclic C₃₀ triterpenoids 82 and 83 originally detected in the BCF by Summons et al. (1988). The investigation of the mass spectra of their pseudo homologous series revealed a cholestane-side chain, leaving only compounds with the dammarane, lanostane, cucurbitane or cycloartane-skeleton as plausible biological precursors. A series of experiments established the absence of a double bond or propyl-ring, confirming the pentacyclic nature. Cycloartenol seems to be most likely biogenic precursor considering the similarity of the mass spectra of cycloartane and the unknown triterpanes, and the potential mechanism of bridge formation involving a radical intermediate created from cleavage of the propyl-ring. Potentially reflecting the oldest, most primitive steranes we propose to call 82 and 83 protosteranes.

Two types of tetracyclic compounds, 1, 2 with, and 93 without a m/z 191 fragment can also be recognised in the biodegraded oil and may well represent protosterane ring-opening products. 1 and 2 may reflect highly unusual 8,14-cleaved dispiro-steranes, the occurrence of which can be readily explained by microbially mediated 8,14-cleavage which is likely to explain the occurrence of a variety of 8,14-secotriterpenoids in the BCF. 1 and 2 may also reflect dammarane stereoisomers of the protostane-type however. 93 is either not related to the protostanes are should represent a novel ‘cucurlanorane’ with unprecedented 9,10,13-methylation. A protostane- or cycloartane-type precursor thus both seem plausible for the BCF protosteranes.

9.3.2. BCF steranes and artificial maturation experiments

9.3.2.1. Lanostanes

The discovery of the protosteranes in the BCF indicates that other primitive triterpenoids may thus far have evaded identification in the Barney Creek or other Proterozoic
formations. Such compounds may be overlooked in most biomarker analyses due to their unusual structures or rarity in Phanerozoic settings. This triterpenoid-evasion-hypothesis is supported by the recent discovery of a novel 26-methylcholestane (‘cryostane’) from Neoproterozoic sediments around the world (Brocks et al., 2015), demanding a search for other unusual structures. Considering hydrocarbons with similar structures as the protosteranes, the mass spectrum of cucurbitane is unknown, strongly hampering its detection in geological samples. Cycloartane was never discovered in the geological record, while dammarane and lanostane are only known from a handful of Phanerozoic settings and are not routinely looked-for. It was thus attempted in this study to search for these and other unusual steroids or triterpenoids in the BCF.

In this quest, a number of peaks with elution times slightly earlier than the C30 α,β-hopane were detected in the m/z 259 partial ion chromatograms of some BCF samples. In these samples, there are two peak doublets (94 to 97) and a number other peaks (Figure 9-25) in the MRM m/z 414 → 259 transition, suggesting C30 compounds. Although it is difficult to obtain clean mass spectra, a lanostane-like fragmentation with M+ 414 and prominent fragments of m/z 259, 190 and 274 is apparent for 94 to 97 (Figure 9-26). In 1989, Chen and colleauges described the first geologic record of lanostane. They confirmed the presence of lanostane in Eocene sample Y2 (2036 m) from the Biyang Basin, China, through a co-elution experiment with authigenic 8β(H),9α(H)-lanostane (95% purity) purchased from Chiron (Chen et al., 1989). An aliquot of the original sample Y2 was kindly provided by J.H. Chen (Geoscience Australia) to test for the presence of lanostane in the BCF. In the m/z 414 → 259 transition of BCF samples, 97 shows the same elution time as lanostane in Y2 (Figure 9-27A & B) and yields the same mass spectrum (Figure 9-28 ). A co-injection experiment (Figure 9-27C) confirmed that 8β(H),9α(H)-lanostane detected in Y2 by Chen et al. (1989) is also present in the BCF.
Figure 9-25. MRM m/z 414 → 259 transitions of A) BCF sample B03178 (199.08 m, GR7) with 81 = C30 α,β-hopane, B) products of cycloartenol 300°C, 1 h pyrolysis experiment with subsequent hydrogenation, C) products of experiment repeated with lanosterol.

Figure 9-26. Mass spectra of BCF peaks 94 to 97 in sample B03178 (GR7, 199.08 m). 87 has the same elution position as 8β(H),9α(H)-lanostane of Chen et al. (1989).
Figure 9-27. M/z 259 partial ion chromatograms of A) Eocene Biyang Basin 1/10 1/10 of saturates aliquot from Y2 (2036 m), 8β(H),9α(H)-lanostane confirmed by Chen et al. (1989) by co-elution with authigenic Chiron standard (see Fig. 3 in Chen et al., 1989); B) BCF B03178 (199.08 m, GR7, 1/10 of saturate fraction), C) co-elution experiment: black = Y2 (Chen), blue = Y2 + 1/10 B03178, red = Y2 + 3/10 B03178.

Figure 9-28. Mass spectrum of A) BCF compound 97 eluting at the same time as lanostane in Y2 (B03178, 199.08 m, GR7), B) 8β(H),9α(H)-lanostane confirmed in Eocene Biyang Basin sample Y2 (2036 m) by Chen et al. (1989) by co-elution with authigenic Chiron standard (see Fig. 3 in Chen et al., 1989).
9.3.2.2. Pyrolysis experiments

Chen and colleagues (1989) attribute lanostane to a lanosterol precursor (XXX), but theoretically cycloartenol (XXIV) is also a potential biological precursor. Cleavage of the propyl-ring in cycloartenol may result in the formation of lanostane- or cucurbitane-type products (see XXXI for cucurbitane-type structure). Commercially available cycloartenol, cucurbitadienol (XXXI) and lanosterol were subjected to pyrolysis experiments with active carbon, clay or lime as catalysts, followed by hydrogenation with H₂ gas. These experiments aim to simulate the geological alteration of these biomolecules to unfunctionalised hydrocarbons. Pyrolysates and hydrogenated pyrolysates were then analysed by GC-MS in full scan or multiple reaction monitoring mode and compared to BCF sediment extracts and reference oils. Due to time constraints, cucurbitadienol pyrolysates were not yet hydrogenated and only lanosterol pyrolysates of two experiments (300°C, 1 h and 330°C, 18 h, active carbon) were hydrogenated. The non-hydrogenated pyrolysates are nevertheless useful to test for the potential formation of protosteranes or aromatic steroids.

The artificial maturation of lanosterol and cycloartenol with an active carbon catalyst followed by hydrogenation under H₂ gas with PtO₂ catalyst yielded a white array of compounds with a molecular mass of 414 Dalton and a lanostane-type fragmentation. This is exemplified by the MRM m/z 414 → 259 transition of cycloartenol (Figure 9-25B) and lanosterol (Figure 9-25C) pyrolysis at 300°C and subsequent hydrogenation. These experiments revealed peaks in the same elution positions as BCF compounds 94-97 indicating that all of these BCF compounds are also produced in the pyrolysis. Like in the BCF, 97 (8β(H),9α(H)-lanostane) has the highest intensity of the four isomers, but the intensity of the other peaks varies. 2 which has a similar intensity as 8β(H),9α(H)-lanostane seems to be potentially produced in trace amounts in the cycloartenol experiment but is present in the lanosterol experiment, while a peak with the same elution position as 1 can be clearly distinguished in the cycloartenol experiment but it is unclear if it is present in the lanosterol pyrolysate. The variety of compounds with similar mass spectra in these experiments is exemplified in Figure 9-29. The pyrolysates are dominated by aromatic compounds (see chapter 10 and it is difficult to obtain clean mass spectra for the saturates. Comparison of the m/z 259 trace of a hydrogenated
cycloartenol pyrolysate (Figure 9-29B) with the TIC of the hydrogenated products of cycloartenol treatment with sulphuric acid reveals however, that the m/z 259 peaks (near) quantitatively dominate as cycloartenol ring opening products. The mass spectra (Figure 9-30) confirm that all the compounds yield mass spectra that are very similar to lanostane (Py7).

Figure 9-29. Comparison of A) main cycloartenol ring opening products in total ion chromatogram (H₂SO₄, hydrogenated) and B) main peaks in m/z 259 trace of cycloartenol pyrolysate (300°C, 1 h, PtO₂ hydrogenation).

The pyrolysis and cycloartenol-ring opening experiments show the wide variety of lanostane-isomers that can be produced from cycloartenol and lanosterol. Since the mass spectra are very similar and most compounds are produced from cycloartenol as well as lanosterol pyrolysis (compare Figure 9-25B & C), it is likely that the majority of compounds are configurational lanostane isomers. Compounds that are only produced from cycloartenol may potentially be cucurbitanes. However, the mass spectrum of cucurbitane is currently unknown and hydrogenation of cucurbitadienol pyrolysates could not yet be conducted due to time constrains. Once the mass spectrum and elution position of cucurbitanes are established, we can search for them in the BCF extracts.

Since at least 95 and 96 are also produced from lanosterol, it is unlikely that they are cucurbitanes and instead most likely constitute configurational lanostane isomers. One may speculate that 94 which has much higher yields in the cycloartenol compared to
lanosterol pyrolysate may potentially be cucurbitane, but further analyses are needed to establish the exact nature of this compound.

The pyrolysis experiments show that both lanosterol and cycloartenol are suitable biogenic precursors for the $8\beta$(H)$_2$9α(H)$_2$-lanostane (97) and the other BCF isomers (94 to 96) with a lanostane-type fragmentation. The occurrence of other potential cycloartenol-products in the BCF supports our interpretation of the protosteranes as possessing structure XXXVII being derived from propyl-ring opening and subsequent bridge formation from a cycloartenol precursor. Nevertheless, protosteranes were not yet detected in any of the pyrolysis experiments using a variety of experimental conditions. It is envisaged that particular conditions are needed for the radical-reaction to occur that are not adequately simulated in our pyrolysis experiments. Pyrolysis
experiments rather simulate catagenetic processes, while protosterane formation may be related to (potentially microbially mediated) early diagenetic processes. Protosteranes were also not produced from the biogenic compounds with related structures, lanosterol and cucurbitadienol, in our pyrolysis experiments, supporting the assumption that their formation requires specific (diagenetic) conditions.
9.4. Conclusions and outlook

It was established in this chapter that the unknown C₃₀ pentacyclic triterpanes that were found by Summons et al. (1988) to be characteristic for the BCF are probably novel pentacyclic steranes with a bridge across one of the six-rings. Most plausible is structure XXXVII that deviates from a lanostane-like skeleton by the methyl group at C-13 forming a bridge across ring-C to C-9, with bridge-formation being initiated from cyclopropyl cleavage in cycloartenol. This is supported by the occurrence of 8β(H),9α(H)-lanostane and at least three other related compounds that were detected for the first time in the BCF and that indicate the potential presence of cycloartenol in the BCF. In our experiments, 94 is mostly or only produced in cycloartenol pyrolysis, supporting a cycloartenol source of the BCF compound. 95 is mostly or only produced in lanosterol pyrolysis. It was however also shown in this study, how easily and quantitatively the propyl-ring is cleaved in cycloartenol and cycloartane, so that cycloartenol must be expected to be converted to lanosterol and related compounds during diagenesis. Thus, lanosterol-like signatures do not point against cycloartenol precursors, while cycloartenol-specific signals point against lanosterol precursors as cycloartenol cannot plausible form diagenetically from lanosterol-type precursors. Considering the variety of BCF compounds, and particularly the high concentrations of 94 compared to the other lanostane isomers and the occurrence of protosteranes 82 and 83 of a probably cycloartenol-source, it seems likely that cycloartenol contributed to the BCF sterane signature. This is consistent with the hypothesis about an ancestral cycloartenol route in sterol evolution (Desmond and Gribaldo, 2009, Ourisson and Nakatani, 1994, Bloch, 1991). It is also possible however, that lanosterol may have evolved first. The deep branching of sterol-bifurcation in the eukaryotic tree indicates that this event occurred very early in eukaryotic history and it is thus possible that cycloartenol as well as lanosterol-producing eukaryotes existed during BCF times.

The detection of clearly syngenetic (see also chapter 7) steranes in the BCF extends the known geologic records of steranes by more than 800 Million years doubling the likely eukaryotic molecular fossil record. Prior to this study the oldest clearly syngenetic steranes were cholestane and a novel 26-methylsterol from the 800-740 Ma Visingsoe Group of Sweden, the 780 Ma Kampa Formation from Australia, and the 740 Ma Chuar
group from the USA (Brocks et al., 2015) and most recently the ~820 Ma Johnnys Creek Formation of the Australian Bitter Springs Group (Brocks et al., 2017). The conspicuously low concentrations of steranes in pre-Ediacaran rocks was one of the greatest puzzles in Proterozoic geology. It is often attributed to low eukaryotic activity (e.g. Brocks et al., 2005). This may in turn be related to hydrogen sulphide poisoning and draw down of bio-essential trace metals in sulphidic waters (Anbar and Knoll, 2002). The dominance of ferruginous waters throughout the Proterozoic as revealed by more recent ion speciation studies (e.g. Poulton and Canfield, 2011, Planavsky et al., 2011) and the prevalence of such conditions in most of the BCF stratigraphy across the McArthur basin as revealed in this study suggest that water chemistry is unlikely to explain the restricted activity of eukaryotes however. Frequent fluctuations between ferruginous and euxinic conditions may provide one potential explanation for eukaryotic restriction, but most of the times an oxygenated upper layer was likely maintained where eukaryotes may have thrived even during intermittent euxinic periods.

Some palaeontologist argue for abundant eukaryotes throughout the Proterozoic and a mat-seal hypothesis was proposed to explain the paucity of steranes and other features of pre-Ediacaran biomarker assemblages (Pawlowska et al., 2013). One of the fundamental arguments is the paucity of steranes compared to bacterial hopanes that should have a similar stability. It was thus envisaged that microbial mats may provide an impermeable seal for planktonic eukaryotic biomarkers, while bacterial hopanes produced in the mat would be preserved. However, firstly eukaryotic communities inhabit most modern microbial mats, and secondly the detection of clearly indigenous steranes in the BCF strongly challenges this hypothesis. It appears that at least in the BCF, but possibly in other Proterozoic formations, steranes are not absent due to a taphonomic bias but instead they are different to the typical Phanerozoic steranes that organic geochemists usually look for. Thus, the BCF sterane assemblage was overlooked for two decades of biomarker research due to a contamination overprint of Phanerozoic steranes on the one hand, and when this was finally recognised (Brocks et al., 2008), the unusual structures of BCF steroids. While unusual C30 triterpanes where recognised in BCF extracts almost 20 years ago (Summons et al., 1988), their recognition as protosteranes was not possible until the discovery of the pseudo-homologous series in a
biodegraded oil in this study. Similarly, lanostanes have rarely been reported and are not routinely searched for, and could thus evaded recognition in previous biomarker studies.

The BCF steranes are likely the saturated equivalents of the unusual, dominantly 4-methylated triaromatic steroids, previously reported from the BCF. In general, steranes are always present when aromatic steroids are found, so the apparent absence of saturated equivalents of the triaromatic BCF steroids was one of the great mysteries in Precambrian biomarker research – and is now solved. The newly recognised BCF steranes also indicate that the aromatic steroids are derived from the same protosterols precursors as the steranes, namely lanosterol and/or cycloartenol.

The probable BCF biological sterane precursors are the first products in the formation of any sterols. The absence of any clearly indigenous evolved steranes from the BCF and apparently any geological formations older than ~820 Ma suggests that the BCF may record some of the earliest stages in sterol evolution. Biogenic precursors of the BCF steroids and triterpenoids and the implications for the BCF ecology and eukaryotic evolution are discussed in more detail in chapter 11. The combination of a cleaned-up Precambrian biomarker record and the new BCF sterane discoveries could fundamentally change our views of early eukaryotic evolution and the ecological role that our ancient ancestors played throughout Earth’s Middle Ages.
9.5. References


CRICK, I., BOREHAM, C., COOK, A. & POWELL, T. 1988. Petroleum geology and geochemistry of Middle Proterozoic McArthur Basin, northern Australia II:


10. **BCF aromatic steroids**

10.1. **Introduction**

Sterols, the typical biomarkers of eukaryotes, can be diagenetically and catagenetically converted into saturated and aromatic steroids. The most common aromatic steroids are mono- and triaromatic steroids, but diaromatic steroids can also form (see e.g. Peters and Moldowan, 1993). The BCF contains a very unusual steroid assemblage (Figure 10-1). Regular steranes were found to be of allochthonous origin in the BCF (Brocks et al., 2008).

Unlike any other formation, triaromatic steroids XXVII and XXVIII (Figure 10-1) were the only clearly indigenous steroids reported to date. Brocks et al. (2005) reported that more than 90% by concentration of the triaromatic steroids were methylated at C-4 (XXVIII) and none were alkylated at C-24 (Brocks et al., 2005). The saturated counterparts of the aromatic steroids could not previously be detected but were reported in this thesis (chapter 9). They comprise tentatively identified protosteranes, compounds with a cholestane-type side chain and probably a bridge across ring-C, 8β(H),9α(H)-lanostane (XXXIII, Figure 10-1) and unknown lanostane isomers. The unusual structural features and rarity in Phanerozoic sediments and oils explains why these compounds have previously not been recognised. All the steranes may be derived from cycloartenol (XXIV) or lanosterol (XXX) (see Fig. 6-2). Therefore, these compounds are also plausible precursors of the BCF triaromatic steroids (TAS) considered to be the aromatic derivatives of the protosteranes and lanostanes. The detection of previously unrecognised steranes in the BCF sediments demands a re-examination of the aromatic fractions for other as yet unrecognised sterol derivatives, which is the goal of this chapter. As the first sterol-products in the biosynthesis of all other sterols, cycloartenol and lanosterol are also referred to as protosterols (Summons et al., 2006).

We hypothesised that the BCF TAS may form dia- and/or catagenetically from protosterols or the structurally similar cucurbitadienol (XXXI). Therefore, artificial maturation experiments were conducted with these compounds. The pyrolysis products were compared to BCF extracts to see if any of the known BCF steroids were generated, or if additional steroids could be detected in the BCF.
Figure 10-1. BCF steroids. A) lanostane (XXXIII), B) triaromatic steroids (TAS) differing in core methylation: \textbf{XXVII} = C_{26} cholesterol, \textbf{XXVIII} = 4-methyl C_{27} cholesterol, \textbf{XLVI} = unknown C_{28} triaromatic steroid.
10.2. Samples and methodology

The focus of this chapter is on marginally mature BCF samples from the upper ~300 m of drill core GR7 from the Glyde River region. Also analysed was a strongly biodegraded bitumen from drill core MY4 of the Myrtle area. In MY4, we discovered solid bitumen filling an open vug in the Coxco dolomite directly underlying the BCF (see chapter 9).

Regular biomarker analysis of drill core GR7 is supported by interior-exterior experiments on selected samples for syngeneity assessment (chapter 7) that confirmed the syngeneity of all major steroid classes presented here. Further, in an attempt to produce some of the BCF steroids from biological precursors, artificial maturation experiments were conducted on lanosterol, cucurbitadienol and cycloartenol by heating in evacuated glass tubes with a clay, lime or in most experiments active carbon catalyst. Further details of the methodology can be found in chapter 2.
10.3. Results and Discussion

10.3.1. Comparison of different catalysts in pyrolysis experiments

Three different catalysts, lime, active carbon and clay were tested in artificial maturation experiments. All catalysts lead to very different reaction products. This is illustrated for lanosterol pyrolysis (330°C, 18h) in Figure 10-2. The reaction products were tentatively identified based on the mass spectral characteristics (see also section 10.3.2).

![Figure 10-2. Influence of different catalysts on lanosterol during pyrolysis (330°C, 18h): A) active carbon, B) clay, C) lime.](image)

10.3.1.1. Lime catalysis

All pyrolsates of lime catalysed experiments are dominated by high molecular weight compounds. For example, the lanosterol pyrolysate (Figure 10-2B) is dominated by more than ten different M+ 408 compounds, tentatively identified as C30 monoaromatic lanosteroids. Aromatisation of the B-ring of lanosterol would require the shift of only one (C-19) methyl-group. The most likely lanosterol aromatisation product would thus be a B-ring monoaromatic steroid where the C-19 methyl group is shifted from C-10 to C-1 (XLVII, Figure 10-2B). The high number of M+ 408 compounds indicates however 280
that other (positional) isomers are also formed. Lanosterol pyrolysis experiments with lime catalyst also resulted in a number of compounds with molecular masses of M* 428, 426 and 424. Due to their molecular weight and by comparison with the NIST database, these compounds are tentatively identified as lanostenols, lanostenones and lanostadienones respectively.

The dominance of C30 compounds indicates that lime has a very mild catalytic effect, leading to the preservation of most structural characteristics. The products indicate that methyl shifts are favoured over methyl-losses during lime-catalysed aromatisation.

10.3.1.2. Clay catalysis

Clays (Montmorillonite K10) act as harsh catalyst on sterols as can be seen in Figure 10-3. At 330°C (18 h), the sterols are almost quantitatively converted to alkylated naphthalenes and phenanthrenes, with no peaks in the high elution range. The lanosterol (Figure 10-3A) and cycloartenol (Figure 10-3B) pyrolysate chromatograms look very similar and are dominated by tri- and tetra-methylated naphthalenes. A series of alkylated phenanthrenes is also present, dominated by tri- and tetra-methylated compounds.

![Figure 10-3. Comparison of A) lanosterol and B) cycloartenol clay-catalysed pyrolysis (330°C, 18 h).](image)
10.3.1.3. **Active carbon catalysis**

The low-boiling range looks similar for different educts and is dominated by various alkylated naphthalenes. In the high boiling range, there are distinctive differences between cucurbitadienol and the other pyrolysates as shown in Figure 10-4 (A-C). Molecular masses of 376, 390 and 404 Dalton, are consistent with C_{28} to C_{30} diaromatic steroids. The high boiling range yields the same products only for the cycloartenol (Figure 10-4B) and lanosterol (Figure 10-4C) experiment. The products of the cucurbitadienol experiment have slightly shifted elution times (Figure 10-4A). In the lanosterol experiment, M\(^+\) 404, 390 and 376 compounds were produced that according to regular elution intervals should be pseudo-homologs (Figure 10-4E – F) and accordingly share the same skeleton with different degrees of alkylation.

![Figure 10-4. Comparison of pyrolysis results (330C, 18h, active carbon) for A) cucurbitadienol, B) cycloartenol and C) lanosterol (TIC), also showing the partial ion chromatograms in the lanosterol experiments of D) m/z 404, E) m/z 390 and F) m/z 376.](image)

A comparison of the diaromatic steroids produced during cycloartenol and cucurbitadienol pyrolysis is given in Figure 10-5. The mass spectra of the diaromatic steroids produced during cycloartenol and lanosterol pyrolysis are identical and all isomers in these experiments show very similar spectra. In contrast, the mass spectra of the diaromatic steroids produced during cucurbitadienol pyrolysis look very different.
Figure 10-5. Comparison of mass spectra of diaromatic steroids produced during cycloartenol and cucurbitadienol pyrolysis (330°C, 18h, carbon) (A – F) and potential structures and mass fragmentation of C₂₈ diaromatic steroids (G – I). G) XLVIII 4-methyl diaromatic lanosteroid H) XLIX favoured structural interpretation for C₂₈ lanosterol- and cycloartenol-derivatives and mass fragment from retro-Diels-Alder cleavage of the C-ring. I) L structure favoured for C₂₈ cucurbitadienol-derivatives.
The cucurbitadienol products are dominated by one mass fragment of 195 (C\textsubscript{28}), 209 (C\textsubscript{29}) and 223 (C\textsubscript{30}) Dalton respectively. These masses are consistent with fragmentation through the C- and D-ring in an A,B-aromatised compound, with the ion retaining the A- and B-ring. This is illustrated for the M\textsuperscript{+} 376 compounds in Figure 10-5 (G-I). Such a fragmentation was proposed by Jacob et al. (2007) for compounds with similar mass spectra, that were tentatively identified as diatomic lanosteroids. The formation of such compounds from cycloartenol and lanosterol strongly supports this interpretation.

The characteristic, but uncommon, m/z 195 fragment is consistent with a C\textsubscript{15} fragment containing three rings. In diatomic lanosteroids, such a fragmentation is however not possible. Instead, the aromatisation of the B-ring can initiate a retro-Diels-Alder reaction with the charge remaining with the diene portion (Budzikiewicz et al., 1963) as proposed for B-ring aromatic arborane-derivatives (Hauke et al., 1992a), and similarly for inferred diatomic lanosteroids (Jacob et al., 2007) that yield a C-D-ring cleavage.

It appears that the aromatisation of cucurbitadienol results in the formation of different isomers than that of cycloartenol and lanosterol. As lanosterol, cycloartenol and cucurbitadienol share a common stereochemistry, cucurbitadienol pyrolysates probably contain different positional isomers. The difference between the cucurbitane and lanostane-skeleton lies in the position of C-19 that is attached either to C-10 or C-9. During aromatisation of the B-ring, the methyl at C-10 in lanostane is expected to jump to C-1 (XLI\textsubscript{X}, Figure 10-5H), whereas the methyl at C-10 in cucurbitane is expected to jump to C-11 (L, Figure 10-5l), providing a plausible explanation for the occurrence of different isomers. The C-ring methylation in the cucurbitadienol fragments may change fragmentation behaviour at the C-ring, with the retro-Diels-Alder reaction becoming the dominant fragmentation mechanism in cucurbitadienol-derived diatomic steroids. It is surprising that the M\textsuperscript{+} 376 diatomic steroids seem to be methylated at C-1 and C-11 respectively, as one of the C-4 methyl groups is usually preserved during aromatisation of triterpenoids (see e.g. Laflamme and Hites, 1979, Wolff et al., 1989). Considering the presence of an unsaturation in the B-ring in lanosterol and cucurbitadienol, and the probably formation of a B-ring unsaturation upon propyl-ring cleavage in cycloartenol, as well as the fact that B-ring aromatisation only requires a single methyl-shift, it is envisaged that in the 13,14-methylated sterols, aromatisation starts in the B-ring (with an additional unsaturation being provided by dehydration at C-3) and proceeds to ring
A. If both C-4 methyl groups are lost during aromatisation of the C-ring, the C28 M+ 376 diaromatic steroids would form, while loss of one of the C-4 methyl groups would result in formation of the C28 M+ 390 diaromatic steroids. Shift of one of the C-4 methyl groups (probably to C-3) would result in the formation of C30 M+ 404 diaromatic steroids. It is noteworthy, that not only the main peaks shown in Figure 10-4 were produced in the cycloartenol pyrolysis experiment, but that at least 32 different C28 to C30 diaromatic steroids formed, many of them being C30 isomers (Appendix 10), that may potentially be found in sediments in the future. Due to time constraints, only a limited number of pyrolysis experiments could be conducted in this study and active carbon catalysis was favoured as it showed a catalytic effect intermediate to clay and lime, so there would not be a strong bias towards one type of reaction products and we thus hoped to produce the largest variety of potential BCF isomers. In the following, the aromatic steroid content of the BCF is investigated and compared to pyrolysis experiments.

10.3.2. BCF aromatic steroids and comparison to pyrolysis experiments

10.3.2.1. Triaromatic steroids (TAS)

The distribution of triaromatic steroids of a representative BCF sample (47.5 m, GR7) is illustrated in Figure 10-6. The high molecular weight (HMW) range of the TIC (bottom) is dominated by four peaks, two of which (101 & 102) correspond to C27 4-methyl triaromatic cholesteroids (XXVIII, Figure 9-2) (Brocks et al., 2005). Although visible in TIC of most aromatic fractions, the C26 (103 & 104, Figure 10-7; XXVII, Figure 9-2) and C28 TAS (98 -100, XLVI, Figure 10-1) are usually <10% by concentration of the 4-methyl cholesteroids (Brocks et al., 2005). Concentrations of the triaromatic steroids in the BCF have been determined as 60-130 ppm (Brocks et al., 2005).
10.3.2.1.1. **M/z 231 triaromatic cholesteroids (C26)**

Figure 10-7 shows the comparison of the m/z 231 traces of the BCF, the AGSO-II standard, the lanosterol and cycloartenol pyrolysis products. In contrast to the AGSO standard that is dominated by triaromatic ergo- and stigmasteroids yielding a methyl- and ethyl-substitution at C-24, the BCF only contains C26 triaromatic cholesteroids without alkylation at C-24 (103, 104; XXVII). C26 triaromatic cholesteroids (XXVII) were previously reported from the BCF, but are generally about an order of magnitude lower than the 4-methylated counterparts (XXVIII) (Brocks et al., 2005). The appearance of a similar doublet in the m/z 231 trace of lanosterol and cycloartenol maturation experiments (Figure 10-7) indicates that these compounds can be produced from 4-methylated C30 steroids upon thermal maturation.
10.3.2.1.2. M/z 245 4-methyl-triaromatic steriods (C₁₉ to C₂₆)

Similar to the distribution of the m/z 231 series, the m/z 245 partial ion chromatogram of the AGSO-II standard yields a variety of dimethylated triaromatic steroids with the second methyl group at C-2, C-3 and C-4, as well as 4-methylated dinosteroids (Brocks et al., 2015). The dinosteroids are not present in the BCF and the m/z 245 trace (Figure 10-8) is almost quantitatively dominated by the two 4-methylated triaromatic steroid isomers (XXVIII). This is a characteristic pattern of the BCF that has never been reported for any other formation (Brocks et al., 2005). Traces of 2- and 3-methyl TAS may also be present but are at least an order of magnitude lower and are therefore likely to represent re-arrangement products of 4-methyl steroids. The entire homologous series of 4-methylated cholesterol is present in the BCF as shown in Figure 10-8. While the molecular ions are very small, the expected masses are clearly present in each spectrum. Note that homologues with C₂- and C₇-loss are absent or in small concentration. This is
the typical pattern of a cholestane-like side chain. Homologues with more than an isopropyl side chain (M+ > 288) exhibit double peaks, as C20 becomes a chiral centre upon side chain extension. The presence of side chain degraded triaromatic products in the BCF may be the result of diagenetic, microbial or thermal degradation of side-chain intact triaromatic steroids.

Figure 10-8. Homologues series of 4-methylated triaromatic cholesteroids in BCF. M/z 245 selected ion chromatogram highlights the homologous series. 102 & 102 C27 (M+358), 105 & 106 C26 (M+344), 107 & 108 C25 (M+316), 109 & 110 C24 (M+302), 111 C22 (M+288), 112 C21 (M+274), 113 C20 (M+260). Inset: Mass spectra for each member of the series & inferred structures.

Thermal generation of 4-methyl triaromatic cholesteroids from C30 sterols could not be observed in my pyrolysis experiments. In the lanosterol pyrolysis experiment, two peaks are in the same elution positions in the m/z 245 trace as the 4-methyl TAS in the BCF, but mass spectral confirmation was not possible due to low concentrations. Based on the high yields of diaromatic steroids in some of my pyrolysis experiments, and the success of forming regular TAS over MAS in pyrolysis experiments with sulphur-addition (Abbott et al., 1984), I predicted that the 245-TAS would probably form under slightly
more aromatising conditions, like in the presence of elemental sulphur. The 245-TAS series was indeed produced by Julien Langley during lanosterol pyrolysis at 330°C for 18 h with 90% charcoal and 10% elemental sulphur (J. Brocks, personal communication 2017), so the prominent BCF TAS can indeed form cata- and/or diagenetically from protosterols.

10.3.2.1.3. M/z 259 triaromatic steroids (C_{28})

In addition to the m/z 231 and 245 series shown in Brocks et al. (2005), the BCF sediments analysed by Brocks and colleagues also contain a m/z 259 series with additional methylation in the ring system (Figure 10-9, XLVI). Figure 10-9B shows the typical triplet of C_{28} triaromatic steroids in the aromatic fraction of the BCF as separated on a DB5MS-GC column. Peak 99 is actually a doublet that is further separated on a DB1 column. To date, the structure of the C_{28} triaromatic steroids is unknown. In the current study, the triaromatic steroids were generated from biological precursor compounds (lanosterol and cycloartenol) in artificial maturation experiments (Figure 10-9). The lanosterol and cycloartenol maturation experiments (Figure 10-9) yield two compounds that have the same elution position as the two later eluting BCF compounds and traces of the earlier eluting BCF isomer may also have been produced (Figure 10-9C).

The mass spectra of 99 in the BCF and from peaks with the same elution positions in the lanosterol- and cycloartenol pyrolysates are shown in Figure 10-10. The spectra confirm that the same type of steroids that are present in the BCF extracts are also produced in the C_{30} sterol maturation experiments. Due to focusing of the charge on the aromatic rings, there are few characteristics fragments that could be used for structural inferences. The m/z 259 base ion is formed due to side chain loss. Additionally, the loss of two methyl groups from this fragment is also indicated by two ions of 244 and 229 Dalton. In analogy to the C_{27} triaromatic cholesteroids, it is envisaged that the methyl-group from C-13 jumped to C-17 in the aromatisation process and that a methylation is also present at C-4 (see inset in Figure 10-9).
Figure 10-9. Selected ion chromatogram of m/z 259 trace of A) lanosterol pyrolysis experiment (330°C, 18 h, active carbon compared to B) BCF (B03166, GR7, 67.14 m) and C) cycloartenol pyrolysis experiment (330°C, 18 h, carbon).

Figure 10-10. Comparison of mass spectra of A) lanosterol pyrolysate (330°C, 18 h, active carbon), B) BCF peak 99, C) cycloartenol lanosterol pyrolysate (330°C, 18 h, active carbon). Inset: inferred structure and main mass fragmentation. -Me indicates methyl-loss during electron impact.
The position of the third methyl-group is unknown, and based on a protosteroid-precursor, could conceivable be C-1, C-3 or C-15. A methyl shift during progressive aromatisation was also observed in pyrolysis experiments with monoaromatic cholesteroids (Abbott et al., 1984), making a C-1 methylation likely. Similarly, a shift from C-4 to C-3 occurs in the aromatisation of triterpenoids, resulting e.g. in the formation of DAPH 2 from arborane/fernane-type precursors (Auras et al., 2006). 98 to 100 may thus plausibly reflect two R and S isomers couplets of TAS yielding methylation at C-1 and C-3, in addition to C-17.

10.3.2.2. Diaromatic steroids (DAS)

10.3.2.2.1. C₂₈ diaromatic steroids of the m/z 376 series

The high boiling range of many BCF aromatic fractions is dominated by two other peaks (116 & 117) eluting between the two 4-methylated TAS isomers (101 & 102, Figure 10-11). 116 and 117 have similar mass spectra (117 shown in Figure 10-12A), and their molecular mass (M⁺ 376) is consistent with a C₂₈ diaromatic steroid. 116 and 117 elute at the same time as 376_1 and 376_2 produced during the artificial maturation of cycloartenol (Figure 10-11) and lanosterol (Figure 10-4) and the mass spectra are undistinguishable (Figure 10-12). As discussed in section 10.3.1.3, methylation at C-4, C-14 and C-15 can be expected for C₂₇ diaromatic steroids derived from lanosterol and cycloartenol because these methyl groups can be retained during aromatisation. The structure expected from aromatisation of a lanostane-type precursor that is shown in Figure 10-11 (XLVIII) is consistent with the observed mass fragmentation (see Jacob et al., 2007), Figure 10-12). Based on the differences in cucurbitadienol versus lanosterol and cycloartenol pyrolysis experiments, methylation at C-1 seems to be more likely for 116 and 117 (R and S isomers of XLIX), making 114 and 115 likely 4-methylated isomers (XLVIII). On the other hand, in the aromatisation of most triterpenoids, a C-4 methylation is retained (Wolff et al., 1989, Laflamme and Hites, 1979). Considering further the dominance of 4-methylated TAS in the BCF, it seems more likely that the dominant m/z 376 compounds are also 4-methylated.
In the BCF, there are more than 20 peaks in the \( m/z \) 376 selected ion chromatograms that could also represent diaromatic lanosteroid isomers. At least the six best distinguishable mass spectra are very similar, supporting the hypothesis of a diaromatic lanosteroid skeleton for most of these compounds. The additional peaks could correspond to rearrangement products encompassing additional methyl-shifts and possible isomerisation at C-13, C-14 and C-17, in addition to C-20 R and S isomerisation.

Diaromatic steroids have rarely been reported in the literature (Adam, 1991). Their scarcity is attributed to the slow pace of B-ring aromatisation, combined with the considerable speed of subsequent A-ring aromatisation (Yang et al., 2013), so that the B-ring is either not aromatised resulting in C-ring monoaromatics, or that the aromatisation reaction proceeds to form triaromatic steroids. One of the few publications on related diaromatic compounds is a series of unusual 14-methyl \( A + B \)-ring diaromatic steroids described by Lemoine et al. (1996) for a biodegraded seep oil from Pakistan. Lemonine et al. (1996) proposed that the methyl substituents at the \( C/D \)-ring junction hinder further aromatisation as observed previously for \( B \)-ring aromatised isoarborinol.

Figure 10-11. Elution range of diaromatic steroids of A) \( m/z \) 376 partial ion chromatogram of cycloartenol pyrolysis (330°C, 18h, active carbon), B) \( m/z \) 376 partial ion chromatograms of BCF sample B03166 (GR7, 67.14 m) and C) TIC of B03166. Inset: potential structure of C28 diaromatic lanosteroids.
and fernene-derivatives (Hauke et al., 1992b). Similarly, the methylation at C-13 and C-14 in the protosterols can be expected to hinder aromatisation, explaining the abundance of diaromatic steroids in the BCF extracts. Removal of the C-13 or C-14 methyl group by diagenetic processes may be required for aromatisation to proceed to the C-ring, which may explain why the BCF extracts contain similar concentrations of tri- and diaromatic steroids, whereas the pyrolysates are dominated by di- and monoaromatic steroids. Considering the near quantitative conversion of mono- to triaromatic steroids during thermal maturation (Peters et al., 2005), it is also possible that higher pyrolysis temperatures or longer experiment durations are needed for a higher yield of triaromatic steroids from C30 sterols, and elemental sulphur also increases the relative yield of TAS.

Figure 10-12. Mass spectra for M+376 peaks A) 117, B) 116 of BCF sample B03166 (GR7, 67.14 m), C) 376_2 in cycloartenol pyrolysis (330°C, 18h, activated carbon), D) 114 and E) 115 of sample B03166.
The aromatic fractions of most BCF samples also yielded a series of later eluting peaks that can be distinguished in TIC and have a molecular ion of 390 Dalton. The m/z 390 partial ion chromatograms of many BCF samples show four main peaks (119, 122 – 124) and a few smaller peaks (e.g. 188, 120, 121, Figure 10-13). Figure 10-14 compares the mass spectra of the three BCF peaks (122 – 124) for which a relatively clean mass spectrum could be obtained to peaks with a similar retention time in the cycloartenol pyrolysate (330C, 18h, active carbon). All spectra are very similar and show the same main mass fragments. This shows that the same kind of compounds that were produced during cycloartenol and lanosterol pyrolysis are present in the BCF, and that they all have a very similar structure. BCF peak 124 can be most confidently interpreted to represent the same compound as 390_2 in the pyrolysate.

The spectra are also very similar to those of the C28 DAS of the m/z 376 series, but the major mass fragments are 14 units heavier, with m/z 209 forming the dominant fragment, consistent with an additional methylation at the ring-system. The M+ 390 compounds are thus interpreted as C29 diaromatic lanosteroids with the possible structures shown in Figure 10-15. Since the compounds form from cycloartenol and lanosterol, methylation at C-4, C-13 and C-14 is expected, while the fourth methyl-group may be attached to C-1 or C-3 in the most abundant isomers. As typical for geological sterol-derivatives, the occurrence of 20-R and -S isomers can be expected. Further methyls-shifts and/or isomerisation at C-13, C-14 and C-17 can explain the occurrence of additional peaks. As shown in Figure 10-13, based on similar peak heights and relative distances, there could be three major 20-R and -S couplets in the BCF extract. Considering the original methylation of potential C30 precursor sterols, 123 and 124 may correspond to 1,3-methylated derivatives, while the closer elution position of the two other couplets may argue for structurally more similar 1,3- and 1,4-methylated derivatives. Of these, 1,4-methylated derivatives seem more likely to form from the precursor sterols and may thus be represented by the higher peaks 119 and 122, while 1,3-isomers may be represented by peaks 118 and 121. It is unclear, why the pyrolysate shows high concentrations of a different isomer (390_1). It seems unlikely, that the two highest peaks in the m/z 390 traces of pyrolysates are S and R isomers since the elution difference is
relatively large. As a methyl-shift from C-13 to C-17 often occurs during aromatisation of a steroid C-ring, it is possible that 390_1 represents such a re-arranged isomer as a likely intermediate in a diagenetic pathway leading to triaromatic steroids, although greater mass spectral differences than those between 390_1 and 390_2 that yield almost indistinguishable spectra may be expected. The pyrolysates also contain several M+ 390 peaks that are not present in the BCF, indicating that additional C29 diaromatic lanosteroid isomers can form by thermal maturation processes and may be found in geological samples in the future.

While the exact configurations of the different isomers cannot currently be determined, the pyrolysis experiments confirm that the M+ 390 BCF compounds are C20 diaromatic steroids that can form from cycloartenol and lanosterol precursors.

![Figure 10-13. M+ 390 partial ion chromatograms of A) BCF aromatic fraction (B03166, GR7, 67.14 m), versus artificial maturation of B) cycloartenol (330°C, 18h carbon). Potential 20-R and -S couplets are highlighted.](image-url)
Figure 10-14. Comparison of the mass spectra of BCF peaks 122, 123 & 124 (B03166, GR7, 67.14 m) versus those of cycloartenol pyrolysis (330°C, 18 h carbon) peaks 390_1, 390_3 & 390_4.
10.3.2.2.3. $C_{30}$ DAS of the m/z 404 series

Some of the cycloartenol and lanosterol pyrolysates (e.g. active carbon, 330°C, 18 h) yield high concentrations of $M^+$ 404 compounds dominated by two peaks 404.1 and 404.2 (Figure 10-16B). The mass fragmentation (Figure 10-16C) is similar to $C_{29}$ diaromatic lanosteroids, suggesting that these compounds are $C_{30}$ diaromatic lanosteroids. This assumption is supported by the elution positions, forming a pseudo-homologous series with the $C_{28}$ and $C_{29}$ diaromatic steroids (Figure 10-4) The hypothesised structures are similar to the $C_{29}$ counterparts (Figure 10-15) with additional methylation at the ring system. From a $C_{30}$ sterol precursor, 1,3,4,13,14-methylation is most plausible and one or both (as 20-R and -S isomers) of the two main peaks (404.1 and 404.2) are expected to reflect such a configuration.
Figure 10-16. Comparison of partial ion chromatograms of A) BCF sample B03179 (GR7, 218.1 m) and B) cycloartenol pyrolysate (330°C, 18h, active carbon), C) peak 404_3 in cycloartenol pyrolysis (m/z 277 is at least partly due to co-elution) and D) peak 126 in B03179.
The BCF samples lack peaks in the elution positions of 404.1 and 404.2 in the m/z 404 chromatograms. In a few samples such as B03179 (GR7, 218.1 m depth), there are instead three peaks in the m/z 404 trace that elute ~10 minutes earlier (Figure 10-16A). Although the pyrolysis extract is dominated by the two late eluting M+ 404 peaks with the typical lanostane-type DAS fragmentation (404.1 and 404.2), there are also a number of different isomers. One of these isomers (404.3) elutes at the same time as 126. Despite the low concentrations that result in low signal to noise ratios, the spectra are very similar (Figure 10-16C & D) indicating that these may be the same compounds. 125, 126 and 127 also show the typical DAS mass fragments of 207, 221 and 235 Dalton, indicating that there are likely three different C30 DAS isomers in this BCF sample. The large elution distance to the main M+ 404 pyrolysis products indicates that the BCF contains significantly altered isomers. It should be noted that some of the minor peaks in the m/z 376 chromatograms of the pyrolysates show a similar elution distance to the main M+ 376 pyrolysis products, supporting the formation of such altered isomers from C30 sterols. Methyl shifts and transalkylation reactions that take place during thermal maturation of aromatic hydrocarbons (see e.g. van Aarssen et al., 1999) provide suitable mechanisms for the formation of a variety of C27 to C30 aromatic steroid isomers from protosteroids precursors.

10.3.2.2.4. C27 DAS of the m/z 362 series

In some BCF samples, two M+ 362 peaks, 128 and 129, elute slightly before the two main C28 DAS peaks 116 and 117 (Figure 10-17). The molecular mass is consistent with C27 diaromatic steroids and the fragmentation pattern is very similar to that of the C28 DAS of the m/z 376 series (Figure 10-17). In contrast to the other DAS that show a continuous shift in the most abundant mass fragments from C30 to C28, the most abundant fragments of 128 and 129 are m/z 195, m/z 207 and m/z 221, the same as in the C28 DAS. Preservation of these ring-fragmentation fragments, particularly of the C19 C-ring cleavage fragment m/z 249, indicate preservation of the ring system (see fragmentation in Figure 10-5G) and accordingly, a tri-methylated diaromatic steroid structure like XLVIII, but with additional methyl-loss from the side chain rather than the ring-system. The most likely structures are shown in Figure 10-17B. 128 and 129 probably reflect either 20-R and -S
isomers of LXVI, or the two peaks represent two different side-chain cleavage products, one likely being an isomer of LXVI and the other of LXVI. Such compounds have not been observed in the pyrolysates.

The elution positions of 128 and 129 correspond to two clearly visible peaks in the TIC of some BCF samples (Figure 10-17). The peak intensity is probably too high for these compounds to represent thermal maturation products of 116 and 117 and they are more likely diagenetic degradation products these. Side-chain methyl-loss may have been facilitated by a free double bond in the side-chain, for example through oxic decarboxylation similar to arylisoprenoid degradation. Although speculative at this point, the relative concentrations of sterol side-chain degradation products like 128 and 129 could potentially record paleo-redox conditions similar to for example arylisoprenoid ratios. $C_{28}/C_{29}$ DAS ratios determined as $(128 + 129)$ from $m/z$ 362 divided by $(116 + 117)$ from $m/z$ 376 does not correlate with Pr/Ph, and there is no significant correlation with most BCF ecological proxies. However, in the upper 220 m of GR7 it does show some correlation with the arylisoprenoid ratio AIR-P ($R^2 = 0.53$, $n = 14$, $p < 0.05$), so the ratio may have some sensitivity towards post-depositional oxidation. A potential oxygen-control on side chain degraded versus intact steroids may be investigated in future studies.
Figure 10-17. A) m/z 362 partial ion chromatogram and B) TIC, mass spectra of peaks C) 116, D) 128, E) 129 in BCF sample B03166 (GR7, 67.14 m).
10.3.2.3. Monoaromatic steroids (MAS)

C₃₀ monoaromatic steroids were not detected in the BCF, but high concentrations of M⁺ 394 compounds with similar fragmentation patterns then C₂₉ MAS were observed in cycloartenol pyrolysis experiments (Appendix 10). They could potentially be preserved in thermally less mature formations and maybe be detected in future studies.

10.3.2.3.1. C₂₉ monaromatic steroids with m/z 379 base ion

Many BCF samples from the upper 300 m in GR7 contain at least two M⁺ 394 compounds (131, 133, Figure 10-18) that yield mass spectra consistent with expected fragmentation of C₂₉ monoaromatic steroids (MAS) (Figure 10-19). The fragmentation pattern is similar to that of diaromatic steroids (Figure 10-14), but all major fragments are shifted four units to higher masses, consistent with the presence of only one aromatic ring. The similarity of the fragmentation to those of the diaromatic steroids suggest aromatisation of the B-ring resulting in C- and D-ring cleavage analogous to the C₃₀ diaromatic steroids (Figure 10-15), whereas C-ring monoaromatic steroids are dominated by side-chain cleaved products (Peters et al., 2005). These compounds may thus constitute B-ring C₂₉ monoaromatic steroids.

Compounds with mass spectra (Figure 10-19C) undistinguishable from those of the BCF compounds 131 and 133 (Figure 10-19A, B) are cycloartenol and lanosterol derivatives that are present in the pyrolysates only after hydrogenation (Figure 10-18D), not in the untreated pyrolysates (Figure 10-18E). These compounds may thus represent hydrogenation products of C₃₀ diaromatic steroids. The compounds in the hydrogenated pyrolysates show however a very different elution pattern, indicating that different isomers are produced in the pyrolysis experiments than present in the BCF. There are also a few minor peaks in the pyrolysates that yield similar spectra, but it is currently unclear if any of these compounds are present in the BCF. It is further unclear why the pyrolysates yield such a different isomer distribution. Considering the occurrence in the hydrogenated pyrolysates, it is possible that BCF compounds represent intermediates in the aromatisation pathway of C₃₀ sterols towards di- and triaromatic steroids, whereas the experimental compounds represent hydrogenation products after thermal diaromatic steroid formation.
Figure 10-18. Partial ion chromatograms of A) m/z 225 hydrogenated (4h, Pt0) lanosterol pyrolysate (330°C, 18h, active carbon), B) m/z 225 BCF sample B03166 (GR7, 67.14 m), C) m/z 379 of B03166, D) m/z 379 of hydrogenated and E) of non-hydrogenated lanosterol pyrolysate.
The identity of the putative C$_{29}$ MAS B-ring aromatic lanosteroids (130 and 132) was confirmed by comparison with an authentic standard. B-ring aromatic lanosteroids were kindly provided by Pierre Adam (Universite Louis Pasteur, Strasbourg), who converted lanost-8(9)-ene through S$_8$ induced dehydrogenation and subsequent hydrogenation (H$_2$, PtO$_2$) of the C$_1$ and C$_{11}$ double bonds that formed upon S$_8$ treatment in addition to 304
the aromatisation of the B-ring (Pierre Adam, personal communication). Lanostene should have the same configuration as biological lanosterol (XXX), allowing to infer the stereo-configuration at C-13 and C-14 of the MAS. 133 clearly co-elutes with the main peak of the lanosteroid standard (379_3, Figure 10-20) and yields an identical mass spectrum (Figure 10-21), confirming the presence of the C29 B-ring monoaromatic lanosteroids in the BCF. The presence of the first eluting BCF monoaromatic steroid (131) cannot be unambiguously confirmed in the lanosteroid standard due to the low concentration, but in the standard a small peak (379_2) elutes at the same time as 131. It is inferred that 131 and 133 represent 20-R and S isomer of the B-ring monoaromatic lanosteroid with structure LVI. The only previous report of C29 MALS is from Oligocene evaporites in France in which putative lanostane was tentatively identified based on mass spectral interpretation (Adam, 1991).

![Image](image.png)

**Figure 10-20.** C29 B-ring MAS steroid co-injection experiment in m/z 379 partial ion chromatograms. Black: BCF (B03163, GR7, 47.55 m), red: BCF extract co-injected with steroid standard. Insets: BCF (bottom), standard (middle) and close-up of standard (top), and structure of MAS standard (LVI).
10.3.2.3.2. $C_{28}$ monoaromatic steroids with m/z 365 base ion

In addition to 131 and 133, some BCF samples also yield two $M^+$ 380 peaks (132, 134) eluting slightly earlier (Figure 10-18B). Comparison of the mass spectra reveals that these are monoaromatic lanosteroids after loss of an additional methyl group from the ring system. Methyl-loss from the core and not the side chain is indicated by a 14 Dalton decreases of the major mass fragments (e.g. LVII, Figure 10-19E). Elution position (Figure 10-18A, B) and mass spectra (Figure 10-19D, E) indicate that 132 is potentially produced during lanosterol pyrolysis.

10.3.2.4. Pentacyclic monoaromatic triterpenoids in a biodegraded oil

Aromatic steroids are highly resistant towards biodegradation and are strongly depleted only in the most severely biodegraded oils in category 10 of Peters and Moldowan’s 306
biodegradation scale (Peters et al., 2005). Although abundant in most thermally well preserved BCF samples, aromatic steroids were below detection limits in strongly biodegraded oil extracted from a vug in drill core MY4, where the saturated fraction is dominated by a doublet of arcane pentacyclic triterpanes with cholestane-like side-chain and an enormous unresolved complex mixture (UCM) as discussed in see chapter 9. The aromatic fraction of the oil also yields an enormous UCM and additionally contains only three major peaks with molecular ions M+ 406 (136), 364 (135) and 350 (134, Figure 10-22).

Thee molecular masses of these peaks are consistent with C30, C27 and C26 pentacyclic monoaromatic compounds. Figure 10-22 also shows the mass spectra and potential structures. All spectra indicate the loss of a C14H24 unit corresponding to a tricyclic fragment. This suggests that the three compounds form a pseudo-homologous series of pentacyclic monoaromatics. The C16H22 fragment (m/z 214) in the C30 compound should correspond to a bicyclic monoaromatic unit. The mass spectrum of 136 further indicates that a C3-unit, probably an isopropyl-chain, is lost from the monoaromatic fragment. If the propyl-side chain was instead attached to the non-aromatic ring, one would expect a much bigger propyl-loss fragment like for example displayed by 8,14-monoaromatic hopanoids (X, chapter 8). The mass difference between the M+ 406 and M+ 364 compounds suggest that the isopropyl-chain that is lost in the fragmentation of the C30 compound during electron impact is missing in the C27 compound. A pentacyclic triterpenoid structure with isopropyl- and a methyl-group attached to an aromatic ring is shown for a benzolanosteroids with aromatised side-chain in Figure 10-22D. While the structure could potentially allow for the observed mass fragmentation, on would expect a different cleavage upon electron impact (red arrow in Figure 10-22B) resulting in a much more stable cation. For the structures shown in Figure 10-22, the base ion should be a phenyl cation with the positive charge in α- and not β-position to the aromatic ring, strongly arguing for a different structure.
The strong enrichment in the severely biodegraded oil indicates that the novel aromatics may be among the most biodegradation resistant biomarkers discovered to date. The
enrichment in the biodegraded oil could be the result of an unusual resistivity towards biodegradation (selective enrichment), or the compounds may be products of the biodegradation process. Selective enrichment is corroborated by significant (visible in TIC) concentrations of the three compounds in other, clearly not biodegraded BCF samples (e.g. from 199.08 m and 218.1 m depth in GR7). The co-incidence of 134 to 136 enrichment together with enrichment of 82 and 83 in the saturate fractions can be explained if the aromatics were ring-opening products of the protosteranes, which were presumably enriched in the oil due to their unusual structural feature – a carbon bridge across the steroid ring-system. 134 to 136 may thus reflect compounds that formed upon ring-opening of protosteranes, during or after biodegradation. 8,14-seco-triterpenoids are abundant in the BCF (chapter 7). Such compounds are often enriched in biodegraded oils and may even be produced during biodegradation, thus 8,14-cleavage is a plausible ring-opening process for the formation of the aromatic compounds.

If the 8,14-bond is cleaved in a sterane with a bridge in ring-C, then the bridge would become part of a new C-ring as shown in potential diagenetic schemes for cycloartenol or dammarane/protostane-type precursors (Figure 10-23). The products of both schemes seem to be consistent with the mass spectra of 134 – 136. A cycloartenol precursor is favoured by the occurrence of potential saturated equivalents of LVIII to LX in the saturate fraction of the oil, and the plausible formation pathway via a carbocation-intermediate upon propyl-ring cleavage. In consideration of the saturated fraction of the biodegraded oil and the overall high abundance of plausible protosterols-derivatives in the BCF, peaks 134 – 136 are likely side-chain aromatised equivalents of protosteranes and likely share with these a common cycloartenol source. They are tentatively interpreted to possibly reflect side-chain aromatised proto-spirosteranes with structures LVIII to LX. Authigenic standards or elaborative isolation and structure elucidation by NMR analyses would be required to unambiguously identify these novel compounds.
Figure 10-23. Two potential diagenetic schemes and structures of compounds corresponding to BCF peaks 134, 135, 136. Scheme 1 is based on a cycloartenol-, and scheme 2 on a dammarane- or protostane-type precursor.
10.4. Conclusions and outlook

A variety of aromatic steroids were detected for the first time in the BCF. Most of these compounds have rarely or never been reported in the literature. The only other report of possible C\textsubscript{28} diaromatic lanosteroids encompasses extracts from thermally immature Halocene sediments from Lake Caco (Jacob et al., 2007). In this chapter, it was shown that these compounds can form from lanosterol and cycloartenol, confirming that they are diaromatic lanosteroid derivatives. For the first time detected in a geological sample were further C\textsubscript{27}, C\textsubscript{29} and potentially even C\textsubscript{30} diaromatic lanosteroid derivatives. The C\textsubscript{29} B-ring monoaromatic lanosteroids, unambiguously identified in the BCF by co-injection experiments with an authigenic standard, were previously only reported from Oligocene evaporites. C\textsubscript{28} monoaromatic lanosteroid derivatives were also tentatively identified and were likely also produced in the lanosterol and cycloartenol pyrolysis experiments. The current study thus extends the record of mono- and diaromatic lanosteroids by 1.6 Ga. It was also found that the C\textsubscript{26}, C\textsubscript{27} and C\textsubscript{28} TAS can be formed from lanosterol and cycloartenol precursors. All BCF triaromatic steroids may thus plausibly have a protosteroid source.

Severely biodegraded oil from a vug in the Coxco Dolomite underlying the BCF in MY4, yielded a series of unusual monoaromatic pentacyclic triterpenoids. These compounds were tentatively identified as side chain cyclised and aromatised monoaromatic lanosteroid derivatives, likely with a highly unusual di-spio configuration of the C-ring. Together with the protosteranes, the aromatic derivatives are likely among the most biodegradation resistant compounds discovered to date.

All aromatic steroids occurring in the BCF, including the inferred side-chain aromatisation products, can potentially derive from protosteroids through diagenetic and catagenetic processes. The artificial production of many of the BCF aromatic steroids from protosterols constrains the structures of these compounds reasonably well, but isolation from the complex bitumen is required to confirm the exact structures of most of these compounds by NMR. In the future, isotopic analyses are also expected to provide additional insights into plausible biogenic precursors and source organisms, particularly regarding methanotrophic bacteria.
Another aspect for more detailed future analyses relates to the artificial production of thermally stable, yet diagnostic, degradation products of protosteroids. Steroid degradation, catalysed by clay minerals, may be partly responsible for a large proportion of the (poly-)methylated naphthalenes and phenanthrenes abundant in many, particularly thermally mature, BCF sediments. A more detailed investigation of the low molecular weight pyrolysis products may eventually allow attributing some of them to potential steroid precursors. This could potentially provide a means of inferring the former presence of such compound even in thermally overmature sediments.

We are excited by the large variety and quantity of aromatic steroid that could be newly discovered in this study. A sound understanding of the sterol inventory of Proterozoic sediments may be crucial for elucidating the evolutionary advance and ecological roles of eukaryotes in Earth’s Middle Ages. A more detailed discussion of biogenic precursors, diagenetic schemes and ecological implications of the BCF (aromatic) steroids is provided in chapter 11.
10.5. References


11. Biogenic precursors, diagenetic pathways and ecological implications of BCF triterpenoids

11.1. Introduction

The BCF provides the only preserved molecular window into Paleoproterozoic ecosystems, providing unique insights into the state of evolution more than one and a half billion years ago. Due to their structural complexity, cyclic triterpenoids are among the most informative molecular fossils. The known BCF triterpenoid inventory that encompasses homohopanes, 2- and 3-methyl derivatives, and enigmatic triaromatic steroids was greatly expanded in this work. In addition to a variety of hopanoids, including 8,14-secohopanes and aromatic derivatives including those with fluorene and acenaphthene moieties, a variety of tri- and particularly tetra aromatic triterpenoids were detected that yield methyl-, ethyl- or isopropyl-substitutions at ring E. We further reported the first Precambrian arborane-or fernane-derivatives (MAPH and DAPHs) that probably share biogenic precursors such as isoarborinol with at least a proportion of the aromatic triterpenoids.

For the first time, the saturated counterparts of the BCF triaromatic steroids, which are mostly methylated at C-4, could be identified: C30 lanostanes and novel protosteranes with an unusual carbon bridge that may be derived from diagenetic reactions involving the opening of the propyl-ring in cycloartenol. Furthermore, the BCF was found to host a large variety of previously unrecognised mono- and di-aromatic steroids, as well as side-chain cyclised and aromatised derivatives that may all be derived from the protosterols cycloartenol and/or lanosterol. Also present in some samples were a variety of tentatively identified (poly-)methylated picenes that are probably derived from tetrahymanol or similar triterpenoids with five hexacyclic rings. The BCF further yields dammaranes that are rarely described in the literature.

In contrast to the wide variety of steroid derivatives with a probable protosterols source, regular steranes like cholestane, ergostane, stigmastane and dinosteranes were only found in trace concentrations and are elevated on exterior surfaces, a pattern indicative of later contamination. The BCF thus yields a unique triterpenoid distribution that allows important insights into the evolution of triterpenoids and their bacterial and
eukaryotic source organisms. This chapter explores how such a unique molecular signature could come about.
11.2. Methods and samples

This chapter largely summarises and discusses in more detail the biomarker observations from the triterpenoid and steroid chapters with a focus on biogenic precursors, diagenetic pathways, source organisms and ecological implications. For details of methods and samples refer to chapters 7, 8 and 9. Samples employed for triterpenoid quantitation are all from drill core GR7, from the following depths: 47.55, 67.14, 71.65, 82.95, 90.3, 106.28, 162.85, 199.08 and 218.1 m.

To improve GC-MS quantitation of compounds with vastly different fragmentation patterns, we developed ion response factors that correct for differences in fragmentation between different compounds upon electron impact. For this, background subtracted clean mass spectra were obtained and the base ion, employed for quantitation, was divided by the intensity of all ions in the spectrum to obtain an ion factor (IF). The same was done for the internal standard (IS) and the resulting IS-ion factor was divided by that of the compound of interest to obtain an ion response factor (IRF) relative to the internal standard. Concentrations were then calculated as usual dividing the area of the peak of interest by that of the internal standard (each in base ion trace) multiplied with the amount of IS, but results were then multiplied with the ion response factor. The IFs and corresponding IRFs calculated for selected compounds of each major BCF triterpenoid class are shown in Appendix 11. The different fragmentation behaviour of different compound classes can thus be accounted for, resulting in greatly improved concentration estimates for diverse compound classes. When fragmentation correction factors are applied, our concentration estimates should be comparable to those from co-elution-free TIC integrations. There still remain a number of uncertainties in concentration determinations, such as variations in ionisation efficiency, neutral mass fragments and small mass fragments (we only measured m/z ≥ 57). Therefore, absolute concentrations are still to be treated with care, but relative abundances of different compound classes are much more comparable in our analyses. To calculate abundances of different types of triterpenoids, in this chapter, 245-TAS concentrations are calculated from peaks 101 & 102 and 105 – 113 for side-chain degradation products, 259-TAS from 98 – 100, 231-TAS from 103 & 104, MAS 131 & 133, C28 DAS 114 & 115, C29 DAS 118 – 120. Arboranoids concentrations are calculated from 65 – 70, 8-14 secoaromatic hopanoids...
with fluorene moiety from 43 regular 8,14-seco aromatic hopanoids from 46 & 47, protosteranes 82 & 83, lanostanes 94 – 97, dammaranes 3 & 5, C27 – C35 hopanes from C27 to C35 α,β-hopanes. 309-TeATs (III) were principally calculated from peaks 20 – 26, 295-TeATs (II) from 14 – 19, and 281-TeATs (I) from 10 -13, but a few non-labelled peaks from these mass traces were also used (see Appendix 11). Appendix 11 shows peak labels, retention times and concentrations of all relevant peaks, as well as summed concentrations employed for the figures in this chapter.
11.3. Results and discussion - the BCF triterpenoid inventory and plausible biogenic precursors

In this study, we could show that the BCF yields a large variety of triterpenoid derivatives. Nevertheless, we recognised a limited number of basic triterpenoid motifs from which all the BCF triterpenoids could be derived via dia- and catagenetic processes. Among the BCF triterpenoid motifs are those of lanostane, regular and 2- and 3-methylated hopanes, arborane or fernane, dammarane and gammacerane. Functionalised biogenic derivatives of these few basic structures may have been converted by geological processes into the diverse triterpenoid derivatives detected in the BCF. For a variety of BCF steroid derivatives, we could show with artificial maturation experiments that they can form from cycloartenol and lanosterol through catagenetic processes. It can be envisaged that similar dia- and catagenetic processes allow the formation of the other BCF triterpenoids from only around a dozen biogenic precursor molecules. The wide variation in mass spectral fragmentation of the BCF triterpenoids hampers accurate quantifications. It is thus difficult to compare the (relative) concentrations of different BCF triterpenoid derivatives. This prompted us to introduce mass fragmentation factors for improved quantification measurements that correct for variations in fragmentation behaviour. We first consider triterpenoid abundances, before discussing the most plausible biogenic precursors and diagenetic pathways leading to the BCF triterpenoids.

11.3.1. Triterpenoid quantification

The sterane/hopane ratio is often used as a rough indicator of eukaryotic versus bacterial contributions. For this ratio, C27, C28 and C29 ααα (20S + R) steranes are commonly integrated in (M⁺ → 217) transitions or m/z 217 traces. Similarly, C29 to C33 17α-hopane pseudo-homologs are integrated in M⁺ → 191 transitions or m/z 191 traces. Generally, high sterane concentrations with sterane/hopane ≥1 are typical for marine organic matter with major contributions from planktonic and/or benthic algae. On the other hand, low sterane/hopane ratios are more indicative of terrigenous and/or microbially strongly reworked material. This results in very low (close to zero) sterane/hopane ratios in many
non-marine oils (Peters et al., 2005 and references therein). A compilation of 482 crude oils from Upper Jurassic source rocks showed that there is a latitude effect with low latitude Jurassic source rocks often yielding sterane/hopane ratios between ~0.2 and 0.5, whereas high latitude samples can have ratios close to 1 (Andrusevich et al., 2000). This relationship suggests relatively more bacterial input in equatorial carbonates (Peters et al., 2005 and references therein).

Since there are no regular syngenetic steranes in the BCF, regular hopane/sterane ratios cannot be constructed. One may aim however, to construct protosteroid to hopanoid ratios in order to obtain some information about the relative ecological importance of hopanoid and steroid producing microorganisms. Finding a meaningful ratio that can be compared to the Phanerozoic record is complicated however. The BCF contains unusually high abundances of aromatic derivatives, and the tetraaromatic triterpenoids (TeATs) could stem from biogenic fernane/arborane-type as well as ≤C₃₀ hopanoid precursors. Further, the mass fragmentation patterns vary greatly between different types of triterpenoids, particularly for the aromatic derivatives, causing large errors in compound quantification. For example, C₂₈ diaromatic steroid peaks (116 and 117) are usually slightly higher than the C₂₇ triaromatic steroid peaks (101 and 102) in total ion chromatograms of BCF samples (Figure 11-1). Yet, as shown in Figure 11-2, if concentrations are calculated using the base ions m/z 245 for the TAS and m/z 376 for the DAS, the TAS are on average (n = 9, <220 m GR7) 4.5 times more abundant than DAS. Similar effects can be seen between other aromatic hydrocarbons or when saturated triterpanes with complex fragmentation patterns are compared to the TAS, whose mass spectra are strongly dominated by side-chain cleavage-fragments. Low concentrations of some compounds and co-elution problems preclude simply using GC-MS total ion chromatograms or GC-FID chromatograms for quantifications.
Figure 11-1. Relatively abundance of aromatic steroids relative to triterpenoids illustrated in full scan chromatograms of three different aromatic BCF extracts from drill core GR-7: A) B03163 (47.55 m), B) B03166 (67.14 m), C) B03167 (71.65 m). The steroids are dominated by 4-methylated triaromatic cholestanoids (101 & 102) and C28 diaromatic lanosteroids (116 & 117). The dominant triterpenoids are isopropyl-substituted dimethylated TeATS (19) and ethyl-substituted monomethylated TeAT (11).

Figure 11-2. Concentrations of A) TAS and B) DAS as determined from TIC (back) and base ion (front) peak integrations.
11.3.2. Comparison of total triterpenoid concentrations

As can be seen in Figure 11-1, steroid concentrations in the aromatic fractions are very high and in the same order of magnitude as the aromatic triterpenoid derivatives. Figure 11-3 and Figure 11-4 illustrate that, in most BCF samples, the saturated triterpane concentrations are rather insignificant compared to the aromatic derivatives. On average (n = 9, <220m, GR7), the triterpanes (C_{27}-C_{29} hopanes, dammaranes, protosteranes) contribute ~9% to the total triterpenoid pool. Only in a single sample (199.08 m, GR7) do hopanes (20%) show a similar relative abundance as aromatic steroids (41%) and tetraaromatic triterpenoids (27%). In all other samples, triterpenoids are strongly dominated by tetraaromatic triterpenoids and aromatic steroids (both on average ~41% of total triterpenoids).

Our concentration estimates show that aromatic steroids and TeATs are generally the most abundant triterpenoid classes. We only identified the side chain-degradation series of the m/z 245 TAS series, so total triterpenoids concentrations would be even higher if side-chain degradation products of all aromatic steroids were considered. The ratio of protosteranes/(C_{27}-C_{30}) hopanes varies from 0.02 to 0.53 with an average of 0.2. This value is similar to Phanerozoic sterane/hopane ratios and is within the range of tropical marine sediments (Andrusevich et al., 2000).

The ratios of total steroids/total hopanoids, including C_{28} and C_{30} aromatic secohopanoids, C_{30} secohopanoids with fluorene-moiety and TeATs, ranges from 0.4 to 1.9 with an average of 1.0. Total steroid to hopanoid ratios are thus even higher than the saturated protosterane to hopane ratios, pointing towards a significant steroid contribution that is in the same order of magnitude as in Phanerozoic marine sediments. As discussed, the most abundant BCF triterpenoid class, the TeATs, are not necessarily derived from hopanoids, but are likely at least partially derived from arborane/fernane-type precursors. Without the TeATs, the total steroid/total hopanoid ratio would be much higher still, ranging from 1.8 to 8 with an average of 4.2. Aromatic steroids are not only high compared to hopanes and other triterpenoids, but also compared to other hydrocarbons, as they form prominent peaks in full scan chromatograms of many aromatic fractions (Figure 11-1) and are sometimes among the highest peaks in the entire
aromatic fraction (e.g. at 67.14 m in GR7). All triterpenoid concentrations are tabulated in Appendix 11.1.

**Figure 11-3.** Relative concentrations of major triterpenoid classes in BCF samples from GR7. From the inside to the outside: 47.55, 67.14, 71.65, 82.95, 90.3, 106.28, 162.85, 199.08 and 218.1 m depth. Appendix 11 shows peaks included for each compound class. FluoreneAH: C32, 8,14-secohopanes with fluorene moiety (43), SecoAH: C29 & C30 monoaromatic 8,14-secohopanoids (46 & 47).

**Figure 11-4.** Average concentrations (in % of total triterpenoids and ng/g of rock) of major BCF triterpenoid classes in the upper 220 m of GR7 (n = 9, samples & details as in Figure 11-3).

In conclusion, in the BCF, sterols derivatives have a similar or higher abundance than hopanol derivatives, and sterane/hopane ratios are comparable to those of Phanerozoic
marine sediments. In inference, sterol producing organisms must have played a significant ecological role.

11.3.3. Comparison of TeAT concentrations

Figure 11-5 shows the concentrations of total TeATs, monomethyl-, dimethyl- and trimethylated TeATs for nine samples from drill core GR7. On average, the TeATs are dominated (46%) by derivatives with demethylation at the aromatic ring system. Within the dimethyl-TeATs, those with E-ring isopropyl-substitution are dominating (54%). In contrast, monomethyl-TeATs are strongly dominated by E-ring ethyl-substituted derivatives (81%). Trimethylated TeATs in turn are dominated by E-ring methyl-substituted derivatives (54%). TeAT distributions for different sample depths are shown in Figure 11-6. TeAT distributions are broadly similar for most samples. Somewhat elevated concentrations in isopropyl-substituted TeAT are observed in the shallowest sample (47.6 m: 49.9% for I-III), two of the samples towards the top of the ‘green endmember excursion’ (chapter 5; 71.7 m: 53.7% and 83 m: 41.9 % for I-III) and in the two deepest quantified samples (199.1 m: 73.4% and 218.1 m: 68.5% for I-III). There could thus be some link to the depositional redox conditions, but there is no significant correlation with the ecological proxies, and isopropyl-dominance is not a characteristic feature of the green ecological endmember (chapter 5). Elevated concentrations in the slightly deeper samples may also indicate a potential maturity influence. For example, intact isopropyl-substituted TeATs (maybe due to C-30-functionalisation) may have been incorporated into kerogen during early diagenesis and may therefore be preferentially released towards the main phase of petroleum generation.
It is unclear why different TeATs with different methylation at the aromatic ring system show different E-ring substitutions. The most plausible explanation would be different biogenic or diagenetic precursor compounds for the different TeATs. For example, monomethyl-TeATs may be largely derived from 28,30-bisnorhopane precursors potentially produced by bacteria dwelling at or below the chemocline as proposed by Sinninghe Damsté et al. (2014). Monomethyl E-ring-ethyl-substituted tetra-aromatics are the end products in the proposed aromatisation pathway of 28,30-dinorhop-(13,18)-ene (Sinninghe Damsté et al., 2014), potentially explaining the dominance of E-ring ethyl-substitutions (Ib) in the core-monomethylated TeAT fraction (I). The majority of BCF TeATs, however, show isopropyl substitution (average 47.5%, n = 9) and must thus be derived from isopropyl-substituted biogenic precursors such as C30 hopanoids, isoarborinol, adriaticol or eudoraenol.
Figure 11-6. Comparison of side-chain (a = methyl, b = ethyl, c = isopropyl) patterns in mono- (I), di- (II) and tri- (III) core-methylated tetra-aromatic triterpenoids (TeaTs). From the inside to the outside: 47.55, 67.14, 71.65, 82.95, 90.3, 106.28, 162.85, 199.08 and 218.1 m (GR7).
11.3.4. Comparison of aromatic steroid concentrations

Figure 11-7 shows the concentrations of aromatic steroids. Concentrations of DAS are slightly higher than those of TAS and both are much higher than MAS. For the abundant m/z 245 TAS, side-chain degradation products can also be quantified as the entire homologous series is visible in m/z 245 chromatograms. Side chain degradation products are very significant even in the thermally well-preserved samples (GR7 < 200 m) and on average (n = 9) yield similar concentrations (273.4 ng/g) as the sum of all intact TAS. The TAS are almost 90% methylated at C4, while cholesterol without methyl group at ring A occur only in trace concentrations (Figure 11-8).

![Figure 11-7](image)

**Figure 11-7.** Average concentration (ng/g of rock) of aromatic steroids with intact side-chains of 9 BCF samples (see Appendix 11, samples as in Figure 11-6).

![Figure 11-8](image)

**Figure 11-8.** Average concentration (ng/g of rock) of main isomers of C26, C27 and C28 TAS cholesterol of 9 BCF samples (see Appendix 11, samples as in Figure 11-6).
11.3.5. Biogenic precursors and diagenetic products of BCF triterpenoids

To account for the diverse BCF triterpenoid derivatives with cheilanthane, gammacerane, fernane or arborane, protosterane, lanostane, dammarane and different hopane structures, there must have been a variety of biogenic precursor compounds. In the following, we explore plausible biogenic precursors and source organisms.

11.3.5.1. Cheilanthanes

The BCF yields a homologous series of cheilanthanes extending from C19 to at least C25 and probably C28. Cheilanthanes are likely degradation products of higher homologues of tricyclic terpanes with isoprenoid side-chain. Due to the widespread occurrence of tricyclic terpanes and the molecular properties of tricyclohexaprenol, it was proposed that tricyclohexaprenol (Figure 11-9) is the parent compound for cheilanthanes with 30 or fewer carbon atoms (Ourisson et al., 1982). Diagenetic and/or catagenetic processes can saturate the biogenic precursors and cleave the tricyclohexaprenol side-chain, resulting in shorter homologues (Figure 11-9). Sediment extracts and oils are often dominated by degraded homologues, with C23 typically being the most abundant. Oils and bitumens from carbonate-rich source rocks usually have lower concentrations of >C26 terpanes. Cheilanthane concentrations are often high in Tasmanite source rocks, so these algae have been proposed as potential source organisms. Other algal or bacterial sources are also possible, but the source organisms remain unknown (see Peters et al., 2005 and references therein).

![Diagram of tricyclohexaprenol and cheilanthanes](image)

**Figure 11-9.** Potential diagenetic pathway from tricyclohexaprenol to (≤ C26) cheilanthanes (R = isoprenoid side-chain).
11.3.5.2. **Gammacerane and picenes**

Low concentrations of gammacerane are discernible in $m/z$ 191 chromatograms of many BCF extracts. Gammacerane typically forms via reduction of tetrahymanol (see Figure 11-10). Gammacerane can form through dehydration of tetrahymanol to gammacer-2-ene followed by hydrogenation, or by sulphurisation of tetrahymanol and subsequent sulphur-removal (see Peters et al., 2005 and references therein).

Other BCF biomarkers that may be derived diagenetically and/or catagenetically from tetrahymanol are (methylated) picenes. Methylated picenes may theoretically form from nucleation and transalkylation reactions of different polyaromatic hydrocarbons. However, a greater number of positional isomers than observed in the BCF extracts would be expected if this was the main formation mechanism. Methylated triterpenoids are usually interpreted to form through the aromatisation of biogenic triterpenoids, which is also the most plausible explanation for BCF picenes. Methylated picenes have been postulated to form from oleanane-type precursors, with aromatisation being initiated by loss of the hydroxyl-group in ring A (e.g. Chaffee and Fookes, 1988). While oleanane was not detected in the BCF, the typical gammacerane precursor tetrahymanol differs from oleanane only in the methylation positions in ring D, and we therefore expect it to also form methylated picenes upon diagenetic aromatisation. Aromatisation and removal of methyl-groups by dia- and or catagenetic processes could result in picenes with various degrees of methylation (Figure 11-10).

![Inferred transformation of tetrahymanol to gammacerane and methylated picenes.](image-url)
Tetrahymanol is a lipid that replaces steroids in phylogenetically diverged eukaryotes living under oxygen-poor conditions such as many ciliates, some Excavata like *Andalucia incarcerate* and even a Polychaeta animal, and is also produced by some bacteria such as *Rhodopseudomonas palustris* (Takishita et al., 2012). In sediments, tetrahymanol derivatives are mostly attributed to marine or freshwater ciliates such as *Tetrahymena* in which it was first identified. The tetrahymanol-producing bacterivorous ciliates live at the oxic-anoxic interface in stratified water columns, explaining the utility of gammacerane as a stratification-marker (Sinninghe Damsté et al., 1995). Ciliates are important ecological players of modern aquatic communities and may be the dominant group of eukaryotic heterotrophic plankton in temperate coastal waters, consuming 30-50% of the primary production (Pierce, 1992). Many ciliates also produce hopan-3β-ol in similar quantities as tetrahymanol (Harvey and McManus, 1991). In addition to ciliates, tetrahymanol was also identified in the anaerobic rumen fungus *Piromonas communis* (Kemp et al., 1984), the purple non-sulphur anoxygenic phototrophic bacterium *Rhodopseudomonas palustris* (Kleemann et al., 1990, Rashby et al., 2007), as a trace constituent of the lipidome from the fern *Oleandra wallichii* (Zander et al., 1969), and the nitrogen-fixing bacterium *Bradyrhizobium japonicum* (Bravo et al., 2001). Takishita et al. (2012) showed that genes encoding tetrahymanol synthesising squalene-tetrahymanol cyclases (STC - all enzyme abbreviations are summarised in Table 11-1) occur in several phylogenetically diverged eukaryotes living in oxygen-poor environments. These data suggest that the genes were laterally transferred among these eukaryotes and that tetrahymanol functions as a sterol surrogate, playing an important role in phagocytosis under oxygen-poor conditions. In addition to ciliates, the anaerobic/microaerophilic free living protists *S. marylandensis*, *A. incarcerate* and *T. pyriformi*, which belong to the eukaryotic ‘super-group’ Excavata, also produce tetrahymanol. STC genes were also detected in *A. pompejana*, a polychaete worm found only at deep-sea hydrothermal vents under often hypoxic/anoxic conditions. A putative original donor of the eukaryotic STC gene could not be identified, but the STC of the phylogenetically diverse eukaryotes form a monophyletic group and seem to branch deep within the bacterial squalene-hopene cyclase (SHC). The monophyletic grouping of eukaryotic STC suggests that the genes were either inherited from a common ancestor and lost in numerous eukaryotic lineages or, far more likely, acquired through
horizontal gene transfer (HGT), possibly from an original bacterial donor (Takishita et al., 2012).

**Table 11-1.** Abbreviations of triterpenoid synthase enzymes used in this chapter.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHC</td>
<td>Squalene-hopene cyclase</td>
</tr>
<tr>
<td>OSC</td>
<td>Oxidosqualene cyclase</td>
</tr>
<tr>
<td>EUS</td>
<td>Eudoraenol synthase</td>
</tr>
<tr>
<td>STC</td>
<td>Squalene tetrahymanol cyclase</td>
</tr>
<tr>
<td>LAS</td>
<td>Lanosterol synthase</td>
</tr>
<tr>
<td>Ths</td>
<td>Tetrahymanol synthase</td>
</tr>
</tbody>
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In 2015, it was shown by Banta et al. that the genetic make-up for tetrahymanol-synthesis is much more wide-spread in bacteria than previously known, with aerobic methanotrophs, nitrite-oxidisers, sulphate reducers and a subset of aquatic and terrestrial metagenomes yielding tetrahymanol synthesis genes. However, these bacteria employ a newly discovered tetrahymanol synthase (Ths) that is not encoded in any eukaryotic genome, nor is it homologous to the eukaryotic STC. Bacteria couple the cyclisation of squalene to a hopane-intermediate produced by SHC, with a subsequent Ths-dependent ring expansion to form tetrahymanol. Bacteria and eukaryotes thus seem to have evolved distinct biochemical mechanisms for producing tetrahymanol. The environmental genome data available to Banta et al. (2015), with few marine bacteria encrypting Ths genes, suggested that ciliates are the predominant source of tetrahymanol in modern marine environments, whereas bacterial sources may be significant in terrestrial or lacustrine ecosystems, but these conclusions could also be caused by a sampling bias towards terrestrial bacteria (Banta et al., 2015).

Based on recent molecular clock estimates (Eme et al., 2014), it is unlikely that extant tetrahymanol producing eukaryotic groups emerged before 1.6 Ga. Thus, the BCF tetrahymanol derivatives are more likely derived from bacteria, either employing Ths for expansion of the E-ring of hopane-precursors, or an ancient bacterial STC homologue. To account for high picene concentrations however, marine tetrahymanol-producing bacteria must have been much more important than in modern marine ecosystems.
Not only is STC restricted to relatively few eukaryotic groups, but there is also a low number of Ths homologs identified in marine genomes – the former arguing for a late transfer of STC to eukaryotes, the later rather arguing for a eukaryotic source of significant tetrahymanol in modern marine environments. The fact that STC is transferred between phylogenetically diverse eukaryotes living under low-oxygen conditions indicates that modern eukaryotes acquired STC several times independently, presumably from unknown bacteria. It is thus plausible that stem-group eukaryotes also acquired this capacity independently and may well be the source of the picenes, analogous to the predominance of eukaryotic-derived gammacerane in modern marine sediments. Since many ciliates also produce hopan-3β-ol, and tetrahymanol producing eukaryotes form a monophyletic group in the cyclase tree of Takishita et al. (2012), it is likely that the original STC would also have produced both tetrahymanol and hopan-3β-ol, which could explain the co-occurrence of high concentrations of TeATs and methylated picenes in the BCF extracts. This idea is consistent with the speculations of Takishita et al. (2012) that, like modern eukaryotes inhabiting low-oxygen environments, Proterozoic eukaryotes may also have acquired the STC gene from bacterial donors and produced tetrahymanol instead of sterols, and that such eukaryotes may have been more abundant both in terms of diversity and biomass than their extant descendants. Thus, the gammacerane and abundant picenes in the BCF may be of heterotrophic eukaryotic origin. However, an abundant bacterial source cannot be excluded at present.

11.3.5.3. Aromatic arborane/fernane derivatives, TrATs & TeATs

Based on elution patterns and mass spectra, the aromatic hydrocarbons C29 MAPH, C28 DAPH 1 and C29 DAPH 2 were tentatively identified in many BCF extracts. Some samples may potentially even contain a C30 homologue of the DAPH series. MAPH and DAPHs are diagenetic products of triterpenoid precursors with either fernane or arborane skeleton (Peters et al., 2005) as they retain the characteristic 13,14- and 17-methylation pattern. The B-ring aromatised MAPH additionally retains the 4,4-dimethylation of the A-ring. The carbon skeletons of the arborane and fernane series only differ in the orientation around optical centres, so their precursors cannot be determined by GC-MS alone (Peters et al., 2005). Additionally, C25 (69) and C24 (70) were
tentatively identified as des-E arborane/fernane-type monoaromatics as reported by (Hauke et al., 1993). The des-E aromatic compound (69) was interpreted to be derived from an arborane/fernane-type precursor (Borrego et al., 1997), but can theoretically also be derived through the re-arrangement of hopanoids or similar triterpenoids.

The presence of typical fernane/arborane-derivatives in the BCF supports the hypothesis that a large proportion of the TrATs and TeATs may also have a fernenol/arboranol source. Numerous particularly isopropyl-substituted aromatic triterpenoids are rarely reported in the literature, despite the ubiquity of hopanes in most oils and bitumens. Most biogenic hopanoids are not functionalised at the ring system, hampering aromatisation in comparison to e.g. plant triterpenoids. The low abundance of common aromatic derivatives of hopanepolyols such as benzohopanes, and the large proportion of aromatic triterpenoids compared to saturated equivalents in the BCF, strongly argue for a non-hopanoid source of TrATs and TeATs. In light of higher fernane/arborane-homologues detected in the BCF compared to benzohopanes, arborane or fernane type precursors such as isoarborinol (LXVI), adriaticol (LXIV), eudoraneol (LXV) or ferneol (LXVII) are thus likely precursors of BCF TrATs and TeATs (Figure 11-11).
Saturated 8,14-secohopanes were previously found to have similar carbon number distributions as regular hopanes (Fazeelat et al., 1994), so can probably form from hopanoid precursors. Regular saturated and aromatic ≤C₃₀ 8,14-secohopanoids may however also be partly derived from fernane/aborane-type precursors. The high ratio of C₃₀/C₃₁ monoaromatic 8,14-secohopanoids in the BCF (Figure 8-18) indicates that they are likely not sourced from hopanopolyols, instead pointing towards an additional input from C₃₀ precursors. Cleavage of the 8,14-bond could be facilitated by the presence of the double bond in ring-C that is found in potential precursor compounds such as isoaborinol, adriaticol, fernenol and eudoraenol. According to Vliex et al. (1994), monoaromatic 8,14-secohopanoids may originate from fernene/abororene-type compounds (Vliex et al., 1994). Under the experimental conditions employed by Hayatsu and colleagues, aromatic 8,14-seco-hopanoids were not produced from fernenes (Hayatsu et al., 1987), but that does not exclude that other diagenetic processes involving methyl-shifts lead to their formation. The isomerisation of fernenes under acidic conditions leads to a mixture of neo-hop-13(18)-ene and hop-17(21)-ene (Ageta et al., 1987) that are exactly the intermediates expected in the transformation of side-chain poly-functionalised hopanoids into the D-ring aromatic 8,14-seco series (Vliex et al., 1994). Intriguingly, Vliex et al. (1994) found monoaromatic 8,14-secohopanoids in similar concentration as other arborane/fernane-products such as DAPH 2, whereas aromatic hopanoids such as benzohopanes were not present in high concentrations (see Fig. 5 in Vliex et al., 1994), suggesting that arborane/fernane-type compounds are indeed plausible sources for monoaromatic 8,14-seco-hopanoids. In light of the presence of abundant other potential fernane/arborane products and the high C₃₀/C₃₁ ratio, ≤C₃₀ regular monoaromatic 8,14-secohopanones are likely also largely derived from fernane/arborane precursors in the BCF.

Since no saturated equivalents of the arborane/fernane aromatics were detected, a 3-hydroxylation that facilitates aromatisation is expected and isoaborinol, adriaticol or fernenol are thus more likely precursors than non-hydroxylated equivalents such as fernene. The aromatic derivatives are thus likely derived from compounds such as adriaticol via diagenetic process as shown in Figure 11-11. A large proportion of the BCF
Precursors, pathways & ecological implications

Tri- and tetra-aromatic triterpenoids may also have been produced from the same precursors through progressive diagenetic aromatisation reactions (Figure 11-11). The most abundant triaromatic triterpenoids show a suspicious absence of M⁺ -15 Dalton fragments that are expected to be abundant in 4,4-dimethylated compounds, and thus A-ring aromatisation is likely. Aromatisation starting in ring-A is in turn indicative of 3-hydroxylation (see e.g. Hayatsu et al., 1987) as found in triterpenoids such as adriaticol.

The oldest current report of the arborane derivatives is from the Permian, while probable fernane derivatives have been isolated from a Carboniferous coal (Vliex et al., 1994, Hauke et al., 1995). This study thus extends the geologic record of these triterpenoids by ca. 1.3 billion years. An arborane/fernane skeleton can only be distinguished for these compounds upon isolation and optical rotation measurements or HPLC chiral separation analyses. Fernane-type compounds in the Carboniferous coals are likely derived from land plants (cordaites) (Auras et al., 2006). Fernenes were once thought to be derived only from ferns but can probably also be sourced by bacteria (Brassell and Eglinton, 1981). In some Recent sediments from saline Antarctic Ace Lake, fern-7-ene is associated with methanogenic biomarkers and anoxic depositional environments, and interpreted to be derived from purple non-sulphur bacteria (Volkman et al., 1986). Hydroxylated fernane-derivatives are also known from some lichens. While lichens were probably not present in the Paleoproterozoic (see e.g. Lücking et al., 2009), it is possible that the fernane-derivatives of lichens are produced by bacterial symbionts with potential Paleoproterozoic relatives.

Hauke et al. (1995) convincingly demonstrated a probable isoarborinol source for the arborane-type compounds in the Permian and Triassic sediments based on optical rotation measurements and HPLC chiral separation analysis, and comparison to Messel extract where both intact isoarborinol and an aromatic product are present. The only known extant sources of isoarborinol are certain flowering plants (Vorbrüggen et al., 1963, Ohmoto et al., 1970). Isoarborinol was isolated from the lichen Cladina macronesica (González et al., 1991), but this cannot explain the occurrence in aquatic environments. The occurrence of arboranoids in the Permian, before the accepted first appearance of flowering plants, and in other settings with little plant input strongly suggests a microbial source for a large proportion of sedimentary arborane-derivatives (Hauke et
The amino acid sequence of isoarborinal synthase was newly identified in rice and it was shown that monocot (flowering plants) isoarborinol cyclase clade was derived recently by convergent evolution (Xue et al., 2012). Thus, flowering plants or their earlier Phanerozoic ancestors cannot account for ancient isoarborinol-derivatives. High concentrations of isoarborinol (~20% of diploterol) in sediments from a small pond near Strasbourg and other young sediments may also be derived from microbial sources (Rohmer et al., 1980 and references therein).

Isoarborinol producing microorganisms remain unknown and it was proposed that either bacteria or algae were the producers of ancient isoarborinol (Hauke et al., 1995), similar to the prediction of Ourisson et al. that an as yet unknown aerobic (inferred from the 3-hydroxylation) bacterium may be the sources of isoarborinol in many ancient and modern sediments (Ourisson et al., 1982), although the hydroxylation of tetrahymanol does not depend on molecular oxygen (Caspi et al., 1968). Isoarborinol forms a primitive cyclisation product of oxidosqualene and was considered a likely intermediate between the hopanol and sterol biosynthetic pathway (Ourisson et al., 1987). The triterpenoid evolution pathways hypothesised by Fischer and Pearson (2007) suggests a close association with steroid evolution, consistent with the 3-hydroxylation typical for eukaryotic oxido-squalene substrates.

The prediction of an important structural role of hopanoids, which were found to be ubiquitous in geological sediments, in bacterial cell membranes was followed by the discovery that many bacteria indeed employ hopanols as sterol-equivalents, and constitutes one of the greatest success stories of organic geochemistry, forming the “molecular analogue of the discovery of the coelacanth” (Ourisson et al., 1982). Intriguingly, the prediction of a microbial source of arborane-type lipids was also recently confirmed by bioinformatics coupled to lipid analyses and protein characterisation (Banta et al., 2016). The bacterium *Eudoraea adriatica* was found to produce three triterpenoids: 1.) trace concentrations of lanosterol, 2.) adriaticol, an isomer of isoarborinol with shifted position of the double bond, 3.) eudoraenol, an isoarborinol isomer where the methyl group at C-13 is shifted to C-8. We would expect C₃₀ monoaromatic 8,14-secohopanoids, which we observed in the BCF in higher concentrations than >C₃₀ homologous, to readily form from eudoraenol Figure 11-11.
In *E. adriatica*, eudoraenol and adriaticol were produced by a novel oxidosqualene synthase termed eudoraenol synthase (EUS). *E. coli* expression experiments confirmed that the triterpenols are synthesised directly from an oxidosqualene precursor. The enzyme does not seem to be related to the isoarborinol synthase in plants and accordingly was most likely not acquired by horizontal transfer between plants and *E. adriatica*. In a phylogenetic tree of (oxido)squalene cyclases, EUS falls between bacterial squalene hopane cyclase (SHC) and bacterial oxidosqualene cyclase (OSC) (Figure 11-12, from Banta et al. (2016)). It either derived from a lanosterol synthase (LAS), consistent with the evolutionary scheme proposed by Fischer and Pearson (2007), or from a squalene-hopene cyclase, consistent with the proposals of Ourisson et al. (1987) (Banta et al., 2016).

![Figure 11-12. Oxidosqualene Cyclase Diversity as presented in Banta et al. (2016).](image)

Much has yet to be learned about the ecological distribution of the EUS or related enzymes, but according to initial analyses it can be found in several bacteria from marine and lacustrine environments (Banta et al., 2016). An ancient EUS, producing 3-
hydroxylated triterpenols such as adriaticol and eudoraenol from an oxidosqualene precursor, can explain a large proportion of the unusual BCF triterpenoid distribution, including MAPH, DAPHS, some 8,14-secohopanoids, TrATs and TeATs. As in isoarborinol, the double functionalisation of the ring system makes aromatisation much more likely than in hopanoid precursors, consistent with the high degree of aromatisation of BCF triterpenoids.

In view of the recent discovery of Banta et al. (2016), a bacterial source is likely, but the occurrence of a similar cyclase in (potentially extinct) eukaryotic organisms cannot be excluded at this point. *E. adriatica* branches from the oxidosqualene cyclase tree just before extant eukaryotes (Wei et al., 2016), so it is quite possible that some groups of (possibly now extinct) eukaryotes possessed EUS equivalents. They may have acquired these via horizontal gene transfer from bacteria, but early eukaryotes may as likely have invented EUS and CAS synthesis and transferred the genes to a limited number of (Group 2 in Wei et al., 2016) bacteria. Since sterol synthesis form EUS only requires substitution of four amino acid residues (Banta et al., 2016), eukaryotic sterol synthesis may even be derived from an EUS precursor either in (Group 2) bacteria or eukaryotes.

11.3.5.4. *Hopanes, seco-hopanes and aromatic derivatives*

Several saturated and aromatic BCF triterpenoids are plausibly derived from hopanepolyols, 2- and 3-methylated hopanoids and related compounds. The hopanol-derived BCF triterpenoids encompass homohopanes ranging from C₃₁ to C₃₉, C₃₀ hopanes and de-methylated derivatives, a homologous series of C₃₁ to C₃₆ 2- and 3-methyl hopanes, 8-14-secohopanes and monoaromatic derivatives, including those with fluorene- and acenaphthene-moiety, benzo hopanoids, and a proportion of tri-and tetra-aromatic triterpenoids.

The diagenetic scheme that may lead to these products is shown in Figure 11-13. Defunctionalisation and hydrogenation of hopanoids results in different types of hopanes. All hopanes >C₃₀ are presumably derived from hopanepolyols, with 2- and 3-methylhopanes forming from 2- and 3-methylated hopanepolyols. Cleavage of the 8,14-carbon bond (likely facilitated by unsaturation) would have been partly followed by
side-chain cyclisation and aromatisation to form the fluorene and acenaphthene derivatives. Removal of heteroatoms and aromatisation results in the formation of aromatic hopanoids.

Some BCF samples also contain low concentrations of benzohopanes. Benzohopanes form through cyclisation of the extended side chain of hopanepolyols during early diagenesis, consistent with their carbon number range from C_{32} to C_{35}. Concentrations are highest in oils and bitumens from evaporitic and carbonate source rocks, but they occur in trace amounts in most oils and source rocks (Peters et al., 2005 and references therein). Despite the high carbonate content and the abundance of extended saturated hopanes in many BCF samples, benzohopanes generally only occur in trace amounts and are below detection limits in many samples. This indicates that the aromatisation of the side-chain of extended hopanoids was not favoured during the deposition and burial of BCF sediments despite the high degree of aromatisation generally observed in BCF triterpenoids.

Secohopanoids are often enriched in biodegraded oils suggesting that they are highly bio-resistant and/or form through the microbial alteration of bitumen. They are not restricted to biodegraded oils though, suggesting a direct biological precursor or formation during early diagenesis (Peters et al., 2005 and references therein). Compared to C_{29}, the 28-nor monoaromatic 8,14-secohopanoids (XI) yield high concentrations of the C_{30} compound that would correspond to a C_{31} precursor hopanoid (Figure 8-20). A hopanepolyol source thus seems most likely for the demethylated secohopanoids.

Secohopanoids with fluorene moiety (VIII) also occur in significant concentrations in many BCF sediments. They are rarely reported in the literature, with the previously oldest published occurrence in the Early Permian (Oba et al., 2009). However, these compounds have also recently been detected in ca. 1.1 Ga sediments from Mauretania (Gueneli, 2016). The secohopanoids (VIII) may form during early diagenesis through cleavage of the bond between C-8 and C-14 as a result of aromatisation, coupled with the cyclisation and aromatisation of the functionalised side chain of a hopanoid precursor. Their formation may potentially relate to specific depositional conditions (Sinninghe Damsté et al., 1998). Oba et al. (2009) found that in early Permian samples, the concentrations of VIII are similar to those of side-chain aromatised benzohopanes,
suggesting that either the benzohopanes are precursors of the 8,14-secohopanoids, or that both were generated from a common precursor.

Figure 11-13. Proposed diagenetic scheme for most of the BCF hopanoids. Note: The \( m/z \) 207 fragment of VIII could also be derived from the fluorene moiety.
The acenaphthene hopanoids reported from some Mexican crude oils occur in a series of C\textsubscript{30} to C\textsubscript{34} homologues (Carrillo-Hernández et al., 2001) as often observed for benzohopanes. Carrillo-Hernández et al. (2001) therefore proposed that these compounds are generated during thermal maturation from benzohopanes involving cleavage of the 8,14-carbon bond and formation of the additional aromatic ring by hydrogen transfer reactions.

Some of the tri- and tetra-aromatic triterpenoids may also be derived from ≤C\textsubscript{30} hopanoid precursors. However, if common hopanoids like diploptene or diplopterol were the precursors, we would expect these compounds to be much more common in geological samples. While fernane/arborane-type triterpenoids are the most plausible source for the majority of TrATs and TeATs, unusual ring-functionalised hopanoids may also have contributed to the signatures. In particular, 28,30-dinorneohop-13(18)-enes, potentially produced by certain bacteria dwelling at or below the chemocline are plausible precursors for a large proportion of ethyl-substituted TrATs and TeATs as previously proposed for Cretaceous black shales (Sinninghe Damsté et al., 2014). These yet to be identified bacteria may thus have been important ecological players during BCF times.

The BCF triaromatic triterpenoids are likely dominantly A-ring aromatised which would strongly suggest that a biogenic functionalisation of the A-ring was responsible for the initiation of the aromatisation. There is one known A-ring functionalised hopanoid that occurs in high concentrations in some organisms: hopan-3β-ol. There are currently only very few known source organisms of hopan-3β-ol. It was detected in certain plants (Tsopmo and Kamnaing, 2011) that cannot have contributed to Proterozoic organic matter. However, many marine ciliates produce similar concentrations of hopan-3β-ol and tetrahymanol (Harvey and McManus, 1991). As discussed for picenes, ancient squalene tetrahymanol cyclases (STC) may also have produced both, tetrahymanol and hopan-3β-ol. Since known bacteria do not employ STC, the source organisms may have been primitive eukaryotes that replaced sterols with tetrahymanol and hopan-3β-ol under low-oxygen conditions.

2α- and 3β-hopanes derive from A-ring methylated hopanoids produced by certain bacteria. Some of these bacteria may synthesize either regular or methylated hopanoids depending on their metabolic state (Peters et al., 2005 and references therein).
hopanes are in low relative concentrations in BCF extracts (Brocks et al., 2005). 2α-hopanes were considered as biomarkers for cyanobacteria in the past (Summons et al., 1999), but are now known from a variety of sources and likely evolved in alphaproteobacteria (Ricci et al., 2015). They may play an important role in stress reduction by increasing the rigidity of bacterial cell membranes (Wu et al., 2015).

High relative concentrations of 3β-hopanes were reported from BCF sediments (Brocks et al., 2005). 3-methylated hopanoids are mostly produced by microaerophilic proteobacteria, particularly type I methanotrophs (Neunlist and Rohmer, 1985, Summons et al., 1994) that often thrive in low sulphate environments (Brocks et al., 2005). The BCF hopanoids can thus be expected to have been produced by a large variety of different bacteria, including cyanobacteria, type I methanotrophs and as yet unknown bacteria dwelling at or below the chemocline.

11.3.5.5. Dammaranes

Dammaranes were identified based on their mass spectral characteristics and confirmed by a co-elution experiment with an authentic standard. These molecular fossils may derive from dammarene or dammarenediol precursors as (Figure 11-14). We did not detect any aromatic derivatives of dammaranoids. The main TAS isomers yield a 17- and main DAS and MAS isomers a 13,14-dimethylation that is inconsistent with a dammarane-type precursor, but minor unknown TAS isomers could potentially derive from dammaronoids. In the geological record, it is unclear why dammarenes are found in many immature sediments, but dammarane occurrences in thermally more mature sedimentary rocks are rare. It was proposed that dammarenes may be diagenetically transformed into derivatives that do not retain a dammarane skeleton. Dammarenes and dammaranes are known from Pleistocene to Jurassic sediments (Meunier-Christmann et al., 1991), so the BCF discovery extends the geologic record by more than 1.4 billion years.
Dammarane-type triterpenoids were first identified in dammar resins (Mills and Werner, 1955) and are only known from land plants. However, the occurrence of dammarenes and dammaranes in diverse marine sediments strongly suggests an additional microbial source. This is supported by $\delta^{13}$C values of dammarenes from an upper Maastrichtian/Paleocene shale near Timahdit (Morocco) that are similar to the bulk organic matter and bulk nonaromatic hydrocarbon fraction. In the Maastrichtian sediments, the organic matter is essentially derived from algal and bacterial precursor material (Meunier-Christmann et al., 1991), pointing to a microbial source of the dammarenes.

Triterpenoids with a dammarane-skeleton are structural intermediates between hopanoids and steroids, and are thus likely evolutionary intermediates in the sterol evolution pathway. Based on amino acid sequence analysis, minimum evolution and biochemical energetics, it appears that dammaranoid-cyclases are the precursors of the other triterpenoid cyclases (Fischer and Pearson, 2007). However, Fischer and Pearson (2007) inferred poor fitness of dammaradiene that lacks amphiphilic properties which may have prompted rapid evolution of the cyclase towards steroid and hopanoid products (Fischer and Pearson, 2007). On the other hand, geological dammarenes and dammaranes could also be derived from oxygenated precursors (Meunier-Christmann et al., 1991) with amphilic properties that would make them more useful membrane lipids. Dammarane cyclases in inferred modern day microbial source organisms of dammaranes could be direct descendants of the Paleoproterozoic dammarene-cyclases, or dammarene-biosynthesis could have been re-invented by Phanerozoic microorganisms. The occurrence of dammaranes in the context of the BCF triterpenoid assemblage points to the presence of evolutionary early triterpenoid cyclases.
11.3.5.6. **Protosteroid derivatives**

A variety of BCF triterpenoids can plausibly be attributed to a protosteroid source such as lanosterol and cycloartenol (Figure 11-15). The inferred geological protosterol products include:

- triaromatic C$_{26}$ sterols, C$_{27}$ 4-methyl sterols and C$_{28}$ dimethyl sterols
- diaromatic C$_{27}$, C$_{28}$, C$_{29}$ and potentially C$_{30}$ lanosteroids
- B-ring monoaromatic C$_{28}$ and C$_{29}$ lanosteroids
- tentatively identified C$_{26}$, C$_{27}$ and C$_{30}$ side-chain aromatised sterols and their potential ring-opening products
- protosteranes
- lanostanes

![Diagram of sterol derivatives](image)

**Figure 11-15.** Proposed diagenetic reaction products of biogenic C$_{30}$ protosterols lanosterol and or cycloartenol in the BCF.
Precursors, pathways & ecological implications  Chapter 11

C26 and C27 TAS were already identified in the BCF more than ten years ago (Brocks et al., 2005). The vast majority (~86%) of the TAS are 4-methylated (4-methylation almost 100% in m/z 245 series), and in the upper BCF (n = 9), C26 cholesteroloids contribute on average only ~4% to the TAS, while a new m/z 259-series shows additional (di)methylation and contributes ~10% to the TAS (Figure 11-8). Aromatic steroids consist of ~10% MAS, 40% TAS and 50% DAS (Figure 11-7). No saturated cholestanes or 4-methylcholestanes were detected in the BCF. Detected, however, were C30 4,4-dimethylated protosteranes and lanostanes.

As in Phanerozoic oils, the C26 BCF triaromatic cholesteroloids could theoretically stem from C27 steroids such as zymosterol or cholesterol, but no indigenous saturated equivalents could so far be detected. TAS steroids, including 4-methylated cholesteroloids are ubiquitous in Phanerozoic oils and bitumens. However, the BCF distribution with a strong dominance of 4-methyl derivatives and the absence of indigenous 24-alkylated homologues is unique (see Fig. 5 in Brocks et al., 2015 for typical Phanerozoic TAS assemblage). The BCF TAS must therefore have different biogenic sources. To account for the high degree of 4-methylation in TAS, 4- or 4,4-dimethylated cholesteroloids are more plausible precursors. 4,4-dimethylated protosteroids are thus obvious source candidates. In chapter 10, I showed that C26, C27 and C28 TAS are formed during pyrolysis of cycloartenol and lanosterol, strongly suggesting that C26 TAS can indeed form through the thermal maturation of protosteroid precursors. The low relative concentrations of the C26 versus C27 TAS in the BCF also indicate that the former are likely degradation products of the latter. In addition, DAS and MAS isomers were also produced during pyrolysis, and co-injection with an authigenic standard confirmed that BCF MAS are B-ring lanosteroids. Since saturated cholestanes and 4-methyl cholestanes were not detected, following Okham’s razor, TAS, including C26 cholesteroloids, are best explained as products of protosterol diagenesis.

The pyrolysis experiments also confirmed that a variety of lanostane isomers can form from lanosterol as well as cycloartenol precursors. All four major BCF compounds with a lanostane-like fragmentation (94 to 97) seem to have been produced in our pyrolysis experiments. The BCF lanostane pattern appears to be intermediate between that of cycloartenol and lanosterol maturation with high ratios of lanostane isomers 96/97 (as
e.g. in the 300°C, 1h cycloartenol experiment) and high concentration of compound that was only produced in significant quantities in the lanosterol pyrolysis (300°C, 1h) experiment (see Fig. 25 in chapter 8). While this may be an artefact of pyrolysis conditions, the BCF lanostane isomer distribution may potentially provide circumstantial evidence for the presence of both cycloartenol and lanosterol type precursors.

The apparently bridged protosteranes may be diagenetic products of either cycloartenol, lanosterol or terpenoids with the dammarane skeleton. However, only cleavage of the cyclopropyl-ring of cycloartenol provides a plausible driving force for formation of the inferred bridge across the sterane-ring system. Cycloartane-derivatives have never been reported from geological samples, arguing for a rapid conversion of these compounds that are produced by all algae and plants as intermediate products during phytosterol synthesis. Acid treatment, hydrogenation and heating experiments on cycloartenol confirmed the instability of the propyl-ring and the (near) quantitative conversion to lanostane and aromatic derivatives without formation of cycloartane. Therefore, cycloartane or other derivatives with cyclopropyl-group are not expected to be preserved in ancient sediments, but the former presence of a cycloartane ring could be reflected in the carbon-bridge in protosterane.

Lanostanes are rare in the geological record and the BCF lanostane signature is unique. The few reported lanostane occurrences include C₃₀ and 24-methylated C₃₁ lanostanesulphides from an immature Paleocene crude oil (Peng et al., 1998), a series of C₃₀ to C₃₂ lanostanes in Eocene lacustrine deposits with side-chain alkylation at C-4 (Chen et al., 1989, Chen and Summons, 2001), 4-monomethyl norlanostane, C₃₀ lanostane and 24-methyalted C₃₁ lanostane from Miocene methane seep carbonates (Peckmann et al., 2004, Birgel and Peckmann, 2008), functionalised C₂₄-methylated nor- and bis-norlanostane derivatives in a ca. 2 Ma plant fossil (Murae et al., 1990), side-chain degraded C₂₄, C₂₅, and regular C₃₀ lanostanes in Tertiary sulphur-rich crude oil that is rich in 4-methyl and 4,4-dimethyl steranes (Lu et al., 2011), C₂₉ and C₃₀ lanostanes from Cambrian organic matter (Parfenova, 2011), as well as tentatively identified lanostane co-occurring with B-ring monoaromatic lanosteroids (LVI) in Oligocene evaporites (Adam, 1991). In the majority of cases, methyl- or higher alkylated side-chain derivatives also occur or 4- and
4,4-diethyl steranes are dominant, indicating a significantly different source compared to the BCF. Only the Oligocene and Cambrian bitumens seem to only contain C\textsubscript{30} lanostanes and potential degradation products such as those encountered in the BCF. As methylotrophic bacteria such as *Methylococcus capsulatus* are the only known major modern sources of lanosterol, lanostanes are usually attributed to these bacteria (e.g. Chen and Summons, 2001), and such a source is consistent with the very low carbon isotopic composition of lanostanes in the Miocene seep carbonates (Peckmann et al., 2004). Methanotrophic bacteria were also proposed as a probable source of the BCF 4-methylated triaromatic steroids (Brocks et al., 2005).

Recent studies showed that a number of different bacteria can also produce protosteroids. Currently identified species that only produce lanosterol and/or cycloartenol and no demethylated-derivatives are *Cystobacter* species such as *Cystobacter fuscus*, and *Stigmatella aurantica* both of the myxobacteria, *Fluviicola taffensis* of the bacteriodetes, *Methyloceanibacter caenitepidi* of the \(\alpha\)-proteobacteria and *Gemmata obscuriglobus* of the Planctomycetes, so these bacteria could be potential sources of the BCF steroids. In addition, other members of these groups also produce 14,\(-\)4,\(-\) and 4,4- and demethylated derivatives down to the cholesterol isomer zymosterol (see Wei et al., 2016 and references therein), but since these were not detected in the BCF, such bacteria are unlikely sources.

Lanosterol or cycloartenol are also produced by most eukaryotes as intermediates in their sterol biosynthetic pathways (Summons et al., 2006). The high abundance of BCF steroids indicates that these were membrane lipids and not just intermediate products. The absence of any downstream products in eukaryotic sterol synthesis strongly argues against crown group eukaryotes as biological sources of BCF steroids.

Reports of diaromatic steroids are very rare in the literature. Only 4,4-demethylated 13,14-methyl diaromatic steroids have been unambiguously identified from biodegraded oil seeps (Lemoine et al., 1996), but the BCF diaromatic steroids dominantly yield additional methylations and thus must have different precursors as these 4,4-demethylated compounds. Based on the mass spectral characteristics, 4-methyl diaromatic steroids were tentatively identified from euxinic sediment of the Qianjiang Formation (Grice et al., 1998). In contrast to the BCF assemblage, these steroids lack
methylation at C-14 and are thus not lanosteroids. In addition, they also yield a 24-ethylation. An inferred 4-monomethylated B-ring monoaromatic counterpart is also present in the Qianjiang extracts and a 4-methyl precursor is confirmed by the close isotopic similarity to abundant C30 4-methylsteranes in these samples (Grice et al., 1998). The Qianjiang DAS must thus have different biogenic sources than the BCF steroids. Based on mass spectral characteristics, diaromatic steroids with an inferred lanostane-skeleton were tentatively identified for the first time by Jacob et al. (2007) in recent (up to 20 ka) sediments of tropical Lake Caco. Functionalised derivatives with ketone, alcohol and methyl-ether groups were also tentatively identified (Jacob et al., 2007). The triterpenoids from Lake Caco can be attributed to a plant source, likely Gramineae, (Jacob et al., 2005) and can thus not be related to the BCF sources. Thus, there is no known occurrence of BCF-like diaromatic steroid patterns, highlighting the peculiarity of the BCF steroid signature.

B-ring monoaromatics of the lanostane-type have only been described from Oligocene evaporites co-occurring with tentatively identified lanostane, and were tentatively attributed to methanotrophic bacteria, the only lanosteroid producing microorganisms known at the time (Adam, 1991). In contrast to the BCF, these steroids are however only minor constituents of the triterpenoid pool, abundant 4-methyl TAS and DAS were however not reported by Adam (1991).

The high concentrations and diversity of protosterol derivatives discovered in the BCF extracts is unique in the geological record, suggesting a completely different ecosystem than in any known examples throughout Earth’s history. The unique restriction to lanostane-type motifs before the emergence of downstream biosynthetic derivatives in the rock record is best explained by the activity of abbreviated (“primitive”) sterol biosynthetic pathways. These could have been present in certain protosterol-producing bacteria such as those discussed above. Considering the high abundance of BCF steroids and relatively high protosterane/hopane ratios, such bacteria must have been much more important at BCF times than throughout the Phanerozoic. Alternatively, the BCF steroids could have been produced by eukaryotes lacking the downstream modification capabilities. Since LECA already had the potential to produce modified sterols
(Desmond and Gribaldo, 2009), such eukaryotes in a Paleoproterozoic setting would have to be attributed to the stem lineage of the eukaryotic domain.

11.3.6. Summary of biogenic precursors and diagenetic summary scheme

The BCF yields more than 40 different triterpenoid and steroids structures (Figure 11-16 and Figure 11-17), many of which were not previously recognised. They can all be plausibly attributed to only about a dozen biogenic precursor compounds (or close structural relatives): tricyclohexaprenol, dammarane or dammaradienol, tetrahymanol, diploptene or diplopterol, 28,30-bisnorhopenes, hopanopolyols and 2- & 3-methyl hopanols, lanosterol and/or cycloartenol, isoarborinol and/or adriaticol, and possibly eudoraenol and hopan-3-ols. The potential biogenic precursors and important diagenetic routes to the different BCF triterpenoid products are illustrated in Figure 11-16 and Figure 11-17. If the inferred precursors are correct, they would have very important implications for interpretation of plausible source organisms. The exact functionalisations of the biogenic precursor compounds are of course not known, but the structures proposed in the diagenetic scheme are found in modern organisms and can plausibly be diagenetically and catagenetically transformed to the BCF molecular fossils.
Figure 11-17. Proposed diagenetic scheme for BCF triterpenoids part 2.
11.4. Discussion of source organisms and ecological implications

Although triaromatic steroids were previously recognised to occur in significant concentrations in the BCF (Brocks et al., 2005), it is still surprising how high total steroid concentrations are in BCF extracts. Steroids usually form prominent peaks – sometimes even being among the highest peaks – in the full scan chromatograms of aromatic fractions, and steroid concentrations are significant compared to any biomarkers from $n$-alkanes (e.g. largely fatty acid derived) to Pr and Ph (e.g. largely chlorophyll derived) to arylisoprenoids (e.g. derived from pigments of anoxygenic phototrophs). Particularly, steroid concentrations are significant compared to hopanes or hopanoids, even if TrATs and TeATs are assumed to be diagenetic hopanoid derivatives. Low concentrations of steranes, even in samples with abundant hopanes, are thought to be a typical feature of pre-Ediacaran bitumens (Pawlowska et al., 2013) but here we could show for the first time that total steroid concentrations can be higher than total hopanoid concentrations in Precambrian sediments. The high relative abundance of steroids to hopanoids indicates a high activity of steroid-producing organisms.

Hopanoids are generally interpreted as biomarkers of bacteria, and steroids as biomarkers of eukaryotes (see e.g. Peters et al., 2005). However, the picture becomes more and more complicated as we learn more about the metabolic capabilities of different organisms and their phylogenetic relationships. For example, Pearson and colleagues recently estimated that fewer than one in 10 bacterial cells in soils (Pearson et al., 2007) and fewer than one in 20 bacterial cells in the ocean, contain copies of SHC genes (Pearson and Rusch, 2009) and are thus capable of the de-novo hopanoid synthesis. At the same time, it has become apparent that a variety of bacteria can produce sterols and more than 30 bacterial species are now known to contain OSC gene copies (Wei et al., 2016), as do around 300 metagenome sequences (Banta et al., 2016).

Broadly, there are three groups of bacteria with OSC gene copies: the first group (bacterial group 1 of Banta et al., 2016) contains eudoraenol gene copies (EUS) that branch most closely to bacterial SHC in a maximum likelihood phylogenetic tree of OSC homologs (Figure 11-12, from Banta et al., 2016). The second group (bacterial groups 2 to 4) branches between EUS and eukaryotic OSCs, while the third group (bacterial group
5) branches within eukaryotic OSCs and thus seems to have acquired their genes via HGT from eukaryotes (Figure 11-12).

Currently, it is not possible to distinguish whether bacteria or eukaryotes invented sterol synthesis. Either eukaryotes obtained sterol synthesis from bacteria via HGT or from the bacterial symbiont of the original archaeon/bacterial fusion, or bacteria obtained OSC genes via HGT from stem group eukaryotes. The fact that OSC is sparsely distributed in bacteria suggests several independent HGT events from eukaryotes to bacteria occurred. Thus, the horizontal transfer of OSC to bacteria is a relatively likely event. The feasibility of transfer of OSC from eukaryotes to bacteria is recorded by the presence of OSC in bacterial group 5 branching within the eukaryotic OSC groups (Figure 11-12). Thus, it is plausible, or at least possible, that bacterial groups 2 to 4 obtained their sterol biosynthetic capabilities from stem group eukaryotes. Conversely, it is unclear whether eukaryotes would likely have obtained such gene copies from bacteria.

As LECA existed some 1 to 1.9 Ga ago (Eme et al., 2014), LECA already possessed an essentially complete sterol biosynthetic capacity (with nearly 20 enzymatic steps leading to cholesterol) (Desmond and Gribaldo, 2009) and OSCs of bacterial groups 2-4 branch basal to eukaryotic OSCs (Banta et al., 2016, Wei et al., 2016), it is very likely that the first transfer of OSC between eukaryotes and bacteria occurred before 1.64 Ga ago. This means that both stem group eukaryotes and some bacteria were probably able to produce sterols at the time the Barney Creek Formation was deposited. To interpret the BCF steroids, we thus need to find ecological arguments to determine what organisms likely left such a prominent steroid signal in sediments deposited over hundred thousands of years in a relatively shallow sea.

11.4.1. BCF steroids in light of the geological rock record

Figure 11-18A outlines a potential scenario of sterol evolution. As discussed, either bacteria or stem groups eukaryotes may have invented sterol synthesis and transferred OSC to the other domain. One of the bacterial symbionts in one of the archaeal/bacterial fusions leading to the eukaryotic nucleus and mitochondrion may have introduced an early triterpenoid cyclase to the new organism, and this may likely have been a squalene-
hopene cyclase (SHC), eudoraenol cyclase (EUS) or an oxidosqualene cyclase (OSC) of the cycloartenol (CAS) or lanosterol (LAS) type. So, either eukaryotes inherited OSC from bacteria during fusion (bottom dashed arrow in Figure 11-18A) or through HGT (3. arrow), or eukaryotes inherited SHC (bottom arrow) that subsequently developed into OSC and was later transferred back to bacteria (2. arrow). So, directly after the OSC transfer, there would have been stem group eukaryotes and bacteria that produced the protosterols cycloartenol and/or lanosterol (dark blue squares in Figure 11-18A) by enzymatic cyclisation of epoxysqualene. However, these organisms did not yet possess the capacity to demethylate sterols at C-4 and C-14, or alkylate the side chain. It is these products that are found in the BCF.

It is currently not possible to directly differentiate between eukaryotic and bacterial protosterol products in the BCF. However, based on circumstantial evidence I suggest that a stem group eukaryotic source is more likely. There are no typical marine Phanerozoic biomarkers occurrences where typical bacterial sterols play a role (see e.g. Peters et al., 2005) – there are in fact hardly any reports of such protosteroids at all, and then only in unusual settings such as high salinity environments (Chen et al., 1989) or methane-seeps (Peckmann et al., 2004). Even in anoxic settings like the Black Sea that may be in many ecological aspects analogous to Proterozoic environments, there is no known record of significant quantities of bacterial steroids. Thus, bacteria that possess OSC and produce protosterols are not quantitatively dominant ecological players in oxic and anoxic marine environments. There is no known reason why this should be different in the BCF. Therefore, although it cannot be ruled out, it does not appear likely that such bacteria could have had an overwhelming ecological presence in the BCF, in the green and purple ecological endmember states. We thus propose that, like throughout the Phanerozoic, most Proterozoic steroids can plausibly be attributed to a eukaryotic source. Steroid abundances, and in particularly steroid/hopanoid ratios broadly comparable to tropical marine sediments in the Phanerozoic, show that stem group eukaryotes were ecological important.
Figure 11-18. A) Potential triterpenoid evolution in a hypothetical schematic rDNA tree with triterpenoid highlighted triterpenoid cyclases. The last eukaryotic common ancestor (LECA) = orange circle. LECA probably produced cholesterol and 24-alkylated cholesterols (Desmond and
Gribaldo, 2009). Bacterial SHC is at the root of hopane-synthesis (SHC). If sterol-synthesis was invented by eukaryotes, it was transferred early to some bacteria via HGT (OSC bacteria 1 – 4). There was at least one, and possibly multiple, HGTs from eukaryotes to bacteria (OSC bacteria 5) after emergence of LECA. Coloured branches indicate production of specific triterpenoids: purple = arborane (EUS), dark blue = only protosterols cycloartenol and/or lanosterol, medium blue = 4,4-dimethyl-, light blue = 4-methyl, red = cholesteroids, brown = ergosteroids, green = stigmastereoids. The biomarker record can be interpreted in terms of Bloch’s hypothesis of sterol evolution going from cycloartenol or lanosterol via 4,4-dimethyl, 4-methyl- to cholestane. 24-alkylation possibly developed later through emergence of a methyl-transferase (SMT). Alkylation have proceeded from methyl (ergostane) to ethyl (stigmastane) to n-propyl-cholestane, controlled by number of SMT copies (Gold et al., 2016). B) Current record of eukaryotic evolution based on molecular fossils (red) (Butterfield, 2015) biomarkers (orange) (Brocks et al., 2015, this study, Love et al., 2009) and molecular clock estimates indicating LECA 200 – 300 Ma within crown group divergence (Eme et al., 2014). Right: plausible LECA range based on molecular clocks (Hedges et al., 2004, Eme et al., 2014).

As detailed in chapter 9, Konrad Bloch hypothesised that the sequence of sterol biosynthetic steps parallels sterol evolution and that natural selection over long timescales led to stepwise improvements of sterol properties with regards to membrane function (Bloch, 1983). Following this theory, one would expect to find in the geologic record the occurrence of more and more sophisticated sterane fossils that would have provided improved membrane fitness to early eukaryotes. Previously, it was thought that evolved steranes had appeared by 2.7 billion years ago (e.g. Brocks et al., 1999, Waldbauer et al., 2009) and that more primitive sterane inventories are not preserved in even older rocks. However, the ‘cleaning up’ of the Precambrian rock record (see Pawlowska et al., 2013 and references therein) now provides us with the opportunity to test the Bloch hypothesis in the rock record. The absence of clearly indigenous steranes in the entire mid-Proterozoic rock record even when hopanes with similar thermal stabilities are preserved (Pawlowska et al., 2013) could mean that modern sterols were simply not evolved yet.

The detection of indigenous lanostanes and protosteranes in this study provides a critical puzzle in the interpretation of sterol evolution. It shows that steranes can be abundant in pre-Ediacaran sediments and are not generally taphonomically excluded as suggested by Pawlowska et al. (2013). The greatly broadened BCF steroid inventory can be fully explained by biogenic protosterol precursors, as supported by our thermal maturation experiments on lanosterol and cycloartenol. During deposition of BCF in the McArthur Basin, protosterols were the only sterols produced by marine organisms in detectable quantities. Since clearly indigenous trifecta steranes do not appear in the rock record until ~820 Ma (see also Figure 11-18B), it is possible that 24-alkylated sterols were not evolved at 1.6 Ga.

356
Currently, it is not clear what order of sequences is expected if the Bloch hypothesis of cholesterol evolution is expanded to phytosteros. C-24 alkylation mostly takes place after removal of the C-14 methyl group and at least one C-4 methyl group (Nes and McKean, 1977). In contrast, in many fungi 24-alkylation occurs as the first step in the conversion of lanosterol to ergosterol, but then some fungi also produce C27 sterols such as cholesterol (Weete, 1980). Based on sterol biosynthetic pathways in many extant eukaryotes (Summons et al., 2006), duplication events of genes coding for the 24-methyl sterol transferase (SMT) in eukaryotes, and the fact that the degree of 24-alkylation in most eukaryotes seems to correlate with the number of SMT gene copies (Gold et al., 2016), the order of sterol evolution depicted in Figure 11-18A is a plausible scenario. While it is clear that the first defining step in the evolution of modern sterols was the evolution of an oxidosqualene cyclase, the following demethylations and alkylation followed a now unknown order, but that may be detectable in the rock record. The new data presented in this thesis shows that the defining step in sterol evolution, emergence of an oxidosqualene cyclase, happened prior to 1.64 Ga.

Key points of the Precambrian sterane record are summarised in Figure 11-18B. It was suggested that cycloartenol has a bent conformation that ‘hides’ the methyl group at C-14, making it a suitable membrane lipid and was therefore at one point the end of the sterol biosynthetic pathway. Lanosterol in contrast “has a rigid planar conformation in which the methyl group protrudes unavoidably, and therefore can never substitute for cholesterol” (Ourisson and Nakatani, 1994). This assumption was confirmed in experiments where lanosterol performed poorly as a membrane lipid, while cycloartenol was a reasonable cholesterol surrogate (e.g. Bloch, 1983 and references therein). According to Ourisson and Nakatani (1994), the unavoidable metabolic degradation of cycloartenol would have resulted in some sterols that were also suitable sterane reinforcingers and the same enzymatic steps could then have been used to convert lanosterol into suitable membrane sterols.

It is thus possible that some intermediate products were poor membrane sterols and were thus never produced in significant quantities and accordingly may not be expected to be found in ancient sediments. The absence of intermediate sterane motifs from the Precambrian rock record may however also be due to the strong patchiness of the
pre preserved biomarker record and it is also possible that a more concerted effort will eventually lead to the detection of such products. The recently ‘cleaned up’ Precambrian biomarker record indicates that the 4,4,14-demethylation of protosterols occurred prior to ~820 Ma (Brocks et al., 2017). 24-ethylolation of cholesterols occurred prior to 635 Ma. By 635 Ma 24-propyl cholestanes were also evolved (Love et al., 2009). 24-n-propyl cholestanes in the rock record may reflect activity of the crown group eukaryotic groups pelagophyte algae or foraminifera, while 24-isopropyl cholestanes may even witness the emergence of metazoa in the form of demosponges (Love et al., 2009, Grabenstatter et al., 2013). Ecological tinkering with sterol structures, potentially as a response to predation pressure, seems to also have happened prior to ~820 Ma (Brocks et al., 2017).

One way of interpreting the Precambrian biomarker record is to consider sterane occurrence to closely reflect emergence of the sterol biosynthetic capabilities according to Bloch’s hypothesis of sterol evolution. The last eukaryotic common ancestor (LECA) could already produce a wide array of modified sterols, including 24-methylated and potentially 24-ethylated products (Desmond and Gribaldo, 2009), so in theory all steranes >635 Ma could still be derived from stem-group eukaryotes. Based on a literal reading of the biomarker record, extant eukaryotic groups may thus have evolved as late as 820 or even 635 Ma.

Microfossils are relatively confidently interpreted as eukaryotic from ~1.8 Ga (Knoll, 2014) and very confidently from ~1.6 Ga (Butterfield, 2015). Thus, eukaryotes clearly evolved before BCF times. Bangiomorpha red algae are the earliest fossils that can be confidently ascribed to crown group eukaryotes and are probably~1.1 Ga based on sequence comparisons (Butterfield, 2015). Other Paleo- and Mesoproterozoic microfossils cannot be attributed with confidence to any known eukaryotic group and may belong to now extinct stem lineages (see e.g. Knoll, 2014) although some palaeontologist argue that the size and complexity displayed since ~1.6 Ga by some acritarchs such as Tappania and Shuiyousphaeridium is “a clear and conservative measure of crown-group eukaryoticity” (Butterfield, 2015).

Differing model assumptions for molecular clocks result in a billion-year extrapolation errors, precluding any consensus on the timing of LECA (Butterfield, 2015). One extreme is an estimate of >2.3 Ga, placing LECA around the great oxidation event and the
addition of plastids to around 1.5 Ga (Hedges et al., 2004), close to BCF times. At the other end of the scale is an extreme and controversial estimate for the timing of LECA of ~1085 Ma (950-1259 Ma) (Douzery et al., 2004). A calibration including more Phanerozoic fossil-constraints estimates the age of LECA to be ~1126 Ma (948-1357) (Berney and Pawlowski, 2006). Analysis of a taxon-rich multigene dataset gave an age of 1679-1866 Ma (Parfrey et al., 2011). A larger dataset gave a larger range of estimates between 1007 (943-1102) Ma and 1898 (1655-2094) Ma, but in all analysis the eukaryotic supergroups diverged rapidly (i.e. within 300 Ma of LECA) (Eme et al., 2014). Based on molecular clocks, LECA thus lived most likely sometime between ~1.9 and 1 billion years ago and most crown group eukaryotic groups emerged shortly thereafter. Bangiomorpha fossils indicate the presence of crown group eukaryotes by at least ~1.1 Ga and according to the rapid divergence modelled by Eme et al. (2014), LECA could have lived between ~1.4 and 1.1 Ga. In contrast, the biomarker record yields the first convincing evidence for crown group eukaryotes around 800 Ma (Brocks et al., 2015, Butterfield, 2015, Brocks et al., 2017), so in environments where biomarkers can be preserved, crown group eukaryotes were not ecologically important. It should be noted however, that convergent evolution of stem group eukaryotes may in theory also explain the appearance of fossils such as Bangiomorpha (Berney and Pawlowski, 2006).

Rather than seeing sterol evolution as a straight line of sequential improvements, more likely many now extinct lineages branched off the eukaryotic stem lineage, and some stem groups may well have outlived LECA. They were only later in Earth’s history outcompeted and probably eventually eliminated (Butterfield, 2015) (see Figure 11-18A). Similarly, many crown groups may not have left a significant biomarker record for a long time after initial emergence, and rather than indicating the initial evolution of certain sterol modification capabilities or the emergence of crown groups, the subsequent emergence of trifecta steranes in the rock record (Figure 11-18B) is more likely to reflect the rise to ecological importance of specific eukaryotic groups like red and green algae.

The current study is the first to find high quantities of likely eukaryotic steroid biomarkers in a mid-Proterozoic setting. Our findings can thus reconcile the biomarker- and microfossil record and suggest that both undifferentiated microfossils and steroids
derive from stem-group eukaryotes. This discovery could thus revolutionise our perception of early eukaryotic evolution and the ecological role that our early eukaryotic ancestors played during the Middle Ages of Earth’s history.

11.4.2 Molecular oxygen and BCF steroids

The high ratio of aromatic versus saturated steroids and high concentrations of A,B-ring aromatised diaromatic steroids strongly suggest the presence of A-ring functionalisation in the biogenic sterols, as this would probably have initiated aromatisation as is observed in plant-triterpenoids (Wolff et al., 1989) and also explains the high degree of steroid aromatisation. This conclusion is strongly supported by the similarity of the BCF steroid derivatives to those formed during our thermal maturation experiments, which also employed 3-hydroxylated protosterols. Therefore, molecular oxygen-requiring oxidosqualene, is the probable substrate of BCF steroids. The abundance of 3-hydroxylated triterpenoid derivatives in the BCF may provide some of the first geological evidence for the assimilation of molecular oxygen into the cell constituents of organism and not just production or respiration of molecular oxygen.

The abundance of protosterols and absence of more derived sterols in the BCF and potentially up to ~820 Ma ago (Brocks et al., 2017) may be directly related to low Mid-Proterozoic oxygen concentrations, but may potentially also be used to constrain them. Mid-Proterozoic oxygen concentrations may have been as low as 0.02% (Planavsky et al., 2014) and such low oxygen levels may have prevented the oxygen-intensive downstream reactions in sterol formation (Summons et al., 2006). If oxygen concentrations were limiting sterol biosynthesis in the Proterozoic at least three evolutionary scenarios are possible:

1) Sterol modifications and crown-group eukaryotes may not have evolved – partly or fully due oxygen limitations

2) Crown group eukaryotes were already evolved and possessed sterol modifying capabilities but almost exclusively produced protosterols due to the lower oxygen requirements.

3) Crown group eukaryotes were already evolved but were not as fit as stem group eukaryotes under the low oxygen conditions. The lower oxygen requirements of
stem group protosterol synthesis may have been one of the reasons for their greater ecological fitness.

Thus, low oxygen availability may have been the prime cause for the limited mid-Proterozoic sterol inventories and may have favoured stem group eukaryotes that employed less oxygen for sterol biosynthesis. This may not only have resulted in faster synthesis rates as organisms would not need to accumulate as much oxygen, but the rare molecular oxygen could also be used for respiration or other essential assimilation reactions that conveyed greater ecological fitness – offsetting the potential disadvantages of less-streamlined membrane lipids.

The strong predominance of protosterols may also inform on the oxygen content of the Proterozoic ecosystems and may provide independent evidence for low atmospheric oxygen levels. For example, S. cerevisiae yeast cells almost double squalene epoxidase activity at 0.03 vol% and almost triple it at 0.3 vol% O₂ where they almost reach maximum values (Jahnke and Klein, 1983). This attest to the efficiency of protosterol formation at probable mid-Proterozoic oxygen concentrations since the cyclisation of squalene epoxide to protosterols does not require additional molecular oxygen (Summons et al., 2006). However, additional molecular oxygen is required for most downstream modifications with the notable exception of 24-methylation of protosterol as seen in the plant pathway (Summons et al., 2006), so that from an oxygen-perspective the formation of 24-methylene protosterols would be the logical next step in sterol evolution. If this sequence of events can be confirmed in the rock record, it may provide additional evidence for the potential effect of low atmospheric oxygen concentrations on sterol evolution.

Much more molecular oxygen is required for the synthesis of evolved steroids such as ergosterol and in the yeast experiment ergosterol concentrations rise dramatically only between 0.3 and 2 vol% O₂ (Jahnke and Klein, 1983). The absence of evolved steroids from the rock record >820 Ma would thus suggest low atmospheric oxygen contents (likely <<2%). The absence from the known biomarker record does not require that evolved steroids emerged this late in Earth’s history (rather unlikely considering microfossil evidence such as Bangiomorpha and molecular clock estimates), but the source
organisms may have initially been largely restricted to highly oxygenated ecological niches. Reversely, an often inferred major Neoproterozoic oxygenation event that may e.g. have caused shifts in Cr records between ~0.8 and 0.75 Ga (Planavsky et al., 2014) may then have triggered the ecological success of oxygen-intensive sterol biosynthesis and the related rise of modern-like algae (Brocks et al., 2017).

11.4.3 BCF triterpenoids in light of the evolution of triterpenoid cyclases

The triterpenoid inventory of the BCF encompasses an entire array of tetra- and pentacyclic triterpenoids with dammarane, hopane, arborane, sterane and gammacerane skeletons. The respective cyclases may have diverged from a common ancestor, likely a malabaricane proto-cyclase or a tricyclohexaprenol cyclase (Fischer and Pearson, 2007). The presence a large number of basic triterpenoid motifs in the BCF indicates that the divergence of all major triterpenoid cyclases already occurred prior to 1.64, providing a minimum age estimate for the divergence of all basic triterpenoid cyclases.

Historically, hopane-producing squalene-cyclase (SHC) was considered to be the probable precursor of the steroid-producing oxido-squalene cyclase (OSC) (Ourisson et al., 1982). More recent considerations favour the divergence of hopanoid and steroid cyclases from a common ancestor (Fischer and Pearson, 2007). According to Fischer and Pearson (2007), different scenarios of cyclase evolution should be testable in the geologic record. The two most parsimonious scenarios described by Fischer and Pearson (2007) are generally consistent with the BCF biomarker record.

According to three main evolutionary characters of triterpenoid formation, (i) number of anti-Markovnikov ring closures (ii) CCC- versus CBC-conformation and (iii) use of squalene versus 2,3-oxidosqualene substrate (Figure 11-19), four competing phylogenies for the evolution of polycyclic triterpenoids can be constructed (Fischer and Pearson, 2007). The two most parsimonious trees suggest that hopanoid cyclases and steroid cyclases diverged from a common ancestor, probably a dammaranoid cyclase (Figure 11-19). In the first scenario, hopanoids diverged before steroid-cyclases either with an original tricyclohexaprenol cyclase as depicted in Figure 11-20A, or with a malabaricatriene cyclase as shown by Fischer and Pearson (2007). In the second scenario steroid- diverged before hopanoid-cyclases (Figure 11-20B). Any firm inferences on the
topology of the tree would require yet older biomarkers. However, the presence of all the triterpenoid motifs in microorganisms at 1.64 Ga suggests that one of the evolutionary scenarios may indeed be correct and that ancestral and sister cyclases of OSC and SHC existed in the late Paleoproterozoic.

Figure 11-19. Major categories of polycyclic triterpenoid hydrocarbon skeletons arranged according to three fundamental characters (i) the ability to promote and stabilise unfavourable anit-Markovnikov carbocations (A, A’, A’, A’’), (ii) enforcement of CBC backbone stereochemistry, and (iii) the evolution of an oxygen containing substrate reproduced from (Fischer and Pearson, 2007).

Figure 11-20. Most plausible scenarios for the evolution of the major classes of polycyclic triterpenoid cyclases following Fischer and Pearson (2007) taking into consideration the new BCF discoveries. Character symbols: square, CCC-fold, star: CBC-fold; circle, 2,3-oxidosqualene substrate; polygon, hexaprenol substrate; bar(s), anti-Markovnikov carbocation(s). T, tricyclohexaprenol
According to Fischer and Pearson (2007), the most likely candidates for being the most ancient triterpenoid cyclases are malabaricanoid or, alternatively tricyclohexaprenol cyclases, which were likely succeeded by dammaranoid cyclases (Fischer and Pearson, 2007). Like malabaricane, tricyclohexaprenol is a C_{30} tricyclic isoprenoid that can be formed via the cyclisation of a C_{30} isoprene (i.e. hexaprenol) substrate, analogous to the formation of steranes and hopanes from (oxido)squalene. In geological samples, ≤C_{30} cheilanthanes appear to originate from regular C_{30} isoprenoids like tricyclohexaprenol (Aquino Neto et al., 1981). It is thus possible that the ‘ur-cyclase’ would have produced tricyclohexaprene or tricyclohexaprenol triterpenoids and not malabaricatriene as proposed by Fischer and Pearson (2007).

According to Ourisson et al. (1982), tricyclohexaprenol could be formed anaerobically from the universal cell constituent hexaprenol, and “its shape and polarity would make it a perfect sterol surrogate” in cell membranes. The hexaprenol-cyclase could be related to the hopane-producing squalene-cyclase and potentially be even more primitive (Ourisson et al., 1982). The existence of a protocyclase that was specific to an alternate substrate like hexaprene instead of squalene, resulting in compounds such as cheilanthanes, was also considered by Fischer and Pearson (2007) as a viable alternative to a squalene-protocyclase. Like malabaricatriene (Fischer and Pearson, 2007), tricyclohexaprenol has an energetically favourable all chair conformation (Ourisson et al., 1982) and is thus a plausible product of proto-triterpenoid cyclases. Considering that BCF cheilanthanes are likely diagenetic degradation products of tricyclohexaprenol and that the second evolved triterpenoid cyclase, presumably dammarane cyclase, still seemed to have been ecological important at BCF times, it is possible that also the first evolved triterpenoid cyclase, if indeed a tricyclohexaprenol cyclase, was also still ecologically important at BCF times. Later occurrences of cheilanthanes in the rock record may reflect convergent evolution and if the ‘urcyclase’ produced tricyclohexaprenol, then many mutations that would result in damaged enzymes unable to produce evolved triterpenoids, but where ‘more primitive’ regions would still be intact allowing for production of abbreviated tricyclic products, could result in the
emergence of ‘new’ tricyclohexaprenol cyclases. With the same reasoning, modern malabaricatriene cyclases may also represent convergent evolution.

According to Fischer and Pearson (2007), the transition from chair-chair-chair (CCC) to chair-boat-chair (CBC) pre-folded substrates marks a major geologically detectable divergence in the history of polycyclic triterpenoids, but does not necessarily imply the simultaneous adoption of an O₂-requiring synthetic pathway. The occurrence of steroids and probable arborane-derivatives in the BCF sediments indicates that the evolutionary defining step from CCC to CBC substrate occurred prior to 1.64 Ga.

Since the substrate- and folding-specificity results from different domains in the cyclase enzymes, the switch from a squalene to an oxidosqualene substrate, and from a CCC- to CBC-fold was not necessarily related (Fischer and Pearson, 2007). It is thus theoretically possible that the first lipids with steroid-skeleton were derived from squalene, and lacked the 3-hydroxylation that requires molecular oxygen during biosynthesis. Therefore, molecular oxygen may not necessarily have been required in the formation of the most ancient steroids. At BCF times however, molecular oxygen-requiring oxidosqualene, is the probable substrate for steroid biosynthesis (see 11.4.2). It follows, that the sterol- and aboranol-producing organisms probably lived under at least mildly oxygenated conditions. Considering the low oxygen requirements of protosterols synthesis (Summons et al., 2006), protosterol producers may have been better adapted to low O₂ environments than modern eukaryotes, and low concentrations of molecular oxygen in the atmosphere and surface oceans would probably not (directly) have hampered the geographical distribution of exclusively protosterol-producing eukaryotes.

The presence of dammaranes that are not seen in the geological record until the Jurassic (Meunier-Christmann et al., 1991), but are the intermediates in all other triterpenoid evolutionary pathways as inferred by Fischer and Pearson (2007), suggests that some Paleoproterozoic organisms still used this more primitive triterpenoid pathway. Potentially, these organisms would thus have diverged even before the invention of sterol biosynthesis in the eukaryotic stem-lineage. The BCF source organisms may thus reflect some kind of stem groups of hopanoid producing bacteria, or even ‘proto-stem group-eukaryotes’. Similarly, the source organisms of the BCF cheilanthanes may also
be part of the stem lineage of hopane producers and may have diverged even earlier. Thus, the presence of primitive (proto)steroids, dammarane, cheilanthanes and probable arboranol derivatives suggests that organisms employing primitive cyclases inhabited the BCF waters and played a significant ecological role.
11.5. Synthesis

This study advances our understanding of the BCF steroid and triterpenoid inventory and may have impact on the reconstruction of early eukaryotic evolution. We showed for the first time how abundant BCF steroids are compared to hopanoids, and that there is a very large, previously unrecognised, diversity of sterol-derivatives. Our thermal maturation experiments showed that most of the BCF steroids can be formed from lanosterol and cycloartenol. Theoretically, all known BCF sterol-derivatives are plausible diagenetic or catagenetic products of these protosterols. The absence of any clearly indigenous steranes that are demethylated at C-4 or C-14, or alkylated at the side-chain strongly supports the hypothesis that the entire array of BCF steroids has a protosterol source. Throughout the Phanerozoic, marine steroid signatures are almost exclusively eukaryotic and microfossils clearly indicate eukaryotic communities at 1.6 Ga. Since there are no other typical eukaryotic biomarkers in the rock record until ~820 Ma and protosterols are the expected products of early eukaryotes, the BCF steroids are most plausibly attributed to largely eukaryotic source organisms.

The last common ancestor of all extant eukaryotes (LECA) should have had the capability to produce a variety of sterols, including those with 24-alkylation (Desmond and Gribaldo, 2009), and the lack of the corresponding biomarkers suggests that the BCF eukaryotes where more primitive than LECA. This implies that they belonged to the stem-lineage of the eukaryotic branch in the tree of life. Stem group eukaryotes are in fact consistent with the Paleo- and Mesoproterozoic record of undifferentiated microfossils. The first evidence for more evolved, crown group eukaryotes is Bangiomorpha, widely attributed to the red algal lineage (Butterfield et al., 1990). Bangiomorpha probably occurs ~1.1 Ga, but in the absence of direct dating could theoretically be younger (Butterfield, 2015), and even for these fossils convergent evolution of stem groups is theoretically possible (Berney and Pawlowski, 2006). The BCF steroid assemblage thus allows to finally reconcile the molecular and microfossil record, indicating that both microfossils and steroids dominantly reflect stem-group eukaryotes. With the abundant preservation of steroids in two distinct ecological endmembers, there is further no need to assume taphonomic biases or other ecological factors as an explanation for the discrepancy between the two types of proxy records.
If crown group eukaryotes were ecological significant, they should have left a biomarker record of indigenous trifecta steranes, thus the BCF eukaryotic community seems to have been dominated (near) quantitatively by stem group eukaryotes. It is possible that stem group eukaryotes remained important ecological players long after the emergence of crown group eukaryotes. Both scenarios, pre- and post- 1.64 LECA, are plausible considering the microfossil record and molecular clock estimates. In extreme scenarios, crown group eukaryotes may have evolved much later, potentially as late as the Neoproterozoic. If crown group eukaryotes were ecological significant they should however have left a biomarker record of indigenous trifecta steranes. The current pattern of a cholestane dominance in some pre-Ediacaran formations would rather argue for a late emergence of crown group eukaryotes, and a Neoproterozoic radiation is consistent with some recent molecular clock estimates that place LECA in the late Meso- to Neoproterozoic (Eme et al., 2014). This would require however, that Bangiomorpha is not a red alga, but instead for example a converged stem group fossil. Currently, the minimum timing of crown group eukaryote emergence is most reasonably defined by the Bangiomorpha microfossils and may be pinned down in the future by more accurate dating of fossiliferous formations.

Although a bacterial source can currently not be ruled out for the BCF steroids, it would be very difficult to explain the abundance of bacterial steroids, eukaryotic microfossils and the simultaneous absence of other eukaryotic steranes. The ratio of steroids to hopanoids in many samples is surprisingly high, indicating that stem group eukaryotes likely played an important role and may even have been comparable in abundance to their Phanerozoic counterparts. In contrast to the strong prokaryotic dominance usually inferred for Proterozoic ecosystems (see e.g. Butterfield, 2015) the new data suggest that eukaryotes were important already. At the same time, our data indicate that eukaryotes were stem group representatives, consistent with the microfossil record (Knoll, 2014), but in contrast to earlier biomarker studies (e.g. Brocks et al., 1999, Waldbauer et al., 2009). The inferred arborane-derivatives may potentially also be of eukaryotic origin, in which case eukaryotes would have been even more abundant than inferred from steroids.
The presence of an ancestral dammarane triterpenoid may point towards some kind of proto-stem group eukaryotes that still retained the energetically favourable CCC substrate-configuration in their membrane lipids, although, here again, it is impossible to rule out a bacterial source. The abundance of steroids and other triterpenoids derived from likely 3-hydroxygenated lipids such as isoarborinol would also allow for some of the stem group eukaryotes to potentially have been important primary producers that may have outcompeted cyanobacteria particularly in nutrient-rich near-shore environments, but if Paleoproterozoic eukaryotes possessed the necessary toolkit to carry out photosynthesis is another question that is out of the scope of this thesis.

This thesis may thus provide important insights into the Paleoproterozoic ecology and early eukaryotic evolution. It may change how we look at the Proterozoic biomarker and microfossil records and which biomarkers we consider as probably eukaryotic in ancient sediments. This first clear recognition of the existence and potential abundance of stem eukaryotes in the Palaeoproterozoic also warrants a fresh look at eukaryotic acritarchs and their assignment to crown groups, in particular algae.
11.6. References


12 Highlights, outlook and future work

12.1 Research highlights

Implications of this study range from the interpretation of the palaeoredox conditions and anoxic phototrophic bacterial communities of the BCF waters to base metal mineralisation, the potential dynamic nature of Proterozoic environments, and early eukaryotic evolution. The most important findings and implications are summarised in the following.

12.1.1 Proxy correlations and two-endmember mixing

One of the main outcomes of this research project is the discovery of unprecedented correlations between biomarker and other geological proxies in BCF sediments. Proxy data in drill core GR7 are best explained by a two-endmember mixing model. This has important implications for palaeoecological reconstructions and offers the unique opportunity to learn about the potential small-scale variability of Paleoproterozoic ecosystems. Intriguingly, iron speciation redox proxies correlate with organic redox proxies, and even proxies of microbial community composition – a unique observation in the geological record. A plausible ecological scenario would be a geographic endmember mixing, where the green ecological endmember reflects benthic microbial mats and the purple endmember the water column ecosystems. If this model was correct, it would have profound implications for the interpretation of Precambrian biomarker and redox proxies. However, this scenario is incompatible with the iron speciation proxies and would mean that this widely applied palaeoredox tool cannot be applied in its current form to microbial mat settings and, thus, many Precambrian sedimentary sequences. It remains to be seen in studies of modern microbial mat analogues if microbial mats could be responsible for the green ecological state signature.

Assuming that iron speciation reflects Proterozoic water column chemistry as is the current consent in the palaeo-geochemical community (Poulton and Canfield, 2011, Planavsky et al., 2011), the most plausible ecological model is a temporal mixing model, where the marine ecosystem fluctuates between a ferruginous ecological state
dominated by planktonic purple sulphur bacteria, and a euxinic green state dominated by planktonic green sulphur bacteria. Currently, only planktonic PSB are known to produce okenone making this the most plausible model. The strong correlation between Pr/Ph and the G/(G+P) proxy further indicates that the shift in phototrophic sulphur bacterial communities is due to a shift in the depth of the chemocline. Euxinic conditions that correlate with periods of higher siliciclastic deposition can be explained by enhanced terrestrial sediment and nutrient influx. An increase in nutrient fuelled primary production would in turn have provided enhanced nutrition for sulphate reducing bacteria that produced hydrogen sulphide. When the bacterial sulphide production overwhelmed the influx of reactive iron into the system, at some point all dissolved iron may have been titrated out of the water column, resulting in euxinia. Since the vast majority of samples show a mixed ecological signature, the temporal fluctuations must have occurred at a subsample scale and thus in less than a few hundred or thousands of years. The temporal fluctuations between ferruginous and euxinic water column conditions would thus have been frequent and potentially even seasonal. It is the first time that such frequent redox fluctuations were observed in a Precambrian setting.

These frequent redox fluctuations may be analogous to a sulphidic wedge that pulsed in and out of the Glyde River area. Current models of the Proterozoic marine redox landscape envisage the development of sulphidic wedges along continental margins of ferruginous seas. For the first time, the potential short-term dynamics of such a near-shore marine setting could be illustrated. Furthermore, the BCF correlations provide insights into the potential mechanisms that drive the development of euxinia in mid-Proterozoic marine basins, namely nutrient influx or nutrient redistribution through mixing processes. In the BCF waters, the nutrient influx was likely mostly terrestrial and related to precipitation variability, while possible nutrient redistribution may have been linked to mixing mechanisms such as wind activity. It is thus plausible that the BCF redox conditions, as well as parts of the microbial communities, where controlled by wind and rain activity that fluctuated on small, possibly seasonal, time scales.
12.1.2 Large scale proxy variations and orbital climate cycles

In addition to the inferred sub-sample fluctuations between two distinct ecological endmember states, there are long-term trends in the BCF proxy-correlations. Variations in climatic factors such as wind, rain and evaporation can explain all the BCF proxy variations. The most obvious explanation for the large-scale proxy trends would thus be long-term climate variations. In the Phanerozoic, climate cycles are often controlled by orbital processes and the cyclicity in the proxy data is evocative of orbital climate cycles recorded in many younger marine sediments. Due to the antiquity of the BCF sediments, potential time scales of the two sulphidic excursions can only be very roughly estimated, but they seem to be generally consistent with orbital time scales. It is thus possible that, like in the Phanerozoic, orbital cycles controlled climate and ecology in the Paleoproterozoic, including redox conditions and microbial communities of marginal marine basins. Orbital climate fluctuations were recently advanced to explain changes in the geochemistry of the 1.4 Ga Xiamaling Formation in China. Geochemical fluctuations are interpreted to reflect orbitally forced changes in wind patterns and ocean circulation that influenced rates of organic matter flux, trace metal deposition and sources of detrital material (Zhang et al., 2015), strengthening our BCF proxy interpretations. Climate variability is frequently recorded in modern lakes and restricted marine basins, and it is thus plausible that the sediments of the restricted McArthur Basin record the orbitally controlled climatic variability and resulting ecological perturbances that occurred 1.64 Ga ago.

12.1.3 Implications for base metal mineralisation

The Mt Isa Superbasin is one of the world’s most important regions for economic base metal deposits. There, the BCF in the McArthur Basin hosts one of the world’s biggest stratiform lead zinc deposits, the HYC McArthur River deposit, and other deposits such as Myrtle. The palaeoredox conditions probably played an important role in the formation of these giant deposits. The reconstruction of the BCF redox conditions as advanced in this study can thus offer new insights into the formation of sygenetic base metal deposits. A temporal two endmember mixing model with frequent fluctuations between ferruginous and sulphidic conditions would have profound implications for
syngenetic base metal mineralisation. Formation of major base metal deposits may have been restricted to local exhalative events that coincided with long term euxinic excursions. The identification of euxinic horizons may thus have potential as vectors for base metal prospective horizons. At the same time, subsample fluctuations between ferruginous and sulphidic redox conditions provide an alternative explanation for the intricate small-scale lamination of the HYC ores. Frequent variations in the degree of pyritisation were previously observed at HYC, but it was considered “unlikely that redox conditions in the McArthur basin oscillated from reduced to oxidised millions of times during BCF deposition” and a vault-valve mechanism was proposed instead to trigger frequent influx of oxygenated turbidites from marginal shallow water oxic environments and oscillating release of mineralising brines from the fault (Large et al., 1998). However, we now found that across the McArthur Basin low degrees of pyritisation do not reflect oxic, but instead anoxic ferruginous conditions that could not be recognised before development of the refined iron speciation technique (Poulton and Canfield, 2005). Independent evidence for frequent redox fluctuations in the Glyde River area suggest that variations in the degree of pyritisation observed at HYC may also reflect variations between sulphidic and ferruginous conditions. Thus, the temporal two endmember mixing also provides a potential explanation for the intriguing fine lamination of the stratiform base metal sulphides at HYC. Considering the inferred climatic control of the BCF proxy correlations, it is thus possible that even the economic base metal mineralisation was ultimately controlled by climatic variations that coincided with local exhalation of base metal-rich fluids along fault zones.

We also found that FeHR/FeT is enriched in drill cores from the central basin, which is best explained by enhanced influx of reactive iron from hydrothermal fluids. Future research needs to test how far these exhalative FeHR/FeT signatures extent from the deposits. Potentially, FeHR/FeT could be used as another vector towards base metal deposits and may be particularly useful as exhalation of Fe-rich fluids outlived exhalation of base metal-rich fluids in the basin, and it may thus indicate in accessible higher parts of the stratigraphy proximal exhalative activity in deeper, less accessible stratigraphy.
12.1.4 Aromatic triterpenoids

For the first time, the BCF inventory of aromatic triterpenoids was comprehensively investigated, yielding surprising results. Although benzohopanes, which constitute the most common aromatisation products of hopanoids, were close to detection limits in many BCF samples, a large number of previously unrecognised tri- (TrAT) and particularly tetra-aromatic (TeAT) triterpenoid-derivatives were discovered. The aromatic triterpenoids are more abundant than saturated hopanes and are thus very important for palaeoecological reconstructions. The restriction to methyl-, ethyl- and isopropyl-substitution strongly indicates a C\textsubscript{30} biogenic precursor for most of the aromatic triterpenoids. The scarcity of such triterpenoid patterns in the literature points to an unusual source, and the high degree of aromatisation points towards ring-functionalised precursors. Hopan-3-ols, eudoraenol, adriaticol or isoarborinol are the most plausible biogenic precursors for the majority of TrATs and TeATs, and these may have a bacterial or eukaryotic origin. High relative abundances of some TeATs with ethyl side-chain in the BCF, particularly those with mono-methylation of the core structure are likely derived from 28,30-bisnorhopane-type precursors such as 28,30-dinorneohop-13(18)-ene potentially produced by chemoautotrophic bacteria living at the chemocline (Sinninghe Damsté et al., 2014), and these yet to be discovered bacteria may have been important ecological players in Paleoproterozoic ecosystems.

The co-occurrence in some BCF samples of (poly)methyl-picenes, likely derived from tetrahymanol, and TrATs and TeATs, potentially derived from hopan-3-ol, may indicate the activity of a squalene-tetrahymanol cyclase (STC) because in some modern anaerobic eukaryotes STC produces both tetrahymanol and hopan-3-ol. Modern anaerobic eukaryotes seem to have acquired STC several times independently, possibly from an unknown bacterial source, and it is thus possible that Proterozoic stem-group eukaryotes also independently acquired this capability that seems to offer evolutionary advantages in low-oxygen environments. As in modern marine sediments, most of the BCF tetrahymanol derivatives could thus stem from eukaryotic sources that may have been well adapted to the low oxygen conditions in Proterozoic seas.
12.1.5 Arborane/fernane-derivatives

Some of the BCF aromatic triterpenoids are unlikely to have a hopanoid precursor, but instead appear to be derived from an arborane-or fernane-type biogenic precursor such as isoarborinol. This is a very surprising finding as such compounds are not encountered in the geologic record for over a billion years until the Jurassic. Intriguingly, isoarborinol is the pentacyclic epoxy-squalene cyclisation analogue of tetracyclic lanosterol and was considered the most likely intermediate in the evolution of sterols from hopanoids by Ourreison et al. (1982). More recent considerations by Fischer and Pearson (2007) suggest however, that hopanoid-, isoarborinol- and sterol-cyclases may have diverged from a common ancestor. In the context of the BCF steroid and hopanoid assemblage, isoarborinol appears to be the most likely precursor for the BCF arborane/fernane-type aromatics. From an evolutionary perspective, Paleoproterozoic isoarborinol is compatible with both Ourreisson’s and Fischer’s hypotheses of triterpenoid evolution. Isoarborinol may represent the evolutionary intermediate between hopanoid and steroid synthesis, or reflect a divergence of steroid-, hopanoid- and isoarborinol-cyclases predating the BCF. The most plausible explanation for the BCF arboranes is provided by the recent discovery of bacterial eudoraenol synthase (EUS). It is currently unclear if a similar arborane-type cyclase existed in stem group eukaryotes and the BCF arboranoids may have a bacterial or eukaryotic origin.

12.1.6 Steroids

This study greatly advances our understanding of the BCF steroid inventory and may have a strong impact on the reconstruction of early eukaryotic evolution. Previous reports of more Phanerozoic-like steroid assemblages are likely contamination artefacts (e.g. Pawlowska et al., 2013). Instead, it was recognised that typical Phanerozoic steranes such as cholestane, ergostane and stigmastane are below detection limits in the BCF, while mostly 4-methyl triaromatic steroids are abundant (Brocks et al., 2005).

In this study, it was shown for the first time that mono- and diaromatic steroids also occur, and that the diaromatic steroids are usually even slightly more abundant than the triaromatic steroids. Surprisingly, in many samples, total steroids, including the
aromatics, are in much higher concentration than hopanoids, even if abundant TeATs were derived from hopanoid precursors. The high abundance of steroids points towards a significant ecological role of the source organisms. We further demonstrated that most of the BCF steroids can form through the thermal maturation of the protosterols lanosterol and cycloartenol. The entire array of BCF steroid derivatives is plausibly derived from such protosterol precursors. Our new findings show that previous explanations attributing the paucity of steroids in Proterozoic sediments to a preservation bias (Pawlowska et al., 2013) are false.

Although the protosterol derivatives in the BCF may have a bacterial or eukaryotic source, based on ecological considerations a largely stem eukaryotic source is more likely. This study is the first to find high quantities of steroid biomarkers as well as steranes in a mid-Proterozoic setting, and it is the first direct detection of stem-group eukaryotes. Our findings can thus reconcile the biomarker- and microfossil record suggesting that both undifferentiated microfossils and steroids derive from stem-group eukaryotes. This discovery could thus revolutionise our perception of early eukaryotic evolution and the ecological role that our early eukaryotic ancestors played in the mid-Proterozoic.

12.1.7 Dammaranes

Another important BCF biomarker discovery are dammaranes. Triterpenoids with a dammarane-skeleton are structural intermediates between hopanoids and steroids, and are thus likely evolutionary intermediates in the sterol evolution pathway. Based on amino acid sequence analysis, minimum evolution and biochemical energetics, dammaranoid-cyclases may be the precursors of later triterpenoid cyclases (Fischer and Pearson, 2007). BCF dammaranes may potentially be derived from yet more primitive proto-stem-group eukaryotes or ancestors of hopanoid-producing bacteria.

12.1.8 The combined BCF triterpenoid record

The new BCF triterpenoid record as elucidated in this study encompasses the entire array of basic triterpenoid skeletons of the dammarane, hopane, arborane, sterane and
gammacerane-types. This is consistent with the inferred divergence of the triterpenoid proto-cyclase into cyclases for all these triterpenoids (Fischer and Pearson, 2007). This divergence must have occurred before 1.64 Ga. Historically, steroid producing oxidosqualene cyclase (OSC) was considered to have likely evolved from the hopane-producing squalene-cyclase (SHC) (Ourisson et al., 1982), but more recent considerations favour the divergence of hopanoid and steroid cyclases from a common ancestor (Fischer and Pearson, 2007). Both scenarios are consistent with the BCF biomarker record. According to Fischer and Pearson (2007), the transition from CCC to CBC pre-folded substrates marks a major geologically detectable divergence in the history of polycyclic triterpenoids, but does not necessarily imply the simultaneous adoption of an O2-requiring synthetic pathway. The occurrence of steroids in the BCF sediments indicates that the evolutionary defining step from CCC to CBC occurred prior to 1.64 Ga. The high ratio of aromatic versus saturated steroids strongly indicates the presence of a C-3 hydroxylation and thus an oxidised substrate requiring molecular oxygen for its synthesis.
12.2 Future work and implications for other studies

The discovery of a large variety of previously unrecognised steroids and other triterpenoids in the BCF offers great potential for future studies. There remain additional biomarkers to be discovered. Due to time constraints, many compounds could thus far only be tentatively identified. More rigorous structure elucidations would greatly help to decipher the relationships between the diverse triterpenoid derivatives, and to pinpoint the most plausible ecological sources.

Currently, the greatest obstacle for a more thorough characterisation of the BCF biomarkers is the complexity of the bitumen mixtures. Particularly the high unresolved complex mixture that underlies all BCF chromatograms precludes meaningful isotopic analysis and hampers isolation of individual compounds. For example, Brocks and Schaeffer (2008) attempted to measure the isotopic signature of BCF arylisoprenoids, but even a combination of column chromatography, thin layer chromatography and preparative GC did not sufficiently reduce the UCM (Brocks and Schaeffer, 2008). The characterisation of detailed structures and isotopic makeup of the BCF triterpenoids is thus not an easy task. However, the combination of a variety of organic geochemical techniques, particularly column chromatography, high pressure and gel permeation chromatography, and preparative gas chromatography, should eventually allow to isolate and characterise at least some of the many BCF triterpenoids that have now been discovered. Removal of a large proportion of the unresolved complex mixture would also allow to determine the isotopic composition of the 2,3,6-arylisoprenoids, which may inform on green sulphur bacteria or oxygenic phototrophic (β-carotene-derived) sources.

Furthermore, carbon isotopes may inform which compounds are likely to have a common biogenic source. Isotopic studies might further elucidate if the abundant TeAT are associated with hopanoids or arborane -derivatives or entirely different precursors. For arborane-type precursors, one would expect the tri- and tetra-aromatic triterpenoids to show an isotopic signature most similar to DAPH 1, 2 and MAPH. Further, these compounds could potentially be isolated and optical rotation measurements and chiral separations on HPLC columns with β-cyclodextrin phase may be conducted as
demonstrated on younger material (Hauke et al., 1995). This should allow to distinguish between fernane- and arborane skeletons.

A more thorough quantification of all triterpenoids including picenes is also suggested to gain a better understanding of the relative importance of the source organisms. To this end, aromatic hopanoids, triterpenoids and aromatic steroids should also be included in steroid/hopanoid and steroid/triterpenoid ratios from diverse Phanerozoic marine settings that lack significant terrestrial (plant) input in order to put the Paleoproterozoic ratios into a better perspective. Steroid and triterpenoid abundances should be compared to diverse biomarkers from different sources such as \( n \)-alkanes, pristane and phytane and arylisoprenoids in both, the BCF and representative Phanerozoic samples. This may provide insights into the abundance of the different triterpenoid precursors in Proterozoic ecosystems.

The search for the unusual BCF triterpenoids should further be extended to other Proterozoic formations. I predict that some of the BCF triterpenoids will also be encountered in other mid-Proterozoic basins. Finally, the integration of the indigenous Proterozoic steroid assemblage with microfossil data and the latest insights of the evolutionary relationships of different groups of organisms, as well as robust molecular clock estimates, should allow to constrain the evolution of eukaryotes, and potentially different groups of triterpenoid producing bacteria, in unprecedented detail.

Eventually, the comparison of maturation products of diverse triterpenoid precursors and diverse experimental conditions in combination with detailed structure elucidations and isotopic analyses may allow to confidently assign certain triterpenoids to biogenic precursors. This may in turn give important insights into early (eukaryotic) evolution and the constitution of Paleoproterozoic ecosystems.

The occurrence of arborane/fernane type compounds in the BCF also strongly strengthens the assumption of a microbial source for some of these compounds in some modern settings and I propose a detailed investigation of the lipid inventories of modern ecosystems with respect to identifying such organism. Intriguingly, while writing the thesis, a bacterial source of arborane-type triterpenoids was identified by (Banta et al., 2016). It would nevertheless be valuable to test for the existence of analogous cyclases in
extant eukaryotes, as isoarborinol is also found in dominantly microbially-sourced biomarker signatures.

Regarding our multi-proxy analyses, the two-endmember mixing model proposed for the BCF should be investigated in more detail. In a first step, it should be examined whether the correlations can be replicated in other parts of the basin to test whether this is a regional (basinal) or local signature. More detailed sedimentological studies on the BCF sediments from GR7 may help to distinguish between a temporal and geographical mixing models. It would also be interesting to see if the green endmember biomarker signatures can be the product of diagenetic or microbial alteration of a purple endmember signature – a possibility that is currently considered unlikely but that cannot be excluded. Further, the implications of the BCF redox interpretations (either frequent temporal fluctuations or water column versus microbial mat signature) on base metal mineralisation should be investigated in more detail. To this end, the HYC deposit should be re-visited and the feasibility of the ecological-fluctuation model examined for the mineralised horizons. It should be investigated in detail if iron speciation can assist base metal exploration in the McArthur or other Proterozoic basins. FePy/FeHR may have potential as a vector towards base metal prospective horizons, whereas locally elevated FeHR/FeT ratios may potentially be used as a vector towards exhalative activity even lower in the stratigraphy since in the McArthur Basin exhalation of base metal-rich fluids ceased when iron-rich hydrothermal fluids were still exhaled into the water column.

Hopefully this study can help to interpret the Precambrian biomarker record in a more meaningful and integrated way. Subsamples fluctuations and two-endmember mixing should receive more consideration in paleo-ecological reconstructions and this study may help to recognise two-endmember mixing in other studies. The possibility of sub-sample fluctuations should be considered particularly in paleo-redox reconstructions. The detailed integration of biomarkers with Fe-speciation and other proxies as advanced in this study offers great potential and should be more widely applied.

Generally, more efforts should be put into the identification of microbial mats in Precambrian siliciclastic sediments through geochemical, sedimentological and petrographic analyses. It should be investigated if microbial mats can significantly
influence Fe-speciation signatures. Further, the impact of microbial (mat) reworking on biomarkers such as arylisoprenoid, phytane and hopanes should be investigated in more detail. This may inform on the feasibility of alternative interpretations for the BCF endmember ecologies and would be of great interest for most Precambrian ecological reconstructions.

Lifting the veil of Phanerozoic contamination overprint, we start to see the real picture of Precambrian evolution, particularly with respect to our early eukaryotic ancestors. The common perception of advanced eukaryotic communities existing long before the great oxidation event gives way to a more nuanced picture of much later and more stepwise evolution of different groups of eukaryotes. In my view, stem group eukaryotes may have played an important role throughout much of the mid-Proterozoic and crown group eukaryotes may only have emerged in the Neoproterozoic. In my opinion, the BCF protosterol discovery, particularly the saturated equivalents, is a key finding that greatly strengthens the Bloch hypothesis and constitutes the missing link to explain the apparent discrepancy in the pre-Ediacaran molecular and microfossil record. A more concerted search for intermediate products in the synthesis of evolved sterols may confirm Bloch’s hypothesis of stepwise sterol evolution in the rock record. I am looking forward to seeing the ‘complete’ contamination-free Precambrian biomarker record assembled and interpreted in the light of early eukaryotic evolution.
12.3 References


Appendix 2: Overview of labwest MMA-04 elemental analyses

Table A2-1. Overview of 61 elements analysed by labwest in their microwave assisted total digestion analyses, including detection limits and upper range (table from labwest.net).

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<td>Ca</td>
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<tr>
<td>Fe</td>
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<tr>
<td>Si</td>
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<td>10 ppm</td>
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</table>

This image may contain third party content and was omitted from the digital version of the thesis. Please refer to the hardcopy version deposited in the ANU library or contact the author.
Appendix 5.1 Results of iron speciation & XRPD analyses

Table A5.1-1. Fe-speciation data for drill core Bing Bong^1^  

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<th>depth (m)</th>
<th>name</th>
<th>FeCarb wt%</th>
<th>FeOx wt%</th>
<th>FeMag wt%</th>
<th>FePy wt%</th>
<th>FeT wt%</th>
<th>FeHR wt%</th>
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<td>0.69</td>
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Table A5.1-2. Fe-speciation data from the Lynott Formation in drill core Cow Lagoon 1

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Table A5.1-3. Fe-speciation data for Glyde River drill cores^2^  

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^1^ (n.d.: not determined, avg.: average  
^2^ n.d.: not determined, avg.: average, rep.: repeat  
390
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3 n.d.: not determined, avg.: average, av.s.: average for subsample. Subsamples: Samples collected from drill core store have been divided into 2-3cm intervals and analysed separately. Av.s.u indicates the averaged signal, see “subsamples” for individual results.
### Table A5.1-5. Fe-speciation data for drill core MacArthur\(^4\)

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<th>FeHR wt%</th>
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<th>FePy/FeHR</th>
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Replicates

- B03117 elemental analysis 2.47
- B03117 elemental repeat 2.49

### Table A5.1-6. Fe-speciation data for drill core Myrtle 5\(^5\)

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\(^4\) n.d.: not determined, avg.: average

\(^5\) n.d.: not determined, avg.: average, rep.: repeat
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Replicates

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12Z123 Labwest elemental repeat 2.29

* n.d.: not determined, avg.: average, rep.: replicate

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Table A5.1-7. Fe-speciation data for drill core Myrtle 46

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Figure A5.1-10. FePy/FeHR for drill core LV09001.

Figure A5.1-11. FePy/FeHR for drill core MYS
Figure A5.1-12. FePy/FeHR for drill core MY4.

Figure A5.1-13. FePy/FeHR for drill core McArthur 5.
Figure A5.1-14. FePy/FeHR for drill core Wamara 6.

Figure A5.1-15. FePy/FeHR for drill core BB5.
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**Table A5.1.9. Results of XRD analyses.**
Appendix 5.2: All proxy crossplots from mixing simulation

Table A5.2-1 shows the plot numbers used in the following for mixing plots of different ecological proxies.

Table A5.2-1. Overview of mixing simulations and number of graph in the following plots. Due to the similarity to Ca, not all carbonate plots are shown (X).

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Table A5.2-2 shows the inferred endmember compositions and values obtained if the two endmember values are mixed in the ratio shown in the first column. The following figures contain all crossplots of BCF ecological proxies from the upper 200 m in GR7, as well as mixing lines resulting from the mixing simulation shown in Table A5.2-2. All elemental abundances are in weight %. Biomarker concentrations are expected to be strongly affected by post-mixing alteration like hydrocarbon cracking and expulsion, so absolute concentrations were arbitrary chosen for the simulation and only biomarker ratios considered to be meaningful.
The variables for the endmember states (marked in green and purple) are set and then mixed in the proportions indicated in each mix, the mixing ratios are set in the first column (0.95 meaning 95% green and 5% purple), the post-mixing endmember contribution. The post-mixing variables for the individual variables are then calculated, this is illustrated for Pr and Ph. Ratios (e.g. Pr/Ph) are then calculated form the new variable values in each mix. The proxy values are calculated for the corresponding mixing ratio by dividing the new values of Pr by the Pr(purple state) and the new Prisatn/pristane ratio is then calculated form the formula: ‘mix ratio’ * Pr(green state) / (1 - ‘mix ratio’).

The mixing ratios are in the first column for the correponding mixing ratio: 0.00, 0.05, 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, 0.80, 0.90 and 0.95.

The mixing ratios are in the first column and the corresponding mixing ratios are calculated then mixed into the proportions indicated in each mix.
Figure A5.2-1. Two-endmember mixing simulations for BCF ecological proxies.
## Appendix 8.1 Peak and structure labelling

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**Notes:**
- H2 pyro first MAS
- H2 pyro 2nd MAS
- B-ring monoaromatic steroid
- STD
- side-chain aromatised
- aromatised side-chain
- 'aromatic protospirostereoids'
- adriaticol
- eudoraenol
- isoarborinol
- fernenol
Appendix 8.2: Mass spectra of tetraaromatic triterpenoids (TeAT)

Figure A8.2.1. Mass spectra of tetraaromatic triterpenoids (TeAT).
Tetraaromatic Triterpenoid spectra

Appendix 8.2

B03166 aros, 1/10 EOM (DCM column extract), 60ngD4 + 2ug 18-MEAME, 1/250 ul.
15 Aug 18:32 at23 (16:14) Ch (2125,2128-4127,4129+4119)
Tetraaromatic Triterpenoid spectra

Appendix 8.2

B03166 aros, 1/10 EOM (DCM column extract), 50ngD4 + 2ug 18-MEME, 1/250 uL

13 Aug 18 32 4049 (74.069) Cm (4007-4049-(4052-4055+4044-4045))

Magnet Ei+

17

B03166 aros, 1/10 EOM (DCM column extract), 50ngD4 + 2ug 18-MEME, 1/250 uL

13 Aug 18 32 4089 (75.611) Cm (4089-4083-4064))

Magnet Ei+

18
Peak 19 (IIc)

m/z 295

20
Appendix 8.2

Subtraction artefact
Coeluting 338->295 (?)

23

Coeluting 338(?)

24
Tetraaromatic Triterpenoid spectra

Appendix 8.2
Appendix 10: BCF aromatic steroids

1. Diaromatic steroids

In addition to the main peaks apparent in Figure 10-4, a closer examination of the m/z 376, 390 and 404 mass chromatograms in the cycloartenol pyrolysis experiment revealed a variety of different diaromatic steroid isomers (Figure A10-1). At least 32 different diaromatic steroids were produced in the experiment. The C28, C29 and C30 isomers all yield very similar mass spectra (Figure A10-2, Figure A10-3 & Figure A10-4). At least seven isomers can be distinguished for the C28, 11 for the C29 and 15 for the C30 steroids (Figure A10-1). It is remarkable, that C30 steroids are produced in such abundance and yield such a variety of isomers in the pyrolysates. Aromatisation of the A and B ring requires the loss or shift of two methyl-groups, yet both are retained in the C30 steroids. The variety of isomers in the pyrolysate shows that various methyl-shifts occur during thermal maturation of the steroids in the presence of a carbon catalyst.

Figure A10-1. High boiling range of cycloartenol pyrolysis experiment (330°C, 18h active carbon). A) TIC, selected ion chromatograms: B) m/z 376, C) m/z 390, D) m/z 404.
Figure A10-2. C_{28} diaromatic steroids produced during cycloartenol pyrolysis (330°C, 18h active carbon).
Figure A10-3. C$_{29}$ diaromatic steroids produced during cycloartenol pyrolysis (330°C, 18h active carbon).
Figure A10-4. C$_{30}$ diaromatic steroids produced during cycloartenol pyrolysis (330°C, 18h active carbon).
2. C\textsubscript{30} monoaromatic lanosteroids (C\textsubscript{30} MALS)

Compounds with a molecular mass of 408 Dalton are among the most abundant lanosterol and cycloartenol pyrolysis products. The second biggest peaks in both non- and hydrogenated lanosterol pyrolysates represent a M\textsuperscript{+} 408 doublet similar to that of the M\textsuperscript{+} 394 compounds, and the mass spectra are very similar but shifted by fourteen mass units (Figure A10-5). These compounds thus appear to be C\textsubscript{30} monoaromatic lanosteroids. It is interesting that, like the diaromatic steroids, most monoaromatics retain all methyl-groups during artificial maturation. This requires the shift of one methyl group in B-ring, and 2 methyl groups in A-ring aromatics. Monoaromatic lanosteroids can be expected to be mostly B-ring aromatised as this only requires the shift or loss of one methyl group. Although C\textsubscript{30} monoaromatic lanosteroids were not observed in the BCF, their high abundance in the pyrolysis experiments indicates that they could potentially be preserved in thermally less mature formations and maybe be detected in future studies.

![Figure A10-5](image_url)

*Figure A10-5. Mass spectrum of inferred C\textsubscript{30} monoaromatic lanosteroid produced during lanosterol pyrolysis.*
Appendix 11: Triterpenoid concentrations

The ion response factors (IRF) calculated from base ions relative to the internal standard (IS) 18-MEME in full scan analyses used for triterpenoid quantifications in chapter 11 are shown in Table A11-1. Ion factors (IF) were calculated by dividing the base ion abundance in a mass spectrum (as clean as possible) by the total ion abundance. Ion response factors were then calculated by dividing the \( m/z \) 340 IF of the IS by the IF of the base ion of the compound of interest. Absolute concentrations (ng/g extracted rock) obtained in the usual way by multiplying the peak area of the compound of interest in the base ion trace with the mass of the internal standard (in ng) and, if applicable, with a dilution factor, and dividing by the area of the internal standard in the \( m/z \) 340 trace and the rock mass employed for extraction (in g), were then multiplied with the IRF. Ion response factors correct for fragmentation differences between different compounds relative to the internal standard to obtain more accurate concentration estimates and allow a much better comparison of absolute concentrations of different compound classes. To obtain non-fragmentation corrected triterpenoid concentrations (in ng/g of rock), corrected concentrations in Tables A11-2 to A11-9 would need to be divided by the IRFs of the compound class shown in Table A11-1.
Table A11-1. Overview of ion factors (IF) for base ions and corresponding ion response factors (IRF) relative to 18-MEME (integrated in m/z 340) that were used for triterpenoid concentrations.

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### Appendix 11: Triterpenoid concentrations

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#### Table A11-3. Core-monomethylated TeATs (in ng/g rock, fragmentation corrected).

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Table A11.9: Steranes and triterpenoid concentrations (ng/g rock, fragmentation corrected).
Figure A11-1. Proposed diagenetic scheme for BCF triterpenoids part 1 (also in main text, can be cut out to more easily follow discussions in chapters 8-11).
Figure A11-2. Proposed diagenetic scheme for BCF triterpenoids part 2 (also in main text, can be cut out to more easily follow discussions).