Department of Chemistry

Novel biomarker and stable isotopic approaches for palaeoenvironmental reconstruction of saline and stratified ecosystems:
The modern Coorong Lagoon and Devonian reefs of the Canning Basin

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Svenja Tulipani

Perth, May 20th, 2013

Abstract

Several large mass extinction events have occurred throughout Earth's history and significantly shaped life on our planet by both, wiping out a large variety of species on the one hand, and promoting the evolution of new life forms to fill empty niches on the other. The largest extinction occurred about 252 million years ago (Ma) at the Permian-Triassic boundary, wiping out 80-96% of all marine species. The Late Devonian extinction, which has been further investigated in this PhD project, is also ranked amongst the biggest mass extinction events in the Phanerozoic. Causes and nature of most extinction events are still poorly understood and their investigation seems particularly relevant in the present-day since scientific evidence points to perhaps another imminent mass extinction, this time predominantly driven by human impact such as large scale changes of natural environments and the promotion of climate change (e.g. burning of fossil fuels).

The analysis of sedimentary biomarkers (i.e. molecular skeletons) and their stable isotopic composition bears a great potential for the investigation of recent and ancient palaeoenvironments including extinction horizons since they can be used to help reconstruct environmental and climatic conditions or organic matter (OM) sources in past depositional settings. Although some biomarkers are source specific and have been used as established palaeoenvironmental indicators for several decades, the field of biomarker research is highly dynamic. New relationships between organic constituents of palaeoenvironments and extant organisms are still explored to identify new biomarkers and establish origin or formation pathways of many sedimentary compounds which presently remain unknown.

In this PhD-project a broad range of applications for sedimentary biomarker studies and stable isotope analysis was demonstrated by the reconstruction of palaeoenvironmental changes in (i) the recent Coorong Lagoon, an estuarine ecosystem in South Australia severely impacted by human practices and (ii) ancient marine environments associated with reef-systems affected by the Late Devonian extinction. A primary focus of this project was the reconstruction of salinity and water-column stratification. Both conditions can significantly impact estuarine and marine ecosystems and often correlate with each other. Water-column stratification was particularly widespread in (global) oceanic anoxic events associated with many

mass extinction events and may have also contributed to the Late Devonian extinctions.

In **Chapter 2** of this thesis, recent ecological changes in the northern Coorong Lagoon were investigated by the analysis of distributions and stable isotopic composition ($\delta^{13}C$ and δD) of biomarkers as well as $\delta^{13}C$ of bulk OM in a sedimentary core spanning the past ~5000 years. The aim was to reconstruct palaeoenvironmental conditions such as salinity and OM matter sources in the North Lagoon prior to European settlement, providing a natural baseline to explore the extent and nature of changes in these parameters which occurred as a direct result of human modifications to the complex natural water-regime. The Coorong is part of a terminal lake system at the mouth of the River Murray, which is of great ecological significance but has undergone a severe decline in biodiversity over the past decades largely driven by a significant increase of salinity caused by a progressive isolation of the lagoon complex by human practices as well as a prolonged drought from 2000-2010. Changes in molecular distributions of long-chain *n*-alkanes, hopanoids and steroids as well as an increase in phytoplanktonic biomarkers and a shift in $\delta^{13}C$ of bulk OM and *n*-alkanes indicated significant changes in OM sources and primary production in the North Lagoon after the 1950s, most likely due to the aquatic macrophytes changing towards a major algal and (cyano)bacterial input. δD profiles of *n*-alkanes revealed variable salinities throughout the Holocene but yet with an overall increasing trend. However, the most significant increase occurred over the past decades. The changes in the investigated biomarker parameters were consistently much more pronounced in sediments deposited after the 1950s coinciding with the implementation of substantial water management practices compared to variations in the older sediments representing several thousand years.

Chapter 3 presents a new analytical approach with the potential to elucidate the origin and formation pathway of sedimentary methyltrimethyltridecylchromans (MTTCs). MTTCs are isoprenoid-substituted aromatic compounds and the "chroman ratio" between different isomers has been frequently used as a palaeosalinity indicator. It is still debated whether these compounds are directly biosynthesised by phytoplankton organisms or represent early diagenetic products from condensation reactions of phytol (predominantly algal-derived) with alkyl phenols (presumably higher plant-derived). In the latter case their isoprenoid and phenolic subunits would largely originate from different source organisms, which may also be reflected by differences in the stable isotopic compositions of these moieties. Therefore a pyrolysis - gas chromatography - isotope ratio mass spectrometry (Py-GC-irMS) method was developed in this project to analyse the $\delta^{13}C$ value of chroman fragments (i.e. the respective alkylphenol and pristenes). High accuracy and reproducibility of the method was demonstrated by analysis of an authentic 5,7,8-trimethyl-MTTC standard. However, matrix effects prevented the formation of the alkylphenol when isolates of this chroman from natural samples were analysed. Although sample preparation and/or pyrolysis conditions still require further optimization for the analysis of chromans in geological samples, the development and demonstration of this straight-forward method showed great potential for future applications to investigate MTTC-formation. A better understanding of the origin of MTTCs would also help to interpret variations in the chroman ratio with regards to more accurate salinity reconstructions.

In **Chapter 4** a novel conceptual model utilising MTTCs in combination with other molecular indicators for the reconstruction of freshwater incursions in marine palaeoenvironments was introduced and applied for the reconstruction of palaeoenvironmental conditions in a Devonian (Late Givetian-Early Frasnian) marine setting. The disposition of reef systems in this time period restricted water-exchange between the somewhat enclosed embayment and the open ocean. A freshwater lens predominantly from riverine input was overlying the more saline bottom waters leading to persistent density stratification of the water-column with a sharp chemo-, pycno- and halocline at the interface. The high abundance of specific *Chlorobi* biomarkers indicated the presence of photic zone euxinia (PZE) which was promoted by the stagnant water column. PZE describes a condition where anoxia and high concentrations of hydrogen sulfide produced by anaerobic sulfate reducing bacteria in sediments or the lower water-column reach the photic zone. It represents elevated levels of biotic stress and has been associated with various mass extinction events. High chroman ratios (> 0.9) reflected the freshwater conditions in the epilimnion whereas pristane/phytane ratios < 1 were indicative of anoxia and higher salinities in the hypolimnion and sediments. The correlation of MTTC abundances with perylene, a polycyclic aromatic hydrocarbon (PAH) presumably originating from pigments in wood degrading fungi, may be indicative of a common terrigenous source, supporting the formation of MTTCs during early diagenetic condensation reactions of higher plant derived alkylphenols with phytol. This formation pathway would also account for the similar $\delta^{13}C$ values of phytane (phytol-derived) and MTTCs (*n.b.* due to the significantly higher number of carbon atoms in the isoprenoid subunit of MTTCs, this part of the molecule largely determines the overall $\delta^{13}C$ value).

In **Chapter 5** a sedimentary core from the Canning Basin (WA) spanning the time period from close to or at the Givetian-Frasnian (G-F) boundary until later in the Frasnian was investigated with a comprehensive analytical approach which included molecular, elemental (C, N and S) and stable isotope ($\delta^{13}C$ of OM, carbonates and biomarkers, $\delta D_{kerogen}$ and $\delta^{34}S_{pyrite}$) analysis to reconstruct palaeoenvironmental changes associated with the Late Devonian extinctions. Although the most pronounced loss of species in the Late Devonian occurred at the Frasnian-Famennian (F-F) boundary, there have also been major biotic crises in the Late Givetian and possibly Frasnian as well as at the end of the Famennian which have been explored to a lesser extent. **Chapter 5** analysed the same sediments studied in **Chapter 4**. Whilst the research in **Chapter 4** mainly focussed on the basinal part of the core, **Chapter 5** also investigated palaeoenvironmental settings later in the Frasnian, corresponding to the upper core section, and was mainly focussed on variations of the investigated parameters throughout the core for a potential correlation with extinction events in that time period. It also included additional elemental, biomarker and stable isotope parameters. Whereas the palaeoenvironment close to or at the G-F boundary represented a time period of elevated biotic stress and was characterised by a stratified water-column (enhanced gammacerane indices), anoxia (e.g. low Pr/Ph ratios) and PZE (abundance of *Chlorobi* biomarkers), the setting in the Frasnian appeared to be less restricted with more oxic conditions (e.g. Pr/Ph > 1) and no indications of severe biotic crisis (e.g. low abundances of gammacerane and absence of the *Chlorobi* derivatives palaeorenieratane and isorenieratane). Variations in hopane and sterane distributions indicated significant changes in bacterial and algal populations with an algal predominance in the lowermost part of the core and a significantly higher bacterial contribution in the upper section. Distinct sterane distributions in sediments deposited close to or at the G-F boundary may furthermore reflect input from freshwater algae or algal blooms due to terrigenous nutrient input.

Sediments throughout the whole core exhibited an exceptionally low thermal maturity which was evident in a low degree of biomarker isomerization as well as in low T_{max} values from Rock Eval pyrolysis (< 421 °C) and palynological analyses of the spore colour (pale to mid yellow). The sediments also contained the presumably oldest preservation of biologically sourced C/N ratios. This further demonstrated the remarkable preservation of OM and biomarkers in the Gogo Formation, which represents the Givetian-Frasnian basin facies of the reef systems in the Canning Basin.

This thesis presents the successful application of integrated biomarker, elemental and stable isotope approaches to reconstruct recent and ancient marine or estuarine depositional environments affected by significant ecological and environmental changes. It also further investigated or introduced various parameters for reconstruction of palaeosalinity and water-column stratification including δD analyses of *n*-alkanes and kerogens as well as a novel conceptual model based on abundances of MTTCs. Furthermore, a new analytical method which may be applied to investigate origin and formation pathway of MTTCs was introduced.

Acknowledgements

This PhD has been a long journey full of many great experiences but it has also had its testing moments. I was lucky to have the support of many great people who helped, inspired and encouraged me along the way. Without you this project would not have been possible!

Firstly, I would like to thank my supervisor Prof Kliti Grice for giving me the opportunity to work on this great project and always supporting me with her scientific enthusiasm, feedback on data and mentoring. She provided me with excellent guidance whilst still allowing me creative freedom in my scientific work. I am very thankful for all the opportunities she gave me during my PhD, including visits of several national and international conferences, a trip to Indiana University to work in the laboratory of Assoc/Prof Arndt Schimmelmann, an unforgettable field trip to the Western Australian Kimberleys and of course for providing her excellent laboratory facilities (all supported by ARC Infrastructure grants).

I would also like to thank my co-supervisor Assoc/Prof Paul Greenwood for always being available for scientific advice and discussions, for technical support with pyrolysis GC-MS and his valuable and prompt contributions to the writing of this manuscript and for cheering me up in my more challenging times.

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Of course I also thank my fellow students and staff members at WA-OIGC for making Curtin University a nice and fun place to work and for the mutual support with labwork and other scientific questions: Anais Pagès, Ines Melendez, Caroline Jaraula, Mojgan Ladjavardi, Jeffrey Dick, both Christianes (Eiserbeck and Vitzthum von Eckstaedt), Birgit Nabbefeld, Pierre. Le Métayer, Tobias Ertefai, Dawn White, Muhammad Asif, Robert Lockhart, Lyndon Berwick, Amy Bowater, Ken Williford, Melissa Berke, Alex Holman, Alison Blyth, Pia Atahan and Ercin Maslen. This also includes Geoff Chidlow, who provided excellent technical support and scientific advice, mainly on GC-MS analysis (also including pyrolysis) and Stephen Clayton for his support with GC-irMS analyses. Thanks also to Assoc/Prof Kate Trinajstic for discussions on the Geology of the Devonian Canning Basin and to Tanya Chambers and other administrational staff for doing a very good job and being patient.

Furthermore, I would like to thank everyone involved in the field trip to the Canning Basin in which most of the samples included in this PhD were collected. It was a great experience for me and I really enjoyed camping in the remote area with great company (except for maybe the part where we almost got washed out by the rain!) and learning more about Aboriginal culture, the geology of the Canning Basin and also how to work a small drilling rig (including all the problems with technical failures…). I particularly would like to thank Dr Ted Playton, Dr Peter Haines, Assoc/Prof Eric Tohver, Ray Addenbrooke, Prof Joseph Kirschvink, Dr Roger Hocking, Assoc/Prof Kate Trinajstic, Prof Kliti Grice and all Chevron staff who were involved in the organisation of the trip and/or the operation of the Winkie drill. Furthermore, I am very thankful to Maria, Colin and Marc from the Wundargoodie team for making the stay as comfortable as possible, the nice food mainly cooked on the campfire and the many interesting talks about Aboriginal culture, stories and art. I also wish to thank the local Mimby community for taking us to the impressive Mimby caves and other locations, speaking about their culture, supporting our work and the company at the campfire.

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Furthermore, I wish to acknowledge Dr Evelyn Krull for providing the samples from the Coorong Lagoon and for very helpful discussions on the fate of the Coorong and her scientific input towards the publication presented in Chapter 2. Thanks also to Dr Andrew Revill for providing data for the MS on the Coorong Lagoon and for his scientific input.

I thank Prof Roger Summons for MRM-GC-MS analysis of my samples as well as Prof Michael Böttcher for δ^{34}S analysis of my samples and I thank both for scientific input. Thanks to Dr Greg Skrzypek and Douglas Ford for their support with δ^{13}C analysis of OM and carbonates. Thanks also to Prof Clinton B. Foster for providing palynological data and advice for interpretation.

I would like to acknowledge Curtin University for a CSIRS scholarship and The Institute for Geoscience Research (TIGeR) for a top-up scholarship. I also acknowledge the ARC for funding (QEII Discovery and a Linkage grant) awarded to my supervisor to help pursue the research of a larger project on several extinction events and their association with sulfide-rich environments.

Many thanks go to my loving family and my beautiful friends, in Perth as well as in Germany, for supporting me in every possible way throughout my PhD and being very understanding and patient. And last but certainly not least I would like to thank Jodie Buswell who was always there for me, helped me in every way a person can, gave me laughter, joy and kept me sane.

Primary publications

This thesis is assembled by publications, either accepted with minor revisions, submitted or in preparation, which form the individual chapters and are listed below.

Chapter 2

Tulipani, S., Grice, K., Krull, E., Greenwood, P., Revill, A.T. Salinity variations in the Northern Coorong Lagoon, South Australia: Significant changes in the Ecosystem following human alteration to the natural water regime. *Organic Geochemistry*, submitted (2013) impact factor 2.8

Chapter 3

Tulipani. S., Grice, K., Greenwood, P., Schwark, L. 2013 A pyrolysis and stable isotopic approach to investigate the origin of methyltrimethyltridecylchromans (MTTCs). *Organic Geochemistry* 61, 1-5. impact factor 2.8

Chapter 4

Tulipani, S., Grice, K., Greenwood, P., Schwark, L., Böttcher, M.E., Summons, R.E., Foster, C.B. Geochemical evidence for freshwater incursions into stratified marine palaeoenvironments: Origin of methyltrimethyltridecylchromans (MTTCs). In preparation for submission to *Nature Geoscience*, impact factor 12.4

Chapter 5

Tulipani, S., Grice, K., Greenwood, P., Haines, P., Sauer, P., Schimmelmann, A., Summons, R.E., Foster, C.B., Böttcher, M.E., Playton, T., Schwark, L. Changes in palaeoenvironmental conditions recorded in Late Devonian Reef systems from the Canning Basin, WA: A biomarker and stable isotope approach. In preparation for submission to *Earth and Planetary Science Letters*, impact factor 4.2

Statement of contributions of others

The work presented in this thesis was primarily designed, experimentally executed, interpreted, and the individual manuscripts were prepared by the first author (Svenja Tulipani). Contributions by co-authors are described below.

Chapter 2

Evelyn Krull provided the samples. TOC and bulk $\delta^{13}C$ analyses were performed by Andrew T. Revill at CSIRO Marine and Atmospheric Research, Hobart, Australia. Svenja Tulipani and Kliti Grice designed experiments. This chapter also includes some work performed by Svenja Tulipani as part of her "Master of Science" degree which was sample preparation as well as GC-MS and GC-irMS analyses of aliphatic fractions. The substantial processing of GC-MS analyses including hopanoid, steroid and chloro-*n*-alkane identifications as well as all peak integrations and calculation of ratios and relative abundances were performed by Svenja Tulipani exclusively as part of this PhD project. Furthermore, this chapter includes additional GC-irMS results processed by Svenja Tulipani and GC-MS analyses of aromatic fractions performed by Svenja Tulipani. The writing of the manuscript representing **Chapter 2** was also carried out during the PhD project by Svenja Tulipani with contributions from all other co-authors, all of whom also provided additional intellectual input in the discussions of results. General interpretations and format differed substantially from a preliminary report which formed the Master thesis. Kliti Grice provided analytical facilities. Infrastructural support was provided by John de Laeter Centre and financial support by The Institute for Geoscience Research (TIGeR).

Chapter 3

The 5,7,8-trimethyl-MTTC standard was synthesized by Lorenz Schwark (who also provided the starting products 2,3,5-trimethylphenol and phytol) at the Institute of Geoscience, Kiel University, Germany. The geological samples originated from a core which was collected within an ARC Linkage project supported by Chevron Energy Technology Company and the Geological Survey of Western Australia. Svenja Tulipani and Kliti Grice helped with sample collection and operation of the drill. Experiments were designed by Svenja Tulipani, Kliti Grice, Lorenz Schwark and Paul Greenwood and executed by Svenja Tulipani with some technical support from Geoff Chidlow, Steven Clayton and Paul Greenwood for the setup of the pyrolysis units. EA-irMS analyses were performed by Douglas Ford and Greg Skrzypek at the School of Plant Biology, University of Western Australia, Australia. Svenja Tulipani wrote the manuscript with contributions from all other co-authors, all of whom also provided intellectual input in discussions. Kliti Grice provided analytical facilities except where mentioned otherwise. This research was funded by QEII and ARC Linkage grants (Kliti Grice).

Chapter 4

The samples originated from the same core as the sediments analysed in **Chapter 3**. Experiments were designed by Svenja Tulipani and Kliti Grice. Sample preparation, including decarbonation for Rock Eval pyrolysis and elemental analysis as well as all preparations for bulk $\delta^{13}C$ analysis of organic matter and carbonates (including weighing of samples for analysis) was performed by Svenja Tulipani. Elemental analysis and Rock Eval pyrolysis were performed by Lorenz Schwark at the Institute of Geoscience, Kiel University. $\delta^{34}S$ analyses were performed by Michael E. Böttcher at the Marine Geology Department, Geochemistry & Isotope Geochemistry Group, Leibniz-Institute for Baltic Sea Research, Warnemünde, Germany. Roger Summons performed MRM-analyses of hopanoids and steroids at Department of Earth, Atmospheric and Planetary Sciences, MIT, Boston, USA. Clinton Foster performed palynological analyses at Geoscience Australia, Canberra. $\delta^{13}C$ analysis of organic matter (OM) and carbonates were performed by Douglas Ford and Greg Skrzypek at the School of Plant Biology, University of Western Australia, Australia. Svenja Tulipani performed all remaining experiments, processed and interpreted the data and wrote the manuscript. Kliti Grice, Paul Greenwood, Lorenz Schwark, Roger Summons and Michael E. Böttcher also contributed to the writing and provided intellectual input in discussions. Kliti Grice provided analytical facilities except where indicated otherwise. This research was funded by QEII and ARC Linkage grants (Kliti Grice).

Chapter 5

The samples originated from the same core which was also analysed in **Chapter 3** and **Chapter 4**. Ted Playton and Peter Haines also helped with planning of the field trip and sample collection. Svenja Tulipani and Kliti Grice designed all experiments except for the isolation of kerogens for δD analysis, which was designed by Arndt Schimmelmann. Sample preparation, including decarbonation for Rock Eval pyrolysis and elemental analysis as well as all preparations for bulk $\delta^{13}C$ analysis of organic matter and carbonates (including weighing of samples for analysis) and isolation of kerogens and weighing of the latter into silver capsules for δD analysis was performed by Svenja Tulipani. Elemental analysis and Rock Eval pyrolysis were performed by Lorenz Schwark at the Institute of Geoscience, Kiel University, Germany. $\delta^{34}S$ analyses were performed by Michael E. Böttcher at Leibniz-Institute for Baltic Sea Research, Warnemünde, Germany. Roger Summons performed MRM-analyses of hopanoids and steroids at Department of Earth, Atmospheric and Planetary Sciences, MIT, Boston, USA. Clinton Foster performed palynological analyses at Geoscience Australia. $\delta^{13}C$ analysis of organic matter and carbonates were performed by Douglas Ford and Greg Skrzypek at the School of Plant Biology, University of Western Australia, Australia. δD analysis of kerogens was carried out by Peter Sauer at the Department of Geological Sciences, Indiana University, USA. Peter Haines provided the sedimentary log. Svenja Tulipani performed all remaining experiments, processed and interpreted the data and wrote the manuscript. Kliti Grice, Paul Greenwood and Ted Playton also contributed to the writing. All Co-authors provided intellectual input in discussions. Arnd Schimmelmann provided analytical facilities for the preparation of kerogens for δD analysis. Kliti Grice provided analytical facilities for the remaining analyses. This research was funded by QEII and ARC Linkage grants (Kliti Grice).

Secondary publications

Manuscripts and abstracts based on research that was conducted during the preparation of this thesis.

Peer reviewed journal articles not part of thesis research

Grice, K., Lu, H., Atahan, P., Asif, M., Hallmann, C., Greenwood, P., Maslen, E., **Tulipani, S.**, Williford, K., Dodson, J. (2009) New insights into the origin of perylene in geological samples. *Geochimica et Cosmochimica Acta* 73, 6531-6543. Impact factor: 4.3

Conference abstracts

*Directly related to PhD

***Tulipani, S.**, Grice, K., Greenwood, P., Haines, P., Sauer, P., Schimmelmann, A., Summons, R.E., Foster, C.B., Böttcher, M.E., Playton, T., Schwark, L. A novel biomarker approach for the reconstruction of freshwater incursions in stratified marine palaeoenvironments: Origin of methyltrimethyltridecyl chromans (MTTCS). "26th International Meeting of Organic Geochemistry" (IMOG), Tenerife, Sept. 2013, oral presentation.

Grice, K., **Tulipani, S.**, Jaraula, C.M.B., Melendez' I., Böttcher, M.E., Schwark, L., Foster, C.B., Twitchett, R. Consistent changes in biomarkers (microbes and flora) and stable isotopes across three major extinction events of our planet "26th International Meeting of Organic Geochemistry" (IMOG), Tenerife, Spain, Sept. 2013.

***Tulipani, S.**, Grice, K., Greenwood, P.F., Haines, P., Summons, R.E., Böttcher, M.E., Foster, C.B., Woltering, M., Playton, T. (2013) Changes in palaeoenvironmental conditions in Late Devonian Reef systems from the Canning Basin, WA: A biomarker and stable isotope approach. "West Australian Basins Symposium", (WABS) 2013, poster presentation.

Playton. T., Hocking, R., Katz, D., Haines, P., Hillbun, K., Tohver, E., Trinajstic, K., Yan, M., Hansma, J., Pisarevsky, S., Kirschvink, J., Montgomery, P., Cawood, P., Grice, K., **Tulipani, S.**, Ratcliffe, K., Wray, D., Caulfield-Kerney, S., Ward, P., Playford, P. (2013) Insights on Upper Devonian Reef Complex Depositional Heterogeneity and Architecture Unraveled Through Integrated Chronostratigraphy: Lennard Shelf, Canning Basin, Western Australia “West Australian Basins Symposium”, (WABS) 2013.

Playton, T., Hocking, R., Montgomery, P., Tohver, E., Hillbun, K., Katz, D., Haines, P., Trinajstic, K., Yan, M., Hansma, J., Pisarevsky, S., Kirschvink, J., Cawood, P., Grice, K., **Tulipani, S.**, Ratcliffe, K., Wray, D., Caulfield-Kerney, S., Ward, P., Playford, P. Development of a Regional Stratigraphic Framework for Upper Devonian Reef Complexes Using Integrated Chronostratigraphy: Lennard Shelf, Canning Basin, Western Australia “West Australian Basins Symposium”, (WABS) 2013.

***Tulipani, S.**, Grice, K., Greenwood, P., Haines , Summons, R.E., Böttcher, M.E., Foster, C.B., Playton, T., Schwark, L., Sauer, P., Schimmelmann, A. Changes in palaeoenvironmental conditions in Late Devonian Reef systems from the Canning Basin, WA: A biomarker and stable isotope approach "AAPG Hedberg Research Conference” Beijing, China, April 2013.

Grice, K., Jaraula, C.M.B., Williford, K., **Tulipani, S.**, Nabbefeld, B., Summons, R.E., Böttcher M.E., Twitchett, R. (2012) Consistent changes in biomarkers (microbes and flora) and stable isotopes across several major extinction events of our planet “Australian Organic Geochemistry Conference” (AOGC), 2012, Sydney, Australia, oral presentation, Dec 2012.

Grice, K., Jaraula, C.M.B., Williford, K., Melendez, I., **Tulipani, S.**, Nabbefeld, Summons, R.E., Böttcher M.E., Woltering, M.[1], Twitchett, R. (2012) Consistent changes in biomarkers (microbes & flora) & stable isotopes across several major extinction events of our planet, “34th International Geological Congress” (IGC), Brisbane, Australia, Aug 2012.

Grice, K., Nabbefeld, B., Maslen, E., Jaraula, C., Holman, A., Melendez, I., **Tulipani, S.**, Twitchett, R., Hays, L.E., Summons, R.E., Mella, L., Williford, K.H., McElwain, J., Böttcher, M. (2011) Exploring mass extinction events and their association with global warming events from muliproxy biomarker and isotopic approaches, "American Geophysical Union (AGU) Fall Meeting" San Francisco, USA, Dec. 2011.

***Tulipani, S.**, Grice, K., Greenwood, P.F., Asif, M., Williford, K.H., Lockhard, R., Schimmelmann, A. (2011) The significance of novel A-*nor*steranes and perylene in Devonian reefs and crude oils "25th International Meeting of Organic Geochemistry" (IMOG), Interlaken, Switzerland, poster presentation, Oct. 2011.

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Tulipani, S., Grice, K., Greenwood, P.F., Asif, M., Williford, K.H. (2010) The significance of perylenequinones and their diagenetic alteration products in Devonian reefs (Canning Basin, WA). "Australian Organic Geochemistry Conference" (AOGC), 2010, Canberra, oral presentation, Dec 2010.

Tulipani, S., Grice, K., Greenwood, P.F., Asif, M., Williford, K.H., Maslen, E., Busetti, F. (2009) The significance of perylenequinones and their diagenetic alteration products in Devonian reefs (Canning Basin, WA). "10th Australasian Environmental Isotope Conference & 3rd Australoasian Hydrogeology Research Conference", Perth, Western Australia, poster presentation, Dec 2009.

Tulipani, S., Grice, K., Krull, E., Revill, A.T. (2009) Biogeochemistry of the Coorong Lagoon using a biomarker and compound specific isotope approach. "10th Australasian Environmental Isotope Conference & 3rd Australoasian Hydrogeology Research Conference", Perth, Western Australia, oral presentation, Dec 2009.

Tulipani, S., Grice, K., Greenwood, P.F., Asif, M., Williford, K.H., Maslen, E., Busetti, F. (2009) The significance of perylenequinones and their diagenetic alteration products in Devonian reefs (Canning Basin, WA). “24th International Meeting of Organic Geochemistry” (IMOG), Bremen, Germany, poster presentation, Sept. 2009.

***Tulipani, S.**, Grice, K., Krull, E., Revill, A.T. (2009) Biogeochemistry of the Coorong Lagoon using a biomarker and compound specific isotope approach. “24th International Meeting of Organic Geochemistry” (IMOG), Bremen, Germany, poster presentation, Sept. 2009.

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Abbreviations

ACL	average chain length
AMS	accelerator mass spectrometry
ASE	accelerated solvent extraction
BNH	bisnorhopane
BSIA	bulk stable isotope analysis
CD	Canyon Diablo Troilite
CF	continuous-flow
CPs	chloro-paraffins
CPI	carbon preference index
CSIA	compound specific stable isotope analysis
DCM	dichloromethane
DIC	dissolved inorganic carbon
EA	elemental analysis
irMS	isotope ratio mass spectrometry
FID	flame ionisation detection
F-F boundary	Frasnian–Famennian boundary
Fm.	formation
GC-MS	gas chromatograpy-mass spectrometry
G-F boundary	Givetian-Frasnian boundary
GSB	green sulfur bacteria
HBI	highly branched isoprenoid
HI	hydrogen index
HPLC	high performance liquid chromatography
MRM	multiple reaction monitoring
MSR	microbial sulfate reduction
MTTCs	methyltrimethyltridecylchromans
OAE	oceanic anoxic events
OC	organic-carbon
OM	organic matter
OI	oxygen index
PI	production index
Pr/Ph	pristane/phytane ratio
P-T boundary	Permian-Triassic boundary
PSB	purple sulfur bacteria

Py	pyrolysis
PZE	photic zone euxinia
R_0 %	vitrinite reflectance
SI	supplementary information
SRB	sulfate reducing bacteria
TC	thermochemical conversion
TIC	total ion chromatogram
TLC	thin layer chromatography
TLE	total lipid extract
TRIS	total reducable inorganic sulphur
TCA-cycle	tricarboxylic acid-cycle
TMP	trimethylphenol
Tm	17α 22,29,30-trisnorhopane
Tm-β	17β 22,29,30-trisnorhopane
triMeMTTC	trimethyl-methyltrimethyltridecylchroman
Ts	18α 22,29,30-trisnorhopane
VPDB	Vienna Pee Dee Belemnite
VSMOW	Vienna Standard Mean Ocean Water

Chapter 1

Introduction and overview

Mass extinctions in Earth's history (and in the near future?)

Since life evolved on Earth there have been several major extinction events which wiped out a variety of life-forms but at the same time also left empty ecological niches and habitats for new and often more complex organisms to evolve and diversify. One of the first great biotic crises occurred in the Paleoproterozoic between ~2.5 and ~2.2 billion years ago with the oxygenation of the atmosphere driven by the rise of cyanobacteria which produced O_2 as a product of oxygenic photosynthesis (e.g. Bekker et al., 2004; Catling and Claire, 2005). Although many organisms today have a metabolism depending on free oxygen, it was highly toxic to the majority of the mainly anaerobic life-forms present at that time. In addition the oxygenation of the atmosphere and the consequential removal of greenhouse gases was also most likely linked to a dramatic global cooling which created the Paleoproterozoic "Snowball-earth"-event (Pavlov et al., 2000; Kopp et al., 2005).

Throughout the Phanerozoic, various biotic crises took place with the 5 biggest extinction events occurring in the Late Devonian and at the Ordovician–Silurian, Permian–Triassic, Triassic–Jurassic and Cretaceous–Tertiary boundaries as illustrated **Figure 1.1** (e.g. Sepkoski 1986, 1993). The reconstruction of possible causes and extents of these mass extinctions and their often selective effects on different species are issues of ongoing investigation and many questions still remain to be solved (e.g. Bambach, 2006). It is now largely accepted by most scientists that the famous End-Cretaceous extinction (65 Ma), which wiped out the dinosaurs, is associated with a large bolide impact (e.g. Alvarez et al., 1980; Kring, 2007). In contrast, the by far biggest of all extinctions at the Permian–Triassic boundary was most likely caused by the combined effects of different terrestrial events; i.e. dramatic global warming started by increased volcanic activity and further enhanced by outgassing of methane hydrates combined with persistently anoxic and sulfidic oceans emitting toxic gases such as H_2S into the atmosphere, which impacted terrestrial as well as marine organisms (Grice et al., 2005a; Kump et al., 2005; Racki and Wignall, 2005; Nabbefeld et al., 2010a). However, a detailed reconstruction of incidents leading to many other extinction events including the Late-Devonian and Triassic-Jurassic extinctions has not been established so far. It also remains difficult to determine accurate duration and intensities of the Phanerozoic extinction events (e.g. Bambach, 2006).

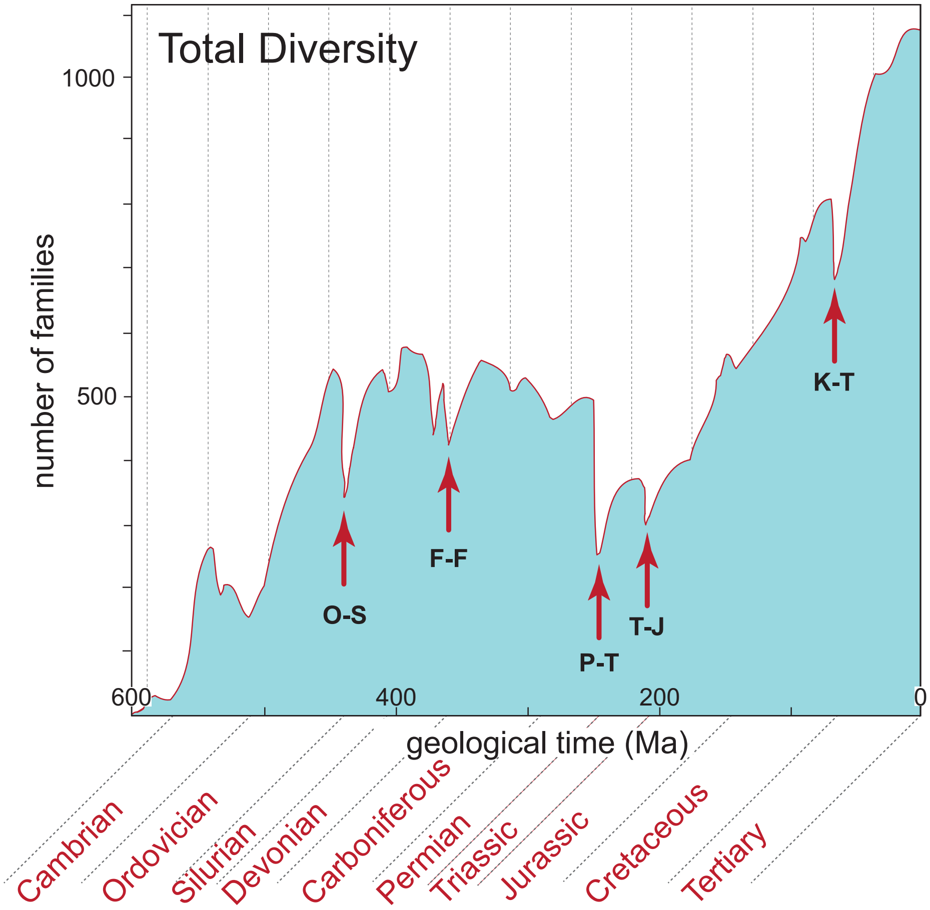

Figure 1.1: Total biodiversity of marine species throughout the Phanerozoic (after Sepkoski, 1993). Arrows mark the five major mass extinction events of Earth's history.

Detailed investigations of causes, nature and effects of these events appear to be especially relevant for the present-day given the many scientific predictions of another imminent mass extinction which is believed to be largely driven or at least accelerated by anthropogenic activities such as large scale changes of natural environments and ecosystems, decimation of species or increased emission of greenhouse gases (especially CO_2) as a result of fossil fuel burning and deforestation, ultimately leading to climate change (e.g. Myers, 1990; Dirzo and Raven, 2003; Wake and Vredenburg, 2008; Barnosky et al., 2011).

This PhD-project demonstrates the great potential of sedimentary biomarker studies combined with stable isotope analysis for the investigation of conditions in recent and ancient palaeoenvironments and the reconstruction of changes which occurred in past ecosystems. Both of these techniques are frequently applied in organic geochemistry and their operation and application will be explained in detail throughout the following sections of this introduction. Recent environmental changes were investigated in the Coorong Lagoon, an ecosystem which has been severely affected by human practices, as well as conditions in palaeoenvironments associated with the Late Devonian extinctions, to show the broad range of applications of these organic geochemical and stable isotope techniques.

Late Devonian extinctions

The Devonian was a relatively warm time period in which extensive reef-systems mainly built by ancient rugose corals, stromatoporoids (extinct calcareous sponges) and calcareous algae and bacteria flourished in tropical, shallow waters (e.g. Playford et al., 2009). A Devonian timescale is shown in **Figure 1.2** and a map of the Early Devonian world is displayed in **Figure 1.3**. Fish, in particular the extinct placoderms, continued to diversify rapidly and developed many unique morphological variations (Long and Trinajstic, 2010). Furthermore, the first lung-fish and early tetrapods evolved over the time period from the Givetian to the Late Famennian (Ahlberg et al., 1994; Clack, 2007). However, the most drastic environmental changes were caused by the rise of terrestrial plants. Primitive vascular land plants, which first occurred globally in the Upper Silurian (Steemans et al., 2009), started to diversify rapidly throughout the Devonian and were the first complex organisms to colonize terrestrial environments (Chaloner, 1967). The development of more diversified tissues including deeper roots, led to enhanced weathering of rocks and the first significant formation of soil organic matter (e.g. Algeo and Scheckler, 2010). By the Givetian, primitive woody plants had evolved, and were forming widespread forests (Grice et al., 2009; Meyer-Berthaud et al., 2010; Mintz et al., 2010). This was accompanied by a decrease of atmospheric CO_2 since the increasing terrestrial biomass acted as a carbon-sink. Furthermore, there were significant changes in sediments and in the cycling of nutrients and water (Walliser, 1996; Algeo and Scheckler, 2010). The first direct ancestors of seed bearing plants were reported from the Givetian (Gerrienne et al., 2004) and by the Famennian seed bearing plants were already quite common (e.g. Chaloner, 1967; Fairon-Demaret, 1996).

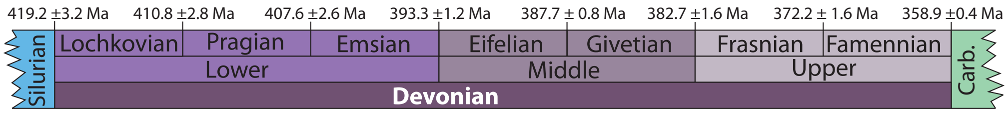

Figure 1.2: Devonian timescale after International Commission of Stratigraphy (ICS) 2013

The Late-Devonian extinction at the Frasnian–Famennian (F-F) boundary (Upper-Kelwasser event) is regarded as one of the "big five" mass-extinction events described in the previous section (Sepkoski, 1986, 1993). However, it was not the only major biodiversity crisis occurring towards the end of the Devonian era; extinction rates were generally high and origination of new species low from the Middle Givetian until the Late-Famennian (Bambach, 2006). There were distinct extinction events in the Late Givetian (Walliser, 1996; Aboussalam and Becker, 2001) and also the Famennian was closed by the Hangenberg-event, which almost reached the intensity of the F-F extinctions (Walliser, 1996; Caplan and Bustin, 1999; Streel et al., 2000). These biotic crises mainly diminished organisms in shallow marine environments, in particular rugose corals and stromatoporoids, whereas terrestrial ecosystems were only marginally affected (Copper, 1986; Fagerstrom, 1994; McGhee, 2005).

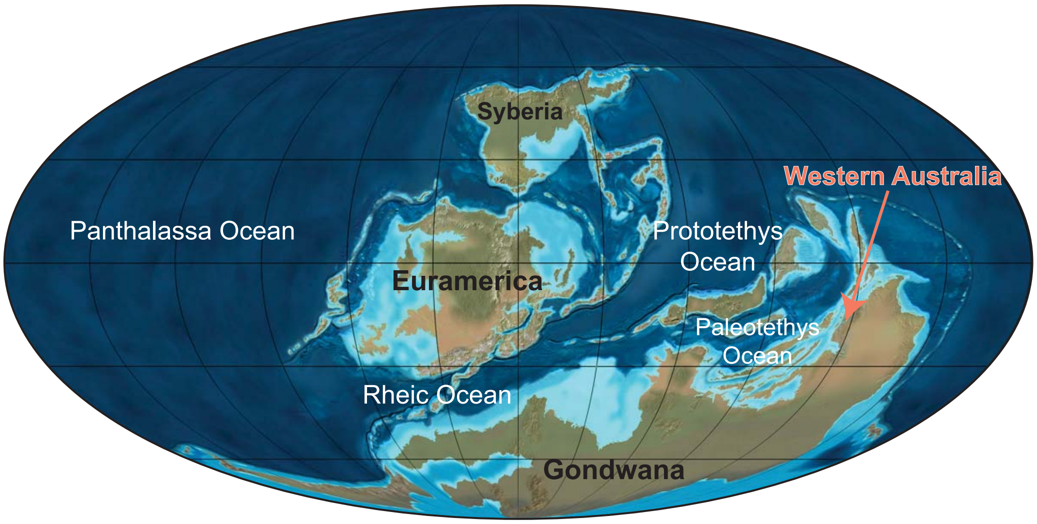

Figure 1.3: Map of the Devonian world at 370 Ma. Modified after Ron Blakey, Colorado Plateau Geosystems, Arizona USA; http://cpgeosystems.com/paleomaps.html

There are still many ongoing arguments about intensity, nature and causes of the Late Devonian biotic crises. One or several bolide impacts have been suggested as possible triggers (McLaren, 1970; Sandberg et al., 2002; Ellwood et al., 2003), however a combination of terrestrial causalities seems more plausible at this point since timing and intensities of potential impacts generally do not match the geological/paleontological records, although some local influence of impacts cannot be excluded (McGhee, 2005; Racki, 2005). Algeo and Scheckler (2010) suggested some major environmental changes were linked to the rise of terrestrial plants, e.g. global cooling as a result of reduced atmospheric CO_2 and anoxia/eutrophication in the ocean generated from increased weathering and nutrient input. Other potential causes include volcanism as trigger for climate change or rapid sea-level fluctuations possibly linked to glaciations and combinations of all (Caputo and Crowell, 1985; Stanley, 1988; Murphy et al., 2000; Courtillot and Renne, 2003; McGhee, 2005). There is also evidence of at least episodically euxinic (i.e. anoxic and sulfidic) epicontinental oceans in different parts of the world from the Late Givetian until the early Carboniferous (Summons and Powell, 1986; Requejo et al., 1992; Joachimski et al., 2001; Brown and Kenig, 2004; Marynowski et al., 2000, 2011; Maslen et al., 2009; Melendez et al., 2013a, 2013b).

Geological settings in the Canning Basin, Western Australia

In the Canning Basin located in the Western Australian Kimberleys, a well exposed large Devonian barrier-reef extends over ~350 km. It is probably the most well preserved reef system from that time period which was largely unaffected by tectonic movements and other geological alteration (Playford et al., 2009). Furthermore, it is well known for its exceptionally well preserved fossils (including soft tissue) in calcareous nodules deposited within the Gogo Formation (e.g. Long and Trinajstic, 2010; see **Figure 1.4** for geological formations). Besides conventional fossils, some of these nodules also contain remarkably well preserved biomarkers, which are molecular fossils of biolipids in the specimen as well as of micro-organisms present in the time of deposition and involved in the nodule-formation (Melendez et al. 2013a, 2013b).

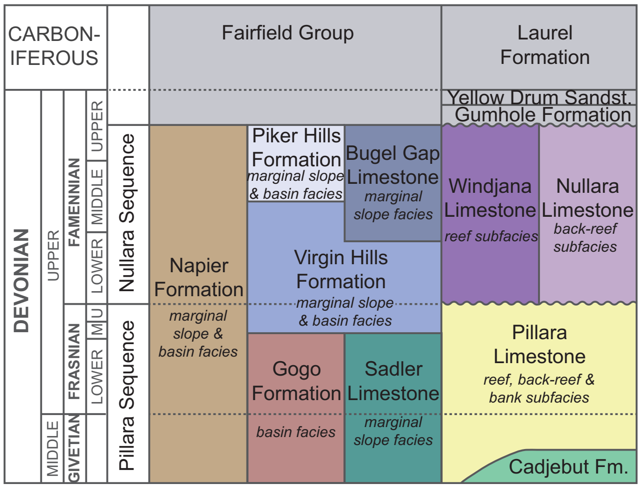

Figure 1.4: Formations in the Devonian reef systems of the Canning Basin modified after Playford et al. (2009)

The geology of the reef-systems in the Canning Basin is schematically illustrated in **Figure 1.4** and described in detail by Playford et al. (2009). The different facies associated with reef-systems in general are displayed in **Figure 1.5**. As a basic summary: reef-building in the Canning Basin commenced in the Late-Givetian and can be divided into two sequences (i) the Pillara sequence laid down over Precambrian and Ordovician rocks from the Late-Givetian until the Late-Frasnian and (ii) the Famennian Nullara sequence laid down largely unconformably over the Pillara sequence as a result of a significant drop in sea-level.

The Pillara sequence comprises the Pillara Formation (platform facies), the Sadler Formation (marginal slope facies) and the Gogo Formation (basin facies). Especially the Gogo Formation is organic rich in most parts and gave rise to the high quality oils of the Canning Basin (Cadman et al., 1993; Barber et al., 2001; Greenwood and Summons, 2003; Maslen et al., 2009, 2011). Also the Pillara formation contains relatively well preserved organic matter (OM) compared to later platform facies. The Nullara sequence mainly consists of the Windjana and Nullara Formations (Famennian platform facies), the Virgin Hills Formation (Upper-Frasnian to Middle Famennian marginal slope and basin facies) and the Piker Hills and Bugle Gap Formations (Middle to Upper-Famennian marginal slope and basin facies).

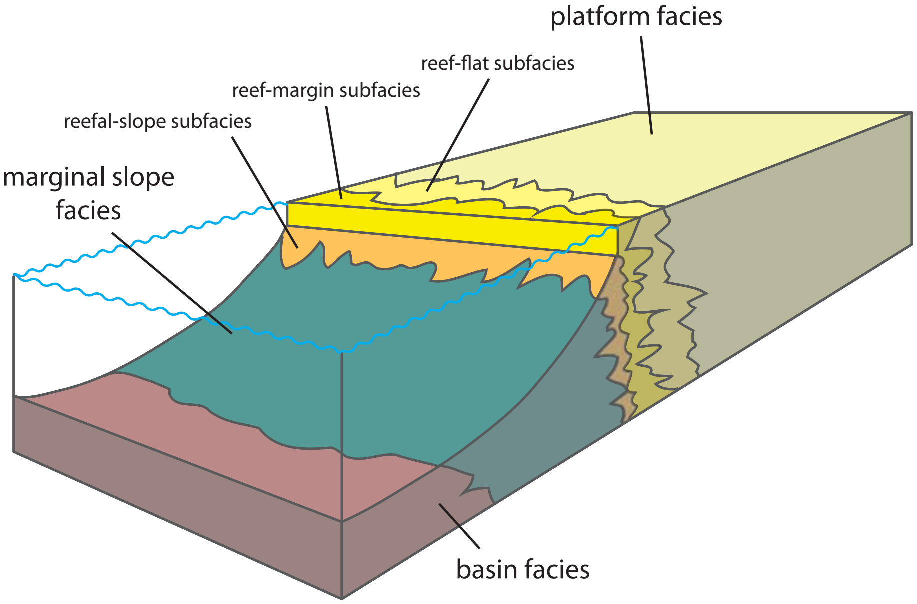

Figure 1.5: Different facies associated with reef systems; after Playford et al. (2009)

Throughout the Givetian and until the Middle Frasnian rising sea-levels led to advancing reef platforms, whereas a global fall in sea-level in the latest Frasnian and Famennian caused retreating and backstepping of the reef-systems. The main reef-builders throughout the Givetian and Frasnian were ancient rugose corals, stromatoporoids and calcareous bacteria. Whereas the latter were relatively unaffected by the F-F extinction, corals and stromatoporoids were almost completely wiped out at that point, however they had already started declining in diversity and abundance earlier in the Frasnian (Becker et al., 1991; Fagerstrom, 1994; Wood, 2004; Playford et al., 2009). Famennian reef-systems in the Canning Basin therefore largely consisted of stromatolitic bacteria. In the end of that epoch, reef building ceased altogether and throughout the Carboniferous and Permian the area was covered by large continental glaciers.

Environmental conditions in the present day Coorong

The Coorong comprises two shallow coastal lagoons (North and South Lagoon) and is located in South Australia. It is part of a terminal lake system at the mouth of the River Murray; a formerly complex ecosystem with a particularly high significance for migratory and native water-birds (Paton et al., 2009). The region is unfortunately a classical example of the detrimental effects human interference in natural processes and exploitation of resources can have. Furthermore, it is also representative of many other estuarine ecosystems in South Australia and around the world which have been similarly impacted by anthropogenic activities. However, the recent end of a severe drought and the associated flood has led to a short term improvement of the ecological situation. In addition, various remediation strategies have been recently implemented and future monitoring will evaluate their success. To be able to fully evaluate both the ecological deterioration following development of European settlements and the apparent ecological recoverability with the recently improved hydrological situation, the environmental conditions in the ecosystem (e.g. salinity or type of primary production at the base of the food-web) prior to human influence first need to be established.

Human influence in the region began about 100 years ago. Prior to this water levels and salinities in the Coorong fluctuated dynamically as a result of seawater input from the Murray Mouth and freshwater input from the connected Lake system (which is supplied with water from the River Murray). This created a variety of different habitats in and around the lagoons supporting a diverse ecosystem (Boon, 2000; Geddes, 2003; Webster, 2005). However, the almost complete isolation of the Coorong from the other lakes through newly installed barrages (beginning in the 1940s and intensifying in the 1950s), and an additional reduction of inflow from the River Murray as a result of excessive water abstraction and a severe drought from 2000-2010, led to an increase of salinity, particularly in the South Lagoon, and a progressive siltation of the connection to the ocean. The overall consequences were the extinction of key species such as macrophytes, which were important primary producers at the base of the food-web, and a dramatic decline of biodiversity in fish and bird populations (Boon, 2000; Nicol, 2005).

Significance of biomarkers in organic geochemistry

Biomarkers are "molecular fossils" preserved in sediments or petroleum which originated from biolipids of once living organisms (**Figure 1.6)**. Usually the more reactive functional groups of biolipids are lost after burial whereas the basic carbon skeleton often gets preserved (Peters et al., 2005, see also following sections for more details about biomarker formation and preservation).

The identification and quantification of biomarkers in geological samples is of great significance for the reconstruction of recent and ancient environments since the biomarker composition may reflect the ecological communities and OM sources in the depositional environment. Some biomarkers are specific for a particular species, group of organisms or metabolic pathway whereas others have multiple sources from biolipids abundant in a variety of different organisms. Particularly the source specific biomarkers may also provide information about environmental conditions at the time of deposition such as salinity (ten Haven et al., 1985, 1988), water-column stratification (Sinninghe Damsté et al., 1995) or euxinia (Summons and Powell, 1986) since many organisms prefer particular environments or produce certain molecules as a result of environmental stress. Exemplary applications of biomarker studies for the investigation of past environments are described from page 30 onwards. Much ongoing research aims to establish the origin and formation pathways of novel biomarkers which often involves correlation to, and thus analysis of, lipids in extant organisms (e.g. Volkman et al., 1998; Grice et al., 2009). Further applications of biomarker analysis are in the field of petroleum exploration and include oil-oil and oil-source rock correlations and estimation of quality and economic value of oil reservoirs (e.g. Hughes and Holba, 1988; Grosjean et al., 2009; Maslen et al., 2009).

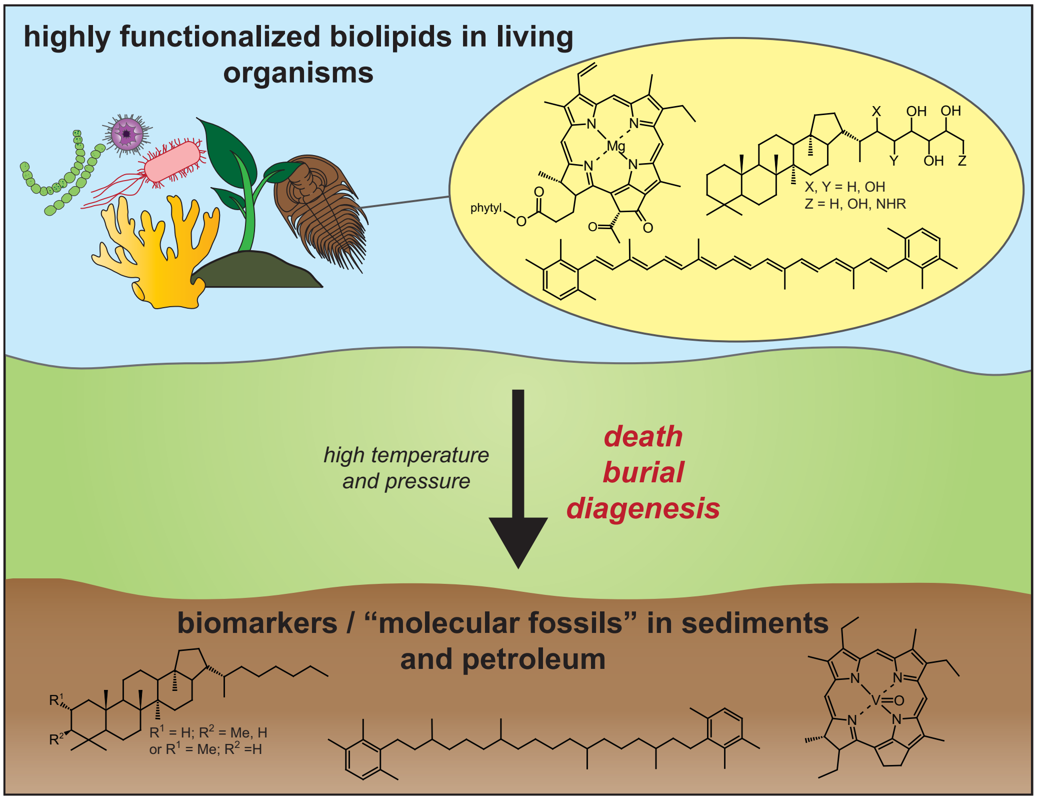

Figure 1.6: Simplified illustration of biomarker formation

Organic matter (OM) deposition and preservation in sediments

Biomass is formed by autotrophic primary producers which assimilate inorganic components such as CO_2 into a large variety of biomolecules. The vast majority of biomass is generated by algae, cyanobacteria and terrestrial and aquatic plants *via* oxygenic photosynthesis. These primary producers form the base of the food-web and support a great diversity of heterotrophic organisms. Most of the detrital OM from dead organisms gets recycled by decomposers and is re-introduced into the food-web. A small part, however, accumulates in sediments together with inorganic debris.

Sediments are deposited in various environments which largely influence their composition and properties. Marine sediments can for example be deposited in deep or shallow water settings, and can be associated with reef-systems or in deltaic settings. All of these show significant differences with regard to the type of organic and inorganic debris typically present in the particular environment as well as other conditions such as water movement or currents. Continental depositional settings include lakes, rivers, lagoons or swamps but also glaciers or wind can transport particles and accumulate sediments. Sedimentary OM can be autochthonous, i.e. produced *in situ*; or allochthonous, i.e. transported from a different source; e.g. terrestrial OM transported into the oceans or lacustrine settings *via* rivers or surface runoff. Estuarine or deltaic ecosystems commonly have a large variety of OM-sources whereas in many deep marine settings a large part of the deposited OM is autochthonous.

After the accumulation of OM in sediments it continues to be subject to degradation and remineralisation. Only less than 0.1% of total biomass gets preserved over geological timescales (Holser et al., 1988). The best OM preservation is commonly achieved in anoxic depositional environments since anaerobic degradation processes are typically not as efficient as aerobic processes (Claypool and Kaplan, 1974; Moodley et al., 2005). Anaerobic conditions often develop in the bottom of stagnant and stratified water-columns where oxygen in the lower part gets consumed by aerobic decomposers and mixing with the oxygenated top water is prevented. This occurs for example during density stratification of a warm water layer over cold bottom waters or (riverine) freshwater over more saline water, resulting in the formation of a thermocline or halocline, respectively (e.g. Kling, 1988; Grice et al., 1998a). OM preservation in sediments is also influenced by physical properties such as sedimentation rate and grain size (Huc, 1988). During the big mass extinction events large amounts of detrital material was created, often leading to global anoxia in the oceans and high rates of burial. The large amount of well-preserved OM often generated big petroleum reservoirs.

Transformation of sedimentary OM

The transformation of sedimentary OM after burial is often referred to as thermal maturation. In the beginning these processes are largely driven by biological and chemical transformations (i.e. diagenesis) whereas with increasing burial depth and time thermal alteration become predominant (i.e. catagenesis). A simplified version of these processes and their relationship to fossil hydrocarbon generation is shown in **Figure 1.7**.

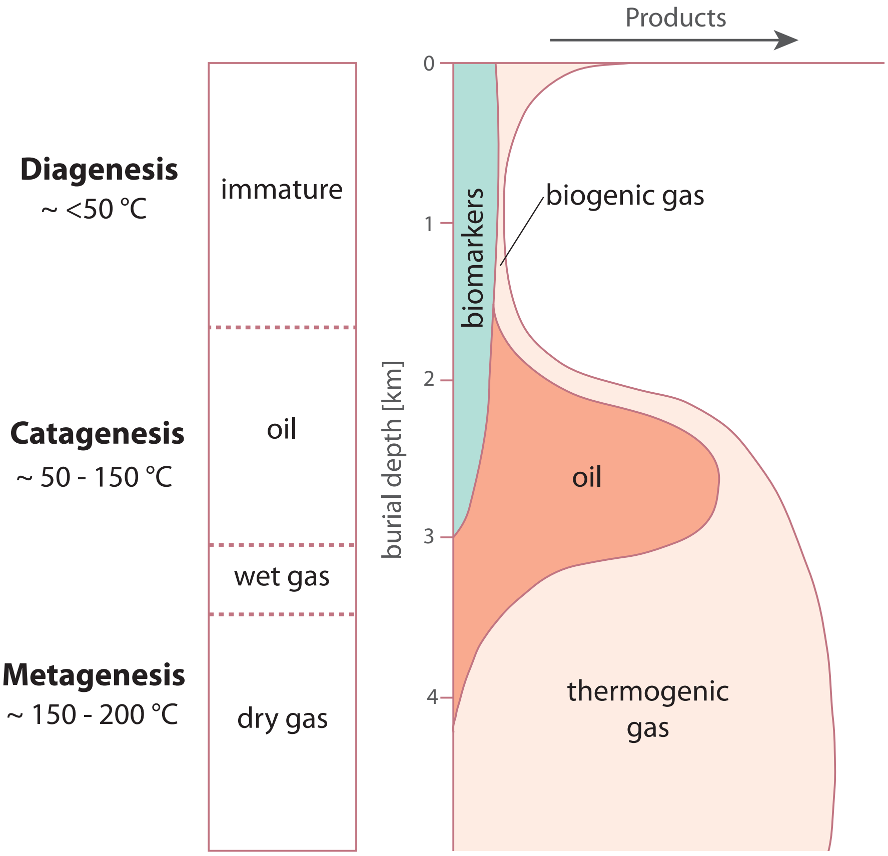

Figure 1.7: Transformation of sedimentary OM after burial (modified after Peters et al., 2005)

Diagenesis

Diagenesis is defined as the combination of complex biological and chemical processes which transform biomolecules before significant thermal alteration takes place (< 50 °C; e.g. Peters et al., 2005, Killops and Killops, 2013). Diagenesis already starts in the water-column and continues in the sediment where it is enhanced by the increasing temperatures with deeper burial. The processes during diagenesis are highly diverse and are strongly influenced by conditions in the depositional environment such as O_2 or H_2S levels and many other biochemical parameters (Brocks and Grice, 2011 and references therein). In some cases the type and abundances of the generated biomarkers can be indicative of diagenetic reactions specific to particular environments. Two examples are the preferential formation of phytane (**I**, see **Figure A1.1** for chemical structures) over pristane (**II**) from the phytol side-chain of different chlorophylls (**III**), which occurs under anaerobic conditions (Brooks et al., 1969) or the enhanced formation of diasteranes (**IV**) in the presence of clay minerals (Rubinstein et al., 1975; van Kaam-Peters et al., 1998; Nabbefeld et al., 2010b).

During diagenesis a highly complex macromolecular matrix (kerogen) is formed by enrichment of resistant biological (macro)molecules (e.g. algaenan, cellulose or diamondoids) due to selective degradation (Largeau and Derenne, 1993; Grice et al., 2000, 2003) and the incorporation of these macromolecules and other smaller compounds in sedimentary OM through condensation and cross-linking reactions (Tissot and Welte, 1984).

Catagenesis

Catagenesis takes place at temperatures between ~50 to 150 °C and can last several million years. The kerogen is thermally altered, bonds are cracked and lower-molecular-weight hydrocarbons are released to form liquid bitumen (e.g. Peters et al., 2005, Killops and Killops, 2013). Bitumen is operationally defined as the free OM which is indigenous to the rock and extractable with organic solvents. It consists mainly of defunctionalised and isomerised saturated and aromatic hydrocarbons, including many biomarkers, as well as some polar compounds (Brocks and Grice, 2011). With increasing pressures the bitumen will start to migrate through porous rocks. If it reaches an impermeable cap rock it may accumulate and form a petroleum reservoir.

The nature of kerogens and their potential for petroleum formation are determined by the type of source OM and the environmental conditions during early diagenesis. Several different types of kerogens can be distinguished on this basis:

- Type I kerogens typically originate from algal rich OM with a low sulfur content, which was deposited in lacustrine or brackish environments. They are highly oil prone and form very high quality oils.
- Type II kerogens usually originate from mainly marine OM and typically have higher sulfur contents and larger contributions of aromatic and ketone structures compared to Type I kerogens. They are also oil prone.
- Type II-S kerogens represent Type II kerogens with a high sulfur content
- Type III kerogens originate from terrestrial OM mainly composed of vascular plants, typically deposited in a deltaic environment. They yield less hydrocarbons compared to the other kerogens and are gas prone.
- Type IV kerogens do not yield any hydrocarbons.

Metagenesis

Metagenesis is the process in which hydrocarbons (including biomarkers) are extensively cracked and transformed into gases at temperatures between ~150 and 200°C (e.g. Peters et al., 2005, Killops and Killops, 2013). During this process gas reservoirs might develop.

Maturity parameters

The state of thermal maturity of a (potential) source rock indicates if it may have already generated oil or gas. Rocks that have undergone diagenesis but have not yet been thermally altered are referred to as immature. Rocks in and after the oil-generative window (**Figure 1.7**) are defined as mature and post-mature, respectively. During the process of diagenesis and thermal maturation, sedimentary biolipids undergo various transformations including the loss of the more reactive functional groups and unsaturated bonds, leaving only the basic hydrocarbon skeleton. Another characteristic alteration which commonly occurs in biomarkers is a change in stereochemistry from the biological configuration, created by enzymes during biosynthesis, into the configuration with the greatest thermal stability. As an example, biological (thermally unstable) and geological (thermally stable) isomers of hopanes derived from bacterial membrane lipids (Rohmer et al., 1984; Ourisson and Albrecht, 1992), are illustrated in **Figure 1.8**. Isomerisation at C-17 and C-21 occurs in all hopanes whereas isomerisation at C-22 can only take place in homohopanes ($> C_{30}$) since an asymmetric centre at C-22 does not exist in the lower-molecular-weight homologues. Hopanes with the 17α (H), 21β (H) configuration (αβ) exhibit the greatest thermal stability and are therefore dominant in oils and mature source rocks. In contrast, ββ-hopanes are the least thermally stable and are therefore only found in very immature sediments. 22S and 22R isomers are both abundant in mature samples with 22S showing a slightly higher thermal stability. The abundance ratio of 22S/(22S + 22R) is frequently used to assess thermal maturity and approaches values of ~0.6 (for the C_{31}-homologue) in endpoint mixtures of very mature crude oils (Seifert and Moldowan, 1980; Zumberge, 1987)

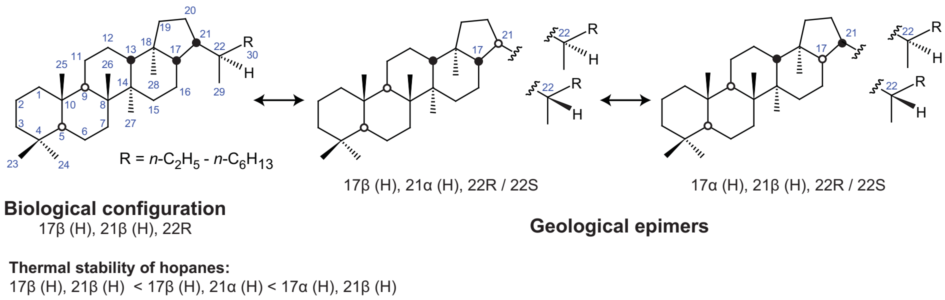

Figure 1.8: Isomerisation of hopanes during diagenesis and thermal maturation

Other biomarker parameters commonly used to determine thermal maturity are for example βα-hopane/αβ-hopane, the ratio of the trisnorhopanes: Ts/(Ts + Tm) (**V, VI**) or the 20S/(20S + 20R) ratios of steranes (**VII**). However, there are many more biomarker ratios which can be used as maturity parameters, most of these are listed in Peters et al. (2005).

Another frequently used maturity indicator is the methylphenanthrene (**VIII**) index which has been used in several slightly modified versions (e.g. Radke et al., 1982; Boreham et al., 1988; Cassani et al., 1988). Due to the higher thermal stability of the phenanthrenes (**VIII**) it can also be used at later stages of maturation when conventional biomarkers are already affected by metagenesis.

Non-molecular maturity indicators are vitrinite reflectance (R_0 %) and parameters determined by Rock-Eval pyrolysis (T_{max} and production index (PI)). Vitrinite is derived from tissue of terrestrial plants and is an important component of many kerogens. With increasing thermal maturity it becomes more reflective and therefore the measured vitrinite reflectance (R_0 %) can be used as a maturity parameter (Dow, 1977). Values < 0.6 % and > 1.35 % correspond to immature and overmature sediments, respectively (Peters and Cassa, 1994). Rock Eval is a stepwise pyrolysis technique which is used frequently as a screening method to evaluate maturity and quality of source rocks. The analysed parameters include S1 and S2, which refer to the amount of volatile organic compounds in a rock and the amount of organic compounds generated from cracking of the kerogen, respectively. The frequently used parameter T_{max} is the oven temperature corresponding to the maximum of hydrocarbon generation from a sample (maximum of the S2 peak) during Rock Eval analysis. PI is defined as S1/ (S1+S2). T_{max} of < ~435°C and PI < 0.1 indicate immature OM whereas values of ~470°C and ~0.4 for T_{max} and PI, respectively, are indicative of the wet gas window (**Figure 1.7**). For more details on Rock Eval analysis see Espitalié et al. (1977) and Peters (1986).

Biomarker analysis

This section is intended to give a brief overview of analytical techniques and basic steps commonly used for the analysis of sedimentary biomarkers. Some of these are illustrated in **Figure 1.9**. However, this discussion is not exhaustive and there are other means of analytical chemistry and preparation techniques which are applied in biomarker studies but are not mentioned here.

The first step usually is to extract the free OM from the powdered sample. Typically a mixture of dichloromethane (DCM) and methanol is used for this purpose but solvents with a higher polarity might be more suitable in some cases including recent samples. Depending on the type of sample and availability of equipment either Soxhlet extraction, accelerated solvent extraction (ASE) or ultrasonic extraction can be performed. ASE is an automated extraction technique in which the sample is loaded in a metal cell and extracted in a relatively quick timeframe under elevated temperature and pressure.

In the next step the obtained total lipid extract (TLE, typically bitumen) is further fractionated in order to make it more amendable to gas chromatograpy mass spectrometry (GC-MS) and avoid co-elutions. This can for example be achieved by thin layer chromatography (TLC) or silica gel / alumina column chromatography with solvents of different polarities. Often various procedures are applied to further clean-up or separate the obtained fractions. An example for this is a 5Å-molecular sieving procedure which separates aliphatic fractions into *n*-alkanes and branched / cyclic compounds in (Grice et al., 2008b). Furthermore, polar compounds have to be derivatised if GC analysis is intended.

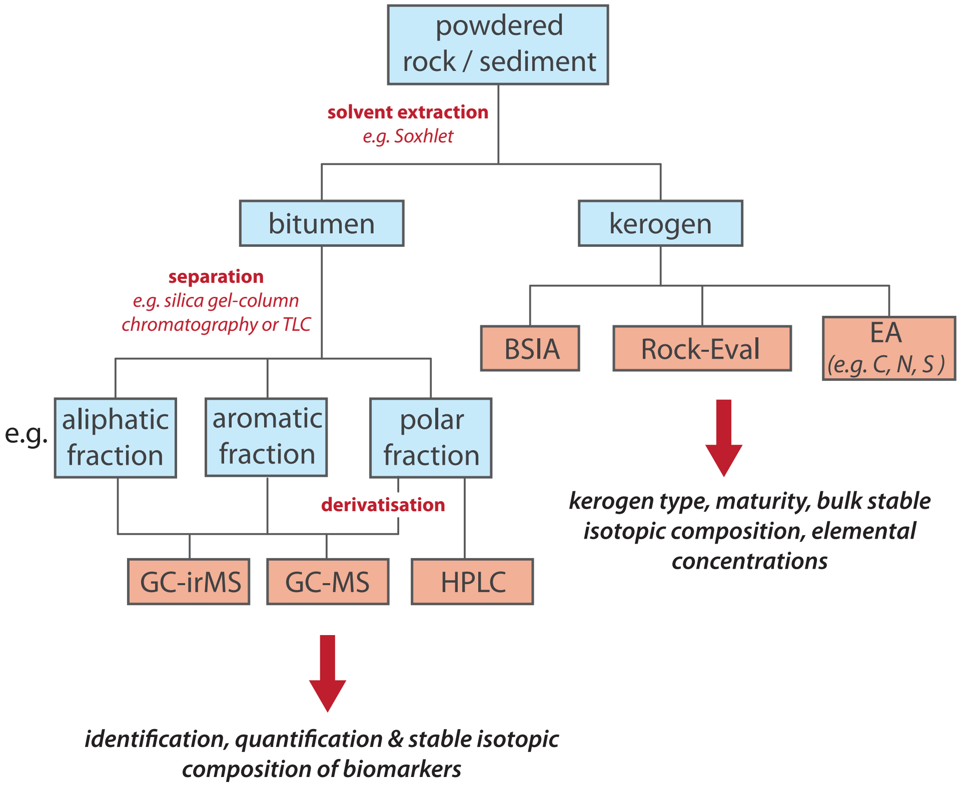

Figure 1.9: Flowchart of basic sample preparation and analyses steps performed in many biomarker studies. GC-irMS = gas chromatography-isotope ratio mass spectrometry; GC-MS = gas chromatography-mass spectrometry; HPLC = high performance liquid chromatography; BSIA = bulk stable isotope analysis; EA = elemental analysis

The obtained fractions are usually analysed by GC-MS (used the most frequently), GC-flame ionisation detection (FID) or high performance liquid chromatography (HPLC) for identification and quantification of biomarkers and other compounds. Furthermore, they can be analysed by GC-isotope ratio mass spectrometry (irMS) to obtain the stable isotopic composition of biomarkers (see following sections).

Bound biomarkers in the extracted residue (kerogen) can be released with various on- and off-line pyrolysis techniques and subsequently analysed by GC-MS. Furthermore, it is becoming more common to extract a second bitumen trapped in the inorganic matrix after acid digestion of carbonates and silicates with hydrochloric and hydrofluoric acid, respectively (e.g. Sherman et al., 2007; Nabbefeld et al., 2010b; Holman et al., 2012).

Finally, biomarker studies are frequently complemented with the analysis of bulk parameters such as elemental analysis (EA), Rock Eval pyrolysis or bulk stable isotope analysis (see following section for the latter).

Significance of stable isotopes in organic geochemistry

Conventional biomarker analysis in sediments and petroleum provides valuable information for detailed reconstructions of palaeoenvironments or oil–source rock correlations. However, the precursor lipids of many sedimentary hydrocarbons (e.g. *n*-alkanes or aryl isoprenoids) are abundant in many different types of organisms and thus their potential use as source specific biomarkers is often limited (e.g. Collister et al., 1994a; Grice et al., 1996a; Koopmans et al., 1996a; Volkman et al., 1998). Furthermore, the source organism or formation pathways of many biomarkers still remain unclear (e.g. Sinninghe-Damsté et al., 1987; Grice et al., 2009). Some of these problems can be overcome by complementing biomarker studies with stable isotope analysis.

Most elements of geochemical interest have two to four non-radioactive (i.e. stable) isotopes which occur in relatively constant natural abundances. Stable isotopes of the major elements in biolipids are displayed in **Table 1.1**. The light isotopes are generally much more abundant than their heavier counterparts. C and H isotopes are used the most frequently in biomarker studies since they form the backbone of all organic compounds. In this PhD project mainly stable isotope analysis of carbon, hydrogen and sulphur were performed. Therefore descriptions in the following sections focus only on these elements.

Table 1.1: Stable isotope abundances (atom %, Faure and Mensing, 2005 and references therein)

Carbon		Hydrogen		Sulfur		Oxygen		Nitrogen	
^{12}C	98.899 %	^{1}H	99.985 %	^{32}S	95.02 %	^{16}O	99.762 %	^{14}N	99.9634 %
^{13}C	1.111 %	D	0.015 %	^{33}S	0.75 %	^{17}O	0.038 %	^{15}N	0.3663 %
				^{34}S	4.21 %	^{18}O	0.200 %		
				^{36}S	0.02 %				

Isotopic fractionation

Although the overall abundance of stable isotopes remains constant, most physical, chemical and biological processes, such as phase transfer, chemical reactions or steps in metabolic pathways, lead to characteristic isotopic fractionations as a result of slightly different physicochemical properties of heavier and lighter isotopes. Lighter elements generally show much more significant isotope effects as a result of the bigger relative mass difference between the isotopes.

Equilibrium isotope effects are defined as the isotopic fractionations which take place between different phases or compounds in a closed system in the state of thermodynamic equilibrium. The intensity of fractionation is temperature dependent and the effects are reversible. Since heavier isotopes tend to form stronger bonds they usually are enriched in the phase with stronger intra-molecular interactions or in molecules with stronger covalent bonds (Urey, 1947). An equilibrium isotope effect for example occurs between the liquid and vapour phase of water in a closed system where deuterium gets enriched in the liquid phase and depleted in the vapour. Kinetic isotope effects in contrast are unidirectional processes which lead to irreversible isotopic fractionations and a net change of the stable isotopic composition in a system. These processes for example include isotopic fractionation in metabolic pathways or diffusion reactions. Since heavier isotopes generally have slower reaction rates they usually become depleted in the products (Bigeleisen and Wolfsberg, 1958).

The stable isotopic composition of a particular sample or compound can often provide diagnositic information about its origin and geological/environmental fate. Therefore stable isotope analysis has a wide field of applications in various disciplines including forensic sciences (e.g. Benson et al., 2006), environmental science (e.g. tracing the origin or monitoring diffusion and degradation of environmental contaminants; Schmidt et al., 2004; Vitzthum von Eckstaedt et al., 2011, 2012) or organic geochemistry (e.g. origin of biomarkers and reconstruction of palaeo-climates; Summons and Powell, 1986; Polissar and Freeman, 2010).

Notation, standards and analysis

Isotope abundances in samples are not measured in absolute values but as ratios of the heavier to the lighter isotopes relative to reference standards with a known isotopic composition. This is essential to avoid analytical errors since the abundances of heavier isotopes are very low and differences in isotopic compositions are minuscule. Stable isotopic compositions are reported in the δ-notation in units of ‰ relative to international reference materials.

$$\delta E_{\text{Sample}} = \left(\frac{R_{\text{sample}}}{R_{\text{reference}}} - 1 \right) \times 1000\ ‰$$

R stands for the ratio of the heavier to the lighter isotope (e.g. $^{13}C/^{12}C$).

$\delta^{13}C$ values are reported against Vienna Pee Dee Belemnite (VPDB), which is an international reference standard calibrated against the original marine limestone from the Pee Dee formation in South Carolina (USA). For δD analyses, Vienna Standard Mean Ocean Water (VSMOW) is used as an international standard and $\delta^{18}O$ can be reported relative to either of the before-mentioned standards. For $\delta^{34}S$ and $\delta^{15}N$ Canyon Diablo Troilite (CD) and atmospheric nitrogen are used as international standards, respectively.

Stable isotope analysis is performed on isotope ratio mass spectrometers (irMS). The main difference to conventional mass spectrometry is the utilization of fixed collector cups which simultaneously monitor abundances of selected ions at set *m/z* ratios. This is required to achieve the high precision and accuracy needed to detect the minor variations of isotope ratios in the analytes from different samples. To make a sample amenable to irMS it is first converted into a suitable gas (CO_2, N_2, SO_2 and H_2 for C-, N-, S- and H-analysis, respectively) on which the isotope ratios are measured. With $\delta^{13}C$ analysis, for example, the traces of *m/z* 44 ($^{12}CO_2$), 45 ($^{13}CO_2$) and 46 ($^{12}C^{18}O^{16}O$) are monitored whereas the detectors for δD-analysis are set to *m/z* of 2 (H_2) and 3 (DH). All abundances are measured relative to reference gas pulses of known isotopic composition which are measured shortly before and/or after the gas created from the analytes enters the irMS.

A problem which occurs in δD-analysis is the formation of H_3^+ as a by-product in the ion source. Since this creates an isobaric interference with DH, the H_3^+-factor has to be determined for appropriate correction of the measured isotope ratios. A detailed description of this correction method can be found in Sessions et al. (2001).

Bulk stable isotope analysis (BSIA)

In BSIA the average isotopic composition of the whole sample, often a complex mixture of different compounds, is measured. Stable isotope ratios are determined in a continuous-flow (CF) system with an elemental analyser (EA, for C, N and S) or a Thermochemical conversion (TC)-EA (for H and O) connected to an isotope-ratio-mass-spectrometer (irMS). The basic steps involve conversion of the sample into the suitable gas (see previous section), purification steps to remove by-products and analysis in an irMS relative to a reference gas of known isotopic composition.

For $^{13}C/^{12}C$ and $^{15}N/^{14}N$ analysis the samples are weighed into tin capsules and oxidized in a high temperature combustion furnace (~1050°C). This is followed by several clean-up steps including a reduction furnace for the removal of remaining oxygen and reduction of NO_x-compounds and a nafion-membrane or cold-trap to remove H_2O. $^{34}S/^{32}S$ is analysed by conversion into SO_2 using a similar setup but with a slightly different initial purification step.

$^{13}C/^{12}C$ of carbonates can be analysed with a gas bench sample preparation device connected online to an irMS. With this procedure carbonates in a sample are selectively digested and converted into CO_2 by the addition of phosphoric-acid under a helium atmosphere. The generated CO_2 is then diverted into the irMS. Organic-carbon (OC) is not affected by this acid treatment.

For D/H and $^{18}O/^{16}O$ analysis the samples are weighed into silver capsules, pyrolysed in a glassy carbon furnace (~1300°C) and converted into H_2 and CO, which are separated by gas-chromatography (GC) and diverted into the irMS. Schimmelmann (1991) developed a technique to account for exchangeable hydrogen in geological samples which involves equilibration of duplicate samples with two different D-enriched waters of known δD (one each) prior to TC/EA-irMS analysis and the subsequent application of a mass balance. For examples H bound to aromatic C is considered readily exchangeable and therefore does not reflect the true isotopic composition of the original compound/sample (e.g. Schimmelmann, 1991).

For BSIA of geological sediments the samples are often pre-treated to analyse particular fractions. For example selective $\delta^{13}C$ or $\delta^{18}O$ analysis of organic carbon (kerogen) can be achieved following the removal of carbonates with hydrochloric acid. BSIA is generally a robust and straight forward technique and is frequently applied in geological studies (e.g. Joachimski et al., 2001; Pancost et al., 2004; Dawson et al., 2007; Nabbefeld et al., 2010c). Compared to biomarker analysis or compound specific isotope analysis (following section) the effort for sample preparation is low. The application of BSIA in environmental studies is however limited to a single value representing the average stable isotopic value of all components in the analysed sample.

Compound specific stable isotope analysis (CSIA)

CSIA enables the determination of stable isotope ratios in individual compounds which can often provide a much more detailed insight into biogeochemical processes compared to BSIA. This section will focus on carbon (C-) and hydrogen (H-) CSIA since these were the techniques used in the current PhD project and are generally the most common stable isotopes measured in biogeochemical studies. However, methods enabling nitrogen, oxygen and sulfur CSIA have also been developed (Brand et al., 1994; Amrani et al., 2009).

CSIA is commonly performed with a GC connected to an irMS *via* a suitable interface which quantitatively converts the chromatographically separated compounds into gases amenable to irMS. More recently high performance liquid chromatography (HPLC)–irMS facilities have been developed although various analytical problems still remain to be solved before it will be suitable for a broader range of applications (Godin et al., 2005; Kujawinski et al., 2012). S-CSIA has been realised by interfacing a GC to high resolution (e.g., multicollector) ICP-MS (Amrani et al., 2009).

For C-CSIA the analytes are converted into CO_2 and H_2O at ~850 – 940 °C in a combustion furnace containing CuO (and in some systems also NiO) as oxidant. H_2O is subsequently removed by a liquid nitrogen-trap or a nafion-membrane. For H-CSIA, compounds are reduced to H_2, C and CO by high temperature conversion at ~1450°C (without a catalyst) or ~1050°C (with a chromium catalyst).

Since the development of carbon (C-) CSIA by Matthews and Hayes (1978) it has been widely used in geochemical studies for a range of applications including the elucidation of biomarker origins or reconstructions of changes in the carbon cycle (e.g. Summons and Powell, 1986; Grice et al., 2005a; Nabbefeld et al., 2010a). The technique for hydrogen (H)-CSIA was developed more recently (Burgoyne and Hayes, 1998; Hilkert et al., 1999) but is becoming similarly popular mainly due to its great significance in climate and salinity reconstructions (see "Significance of δD-analysis" page 27).

Significance of δ^{13}C-analysis

Together with hydrogen, carbon is the most common element in biomolecules (e.g. DNA, lipids, proteins, polysaccharides) and therefore an abundant constituent in biomarkers and fossil fuels. It also forms the greenhouse gases CO_2 and methane, which are only minor components of the atmosphere but have a great significance for the climatic conditions on Earth. Over geological timescales variations in their concentrations have been associated with major climate change (e.g. Kopp et al., 2005; Racki and Wignall, 2005). Furthermore, the carbonate system between atmospheric CO_2, dissolved bicarbonate and carbonate precipitation and solution plays an important role in controlling the pH of oceans and other water bodies.

Figure 1.10 illustrates basic processes in the global carbon cycle and shows average stable isotopic compositions of different carbon reservoirs. This model was designed to give a general overview of the isotopic fractionations caused by physical, chemical, biological, and anthropogenic processes occurring in nature. However, these values represent the estimated global average and do not consider local variations, which can be quite large particularly in $\delta^{13}C$ of biomass. These values are also representative of the present day environment and do not take into account variations which occurred throughout Earth's history especially during mass extinction events, which were often accompanied by oceanic anoxic events and major perturbations in the carbon cycle.

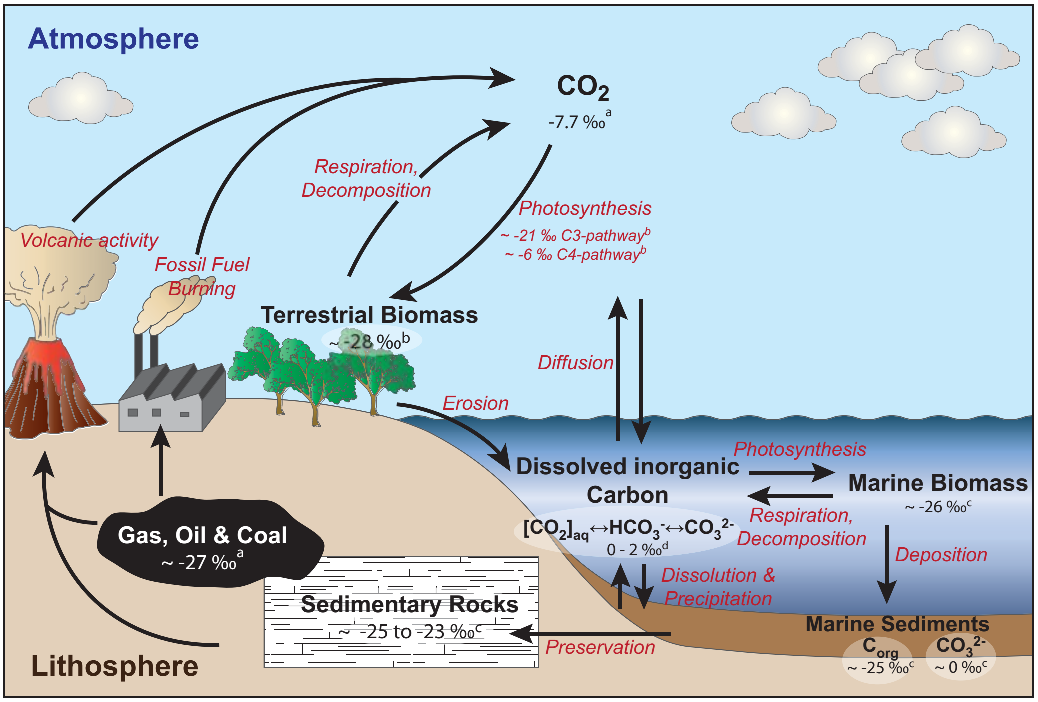

Figure 1.10: Global carbon cycle including average $\delta^{13}C$-values in major carbon reservoirs. The most significant isotopic fractionation takes place during carbon fixation (mainly photosynthesis); isotopic fractionations in heterotrophic pathways are comparatively minor (e.g. Peterson and Fry, 1987) [a] from Hoefs (1997), [b] from Peterson and Fry (1987) and references therein, [c] from Schidlowski (1988), [d] from Kroopnick (1985)

The stable carbon isotopic composition of atmospheric CO_2 is globally relatively constant at -7.7 ‰ although minor seasonal and regional variations are common (Keeling et al., 1989). The enhanced burning of ^{13}C-depleted fossil fuels may have also caused a slight decrease in this value (e.g. Friedli et al., 1986). The most significant isotopic fractionations in the carbon cycle occur (i) in the equilibrium between atmospheric CO_2 and aqueous dissolved inorganic carbon (DIC; Mook et al. 1974) and (ii) during CO_2 fixation *via* photosynthesis.

The isotopic fractionation between gaseous CO_2 and the marine carbonate system ($CO_{2,\ gas} \leftrightarrow CO_{2,aq} \leftrightarrow HCO_3^- \leftrightarrow CO_3^{2-}$) is temperature dependent and leads to relatively ^{13}C-enriched marine DIC with δ^{13}C values of up to 2 ‰ in surface oceans (Mook et al., 1974; Kroopnick, 1985; Zhang et al., 1995). Due to the input of isotopically light CO_2 from respiration and decomposition of OM, the DIC in samples from greater depths as well as marine carbonates deposited in sediments, become slightly more depleted in heavier isotopes with δ^{13}C values around 0. The largest isotopic fractionation of this type occurs during the formation of bicarbonate from CO_2. The precipitation of carbonate minerals only causes very minor isotope effects (Zhang et al., 1995); however secondary effects during diagenesis may significantly alter δ^{13}C values of carbonates (e.g. Scholle, 1995). In more isolated systems, such as lacustrine settings, δ^{13}C values of components in the carbonate system can show large variations depending on factors such as the amount of weathering from enriched carbonate rocks or depleted allochthonous carbon sources derived from respiration or decomposition in soils (e.g. Rounick and Winterbourn, 1986; Rau, 1978; Drummond et al., 1995).

The largest isotopic fractionation in the carbon cycle is however caused by the preferred assimilation of ^{12}C over ^{13}C in photosynthetic CO_2 fixation. The different biosynthetic pathways used by primary producers greatly vary in the extent of isotopic fractionation. Typical δ^{13}C ranges of biomass generated by several types of primary producers are illustrated in **Figure 1.11**. The Calvin cycle (C3 pathway), which is carried out by the majority of terrestrial plants and almost all aerobic marine primary producers, causes a strong isotopic fractionation and generates biomass depleted by an average of ~21 ‰ from the carbon source. Typically δ^{13}C in biomass produced by terrestrial plants using the C3 pathway ranges from -34 to -23 ‰ and shows a clear distinction to the less ^{13}C-depleted biomass generated by plants using the C4 pathway, which mainly include grasses, saltmarsh or desert plants (**Figure 1.11**). δ^{13}C signatures of phytoplanktonic biomass and of plants using the CAM pathway exhibit a comparatively broad range (**Figure 1.11**). Carbon fixation *via* the (reductive) tricarboxylic acid (TCA)-cycle, which occurs predominantly in some facultatively and obligately anaerobic autotrophs including green sulfur bacteria (*Chlorobiaceae*; Sirevåg et al., 1977; Quandt et al., 1977) as well as several sulfur-dependent archaebacteria and eubacteria (e.g. Beh et al., 1993), leads to comparatively minor isotopic fractionations reflected in less ^{13}C-depleted biomass (Quandt et al., 1977; Sirevåg et al., 1977; Schidlowski, 1988; Scott et al., 2006). Different metabolic pathways used for biosynthesis can also lead to distinct isotopic values between compound classes within the same organism (e.g. Sakata et al., 1997). Long-chain *n*-alkanes in many terrestrial plants for example show a distinct zig-zag pattern, with a depletion of even-carbon numbered *n*-alkanes compared to their odd-numbered homologues (Grice et al., 2008b; Zhou et al., 2010).

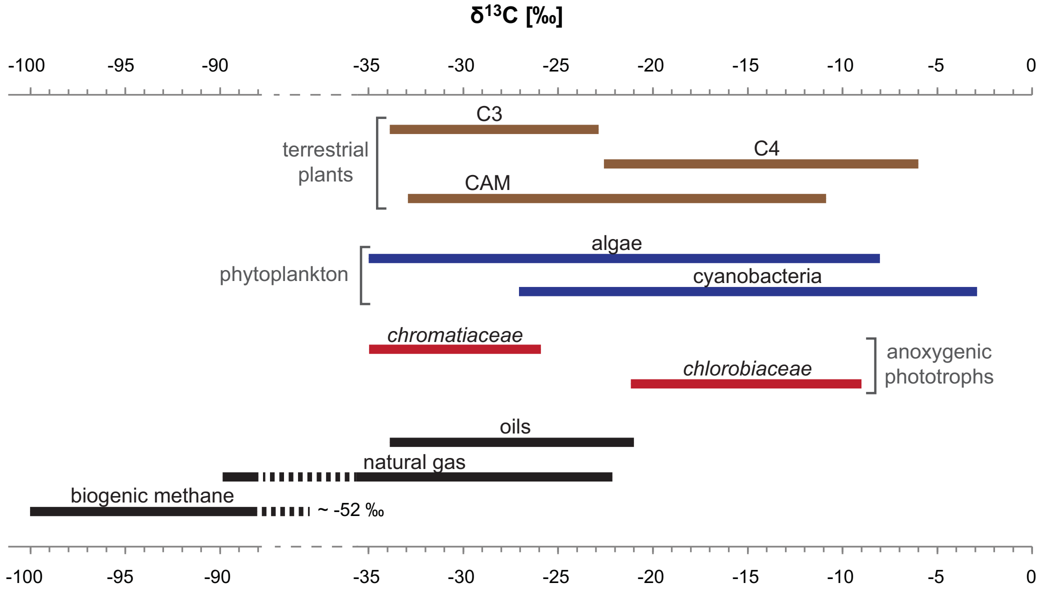

Figure 1.11: Stable carbon isotope ratios in different primary producers and fossil fuels. Adapted from Schidlowski (1988) and Peters et al. (2005)

Besides the type of metabolic pathway, the stable isotopic composition of biomass is also dependant on the δ^{13}C and availability of the carbon source. Isotopic fractionation during biosynthesis is generally highest in an open system with unlimited access to a substrate. If the substrate is limited in abundance, less isotopic fractionation occurs during fixation, leading to comparatively enriched biolipids (Takahashi et al., 1990; Freeman and Hayes, 1992). If all the substrate in a closed system was consumed, logically no fractionation would occur. The accessibility of $[CO_2]_{aq}$ can for example be low during phytoplankton blooms (e.g. Grice et al., 1996b; Pancost et al., 1997). Furthermore, an increase in salinity or pH will lead to lower CO_2 abundances and a ^{13}C-enrichment in organisms (Schidlowski et al., 1984; Grice et al., 1998b; Andersen et al., 2001). Growth forms of bacteria (e.g. planktonic or in microbial mats) and plant morphologies can also have an influence on the extent of isotopic fractionation (e.g. Freeman et al., 1994; Logan et al., 1999). The isotopic fractionation in metabolic pathways of heterotrophic organisms is comparatively minor and δ^{13}C values therefore largely resembles the values in their diet (e.g. Rounick and Winterbourn, 1986; Grice et al., 1998c; Kohn, 1999).

The δ^{13}C signatures of fossil fuels are generally depleted and largely reflect their biological origin (e.g. Hoefs, 1997; see **Figure 1.11**). During maturation the lighter isotopes become enriched in bitumen, accordingly leading to heavier isotopic values of residual kerogen (Peters et al., 1981). Geothermal natural gas can reach very depleted δ^{13}C values as low as -90 ‰ (Peters et al., 2005). Biogenic methane may be even more depleted as a result of fractionation effects in the metabolism of methanogens (Blair et al., 1993; Whiticar, 1999).

Significance of δD-analysis

Hydrogen is a significant element in geochemical studies since it is present in water as well as in all biomolecules. Due to its light atomic weight it shows the largest relative mass difference between its two stable isotopes and therefore also the biggest isotope effects. Whereas fractionation effects in the hydrological cycle are well known (**Figure 1.12**), isotope effects caused by biosynthetic pathways are just beginning to be investigated (e.g. Sessions et al., 1999, 2002; Chikaraishi and Naraoka, 2003; Bi et al., 2005; Smith and Freeman, 2006; Zhou et al., 2010).

In the hydrological cycle significant isotopic fractionations take place during phase transfer processes, which lead to a progressive D-depletion in meteoric waters (i.e. water derived from precipitation) with increasing distance from the ocean and amount of precipitation (**Figure 1.12**). This is largely driven by the lower vapour pressure of water molecules containing heavy isotopes, leading to a D-enrichment in the liquid phase during evaporation as well as precipitation (Gat, 1996; Majoube, 1971). Furthermore, δD signatures in meteoric waters are strongly influenced by factors such as temperature or latitude (Craig, 1961; Dansgaard, 1964; Kehew, 2001). δD values in oceanic waters are close to 0, as defined by the international standard VSMOV (see page 20), while meteoric waters can be depleted by more than 400 ‰ under extreme conditions (e.g. Dawson, 1993 and references therein). The isotopic composition in meteoric waters is largely reflected in δD values of biolipids in local primary producers, which makes H-CSIA of biomarkers an excellent tool for reconstructions of palaeo-climates and hydrological conditions at the time of deposition (e.g. Sessions et al., 1999; Xie et al., 2000; Andersen et al., 2001; Dawson et al., 2004; Sachse et al., 2004a; Sauer et al., 2001; Schefuß et al., 2005;). However, fractionation effects during biosynthesis can show large variations between organisms using different metabolic pathways or plants with different growth form or leaf morphologies (Chikaraishi and Naraoka, 2003, 2007; Sachse et al., 2006; Hou et al., 2008; Pedentchouk et al., 2008; Polissar and Freeman, 2010;; Smith and Freeman, 2006). Nevertheless, changes in D/H ratios of higher plant biomarkers, which correlate with the isotopic composition in local precipitation (Sachse et al., 2004; Sachse et al., 2006; Smith and Freeman, 2006; Hou et al., 2008; Mügler et al., 2010; Polissar and Freeman, 2010), have been successfully used in various climate proxies including for example the reconstruction of African monsoon-patterns throughout the Late Quarternary (Schefuß et al., 2005; Tierney et al., 2011). δD values of biolipids in aquatic primary producers, such as phytoplankton or aquatic plants, reflect the D-abundance in the water-body (Sachse et al., 2006; Mügler et al., 2010), which is in turn largely controlled by the amount of external freshwater input including precipitation (mostly D-depletion) and evaporation (D-enrichment; Gat, 1996; Ingram et al., 1996). In many aquatic settings, in particular those with a marine influence, the same parameters also cause variations in salinity, which therefore often correlate with changes in δD signatures of biomarkers derived from aquatic organisms (Andersen et al., 2001; McKirdy et al., 2010; Mügler et al., 2010). In addition, salinity can also affect D/H ratios in some biolipids independently from the isotopic composition of the source water (Schouten et al., 2006, Sachse and Sachs, 2008). This effect has been used for the development of a salinity proxy based on δD variations in long-chain alkenones derived from haptophyte algae (Schouten et al., 2006; van der Meer et al., 2008).

However, δD analyses of biomarkers and kerogens for environmental reconstructions are restricted to relatively immature sediments since hydrogen exchange with formation water takes place during thermal maturation, which significantly alters the original D/H composition (Schimmelmann et al., 1999, 2001; Leif and Simoneit, 2000; Sessions et al., 2004; Dawson et al., 2005; Nabbefeld et al., 2010c).

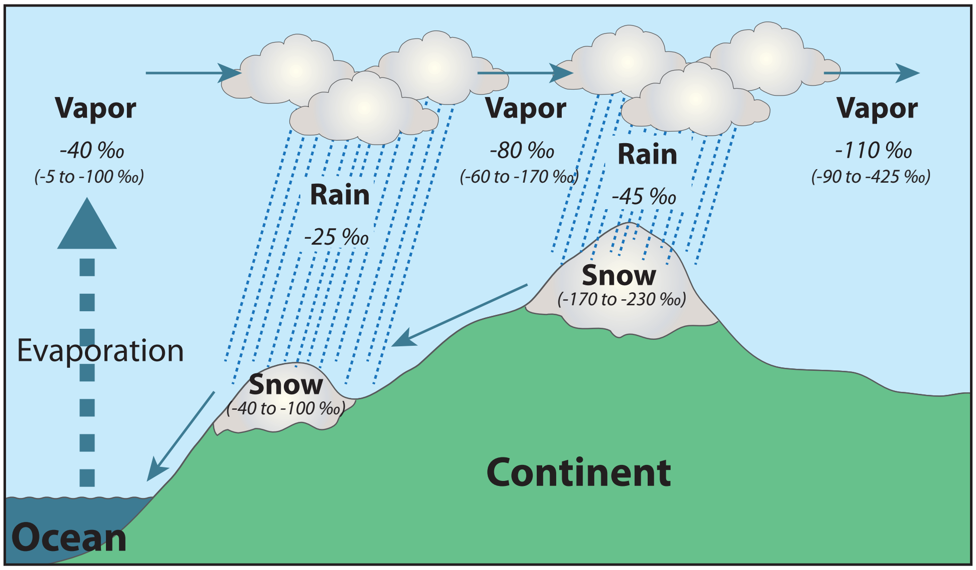

Figure 1.12: Simplified hydrological cycle including approximate stable hydrogen isotopic compositions at mid-latitudes; values in parentheses represent potential ranges inferred from literature reports at different latitudes and temperatures. Modified after Dawson (1993).

Significance of δ^{34}S-analysis

Sulfur occurs in nature in many forms with oxidation states ranging from -2 to +6. It is abundant in the atmosphere (SO_2), geosphere (metal sulfides, elemental sulfur) and oceans (mainly SO_4^{2-}) as well as in a variety of organosulfur compounds present in the biosphere, sediments and petroleum. Sulfur has four stable isotopes (see **Table 1.1**) and is measured as $^{34}S/^{32}S$, since ^{34}S is the most abundant of the heavier isotopes and the relative mass difference to ^{32}S is sufficient to cause significant isotope effects.

The most significant isotopic fractionation in sulfur cycling takes place during bacterial sulfate reduction, which commonly occurs in anoxic marine sediments. Under laboratory conditions this process has caused a ^{34}S-depletion in the generated sulfides by up to 30 ‰ depending on sulfate availability (Rees, 1973; Habicht et al., 2002). In the natural environment even greater differences of up to ~50 ‰ between sulfate and associated pyrite (predominant inorganic sulfur species in sediments) have been measured (Canfield and Thamdrup, 1994; Passier et al., 1999; Grice et al., 2005a; Nabbefeld et al., 2010a). Light $\delta^{34}S$ signatures in marine sedimentary pyrite can be indicative of widespread euxinia in the depositional environment. For more details about sulfur species and their stable isotopic compositions in euxinic marine environments see "Evidence of photic zone euxinia (PZE)" page 30*ff*.

$\delta^{34}S$ values of petroleum, which often contain bound sulfur in significant amounts, can vary over a wide range (-8 to 32 ‰; Faure and Mensing, 2005). Although these values only contain limited information about OM-sources and palaeoenvironmental conditions (due to significant isotopic fractionations by relatively unknown reactions during S-incorporation) $\delta^{34}S$ analysis of petroleum can be very useful in oil-oil correlations (e.g. Gaffney et al., 1980). Furthermore, $\delta^{34}S$ analysis of metal sulfides finds applications in the investigation of ore deposits (e.g. Kajiwara and Krouse, 1971; Rye and Ohmoto, 1974).

S-CSIA has only been developed recently (Amrani et al., 2009), therefore investigations of $\delta^{34}S$ compositions of natural organosulfur compounds are still in very early stages (e.g. Amrani et al., 2012).

Reconstruction of palaeoenvironments using biomarkers and stable isotopes

There are numerous source specific biomarkers or biomarker ratios and stable isotope parameters which can be indicative of different OM-sources, environmental conditions, maturities or depositional ages. Many of these have been summarized and reviewed by Peters et al. (2005), Brocks and Grice (2011) and Grice and Brocks (2011). This section describes selected environmental and source indicators (including biomarkers as well as compound specific and bulk stable isotopic compositions) for ecological conditions of particular relevance to the present research project.

Evidence of photic zone euxinia (PZE)

Photic zone euxinia (PZE) is a condition which can develop in marine settings with a stagnant water-column, where anoxia and high concentrations of toxic H_2S produced by anaerobic sulfate reducing bacteria (SRB) in or near sediments (**Reaction 1.1**) extend to the photic zone (i.e. zone with enough light penetration for photosynthesis to occur; see **Figure 1.13**). In the present day this phenomenon only occurs persistently in very few locations such as some isolated deep Fjords (e.g. Anderson et al., 1988; Millero, 1991) and in the Black Sea, which exhibits a sharp chemocline as a result of permanent density stratification with freshwater over saline bottom waters (Murray et al., 2007). During some periods in the past, however, PZE prevailed globally in oceanic anoxic events (OAE) associated with several big mass extinctions including the Permian–Triassic (P-T) extinction (Grice et al., 2005a; Summons et al., 2006; Hays et al., 2007; Nabbefeld et al., 2010a) and several Mesozoic events (Sinninghe Damsté and Köster, 1998; Pancost et al., 2004; Jaraula et al., 2013). In some cases, when H_2S in bottom waters exceeds a certain threshold, the chemocline can abruptly move upwards and rise to the water surface, leading to outgassing of toxic H_2S (Kump et al., 2005). This phenomenon has been observed periodically in present day fjords (Skei, 1988; Meyer and Kump, 2008) and most likely also occurred persistently at the P-T extinctions (Grice et al., 2005a; Kump et al., 2005; Nabbefeld et al., 2010a). Evidence of PZE has also been reported in Middle to Late Devonian sediments from different parts of the world, which may have potentially been a driver in the Late Devonian extinctions (see "Late Devonian extinctions" page 3 *ff.*). However, at least some of these events in the Devonian were only episodical as indicated for example by bioturbation in sediments (Brown and Kenig, 2004).

$$2CH_2O + SO_4^{2-} \rightarrow 2HCO_3^{-} + H_2S \qquad \textbf{(1.1)}$$

Green and brown pigmented *Chlorobiaceae* (both referred to as "green sulfur bacteria", GSB) are obligate anaerobic phototrophs, which fix CO_2 *via* anoxygenic photosynthesis utilising H_2S generated by SRB as an electron donor (**Reaction 1.2**). Therefore they can only thrive under PZE conditions or occasionally in thick microbial mats in shallow waters, where euxinic microenvironments are created within the mat structures (Nicholson et al., 1987; Brocks and Pearson, 2005). Since *Chlorobiaceae* utilize the TCA-cycle for carbon fixation, their biolipids are less ^{13}C-depleted compared to those of most other marine phytoplankton (see "Significance of δ13C-analysis" page 23).

$$2H_2S+CO_2 \xrightarrow{h\nu} 2S+CH_2O+H_2O \tag{1.2}$$

There are several source specific biomarkers derived from unique carotenoids and bacteriochlorophylls in the photosystem of *Chlorobiaceae*, which provide unequivocal evidence for PZE in the depositional environment if significant input from microbial mats can be excluded. These include for example isorenieratane (**IX**), palaeorenieratane (**X**), chlorobactane (**XI**), specific maleimides (**XII**) or a suite of relatively ^{13}C-enriched 2,3,6-aryl isoprenoids (**XIII**) (Summons and Powell, 1986, 1987; Requejo et al., 1992; Grice et al., 1996a; Grice et al., 1996b; Koopmans et al., 1996b). 2,3,6-Aryl isoprenoids (**XIII**) can however also be derived from β-carotene, which is a non-specific biomarker, abundant in many organisms (e.g. Koopmans et al., 1996a). Especially if no intact C_{40} carotenoids (e.g. isorenieratane; **IX**) or other specific biomarkers for *Chlorobiaceae* are present in the same sample, the origin of 2,3,6-aryl isoprenoids (**XIII**) should be confirmed by CSIA.

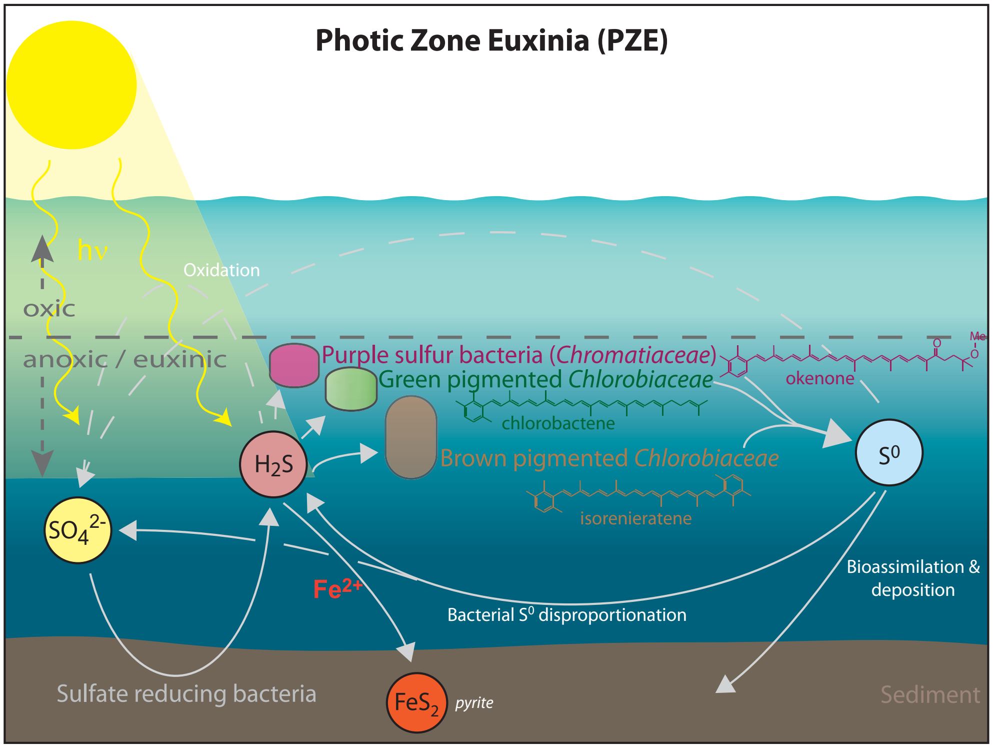

Figure 1.13: Illustration of photic zone euxinia with associated organisms, typical pigment biomarkers and some basic steps of sulfur cycling in a euxinic marine environment. Purple sulfur bacteria and green pigmented *Chlorobiaceae* usually thrive in water depths up to ~13 and 20 m, respectively, whereas brown pigmented *Chlorobiaceae* require less light and grow in depths of up to 80 m (Brocks and Pearson, 2005 and references therein)

Typical organisms present at PZE, their interactions and some associated pigments yielding characteristic biomarkers are displayed in **Figure 1.13**. Purple sulfur bacteria (PSB, *Chromatiaceae*) are also anoxygenic phototrophs which flourish under these conditions and usually grow in a layer above the green pigmented *Chlorobiacea* on the upper end of the chemocline. Brown pigmented *Chlorobiaceae* require less light for photosynthesis than the other species and can grow in depths of up to 80 m (Brocks and Pearson, 2005 and references therein). However, unlike *Chlorobiaceae*, many species of PSB are facultative anaerobes and capable of using a wider range of electron donors for photosynthesis if required (de Wit, 1992 and references therein). Therefore PSB derived biomarkers such as okenane (**XIII**) have to be interpreted accordingly.

A further indication of widespread euxinia in marine environments can be a ^{34}S-depletion in sedimentary pyrite (FeS_2) and high S/C ratios (e.g. Grice et al., 2005a; Meyer and Kump, 2008). In present day conditions SO_4^{2-} in ocean water exhibits relatively constant δ^{34}S-values around +20 ‰ (Canfield and Teske, 1996; Faure and Mensing, 2005) although alterations of this value have been reported in persistently euxinic environments (values $<$ 20 ‰ in upper water-column, values $>$ 20 ‰ in lower water-column; e.g. Anderson et al., 1988). Similar varitions have also occurred throughout Earth's history, in particular during mass extinction events (e.g. Newton et al., 2004; Kampschulte and Strauss, 2004; Wortmann and Paytan, 2012). A major isotopic fractionation takes place during the reduction of SO_4^{2-} to H_2S by SRB in surface sediments or in the water column with unlimited access to SO_4^{2-}. In deeper sediments, which represent a more closed system, significantly less fractionation occurs during H_2S formation. In the case of an oxic/non-euxinic water-column, H_2S formation is restricted to sediments and therefore less ^{13}C-depleted isotopic signatures occur. Some of the H_2S in the water column as well as in sediments is scavenged by Fe^{2+} species, which are commonly present in anoxic seawater and originate from dissolution of iron oxides or hydroxides (Passier et al., 1999), and deposited mainly as pyrite without any major isotopic fractionation (Faure and Mensing, 2005). In laboratory experiments with cultured and natural SRB, fractionations during SO_4^{2-} reduction (under high $[SO_4^{2-}]_{aq}$) led to a ^{34}S-depletion in the product of up to ~30 ‰ (Rees, 1973; Habicht et al., 2002). However, in natural environments even greater depletions of ~50 or 60 ‰ from average ocean water SO_4^{2-} is often measured in associated sulfides (Canfield and Thamdrup, 1994; Canfield and Teske, 1996; Passier et al., 1999). Under persistent PZE the sulfide pool in seawater can become more depleted if the sulfur produced by *Chlorobiaceae* is repeatedly re-oxidized through anaerobic bacterial sulfur disproportionation (**Reaction 1.3**; leading to only relatively minor ^{34}S-enrichment in sulfate) or in the oxygenated zone and reduced again by SRB, leading to a further depletion of H_2S (Jørgensen, 1990; Canfield and Thamdrup, 1994; Canfield and Teske, 1996). However, reservoir effects in the SO_4^{2-} pool, particularly at greater depths, as a result of enhanced burial of isotopically light pyrite or outgassing of depleted H_2S can also have the opposite effect in some environments and lead to a ^{34}S-enrichment in the remaining SO_4^{2-} (Anderson et al., 1988; Newton et al., 2004; Grice et al., 2005a).

$$4S^0 + 4H_2O \rightarrow 3H_2S + SO_4^{2-} + 2H^+ \qquad \textbf{(1.3)}$$

Reconstruction of salinity

Changes in the salinity of an aquatic ecosystem can have a profound impact on biodiversity and the types of organisms present in a particular environment. For example in the Coorong a significant increase in salinity, caused mainly by human alterations to the natural water regime, was likely the main driver of the extinction of key seagrass species and significant alteration of *in situ* primary production (see "Environmental conditions in the present day Coorong" page 8 and **Chapter 2**). Changes in salinity over time can also be an indication of fluctuating water levels and climatic conditions since they co-vary with the amount of evaporation and precipitation. Furthermore, high salinities in combination with freshwater input can also lead to density stratification promoting anoxia or euxinia in the bottom waters (see previous section).

As described previously in "Significance of δD-analysis" (page 27), changes in δD signatures of sedimentary biomarkers derived from aquatic primary producers are often indicative of salinity variations. Generally, higher salinities are reflected in a D-enrichment in water and phytoplankton (page 28). The $\delta^{13}C$ values of phytoplanktonic biomarkers may also be indicative of salinity variations which influence the solubility of CO_2, the carbon source of most phytoplankton. A significant increase in salinity can therefore be reflected in a ^{13}C-enrichment in primary producers (for a more detailed explanation see page 26). However, there are many more parameters which can have a bigger influence than salinity on the δ^{13}C of primary producers. Variations in the stable isotopic composition of biolipids as a result of metabolic pathways are generally more pronounced in δ^{13}C compared to δD (e.g. Chikaraishi and Naraoka, 2003; Polissar and Freeman, 2010).

Another phenomenon which is often associated with hypersaline depositional environments is an even-over-odd predominance in *n*-alkanes, often with an unusually high relative abundance of the C_{22} *n*-alkane (ten Haven et al., 1985, 1988). Accordingly ten Haven et al. (1988) introduced the R_{22}-index ($2\times n\text{-}C_{22}/(n\text{-}C_{21}+n\text{-}C_{23})$) as an indicator of hypersalinity. Furthermore, the enhanced relative abundance of an extended hop-17(21)-ene (**XVII**) series (up to C_{35}) and regular extended αβ-hopanes (ten Haven et al., 1985, 1986, 1988) are an indication for hypersaline palaeoenvironments.

Pristane (**II**) and phytane (**I**) in geological samples are predominantly derived from the phytol side-chain of chlorophyll *a* (**III**) in oxygenic phototrophs (Brooks et al., 1969). However, there are also other known sources of these isoprenoids including bacteriochlorophylls in PSB, archaeal membrane components (Chappe et al., 1982; Rowland, 1990), zooplankton (Blumer et al., 1963) or the diagenetic formation of pristane (**II**) from compounds such as tocopherols (**XV**), or methyltrimethyltridecylchromans (MTTCs; **XVI**; Goossens et al., 1984; Li et al., 1995). The pristane/phytane ratio is commonly used as an indicator for the redox potential of depositional environment with values < 1 indicative of reducing conditions (Didyk et al., 1978). However, this ratio also depends on salinity, with lower values generally occurring in more saline environments (ten Haven et al., 1985; Schwark et al., 1998).

The MTTC ratio of 5,7,8-trimethyl-MTTC/total MTTCs has proven to be a reliable indicator for palaeosalinities (Sinninghe-Damsté et al., 1987, 1993; Grice et al., 1998d; Schwark et al., 1998). In freshwater settings the 5,7,8-trimethyl-MTTC is predominant and the 8-methyl-MTTC is absent whereas in hypersaline settings mono- and dimethyl-MTTCs are a lot more abundant. However, in depositional settings with a stratified water-column the application of the chroman ratio to infer palaeosalinities might be less clear. The chroman ratio seems to reflect the salinity in the surface water in these cases (Sinninghe Damsté et al., 1993). Although the relation of the chroman ratio to salinity in the palaeoenvironment has been well-studied, the origin and formation pathway of MTTCs (**XVI**) remain unclear (see following section).

Gammacerane (**XVIII**) is not a direct indicator of salinity, however its presence reflects a density stratification of the water column, which is often the result of saline/hypersaline bottom waters overlain by freshwater (e.g. Sinninghe Damsté et al., 1995; Grice et al., 1998a). The predominant source of gammacerane (**XVIII**) in sediments is tetrahymanol (**XIX**), a membrane component of bacteriovorous protozoans living at the oxic/anoxic interface in the water-column (Sinninghe Damsté et al., 1995). Gammacerane (**XVIII**) is often quantified relative to the regular αβ-hopane ("gammacerane index" = gammacerane/(gammacerane + αβ-hopane)).

Methyltrimethyltridecylchromans (MTTCs)

As mentioned previously, the abundance of 5,7,8-trimethyl-MTTC relative to other MTTCs (**XVI**) is an excellent indicator for palaeosalinities. However, it remains unclear how these compounds are formed or why their ratio reflects salinity. MTTCs (**XVI**) have been reported in significant abundances in many sediments and crude oils from various depositional environments (Sinninghe-Damsté et al., 1987; Schwark and Püttmann, 1990; ten Haven et al., 1990; Li et al., 1995; Grice et al., 1998a; Schwark et al., 1998). Only certain isomers of mono-, di- and trimethyl-MTTCs occur in geological samples (see **Figure A1.1**; Sinninghe-Damsté et al., 1987). So far, MTTCs (**XVI**) could not be isolated from any organisms and there is an ongoing debate whether these compounds are true biomarkers with a biological origin or if they are formed in condensation reactions during (early) diagenesis (Sinninghe Damsté et al., 1993; Li and Larter, 1995; Li et al., 1995; Sinninghe-Damsté and De Leeuw, 1995; Zhang et al., 2012). Despite a structural similarity to tocopherols (**XV**), these are not thought to be feasable precursors due to the hydroxyl group at C-6 (Sinninghe-Damsté et al., 1987; Li et al., 1995). On account of the limited number of MTTC isomers present in geological samples (**XVI**) and the influence of salinity in surface water on the chroman ratios, Sinninghe Damsté et al.

(1993) suggested a direct biosynthetic origin from phytoplankton. On the contrary, Li et al. (1995) proposed the formation of MTTCs (**XVI**) through condenstion reactions of presumably higher plant-derived alkylphenols (e.g. bound in lignin) with phytol from the sidechain of chlorophylls (most commonly derived from phytoplakton). They demostrated the generation of MTTCs (**XVI**) in various condensation reactions between several free or bound alkylphenols with phytol or chlorophyll *a* (**III**). Furthermore, they showed that pristenes were generated from pyrolysis of MTTCs (**XVI**) and therefore suggested these compounds as a likely source of pristane (**II**) in mature geological samples.

Perylene

Perylene (**XX**) is an unsubstituted PAH which is abundant in many Mesozoic and Cenozoic sediments (e.g. Blumer, 1960; Orr and Grady, 1967; Aizenshtat, 1973; Laflamme and Hites, 1978) and occasionally also occurs in samples as old as the Devonian (Grice et al., 2009). Whereas the majority of unsubstituted PAHs originate from incomplete combustion processes or anthropogenic contamination, perylene (**XX**) has a different, but still unclear origin. Only small amounts of this compound are formed during combustion of organic materials (Kawka and Simoneit, 1990; Jenkins et al., 1996) and its abundance profiles usually differ to those of other combustion PAHs (Jiang et al., 2000; Atahan et al., 2007; Grice et al., 2009; Suzuki et al., 2010). Perylene (**XX**) is almost exclusively found in sediments deposited under reducing conditions (Aizenshtat, 1973; Wakeham et al., 1980). Furthermore, a diagenetic origin of perylene (**XX**) has now been widely accepted, based on its increasing abundances with burial depth which commonly occurs in recent settings (Orr and Grady, 1967; Aizenshtat, 1973; Wakeham et al., 1980; Gschwend et al., 1985).

A likely source seems to be quinone pigments (**XIX**) abundant in a variety of extant organisms including wood-degrading fungi, crinoids and some plants and insects (Jiang et al., 2000; Grice et al., 2009; Suzuki et al., 2010). Suzuki et al. (2010) found loosely correlated perylene (**XX**) abundances with the amount of terrigenous OM input in Cretaceous to Paleogene sediments and suggested a fungal origin based on distinct $\delta^{13}C$ signatures of perylene (**XX**). In addition, perylene (**XX**) has been linked to wood/lignin degradation in Holocene sediments by its appearance in intervals with woody vegetation and an inverse correlation to the lignin biomarker guaiacol, all described in Grice et al. (2009). They furthermore analysed various sediments and crude oils from different time periods and did not find perylene (**XX**) in samples deposited prior to the rise of vascular plants or in exclusively marine depositional environments. However, despite its structural similarities to quinone pigments and its association with fungal wood degradation, a chemical formation pathway has not been proposed so far. Nevertheless, high abundances of perylene (**XX**) imply an anoxic depositional environment and in many cases also terrestrial input and the presence of (wood degrading) fungi or possibly other quinone pigment bearing organisms such as crinoids.

Aims of the thesis

The main objectives of this thesis were to explore biomarker and stable isotope approaches for the reconstruction of environmental and ecological changes in recent and ancient depositional settings with a particular focus on reconstructions of palaeosalinity and water-column stratification. A specific interest was to further investigate the origin and formation pathway of MTTCs in geological samples and their significance in palaeoenvironmental reconstructions, not only as palaeosalinity indicators but also in novel applications as potential markers for terrigenous input and riverine freshwater incursions (possibly leading to water-column stratification). Another aim was to use comprehensive biomarker, elemental and stable isotope studies to gain a more detailed knowledge on environmental changes (i) in a depositional setting associated with ancient reef systems, which were severely affected by the Late Devonian extinctions, and (ii) in the recent Coorong Lagoon, which is representative of modern ecosystems undergoing severe ecological declines as a result of human impact. In both environmental settings salinity and freshwater incursion were major parameters influencing the ecosystem conditions.

Chapter 2

The Coorong region is an estuarine ecosystem in South Australia which has been significantly affected by human alterations to its natural water-regime (page 8).

Previous studies of the Coorong Lagoons indicated significant changes in primary production (Krull et al. 2009; McKirdy et al. 2010) as well as an increase in salinity (e.g. Webster 2005) which occurred as a direct result of the human water-management and a prolonged drought. The objectives of **Chapter 2** were to investigate OM sources and salinity in the northern Coorong Lagoon prior to human influence and further explore the nature and extent of changes in these parameters over the past several decades. For this purpose, biomarker distributions and their stable isotopic compositions ($\delta^{13}C$ as well as δD) as well as $\delta^{13}C$ of bulk OM were analysed in a core from the North Lagoon spanning more than 5000 years.

Chapter 3

More knowledge about the origin and formation pathway of MTTCs in geological samples may significantly enhance their significance in palaeoenvironmental reconstructions and might enable their application in proxies for freshwater-incursions or terrigenous input (pages 35-37). Therefore the purpose of **Chapter 3** was the development of a pyrolysis-GC-irMS method which enables the measurement of individual $\delta^{13}C$ values of the isoprenoid- and alkylphenol subunits of MTTCs in order to possibly distinguish an origin of these compounds from (i) direct biosynthesis or (ii) early diagenetic condensation reactions of higher plant-derived alkylphenols with predominantly phytoplankton-derived phytol. In the case of MTTC formation *via* condensation reactions, these individual $\delta^{13}C$ values may reflect the different source organisms of the MTTC moieties (i.e. higher plants and phytoplankton).

Chapter 4

The main intention of **Chapter 4** was to further investigate the origin of MTTCs as well as their significance in palaeoenvironmental reconstructions and possibly develop a novel biomarker approach for the reconstruction of freshwater incursions in marine palaeoenvironments. Therefore the relations of chroman ratios and MTTC abundances to other molecular and stable isotopic indicators of water-column stratification, salinity, terrigenous input, anoxia and photic zone euxinia were investigated in sediments of a restricted marine Late Devonian palaeoenvironment associated with reef systems. The main aims were (i) the establishment of the relation between chroman ratios and palaeosalinities, particularly in a stratified water-column and (ii) to explore the potential link of MTTC abundances to terrigenous input and (riverine) freshwater-incursions.

Chapter 5

Although the Late Devonian extinctions rank amongst the biggest mass extinction events in Earth's history their nature and caus(es) remain unclear (pages 3-5). The biggest and most famous of the associated events occurred at the F-F boundary; however there were also less intensively studied extinction pulses throughout the time period from the Middle Givetian until the end of the Famennian. In **Chapter 5** the main intention was to gain a more detailed insight into palaeoenvironmental conditions in marine depositional settings associated with reef systems close to or at the G-F boundary as well as later in the Frasnian to identify periods of elevated biotic stress and further explore potential events related to the Late Devonian extinctions. A secondary interest was the investigation of conditions supporting the exceptional OM and biomarker preservation in the Gogo Formation of the Canning Basin. To achieve these goals an integrated biomarker, elemental and stable isotope approach combined with palynolgical data and Rock Eval pyrolysis was used to analyse sediments in a core from the Canning Basin, WA spanning the before-mentioned time periods.

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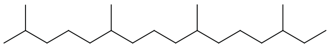
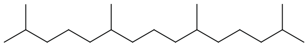
Appendix 1

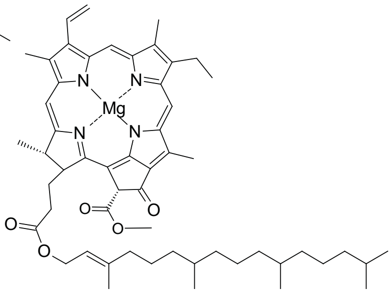
Phytane (**I**)



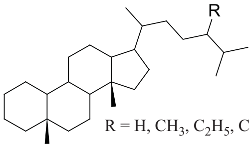
Pristane (**II**)



e.g. Chlorophyll a (**III**)

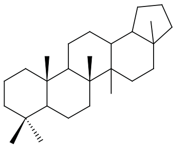


Diasteranes (**IV**)

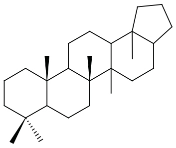


R = H, CH_3, C_2H_5, C_3H_7

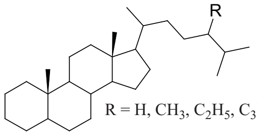
Ts (**V**)



Tm (**VI**)

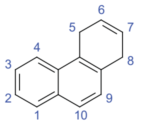


Steranes (**VII**)

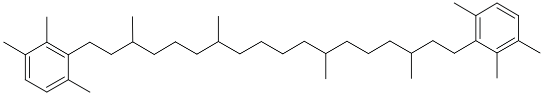


R = H, CH_3, C_2H_5, C_3H_7

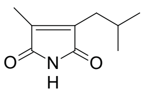
Phenanthrene (**VIII**)



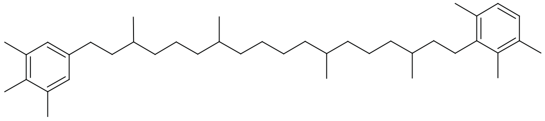
Isorenieratane (**IX**)



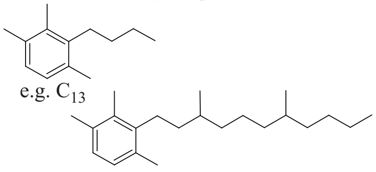
e.g. Methyl-isobutyl maleimide (**XII**)



Paleorenieratane (**X**)



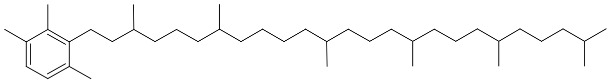
2,3,6-Aryl isoprenoids (**XIII**)



e.g. C_{13}

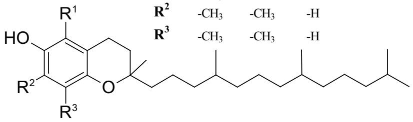
e.g. C_{22}

Chlorobactane (**XI**)

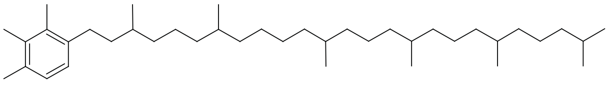


Tocopherols (**XV**)

	α	γ	δ
R^1	$-CH_3$	-H	-H
R^2	$-CH_3$	$-CH_3$	-H
R^3	$-CH_3$	$-CH_3$	-H

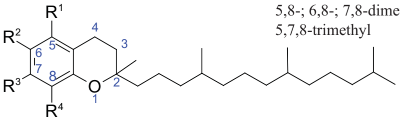


Okenane (**XIV**)

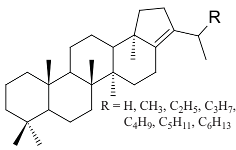


MTTCs (**XVI**)

6- 8-methyl
5,8-; 6,8-; 7,8-dimethyl
5,7,8-trimethyl

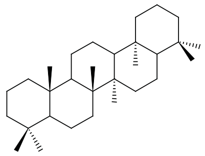


Hop-17(21)-enes (**XVII**)

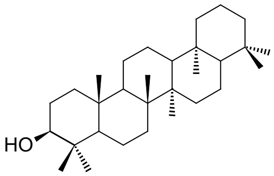


R = H, CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}

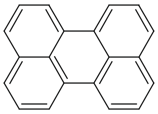
Gammacerane (**XIX**)



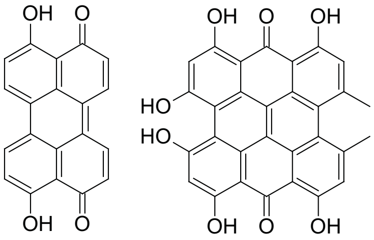
Tetrahymanol (**XIX**)



Perylene (**XX**)



Quinone pigments (**XXI**)



e.g. Perylenequinone e.g. Hypericin

Figure A1.1: Structures referred to in the text

Chapter 2

Salinity variations in the Northern Coorong Lagoon, South Australia: Significant changes in the ecosystem following human alteration to the natural water regime

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***Organic Geochemistry*, submitted**

Abstract

European settlement and drought have significantly impacted the hydrology of the Coorong, a shallow coastal lagoon complex in South Australia, which is part of a terminal wetland at the Mouth of the River Murray. An increased salinity associated with lower water levels and progressive isolation from ocean flushes contributed to a severe decline in ecological diversity over the last several decades. Here we have conducted a molecular and stable isotopic study of a sedimentary core from the northern Coorong Lagoon spanning more than 5000 years to investigate the recent palaeoenvironmental history of the ecosystem. Major alterations were evident in many biogeochemical parameters in sediments deposited after the ~1950s coinciding with the beginning of intensified water regulations. The most prominent shift occurred in $\delta^{13}C$ profiles of long chain *n*-alkanes (C_{21}-C_{33}) from average values of -22.6 ‰ to an average of -29.5 ‰. Further changes included decreases in carbon preference index (CPI) and average chain length (ACL) of the *n*-alkane series as well as significant increases in algal (e.g. C_{20}HBI, long chain alkenes and C_{29}-alkadiene) and bacterial (e.g. ^{13}C depleted short-chain *n*-alkanes and hopanoids, $\delta^{13}C$ values: -35.9 to -30.1 ‰) derived hydrocarbons. Long-chain *n*-alkanes with a strong odd-over-even predominance as observed here are typically attributed to terrestrial plants. In the Coorong however, terrestrial input to sedimentary OM is only minor. Therefore changes in the before mentioned parameters were attributed to a source transition from predominantly aquatic macrophytes towards a major contribution from microalgae and bacteria.

δD values of long chain *n*-alkanes showed a general trend towards less depleted values in younger sediments, indicating an overall rising salinity. However, the most pronounced positive shift in these profiles again occurred after the 1950s. Altogether this study demonstrates that the recent human induced changes of the Coorong hydrology, compounded by a severe drought led to an increase in salinity and alterations of primary production which have been much more significant than natural variations occurring throughout the Holocene over several thousands of years.

Keywords: Coorong Lagoon, δD, salinity, organic matter, perylene, 1-chloro-*n*-alkanes

Introduction

The Coorong is part of a terminal lake system in South Australia at the mouth of the River Murray. It consists of two narrow coastal lagoons (North and South Lagoon) which are connected through a small channel and together extend for more than 130 km in length (**Figure 2.1**). The wetland is of great ecological significance, in particular for water birds (migratory and native; Paton et al., 2009), and is therefore listed under the Ramsar Convention on Wetlands and is protected as a national park. However, substantial water regulations imposed on the River Murray and the lake system in the second half of the 20th century, compounded by a severe drought from 2000-2010, have significantly altered the complex and dynamic hydrology of the ecosystem leading to a marked increase of salinity in the Coorong and a severe decline of ecological diversity over the past several decades (e.g. DEH, 2000; Shuttleworth et al., 2005; Paton et al., 2009; Dick et al., 2011). Although recent floods in 2010 temporarily improved the ecological state of the Lower Lakes and led to a short term recovery of the freshwater levels in the Coorong, the long term issue of providing continuous riverine input (especially under future drought conditions) has not been resolved and an extensive water management plan is required to further restore and maintain the diverse ecosystem of the Coorong (Kingsford et al., 2011).

Estuarine systems like the Coorong have multiple organic matter (OM) sources including *in situ* primary production by organisms such as phytoplankton, microbial mats, macroalgae or aquatic and benthic plants as well as allochthonous input from terrestrial plant debris and soil OM which enter the system mainly through riverine inflow (e.g. Shuttleworth et al., 2005; Volkman et al., 2008). Changes in the type of OM – for example caused by different flow regimes – can have a profound impact on the ecosystem since the base of the food-web is affected (Krull et al., 2009). Efficient remediation measures for the Coorong require a profound understanding of the effects of alterations in parameters such as water-level, salinity or quantity of riverine input (all of which have occurred as an immediate result of human water management) on the ecosystem. This also involves gaining detailed knowledge about ecology and palaeoenvironmental conditions prior to human influence, and tracking changes in OM sources over time to establish their relationship with the key environmental parameters previously mentioned.

Figure 2.1: Location of the Coorong including the sampling site

The analysis of sedimentary biomarkers in combination with compound specific stable isotope analysis (CSIA) is a powerful analytical tool for this purpose and has been frequently used to elucidate OM sources and/or environmental conditions at the time of deposition (e.g. Freeman et al., 1994; Grice et al., 1996, 2001, 2005a; Volkman et al., 2008; McKirdy et al., 2010). Compound specific δD analysis has a great potential for reconstructions of climate and hydrological conditions in palaeoenvironments since D/H-ratios of biolipids in autotrophic organisms are strongly influenced by the composition of the utilized source water (Sessions et al., 1999; Andersen et al., 2001; Sauer et al., 2001; Dawson et al., 2004; Nabbefeld et al., 2010). δD signatures of biomarkers derived from aquatic or benthic organisms thus reflect D/H ratios in the lake or seawater (Sachse et al., 2006; Mügler et al., 2010) and therefore often strongly correlate with palaeosalinities (Andersen et al., 2001; McKirdy et al., 2010; Mügler et al., 2010). Salinity in estuarine systems such as the Coorong co-varies with δD signatures of lake water since it is largely determined by the balance of evaporation and seawater inflow (both leading to a D-enrichment in the reservoir) and the input of D-depleted meteoric waters from precipitation or riverine influx (Gat, 1996; Ingram et al., 1996).

A detailed investigation of OM sources in the recent Coorong was performed by Revill et al. (2009) who analysed abundances and stable isotopic compositions of pigment and lipid biomarkers in the water-column and surface sediments throughout both lagoons. McKirdy et al. (2010) carried out a comprehensive study which also included bulk and compound specific isotope analysis to reconstruct variations in environmental conditions and OM sources in the Coorong from the mid to late Holocene. However, their main focus was a core from the South Lagoon and only some elemental and bulk isotope data from the North Lagoon was included. Nevertheless their data revealed significant differences between the ecosystems of the North and South Lagoon. Krull et al. (2009) combined elemental, bulk $\delta^{13}C$ and ^{13}C-NMR analyses with radionuclide dating in several cores from both lagoons spanning the past 100 years and found evidence for significant changes in sedimentary OM, which coincided with the beginning of substantial human impact on the water regime.

However, so far there have been no biomarker studies in sediments from the North Lagoon spanning the time periods before and after European settlement. Here we analysed $\delta^{13}C$ of bulk OM as well as abundances and stable isotopic compositions ($\delta^{13}C$ and δD) of lipid biomarkers in a core from the North Lagoon dating back more than 5000 years. The aim was to gain insight into the ecosystem of the North Lagoon in pre-European times, reconstruct salinity variations over an extended timeframe and more robustly scrutinise the recent changes in OM sources observed by Krull et al. (2009). Pollen analysis and different dating techniques performed previously by Krull et al. (2009) and Fluin et al. (2007) in a core nearby allowed for a correlation of our data with recent events in the Coorong.

Analysed samples

Environmental settings

Together with Lakes Alexandrina and Albert the Coorong forms a terminal wetland at the mouth of the River Murray (**Figure 2.1**). The region experiences a Mediterranean climate, with comparatively cool and moist winters and long dry summers. The elongated Coorong covers an area between 150-240 km^2 depending on seasonal changes and features an average depth around 1 m with maximum depths of 4 m (Boon, 2000). In its natural state the North Lagoon used to be connected to Lake Alexandrina which receives freshwater input from the River Murray. The hydrology of the Coorong was determined by seawater exchange through the Murray Mouth and freshwater input from Lake Alexandrina as well as groundwater and surface runoff *via* Salt Creek at the southern end of the lagoons (Boon, 2000; Webster, 2005). Seasonal and tidal changes created various dynamic habitats in and around the lagoons, supporting a diverse range of bird, fish and plant species (DEH, 2000; Boon, 2000).

Human water management with an impact on the Coorong commenced approximately 100 years ago in the form of regulations and diversions from the River Murray for agricultural use and ship navigation (Maheshwari et al., 1995). In 1940 barrages were installed between the Coorong and Lake Alexandrina which largely prevented water exchange and reduced riverine input. However, there was still regular freshwater flow into the North Lagoon until the 1950s as the barrages were regularly opened to prevent flooding of the surrounding areas (Krull et al., 2009). After a major flood event in 1956 the inflow from the River Murray was reduced significantly due to increased abstraction for agricultural and domestic use. A severe drought between 2000 and 2010 has led to a further decline in freshwater input (Webster, 2005, 2010; Kingsford et al., 2011). The reduced inflows contributed to siltation of the Murray Mouth, limiting the seawater exchange with a temporary closure occurring in 1981. During the recent drought at the beginning of this century an open connection has only been maintained by regular dredging (Webster, 2005; Kingsford et al., 2011).

Depending on the amount of freshwater inflow through the barrages the salinities in the northern part of the lagoon close to the Murray Mouth can vary from values approaching zero to seawater levels. Salinity increases with distance from the Murray Mouth, and constant hypersalinity prevails in the South Lagoon during periods of low freshwater inflow such as the drought from 2000 to 2010 (e.g. Boon, 2000; Webster, 2005; Webster, 2010). The salinity increase is usually considered the main cause for the extinction of key species such as the macrophytes *Ruppia megacarpa* and other significant alterations to the ecosystem (Nicol, 2005; Dick et al., 2011). Increased environmental flows after 2010 have caused a slight and temporary improvement of the ecological state in the Coorong (Kingsford et al., 2011) but this is not addressed here since the presently analysed samples were collected in 2005.

Sample collection

The core analysed in this study was part of a suite of samples collected throughout the Coorong in 2005 by University of Adelaide researchers for a project commissioned by the Department of Water, Land and Biodiversity Conservation (DWL&BC) for the Upper South East (USE) Program. The sampling site was located in the middle of the North Lagoon slightly south of the Murray Mouth (**Figure 2.1**) and is equivalent to core "C4" in Krull et al. (2009). Sample collection and preparation of the core are described elsewhere (e.g. Krull et al., 2009; see **Appendix 2**). For this study the core was sampled between the depths of 0 and 115 cm in 5 cm intervals for biomarker analysis and 1 cm intervals for bulk stable isotope and TOC measurements.

Chronology

In the present study we used chronological data obtained by Krull et al. (2009) and Fluin et al. (2007) from a core referred to as "C3" (located in close vicinity to C4) to correlate observed alterations in biomarker parameters and their stable isotopic compositions with significant events in the Coorong. Krull et al. (2009) used a combination of *Pinus* pollen analysis (a species introduced by Europeans) and radionuclide-dating (^{137}Cs and Pu isotopes) in various cores collected throughout the Coorong to establish chronologies over the past 50-60 years. Sediment ages in more basal parts of some of these cores, obtained from ^{14}C–accelerator mass spectrometry (AMS), have been published by Fluin et al. (2007).

The first appearance of *Pinus* pollen in sediments from southeastern Australia is a common indicator for the evolution of European settlement and is often used in combination with other dating techniques (Tibby, 2003). In the Coorong its first occurrence roughly correlates with the 1950s (Krull et al., 2009). In core C3 and core C4 the first *Pinus* pollen were detected at depths of 23 and 27 cm, respectively (Krull et al., 2009). A detailed description of the principles of ^{137}Cs and Pu radionuclide dating can be found in Krull et al. (2009).

Analytical methods

Sample preparation and analytical methods can be found in the supplementary information (SI) included in **Appendix 2**.

Results and discussion

Changes of OM sources in the North Lagoon

Figure 2.2 displays depth profiles of selected parameters indicative of changing OM-sources in the North Lagoon in a chronological context. A pronounced excursion in the TOC profile shortly below a depth of 20 cm marks the increased input of (terrestrial) OM being flushed into the lagoon during a large flood event in 1956. A second excursion at ~ 50 cm might indicate an earlier and non-reported flood.

The first occurrence of *Pinus* pollen at a depth of 27 cm indicates the beginning of European influence in the Coorong region and roughly coincides with the beginning of a restricted freshwater inflow into the North Lagoon through the barrages (see "Chronology" page 67). Sediment ages also included in **Figure 2.2a** were published by Krull et al. (2009) and Fluin et al. (2007). Sediments < 25 cm, deposited after drastic alterations of the Coorong water regime, are hereafter referred to as "recent sediments" and the remaining sediments deposited before a significant European influence as "older sediments".

Evidence from microalgal and bacterial biomarkers

A notable difference in recent sediments of the North Lagoon deposited after the ~1950s was a significant increase in relative abundances of microalgal- and bacterial-derived hydrocarbons including the C_{20} highly branched isoprenoid (HBI; **I**, see **Figure A2.1** for structures), C_{27} - C_{29}-alkadienes and short chain *n*-alkanes (C_{14}-C_{20}, **Figure 2.2a** and **Figure 2.3**). Also steroid and hopanoid distributions showed significant alterations in recent sediments (**Figure 2.4a** and **b** and **Figure 2.5**).

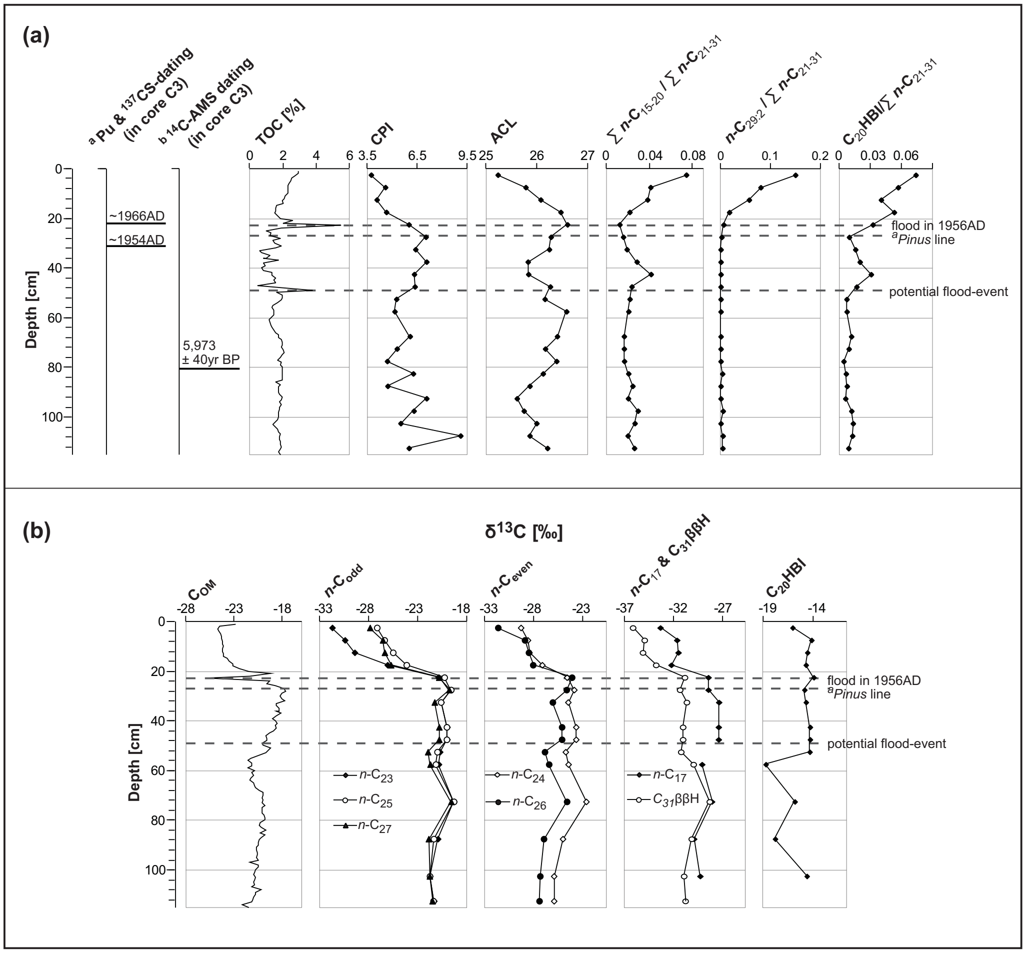

Figure 2.2: Alterations in TOC [%], *n*-alkane distributions (carbon preference index (CPI), average chain length (ACL)) and abundances of selected biomarkers relative to long-chain *n*-alkanes (a) and δ^{13}C- profiles in a core from the northern Coorong Lagoon (b) put into perspective with events in the region and inferred sediment ages from ^{137}Cs, Pu and ^{14}C-AMS dating (dating was performed on a neighbouring core). Flood events were marked according to excursions in the TOC profile. The first occurrence of *Pinus* pollen (*Pinus* line) indicates the beginning of European influence and roughly coincides with the 1950s (Krull et al., 2009). n-C$_i$ = *n*-alkane with chain length “i”; C$_{31}$ββH = 17β,21β-22R-homohopane; CPI = $\sum C_{odd}/\sum C_{even}$ over the range from C$_{21}$-C$_{30}$, where “C$_{odd}$” and “C$_{even}$" are peak areas from TIC chromatograms of *n*-alkanes with odd and even numbered chain lengths, respectively; ACL = $\sum(i\times C_i)/\sum C_i$ where “C$_i$” is the peak area of the *n*-alkane with carbon number “i” over the range from C$_{21}$–C$_{31}$; [a] from Krull et al., (2009), [b] from Fluin et al. (2007). OM = organic matter

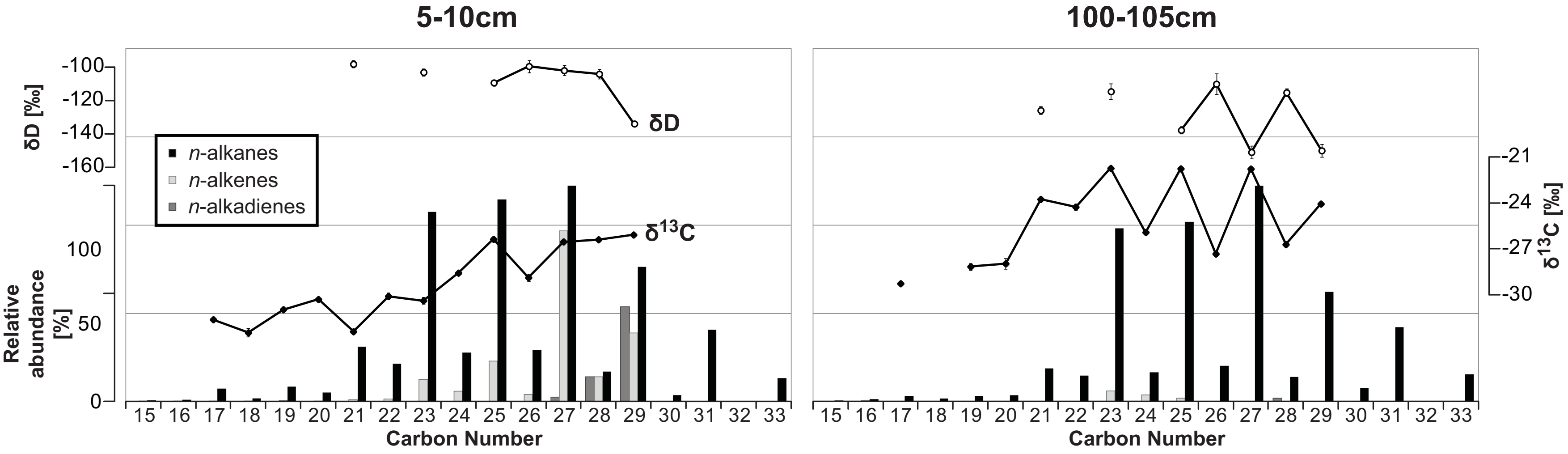

Figure 2.3: Typical distributions and stable isotopic compositions ($\delta^{13}C$, δD) of *n*-alkanes, *n*-alkenes and *n*-alkadienes in sediments from the northern Coorong Lagoon before (depth: 100-105cm) and after (depth: 5-10 cm) human interference with the water-regime. Relative abundances were determined from peak areas in the TIC trace of GC-MS chromatograms and calculated relative to the most abundant compound. Stable isotopic compositions include *n*-alkanes and their corresponding monoenes. Error bars indicate standard deviations of 2 replicate analyses, where error bars are not visible their size is smaller than the symbol.

The C_{20}HBI has been frequently assigned to a diatomaceous origin (e.g. Volkman et al., 1998; Atahan et al., 2007; McKirdy et al., 2010) based on stable isotopic compositions and abundance profiles as well as structural similarities to the C_{25} and C_{30} HBIs (**II, III**), which are established biomarkers for these microalgae (Volkman et al., 1998). However, the C_{20}HBI or potential precursors have, unlike the larger analogues, never been isolated from cultured organisms. The enriched $\delta^{13}C$ values of the C_{20}HBI in sediments from the North Lagoon (-18.8 to -13.9 ‰) were typical of lipids produced by diatoms since many species are capable of assimilating enriched bicarbonate as opposed to more depleted CO_2 (Freeman and Hayes, 1992; Bieger et al., 1997). The C_{20}HBI was the dominant aliphatic product in most sections of a core from the South Lagoon, where conditions such as high salinities and a higher degree of isolation have prevailed throughout the Holocene before being significantly enhanced by the recently employed interferences with the water regime (McKirdy et al., 2010). The elevated abundance of the C_{20}HBI in recent sediments of the North Lagoon indicates important changes in the environmental conditions and water regime since a diatom population similar to that previously present in the South Lagoon became supported.

Furthermore, the uppermost sediments (< 20 cm) contained high concentrations of unsaturated aliphatics, with C_{23}-C_{29} *n*-alkenes and a C_{29} *n*-alkadiene of notably high abundance (**Figure 2.3**). Long-chain *n*-alkenes with a strong odd-over-even predominance are produced by some green microalgae and possibly cyanobacteria (Gelpi et al., 1970; Gelin et al., 1997; Allard and Templier, 2000). The C_{27}-C_{29} *n*-alkadienes were most likely derived from the A race of the green algae *Botryococcus braunii* which is known to produce odd-carbon-numbered C_{27}-C_{31} *n*-alkadienes and minor amounts of the C_{29} *n*-alkatriene (Metzger et al., 1986; Metzger and Largeau, 2005). *n*-Alkadienes of similar chain lengths have also been isolated from some chlorococcales algae (Allard and Templier, 2000). Although *Botryococcus braunii* is a freshwater species, there is reported evidence of its presence in hypersaline environments, mainly due to salinity stratification in the water-column (e.g. Grice et al., 1998a) which also periodically occurs in the Coorong (Webster, 2005). Blooms of this species have previously been observed in the Coorong region (Cane, 1976) and $\delta^{13}C$ signatures of the C_{29} *n*-alkadiene (-24.6 to -22.5 ‰) would also be consistent with an origin from these algae (Grice et al., 1998a). The high relative abundance of the unsaturated aliphatics in recent sediments is in accordance with the high abundance of green algae in the water-column of the present day Coorong (Revill et al., 2009). However, the difference to older sediments, in which these compounds showed very low abundances or were absent, can also be partly attributed to their relatively unstable structures resulting in an early transformation into more stable *n*-alkanes during diagenesis.

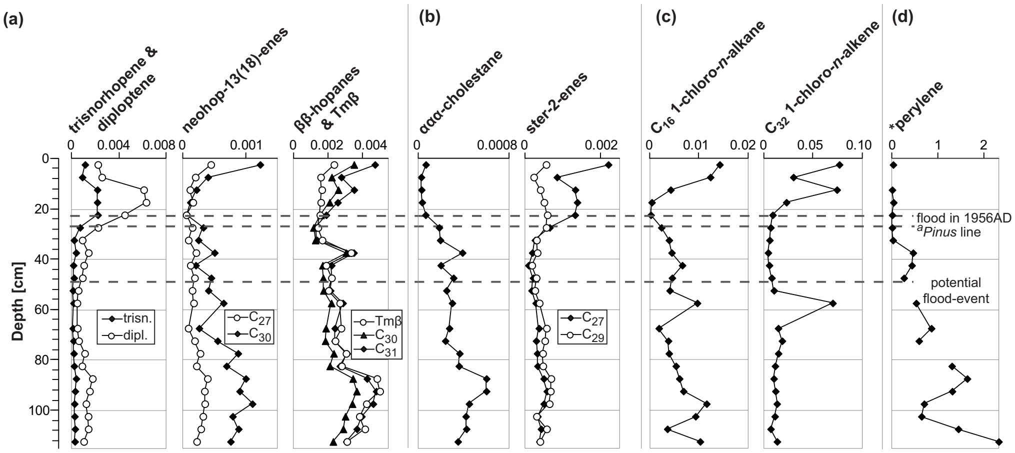

Figure 2.4: Alterations of biomarker abundances relative to $\sum C_{21}$-C_{31} *n*-alkanes in a core from the North Lagoon. *Concentration in dry weight sediment [ng/g]. The first occurrence of *Pinus* pollen (*Pinus* line) indicates the beginning of European influence and roughly coincides with the 1950s (Krull et al., 2009). The corresponding depths of flood-events were determined from excursions in the TOC profile. ^{a} from Krull et al. (2009). Tm-β = . 17β-22,29,30-trisnorhopane

C_{14}-C_{20} *n*-alkanes often maximising at C_{17} are usually attributed to diatoms, green algae or cyanobacteria (Cranwell, 1982; Gelpi et al., 1970; Han and Calvin, 1969). In the most recently deposited sediments from the North Lagoon the C_{17}-C_{24} *n*-alkanes showed an increase of relative abundance (cf. C_{21}-C_{31} *n*-alkanes) and light $\delta^{13}C$ values (-35.3 to -30.0 ‰), which were in the same range as bacterial derived hopanoids (**Figure 2.4b**) indicating a common source. $\delta^{13}C$ signatures of bacterial lipids, in particular those originating from benthic microbial mats, are often more depleted than planktonic microalgal lipids (Collister et al., 1994b; Freeman et al., 1994; Bieger et al., 1997; Logan et al., 1999). Bacterial-derived hopanoids (C_{27}-C_{32}) were abundant in all samples (**Figure 2.4b**) and showed distributions typical of immature sediments with a complex suite of hopenes (hop-17(21)-enes, *neo*hop-

13(18)-enes and hop-22(29)-ene (diploptene)) and high abundances of 17β,21β-hopanes (biological configuration) relative to 17β,21α and 17α21β isomers. Most hopanoids exhibited highest abundances (*cf.* *n*-alkanes) in the uppermost 20 cm indicating an increased bacterial input. The molecular distribution of hopanoids showed slight variations throughout the core, however they were most pronounced in recent sediments presumably reflecting changes in the bacterial populations.

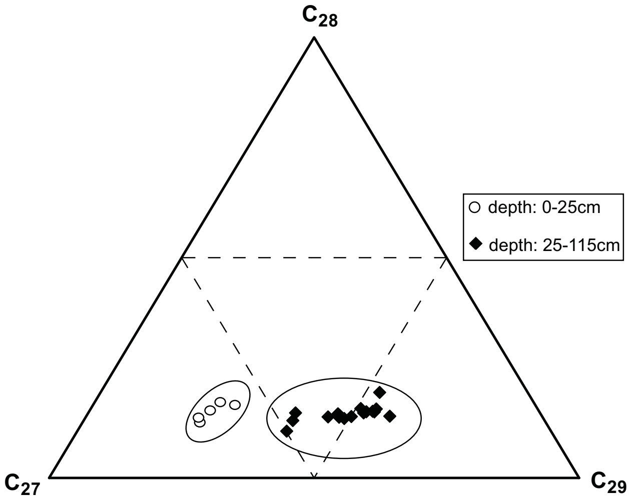

Figure 2.5: Ternary diagram of ster-2-enes and cholestane (only steroids in the aliphatic fractions) in a core from the northern Coorong Lagoon. Sediments between 0-25 cm were deposited after the ~1950s, a period when human interference with the water regime intensified significantly. Deeper sediments were deposited over several thousands of years.

The most abundant steroids in the aliphatic fractions were C_{27}, C_{28} and C_{29} 5α, 14α, 17α, 20R ster-2-enes, which are early diagenetic products of biological steroids (e.g. Mackenzie et al., 1982). We also detected low abundances of 5α,14α,17α 20R-cholestane, a product formed during slightly later stages of diagenesis (Mackenzie et al., 1982), which showed increasing abundances with sediment age typical of a diagenetic product (**Figure 2.4b**).The ternary diagram in **Figure 2.5** showed distinct changes in ster-2-ene distributions in recent sediments, with a significantly increased abundance of cholest-2-ene and other C_{27}-steroids.

Although C_{29} desmethyl-steroids are often assigned to a terrestrial plants origin (e.g. Huang and Meinschein, 1979) they have also been detected in significant amounts in microalgae, seagrasses and sediments without higher plant input (Attaway et al., 1971; Volkman, 1986; Volkman et al., 1998; Grosjean et al., 2009). Revill et al. (2009) attributed all sterols they detected in water-column and surface sediments throughout the Coorong to aquatic organisms, namely different types of green microalgae, diatoms (C_{29}- and C_{28}-sterols) and zooplankton (C_{27}-sterols). The high relative abundance of cholest-2-ene in recent sediments of the North Lagoon may be an indication for an increased population of zooplankton feeding on benthic microbial mats (McKirdy et al., 2010). The primary source organisms of the C_{29} ster-2-ene in the North Lagoon sediments are most likely microalgae and aquatic plants (the latter particularly in older sediments) possibly with a minor contribution from terrestrial plants. The relatively lower abundance of C_{29} ster-2-enes in recent sediments might reflect the recent extinction of aquatic macrophytes due to the rising salinities (Nicol, 2005; Krull et al., 2009; Dick et al., 2011).

Evidence from long-chain n-alkane distributions

The aliphatic fractions were dominated by a suite of mid- to long-chain *n*-alkanes (C_{21}-C_{33}) with a strong odd over even predominance (e.g. **Figure 2.3**). In recent sediments (< 25 cm) their distribution profiles showed significant alterations evident in decreasing carbon preference indexes (CPI, calculated after **Eq. 2.1**) and average chain length (ACL, calculated after **Eq. 2.2**) which both pointed towards a change in the type of source organisms (**Figure 2.2a**).

$$\mathrm{CPI} = \frac{\sum C_{odd}}{\sum C_{even}} \qquad \textbf{(2.1)}$$

where "C_{odd}" and "C_{even}" are peak areas from TIC chromatograms of *n*-alkanes with odd and even numbered chain lengths, respectively over the range from C_{21}–C_{30}

$$\mathrm{ACL} = \frac{\sum (i \times C_i)}{\sum C_i} \qquad \textbf{(2.2)}$$

where "C_i" is the peak area of the *n*-alkane with carbon number "i" over the range from C_{21}–C_{31}

Typically long-chain *n*-alkanes with high CPIs are ascribed to epicuticular waxes of vascular plants (Eglinton and Hamilton, 1967). However, also various aquatic organisms including microalgae, cyanobacteria or aquatic macrophytes are known to produce long-chain *n*-alkanes or long-chain *n*-alkyl precursors such as fatty acids, *n*-alcohols or *n*-alkenes (e.g. Cranwell, 1984; Volkman et al., 1998; Ficken et al., 2000; Jaffé et al., 2001). A major contribution from terrestrial plants to the long-chain *n*-alkanes detected here seems unlikely since several studies (using various analytical approaches) confirmed only a minor terrestrial input to the sediments of the North Lagoon. These included biomarker analysis in surface sediments and the water-column (Revill et al., 2009) as well as elemental (low C/N values; Krull et al., 2009; McKirdy et al., 2010) and ^{13}C-NMR analysis (Krull et al., 2009) in sediment cores. Also the ACL of *n*-alkanes detected in this study (25.2 - 26.6) was lower than the 28 to 33 typical of terrestrial plants (Chikaraishi and Naraoka, 2003). However, the presence of pollen (e.g. *Pinus*, Krull et al., 2009) does indicate a minor terrestrial input.

An alternative source of these hydrocarbons in an estuarine system like the Coorong could be aquatic plants, which mainly synthesise *n*-alkanes of mid-chain lengths typically maximising at C_{21}, C_{23} or C_{25} (Botello and Mandelli, 1978; Cranwell, 1984; Ficken et al., 2000; Jaffé et al., 2001). P_{aq} values (**Eq. 2.3**, where “C” is the peak area of *n*-alkanes), determined according to a proxy to distinguish input from terrestrial *vs*. aquatic plants (Ficken et al., 2000), varied between 0.37 and 0.61, indicating a major contribution from non-emergent aquatic plants to the *n*-alkanes found in the North Lagoon. This is in accordance with the formerly high abundance of aquatic macrophytes, in particular *Ruppia megacarpa,* which used to be a significant source of OM before its recent extinction due to the rising salinity (Krull et al., 2009).

$$\mathrm{P}_{aq} = (C_{23} + C_{25})/(C_{23} + C_{25} + C_{29} + C_{31}) \qquad \textbf{(2.3)}$$

Other potential sources of sedimentary *n*-alkanes in the North Lagoon are aquatic microorganisms such as diatoms, green algae or bacteria. Although most of these organisms commonly produce *n*-alkanes of a shorter chain length (C_{14}-C_{20}, "Evidence from microalgal and bacterial biomarkers", page 67*ff*.), long-chain *n*-alkanes have also been attributed to some of these species (e.g. Cranwell, 1982, 1984; Collister et al., 1994a; Lichtfouse et al., 1994; Volkman et al., 1998; Logan et al., 1999; McKirdy et al., 2010). Whereas diatoms and bacteria typically generate (long-chain) *n*-alkane distributions without a pronounced odd-over-even predominance (Oró et al., 1967; Davis, 1968; Han and Calvin, 1969; Jones and Young, 1970; Volkman et al., 1980; Nichols et al., 1988), green algae often synthesise long-chain *n*-alkenes with odd carbon numbers leading to *n*-alkane distributions with high CPIs (Gelpi et al., 1970; Gelin et al., 1997; Allard and Templier, 2000). The decreasing CPIs observed in the most recent sediments of the North Lagoon (**Figure 2.2a**) could therefore indicate an increasing abundance of cyanobacteria or diatoms consistent with the increase of phytoplanktonic biomarkers observed in this and other studies ("Evidence from microalgal and bacterial biomarkers" page 68*ff*.). Low CPI distributions are also commonly found in mature sediments as the result of degradation processes during diagenesis (Peters et al., 2005) and can therefore be indicative of contamination with petroleum products. However such contamination in the Coorong seems unlikely since there has been no other indication for this in the present project or in previous studies.

Evidence from $\delta^{13}C$ and δD profiles

δ^{13}C profiles of bulk organic carbon and selected biomarkers throughout the analysed core are displayed in **Figure 2.2b**. Most profiles showed comparatively minor variations in older sediments representing several thousand years (25-115 cm), but a marked negative shift in recent sediments (< 25 cm), indicating significant changes in the ecosystem of the North Lagoon since the ~1950s. The bulk δ^{13}C profile also exhibited a -5 ‰ shift coincident with this point but showed an additional sharp negative spike shortly below 20 cm reflecting depleted terrestrial OM being flushed into the system during the large flood event in 1956. The flood was also reflected in the TOC values but its direct effects were not visible in other profiles due to their lower resolution. The bulk δ^{13}C signatures were generally slightly heavier than corresponding values obtained for *n*-alkanes, which is to be expected since lipids in autotrophs are generally more depleted compared to total biomass (Monson and Hayes, 1982).

The most pronounced negative shift (average: 7 ‰) in recent samples occurred in δ^{13}C values of C_{21}-C_{29} *n*-alkanes (+ their monoenes if present, see SI page 93*ff.*). It was most likely caused by a significant change in source organisms, although environmental factors such as increasing salinity can also influence δ^{13}C values in biomarkers and sediments. However, an elevated salinity as reported in the Coorong over the past decades is usually associated with heavier isotopic signatures in biolipids (Schidlowski et al., 1984; Grice et al., 1998; Andersen et al., 2001) and the opposite (lighter isotopic signatures) is observed in the North Lagoon.

Further indications for a change in source organisms were distinct sawtooth patterns with ^{13}C depleted and D enriched even-carbon-numbered *n*-alkanes in older sediments (> 25 cm) whereas these features diminished and became absent in more recent samples (e.g. **Figure 2.3**). Similar sawtooth patterns have been reported in various higher plant waxes due to different metabolic pathways used for the synthesis of odd and even numbered homologues (Grice et al., 2008a; Zhou et al., 2010) as well as in sediments, where this pattern may also be enhanced by or be the result of input from different source organisms such as a microalgal bias to odd numbered *n*-alkanes (Logan et al., 1999; Grice et al., 2001; Dawson et al., 2004).

The isotopic differences evident throughout the studied core were also visible in a crossplot of δ^{13}C *vs*. δD values of long chain *n*-alkanes in representative samples from the North Lagoon well before (105-110 cm) and significantly after (0-15 cm) human interferences had impacted the water regime (**Figure 2.6**). Three groups, namely *n*-alkanes in recent sediments and odd-chain and even-chain *n*-alkanes in older sediments, were clearly separated. The distinction was however somewhat more obvious in δ^{13}C than in δD signatures since isotopic fractionations in different metabolic pathways are usually reflected more strongly by δ^{13}C values (e.g. Chikaraishi and Naraoka, 2003; Polissar and Freeman, 2010). The enrichment in D in the most recent samples is also a result of the rising salinity and decreased freshwater input (see "Salinity variations in the North Lagoon over time" page 83).

δ^{13}C signatures of long chain *n*-alkanes in older sediments are consistent with a predominant origin from aquatic C3 plants such as seagrasses, which produce relatively enriched hydrocarbons compared to their terrestrial counterparts. Chikaraishi and Naraoka (2003) reported an average δ^{13}C value of *n*-alkanes extracted from three marine seagrass species of -22.8 ±1.0 ‰ and slightly more depleted values for aquatic freshwater plants of -25.3 ±1.9 ‰. There may also be a minor contribution from phytoplankton and a small allochthonous input from terrestrial plants potentially including species from halophytes (see “Significance of 1-chloro-n-alkanes” page 81).

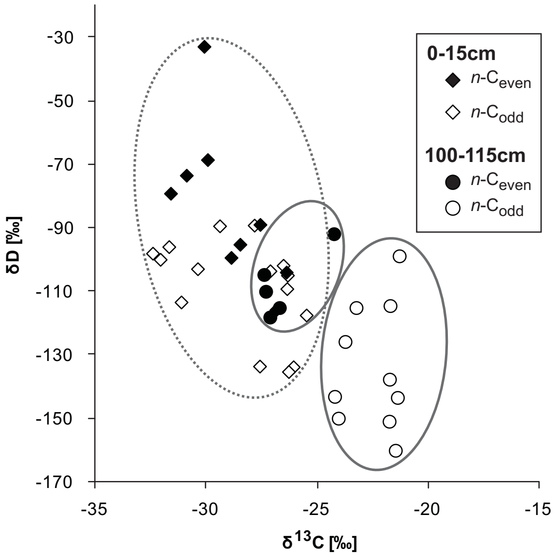

Figure 2.6: Crossplot of δ^{13}C *vs.* δD values of long-chain *n*-alkanes (C_{21}-C_{29}) in representative sediments from the northern Coorong lagoon deposited prior (100-115 cm) and after (0-15 cm) the beginning of human control over the water regime in the Coorong. *n*-C_{even} and *n*-C_{odd} stands for *n*-alkanes with an even- and odd-numbered carbon chain, respectively.

Although the ^{13}C depleted *n*-alkanes in recent sediments of the North Lagoon are in the range of terrestrial C3 plants waxes (e.g. Chikaraishi and Naraoka, 2003), a predominant origin from these organisms seems implausible since a high allochthonous contribution to sedimentary OM in the North Lagoon, especially in recent years, is unlikely (see “Evidence from long-chain *n-alkane* distributions” page 74*ff.*). According to Revill et al. (2009) the primary source of OM in surface sediments of the North Lagoon were benthic diatoms and cyanobacteria whereas OM in the water-column was mainly derived from green algae. Diatom derived lipids often have more ^{13}C-enriched signatures (“Evidence from microalgal and bacterial biomarkers” page 68*ff.*), which makes them an unlikely source of the ^{13}C-depleted *n*-alkanes in the recent sediments from the North Lagoon. McKirdy et al. (2010)

observed a similar shift in $\delta^{13}C$ values of *n*-alkanes in recent sediments of the South Lagoon and attributed it to a primary source change from aquatic macrophytes to bacteria. Such a source transition also seems plausible in the North Lagoon. A depletion would be expected from a higher bacterial contribution to the long-chain *n*-alkanes since also other bacterial derived hydrocarbons such as short-chain *n*-alkanes or hopanoids had very light $\delta^{13}C$ signatures ("Evidence from microalgal and bacterial biomarkers" page 68*ff.*).

In the most recent samples (< 20 cm) longer-chain *n*-alkanes/alkenes > C_{24} were on average 4‰ enriched in ^{13}C compared to their shorter chain homologues, with $\delta^{13}C$ values from -29.3 to -26.1 ‰. This is presumably caused by a contribution from green algae (possibly the A race of *Botryococcus braunii*) to these biomarkers, in particular the *n*-alkenes. It is however more depleted than odd-carbon numbered *n*-alkanes in older sediments which largely originated from aquatic macrophytes. This indicates a mixed source for *n*-alkanes > C_{24} in recent sediments with contributions from bacteria as well as green algae and potentially also a minor input from terrestrial higher plants. Revill et al. (2009) reported depleted values in green algal derived phytol in the water-column sometimes approaching -30 ‰ which they attributed to a slow growth rate.

The $\delta^{13}C$ values of predominantly bacterial-derived C_{17} *n*-alkane and the 17β,21β-22R-homohopane also shifted towards more depleted values in the recent sediments, although the trend was not as pronounced as that evident in (odd-carbon-numbered) long-chain *n*-alkanes (**Figure 2.2b**). A possible explanation for this shift might be a change in the bacterial population, which was also reflected by differences in hopanoid distributions in that core-section ("Evidence from microalgal and bacterial biomarkers" page 68*ff.*). For example an increase of bacterial growth in benthic microbial mats, often favoured in highly saline environments as opposed to planktonic organisms, would likely cause a negative shift in $\delta^{13}C$ values (Logan et al., 1999). However, the observed shifts could also be the result of an increased abundance of dissolved CO_2 caused for example by enhanced heterotrophic activity after algal blooms or by a slight decrease of the pH which would lead to a ^{13}C-depletion in lipids of primary producers. Although the Coorong remains an alkaline system (Revill et al., 2009) temporary stratification with more acidic bottom waters (pH: 6.8) have been observed (Geddes, 2003). With limited supply of $[CO_2]_{aq}$ phytoplanktonic organisms show less discrimination against ^{13}C resulting in significantly enriched biolipids whereas a high abundance of this carbon source leads to an enhanced discrimination and more depleted biolipids (Takahashi et al., 1990; Freeman and Hayes, 1992).

The C_{20}HBI was significantly more enriched in ^{13}C compared to *n*-alkanes and hopanoids and also the sedimentary $\delta^{13}C$ depth profile of the C_{20}HBI was different to those from the other hydrocarbons (**Figure2.2b**). This is a further indication of its diatomaceous origin and the utilization of a different carbon source (i.e. bicarbonate as opposed to $[CO_2]_{aq}$).

Most of the stable isotope and biomarker profiles described were distinctly greater in magnitude than natural variations over thousands of years, thus indicating unambiguous ecological changes in recent sediments (younger than ~1950). However, it should be noted that sediments at greater depths of this core had a very different time resolution (*cf.* radionuclide dates with radiocarbon age) and averaging effects were much more significant for these compared to the recent sediments.

Origin of perylene

The PAH perylene (**IV**) was only present in trace amounts in sediments of the uppermost 30 cm but increased significantly in concentration at greater depths (**Figure 2.4d**). Unlike most other unsubstituted PAHs, which are mainly of a thermogenic or pyrogenic origin, perylene is presumably a diagentic product and typically shows different sedimentary abundances compared to these combustion markers (Jiang et al., 2000; Atahan et al., 2007; Grice et al., 2009; Suzuki et al., 2010). A likely source for perylene seems to be quinone pigments associated with wood degrading fungi in particular but also with other organisms including crinoids and certain plants and insects (Jiang et al., 2000; Grice et al., 2009; Suzuki et al., 2010). A link to fungal wood/lignin degradation is also supported by the absence of perylene in sediments and crude oils predating the evolution of vascular plants and in marine sourced oils (Grice et al. 2009).

In the sediment core from the North Lagoon the increasing abundance of perylene with depth (**Figure 2.4d**) is consistent with a diagenetic origin. Furthermore, ^{13}C-

NMR analyses performed by Krull et al. (2009) showed that a significant lignin source -most likely the macrophyte *Ruppia megacarpa*- was present in older sediments from the North Lagoon (i.e. < 1950s) but absent in more recent sediments. The co-occurrence of perylene and lignin in older sediments from the North Lagoon and the absence of both in recent sediments do concur with the source correlation of perylene to fungal lignin degradation.

Significance of 1-chloro-n-alkanes

The aliphatic fractions also contained a series of C_{10}-C_{25} 1-chloro-*n*-alkanes which can be identified in the *m/z* 91 mass chromatograms (Grossi and Raphel, 2003) illustrated in **Figure 2.8**. This product series may extend to higher MW homologous, but co-elutions with other analytes (i.e. alkenes, hopanoids and steroids) would have compromised their detection. Nevertheless, $C_{30:1}$ and $C_{32:1}$ chloro-*n*-alkenes have been tentatively identified by relative retention times which were consistent with previous reports (Zhang et al., 2011) and by mass spectral features. The abundance profiles of the C_{16}-1-chloro-*n*-alkane (representative of other homologues) and the putative $C_{32:1}$ chloro-*n*-alkene throughout the core are displayed in **Figure 2.4c**. The general similarity of both profiles is indicative of a common source and supportive of our putative identification.

Although a variety of organohalogens are produced biosynthetially, in particular by marine organisms but also by some terrestrial life forms including plants and higher animals (e.g. Gschwend et al., 1985; Winterton, 2000; Gribble, 1996, 2003), reports of mid- to long-chain 1-chloro-*n*-alkanes in sediments are sparse. Zhang et al. (2011) detected $C_{30:1}$ and $C_{32:1}$ 1-chloro-*n*-alkenes, which we also tentatively identified in this study, in sediments from a freshwater crater lake in the Galápagos Islands. A series of long-chain 1-chloro *n*-alkanes (C_{19}-C_{29}) has been isolated from 3 genera (*Suaeda*, *Sarcocornia* and *Halimione*) of halophytic Chenopodidaceae (Grossi and Raphel, 2003). Some of these species (including *Sarcocornia* and *Suaeda*) are also found in the Coorong region (Boon, 2000 and references therein) and represent a potential source for the chlorinated paraffins in the North Lagoon sediments. However, the series detected in this study did not show the odd-over-even carbon number predominance that Grossi and Raphel (2003) had previously observed in halophytes and it also comprised shorter-chain homologues (C_{12}-C_{18}), which have not been detected in the Chenopodiaceae.

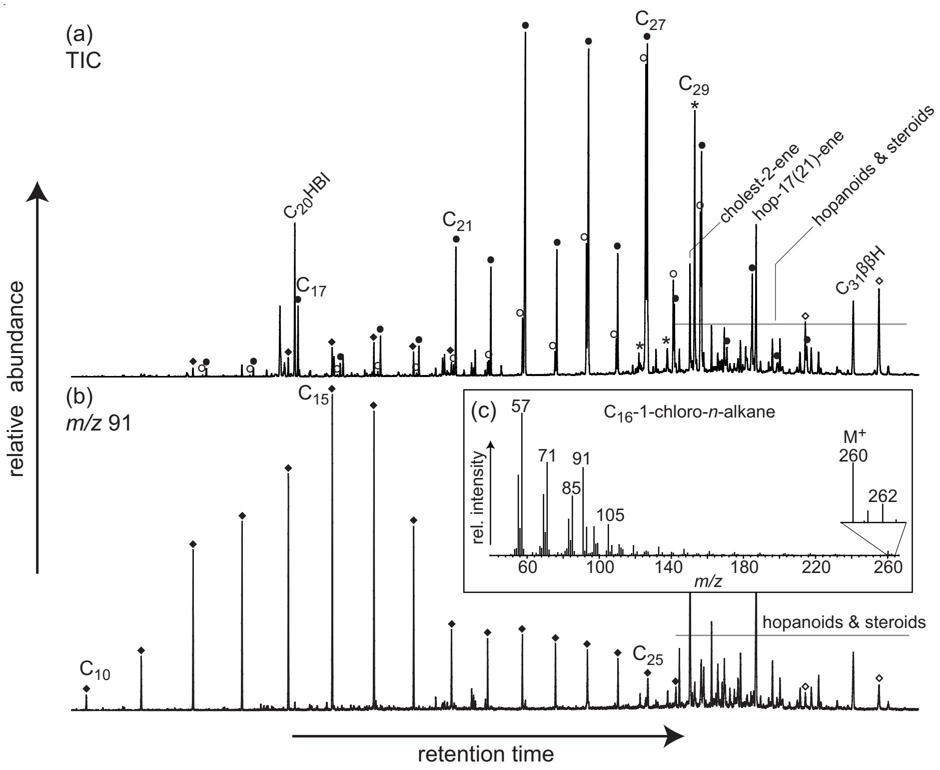

Figure 2.7: Total ion chromatogram (TIC, (a)) and *m/z* 91 extracted ion chromatogram (b) of the aliphatic fraction in sediments from the North Lagoon between depths of 0-5 cm. (c) is the EI mass spectrum of the C_{16}-1-chloro-*n*-alkane. C_i: carbon number of displayed compound classes; : *n*-alkanes; : *n*-alkenes; * : *n*-alkadienes; : 1-chloro-n-alkanes; : tentatively identified 1-chloro-*n*-alkenes; $C_{31}\beta\beta H$ = 17β,21β-22R-homohopane

Other sources of these compounds in the North Lagoon may include algae, seagrasses or cyanobacteria. Although no long-chain-chloro-*n*-alkanes/ *n*-alkenes have been isolated from these organisms so far, they are known to produce a variety of other chloro-organic compounds including volatile 1-chloro-*n*-alkanes in the range from C_1 to C_5 (Mynderse and Moore, 1978; Gribble, 1996, 2003). The increased relative abundances of 1-chloro-*n*-alkanes and 1-chloro *n*-alkenes in the most recent sediments of the North Lagoon (**Figure 2.4**) point towards an algal or cyanobacterial source since these organisms also were significantly more abundant in that part of the core (see “Changes of OM sources in the North Lagoon”). Nevertheless, Chenopodiaceae have a high tolerance to salinity and may have become more abundant in the lagoon catchment with the rising salinities in the Coorong.

Mid- and long-chain chloro-paraffins (CPs; C_{10} - C_{30}) are also known anthropogenic contaminants due to many industrial applications (Tomy et al., 1998; Štejnarová et al., 2005). However, anthropogenically-sourced CPs commonly exhibit complex distributions with various stereoisomers and different degrees of chlorination as a result of the synthesis process (Tomy et al., 1998). Therefore the relative specificity of the distinct series of 1-chloro-*n*-alkanes detected here precludes such an origin.

Table 2.1: $\delta^{13}C$ [‰] ± standard deviation of 2 replicates of 1-chloro-*n*-alkanes/*n*-alkenes throughout a sediment core from the North Lagoon. C_i = carbon number

Depth [cm]	1-chloro-*n*-alkanes			1-chloro-*n*-alkene
	C_{15}	C_{16}	C_{17}	$C_{32:1}$
0-5	-29.0 ±0.0	-29.2 ±0.4	-31.8 ±0.1	-29.8 ±0.1
5-10	-28.1 ±0.1	-26.5 ±0.0	-27.8 ±0.2	-26.6 ±0.3
10-15	-29.1 ±0.2	-30.4 ±0.3		-25.4 ±0.2
15-20				-26.1 ±0.4
25-30		-26.9 ±0.1		
30-35			-24.8 ±0.4	
40-45	-24.5 ±0.3	-27.4 ±0.5		
45-50				
50-55		-26.4 ±0.0		
55-60	-25.1 ±0.2	-25.7 ±0.1	-26.0 ±0.2	
70-75	-25.5 ±0.2	-24.8 ±0.3	-26.4 ±0.1	
85-90		-26.0 ±0.2	-26.6 ±0.1	
100-105	-25.8 ±0.5	-26.1 ±0.2	-27.3 ±0.5	
110-115	-26.3 ±0.5	-26.3 ±0.4	-25.9 ±0.0	

$\delta^{13}C$ values of the mono-chlorinated paraffins in the North Lagoon ranged from -31.8 to -24.5 ‰ (**Table 2.1**) and are consistent with both a C3 plant source (such as the chloro-paraffin containing Chenopodiceae analysed by Grossi and Raphel (2003) and most Chenopodiaceae in the Coorong region) as well as with a bacterial or microalgal origin (*cf.* $\delta^{13}C$ values of algal- and bacterial derived hydrocarbons, "Evidence from microalgal and bacterial biomarkers" 68*ff.*).

Salinity variations in the North Lagoon over time

δD signatures of selected even and odd numbered *n*-alkanes (+corresponding monoenes if present, see SI page 93*ff.*) throughout the analysed core are displayed in **Figure 2.8**. As previously discussed ("Changes of OM sources in the North Lagoon" page 68*ff.*) these hydrocarbons primarily originated from autochthonous *in situ* production and therefore their δD signatures reflect the D/H abundance (and thus also salinity) in the lake water (Sachse et al., 2006; Mügler et al., 2010), with more depleted δD values indicating lower salinities. The δD profiles of all long chain *n*-alkanes showed some variations but a general trend towards enriched δD values in younger sediments, indicating an overall rising salinity (**Figure 2.8**). The most pronounced enrichment was evident in recent sediments (< 20 cm) presumably representing a more pronounced increase in salinity over the past decades than the previous hundreds/ thousands of years represented by the deeper sediments. The *n*-alkanes deposited shortly after the flood event in 1956 showed a slight depletion in δD values, reflecting a temporary freshening of the system. Similar variations in salinity, including the pronounced increase over the past decades, have also been observed in studies of diatom assemblages in the North Lagoon (McKirdy et al., 2010). Relatively large variations of δD values in older sediments also seem consistent with variations in aridity in the Coorong region throughout the Late Holocene e.g. (Ahmad, 1996; Mee et al., 2007). Possibly these changes would have been more pronounced in δD profiles at a higher time resolution.

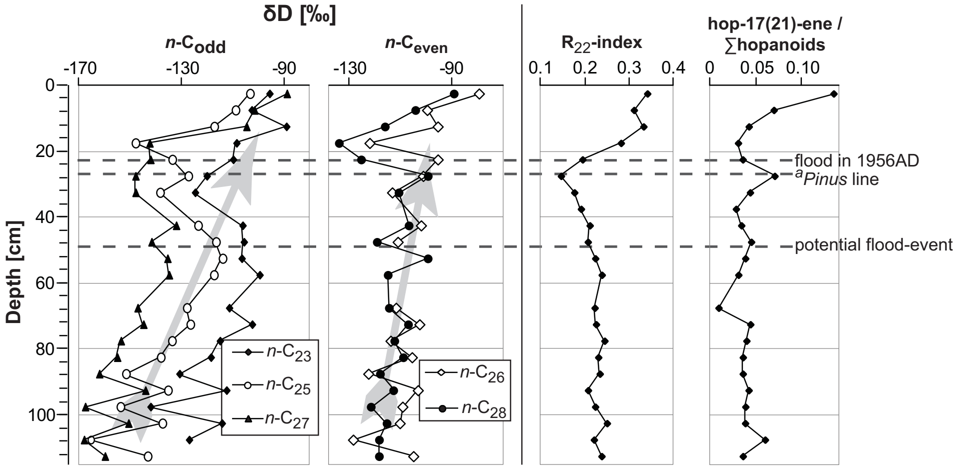

Figure 2.8: δD profiles of representative *n*-alkanes with odd and even chain lengths (*n*-C_{even} and *n*-C_{odd}, respectively) and other potential salinity indicators throughout a core from the North Lagoon. R_{22}index = $2 \times C_{22}/(C_{21}+C_{23})$ where "C_i" is the peak area in GC-MS chromatograms of the *n*-alkane with chain length "i" (ten Haven et al., 1988). The first occurrence of *Pinus* pollen (*Pinus* line) indicates the beginning of European influence and roughly coincides with the 1950s (Krull et al., 2009). The corresponding depths of flood-events were determined from excursions in the TOC profile. [a] from Krull et al. (2009)

Furthermore, the shift in δD values of *n*-alkanes in the recent sediments might also be enhanced by the change in source organisms discussed in "Changes of OM sources in the North Lagoon", page 68*ff*.. The influence of the different source organisms on δD signatures of *n*-alkanes is for example evident in the sawtooth pattern described previously (**Figure 2.3**) which is only present in older sediments prior to a significant human impact.

Another potential parameter reflecting the rising salinity in recent sediments was the increasing abundance of the hop-(17)21-ene relative to other hopanes (**Figure 2.8**). Hop-(17)21-enes seem to be formed during early diagenesis in hypersaline environments in preference to 17α,21β-hopanes (ten Haven et al., 1985; ten Haven et al., 1988). However, longer chain homologues (C_{31}-C_{35}), which are also indicative of high salinities (e.g. ten Haven et al., 1985), were not abundant in the North Lagoon. The R_{22}-index, displaying the abundance of the C_{22} *n*-alkane relative to the C_{21} and C_{23} *n*-alkanes, can also be a marker for hypersaline depositional environments (ten Haven et al., 1985; ten Haven et al., 1988). In the recent sediments from the North Lagoon it increases significantly, as it is expected for rising salinities. However, these recent sediment values of ~0.35 are still much lower than those reported by ten Haven et al. (1985) in hypersaline environments. Despite the increase in salinity evident in the North Lagoon it had not yet progressed to prevalent hypersalinity (e.g. Webster, 2005).

Conclusions

The molecular and isotopic sedimentary record included evidence that human interference with the water regime of the Coorong, namely a drastic reduction of the freshwater inflow due to installation of barrages and extensive water abstractions from the River Murray, was immediately responsible for major changes in the types of primary production, sedimentary OM and salinity in the North Lagoon over the past ~50 years. The magnitude of these parameter changes has been significantly more pronounced than natural variations over thousands of years. Aliphatic and aromatic biomarker analyses (including CSIA, C and H) in a sediment core from the North Lagoon spanning more than 5000 years revealed changes in the populations of primary producers contributing to sedimentary OM from predominantly macrophytes in sediments deposited prior to the 1950s towards bacteria and microalgae in more recent sediments. Furthermore, H-CSIA enabled the reconstruction of salinity variation in the North Lagoon, showing dynamic changes with an overall rise of salinity throughout the Holocene. However, a sharp increase took place shortly after the 1950s presumably due to the restriction of freshwater inflow through the barrages. We also detected an interesting series of mid- to long-chain 1-chloro-*n*-alkanes in these sediments. Potential sources of these compounds could be halophytic Chenopodiaceae, cyanobacteria or microalgae.

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Appendix 2

Supplementary online material

Analytical methods

Sample collection

The core was collected with a field piston corer and sliced vertically using a circular saw and 'dustless' diamond blade and stored at the University of Adelaide in a cold room at 4°C. Subsamples were stored in glass vials at 4°C at CSIRO Land and Water. The core was sampled between the depths of 0 and 115 cm in 5 cm intervals for biomarker analysis and 1 cm intervals for bulk stable isotope and TOC measurements. Ice boxes were used to keep samples cold during transfers between different institutes.

Bulk stable isotope and TOC analysis

Sediment samples were dried at 55 °C overnight, ground, homogenised and weighed into tin cups for analysis. For carbonate removal sulfurous acid was added directly into the tin cups to prevent loss of acid-soluble organic carbon (Verardo et al., 1990). After re-drying the samples were analysed for C content and δ^{13}C using a Carlo Erba NA1500 CNS analyser interfaced via a Conflo II to a Finnigan Mat Delta S isotope ratio mass spectrometer operating in the continuous flow mode. Combustion and oxidation were achieved at 1090 ºC and reduction at 650 ºC. To ensure reproducibility samples were analysed at least in duplicate. Results are presented in the standard delta (δ) notation relative to the international standard VPDB:

$$\delta^{13}C(‰) = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \times 1000\ ‰$$

where R = $^{13}C/^{12}C$. % TOC was calculated from mass spectrometer peak areas using standards with a known C content. The reproducibility of stable isotope measurements was ± 0.2 ‰.

Sample preparation and extraction for biomarker analysis

Samples were dried on a heated sandbath (55 °C) or in a cool oven (45 °C), powdered and extracted with a mixture of 9:1 dichloromethane (DCM): methanol by accelerated solvent extraction (ASE) using a Dionex ASE 200 (Dionex, Sunnyvale, CA, USA). The extracts were desulfurized overnight following addition of activated copper powder. The filtered extracts were successively fractionated by silica gel chromatography (column size: 20 cm x 0.9 cm I.D.) using approximately two column volumes of increasingly polar solvents. Aliphatic and aromatic fractions relevant for this study were eluted with *n*-hexane and 30% dichloromethane in *n*-hexane, respectively. An internal perdeuterated standard (*p*-terphenyl d^{14}) was added to the aromatic fraction to assist semi quantitative analysis. Abundances of hopanoids, sterenes and steranes were calculated from peak areas in *m/z* 191, *m\z* 215 and *m/z* 217 extracted ion chromatograms, respectively and reported relative to peak areas of long-chain n-alkanes (C_{21}-C_{31}) from total ion chromatograms (TIC) without the consideration of response factors.

Gas chromatography mass-spectrometry (GC-MS)

GC-MS analyses were performed on an Agilent 5973 Mass-Selective Detector (MSD) interfaced to an Agilent 6890 gas chromatograph (GC). For the separation of the analytes a capillary column (60 m x 0.25 mm) coated with a 0.25 µm 5% phenyl 95% methyl polysiloxane stationary phase (DB-5MS, J & W scientific) was used. The GC-oven was temperature programmed from 50 °C to 310 °C at a rate of 3 °C/min with initial and final hold times of 1 and 20 minutes, respectively. Samples were injected (split/splitless injector) with a HP 6890 auto-sampler operated in a pulsed-splitless mode at 280 °C. Helium was used as the carrier gas at a constant flow rate of 1.1 mL/min. Full scan (50 - 550 Daltons) 70 *eV* mass spectra were acquired typically with an electron multiplier voltage of 1800 V and a source temperature of 230 °C.

Gas chromatography-isotope ratio mass-spectrometry (GC-irMS)

GC-irMS analyses were performed on a Micromass IsoPrime mass-spectrometer interfaced to either i) an Agilent 6890 GC equipped with a 6890 autosampler for carbon isotope analysis; or ii) an Agilent 6890N GC with 7683 autosampler for hydrogen isotope analysis. The GC- column, carrier gas, injector conditions and oven temperature program were identical to the settings described for GC-MS. For $\delta^{13}C$ analyses an interface consisting of a quartz tube packed with CuO-pellets (4 mm x 0.5 mm, isotope grade, Elemental Microanalysis LTD.) maintained at 850 °C was used to oxidize the organic analytes to CO_2 and H_2O. The latter was subsequently removed by a liquid nitrogen trap at -100 °C. Isotopic compositions were determined by integration of the *m/z* 44, 45 and 46 ion currents of CO_2 peaks from each analyte and reported relative to CO_2 reference gas pulses of known ^{13}C-content. Isotopic values are given in the delta (δ) notation relative to the international standard VPDB.

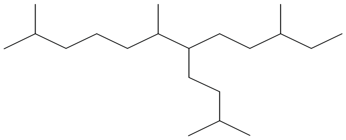
For hydrogen isotope (δD) analysis the gas chromatographically separated analytes were converted into hydrogen gas in a pyrolysis furnace packed with chromium catalyst at 1050 °C. δD values were determined by integration of the *m/z* 2 and 3 ion currents of H_2 peaks generated by the chromatographically separated analytes and reported relative to H_2 reference gas pulses of known D/H content relative to the international standard VSMOW. A correction factor accounting for contributions from H_3^+ produced in the ion source was determined by *m/z* 3 analyses at two different H_2 gas pressures.

Each sample was analysed in duplicate and average values and standard deviations were reported. Standard deviations for C-CSIA in all reported results were < 0.5 ‰ and for H-CSIA always < 7 ‰ and in most cases < 5 ‰. In house standard solutions containing a mixture of *n*-alkanes with a known isotopic composition were regularly (after ~8 analyses) analysed to confirm accuracy of measured isotopic ratios.

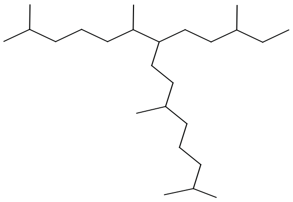
Peaks of corresponding monoenes (if present) were included for the calculation of $\delta^{13}C$ and δD values of *n*-alkanes since co-elution prevented baseline separations. A further separation of the aliphatic fraction by procedures such as 5A-molecular sieving (Grice et al., 2008a) or argentation-thin layer chromatography (TLC) was not possible due to the limited amounts of sample material.

Structures referred to in the text

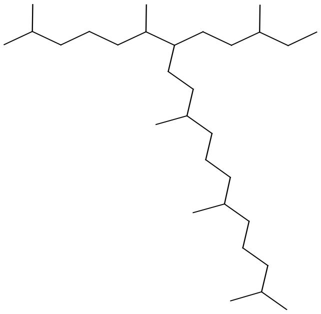
C$_{20}$HBI (**I**)



C$_{25}$HBI (**II**)



C$_{30}$HBI (**III**)



perylene (**IV**)

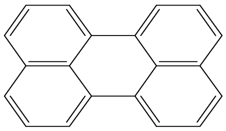


Figure A2.1: Structures referred to in the text

Data included in figures

Table A2.2: TOC % and δ^{13}C of bulk organic matter ($\delta^{13}C_{OM}$) included in **Figure 2.2**

depth [cm]	TOC %	$\delta^{13}C_{OM}$ [‰ VPDB]
1	2.9	-22.8
2	2.9	-24.5
3	2.7	-24.7
4	2.5	-24.6
5	2.5	-24.4
6	2.3	-24.3
7	2.3	-24.3
8	2.3	-24.2
9	2.3	-24.2
10	2.3	-24.1
11	2.1	-24.2
12	2.1	-24.2
13	1.9	-24.1
14	2.0	-24.1
15	1.6	-23.7
16	1.6	-23.5
17	1.5	-23.1
18	1.5	-23.2
19	1.7	-22.4
20	1.9	-21.5
21	2.6	-19.2
22	2.1	-21.4
23	5.5	-25.9
24	1.9	-19.3
25	1.0	-19.7
26	1.3	-18.8
27	1.3	-17.9
28	1.8	-17.6
29	1.4	-18.1
30	1.5	-18.2
31	1.9	-18.0
32	1.6	-17.8
33	0.6	-18.6
34	0.7	-18.6
35	1.2	-18.4
36	1.0	-18.3
36	0.8	-18.7
37	1.7	-18.1
38	0.8	-18.6
39	1.0	-19.0
40	0.7	-18.8
41	0.8	-18.6
42	1.4	-18.8
43	1.3	-18.7
44	1.6	-18.9
45	1.5	-19.3
46	1.5	-19.7
47	0.5	-19.6
48	1.8	-19.6
49	3.8	-20.1
50	1.7	-20.0
51	2.0	-19.3
52	2.0	-19.5
53	1.9	-19.7
54	1.7	-20.5
55	1.4	-21.3
56	1.4	-21.6
57	1.5	-21.2
58	1.5	-21.1
59	1.4	-21.6
60	1.3	-20.9
61	1.2	-21.2
62	1.2	-21.0
63	1.3	-21.3
64	1.3	-21.3
65	1.4	-21.0
66	1.5	-20.9
67	1.8	-20.8
68	1.8	-20.4
69	1.9	-20.0
70	2.0	-20.0
71	1.9	-20.1
72	1.9	-20.0
73	2.1	-20.1
74	2.1	-19.8
75	2.0	-20.1
76	1.8	-20.1
77	1.7	-20.1
78	1.9	-19.9
79	1.8	-19.8
80	2.0	-19.8
81	2.0	-20.3
82	2.0	-20.4
83	2.0	-20.2
84	2.0	-19.7
85	1.9	-20.3
86	1.6	-19.8
87	1.8	-20.2
88	1.8	-20.4
89	1.7	-20.5
90	1.7	-20.5
91	1.8	-20.7
92	2.0	-20.6
93	1.9	-20.6
94	1.8	-20.6
95	1.9	-20.7
96	1.8	-20.5
97	1.8	-21.4
98	1.8	-20.5
99	1.7	-20.5
100	1.7	-20.7
101	1.7	-20.9
102	1.6	-21.0
103	1.4	-20.8
104	1.6	-20.9
105	1.7	-21.0
106	1.8	-20.9
107	1.8	-21.2
108	1.8	-20.2
109	1.8	-20.8
110	1.9	-20.7
111	1.9	-21.0
112	1.9	-21.1
113	1.8	-21.2
114	1.8	-22.1
115	1.8	-21.5
107	1.8	-20.2
108	1.8	-20.8
109	1.9	-20.7
110	1.9	-21.0
111	1.9	-21.1

Table A2.2: Data included in **Figure 2.2** except for TOC % and bulk $\delta^{13}C$ of organic matter. Standard deviations were calculated between two replicate analyses.

medium depth [cm]	CPI	ACL	$\sum n$-C_{15-20}/ $\sum n$-C_{21-31}	n-$C_{29:2}$/ $\sum n$-C_{21-31}	C_{20}HBI/ $\sum n$-C_{21-31}	$\delta^{13}C$ [‰ VPDB] ±standard deviation.							
						n-C_{23}	n-C_{25}	n-C_{27}	n-C_{24}	n-C_{26}	n-C_{17}	$C_{31}\beta\beta$ H	C_{20}HBI
2.5	3.73	25.23	0.07	0.15	0.07	-31.7 ±0.0	-27.1 ±0.0	-27.8 ±0.1	-29.3 ±0.2	-31.6 ±0.2	-33.3 ±0.1	-36.1 ±0.1	-16.1 ±0.1
7.5	4.58	25.78	0.04	0.08	0.06	-30.4 ±0.2	-26.4±0.1	-26.5 ±0.1	-28.6 ±0.1	-28.9 ±0.2	-31.6 ± 0.1	-34. ±0.49	-14.2 ±0.1
12.5	4.07	26.07	0.04	0.06	0.04	-29.4 ±0.3	-25.5 ±0.2	-26.3 ±0.1	-28.4 ±0.5	-28.5 ±0.3	-31.5 ±0.4	-35.1 ±0.5	-14.6 ±0.4
17.5	4.64	26.47	0.02	0.02	0.05	-26.1 ±0.1	-24.1 ±0.1	-25.7±0.1	-27.1 ±0.1	-28.1 ±0.1	-32.2± 0.1	-33.8 ±0.2	-14.7 ±0.3
22.5	5.99	26.60	0.01	0.01	0.03	-20.9 ±0.2	-20.3 ±0.2	-20.8 ±0.2	-24.6 ±0.4	-24.1 ±0.5	-28.4 ±0.4	-30.8 ±0.2	-13.9 ±0.3
27.5	7.01	26.27	0.02	0.00	0.01	-19.6 ±0.0	-19.6 ±0.0	-19.8 ±0.0	-23.9 ±0.2	-24.6 ±0.2	-28.4 ±0.2	-31.3 ±0.1	-14.9 ±0.3
32.5	6.39	26.24	0.02	0.00	0.02	-20.6 ±0.1	-20.6 ±0.0	-21.3 ±0.0	-24.5 ±0.2	-26.1 ±0.1	-27.4 ±0.2	-30.6 ±0.2	-14.7 ±0.4
37.5	7.05	25.82	0.03	0.00	0.02								
42.5	6.31	25.83	0.04	0.00	0.03	-20.0 ±0.0	-20.0 ±0.1	-20.8 ±0.1	-23.7 ±0.0	-25.1 ±0.1	-27.4 ±0.4	-31.0 ±0.2	-14.3 ±0.2
47.5	6.35	26.26	0.02	0.00	0.02	-20.0 ±0.1	-20.0 ±0.1	-20.8 ±0.0	-23.7 ±0.2	-25.1 ±0.1	-27.4 ±0.6	-31.0 ±0.5	-14.3 ±0.4
52.5	5.24	26.15	0.02	0.00	0.01	-20.7 ±0.1	-21.0 ±0.0	-21.9 ±0.0	-24.7 ±0.0	-26.9 ±0.3	n.d.	-31.2 ±0.0	-14.3 ±0.0
57.5	5.14	26.58	0.02	0.00	0.01	-20.8 ±0.3	-21.1 ±0.2	-21.7 ±0.2	-24.5 ±0.4	-26.4 ±0.3	-29.1 ±0.1	-30.0 ±0.3	-18.8 ±0.1
67.5	6.06	26.40	0.02	0.00	0.01								
72.5	5.28	26.16	0.02	0.00	0.01	-19.3 ±0.2	-19.3 ±0.2	-19.6 ±0.3	-22.6 ±0.1	-24.6 ±0.3	-28.1 ±0.2	-28.3 ±0.1	-15.9 ±0.0
77.5	4.69	26.38	0.02	0.00	0.00								
82.5	6.25	26.12	0.02	0.00	0.01								
87.5	4.72	25.86	0.02	0.00	0.01	-20.9 ±0.2	-21.4 ±0.2	-21.9 ±0.3	-25.0 ±0.3	-27.0 ±0.2	-29.9 ±0.1	-30.2 ±0.4	-17.9 ±0.1
92.5	7.05	25.61	0.02	0.00	0.01								
97.5	6.29	25.74	0.03	0.00	0.01								
102.5	5.50	25.99	0.03	0.00	0.01	-21.7 ±0.0	-21.8 ±0.0	-21.8 ±0.1	-25.9 ±0.1	-27.3 ±0.0	-29.3 ±0.0	-30.9 ±0.1	-14.6 ±0.1
107.5	9.09	25.86	0.02	0.00	0.01								
112.5	5.99	26.20	0.03	0.00	0.01	-21.3 ±0.2	-21.4 ±0.2	-21.5 ±0.2	-25.9±0.1	-27.4 ±0.2		-30.8 ±0.4	

Table A2.3: Data included in **Figure 2.3.** Abundance was calculated relative to the largest peak area. Stable isotope values are reported ± standard deviations between two replicate analyses.

depth [cm]	chain length	relative abundance [%]			δ^{13}C *n*-alkanes [‰ VBDB]	δD *n*-alkanes [‰ VSMOW]
		n-alkanes	*n*-alkenes	*n*-alkadienes		
5-10	C_{15}	0.54	0.05	n.d.	n.d.	n.d.
	C_{16}	0.83	0.08	n.d.	n.d.	n.d.
	C_{17}	6.07	0.13	n.d.	-31.6 ±0.1	n.d.
	C_{18}	1.50	0.22	n.d.	-32.5 ±0.3	n.d.
	C_{19}	6.77	0.34	n.d.	-31.0 ±0.0	n.d.
	C_{20}	4.09	0.21	n.d.	-30.3 ±0.1	n.d.
	C_{21}	25.46	0.80	n.d.	-32.4 ±0.1	-98 ±2
	C_{22}	17.59	1.14	n.d.	-30.1 ±0.2	
	C_{23}	87.79	10.20	n.d.	-30.4 ±0.2	-103 ±2
	C_{24}	22.54	4.57	n.d.	-28.6 ±0.1	n.d.
	C_{25}	93.49	18.69	n.d.	-26.4 ±0.1	-109 ±2
	C_{26}	23.73	3.30	n.d.	-28.9 ±0.2	-99 ±4
	C_{27}	100.00	79.14	2.00	-26.5 ±0.1	-102 ±3
	C_{28}	13.84	11.47	11.47	-26.4 ±0.1	-104 ±3
	C_{29}	62.24	31.58	43.88	-26.1 ±0.1	-134 ±0
	C_{30}	2.89	n.d.	n.d.	n.d.	n.d.
	C_{31}	33.25	n.d.	n.d.	n.d.	n.d.
	C_{32}	n.d.	n.d.	n.d.	n.d.	n.d.
	C_{33}	10.88	n.d.	n.d.	n.d.	n.d.
100-105	C_{15}	0.60	n.d.	n.d.	n.d.	n.d.
	C_{16}	1.14	0.12	n.d.	n.d.	n.d.
	C_{17}	2.62	n.d.	n.d.	-29.3 ±0.0	n.d.
	C_{18}	1.30	n.d.	n.d.	n.d.	n.d.
	C_{19}	2.58	n.d.	n.d.	-28.2 ±0.2	n.d.
	C_{20}	2.91	n.d.	n.d.	-28.0 ±0.3	n.d.
	C_{21}	15.34	n.d.	n.d.	-23.8 ±0.1	-126 ±2
	C_{22}	11.95	n.d.	n.d.	-24.3 ±0.2	n.d.
	C_{23}	80.36	4.86	n.d.	-21.7 ±0.0	-115 ±5
	C_{24}	13.59	3.12	n.d.	-25.9 ±0.1	n.d.
	C_{25}	83.17	1.45	n.d.	-21.8 ±0.0	-138 ±2
	C_{26}	16.65	n.d.	n.d.	-27.3 ±0.0	-110 ±6
	C_{27}	100.00	n.d.	n.d.	-21.8 ±0.1	-151 ±4
	C_{28}	11.52	n.d.	1.53	-26.7 ±0.1	-115 ±2
	C_{29}	50.73	n.d.	n.d.	-24.1 ±0.0	-150±4
	C_{30}	6.24	n.d.	n.d.	n.d.	n.d.
	C_{31}	34.44	n.d.	n.d.	n.d.	n.d.
	C_{32}	n.d.	n.d.	n.d.	n.d.	n.d.
	C_{33}	12.71	n.d.	n.d.	n.d.	n.d.

Table A2.4: Relative abundances included in **Figure 2.4** were reported cf. C_{21}-C_{31} *n*-alkanes calculated from peak areas of *m/z* 191 (hopanoids), *m/z* 215 (sterenes), *m/z* 217 (cholestane) extracted ion chromatograms and total ion chromatograms (TIC). Tm-β = . 17β-22,29,30-trisnorhopane

medium depth [cm]	**trisnor-hopane**	**diploptene**	**C_{27} hop13(18)ene**	**hop13(18)ene**	**Tm-β**	**ββ-hopane**	**ββ-22R homohopane**	**ααα-20R cholestane**	**C_{27} ster-2-ene**	**C_{29} ster-2-ene**	**C_{16} 1-chloro-*n*-alkane**	**C_{32} 1-chloro-*n*-alkene**	***perylene**
2.5	0.0022	0.0011	0.0004	0.0012	0.0023	0.0035	0.0047	0.0001	0.0022	0.0006	0.0142	0.0768	0.02
7.5	0.0026	0.0009	0.0002	0.0004	0.0016	0.0022	0.0028	0.0000	0.0008	0.0002	0.0123	0.0298	n.d.
12.5	0.0061	0.0022	0.0001	0.0002	0.0016	0.0026	0.0035	0.0000	0.0013	0.0004	0.0042	0.0743	0.00
17.5	0.0063	0.0022	0.0002	0.0001	0.0016	0.0021	0.0025	0.0000	0.0014	0.0005	0.0003	0.0226	0.03
22.5	0.0045	0.0022	0.0001	0.0001	0.0015	0.0016	0.0019	0.0001	0.0013	0.0006	0.0001	0.0086	0.00
27.5	0.0022	0.0007	0.0001	0.0003	0.0014	0.0011	0.0013	0.0002	0.0006	0.0006	0.0023	0.0065	0.00
32.5	0.0009	0.0002	0.0001	0.0002	0.0016	0.0012	0.0013	0.0002	0.0003	0.0003	0.0039	0.0055	0.02
37.5	0.0014	0.0004	0.0002	0.0005	0.0033	0.0030	0.0035	0.0004	0.0002	0.0003	0.0044	0.0040	0.45
42.5	0.0010	0.0001	0.0001	0.0002	0.0019	0.0017	0.0022	0.0002	0.0001	0.0002	0.0066	0.0051	0.42
47.5	0.0009	0.0002	0.0002	0.0004	0.0022	0.0017	0.0022	0.0003	0.0002	0.0003	0.0045	0.0076	0.25
52.5	0.0006	0.0001	0.0001	0.0004	0.0020	0.0017	0.0021	0.0002	0.0002	0.0003	0.0040	0.0099	n.d.
57.5	0.0005	0.0002	0.0002	0.0006	0.0027	0.0022	0.0028	0.0003	0.0003	0.0004	0.0096	0.0700	0.51
67.5	0.0005	0.0001	0.0001	0.0003	0.0027	0.0019	0.0024	0.0003	0.0004	0.0006	0.0018	0.0143	0.84
72.5	0.0006	0.0001	0.0002	0.0005	0.0024	0.0018	0.0024	0.0002	0.0003	0.0005	0.0037	0.0183	0.57
77.5	0.0011	0.0002	0.0003	0.0009	0.0030	0.0023	0.0031	0.0004	0.0003	0.0005	0.0039	0.0144	n.d.
82.5	0.0009	0.0002	0.0002	0.0007	0.0028	0.0021	0.0027	0.0004	0.0003	0.0005	0.0053	0.0111	1.27
87.5	0.0018	0.0004	0.0004	0.0010	0.0048	0.0034	0.0043	0.0006	0.0005	0.0007	0.0060	0.0098	1.60
92.5	0.0015	0.0003	0.0003	0.0009	0.0050	0.0037	0.0048	0.0006	0.0006	0.0007	0.0068	0.0115	1.28
97.5	0.0012	0.0002	0.0003	0.0011	0.0042	0.0034	0.0046	0.0004	0.0005	0.0006	0.0116	0.0130	0.68
102.5	0.0014	0.0002	0.0003	0.0008	0.0038	0.0030	0.0039	0.0004	0.0003	0.0003	0.0093	0.0109	0.63
107.5	0.0014	0.0003	0.0003	0.0009	0.0041	0.0029	0.0037	0.0004	0.0004	0.0006	0.0035	0.0066	1.41
112.5	0.0010	0.0003	0.0002	0.0008	0.0031	0.0023	0.0031	0.0003	0.0004	0.0004	0.0102	0.0131	2.27

* concentration ng/g sediment

Table A2.5: Peak areas of sterenes and steranes from extracted ion chromatograms (*m/z* 215 and *m/z* 217, respectively) included in **Figure 2.5**

Medium depth [cm]	$\sum C_{27}$ steroids	$\sum C_{28}$ steroids	$\sum C_{29}$ steroids
2.5	613784	125538	211641
7.5	143389	34880	52286
12.5	423477	111844	159943
17.5	261543	83106	110634
22.5	300751	95279	148991
27.5	147588	54276	139184
32.5	30263	13538	39598
37.5	11506	6446	23014
42.5	14313	11274	35996
47.5	29046	18273	57445
52.5	30264	18369	59770
57.5	70899	42911	134701
67.5	123821	100222	265255
72.5	115795	66878	264802
77.5	72253	44394	154829
82.5	136761	81341	271985
87.5	61234	35909	122546
92.5	51114	25395	89124
97.5	129705	69550	235119
102.5	92621	40652	136610
107.5	55558	36194	124201
112.5	142179	63532	220965

Table A2.6: Data included in **Figure 2.8**

Medium depth [cm]	δD of *n*-alkanes [‰ VSMOW]					R_{22}-index	hop17(21)ene / $\sum$hopanoids
	C_{23}	C_{25}	C_{26}	C_{27}	C_{28}		
2.5	-96 ±6	-104 ±2	-79 ±5	-89 ±0	-89 ±1	0.34	0.13
7.5	-103 ±2	-109 ±2	-99 ±4	-102 ±3	-104 ±3	0.31	0.07
12.5	-89 ±4	-118 ±2	-95 ±1	-105 ±0	-116 ±4	0.33	0.04
17.5	-109 ±2	-148 ±4	-122 ±4	-143 ±0	-134 ±2	0.28	0.03
22.5	-110 ±3	-134 ±3	n.d.	-142 ±1	-125 ±0	0.19	0.04
27.5	-120 ±6	-128 ±6	-101 ±3	-148 ±1	-99 ±0	0.15	0.07
32.5	-125 ±3	-139 ±3	-113 ±1	-148 ±3	-110 ±5	0.18	0.04
37.5	n.d.	n.d.	n.d.	n.d.	n.d.	0.19	0.03
42.5	-106 ±6	-124 ±0	-102 ±7	-132 ±0	-107 ±2	0.21	0.03
47.5	-106 ±4	-117 ±2	-111 ±4	-142 ±0	-119 ±0	0.21	0.04
52.5	-107 ±2	-114 ±1	n.d.	-136 ±4	-99 ±4	0.22	0.04
57.5	-100 ±1	-118 ±3	n.d.	-135 ±1	-115 ±3	0.24	0.03
67.5	-112 ±1	-128 ±1	-112 ±0	-147 ±1	-114 ±1	0.22	0.01
72.5	-103 ±2	-127 ±1	-102 ±1	-145 ±0	-107 ±2	0.23	0.04
77.5	-115 ±4	-134 ±3	-114 ±1	-154 ±0	-112 ±0	0.24	0.04
82.5	-119 ±4	-138 ±2	-105 ±5	-155 ±1	-109 ±2	0.23	0.04
87.5	-131 ±5	-152 ±1	-122 ±4	-162 ±1	-118 ±0	0.23	0.04
92.5	-113 ±6	-136 ±2	-103 ±2	-144 ±1	-113 ±1	0.21	0.04
97.5	-142 ±6	-154 ±1	-109 ±5	-168 ±0	-121 ±5	0.22	0.04
102.5	-115 ±5	-138 ±2	-110 ±6	-151 ±4	-115 ±2	0.25	0.04
107.5	-127 ±3	-166 ±6	-128 ±3	-168 ±2	-118 ±4	0.22	0.06
112.5	n.d.	-143 ±3	-105 ±2	-160 ±2	-118 ±4	0.24	0.04

Table A2.7: Data included in **Figure 2.6**

depth [cm]	chain length	δ^{13}C *n*-alkanes [‰ VBDB]	δD *n*-alkanes [‰ VSMOW]
0-5	C_{21}	-32.1 ±0.2	-100 ±1
	C_{22}	-30.9 ±0.1	-73 ±4
	C_{23}	-31.7 ±0.0	-96 ±6
	C_{24}	-29.3 ±0.2	n.d.
	C_{25}	-27.1 ±0.0	-104 ±2
	C_{26}	-31.6 ±0.2	-79 ±5
	C_{27}	-27.8 ±0.1	-89 ±0
	C_{28}	-27.6 ±0.1	-89 ±1
	C_{29}	-27.6 ±0.2	-134 ±1
5-10	C_{21}	-32.4 ±0.1	-98 ±2
	C_{22}	-30.1 ±0.2	n.d.
	C_{23}	-30.4 ±0.2	-103 ±2
	C_{24}	-28.6 ±0.1	n.d.
	C_{25}	-26.4 ±0.1	-109 ±2
	C_{26}	-28.9 ±0.2	-99 ±4
	C_{27}	-26.5 ±0.1	-102 ±3
	C_{28}	-26.4 ±0.1	-104 ±3
	C_{29}	-26.1 ±0.1	-134 ±0
10-15	C_{21}	-31.1 ±0.3	-113 ±1
	C_{22}	-30.0 ±0.5	-69 ±3
	C_{23}	-29.4 ±0.3	-89 ±4
	C_{24}	-28.4 ±0.3	n.d.
	C_{25}	-25.5 ±0.2	-118 ±2
	C_{26}	-28.5 ±0.3	-95 ±1
	C_{27}	-26.3 ±0.1	-105 ±0
	C_{28}	-26.9 ±0.1	-116 ±4
	C_{29}	-26.3 ±0.0	-135 ±1
100-105	C_{21}	-23.8 ±0.1	-126 ±2
	C_{22}	-24.3 ±0.2	n.d.
	C_{23}	-21.7 ±0.0	-115 ±5
	C_{24}	-25.9 ±0.1	n.d.
	C_{25}	-21.8 ±0.0	-138 ±2
	C_{26}	-27.3 ±0.0	-110 ±6
	C_{27}	-21.8 ±0.1	-151 ±4
	C_{28}	-26.7 ±0.1	-115 ±2
	C_{29}	-24.1 ±0.0	-150 ±4
110-115	C_{21}	-23.3 ±0.1	-115 ±4
	C_{22}	-24.4 ±0.1	n.d.
	C_{23}	-21.3 ±0.2	-99 ±0
	C_{24}	-25.9 ±0.1	n.d.
	C_{25}	-21.4 ±0.2	-143 ±3
	C_{26}	-27.4 ±0.2	-105 ±2
	C_{27}	-21.5 ±0.2	-160 ±2
	C_{28}	-27.1 ±0.3	-118 ±4
	C_{29}	-24.2 ±0.2	-143 ±3

References Appendix 2

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Chapter 3

A pyrolysis and stable isotopic approach to investigate the origin of methyltrimethyltridecylchromans (MTTCs)

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***Organic Geochemistry* 61, 1-5 (2013)**

Abstract

Methyltrimethyltridecylchromans (MTTCs) have been widely detected in sediments and crude oils from various depositional settings and are established markers for palaeosalinities. A likely origin of these compounds, which show a distinctive isoprenoid substituted aromatic structure, seems to be condensation reactions of phytol with higher plant-derived alkyl phenols during early diagenesis. However, a direct biological origin from phytoplanktonic organisms cannot be excluded. To further investigate the potential origin from condensation reactions, an online pyrolysis-gas chromatography- isotope ratio mass spectrometry (Py-GC-irMS) method with the capacity to measure $\delta^{13}C$ in fragments (trimethylphenol and pristenes) generated from 5,7,8-trimethyl-MTTC was developed in this study. This straight forward technique poses a great potential for the elucidation of chroman formation in geological samples as it possibly enables the distinction between the different proposed sources of isoprenoid and alkyl-phenol fragments (mainly phytoplankton and higher plants, respectively) based on their stable isotopic compositions. Furthermore, it might be useful for the investigation of products generated from MTTCs during thermal maturation of geological samples.

Keywords: Flash- pyrolysis, CSIA, palaeosalinity, phenols

Introduction

Methylated 2-methyl-2-trimethyltridecylchromans (MTTCs, **I** in **Figure 3.1**) in sediments or crude oils generally occur as distinct isomers of monomethyl, dimethyl and trimethyl homologues. They were first identified in geological samples by Sinninghe-Damsté et al. (1987), who also introduced them as palaeosalinity indicators. MTTCs have since been reported in a great variety of geological samples and the "chroman ratio" (5,7,8-trimethyl-MTTC/total MTTCs) has been established as a powerful tool in salinity reconstructions (e.g. Schwark and Püttmann, 1990; Grice et al., 1998a; Schwark et al., 1998; Sinninghe Damsté et al., 1993). However, their origin and geological formation pathway remain debated (Sinninghe Damsté et al., 1993; Li and Larter, 1995; Li et al., 1995, Sinninghe-Damsté and De Leeuw, 1995). Based on correlation of abundances and chroman ratios with other geological parameters and as an explanation for the limited number of naturally occurring isomers, a biosynthetic origin of MTTCs from phytoplanktonic organisms has been suggested (e.g. Sinninghe Damsté et al., 1993), although to date MTTCs or suitable direct precursors have not been found in organisms. An origin from higher plant tocopherols (**II**, **Figure 3.1**), which bear a strong structural similarity, has been ruled out due to their comparatively low abundances in the geosphere and the presence of a phenolic hydroxyl group at C-6 (Sinninghe Damsté et al., 1993; Li et al., 1995). Li et al. (1995) alternatively proposed that MTTCs might form via early diagenetic condensation reactions of the phytol side chain in chlorophylls with higher plant derived phenols, which would imply largely different source organisms for the isoprenoid and alkylphenol moiety of geological chromans. To further investigate this potential formation pathway, we developed a pyrolysis-stable isotope analytical method for $\delta^{13}C$ determination in isoprenoid and alkylphenol fragments generated from MTTCs, which could possibly be used to establish the relationship to the different proposed source organisms of these fragments on a stable isotopic basis. Furthermore, tocopherols and MTTCs have been suggested as an additional source of pristane in more mature sediments/crude oils (Goossens et al., 1984; Li et al., 1995), which could also possibly be explored with this technique. The method was initially investigated by thermal degradation of an authentic 5,7,8-trimethyl-MTTC (triMeMTTC) standard in order to establish the stable isotopic relationship between the parent compound and the distinctive degradation products. Subsequently, chroman isolates from three Middle to Upper Devonian sediments (Canning Basin, WA) were analysed to demonstrate the applicability of the method in natural samples. Although pyrolysis products of natural and artificial MTTCs and related compounds have been thoroughly investigated by Li et al. (1995), there have been no previous isotopic based studies of these compounds to establish the formation mechanism of MTTCs.

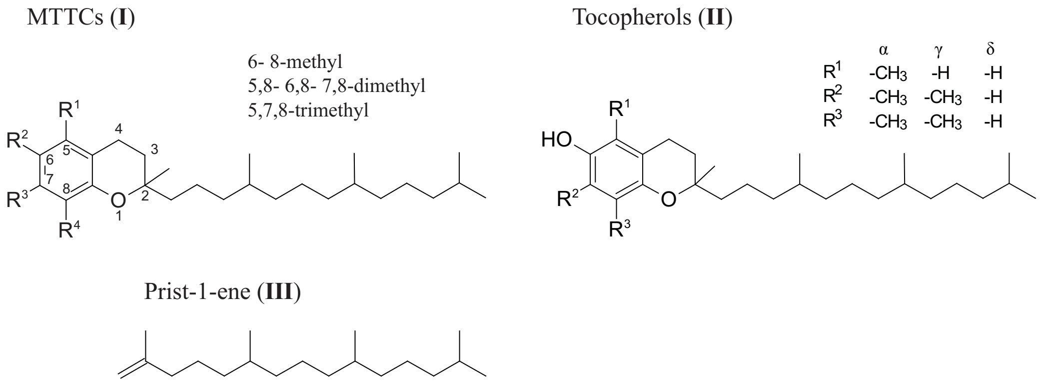


Figure 3.1: Chemical structures referred to in the text

Experimental

The authentic 5,7,8-trimethyl-MTTC standard was synthesised from 2,3,5-trimethylphenol and phytol according to Sinninghe-Damsté et al. (1987).

Sediments (MWR-30.7 m, MWR-40.7 m and MWR-41.2 m) with high triMeMTTC abundances and exceptionally low maturities (e.g. T_{max} 405–413 °C; unpublished data) originated from basin facies associated with Middle to Upper Devonian reef systems in the Canning Basin, Western Australia. The powdered rock was Soxhlet extracted and the total lipid extract fractionated by silica gel column chromatography (for details see Grice et al., 2005a; supplementary material). Unsaturated compounds were separated from the aliphatic fraction by $AgNO_3$ silica column chromatography (10%) using hexane (saturated compounds) and DCM (unsaturated compounds) as eluents. *n*-Alkanes were subsequently removed with ZSM5 molecular sieve (e.g. Audino et al., 2001) to obtain a branched and cyclic fraction. 5,7,8-trimethyl-MTTC was further isolated from the aromatic fractions of MWR-40.7 m and MWR-41.2 m by $AgNO_3$ thin layer chromatography (Eglinton and Murphy, 1969) using hexane as developer and the authentic chroman standard (visualized with rhodamine spray under UV-light) as reference. The MTTC containing silica band was scraped off, extracted with DCM and filtered through a glass sinter funnel under vacuum. The low amount of aromatic compounds in the MWR-30.7 m sample precluded a TLC isolate being obtained.

For bulk $\delta^{13}C$ analysis a Delta V Plus mass spectrometer connected to a Thermo Flush 1112 via Conflow IV (Thermo-Finnigan/Germany) was used. Analytes were combusted at 1020 °C.

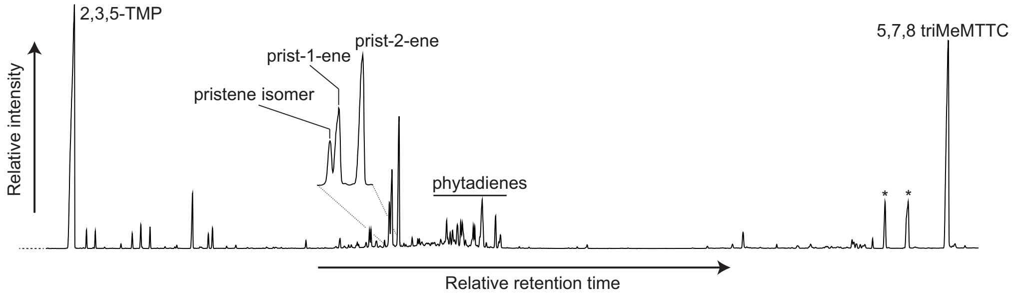
Gas chromatography-mass spectrometry (GC-MS) was performed on an Agilent 5973 GC-MS equipped with a HP 6890 auto-sampler and a DB-5MS capillary column. The GC oven was heated from 40–310 °C or 325 °C at 3 °C/min with initial and final hold times of 1 min and 30 min, respectively. A CDS 5350 Auto-pyroprobe was used for flash pyrolysis (Py)-GC-MS. The pyrolysis chamber and injector were held at 300 °C and pyrolysis was separately performed at temperatures of 550 °C, 650 °C or 750 °C applied for 20 s. The pyrolysates were analysed with a 60:1 split. He carrier gas at a constant pressure of 17.5 psi was used and the GC oven was temperature programmed from -20 °C to 40 °C at 8 °C/min and then to 320 °C at 4 °C/min with initial and final hold times of 1 and 25 min, respectively. All other settings remained unchanged.

Gas chromatography-isotope ratio mass spectrometry (GC-irMS) was performed on a Micromass IsoPrime irMS interfaced to an Agilent 6890N GC fitted with a HP 7683 autosampler. GC parameters were similar to those used for GC-MS. For Py-GC-irMS a CDS-Pyroprobe 5000 was mounted directly on the vaporisation injector of the GC-irMS system. The pyrolysis chamber and injector were set to 300 °C. Analytes were pyrolysed at 650 °C for 20s, injected with a 30:1 split or splitless (for increased sensitivity) and trapped in liquid nitrogen until the end of pyrolysis. The GC oven was programmed from 40–325 °C at 4 °C/min with initial and final hold times of 2 and 15 min, respectively. GC column and all other settings remained unchanged. Reference standards of known isotopic composition were regularly analysed to confirm accuracy of isotope analysis. All $\delta^{13}C$ values reported in this study are the average of at least two replicates and standard deviations were reported.

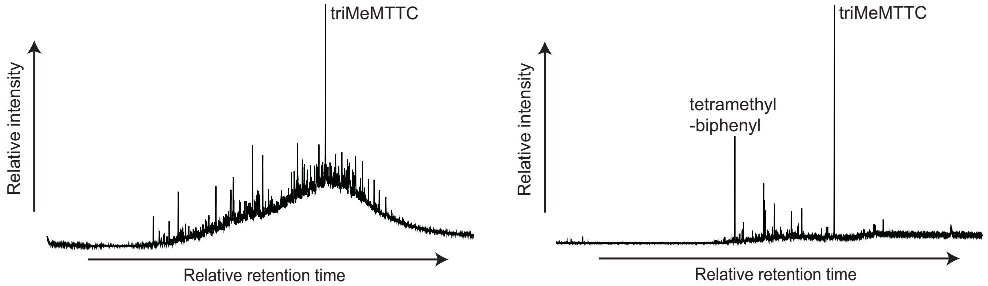
Further details about typical injector, carrier gas and MS/irMS settings as well as GC column, interface (for GC-irMS) and instrument software used for GC-MS/irMS can be found in supplementary materials of Melendez et al. (2013).

Results and discussion

The aim of this study was to develop an online flash pyrolysis-GC-irMS method which would allow stable isotopic correlation of MTTCs and related lower molecular weight products for the elucidation of their sources and formation pathways in geological samples. An authentic 5,7,8-triMeMTTC standard (often the most abundant natural chroman) was first analysed by Py-GC-MS to identify major degradation products of the parent structure and investigate pyrolysis efficiency at different temperatures (550 °C, 650 °C and 750 °C) in separate pyrolysis experiments. The major pyrolysates in all analyses were 2,3,5-trimethylphenol (see appendix for compound identification) and pristenes as well as the intact chroman (e.g. **Figure 3.2a**). The extent of pyrolytic degradation was inferred from the ratio between the abundance (peak area) of the trimethylphenol and all pristene products relative to the original chroman in four replicate analyses. The highest degradation efficiency was achieved at a pyrolysis temperature of 650 °C (ratios of 0.8, 1.6, 1.2 for 550 °C, 650 °C and 750 °C, respectively), which therefore was used in all subsequent analyses. However, the replicates generally showed some variability which is typical of many analytical pyrolysis studies. Li et al. (1995) conducted offline pyrolysis over 65 h at 350 °C on chroman isolates which similarly showed high amounts of pristenes, but contrary to present data generated tetramethylphenol instead of trimethylphenol. This was also the main product we generated in preliminary and unpublished pyrolysis experiments of the 5,7,8-triMeMTTC in sealed glass tubes at temperatures of 310 and 390 °C over 72h. The different product obtained from flash pyrolysis may be the result of the elevated pyrolysis temperatures leading to a different bond cleavage in the chroman. In an earlier study, tocopherols have also been shown to generate significant amounts of pristenes during pyrolysis and have therefore been suggested as a contributor to pristane in geological samples (Goossens et al., 1984).



(a) Pyrolysate of triMeMTTC



(b) MWR-30.7 m

(c) MWR-40.7 m

Figure 3.2: Total ion chromatograms of a typical pyrolysate obtained from an authentic 5,7,8-trimethyl-2-methyl-2-trimethyltridecylchroman (triMeMTTC) standard (a), and triMeMTTC in isolates from natural samples (MWR-30.7 m, aromatic fraction; b, and MWR-40.7 m thin layer chromatography isolate from aromatic fraction (c), which were pyrolysed in subsequent experiments. TMP = trimethylphenol; * = Impurities in triMeMTTC standard.

Precision and accuracy of $\delta^{13}C$ values measured by Py-GC-irMS were tested with five replicate analyses of the 5,7,8-triMeMTTC standard (using split and splitless injections). Standard deviations between 0.2‰ and 0.4‰ for all measured compounds confirmed an excellent precision (**Table 3.1**). $\delta^{13}C$ values reported for prist-1-ene include a coeluting pristene isomer (*cf.* **Figure 3.2a** and **Figure 3.3a, b**). Apart from that, good baseline separations, essential for GC-irMS analysis, were achieved for all remaining products. $\delta^{13}C$ values of trimethylphenol, pristenes and triMeMTTC in pyrolysates were comparable to reference values obtained by elemental analysis (EA)-irMS of the chroman standard as well as the phytol and trimethylphenol utilised for its synthesis (**Table 3.2**). This confirmed both the accuracy of the data and the preservation of the $\delta^{13}C$ signature of source compounds during condensation reactions and pyrolysis. The slight systematic depletion of $\delta^{13}C$ values obtained from EA-irMS in comparison to corresponding values measured on the GC-irMS system (in pyrolysis products as well as in the chroman standard analysed by conventional liquid injection; **Table 3.2**) can be attributed to instrumental bias. Similar systematic variations between different systems for EA- and GC-irMS have previously been reported (e.g. Zwank et al., 2003). Nevertheless, values obtained from both methods are in accordance with the following mass balance equation:

$$\delta^{13}C_{\mathrm{triMeMTTC}} = \frac{9 \times \delta^{13}C_{\mathrm{trimethylphenol}} + 20 \times \delta^{13}C_{\mathrm{phytol/pristenes}}}{29}$$

where "$\delta^{13}C_{\mathrm{phytol/pristenes}}$" stands for $\delta^{13}C$ of phytol (for bulk-irMS) or average $\delta^{13}C$ of all pristenes (for Py-GC-irMS). The calculated $\delta^{13}C$ values for triMeMTTC of -32.5‰ and -33.3‰ for Py-GC-irMS and EA-irMS, respectively, are almost identical to the measured values (**Tables 3.1** and **2**).

Table 3.1: $\delta^{13}C$ values of compounds in the pyrolysate obtained from five replicate analyses of an authentic chroman standard including average $\delta^{13}C$ values ± standard deviation. [a] injection with 30:1 split; [b] splitless injection; * joined peak of prist-1-ene and second, less abundant pristene isomer

	$\delta^{13}C$ [‰ VPDB]					Average
	run 1[a]	run 2[a]	run 3[b]	run 4[b]	run 5[b]	
trimethylphenol	-29.6	-29.9	-29.7	-29.4	-29.6	-29.6 ±0.2
prist-1-ene*	-33.5	-34.2	-34.1	-33.7	-33.9	-33.9 ±0.3
prist-2-ene	-33.4	-34.2	-33.9	-33.4	-34.0	-33.8 ±0.4
5,7,8-triMeMTTC	-32.2	-32.8	n.d.	n.d.	n.d.	-32.5 ±0.4

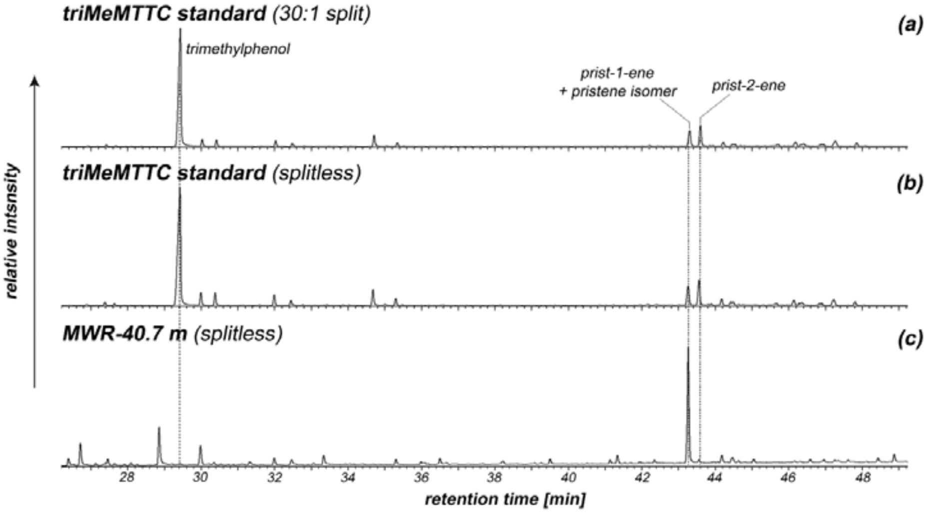


Figure 3.3: Pyrolysis-gas chromatography-isotope ratio mass spectrometry (Py-GC-irMS) chromatograms of authentic 5,7,8-trimethyl-2-methyl-2-trimethyltridecylchroman (triMeMTTC) standard (a and b) and MTTC-isolate from the MWR-40.7 m natural sample (c).

Table 3.2: $\delta^{13}C$ values of the synthesized chroman standard and source compounds ± standard deviations between 3 (^{a}) or 2 (^{b}) replicates obtained by elemental analysis-isotope ratio mass spectrometry (EA-irMS) and gas chromatography (GC)-irMS. n.d. = not determined

	$\delta^{13}C$ [‰ VPDB]		
	2,3,5-trimethylphenol	phytol	5,7,8-triMeMTTC
EA-irMS	-30.6 ±0.0^a	-34.5 ±0.0^b	-33.4 ±0.2^b
GC-irMS	n.d.	n.d.	-32.9 ±0.1^a

Py-GC-irMS was applied to the TLC-isolates from MWR-40.7 m and MWR-41.2 m (**Figure 3.2c**) and the whole aromatic fraction of MWR-30.7 m (containing abundant triMeMTTC – **Figure 3.2b**). **Figure 3.3c** shows a typical GC-irMS trace of pyrolysates obtained from these samples. Notable differences to the pyrolysate distribution of the chroman standard include the absence of trimethylphenol and prist-2-ene, which can probably be attributed to matrix effects, i.e. other compounds present in the TLC isolates/aromatic fraction influencing thermal behaviour, which can alter flash pyrolysis product distributions (e.g. Greenwood et al., 2006). Further optimisation of the pyrolysis conditions for the challenges of geological samples would be useful, but was not done here due to the limited quantity of these samples. The $\delta^{13}C$ values of pristene (most likely prist-1-ene and a second co-eluting isomer) measured by Py-GC-irMS of the three samples was consistently similar to the corresponding values of pristane and phytane obtained from traditional liquid injection GC-irMS. This correlation strongly points to a common source for these products, most likely the phytol side chain in chlorophylls (**Table 3.3**). Furthermore, the traditionally measured $\delta^{13}C$ values of triMeMTTC were also similar to the isotopic signatures of these products, although a very small ^{13}C enrichment was notable (**Table 3.3**). However, since the $\delta^{13}C$ value of the alkylphenol moiety of the chroman standard could not be measured, the suggested formation of chromans by biosynthesis in phytoplanktonic organisms (Sinninghe Damsté et al., 1993) cannot be discounted based on these results.

Table 3.3: $\delta^{13}C$ [‰ VPDB] of selected hydrocarbons in the aliphatic and aromatic fractions as well as pristenes generated by flash pyrolysis of the aromatic fraction ([a]) or isolated chroman ([b]) ± standard deviation of 2 replicate measurements. *Only measured once due to limited sample material.

Sample i.d.	GC-irMS			Py-GC-irMS
	pristane	phytane	5,7,8-triMeMTTC	Pristenes
MWR-30.7 m	-31.3 ±0.2	-29.9 ±0.4	n.d.	-31.2a*
MWR-40.7 m	-33.2 ±0.1	-32.9 ±0.4	-32.7 ±0.2	-33.0 ±0.1
MWR-41.2 m	-32.7 ±0.1	-32.6 ±0.0	-32.1 ±0.0	-32.4 ±0.0

Conclusions and outlook

An online Py-GC-irMS method which enables $\delta^{13}C$ analysis of major thermal breakdown products of triMe-MTTC (trimethylphenol and pristenes) was developed. Initial application to a triMeMTTC standard confirmed high precision and accuracy of the $\delta^{13}C$ data. Furthermore, the isotopic relationship of major pyrolysis products to the parent chroman as well as to the corresponding source compounds used for synthesis of the standard was established. Similar analyses of triMeMTTC in isolates from immature sediments also generated a pristene peak, however, trimethylphenol and prist-2-ene, which were obtained from the standard in the previous analyses, were lacking. A more complete suite of MTTC pyrolysis markers should be achievable with further optimisation of pyrolysis conditions. Nevertheless, the few MTTC products detected in these initial analyses of geological material show a great potential for the application of this analytical method to probe the origin of MTTCs in geological samples.

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Appendix 3

Identification of 2,3,5-trimethylphenol

The identity of 2,3,5-trimethylphenol (TMP) generated by pyrolysis of the 5,7,8-triMeMTTC standard was confirmed by the comparison of retention times with an authentic 2,3,5-TMP standard. For this purpose a CDS-Pyroprobe 5000 was mounted directly on the vaporisation injector of the GC-MS system described in the experimental section. Except for increasing the initial hold time at -20 °C to 2 min, and the utilization of a different GC-column (ZB-5; Phenomenex), all GC-MS conditions were the same as described in the experimental section. Previous studies have shown that 2,3,5-TMP did not co-elute with other TMP isomers at comparable GC conditions (Alexander et al., 2011; Bastow et al., 2005), which enables an unequivocal identification of the generated TMP using this standard. The 5,7,8-triMeMTTC standard was pyrolysed at 650 °C for 20s. For the analysis of the 2,3,5-TMP standard the pyrolysis chamber was kept at 300 °C for 20s. Total ion chromatograms (TIC) of the 2,3,5-TMP standard and the MTTC pyrolysis product are displayed in **Figure A3.1**. The mass spectrum of the TMP generated from MTTC pyrolysis is shown in **Figure A3.2**.

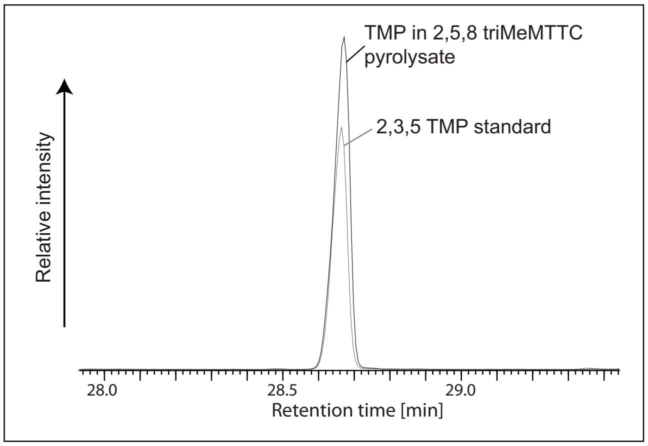

Figure A3.1: Overlain TIC chromatograms of the 2,3,5-trimethylphenol (TMP) standard and the TMP in the pyrolysate of 5,7,8-trimethyl-methyltrimethyltridecylchroman (triMeMTTC) analysed under the same GC-conditions confirming the identity of the latter

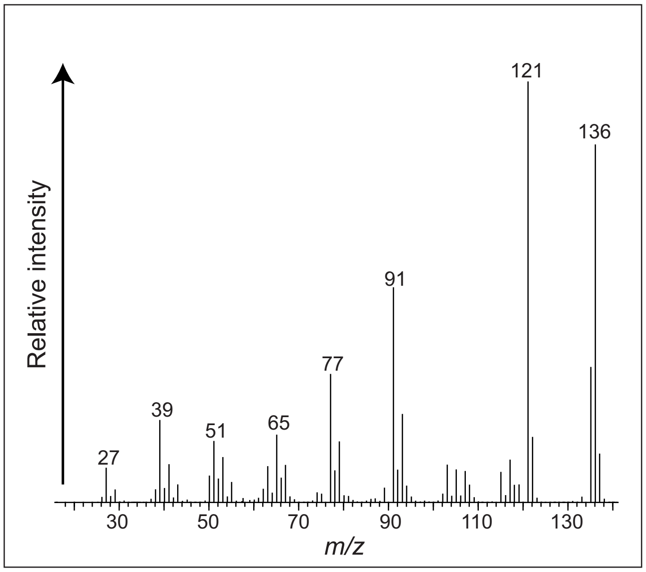

Figure A3.2: Mass spectrum of 2,3,5-trimethylphenol in the pyrolysate of 5,7,8-trimethyl-methyltrimethyltridecylchroman (triMeMTTC)

Supplementary experimental details

Synthesis of 5,7,8-trimethyl-MTTC

The authentic 5,7,8-trimethyl-MTTC standard was synthesised from 2,3,5-trimethylphenol and phytol (Sinninghe-Damsté et al. 1987). In brief, phytol, 2,3,5-trimethylphenol and phosphorus pentoxide were refluxed for 3.5 h in toluene. After cooling, the toluene layer was decanted and the residue was washed with toluene. Diethylether was added to the combined toluene layers and the mixture was washed several times with NaCl-saturated 1M KOH solution and subsequently with a 5% NaCl solution until the pH was neutral. The toluene/diethylether solution was dried over anhydrous $MgSO_4$, evaporated and purified by Al_2O_3-column chromatography using hexane as eluent.

Sample collection, preparation and extraction

The analysed samples originate from a core collected in the Canning Basin, WA (location: McWhae Ridge; see **Appendices 4** and **5**). The core was sliced with a rocksaw (diamond crystal edge with carbon steel centre) and the samples were pre-extracted (ultrasonication) in methanol and dichloromethane (DCM). Subsequently, the powedered rock (stainless-steel rock-mill) was Soxhlet-extracted with 90 % DCM in methanol for 48 h. Activated copper turnings were added to the extracts for elemental sulfur removal. The total lipid extracts were fractionated by silica gel-column chromatography (20 cm x 0.9 cm I.D.) using solvents with increasing polarity. The aliphatic and aromatic hydrocarbon fractions were eluted in hexane and 20 % DCM in hexane, respectively. Subsequently the unsaturated compounds were separated from the aliphatic fraction by $AgNO_3$ silica column chromatography (10%) using hexane (saturated compounds) and DCM (unsaturated compounds) as eluents. *n*-Alkanes were subsequently removed by passing the saturated fraction through a small column (~7 cm x 0.5 cm I.D.) of activated (> 8 h at 250 °C) and pre-rinsed ZSM5 molecular sieve using hexane as a solvent. In this procedure the saturated fraction was transferred onto the column in a minimum amount of solvent and allowed to stand for 2 min before it was eluted with hexane (~2 ml).

5,7,8-trimethyl-MTTC was further isolated from the aromatic fractions by $AgNO_3$ thin layer chromatography using hexane as developer. Silica plates were prepared by exposure to a $AgNO_3$ solution (1 % in methanol/milliQ-water, 1:4 v/v) for 45 s and a subsequent activation for 1 h at 120 °C. The previously synthesised 5,7,8-trimethyl-MTTC standard was used as a reference to identify the MTTC-containing silica band (visualization of the standard with rhodamine spray under UV-light). The MTTC containing silica band was scraped off, extracted with DCM and filtered through a glass sinter funnel under vacuum. During these procedures the exposure of the $AgNO_3$ solution as well the prepared silica plates to light was avoided. Preparation of silica plates and chromatography were performed in a dark room.

Supplementary information on GC-MS conditions

For GC-MS analyses the samples were injected into a split/splitless injector kept at 320 °C in a pulsed-splitless mode. Helium was used as the carrier gas at a constant flow rate of 1.1 mL/min.

For all GC-MS and Py-GC-MS analyses (except for the identification of the TMP, **Appendix 3**) the GC-oven was equipped with a capillary column (60 m x 0.25 mm

I.D.) coated with a 0.25 μm 5% phenyl 95% methyl polysiloxane stationary phase (DB-5MS, J & W scientific). Full scan (typically 50-600 Daltons) at 70 *eV* mass spectra were acquired with an electron multiplier voltage of 1800 V and a source temperature of 230 °C. ChemStation Data Analysis software was used for data acquisition and processing.

Supplementary information on GC-irMS conditions

For GC-irMS analyses carrier gas and injector conditions were identical to the settings for GC-MS analysis. For all GC-irMS and Py-GC-irMS analyses the same GC-column as for GC-MS analyses was used.

The analytes were oxidized to CO_2 and H_2O in a combustion interface (quartz tube packed with CuO-pellets; 4 mm x 0.5 mm, isotope grade) at 850 °C. H_2O was subsequently removed by a liquid nitrogen trap maintained at -100 °C. Isotopic compositions were determined by integration of the *m/z* 44, 45 and 46 ion currents of CO_2 peaks from each analyte and reported relative to CO_2 reference gas pulses of known ^{13}C-content. For data acquisition and processing MassLynx (Micromass Ltd.) was used. Standard solutions containing compounds with a known isotopic composition were analysed after every second sample to confirm accuracy of the instruments.

References Appendix 3

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Chapter 4

Geochemical evidence for freshwater incursions into stratified marine palaeoenvironments: Origin of methyltrimethyltridecylchromans (MTTCs)

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Abstract

Salinity-related density stratification in marine environments is one of the main drivers for the development of persistent anoxic and photic zone euxinic (PZE) conditions and thus presumably contributed significantly to global oceanic anoxic events (OAEs) associated with several mass extinctions in Earth's history. Here we present a new molecular proxy with methyltrimethyltridecylchromans (MTTCs) as indicators of freshwater incursions into a Middle to Late Devonian marine palaeoenvironment with prevailing anoxia (e.g. low pristane/phytane), PZE (*Chlorobi* biomarkers) and water-column stratification (gammacerane abundance). Although ratios between different MTTC isomers have been established as salinity indicators the origin of these isoprenoid-substituted aromatic compounds remains unclear. Similar δ^{13}C values of 5,7,8-trimethyl-MTTC and phytane as well as its co-variations with perylene abundance is consistent with the early diagenetic formation of MTTCs from condensation reactions of phytol with higher plant derived alkylphenols, possibly linking the abundance of MTTCs to predominantly riverine terrigenous input.

Introduction

Persistent salinity-related density stratification in marine environments, as can be observed in the modern Black Sea, has been a significant palaeoenvironmental and palaeoecological feature throughout Earth's history. Such stratification was particularly widespread during global oceanic anoxic events (OAEs) associated with mass extinctions (e.g. Meyer and Kump, 2008). Water-column stratification in these environments is often accompanied by photic zone euxinia (PZE), a condition where enhanced concentrations of toxic hydrogen sulfide produced by anaerobic sulfate reducing bacteria in the sediments or even the water-column, extend to the photic zone (i.e. upper part of the water-column with sufficient light penetration to allow for photosynthesis). The few highly specialized organisms thriving under such conditions typically include the obligately anaerobic green sulfur bacteria (*Chlorobi*), which grow directly at the chemocline since they can access hydrogen sulfide as an electron donor for anoxygenic photosynthesis. Therefore, the presence of characteristic ^{13}C-enriched *Chlorobi* biomarkers derived from carotenoid pigments in their unique photosystem (e.g. isorenieratane, **I** and related aryl isoprenoids, **II, Figure A4.1**) represents unequivocal evidence for palaeoenvironmental PZE (e.g. Summons and Powell, 1986; Grice et al., 2005a). A biomarker presumably indicative of water-column stratification is gammacerane (**III**), which is most likely derived from tetrahymanol (**IV**) in bacterivorous ciliates living at the interface between stratified water layers (Harvey and McManus, 1991; Sinninghe Damsté et al., 1995). Whereas the biomarkers mentioned previously are indicative of the conditions in the lower part of a stratified water-column, other compounds such as methyltrimethyltridecylchromans (MTTCs, **V**) are believed to reflect the conditions above the chemocline (e.g. Sinninghe Damsté et al., 1993). Although specific origin and formation pathway of MTTCs (**V**) in geological samples remain unclear, the ratio between different chroman isomers has been established as reliable indicator for palaeosalinity (e.g. Schwark and Püttmann, 1990; Grice et al., 1998a; Schwark et al., 1998). Hypotheses for the formation of MTTCs (**V**) include direct biosynthesis by phytoplanktonic organisms (Sinninghe Damsté et al., 1993), early diagenetic condensation reactions of phytol with alkylphenols (e.g. from higher plant-derived lignin; Li et al., 1995; Tulipani et al., 2013) or cyclization of alkylphenols (Barakat and Rullkötter, 1997). Here we introduce a new conceptual model with MTTCs (**V**) as indicators of freshwater incursions and possibly also of terrigenous input to a stratified marine palaeoenvironment associated with Middle to Late Devonian reef systems.

Experimental

Samples originated from a core collected in the Canning Basin, WA and comprised Late Givetian-Frasnian age sediments from the marginal slope and basin facies (Sadler- and Gogo Fm.) of ancient reef systems. A description of the geological settings can be found in supplementary information (SI) in **Appendix 4.** Biomarkers and bulk parameters were analysed by conventional methods (see SI in **Appendix 4**).

Results and discussion

Reconstruction of the depositional environment

The palaeoenvironmental setting corresponding to the Late Givetian-Early Frasnian age sediments of the analysed core is schematically displayed in **Figures 4.1a** and **b**. Water-exchange with the open ocean was controlled by the disposition of reef systems forming a somewhat enclosed basin which promoted the development of a stagnant and persistently stratified water-column as indicated by enhanced gammacerane indices (gammacerane/17α, 21β-hopane; 0.55-1.71). The presence of this triterpenoid has been frequently associated with hypersaline palaeoenvironments and more recently has been suggested as a marker for water-column stratification (Sinninghe Damsté et al., 1995). Typically persistent stratification in marine environments is the result of density differences between (hyper)saline bottom waters and an overlying freshwater lens from riverine input, surface runoff or precipitation leading to the development of anoxic conditions in the lower layer with a sharp chemo-, pycno-, thermo- and halocline at the interface (**Figure 4.1a**). A modern analogue for these conditions is the Black Sea, where big rivers from Eastern Europe provide freshwater input and terrigenous runoff, supporting a high primary productivity. The previously described density stratification leads to an oxygenated

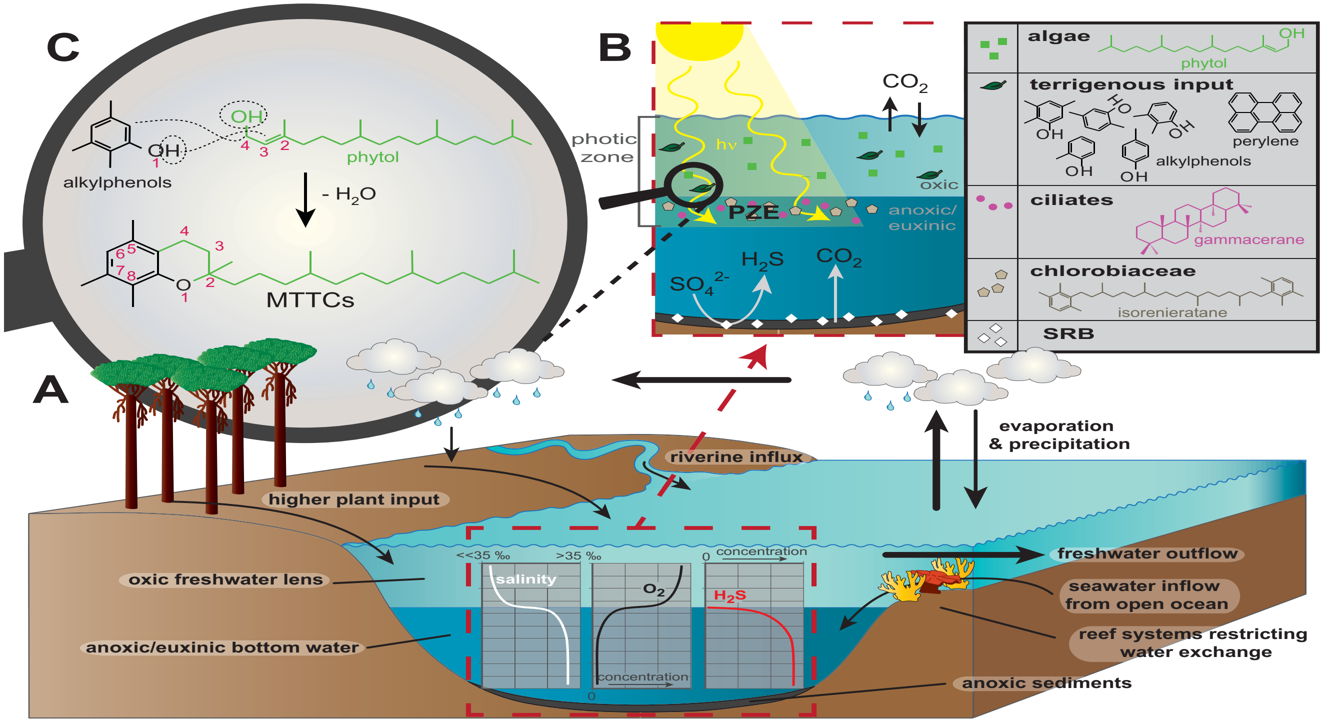

Figure 4.1: (A) Schematic model of the depositional conditions in the stratified Late Givetian-Early Frasnian palaeoenvironment with freshwater incursions overlying more saline bottom waters. (B) Photic zone euxinic conditions in the corresponding water-column and associated microorganisms and organic matter sources with their molecular indicators relevant for this study. MTTCs = methyltrimethyltridecylchromans, PZE = photic zone euxinia, SRB = sulfate reducing bacteria. Water-column stratification and PZE are evident in the abundance of gammacerane and *Chlorobi* carotenoid derivatives (e.g. isorenieratane), repectively. Higher plant input is indicated by the abundance of perylene and MTTCs. (C) Proposed mechanism of chroman formation from predominantly phytoplankton derived phytol with higher plant phenols during early diagenesis.

epilimnion of ~50 m overlying deep sulfidic and anoxic waters (> 100 m; e.g. Murray et al. 2007).

Another molecular indication for a stagnant water-column is the presence of a suite of ^{13}C-enriched *Chlorobi* biomarkers including intact isorenieratane (**I**, **Figure A4.2**) and palaeorenieratane (**VI**, **Table 4.1**), confirming photic zone euxinia (PZE) in the palaeoenvironment (**Figure 4.1b**). These results are further corroborated by the δ^{34}S signatures of pyrite (**Table 4.1**), which indicate that bacterial sulfate reduction was not limited by dissolved sulfate and occurred under essentially open conditions (e.g. Canfield and Teske, 1996; Meyer and Kump, 2008) considering a range for Devonian seawater sulfate between about +15 and +20 ‰ (Kampschulte and Strauss, 2004; Wortmann and Paytan, 2012). The values were in the same range as δ^{34}S signatures of pyrite reported in European Late Devonian sediments corresponding to a euxinic paleoenvironment (Joachimski et al., 2001) and modern euxinic basins of the brackish Baltic Sea (Böttcher and Lepland, 2000; Böttcher et al., 2004). They were, however, less ^{34}S-depleted compared to the Holocene Black Sea (Fry et al., 1991; Böttcher et al., 2004; Jørgensen et al., 2004).

Low pristane (**VII**)/phytane (**VIII**, Pr/Ph) ratios (0.39–1.0) indicate anoxia or relatively high salinities in the lower water-column. In contrast, high chroman ratios (5,7,8-trimethyl-MTTC/total MTTCs; 0.93-0.95) reflect the epilimnetic freshwater conditions (see subsequent sections). The anoxic sediments provided ideal conditions for organic matter (OM) and biomarker preservation. Another significant factor for biomarker preservation is the clay content in sediments since these minerals are known to catalyse various isomerization reactions. Therefore low clay contents lead to more immature biomarker ratios (e.g. Nabbefeld et al., 2010b). Biomarker parameters, such as very low ratios of homohopane (**X**) isomerization (**Table 4.1**), low T_{max} values in Rock Eval analysis (**Table A4.1**) and palynological indicators including pale to mid yellow spore colors (unpublished data) in the presently analysed sediments are all indicative of extremely low thermal alteration. Immature biomarker signatures have also been reported for calcareous nodules from other nearby sections of the Gogo Fm. (Melendez et al., 2013b). However, despite these excellent prerequisites for OM preservation, the total organic carbon content (TOC

%) of the sediments was surprisingly low (**Table 4.1**), pointing towards some dilution by carbonate precipitation and oligotrophic palaeoenvironmental conditions, which is contrary to most euxinic environments (e.g. Grice et al., 2005a). Nevertheless, PZE could prevail in the environment described here due to the stratification of the water-column limiting vertical circulationPerylene (**IX**), which was also present in the analysed samples, is often used as an indicator for terrigenous input. Unlike the pyrogenic origin of most unsubstituted PAHs, perylene (**IX**) presumably forms *via* diagenetic processes under anoxic conditions and has often been linked to fungal wood/lignin degradation (Grice et al., 2009; Suzuki et al., 2010). Whilst an origin from quinone pigments in marine fungi (mainly lignin degraders) or crinoids cannot be excluded, its abundance in the Devonian samples, coinciding with the evolution of the first woody plants and formation of forests (e.g. Meyer-Berthaud et al., 2010), points to a likely terrigenous source.

Table 4.1: Selected elemental, stable isotope and maturity parameters in Late Givetian-Early Frasnian sediments deposited in a stratified and anoxic/euxinic marine environment. Due to the low amount of sample material molecular sieving for the determination of $\delta^{13}C$ of pristane and C_{17} and C_{18} *n*-alkanes was not performed in this study. TOC = Total organic carbon, Carb. = carbonate, C_{31}-hopane S/(S+R) = 17α,21β-homohopane isomerization at C_{22}

Depth [m]	TOC %	Carb. %	C_{31}-hopane S/(S+R)	$\delta^{13}C$ [‰ VPDB]				$\delta^{34}S_{pyrite}$ [‰ VCDT]
				pristane	phytane	*n*-alkanes C_{19-21}	Palaeo-renieratane	
40.2	0.1	62.6	0.23	n.d.	-33.5	-33.0	n.d.	-24.0
40.3	0.3	68.1	0.20	n.d.	-33.6	-32.8	-15.3	-13.8
40.5	0.9	15.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
40.7	0.8	10.5	0.13	*-33.2	*-32.9	-32.3	-15.4	-11.9
41.2	0.7	32.1	0.12	*-32.7	*-32.6	-32.8	n.d.	-15.0
41.9	0.7	13.3	0.11	n.d.	-33.9	-33.4	-15.1	-19.1

*from Tulipani et al. (2013)

Origin of pristane and phytane

The most common source of sedimentary Pr and Ph is the phytol side chain in chlorophyll-*a* (**XI**), which in the marine setting described here would typically be provided by phytoplankton, potentially with some additional terrestrial plant input (e.g. Hayes et al., 1990). However, there are also other potential Pr and Ph sources including archaea (e.g. Rowland, 1990; Grice et al., 1998b) and Pr may be generated during thermal maturation from chromans (Li et al., 1995; Frimmel et al., 2004; Tulipani et al., 2013) or tocopherols (**XII**, Goossens et al., 1984). Similar and relatively depleted $\delta^{13}C$ values measured for Pr and Ph in this sample suite point towards common algal, cyanobacterial or higher plant sources. $\delta^{13}C$ values of short chain *n*-alkanes (C_{19}-C_{21}) were in the same range or slightly more enriched in comparison to the isoprenoids, indicating a mixed *n*-alkanes source from phytoplankton and heterotrophic bacteria (Schouten et al., 1998; Grice et al., 2005a). It could also indicate a contribution of ^{13}C depleted Pr and Ph derived from purple sulfur bacteria (*Chromatiaceae*). However, okenane (**XIII**), which is also indicative of these organisms, was not detected.

Significance of MTTCs

Although the chroman ratio has been established as palaeosalinity indicator, the actual source of MTTCs (**V**) and their formation pathway remain obscure. Sinninghe Damsté et al. (1993) and Sinninghe-Damsté and De Leeuw (1995) suggested a biosynthetic origin from phytoplankton based on abundance profiles and to account for the limited number of naturally occurring MTTC isomers. However, to date neither MTTCs (**V**) nor plausible precursors have been found in any organisms. Whilst tocopherols are structurally similar, the hydroxyl group at C-6 disqualifies them as diagenetic precursor of sedimentary MTTCs (e.g. Sinninghe Damsté et al., 1993). Li et al. (1995) alternatively suggested an origin of MTTCs (**V**) from early diagenetic condensation reactions between bound or free alkyl phenols (most likely predominantly higher plant derived) and phytol from chlorophylls (**Figure 4.1c**). MTTCs (**V**) have almost exclusively been found in geological samples post-dating the evolution of vascular plants, mainly in samples of Permian age or younger (e.g. Sinninghe-Damsté et al., 1987) but also occasionally in Middle to Late Devonian sediments (e.g. Marynowski and Filipiak, 2007). However, they have also once been reported in Neoproterozoic/Early Cambrian crude oils (Dutta et al., 2013) which may in this case be explained by sources of alkyl phenols other than higher plants (e.g. algal derived macromolecular structures or bacteria; Li and Larter, 1995 and references therein). Li et al. (1995) demonstrated that MTTCs (**V**) could be formed by condensation reactions from phytol as well as chlorophyll-*a* with a variety of free and bound alkylphenols including cresols and polyvinylphenol.

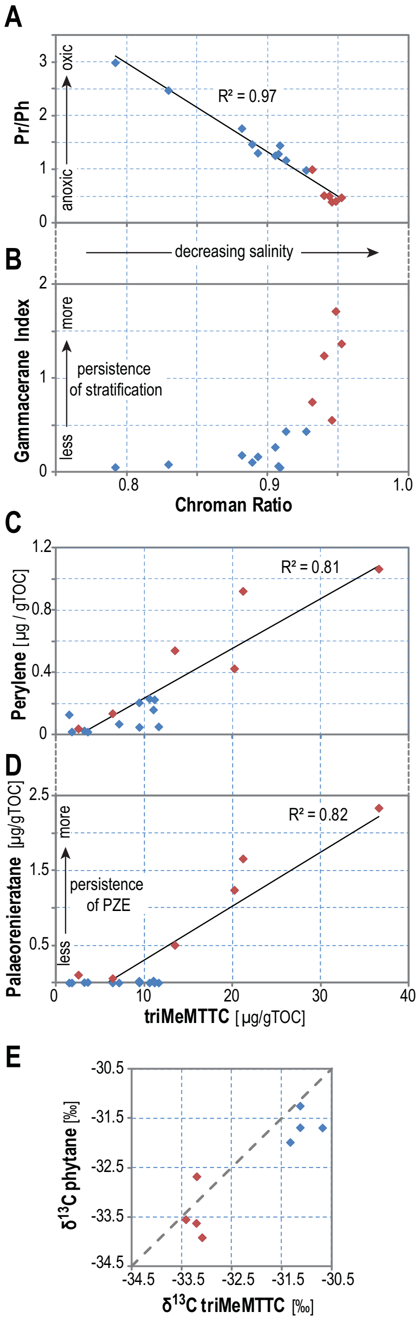

Figure 4.2: Co-variation of characteristic methyltrimethyltridecylchroman (MTTC) parameters with other biomarkers. triMeMTTC = 5,7,8-trimethyl-MTTC, chroman ratio = triMeMTTC/Total MTTC, Pr/Ph = pristane/phytane, PZE = photic zone euxinia. Red diamonds represent sediments deposited in the Late Givetian-Early Frasnian in a stratified, anoxic and euxinic palaeoenvironment whereas blue diamonds represent overlying sediments deposited later in the Frasnian in a less restricted setting. The chroman ratio is an established palaeosalinity indicator with values < 0.5 reflecting hypersaline and values > 0.6 normal marine or freshwater conditions (Sinninghe Damsté et al., 1993). However, their origin or formation pathway remain unclear. The relative ^{13}C depletion of Ph and triMeMTTC in the older samples (E) are consistent with δ^{13}C trends of bulk OM and carbonates (**Table A4.1**) and likely represent enhanced recycling of ^{12}C-rich OM by SRB in the anoxic environments (Küspert, 1982).

Figure 4.2 illustrates correlations of MTTC (**V**) parameters with other molecular indicators in the analysed core. It also includes sediments deposited later in the Frasnian which were laid down in a less restricted setting at higher sea-levels without indications of water-column stratification, anoxia or PZE (i.e. Pr/Ph > 1, low gammacerane index and perylene (**IX**) concentrations and the absence of diaryl isoprenoids e.g. palaeorenieratane, **VI**). The chroman ratio exhibited a strong inverse relation (R^2=0.97) to Pr/Ph (**Figure 4.2a**), contrary to several previous studies in more open marine settings, where an evident positive correlation between these parameters was attributed to the mutual salinity dependency of both ratios (e.g. Schwark et al., 1998). The inverse correlation presently observed indicates that chroman ratios reflected salinity in the epilimnion whereas Pr/Ph was influenced by the conditions in the bottom water or sediments (i.e. higher salinity and anoxia), which is consistent with previous suggestions of chroman formation in the upper water-column (e.g. Sinninghe Damsté et al., 1993).

Further evidence for this, comes from the sharp increase of the gammacerane index in samples with higher chroman ratios (**Figure 4.2b**). The concentration of sedimentary gammacerane (**III**) is largely determined by (i) the persistence of stratification over time and (ii) the abundance of *Chlorobi* and *Chromatiaceae*, the main food source of bacterivorous ciliates at the chemocline. The positive correlation between these parameters reflects the interdependency of salinity levels in the upper water layer (reflected in chroman ratios) and persistence of stratification. The trophic link between ciliates and *Chlorobi* and additionally the promotion of PZE by water-column stratification, account for the co-occurrence of high gammacerane indices (**Figure 4.2b**) and abundant *Chlorobi* biomarkers (e.g. palaeorenieratane, **VI**, **Figure 4.2d**).

The co-variance between 5,7,8-trimethyl-MTTC (triMeMTTC) and perylene (**IX**) concentrations (**Figure 4.2c**) might represent a link to similar higher plant sources, also supporting a diagenetic formation of MTTCs from alkyl phenols with phytol (**Figure 4.1c**). Higher plant input was in addition confirmed by palynological analyses (e.g. comminuted plant debris, resin bodies and microfossils of progymnosperms; unpublished results). However, reducing conditions favoring perylene (**IX**) abundance were stabilized by hyposaline surface waters hindering vertical water mixing. Also the similar $\delta^{13}C$ values of Ph and triMeMTTC observed here (**Figure 4.2e**), and generally similar $\delta^{13}C$ values of Pr or Ph and MTTCs (**V**) reported in several previous studies (van Kaam-Peters et al., 1997; Grice et al., 1998a; Grice et al., 1998d; Hong et al., 2007) concur with a common phytol source of Pr, Ph and the isoprenoidal subunit of MTTCs. The latter largely influences the stable isotopic composition of the whole molecule due to its larger size compared to the phenolic moiety. However, this isotopic relationship could also indicate a direct origin of phytol and MTTCs from similar phytoplanktonic source organisms as suggested by Sinninghe Damsté et al. (1993). To further investigate potentially different sources of the phenolic and isoprenoidal subunits in MTTCs it would be useful to separately measure the $\delta^{13}C$ of these distinct moieties, which might be possible by pyrolysis-GC-irMS (Tulipani et al., 2013).

Conclusions and implications

This study introduces a new conceptual model for the reconstruction of freshwater incursions in a stratified marine palaeo-water-column using chroman ratios in combination with other molecular indicators including gammacerane index, Pr/Ph and *Chlorobi* biomarkers. We demonstrate that chroman ratios in the analysed Middle to Late Devonian sediments were determined by hyposalinity in the upper water column. Physicochemical properties between epi- and hypolimnion differed strongly, minimizing vertical mixing of the water body and favoring growth of floating microbial mats at the interface layer. Grazing of these floating mats by bacterivorous ciliates is indicated by the presence of gammacerane (**III**) and confirmed by strong correlations with other biomarker parameters indicating reducing and euxinic conditions in the epilimnion. The co-variance of triMeMTTC abundance with perylene (**IX**) indicates that MTTCs (**VI**) might be representative of terrigenous/higher plant sources which would be consistent with a diagenetic formation from condensation reactions of higher plant derived alkylphenols with phytol. This study is of interest to the broader geological community since water-column stratification and related anoxia are important factors in OM preservation and often related to biological crises or mass extinction events. A better understanding of source and formation pathway of MTTCs also helps to correctly interpret the chroman ratio with regards to salinity and other palaeoenvironmental conditions.

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Appendix 4

Supplementary information

Supplementary figures

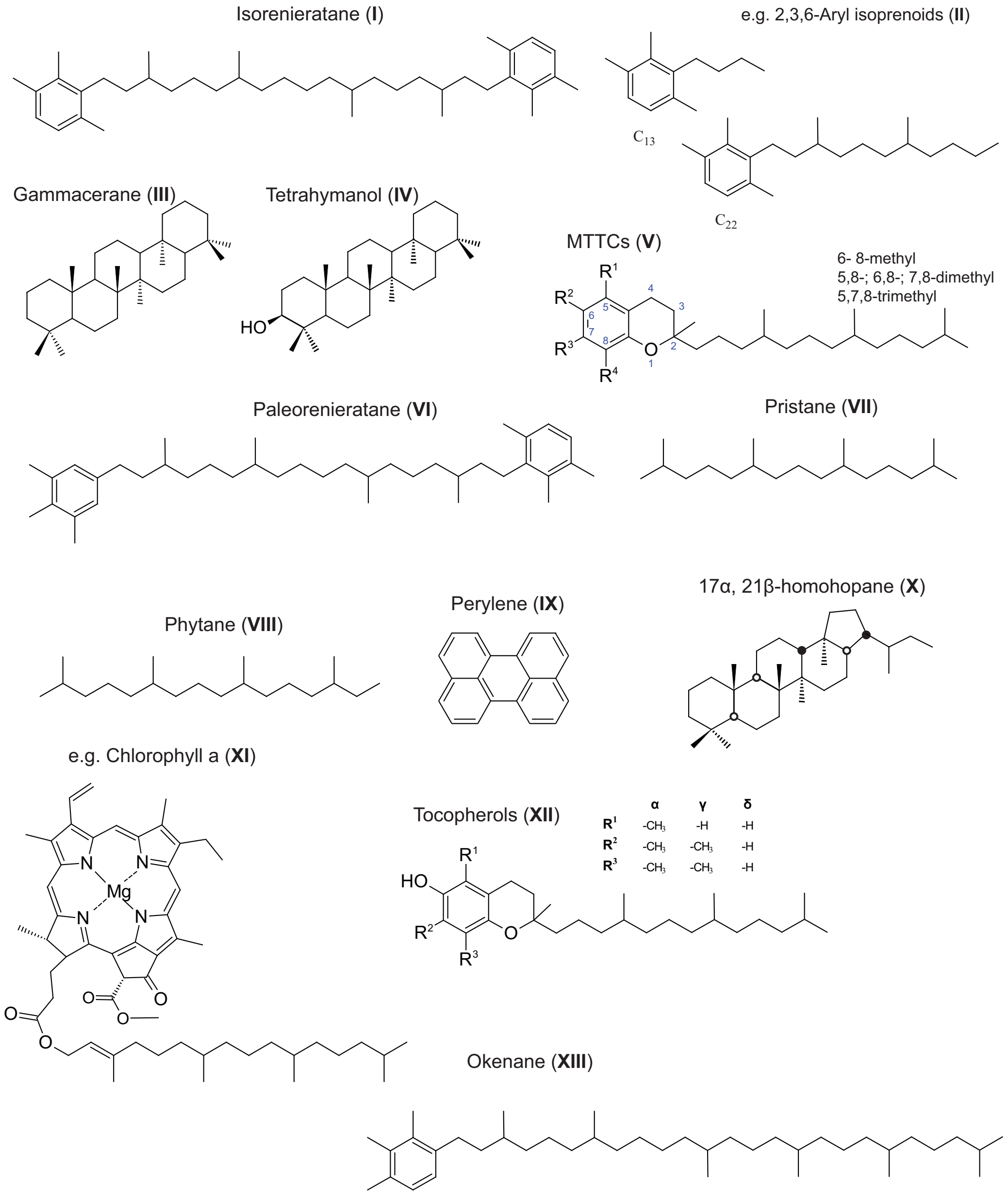

Figure A4.1: Structures referred to in the text

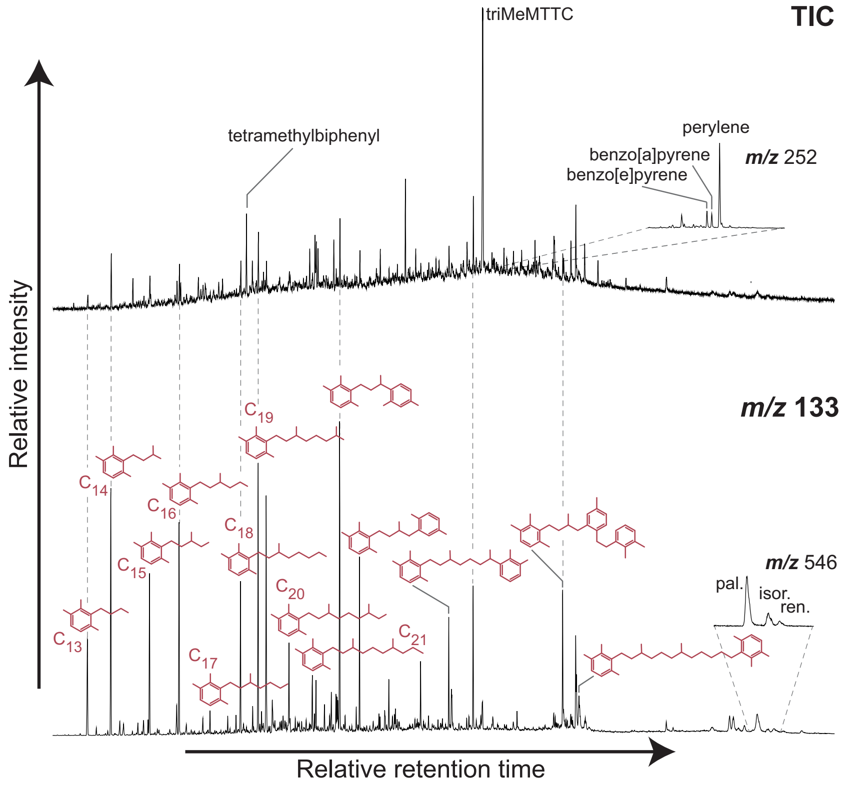

Figure A4.2: Arylisoprenoids, 5,7,8-trimethylmethyltrimethyltridecylchroman (triMeMTTC) and perylene in a typical aromatic fraction from the restricted Late Givetian-Early Frasnian palaeoenvironment. pal. = palaeorenieratane, isor. = isorenieratane, ren. = renieratane

Geological setting

The Canning Basin (Western Australia) is famous for its extensive Middle to Late Devonian reef systems containing exceptionally well preserved macro (Long and Trinajstic, 2010) and molecular fossils (i.e. biomarkers; Melendez et al., 2013). The samples for the present study originate from a core collected at the location of McWhae Ridge (18.72796°S, 126.0682°E) and comprise marginal slope and basin facies (Sadler and Gogo Formation (Fm.), respectively). This study mainly focuses on the lower part of the core from the Gogo Fm. which was deposited in the latest Givetian or earliest Frasnian (dating based on unpublished palynology results). It consists of laminated argillaceous shale and contains fragments of crinoids, stromatoporoids and brachiopods in some parts. Sediments from an overlying section of the core deposited later in the Frasnian were also analysed and some data were included in this study. All samples show an extraordinarily low maturity with very low T_{max} values (Rock Eval analysis, 405-418 °C; **Table A4.1**). For a comprehensive description of the geology in the Canning Basin and more details about the investigated section at McWhae Ridge see Playford et al. (2009).

Throughout most of the Devonian the climate was tropical without polar ice caps. According to Joachimski et al. (2009) temperatures were slightly cooler in the Late Givetian with an average of ~25°C but increased again throughout the Frasnian to ~30°C. Furthermore, there was a global sea-level rise from the Givetian to the Late Frasnian (Johnson et al., 1985; Playford et al., 2009). The expansion of terrestrial plants with the first forests occurring in the Givetian (e.g. Grice et al., 2009; Meyer-Berthaud et al., 2010; Mintz et al., 2010), likely had a major influence on climate and biogeochemical cycling (e.g. Algeo and Scheckler, 2010).

Experimental

Sample collection, preparation and extraction

The core was collected using a Winkie-drill (small portable drilling-rig) without drilling fluids or lubricants, which also previously had never been in contact with the equipment. Samples for biomarker, elemental and stable isotope analyses were cut from one half of the core with a rocksaw (diamond crystal edge with carbon steel centre) and pre-extracted by ultrasonication in methanol and dichloromethane (DCM) to remove potential surface contamination. Subsequently, samples were powdered in a stainless-steel rock-mill and Soxhlet-extracted with 90 % DCM in methanol for 48 hours. Activated copper turnings were added to the round bottom-flask during extraction to remove elemental sulfur. The extracts were separated by silica gel-column chromatography (20 cm x 0.9 cm I.D.) using solvents with increasing polarity. The aliphatic and aromatic hydrocarbon fractions relevant for this study were eluted in hexane and 20 % DCM in hexane, respectively.

Gas chromatography-mass spectrometry (GC-MS)

GC-MS analyses were performed using an Agilent 5973 Mass-Selective Detector (MSD) interfaced to an Agilent 6890 gas chromatograph (GC) equipped with a HP

6890 auto-sampler and a capillary column (60 m x 0.25 mm I.D.) coated with a 0.25 µm 5% phenyl 95% methyl polysiloxane stationary phase (DB-5MS, J & W scientific). For the analysis of aliphatic fractions the GC-oven was heated from 40 °C to 310 °C at 3 °C/min with initial and final hold times of 1 and 30 minutes, respectively. For the analysis of aromatic fractions the final temperature was increased to 325 °C with the rest of the parameters remaining unchanged. Samples were injected into a split/splitless injector kept at 320 °C in a pulsed-splitless mode. Helium was used as the carrier gas at a constant flow rate of 1.1 mL/min. Full scan (typically 50-600 Daltons) at 70 *eV* mass spectra and selected ion monitoring (SIM) spectra (used to achieve a better resolution for quantification of aromatic compounds) were acquired with an electron multiplier voltage of 1800 V and a source temperature of 230 °C. ChemStation Data Analysis software was used for data acquisition and processing. Perylene, palaeorenieratane and isorenieratane were identified by comparison of retention times with authentic standards. For semi-quantitative analyses the aromatic fractions were spiked with a known amount of perdeuterated terphenyl (d^{14}).

Gas chromatography-isotope ratio mass spectrometry (GC-irMS)

GC-irMS analyses were performed on a Micromass IsoPrime mass-spectrometer interfaced to an Agilent 6890N GC fitted with a 7683 autosampler. The GC-column, carrier gas, injector conditions and oven temperature programs were identical to the settings for GC-MS analysis. Analytes were oxidized to CO_2 and H_2O in an interface consisting of a quartz tube maintained at 850 °C packed with CuO-pellets (4 mm x 0.5 mm, isotope grade, Elemental Microanalysis LTD.). H_2O was subsequently removed by a liquid nitrogen trap at -100 °C. Isotopic compositions were determined by integration of the *m/z* 44, 45 and 46 ion currents of CO_2 peaks from each analyte and reported relative to CO_2 reference gas pulses of known ^{13}C-content. Isotopic values are given in the delta (δ) notation relative to the international standard VPDB. For data acquisition and processing MassLynx (Micromass Ltd.) was used. Each sample was analysed at least in duplicate and average values and standard deviations were reported. Standard solutions containing compounds with a known isotopic composition were analysed after every second sample to confirm accuracy of the instruments.

Multiple reaction monitoring (MRM) GC-MS

Selected aliphatic fractions were analysed on a Micromass Autospec Ultima mass spectrometer operating in MRM-mode, which was interfaced to an Agilent 6890 N gas chromatograph fitted with an autosampler and a DB-5MS fused silica capillary column (60 m; 0.25 mm I.D.; 0.25 µm film thickness; J&W Scientific). Helium at a constant flow of 2 ml/min was used as carrier gas. The GC-oven was programmed from 60°C to 150°C at 10°C/min and to 315°C at 3°C/min with initial and final hold times of 2 and 24 min, respectively. The source was operated in electron impact (EI, 70 eV) mode at 250° C, with 8 kV accelerating voltage. Data was acquired and processed using MassLynx 4.0 (Micromass Ltd.) software. Identification of compounds was achieved by comparison with a synthetic mixture of oils (AGSO standard) that contains most common hopanes and steranes. Gammacerane indeces were calculated from peak areas of gammacerane and the 17α,21β-hopane in the chromatogram of the precursor-product reaction of *m/z* 412 to *m/z* 191.

Determination of total organic carbon (TOC) and carbonate content

Small fractions of the non-extracted powdered samples were decarbonated by the addition of hydrochloric acid (~5%), washed, dried and analysed on a VARIO EL-III elemental analyser for the determination of C%. The carbonate content [%] was inferred from the difference of sample weight before and after decarbonation. TOC% was calculated from C% in the decarbonated samples.

RockEval pyrolysis

RockEval pyrolysis was performed on the decarbonated samples (see previous section) due to the low TOC% using a VINCI Rock Eval 2 instrument.

$\delta^{13}C$ of carbonates

$\delta^{13}C$ of carbonates was measured by continuous-flow (CF) analysis on a GasBench II coupled with a Delta XL Mass Spectrometer (Thermo-Fisher Scientific) using the method described in Paul and Skrzypek (2007). In brief, carbonates in the powdered samples were digested by addition of ortho-phosphoric acid in a helium atmosphere.

The generated CO_2 was trapped, purified and diverted to the irMS. Isotope values were reported in the delta (δ) notation relative to the international standard VPDB.

$\delta^{13}C$ of organic carbon

Fractions of the sample residues after Soxhlet-extraction were decarbonated by the addition of ~7N hydrochloric acid. Bulk $\delta^{13}C$ analysis of the washed, dried and homogenized samples was performed on a continuous flow (CF) system consisting of a Delta V Plus mass spectrometer connected to a Thermo Flash 1112 *via* Conflo IV (Thermo-Finnigan/Germany). Combustion and oxidation were achieved at 1700-1800°C and reduction at 650°C. Results were reported in the delta (δ) notation relative to the international standard VPDB. For more details see Skrzypek and Paul (2006).

$\delta^{34}S$ of pyrite

$\delta^{34}S$ of total reducable inorganic sulphur (TRIS, mainly pyrite) was measured in a fraction of the sample residue after Soxhlet-extraction. TRIS was extracted from the sample by treatment with hot acidic chromium (II) chloride (Fossing & Jørgensen, 1989). The generated H_2S was precipitated quantitatively as ZnS, which was then converted to AgS_2. $^{34}S/^{32}S$ ratios were measured by combustion - isotope ratio monitoring - mass spectrometry (C-irm-MS) using a Thermo Finnigan MAT 253 mass spectrometer coupled to an elemental analyser (Thermo Flash 2000) *via* a split interface (Thermo Finnigan Conflo IV). Measured isotope ratios were calibrated with in-house and international reference materials (Mann et al., 2009) and reported in the δ-notation relative to the V-CDT (Vienna Cañon Diablo Troilite) standard.

Supplementary data

Table A4.1: T_{max} (RockEval) and $\delta^{13}C$ values of bulk organic matter (OM) and carbonates (carb.) (reported in ‰ relative to VPDB), in the investigated Devonian samples

Sample depth [m]	27.3	28.1	29.0	30.9	31.9	33.0	33.2	33.4	40.2	40.3	40.5	40.7	41.2	41.9
T_{max} [°C]	413	410	418	405	405	410	415	410	n.d.	415	413	414	410	413
$\delta^{13}C_{OM}$	-29.7	-29.9	-28.3	-28.4	-27.8	n.d.	-28.3	-27.2	-29.0	-29.6	n.d.	-28.7	-29.0	-29.2
$\delta^{13}C_{Carb.}$	1.0	0.7	1.6	1.1	1.4	n.d.	1.9	1.2	0.1	-3.0	n.d.	0.5	-1.6	0.1

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Chapter 5

Changes of palaeoenvironmental conditions recorded in Late Devonian reef systems from the Canning Basin, WA: A biomarker and stable isotope approach

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Abstract

Although the Late Devonian extinctions rank amongst the “big five” mass extinction events in the Phanerozoic, causes, nature and exact timing of the associated events remain poorly understood. Besides the most pronounced biodiversity loss at the Frasnian-Famennian (F-F) boundary and towards the end of the Famennian, there were also less extensively studied extinction pulses in the Middle to Late Givetian and in the Frasnian. Here a combination of elemental, molecular and stable isotope analysis (including $\delta^{13}C$ of carbonates, organic matter (OM) and biomarkers, $\delta^{18}O_{carbonates}$, $\delta D_{kerogen}$ and $\delta^{34}S_{pyrite}$) was used for the investigation of a sedimentary record from this time period (latest Givetian or earliest Frasnian until later in the Frasnian) collected in the Canning Basin (Lennard Shelf), WA.

Depth profiles of most investigated palaeoenvironmental indicators showed distinct variations between sediments corresponding to the time period close to or at the Givetian-Frasnian (G-F) boundary and sediments deposited later in the Frasnian. Alterations in parameters such as pristane/phytane ratios, gammacerane index, abundance of *Chlorobi* biomarkers as well as $\delta D_{kerogen}$ and chroman ratios describe the change from a restricted marine palaeoenvironment with a persistently stratified water-column and prevailing anoxia and photic zone euxinia (PZE) towards a presumably open marine setting with a vertically mixed water-column and oxic to suboxic conditions. Furthermore, simultaneous excursions in $\delta^{13}C$ profiles of carbonates, OM and hydrocarbons reflect changes in the inorganic carbon pool indicating enhanced recycling of ^{13}C-depleted OM by sulfate reducing bacteria in the older sediments, most likely induced by water-column stratification. Elevated abundances of methyltrimethyltridecylchromans (MTTCs) and perylene as well as alterations in sterane distributions likely point to an increased terrigenous input *via* riverine influx. The resulting increase in nutrient input and associated phytoplankton blooms probably represented a crucial factor in the development of anoxia together with the stagnant water-column.

The collected data identify a distinct period of elevated biotic stress close to or at the G-F boundary, particularly for reef-builders and associated organisms, which may be related to a local or global extinction event. It furthermore suggests that PZE may have contributed to the Late Devonian extinctions, consistent with previous studies,

which also reported evidence of this condition in various Middle to Late Devonian sediments. The relation of terrigenous nutrient input and anoxia also supports the hypothesis of a link between the expansion of higher plants and the Late Devonian extinctions.

Introduction

In the Late Devonian some of the biggest mass extinctions in Earth's history ultimately wiped out the extensive reef systems prominent throughout that era (Sepkoski, 1986, 1993; Walliser, 1996; Bambach, 2006;). The most pronounced biodiversity loss occurred at the Frasnian-Famennian (F-F) boundary; however there were also distinct biodiversity crises towards the end of the Famennian time period and in the Middle to Late Givetian and earlier Frasnian (Walliser, 1996; Caplan and Bustin, 1999; Aboussalam and Becker, 2001). The most severely affected organisms were reef-building communities and associated fauna in tropical, shallow marine settings whereas terrestrial ecosystems were only marginally impacted (Copper, 1986; Fagerstrom, 1994; McGhee, 1996). Cause and nature (distinct events, several smaller pulses or rather gradual diversity decline) of the extinctions continue to be debated (e.g. McGhee, 1996; House, 2002; Racki, 2005). Hypotheses range from one or several bolide impacts (McLaren, 1970, 1983; Sandberg et al., 2002; Ellwood et al., 2003) to climate change and rapid sea-level fluctuations in response to various parameters including atmospheric CO_2 reduction due to the rise of terrestrial plants or extensive volcanism (Murphy et al., 2000; Courtillot and Renne, 2003; McGhee, 2005; Algeo and Scheckler, 2010). The expanding terrestrial vegetation and the associated higher nutrient input from enhanced weathering and soil formation may have further contributed to the widespread anoxia and eutrophication in Late Devonian oceans, which have in particular (but not only) been linked to the F-F extinctions (Joachimski and Buggisch, 1993; Bond et al., 2004; Algeo and Scheckler, 2010). Whereas most research focuses on the investigation of the F-F or the End-Famennian extinctions, studies about earlier events in the Late Givetian and Early to Middle Frasnian are comparatively rare.

Several mass extinction events including the Permian-Triassic extinction (Grice et al., 2005a; Summons et al., 2006; Hays et al., 2007; Nabbefeld et al., 2010a) and some Mesozoic events (Sinninghe Damsté and Köster, 1998; Pancost et al., 2004; Jaraula et al., 2013) have been associated with (global) oceanic anoxic events (OAEs) in which a stagnant water-column led to persistent stratification and widespread anoxia and photic zone euxinia (PZE). PZE describes a condition where high concentrations of toxic hydrogen sulfide produced by anaerobic sulfate reducing bacteria (SRB) in or near sediments expand into the photic zone (i.e. the surface water layer with enough light penetration for photosynthesis to occur). Whereas these environments are highly unfavourable for most organisms, the obligately anaerobic green sulfur bacteria (*Chlorobi*) only thrive under these conditions since they use hydrogen sulfide as an electron donor to perform anoxygenic photosynthesis. Therefore the presence of distinctive ^{13}C-enriched sedimentary biomarkers derived from carotenoids in their unique photosystem, such as isorenieratane (**I**, **Figure A5.1**), palaeorenieratane (**II**) or a series of aryl isoprenoids (**III**), represents unambiguous evidence for PZE in the palaeoenvironment (Summons and Powell, 1986, 1987; Requejo et al., 1992; Grice et al., 1996b, 1997; Koopmans et al., 1996b; Schwark and Frimmel, 2004). PZE may have also been a significant factor in the Late Devonian extinctions since evidence of this condition has been reported in Middle to Late Devonian sediments and crude oils from various locations including the Western Canada Sedimentary Basin (Summons and Powell, 1986, 1987; Requejo et al., 1992; Maslen et al., 2009), the Michigan and Illinois Basin, USA (Brown and Kenig, 2004), the Holy Cross Mountains, Poland (Marynowski et al., 2000, 2011; Joachimski et al., 2001; Marynowski and Filipiak, 2007) and in the Canning Basin, Western Australia (Maslen et al., 2009; Melendez et al., 2013a, 2013b; **Chapter 4** this thesis).

The Canning Basin in Western Australia is of high significance for the investigation of Late Devonian extinction events due to the excellent preservation of extensive and well exposed Middle to Late Devonian reefs (e.g. Playford et al., 2009). The Gogo Formation represents the Givetian to Middle Frasnian basin facies of these reef systems and was deposited largely under suboxic, anoxic or euxinic conditions (Maslen et al., 2009; Playford et al., 2009; Melendez et al., 2013a, 2013b). It is of particular significance for studies of macro as well as molecular fossils (i.e. biomarkers) due to the exceptional preservation of soft tissue and original biomarker signatures in calcareous nodules of some sections (e.g. Long and Trinajstic, 2010; Melendez et al., 2013b).

This manuscript presents data from a comprehensive biomarker, elemental and stable isotope approach (S, N, and C %; δ^{13}C of biomarkers, OM and carbonates, $\delta^{18}O_{carbonates}$, $\delta D_{kerogen}$ and $\delta^{34}S_{pyrite}$), complemented with Rock Eval pyrolysis, in sediments from a lower slope/basinal core, which reflect paleoenvironmental changes in the time period from close to or at the Givetian-Frasnian (G-F) boundary until later in the Frasnian. The primary aim was to identify environmental conditions which may reveal causes and timeframes of potential extinction events whilst also extending the general interest in biomarker preservation in the Gogo Formation. **Chapter 4**, which introduces a novel biomarker proxy for freshwater incursions in marine palaeoenvironments based on the analysis of sediments from the lowermost section of this core, revealed evidence of a persistently stratified water-column (freshwater lens overlying more saline bottom water), with prevailing anoxia and photic zone euxinia (PZE) in the corresponding depositional setting. Here a more comprehensive approach was presented including additional parameters and a further extension of palaeoenvironmental reconstructions until later in the Frasnian.

Geological setting

The samples analysed here originate from a core collected at McWhae Ridge (18.72796°S, 126.0682°E), a locality along the Lennard Shelf, Canning Basin, WA located at the southern end of South Lawford Range (**Figure 5.1a**), in which the F-F boundary has been well defined in distal marginal slope/basinal facies. The geological setting at this location is displayed in a cross section in **Figure 5.1b** and has been described in detail elsewhere (e.g. Becker et al., 1991; Playford et al., 2009). In brief, it comprises a faulted reef spine (Pillara-Limestone, Givetian-Frasnian) which was drowned in the early Frasnian and overlain by marginal slope (Sadler Formation, Givetian-Middle Frasnian and Virgin Hills Formation, Frasnian-Famennian) and basin facies (Gogo Formation). Furthermore it was overgrown by a stromatolitic bioherm (now largely eroded) in the latest Frasnian and Early Famennian. The Sadler formation is interfingering with the equivalent basin facies (Gogo Formation) below the surface.

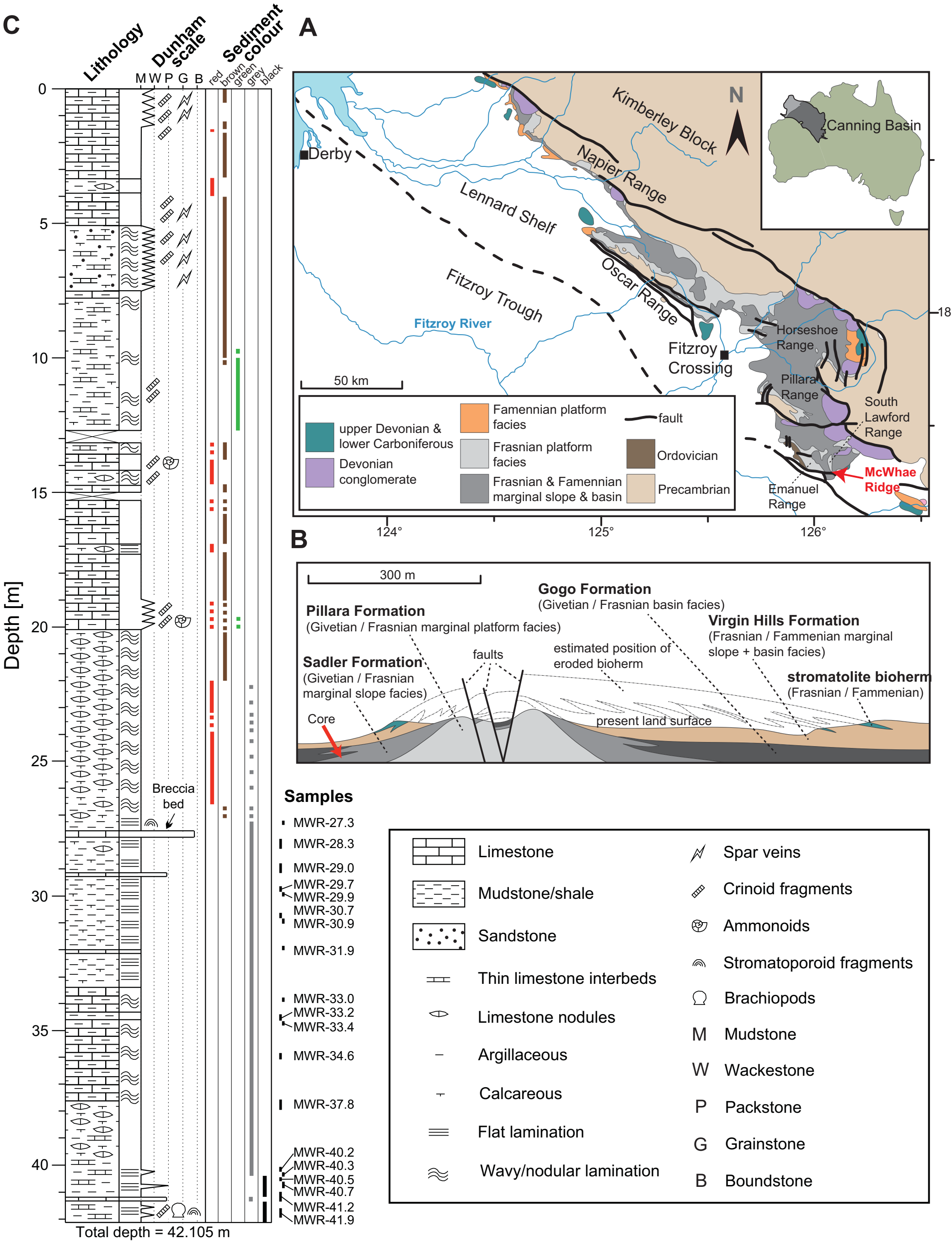

Figure 5.1: Sedimentary log (C) and location (A) of the core collected from McWhae Ridge. (B) Displays the geological setting at that location in a cross section with the red arrow indicating the approximate location of the collected core. Map and cross section were modified after Playford et al. (2009). The graphical illustration of the Dunham scale is used to visualize grain size.

Figure 5.1c shows the sedimentary log of the sampled core. The upper section (< 27.2 m) includes the F-F boundary and consists of the Virgin Hills Formation. However, due to its low OM content this interval is not suitable for biomarker analysis and has not been considered here. In this part of the core beds of red-brown, pale grey-green or pale brown-grey calcareous siltstone partly with abundant micritic nodules are alternating with poorly bedded red, pale red-brown or pale-brown argillaceous wackestone and micrite. There is also an interval of pale brown calcareous and argillaceous, very fine siliciclastic-rich sandstone and argillaceous and sandy, micritic limestone between 5 and 7 m. The more OM-rich section of the core (27.2-42.1 m), which is included in the present study, consists of the interfingering Sadler and Gogo Formations. Depth between 34 and 39 m correspond to conodont zones 1-4 (unpublished results). The lowest part of the sampled interval (40.1-42.1 m) is from the Gogo Formation and of latest Givetian-earliest Frasnian age. Dating of this interval was based on unpublished palynological analysis, in particular the abundance of the plant microfossils of the key taxon *Archaeozonotriletes timanicus* Naumova 1953 which McGregor and Playford (1993) listed as a characteristic element of Australian plant microfossil assemblages assigned to the *optivus-triangulatus* Zone. The core interval consists of laminated argillaceous shales with thin limestone interbeds and some narrow irregular beds of shelly wackestone and fine grained packstone. Brachiopods are abundant throughout the interval and crinoids and stromatoporoid fragments are present in the lowermost 40 cm. The section is followed by an OM-lean interval (33.5-40.1 m) of irregular bedded grey or pale yellowish-grey calcareous siltstone with abundant micrite nodules (37.5-40.1 m) and argillaceous limestone (33.5-37.5 m). The uppermost part of the sampled interval (27.2-33.5 m) consist of medium grey finely laminated calcareous shale and a few narrow beds of nodular and brecciated micrite and fine packstone. Towards lower depths the calcareous content increases whereas lamination decreases.

From the Late Givetian until the Middle to Late Frasnian a long-term global rise in sea-level has been linked to the retreating and backstepping of reef platforms observed in the Canning Basin. This was followed by a decrease in subsidence and dropping sea-levels in the Late Frasnian and Famennian leading to advancing platforms (e.g. Playford et al., 2009). Similar effects were also observed in reef-systems located in other parts of the world (e.g. Western Canada sedimentary Basin; Atchley et al., 2006).

Experimental

Analytical methods and sample preparation are described in the supplementary information (SI) included in **Appendix 5**.

Results and discussion

Aliphatic hydrocarbons

Figure 5.2 displays typical total ion chromatograms (TICs) from gas chromatography-mass spectrometry analysis (GC-MS) analysis of aliphatic fractions in the sediments which were deposited close to or at the G-F boundary (depth > 40 m; e.g. A, MWR-40.7) and later in the Frasnian (depth 27.2-33.5 m; e.g. B, MWR-29.9), respectively. The aliphatic fractions throughout the core, with the exception of the OM-lean interval between 33.5-40 m, contained *n*-alkanes with chain lengths from C_{13}, C_{14}, or (occasionally) C_{15} to C_{33} with distributions typically maximising at C_{21}. *n*-Alkanes in the sample from the OM-lean section (depth 37.8 m) were present at low concentrations and ranged from C_{16} to C_{33}. In most samples a slight odd-over-even predominance in short-chain as well as long-chain *n*-alkanes was evident (see carbon preference indices (CPIs) in **Table 5.1**). The isoprenoids pristane (**IV**) and phytane (**V**) were present in all samples and dominated the aliphatic fractions of all sediments from the lowermost part of the core (> 40 m). Furthermore, the samples contained complex distributions of saturated and unsaturated hopanoids and steroids. The most abundant of these were C_{27} to C_{29} diaster-(13)17-enes (**VI**) highlighted in the *m/z* 257 mass chromatograms in **Figure 5.2**. The hopanoids included C_{29} to C_{35} regular hopanes with 17β,21β (ββ), 17β,21α (βα) and 17α,21β (αβ) configurations (**VII**) as well as 18α 22,29,30-trisnorhopane (Ts), 17α 22,29,30-trisnorhopane (Tm), 17β 22,29,30-trisnorhopane (Tm-β). 29,30 and 28,30-bisnorhopane (BNH; **Figure A5.2**) and also C_{31} to C_{36} 2β- and 3β-methylhopanes were detected in relatively low concentrations (**Figure A5.3**). Hopenes included hop-(13)18-enes and C_{30} to C_{35} hop-(17)21-enes (**Figure A5.4**). Regular steranes were present in the 5α,14α,17α (ααα), 5α,14β,17β (αββ) and 5β,14α,17α (βαα) configurations (**VIII**) from C_{27} to C_{30} as well as 3 and 4 methyl 24-ethylcholestanes (**Figure A5.5**). The C_{30} triterpenoid gammacerane (**IX**) was also only present in significant concentrations in the lowermost samples from depths > 40 m. The identity of hopanes and steranes and also gammacerane (**IX**) were confirmed by multiple reaction monitoring (MRM) gas chromatography-mass spectrometry (GC-MS).

Aromatic hydrocarbons

The most abundant compound in aromatic fractions of all sediments was the 5,7,8-trimethylmethyltrimethyltridecylchroman (**X**; triMeMTTC). The other MTTC isomers which commonly occur in geological samples (**X**; Sinninghe-Damsté et al., 1987) were also present, albeit at much lower concentrations. Sediments at depth > 40 m furthermore contained a series of abundant mono- and diaryl isoprenoids including intact iso- and palaeorenieratane (**I** and **II**; **Figure 5.3** and **5.4**). PAHs were present at relatively low abundances and included typical combustion markers such as benzo[*a*]pyrene (**XI**), benzo[*e*]pyrene (**XII**), coronene (**XIII**), benzo[*ghi*]perylene (**XIV**), fluoranthenes (**XV**) or pyrenes (**XVI**) as well as the presumably fungal-derived perylene (**XVII**; e.g. Grice et al., 2009; **Chapter 2** this thesis).

Evidence of water-column stratification, anoxia and PZE

The palaeoenvironmental setting corresponding to the lowermost part of the core in depth > 40 m (Late Givetian-Early Frasnian) has been described in **Chapter 4**. To summarize, enhanced gammacerane indices indicated a stratified water-column which promoted the development of PZE, evident in the abundance of palaeorenieratane (**II**) and other *Chlorobi* carotenoid derivatives. Low pristane/phytane ratios (Pr/Ph < 1) indicated anoxia and higher salinities in the hypolimnion and sediments whereas high chroman ratios (triMeMTTC/total MTTCs) > 0.9 reflected the low salinities in an overlying freshwater lens from likely predominantly riverine incursions. High abundances of perylene (**XVII)** and MTTCs (**X**) presumably represented significant terrigenous input.

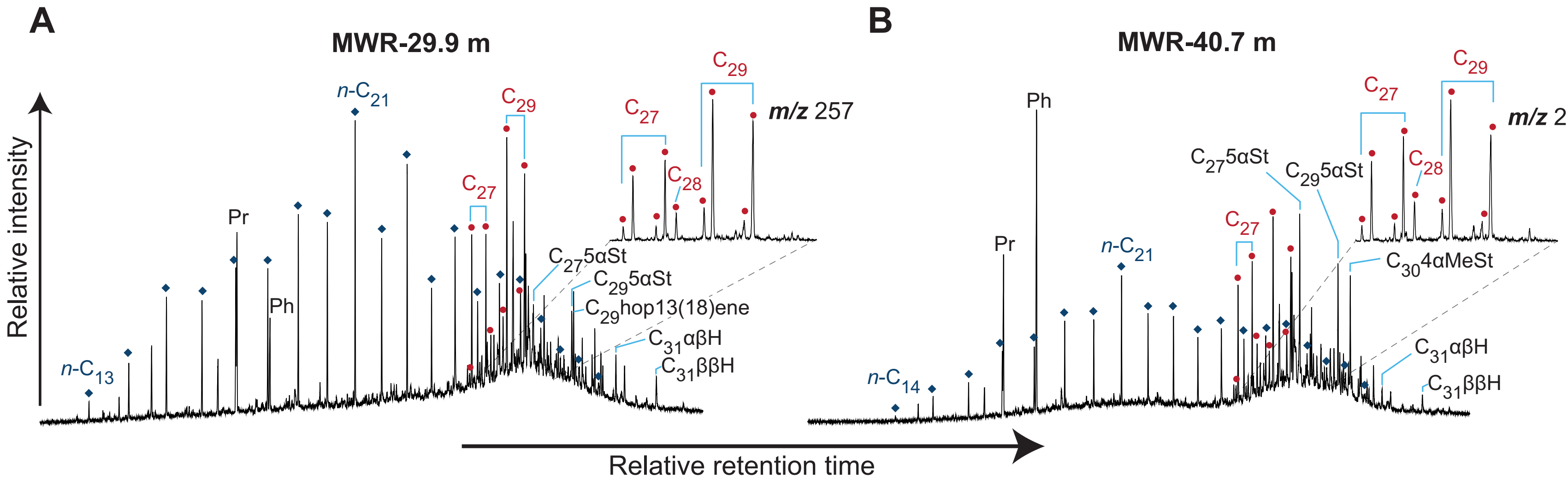

Figure 5.2: Total ion chromatograms showing two representative aliphatic fractions of samples deposited in (A) the earliest Frasnian or latest Givetian under anoxic/euxinic conditions (MWR-40.7 m) and (B) later in the Frasnian in a more oxic setting (MWR-29.9 m), respectively. Extracted ion chromatograms of *m/z* 257 show distributions of C_{27}–C_{29} diaster-13(17)-enes with carbon number C_i. Not all isomers of the C_{28} diaster-13(17)-enes could be identified due to coelutions with more abundant C_{27} and C_{29} isomers. Red dots represent diasterenes, blue diamonds *n*-alkanes. 5αSt = regular 5α,14α,17α-20R-steranes; C_{30}4αMeSt = 4α-methyl-5α,14α,17α-20R-24-ethylcholestane; C_{31}αβH and C_{31}ββH = 17α,21β and 17β,21β-22R-homohopane

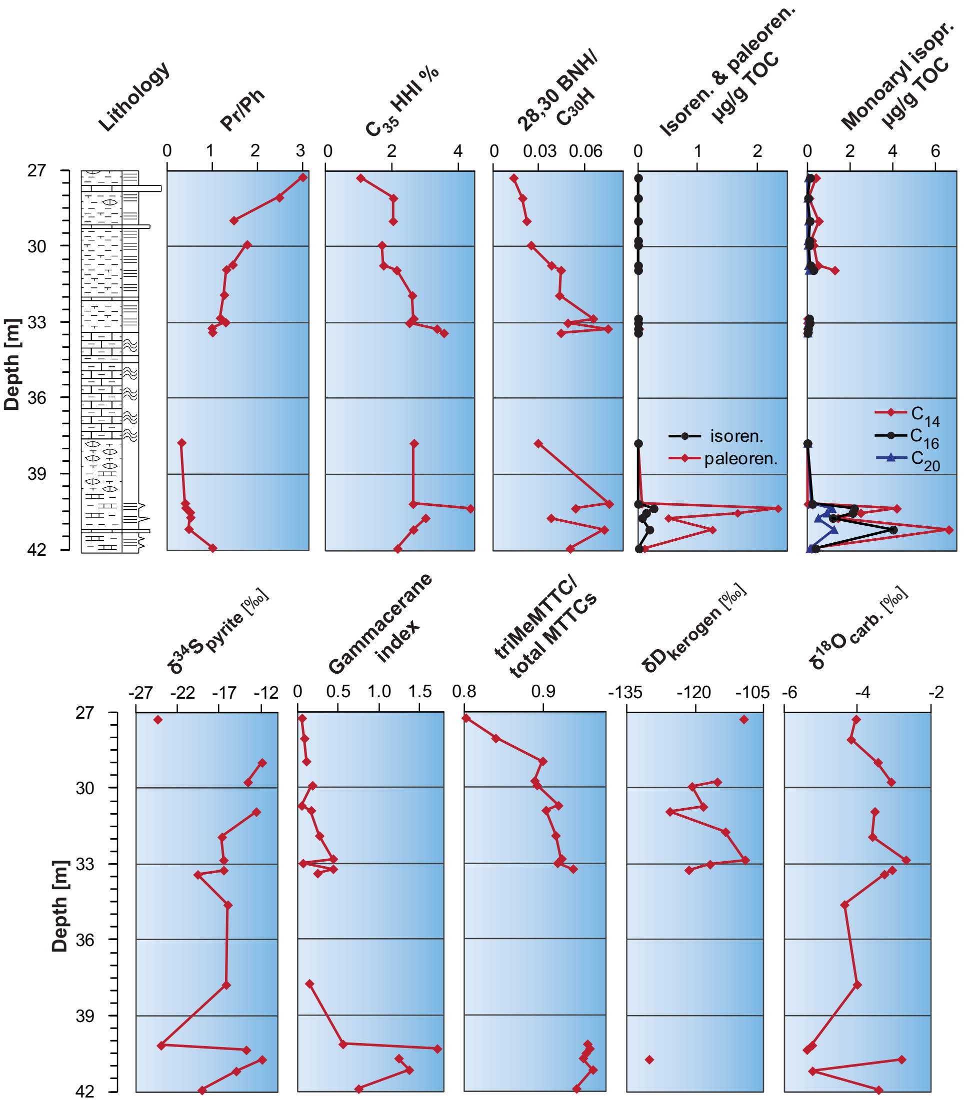

Figure 5.3: Depth profiles of stable isotope and biomarker parameters (potentially) indicative of redox conditions, photic zone euxinia, water-column stratification or salinity throughout the analysed core from McWhae Ridge. Pr/Ph = pristane/phytane; C_{35}HHI % stands for C_{35}-homohopane index and was calculated according to: C_{35}-homohopanes/$\sum(C_{31}–C_{35}$ homohopanes$) \times 100$ %; 28,30-BNH/C_{30}H = 28,30-bisnorhopane/ 17α,21β-hopane; gammacerane index = gammacerane/17α,21β-hopane; the three previous parameters were all calculated from peak areas in suitable MRM-GC-MS transitions. Isoren. = isorenieratane; palaeoren. = palaeorenieratane; isopr. = isoprenoids; triMeMTTC/total MTTC = 5,7,8-trimethylmethyltrimethyltridecylchroman/ total methyltrimethyltridecylchromans, calculated from peak areas of selected ion monitoring traces; carb. = carbonates. δ^{34}S was reported in ‰ relative to VCDT; δD was reported in ‰ relative to VSMOW and has been corrected for exchangeable hydrogen according to Schimmelmann (1991); δ^{18}O was reported in ‰ relative to VPDB. Some of the included data has already been presented in a different context in Chapter 4 (see Table A5.1 for more detail)

Figure 5.3 shows depth profiles of molecular and stable isotope indicators of redox conditions, salinity, stratification and PZE in the analysed core which will be discussed in the following sections.

Indicatators of anoxia

Pr/Ph is a frequently used redox indicator; however it is also influenced by other parameters including salinity (ten Haven et al., 1985; Schwark et al., 1998). Pr/Ph in sediment depths < 33.5 m was higher than in the lowermost part of the core indicating less reducing conditions and lower salinities in the hypolimnion and sediments in the corresponding palaeoenvironment, which would be consistent with a more open setting at higher sea-levels (Playford et al., 2009). In the two uppermost samples Pr/Ph showed a further increase to values approaching 3, reflecting a well-mixed and oxygenated water-column at the time of deposition. A further potential redox indicator is the C_{35} homohopane index (**Figure 5.3**), with higher values indicating the enhanced preservation of extended hopanoids, which is often associated with reducing conditions at the time of deposition and shortly after burial

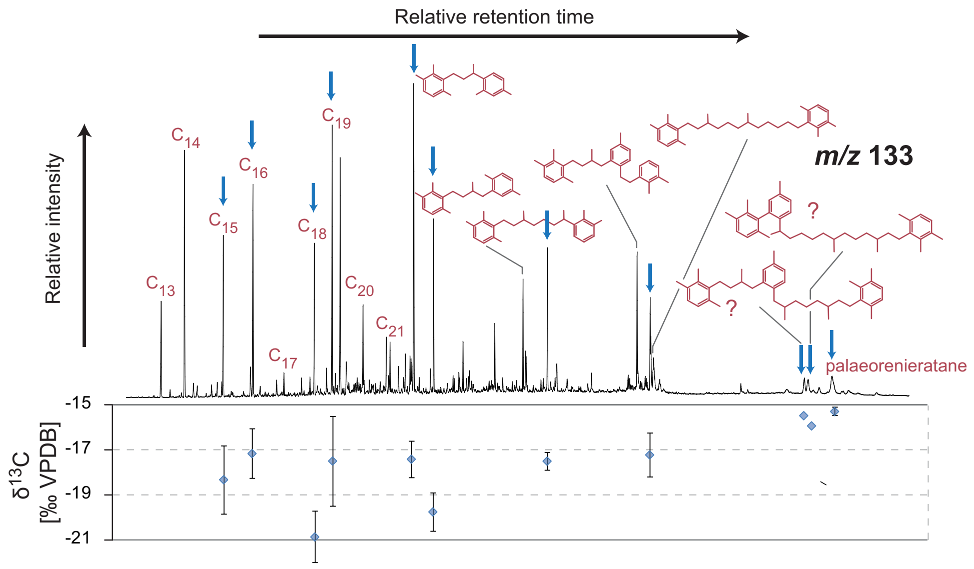

Figure 5.4: *Chlorobi* carotenoid derivatives in the *m/z* 133 trace from selected ion monitoring (SIM) GC-MS of a representative sample in the lowermost part of the analysed core corresponding to the Late Givetian-Early Frasnian (depth 40.7 m). "C_i" mark monoaryl isoprenoids with carbon number "i". $\delta^{13}C$ values represent the average for the respective compound in all samples below 40 m with error bars indicating the standard deviations. The two values without error bars could only be measured in 1 sample. Blue arrows indicate the peak corresponding to the respective $\delta^{13}C$ value. Question-marks indicate tentatively identified structures. The chromatogram as well as the stable isotopic composition of palaeorenieratane is presented in **Chapter 4**.

(e.g. Peters and Moldowan, 1991 and references therein). However, also other parameters such as source organisms, composition of the sediment (e.g. clay contents) or thermal maturity may influence relative abundance and preservation of C_{35}-homohopanes. Some of the trends in the depth profile displayed in **Figure 5.3** are consistent with Pr/Ph ratios although differences between the lowermost (> 40 m) and upper samples (> 33.5 m) were not as pronounced. 28,30-BNH has also frequently been linked to anoxic depositional environments. The *bisnor*-hopane is thought to originate from chemoautotrophic bacteria living at the oxic-anoxic interface, although to date it has not been isolated from a particular source organism (Peters et al., 2005 and references therein). Its sedimentary abundance is also particularly sensitive to changes in bacterial populations and furthermore influenced by parameters such as sediment composition and maturity. Here it showed elevated abundances relative to the αβ-hopane in sediment depths > 40 m consistent with anoxic conditions in the depositional environment. However, it also showed an increase in relative abundance in sediments around a depth of 33 m, which exhibited Pr/Ph ratios > 1.

Chlorobi carotenoid derivatives

The intact carotenoid pigments isorenieratane (**I**), palaeorenieratane (**II**) and renieratane (**XVIII**), which represent unequivocal evidence for the presence of *Chlorobi* and hence PZE (e.g. Summons and Powell, 1986; Schwark and Frimmel, 2004; Grice et al., 2005a), were only abundant in sediments of a depth > 40 m. In contrast, monoaryl isoprenoids (**III**), which do have other sources (e.g. Grice et al., 1996b; Koopmans et al., 1996a), were also present at low concentrations in aromatic fractions from the remaining part of the core. The average $\delta^{13}C$ values of aryl isoprenoids (**III**) in samples > 40 m are displayed in **Figure 5.4** along with a representative SIM trace (*m/z* 133). These data indicate mixed sources of all monoaryl isoprenoids (**III**) and some diaryl derivatives since they were significantly more ^{13}C depleted compared to the exclusively *Chlorobi*-derived palaeorenieratane (**II**), which exhibited heavier $\delta^{13}C$ signatures of around -15 ‰, typical of lipids synthesised *via* the tricarboxylic acid (TCA) cycle used by *Chlorobi* (Quandt et al., 1977; Sirevåg et al., 1977).

δ^{34}S of pyrite

δ^{34}S signatures of total reduced inorganic sulfur (~pyrite) throughout the core ranged from -24.4 to -11.9 ‰ (**Figure 5.3**) which is comparable to data from other Late Devonian sediments obtained by Joachimski et al. (2001). The strong ^{34}S depletion compared to the estimated value for Devonian sea-water (+15 and +20 ‰; Kampschulte and Strauss, 2004; Wortmann and Paytan, 2012) indicate that microbial sulfate reduction took place in surface sediments or even the water-column where it was not limited by sulfate abundance (e.g. Canfield and Teske, 1996; Meyer and Kump, 2008). The values were similar to those in the modern euxinic Baltic Sea (Böttcher and Lepland, 2000; Böttcher et al., 2004), sediments of the hypersaline Basins of the eastern Mediterranean (Ziebis et al., 2000) but less enriched in the light isotope when compared to the Holocene Black Sea (Fry et al., 1991; Böttcher et al., 2004; Jørgensen et al., 2004).

Gammacerane index

Gammacerane indices in the samples deposited later in the Frasnian (< 33.5 m) were low (**Figure 5.3**) suggesting a vertically mixed water-column contrary to the conditions in the Late Givetian-Early Frasnian which exhibited significantly higher gammacerane indices. Gammacerane (**IX**) is presumably sourced from tetrahymanol in bacterivorous cilitates living exclusively at the chemocline and is therefore an indicator for water-column stratification (Harvey and McManus, 1991; Sinninghe Damsté et al., 1995; Grice et al., 1998d; **Chapter 4** this thesis). A vertically mixed water column in palaeoenvironments corresponding to samples from the upper part of the core (in particular the uppermost two samples) is also consistent with lower chroman ratios in these samples reflecting higher salinities in the epilimnion (**Chapter 4**).

δD of kerogen

The δD values of kerogen displayed in **Figure 5.3** represent the isotopic composition of non-exchangeable hydrogen, which was determined according to a method developed by Schimmelmann et al. (1999; 2006) to reduce distorting effects on δD signatures by hydrogen exchange between OM and water during diagenesis.

However, hydrogen that is typically considered as non-exchangeable can also be replaced during later stages of thermal maturation (e.g. Schimmelmann et al., 2004; Dawson et al., 2005, 2007; Pedentchouk et al., 2006; Maslen et al., 2013). Nevertheless, immature kerogens have shown a good preservation of original D/H content and have been frequently used to reconstruct palaeoenvironmental changes (e.g. Hassan and Spalding, 2001; Lis et al., 2006; Nabbefeld et al., 2010c). Since the samples analysed here exhibit a very low thermal maturity ("Thermal maturity and organic matter preservation" page 158), δD values of their kerogens are likely representative of the palaeoenvironment. Generally, δD signatures of biomass are strongly influenced by the D/H composition of the source water used for biosynthesis (Sessions et al., 1999; Dawson et al., 2004; Grice et al., 2008b; Nabbefeld et al., 2010c; Zhou et al., 2011). Therefore the comparatively D-depleted value for the sample in the lowermost part of the core (> 40 m) potentially reflects the freshwater incursions (evident in elevated chroman ratios; **Chapter 4**) which typically lead to a D-depletion in the marine environment. Due to fractionation effects in the hydrological cycle, meteoric waters are more depleted compared to seawater, which exhibits a δD value of ~0 (Gat, 1996). Variations in the δD profile could represent changes in the type of source organisms or growth forms (Nabbefeld et al., 2010c; Polissar and Freeman, 2010), and the more highly depleted values of the deeper sediments may have been influenced by increased input of D-depleted terrestrial biomass (organisms utilizing meteoric waters).

δ^{18}O of carbonates

δ^{18}O values of carbonates in the lowermost part of the core corresponding to the anoxic/euxinic palaeoenvironment with freshwater incursions showed significant variations, but were on average more ^{18}O-depleted than samples in the upper section of the core. δ^{18}O and δ^{13}C values of carbonates showed only a slight correlation ($R^2 = 0.3$, **Figure A5.6**) indicating largely different influences on both parameters and most likely no significant diagenetic control. δ^{18}O signatures of carbonates are typically representative of the stable isotopic composition of the corresponding water (e.g. Sachse et al., 2004b). Therefore the greater ^{18}O-depletion of some samples in the lowermost part of the core might be indicative of freshwater incursions since meteoric waters are depleted in heavy isotopes.

Table 5.1: Selected elemental and molecular maturity parameters throughout the analysed core indicating an exceptionally low thermal maturity. T_{max} was determined by Rock Eval pryolysis. Ts = 18α 22,29,30-trisnorhopane; Tm = 17α 22,29,30-trisnorhopane. $C_{31}H$ = 17α,21β-homohopane; C_{29} ααα St = 5α,14α,17α 24-ethylcholestane; S and R represent the stereochemistry at C22 and C20 for hopanes and steranes, respectively. CPI stands for carbon preference index and was calculated according to $\sum C_{odd}/\sum C_{even}$ over the range of C_{16} to C_{22} and C_{23}-C_{33}, respectively.

Depth [m]	T_{max} [°C]	Ts/(Ts/Tm)	C_{31}H S/(S+R)	C_{29} ααα St S/(S+R)	CPI (C_{15}-C_{22})	CPI (C_{23}-C_{33})
27.3	*413	0.14	0.18	0.14	1.07	1.53
28.1	*410	0.15	0.16	0.15	1.18	1.59
29.0	*418	0.13	0.15	0.12	1.16	1.49
29.7	*409	n.d.	n.d.	n.d.	n.d.	n.d.
29.9	*407	0.14	0.15	0.04	1.18	1.58
30.7	*405	0.14	0.14	0.12	1.12	1.53
30.9	*421	0.14	0.15	0.11	1.20	1.82
31.9	*405	0.16	0.14	0.11	1.11	1.16
32.8	*406	0.18	0.16	0.10	1.02	1.46
33.0	*410	0.18	0.16	0.11	1.10	1.47
33.2	*415	0.19	0.16	0.10	0.93	1.18
33.4	*410	0.18	0.20	0.08	0.97	1.00
34.6	*417	n.d.	n.d.	n.d.	n.d.	n.d.
37.8	n.d.	0.47	0.36	0.19	1.04	1.03
40.2	n.d.	0.22	*0.23	0.08	1.43	1.30
40.3	*415	0.16	*0.20	0.09	1.27	1.20
40.5	*413	n.d.	n.d.	n.d.	1.00	0.89
40.7	*414	0.13	*0.13	0.09	1.04	1.13
41.2	*410	0.10	*0.12	0.09	1.07	1.24
41.9	*413	0.11	*0.11	0.09	1.00	1.16

* also included in **Chapter 4**

Thermal maturity and organic matter preservation

The analysed sediments showed an exceptionally low thermal maturity despite their Palaeozoic depositional age, evident in elemental as well as molecular maturity parameters (**Table 5.1**). T_{max} values from Rock Eval pyrolysis were all < 421 °C indicative of very low thermal alteration (**Table 5.1**; sediments with T_{max} < 435 °C are typically considered immature; e.g. Peters et al., 2005). Furthermore, the aliphatic fractions contained complex hopanoid and steroid distributions typical of immature sediments with high concentrations of biological isomers such as ββ-hopanes and ααα-20R-steranes compared to the more thermally stable geological configuration (**Figure 5.2** and **Figures A5.2** to **A5.5**). This was evident in very low values of frequently used molecular maturity indicators such as the C22 homohopane ratio (22S/ (22S+22R)), ααα 24-ethylcholestane isomerisation at C20 (20S/ (20S+20R)) or Ts *vs.* Tm (Ts/ (Ts+Tm)) displayed in **Table 5.1**. All of these values (with the exception of the organic lean sample at 37.8 m) correspond to vitrite reflectance equivalents < 0.5 % and mostly < 0.4 % (Hulen and Collister, 1999 and references therein) corresponding to burial heating < 80 and < 60 °C, respectively (Barker and Pawlewicz, 1994). The high relative abundances of hopenes and sterenes were also highly unusual for Palaeozoic sediments as these compounds are usually converted to their saturated counterparts during relatively early stages of diagenesis (e.g. Mackenzie et al., 1982; Peters et al., 2005). Regular steranes were generally much more abundant than their corresponding diasteranes (**Figure A5.5**), which is typical of carbonate-rich and clay-poor sediments (e.g. Nabbefeld et al., 2010b). However, diasterenes, which represent diagenetic precursors of diasteranes (Mackenzie et al., 1982) were highly abundant (**Figure 5.2**). A low thermal maturity was also evident in pale to mid to yellow spore colours (thermal alteration index; Staplin, 1969), observed from palynological analysis (unpublished results).

Furthermore, the strong correlation of total organic nitrogen content (TN_{org} %) to TOC % ($R^2 = 0.97$) and relatively low values of the C/N ratio (**Figures 5.5a** and **5.6**) suggest these data are representative of the source OM, which to the best of our knowledge would be the oldest reported preservation of C/N source ratios. The C/N ratio can generally provide a reliable distinction of marine *vs*. terrestrial OM since phytoplanktonic organisms typically have a higher nitrogen-content (C/N: ~4-10) than terrestrial vegetation (C/N > 20; Meyers, 1994 and references therein). However, the ratio can be influenced by the enhanced loss of organic nitrogen over organic carbon during diagenesis, leading to artificially high values of the C/N ratio in affected sediments (e.g. Sampei and Matsumoto, 2001). Furthermore, C/N ratios can be influenced by the contribution of inorganic nitrogen (typically ammonium), particularly in OM-lean sediments with TOC contents < 1 % (e.g. Müller, 1977; Sampei and Matsumoto, 2001). Therefore TN % was corrected to obtain $TN_{organic}$ % by assuming a constant contribution of 0.02% $TN_{inorganic}$, which was determined from the axis intercept of the TN % *vs.* TOC % plot. C/N ratios illustrated in **Figure 5.6** were calculated using $TN_{organic}$ %. The unusual preservation of C/N ratios in the Palaeozoic sediments furthermore points to the unique OM preservation in some sections of the Gogo Formation and a very immature character of the OM.

A low thermal maturity based on molecular ratios has also been reported for a calcareous nodule hosting a well preserved crustacean from a nearby section of the Gogo Formation (Melendez et al., 2013b). However, values for the 24-

ethylcholestane isomerisation at C20 and for Ts/ (Ts+Tm) in the nodule were slightly higher than in most parts of the presently analysed core, and unsaturated steroids or hopanoids were absent. In contrast, other sections of the Gogo Formation, which represent the source rocks of the high quality oils in the Canning Basin, showed significantly higher thermal maturities (Cadman et al., 1993; Barber et al., 2001; Greenwood and Summons, 2003; Maslen et al., 2009, 2011). Nevertheless, the now exposed or near-surface rocks in most parts of the reef systems in the northern Canning Basin (Lennard Shelf) have likely never been exposed to temperatures exceeding 60 to 70 °C during their geothermal history (Playford et al., 2009 and references therein).

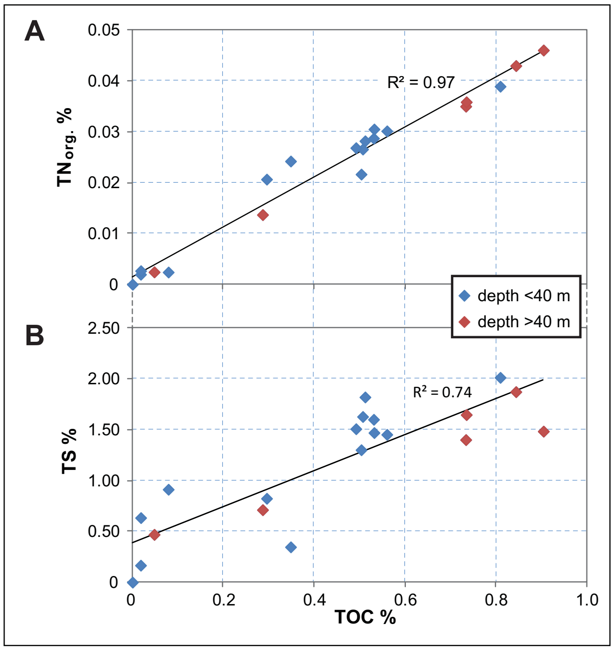

Figure 5.5: Plots showing positive correlations of total organic carbon (TOC %) to (A) total organic nitrogen (TN_{org} %, corrected from TN % assuming a constant contribution of $TN_{inorganic}$ of 0.02 %) and (B) total sulphur (TS %)

The thermal alteration of biomarkers in the studied core seems to be largely independent of the redox conditions at the time of deposition, since all previously discussed parameters indicate similarly low thermal maturities in sediments > 40 m presumably deposited under anoxic/euxinic conditions (see previous section) as well as in overlying sediments deposited under more oxic conditions. However, the measured TOC % was slightly higher in sediments > 40 m (**Figure 5.6**) which would be consistent with enhanced OM preservation under anoxic/euxinic conditions (Claypool and Kaplan, 1974) The estimated original TOC (TOC_{or}, **Figure 5.6**) was calculated according to the following equation developed by Vető et al. (1994).

$$TOC_{or} = TOC + S \times 0.75 \times 1.33$$

This proxy is based on the assumptions that (i) all reduced sulfur was formed syngenetically and (ii) the degradation of OM was essentially due to microbial sulfate reduction (MSR) with the benthic system being more or less closed for dissolved sulfide. The positive correlation between the measured TOC % and TS % (**Figure 5.5b**) may indicate a control of MSR by OM availability. When compared to the relationship established for modern marine sediments (Raiswell and Berner, 1987), all investigated samples contain an excess in reduced sulfur, that may indicate euxinic conditions. Since the degree of OM alteration is small, there is no indication given for a thermal change in the C/S ratios (Raiswell and Berner, 1987).

The slightly higher values for TOC % and TOC_{or} % in the lowermost part of the core may reflect increased phytoplanktonic productivity compared to the other samples due to enhanced terrigenous nutrient input ("Thermal maturity and organic matter preservation" page 158). However, measured as well as calculated TOC % in these samples were still realtively low which can possibly be explained by oligotrophic conditions (despite some terrigenous input) or dilution from carbonate precipitation (**Chapter 4**). The latter is also presumably the main reason for the low TOC% in samples between depths of 33.5-40 m, which had high carbonate contents between 62.6 and 86.2 % (**Figure 5.6**).

Organic matter sources

Whereas relatively low C/N and C/S signatures (Raiswell and Berner, 1987; Meyers, 1994) were indicative of predominantly marine sourced OM, albeit with some terrestrial contribution, high Oxygen Indices (OI) and low Hydrogen Indices (HI) were indicative of Type III kerogens (**Figure 5.6**), which are typically terrestrial derived (also see van Krevelen diagram in **Figure A5.5**). In some cases Type III kerogens may also be derived from oxic or suboxic open marine settings (Peters et

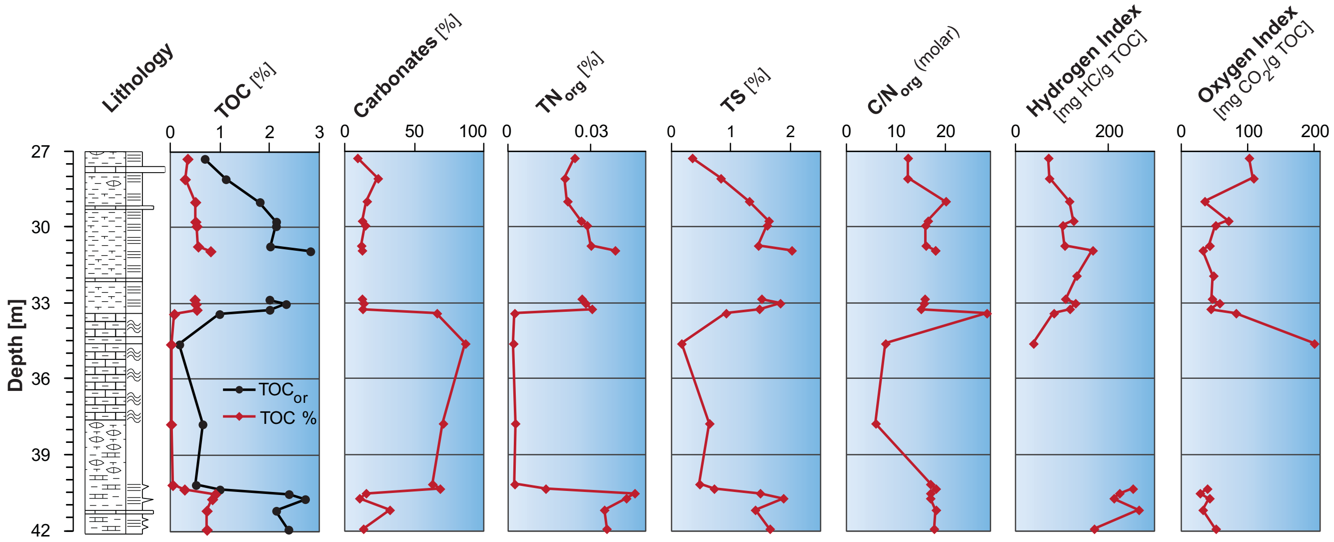

Figure 5.6: Depth profiles of selected parameters determined by elemental analysis and Rock Eval pyrolysis throughout the analysed core from McWhae Ridge. TOC represents the measured total organic carbon content. TOC_{or} refers to the estimated original TOC in sediments at the time of deposition which was calculated after Vető et al. (1994). TN_{org} = total organic nitrogen content calculated from TN assuming a constant contribution of $TN_{inorganic}$ of 0.02 %. TS = total sulphur content.

al., 2005) which would be consistent with the samples deposited later in the Frasnian (depth > 40 m). However, biomarker parameters in the lowermost part of the core (> 40 m) strongly indicate an anoxic and euxinic marine setting, possibly with some terrigenous input. An increase of HIs and a simultaneous decrease of OIs in this core interval reflects more reducing conditions, although the values were still not in the range expected for an anoxic marine setting (**Figure A5.6**). Although Rock Eval pyrolysis is a very useful screening method, it does have some limitations and can give misleading information about kerogen types due to effects from the mineral matrix, which are particularly relevant in immature organic lean samples such as in the presently analysed core and typically lead to artificially low values for HIs (e.g. Katz, 1983; Cowie et al., 1999). Although the samples were decarbonated prior to Rock Eval pyrolysis, matrix effects may have still influenced the results. However, these data could also indicate that periods of persistent water-column stratification accompanied by anoxia and PZE were only episodic.

Significance of $\delta^{13}C$ variations

$\delta^{13}C$ profiles of carbonates, bulk OM and biomarkers generally showed similar trends most likely reflecting variations in the stable isotopic composition of the dissolved inorganic carbon (DIC) pool (**Figure 5.7**). All profiles showed a ^{13}C-depletion (~2-3 ‰ in hydrocarbons and carbonates and slightly less pronounced in OM) in the lowermost part of the core deposited under a stratified water-column (depth > 40 m) compared to the samples deposited in a more oxic palaeoenvironment between the depths of 28.8 and 33.5 m. Negative $\delta^{13}C$ signatures of carbonates reaching values of -3 ‰ in the lowermost part of the core can likely be explained with a model introduced by Küspert (1982). Stratification-induced enhanced degradation of ^{13}C depleted OM (mainly by SRB) led to the accumulation of ^{13}C-depleted DIC in the lower part of the water-column. Some of it escaped to the photic zone and was recycled by phytoplankton leading to a further ^{13}C-depletion of biomass. The fluctuation of $\delta^{13}C_{carbonate}$ in that interval might be explained by alternation of periods with enhanced recycling of OM, leading to a ^{13}C-depletion of DIC and enhanced burial of ^{13}C-depleted OM, leading to a ^{13}C-enrichment.

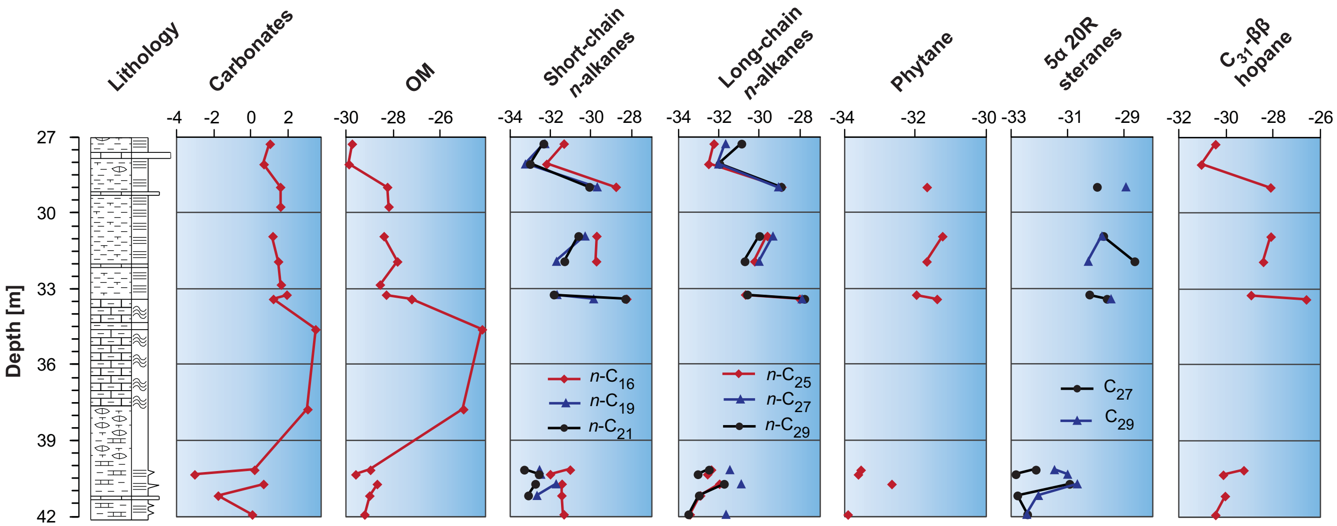

Figure 5.7: δ^{13}C depth profiles (reported in ‰ relative to VPDB) throughout the analysed core of carbonates, bulk organic matter (OM), representative long- and short-chain *n*-alkanes, phytane, selected regular 5α,14α,17α-20R-steranes (5α 20R steranes) and the 17β,21β-22R-homohopane (C_{31} ββ hopane).

However, these shifts in $\delta^{13}C$ values were not evident in the profiles of hydrocarbons or OM.

Carbonates as well as OM exhibited noticeably heavier $\delta^{13}C$ signatures in the OM-lean limestone section of the core between depths of 33.5 and 40 m (**Figure 5.7**). Unfortunately, compound specific $\delta^{13}C$ could not be measured in these sediments due to the low OM content. This shift is likely due to a facies change from Gogo Formation (basin facies) in the lowermost part of the core to the Sadler formation (marginal slope facies). The difference in $\delta^{13}C$ in the OM-lean section may also be the result of diagenetic effects. However, the simultaneous shift in OM and carbonates is evidence against this.

Furthermore, $\delta^{13}C$ profiles of OM, *n*-alkanes and the ββ-homohopane showed a distinctive negative shift in the two uppermost samples (~2 ‰ in OM and ~3‰ in hydrocarbons) deposited under presumably the most oxic conditions (very high Pr/Ph of ~3). Since this excursion was not evident in the $\delta^{13}C$ profile of carbonates it likely represents a change in source organisms, or possibly nutrient-poor conditions that contributed to slower growth of phytoplankton, which typically leads to enhanced fractionation against ^{13}C during biosynthesis due to the higher accessibility of dissolved CO_2, the carbon source for most of these organisms (Freeman and Hayes, 1992). The latter would also be consistent with the lower TOC_{or} % and decreasing biomarker concentrations in these samples.

Changes in populations of primary producers

Variations in steroid and hopanoid distributions

Figure 5.8 shows depth profiles of selected molecular parameters which indicate significant differences between phytoplankton and algal communities in the Late Givetian-Early Frasnian and later in the Frasnian. Elevated sterane/hopane ratios reaching values > 6 in the lowermost part of the core (> 40 m) are suggestive of a strong predominance of eukaryotic algae over bacteria, whilst values < 1 in the OM-lean part of the core (37.8 m) as well as between depths of 27.3-30.7 m indicate relatively higher bacterial input (e.g. Ourisson et al., 1979; Mackenzie et al., 1982; Brocks et al., 1999). Furthermore, changes in algal populations are evident in variations of sterane distributions (see ternary diagram in **Figure 5.9**). Sediments > 40 m showed a significantly enhanced relative abundance of C_{27}-desmethylsteranes, and also slightly more abundant C_{28}-desmethylsteranes. Although C_{29}-desmethylsteroids are often attributed to a terrestrial origin (e.g. Huang and Meinschein, 1979), here they are probably mainly sourced from green algae (Volkman, 1986, 1998; Kodner et al., 2008; Grosjean et al., 2009) which were prominent in the Devonian palaeoenvironment. Nevertheless, higher plant input may also have contributed to the C_{29}-steranes.

C_{27} desmethyl steroids are commonly attributed to zooplankton or red algae, but are also produced by some green algae (Volkman, 1986; Kodner et al., 2008). A higher abundance of red algae, which are predominantly macrophytes, would be consistent with shallower water depth at the comparatively lower sea-level in the Late Givetian-Early Frasnian. Furthermore, Kelly (2009) suggested a relation of the C_{27}/C_{29} sterane ratio in Neoproterozoic-Cambrian sediments to redox conditions in the palaeoenvironment, with higher values under more euxinic conditions due to the higher tolerance of red algae to iron limitation. This would also be in accordance with the presently observed increase in sediments at depths > 40 m.

C_{28}-desmethylsteroids have been frequently attributed to modern green algae and became significantly more abundant in the geological record after the end of the Devonian (Grantham and Wakefield, 1988). Nevertheless, short term increases of C_{28}-steranes relative to C_{29}-steranes have been reported in Late Devonian sediments and were attributed to temporary changes in algal populations with more abundant modern green algae and less contributions from red algae (Schwark and Empt, 2006). Ergosterol (C_{28}) is a common fungal biomarker (Volkman, 2003) and the co-variation of C_{28}-desmethylsterane abundances and perylene (**XVII)** concentrations may be indicative of a common fungal source ("Evidence of terrigenous input and combustion sources" page 170).

Changes in the algal communities in the palaeoenvironments of sediments > 40 m were also evident in enhanced relative abundances of 3- and 4-methyl-24 ethylcholestanes (**Figure 5.8a**). The main source of 4-methylsteranes in geological samples are thought to be dinoflagellates or their ancestors if found in Palaeozoic sediments (Wolff et al., 1986; Peters et al., 2005). However, 4-methylsterols have

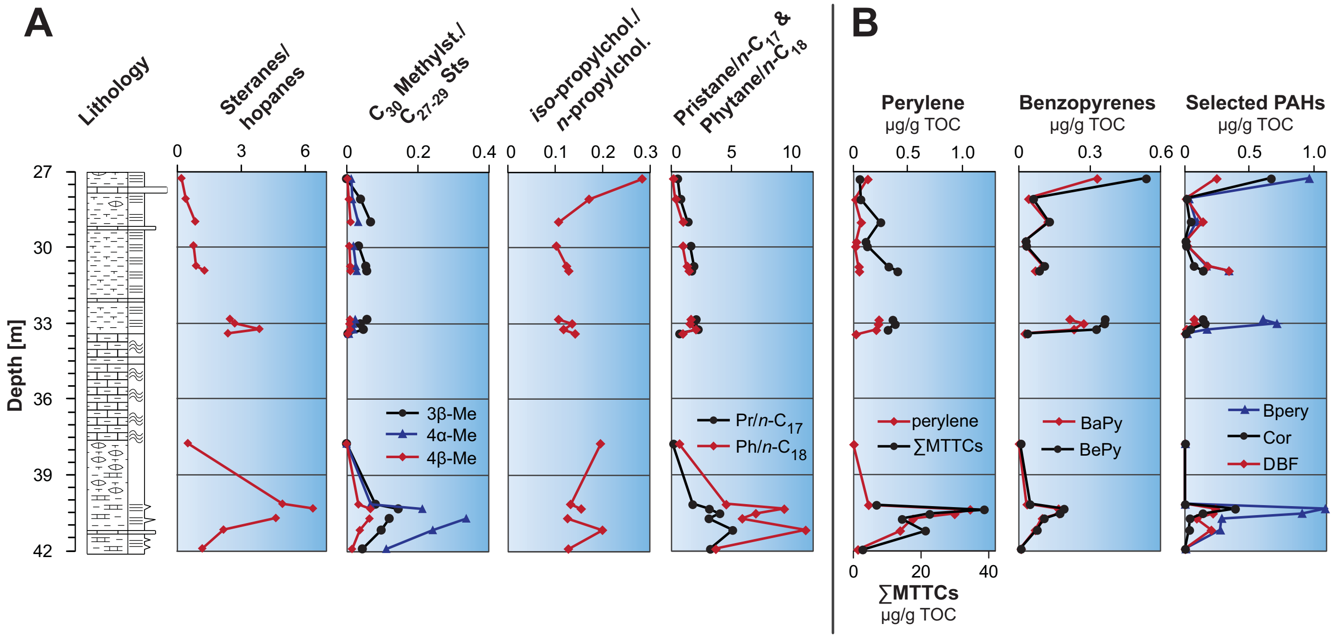

Figure 5.8: Depth profiles of selected molecular parameters indicative of (A) phytoplankton communities and (B) terrigenous OM-input and combustion sources. C_{30} Methylst./C_{27-29} Sts = abundance of selected methylated 24-ethylcholestanes relative to the sum of regular C_{27} – C_{29} 5α14α17α-20R-steranes with 3β-Me, 4α-Me and 4β-Me standing for 3β-methyl, 4α-methyl and 4β-methyl -5α14α17α 20R 24-ethylcholestane, respectively. Propylchol. = propylcholestane; Pr = pristane; Ph = phytane; *n*-C_i = *n*-alkane with chain-length "i"; MTTCs = methyltrimethyltridecylchromans; BaPyr = Benzo[*a*]pyrene; BePyr = Benzo[*e*]pyrene; Bpery = Benzo[*ghi*]perylene; Cor = coronene; DBF = dibenzofuran

also been found in some marine/brackish prymnesiophyte algae (Volkman et al., 1990) and methanotrophs may also represent a potential source (Grice et al., 1998d; Kodner et al., 2008). The $\delta^{13}C$ values of 3- and 4-methyl-24 ethylcholestanes, which could be measured in some samples > 40 m, ranged from -29.1 to -32.5 ‰, consistent with a phytoplanktonic source. An origin from methanotrophs, however, seems unlikely as biolipids of these organisms are typically more ^{13}C-depleted (Kodner et al., 2008). 4-Methylsteranes are common in the Mesozoic sedimentary record and some older sediments, but are largely absent in the Carboniferous and Permian (Peters et al., 2005). Ancestors of dinoflagellates may also be the source of arcritarchs found in the palynological record of the presently analysed core (unpublished results). An association of these microfossils to dinoflagellate cysts has been suggested by several authors (Tappan, 1980; Moldowan and Talyzina, 1998; Schwark and Empt, 2006; Armstrong and Brasier, 2009). Jiamo et al. (1990) investigated various Cretaceous to Tertiary sediments and found generally higher concentrations of 4-methyl-24-ethylcholestanes in freshwater settings as opposed to brackish and hypersaline palaeoenvironments. Their enhanced abundance in sediments > 40 m may reflect differences in algal populations or algal blooms due to the freshwater incursion and terrigenous nutrient input in the Late Givetian-Early Frasnian palaeoenvironment. The covariance of 3β-methyl-24-ethylcholestane and 4-methyl-24-ethylcholestanes concentrations (**Figure 5.8a**) and similar $\delta^{13}C$ signatures are strongly suggestive of a common source for both compound classes. High abundances of 3*β*-alkyl steranes in comparable facies of European Late Devonian sediments were also reported by Marynowski and Filipiak (2007).

The specific sponge biomarker 24-*iso*propylcholestane (McCaffrey et al., 1994; Love et al., 2009) was present at low abundances throughout the core and most likely originated from stromatoporoids, which are classified as sponges and were together with rugose corals and calcareous microbes the main reef-builders throughout the Devonian until the F-F boundary (e.g. Playford et al., 2009). Although stromatoporoids were amongst the most severely affected organisms in the Late Devonian extinctions (e.g. Fagerstrom, 1994; Playford et al., 2009), the relative abundance of 24-*iso*propylcholestane *cf.* 24-*n*-proylcholestane did not show significant variations throughout the analysed core (**Figure 5.8a**).

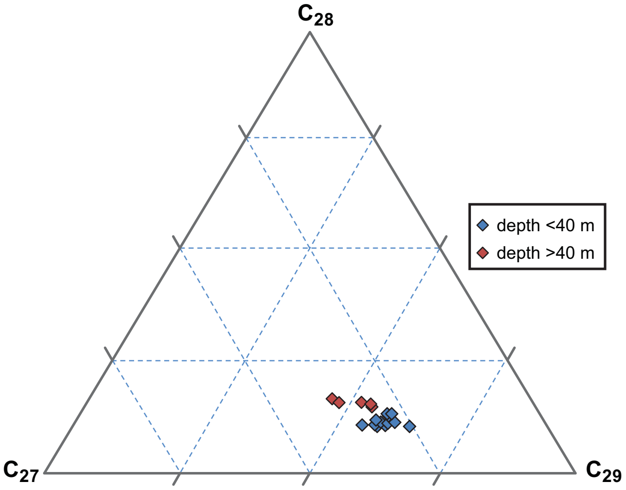

Figure 5.9: Ternary diagram of regular C_{27} – C_{29} steranes in the analysed core from McWhae Ridge. Red diamonds represent samples from a depth > 40 m deposited under anoxic and euxinic conditions and a stagnant, stratified water-column. Blue diamonds represent samples > 40 m presumably deposited under more oxic conditions.

Variations in pristane, phytane and n-alkanes

n-Alkane distributions in sediments corresponding to palaeoenvironments with complex marine and terrigenous OM input, such as the presently analysed core, typically have mixed sources (e.g. bacteria, archaea, algae, terrestrial/aquatic plants; e.g Collister et al., 1994a; Lichtfouse et al., 1994; **Chapter 2** this thesis). $\delta^{13}C$ values of the predominantly phytoplankton-derived phytane were generally in the same range or slightly more depleted than short chain *n*-alkanes (**Figure 5.7**). This indicates a mixed *n*-alkane source from phytoplankton as well as possibly heterotrophic bacteria (**Chapter 4**). Short and long-chain *n*-alkanes in all investigated samples generally showed similar $\delta^{13}C$ signatures (**Figure 5.7**) which may be indicative of a common source. A slight odd-over-even predominance in the *n*-alkanes, particularly > C_{22} but also in shorter chain lengths (**Table 5.1**), would be consistent with high algal input (Gelpi et al., 1970; Volkman et al., 1998; Allard and Templier, 2000) and possibly additional contributions, particularly to longer chain-lengths, from aquatic and terrestrial plants (Eglinton and Hamilton, 1967; Ficken et al., 2000). The *n*-alkanes exhibited similar $\delta^{13}C$ signatures to predominantly algal-derived steranes, whereas the bacterial-derived ββ-20R-homohopane was slightly more ^{13}C enriched (**Figure 5.7**). This furthermore indicates a high algal contribution to *n*-alkanes.

Apart from lower abundances of short-chain *n*-alkanes in the OM-lean part of the core (depth 33.5-40 m), the *n*-alkane distributions showed generally consistent features in parameters such as average chain-length (21.7-23.9) or relative abundances of short-chain *vs.* long-chain *n*-alkanes. The CPI was slightly lower in sediments > 40 m (**Table 5.1**), despite a presumably higher terrigenous input ("Evidence of terrigenous input and combustion sources" page 170), possibly due to an increased contribution from SRB which are known to produce long-chain *n*-alkanes without an odd-over-even predominance (Davis, 1968; Melendez et al., 2013b). The differences in some of these parameters in the OM-lean horizon are presumably the result of diagenesis or biodegradation after the uplift that followed burial, which both would likely have enhanced effects in OM-lean intervals.

The ratios of pristane/C_{17} *n*-alkane and phytane/C_{18} *n*-alkane were significantly higher in the lowermost part of the core (> 40 m, **Figure 5.8**), which may be indicative of changes in phytoplankton/algal populations. Similar variations of these ratios, with higher values in OM-richer sediments with evidence of PZE compared to more OM-lean sections have been reported in European Late Devonian (Famennian) sediments (Marynowski and Filipiak, 2007) and attributed to variations in source organisms.

Evidence of terrigenous input and combustion sources

Figure 5.8b shows concentrations of selected unsubstituted PAHs as well as the total concentration of all MTTCs (**X**) throughout the analysed core. The most common source of many unsubstituted PAHs in sediments is combustion of plant material or fossil fuels (Killops and Massoud, 1992; Jiang et al., 1998; Grice et al., 2005b, 2007; Nabbefeld et al., 2010d). Their abundance has in some cases also been associated with higher thermal maturities and volcanism (e.g. Murchison and Raymond, 1989). On the contrary, perylene (**XVII)** has a diagenetic origin and is most likely derived from quinone pigments in wood degrading fungi and is therefore presumably linked to terrigenous input (Jiang et al., 2000; Grice et al., 2009; Suzuki et al., 2010). Its covariance with chroman abundances (**Figure 5.8b**) may be indicative of a common terrestrial source or influence, with highest abundances in sediments > 40 m pointing towards enhanced terrigenous OM input *via* riverine inflow (**Chapter 4**). This would also be consistent with slightly increased C/N_{org} ratios in the same core-section (**Figure 5.6**). The depth profiles of perylene (**XVII)** and chroman concentrations were distinct to those of the other PAHs, reflecting the different origins.

Pyrogenic PAHs often reach sediments *via* airborne particles and their presence in the analysed sediments may be indicative of wildfires. Benzo[*a*]pyrene (**XI**) and coronene are most likely exclusively formed by pyrogenic processes, whereas other PAHs such as benzo[*e*]pyrene, chrysene or triphenylene may have additional algal sources (Grice et al., 2007; Nabbefeld et al., 2010d). The co-variation of all of these compounds in the present core, with highest concentrations in the uppermost sample as well as at depths around 33 m, strongly indicates a predominant origin from combustion sources (e.g. **Figure 5.8b**). Although benzo[*ghi*]perylene is typically assigned to a pryogenic origin (Blumer and Youngblood, 1975; Killops and Massoud, 1992), it has in some cases shown similar sedimentary distributions similar to perylene (**XVII;** Jiang et al., 1998), implying additional sources of this PAH. Here its depth profile was largely similar to the previously described combustion markers in the upper part of the core, but it also showed an increase in concentration similar to perylene (**XVII)** and MTTCs (**X**) below a depth of 40 m. Dibenzofuran (**XIX**) has been frequently used as indicator of higher plant input (Fenton et al., 2007; Nabbefeld et al., 2010d). However, its depth profile in the core analysed here largely resembled those of the combustion markers (**Figure 5.8b**).

Conclusions and outlook

This organic geochemical based study demonstrates the highly unusual preservation of OM in the Gogo Formation of the Canning Basin, including the oldest known preservation of biological C/N values. This preservation is supported by exceptionally low thermal maturity, consistently evident in elemental and molecular maturity parameters, and largely unaltered biomarker distributions which included unsaturated hopenes and sterenes which are typically highly susceptible to diagenesis and thermal maturity.

Furthermore, molecular and elemental data from the Late Givetian-Frasnian core facilitated a detailed reconstruction of the paleoenvironment over this time period. Evidence for a distinct time interval of elevated biotic stress was found, in particular for reef-building organisms and associated fauna, close to or at the G-F boundary, which may represent a local or global event related to the Late Devonian extinctions. The associated palaeoenvironment was characterised by persistent water-column stratification, freshwater incursions, widespread anoxia and enhanced sulfate reduction resulting in the development of persistent anoxia and PZE in the hypolimnion. Elevated concentration of perylene (**XVII**) and MTTCs (**X**) in sediments from this time interval, likely point towards enhanced terrigenous nutrient input leading to phytoplankton blooms. The latter commonly represent an important factor in the development of anoxia and PZE, particularly in such a stratified palaeoenvironment. In contrast, sediments deposited later in the Frasnian were presumably layed down under more oxic conditions and a largely well-mixed water-column without particular indications of biotic stress. The abundance of combustion derived PAHs, particularly in the younger sediments, may be indicative of wildfires.

The present study adds to the knowledge about conditions in palaeoenvironments associated with the Late Devonian extinctions and might be useful to reconstruct global events if correlated with other datasets. Furthermore, the correlation of anoxia and PZE with markers of terrigenous input may point towards a link between the Late Devonian extinctions and the rise of higher plants. Data from this work combined with some previous studies indicate that PZE likely was a significant factor in Middle to Late Devonian extinction events.

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Appendix 5

Supplementary online material

Experimental

If not indicated otherwise, all analyses were performed in the laboratory of WA-OIGC at Curtin University. All used glassware was pyrolysed at 550 °C for 1 hour and procedural blanks were run regularly.

Sample collection, preparation and extraction

Sample collection and preparation have been described in detail in the supplementary information in **Appendix 4**. To summarize, the core was collected without any drilling fluids or lubricants using a small portable drilling rig. Samples were cut with a rocksaw (diamond crystal edge with carbon steel centre) and pre-extracted in methanol and dichloromethane (DCM) in an ultrasonic bath to remove any potential surface contamination. Samples were ground in a stainless-steel rock-mill and Soxhlet extracted with 90% DCM in methanol for 48 hours. Activated copper turnings were used for the removal of elemental sulfur. The extracts were further separated by silica gel-column chromatography using solvents with an increasing polarity. Aliphatic and aromatic fractions were eluted in hexane and 20% DCM in hexane, respectively. Samples for palynological analyses were not identical to the larger samples for biomarker, elemental and isotope analysis and represented small chips taken throughout the core.

Gas-chromatography (GC-MS)

GC-MS analyses were performed on an Agilent 5973 Mass-Selective Detector (MSD) interfaced to an Agilent 6890 gas chromatograph (GC) fitted with an autosampler, utilizing a DB-5MS capillary column (60 m × 0.25 mm I.D.,0.25 μm film thickness; J & W scientific). The GC-oven was typically heated up from 40 °C to 310 °C (aliphatic fractions) or 325 °C (aromatic fractions) at 3 °C/min with initial and final hold times of 1 and 30 minutes, respectively. Samples were injected into a split/splitless injector at 320 °C in a pulsed-splitless mode. The carrier gas (helium) flow rate was kept constant at 1.1 mL/min. Full scan (50 - 550 Daltons) at 70 *eV* mass spectra and selected ion monitoring (SIM) spectra (used for quantification of aromatic compounds) were typically acquired with an electron multiplier voltage of 1800 V and a source temperature of 230 °C. ChemStation Data Analysis software was used for data acquisition and processing. Perylene, palaeorenieratane (**II**) and isorenieratane (**I**) were identified by comparison of retention times with authentic standards. For semi-quantitative analyses the aromatic fractions were spiked with a known amount of perdeuterated terphenyl (d^{14}).

Multiple reaction monitoring (MRM) GC-MS

MRM-GC-MS analyses were performed at Department of Earth, Atmospheric and Planetary Sciences, MIT, USA on a Micromass Autospec Ultima mass spectrometer interfaced to an Agilent 6890 N gas chromatograph fitted with an autosampler and a DB-5MS capillary column (60 m×0.25 mm I.D.; 0.25 μm film thickness; J&W Scientific). The carrier gas (helium) flow rate was kept constant at 2 ml/min. The GC-oven was programmed from 60°C to 150°C at 10°C/min and to 315°C at 3°C/min with initial and final hold times of 2 and 24 min, respectively. The source was operated in electron impact (EI, 70 eV) mode at 250° C, with 8 kV accelerating voltage. Data was acquired and processed using MassLynx 4.0 (Micromass Ltd.) software. Identification of compounds was achieved by comparison with a synthetic mixture of oils (AGSO standard) that contains most common hopanes and steranes. Semi quantitative analyses were achieved by spiking the samples with a known amount of perdeuterated (d^4) 24-ethylcholestane. The transitions of precursor-product reactions used to monitor the compounds relevant for this study are included in **Figures A5.2**, **A5.3** and **A5.5**.

Compound specific stable isotope analysis

For GC-irMS analyses a Micromass IsoPrime mass-spectrometer interfaced to an Agilent 6890N GC fitted with an autosampler was utilized with GC- column, carrier gas, injector conditions and oven temperature programs identical to the settings for GC-MS analysis. The interface for the conversion of analytes to CO_2 and H_2O consisted of a quartz tube packed with CuO-pellets (4 mm x 0.5 mm, isotope grade, Elemental Microanalysis LTD.) and was maintained at 850 °C. H_2O was removed cryogenically at -100 °C. Isotopic compositions were determined by integration of the *m/z* 44, 45 and 46 ion currents of CO_2 peaks from each analyte and reported relative to CO_2 reference gas pulses of known ^{13}C-content. Isotopic values are given in the delta (δ) notation relative to the international standard VPDB. For data acquisition and processing MassLynx (Micromass Ltd.) was used. Each sample was analysed at least in duplicate and all reported values had standard deviations < 0.5 ‰. To ensure accuracy, in house standard solutions containing *n*-alkanes with a known isotopic composition were analysed after every second sample.

Elemental analysis

Elemental analysis (C, N, S) was performed in the laboratory of the Institute of Geoscience, Kiel University. Prior to analysis fractions of the ground samples were decarbonated with hydrochloric acid (5%), washed, dried and homogenized. The carbonate content [%] was calculated based on weight differences before and after decarbonation. The reported values for TOC %, TN % and TS % have also been corrected for these weight differences. Analyses were performed on a VARIO EL-III elemental analyser. About every second sample was analysed in duplicate or triplicate to ensure reproducibility.

Rock Eval pyrolysis

Rock Eval pyrolysis was performed in the laboratory of the Institute of Geoscience, Kiel University on fractions of the decarbonated samples (previous section) using a VINCI Rock Eval 2 instrument.

$\delta^{13}C$ and $\delta^{18}O$ of carbonates

$\delta^{13}C$ and $\delta^{18}O$ of carbonates were measured in the laboratory of the School of Plant Biology at the University of Western Australia by continuous –flow (CF) analysis using a GasBench II coupled with a Delta XL Mass Spectrometer (Thermo-Fisher

Scientific) after the method described in Paul and Skrzypek (2007). In brief, carbonates in the powdered samples were digested by addition of ortho-phosphoric acid in a helium atmosphere. The generated CO_2 was trapped, purified and diverted to the irMS. Isotope values were reported in the delta (δ) notation relative to the international standard VPDB.

$\delta^{13}C$ of organic carbon

$\delta^{13}C$ of organic carbon was measured in the laboratory of the School of Plant Biology at the University of Western Australia. Residues left after Soxhlet extraction were treated with ~7M hydrochloric acid to remove carbonates, washed, dried and homogenized. $\delta^{13}C$ analyses were subsequently performed on a continuous flow (CF) system consisting of a Delta V Plus mass spectrometer connected to a Thermo Flush 1112 *via* Conflo IV (Thermo-Finnigan/Germany) after the method described in Skrzypek and Paul (2006). The combustion and oxidation temperatures were 1700-1800°C, the reduction temperature 650°C. Results were reported in the delta (δ) notation relative to the international standard VPDB.

$\delta^{34}S$ of pyrite

$\delta^{34}S$ of total reducable inorganic sulphur (TRIS, basically pyrite) was measured at the Marine Geology Department, Geochemistry & Isotope Geochemistry Group, Leibniz-Institute for Baltic Sea Research in a fraction of the sample residue after Soxhlet-extraction. In brief, TRIS was extracted from the sample by treatment with hot acidic chromium (II) chloride (Fossing & Jørgensen, 1989) and the generated H_2S was precipitated as ZnS and subsequently converted to AgS_2. $^{34}S/^{32}S$ ratios were measured by combustion - isotope ratio monitoring - mass spectrometry (C-irm-MS) on a Thermo Finnigan MAT 253 mass spectrometer coupled to an elemental analyser (Thermo Flash 2000) *via* a split interface (Thermo Finnigan Conflo IV). Measured isotope ratios were calibrated with in-house and international reference materials (Mann et al., 2009) and reported in the δ-notation relative to the V-CDT (Vienna Cañon Diablo Troilite) standard.

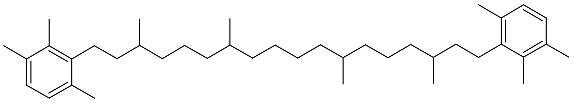
δD of kerogen

δD of kerogen was measured at the Department of Geological Sciences, Indiana University. Kerogens were isolated from the extracted residue by decarbonation with ~7M hydrochloric acid and a subsequent treatment with 25 % hydrofluoric acid to remove silicates and other hydrogen-bearing inorganic phases. Heavy minerals such as acid-insoluble sulfides, titanium oxides and zircon were removed from the kerogen by density separation using aqueous zinc bromide solution (~2.4 g/mL). Zinc bromide was subsequently removed by washing with slightly acidified water. Samples were freeze dried and extracted ultrasonically in DCM for 2 hours to remove any bitumen previously trapped in the mineral matrix. After washing with DCM, drying and homogenization kerogens were weighed into silver capsules for δD analysis.

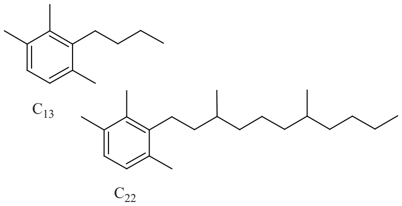
To account for exchangeable hydrogen, aliquots of the kerogens (2 replicates each) were equilibrated in the steam of two different waters with known D/H content for 6 to 10 hours prior to analysis according to a method developed by Schimmelmann et al. (1999; 2006). δD analysis were performed using a Costech elemental analyser (EA). The contents of exchangeable hydrogen were determined using mass balance equations and the measured δD values were corrected accordingly (Schimmelmann et al. 1999; 2006).

Supplementary figures

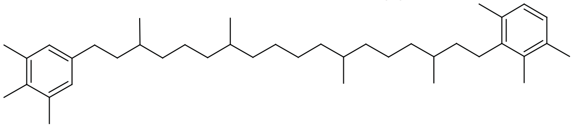
Isorenieratane (**I**)



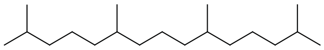
e.g. 2,3,6-Aryl isoprenoids (**III**)



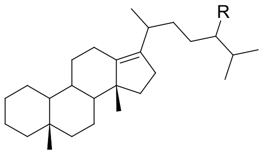
Paleorenieratane (**II**)



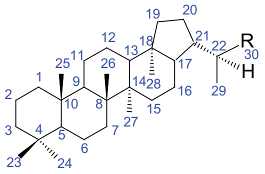
Pristane (**IV**)



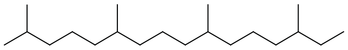
Diaster-(13)17-enes (**VI**)



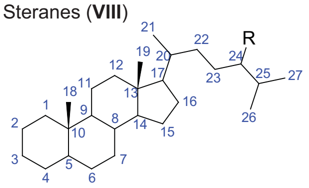
Hopanes (**VII**)



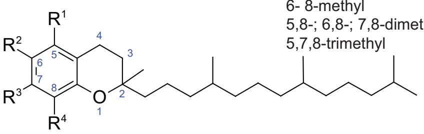
Phytane (**V**)



Steranes (**VIII**)

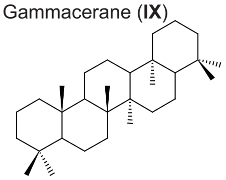


MTTCs (**X**)

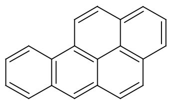


6- 8-methyl
5,8-; 6,8-; 7,8-dimethyl
5,7,8-trimethyl

Gammacerane (**IX**)



Benzo[*a*]pyrene (**XI**)



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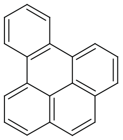
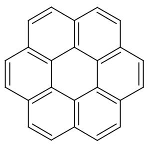
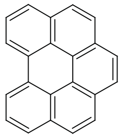


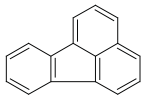
Coronene (**XIII**)



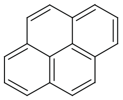
Benzo[*ghi*]perylene (**XIV**)



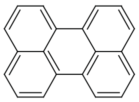
Fluoranthene (**XV**)



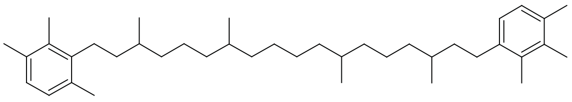
Pyrene (**XVI**)



Perylene (**XVII**)



Renieratane (**XVIII**)



Dibenzofuran (**XIX**)

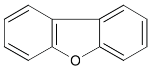


Figure A5.1: Structures referred to in the text

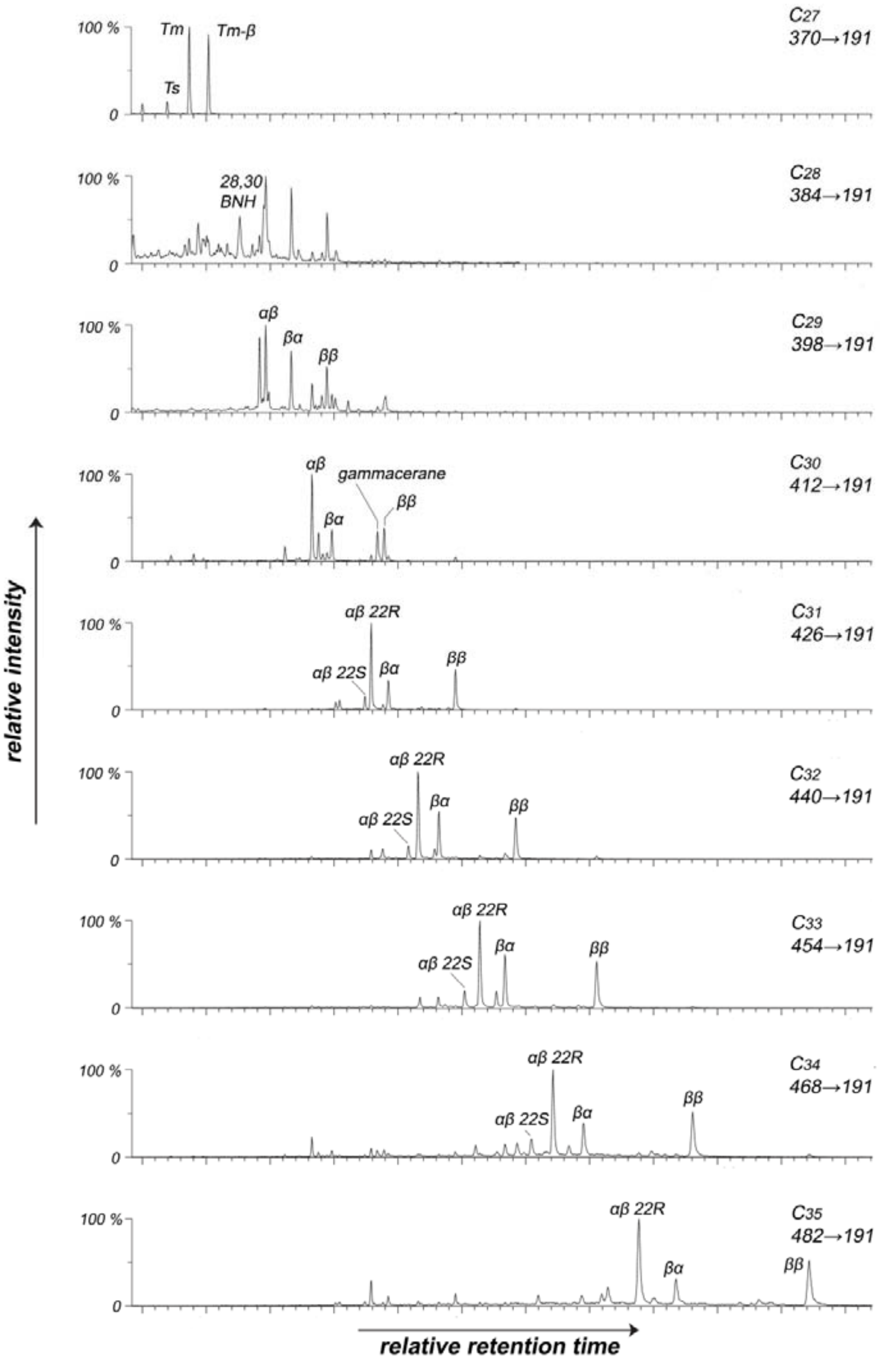


Figure A5.2: MRM chromatograms showing hopanes in a sample from the analysed core at the depth of 40.7 m. S and R stand for the stereochemistry at C22.

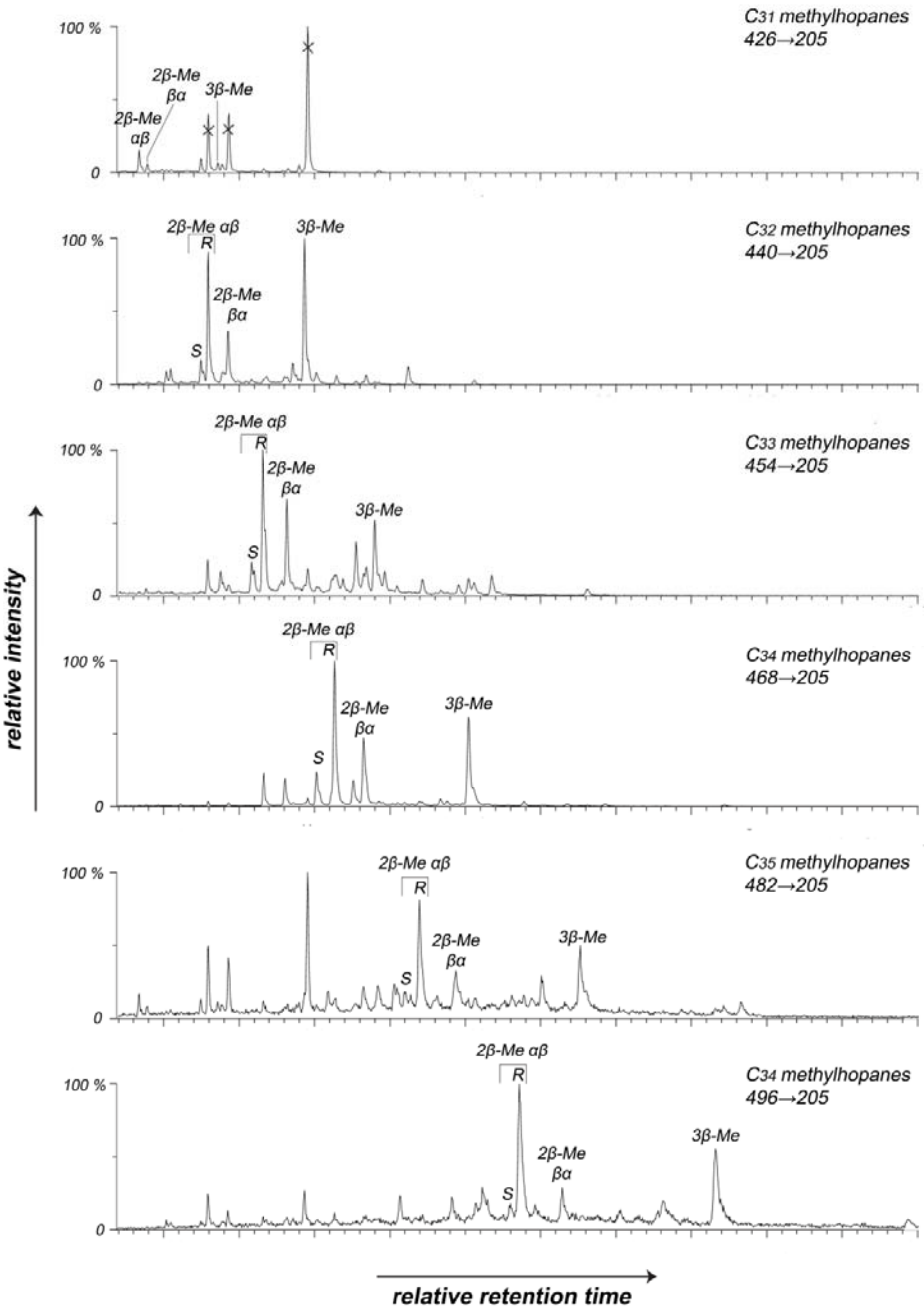


Figure A5.3: MRM chromatograms showing methylhopanes in a sample from the analysed core at the depth of 40.3 m. Crossed out peaks mark cross-talk from regular homohopanes. S and R stand for the stereochemistry at C22. Me = methyl

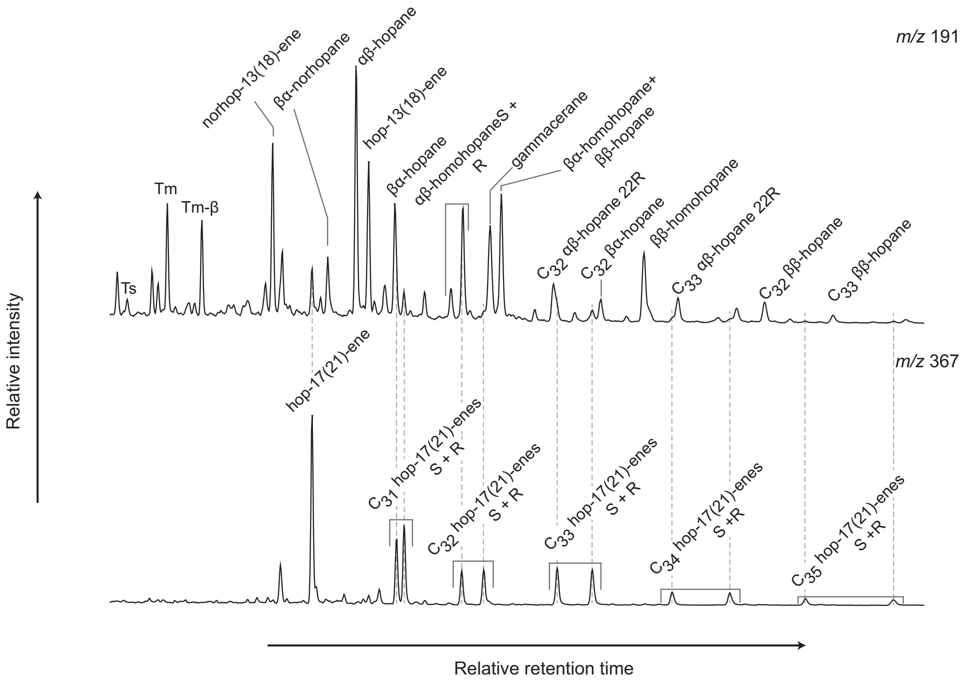


Figure A5.4: Extracted ion chromatograms of a sample from the analysed core at the depth of 40.3 m showing the distributions of hopanes and hopenes in the aliphatic fraction. S and R stand for the stereochemistry at C22

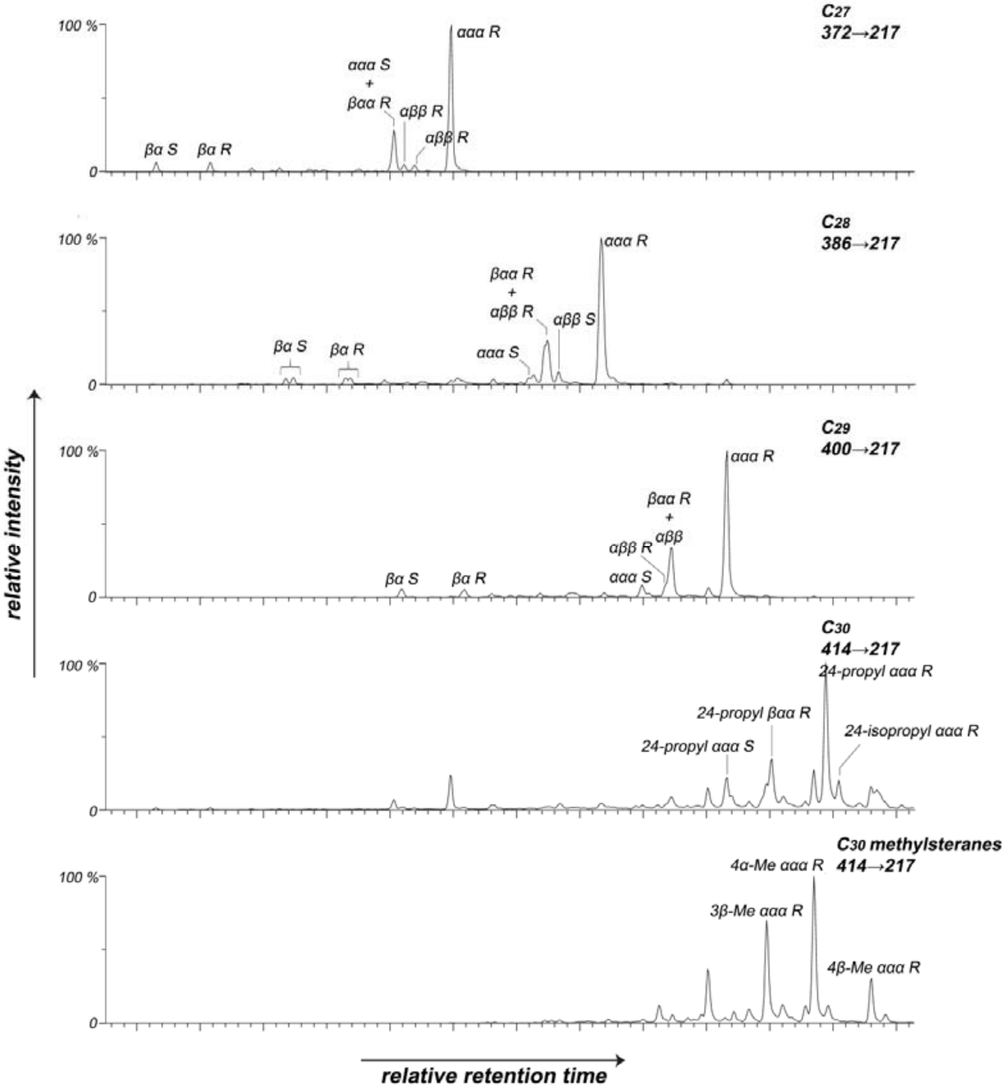


Figure A5.5: MRM chromatograms showing steranes in a sample from the analysed core at the depth of 40.3 m. S and R stand for the stereochemistry at C20. Me = methyl ; βα = 13β,17α-diasteranes

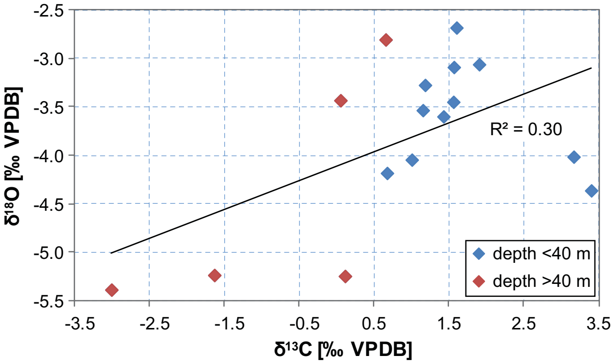


Figure A5.6: Plot of δ^{18}O vs δ^{13}C of carbonates in the analysed core from McWhae Ridge

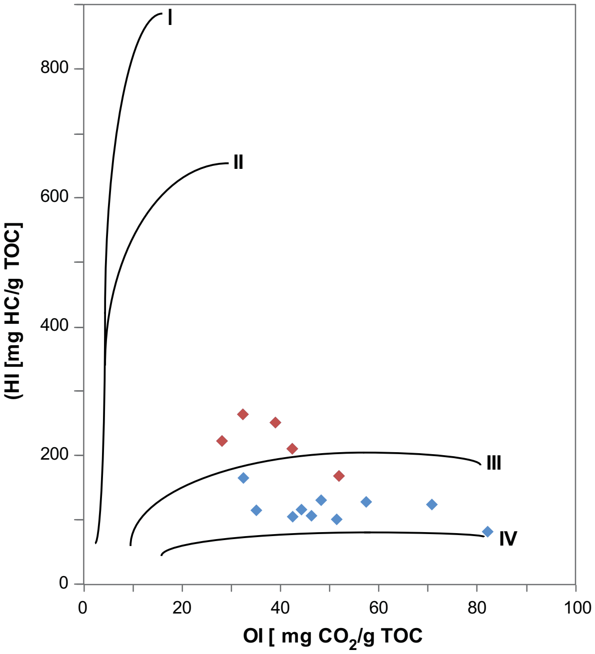


Figure A5.7: Van Krevelen diagram of the analysed sediments indicating the kerogen type.

Data included in figures

Table A5.1: Data included in **Figure 5.3**

Depth [m]	Pr/Ph	C_{35} HHI [%]	28,30 BNH/ C_{30}H	Isor. [µg/g TOC]	Palaeoren. [µg/g TOC]	Monoaryl isopr. [µg/g TOC]			$\delta^{34}S_{pyrite}$ [‰]	Gammacerane index	triMeMTTC/ total MTTCs	$\delta D_{kerogen}$ [‰ VSMOW]	$\delta^{18}O_{carb.}$ [‰ VPDB]
						C_{14}	C_{16}	C_{20}					
27.3	*2.99	1.05	0.01	0.00	*0.00	0.39	0.12	0.03	-24.4	*0.05	*0.79	-109	-4.0
28.1	*2.47	2.04	0.02	0.00	*0.00	0.09	0.03	0.01	n.d	*0.08	*0.83	n.d	-4.2
29.0	*1.47	2.03	0.02	0.00	*0.00	0.53	0.11	0.04	-11.9	*0.11	*0.89	n.d	-3.4
29.7	n.d.	n.d	n.d	0.00	*0.00	0.21	0.08	0.02	-13.6	n.d	*0.88	-115	-3.1
29.9	*1.76	1.70	0.02	0.00	*0.01	0.28	0.09	0.02	n.d	*0.18	*0.88	-121	n.d
30.7	*1.45	1.75	0.04	0.00	*0.00	0.49	0.14	0.04	n.d	*0.05	*0.91	-118	n.d
30.9	*1.31	2.14	0.04	0.00	*0.00	1.27	0.28	0.07	-12.6	*0.16	*0.89	-126	-3.5
31.9	*1.26	2.61	0.04	n.d	n.d	n.d	n.d	n.d	-16.7	*0.27	*0.91	-113	-3.6
32.8	*1.17	2.66	0.07	0.00	*0.00	0.01	0.08	0.03	-16.5	*0.43	*0.91	-109	-2.7
33.0	*1.29	2.53	0.05	0.00	*0.00	0.01	0.11	0.03	n.d	*0.06	*0.91	-117	n.d
33.2	*0.99	3.36	0.07	0.03	*0.02	0.00	0.03	0.04	-16.5	*0.43	*0.93	-121	-3.1
33.4	*1.00	3.57	0.04	0.00	*0.00	0.00	0.01	0.00	-19.6	*0.24	n.d	n.d	-3.3
34.6	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	-16.0	n.d	n.d	n.d	-4.4
37.8	*0.32	2.67	0.03	0.00	*0.00	0.00	0.00	0.00	-16.2	*0.14	n.d	n.d	-4.0
40.2	*0.39	2.64	0.08	0.00	*0.06	0.02	0.22	0.18	*-24.0	*0.55	*0.95	n.d	-5.2
40.3	*0.41	4.36	0.05	0.58	*2.34	4.18	2.19	1.14	*-13.8	*1.71	*0.95	n.d	-5.4
40.5	*0.51	n.d.	n.d.	0.37	*1.66	2.49	2.11	0.90	n.d	n.d	*0.94	n.d	n.d
40.7	*0.51	3.01	0.04	0.13	*0.50	1.42	1.19	0.49	*-11.9	*1.24	*0.94	-130	-2.8
41.2	*0.48	2.65	0.07	0.25	*1.24	6.62	4.01	1.23	*-15.0	*1.37	*0.95	n.d	-5.2
41.9	*1.00	2.17	0.05	0.02	*0.11	0.19	0.38	0.11	*-19.1	*0.75	*0.93	n.d	-3.4

* also included in figures of **Chapter 4**

Table A5.2: δ^{13}C values of the compounds included in **Figure 5.4** in ‰ *vs.* VPDB. “Ci” stands for monoaryl isoprenoids with the carbon number “i”

Depth [m]	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11
	C_{15}	C_{16}	C_{18}	C_{19}	see Fig. 5.4	see Fig. 5.4	?	?	see Fig. 5.4	see Fig. 5.4	palaeorenieratane
40.2	n.d.	n.d.	n.d.	-15.1	-17.8	-20.8	-17.7	-17.0	n.d.	n.d.	n.d.
40.3	n.d.	n.d.	-21.7	-16.8	-18.4	-20.1	-17.0	-16.0	n.d.	n.d.	*-15.3
40.7	-17.2	-16.4	-19.6	-18.6	-16.7	-19.2	-17.9	-17.4	-15.5	-15.9	*-15.4
41.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
41.9	-19.4	-17.9	-21.2	-19.6	-16.7	-18.9	-17.4	-18.4	n.d.	n.d.	*-15.1

* also included in **Chapter 4**

Table A5.3: Data included in **Figure 5.6**

Depth [m]	TOC [wt. %]	TOC_{or} [wt. %]	Carbonates [wt. %]	TN_{org} [wt.%]	TS [wt. %]	C/N_{org} [molar]	HI [mg HC/g TOC]	OI [mg CO_2/g TOC]
27.3	0.3	0.7	9.1	0.02	0.3	12.3	70	102
28.1	0.3	1.1	23.7	0.02	0.8	12.3	72	108
29.0	0.5	1.8	15.8	0.02	1.3	19.9	115	35
29.7	0.5	2.1	12.5	0.03	1.6	16.3	124	71
29.9	0.5	2.1	14.7	0.03	1.6	15.8	101	51
30.7	0.6	2.0	11.9	0.03	1.5	15.9	105	42
30.9	0.8	2.8	12.4	0.04	2.0	17.8	166	32
31.9	n.d.	n.d.	n.d.	n.d.	n.d.	20.4	131	48
32.8	0.5	2.0	12.4	0.03	1.5	15.7	107	46
33.0	0.5	2.3	13.5	0.03	1.8	15.6	128	57
33.2	0.5	2.0	12.8	0.03	1.5	14.9	116	44
33.4	0.1	1.0	65.9	0.00	0.9	28.1	82	82
34.6	0.0	0.2	86.2	0.00	0.2	7.8	38	200
37.8	0.0	0.7	70.3	0.00	0.6	5.9	n.d.	n.d.
40.2	*0.0	0.5	*62.6	0.00	0.5	16.8	n.d.	n.d.
40.3	*0.3	1.0	*68.1	0.01	0.7	17.9	252	39
40.5	*0.9	2.4	*15.2	0.05	1.5	16.8	223	28
40.7	*0.8	2.7	*10.5	0.04	1.9	16.8	211	42
41.2	*0.7	2.1	*32.2	0.04	1.4	18.0	264	32
41.9	*0.7	2.4	*13.3	0.04	1.6	17.6	169	52

* also included in **Chapter 4**

Table A5.4: Data included in **Figure 5.8**

Depth [m]	Steranes/ hopanes	C_{30} Methylst./C_{27-29} Sts			*iso*-propylchol./ *n*-propylchol.	Pristane/ *n*-C_{17}	Phytane/ *n*-C_{18}	Concentration µg/g TOC						
		3β-Me	4α-Me	4β-Me				Perylene	∑MTTCs	BaPy	BePyr	Bpery	Cor	DBF
27.3	0.20	0.00	0.01	0.00	0.28	0.51	0.16	*0.13	*1.83	0.33	0.54	0.96	0.66	0.24
28.1	0.39	0.04	0.02	0.01	0.17	0.77	0.37	*0.02	*2.12	0.04	0.06	0.03	0.01	0.01
29.0	0.84	0.07	0.03	0.01	0.11	1.38	0.97	*0.07	*7.99	0.12	0.13	0.09	0.05	0.14
29.7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	*0.03	*3.60	0.03	0.03	0.01	0.00	0.01
29.9	0.76	0.03	0.02	0.01	0.10	1.61	0.95	*0.02	*4.04	0.03	0.03	0.02	0.01	0.02
30.7	0.88	0.05	0.03	0.01	0.12	1.86	1.30	*0.05	*10.35	0.10	0.11	0.16	0.07	0.17
30.9	1.27	0.06	0.03	0.01	0.13	1.67	1.46	*0.05	*12.99	0.07	0.09	0.34	0.14	0.34
31.9	1.44	0.06	0.02	0.01	0.10	2.63	2.15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
32.8	2.47	0.06	0.02	0.01	0.11	2.04	1.61	*0.23	*11.56	0.21	0.36	0.60	0.14	0.07
33.0	2.69	0.04	0.02	0.01	0.14	1.82	1.53	*0.23	*12.26	0.27	0.36	0.71	0.15	0.08
33.2	3.86	0.05	0.02	0.01	0.12	2.21	2.02	*0.21	*10.12	0.23	0.33	0.17	0.04	0.01
33.4	2.37	0.00	0.01	0.00	0.14	0.66	0.94	*0.02	n.d.	0.02	0.04	0.02	0.01	0.00
34.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
37.8	0.49	0.00	0.00	0.00	0.20	0.16	0.66	*0.00	n.d.	0.00	0.01	0.00	0.00	0.00
40.2	4.94	0.08	0.07	0.03	0.13	1.75	4.54	*0.14	*6.75	0.03	0.05	0.00	0.00	0.00
40.3	6.36	0.15	0.21	0.07	0.15	3.13	9.35	*1.06	*38.54	0.17	0.19	1.08	0.39	0.23
40.5	n.d.	n.d.	n.d.	n.d.	n.d.	4.01	6.99	*0.92	*22.42	0.17	0.17	0.90	0.14	0.22
40.7	4.62	0.12	0.34	0.06	0.13	3.09	5.87	*0.54	*14.29	0.10	0.11	0.28	0.04	0.09
41.2	2.17	0.10	0.24	0.04	0.20	5.07	11.12	*0.42	*21.19	0.07	0.08	0.27	0.03	0.20
41.9	1.17	0.04	0.11	0.02	0.13	3.21	3.67	*0.04	*2.68	0.01	0.01	0.01	0.00	0.00

* also included in **Chapter 4**

Table A5.5: Data included in **Figure 5.9.** Peak areas were inferred from suitable transitions of multiple reaction monitoring-gas chromatography- mass spectrometry (MRM-GC-MS)

Depth [m]	Peak areas		
	∑cholestanes	∑24-methylcholestanes	∑24-ethylcholestanes
27.3	378495	124937	678755
28.1	1993635	707340	3861990
29.0	10189203	3458112	17904085
29.7	n.d.	n.d.	n.d.
29.9	5618845	2099182	10484379
30.7	8955227	3410066	17844243
30.9	20036199	7667875	35901309
31.9	9733470	3956699	20775005
32.8	11028498	4975431	22450442
33.0	24274101	11377345	48922215
33.2	30561811	14750194	64474802
33.4	1022704	417535	2507036
34.6	n.d.	n.d.	n.d.
37.8	326084	102564	511516
40.2	5677128	2771224	9992119
40.3	51521847	25616683	82671752
40.5	n.d.	n.d.	n.d.
40.7	15859006	7138781	19431634
41.2	10344653	4529279	13476443
41.9	2382601	1215802	4162140

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Chapter 6

Conclusions and outlook

This PhD primarily explores and introduces (novel) biomarker and stable isotope approaches for the reconstruction of palaeosalinity and water-column stratification and demonstrates their practical applications in the investigation of palaeoenvironmental conditions in the modern Coorong Lagoon, an estuarine lagoon complex in South Australia and in a Middle to Late Devonian marine setting associated with reef-systems affected by the Late Devonian extinctions. Furthermore, a new analytical approach with a great potential for the investigation of source and formation pathway of the palaeosalinity markers methyltrimethyltridecylchromans (MTTCs) is developed.

Integration of the comprehensive biomarker, elemental and stable isotope data acquired provides an in-depth insight into both the human-induced environmental and ecological changes in the northern Coorong Lagoon as well as changes in levels of biotic stress in depositional settings associated with the Late Devonian extinctions. Changes of salinity in aquatic environments are often of great ecological significance since they typically have a substantial effect on *in situ* primary production, which forms the base of the food-web (e.g. modern Coorong Lagoon, see subsequent section). Furthermore, salinity differences between the epilimnion and hypolimnion in (typically somewhat enclosed) marine settings are often responsible for a persistent density stratification of the water-column. Such conditions commonly lead to prevailing anoxia or euxinia in the hypolimnion, which may also extend to the photic zone, and have been frequently associated with (global) oceanic anoxic events in Earth's history accompanying mass extinction events.

Mass extinction events had a great significance for the evolution of life throughout Earth's history and their investigation may provide a useful insight into present day climate change, particularly given speculation that dramatic anthropogenic impacts on sensitive environmental conditions may represent the beginning of yet another big extinction. The Coorong Lagoon represents such an ecosystem recently impacted by a major anthropogenic influence.

Nature and extent of human-induced changes in the modern Coorong Lagoon

The Coorong Lagoon is part of an ecologically significant wetland located at the Mouth of the River Murray. Similar to many other estuarine environments it has been subject to a dramatic decline in biodiversity over the past several decades as a result of human interference with the natural water-regime, which was compounded by a prolonged drought from 2000-2010.

Despite many previous studies of the present day Coorong, including investigations of hydrology, ecology and organic geochemistry of the water-column and surface sediments, little was known about conditions in the ecosystem of the North Lagoon prior to European settlement and the nature and extent of changes that human water management had produced. The present study (**Chapter 2)** uses biomarker and stable isotope analysis of a sedimentary core from the northern Coorong Lagoon spanning the past ~5000 years to track ecological and environmental changes both prior to European settlement and after the 1950s introduction of substantial water management practices. The newly acquired organic geochemical data clearly demonstrate significant alterations in the deposited organic matter (OM) of the North Lagoon which could be directly related to the introduction of these water management practices. More specifically, there is strong evidence for a change in OM production from a predominant source of aquatic macrophytes prior to the 1950s towards a major input from microalgae and bacteria in more recent sediments. Compound specific δD analysis, a relatively new analytical technique developed in

1998/99, was applied to predominantly *in situ* produced *n*-alkanes, which proves a particularly useful indicator of palaeosalinity. The obtained values indicate a general salinity increase in the North Lagoon throughout the Holocene, despite some variability, and then a pronounced positive shift after the 1950s, indicating a strong elevation of salinity over the past decades. The increase in salinity caused by the new water-management regime and further compounded by the severe drought of recent times, has been generally regarded as one of the major drivers for the ecological decline and extinction of key species. A recent flood in 2010 temporarily improved ecological conditions, but the long-term issue of providing sufficient freshwater input, particularly under future drought conditions has not been fully resolved. The present study extends the knowledge of OM sources and natural salinity levels in the North Lagoon prior to European settlement, and provides an important basis against which the success of remediation measures can be assessed. Furthermore, conditions in the Coorong may also be relevant for other wetlands exposed to similar ecological disturbances. In the future a similar approach may also be used in new sediment cores which include OM deposited after the recent flood in 2010, to track changes induced by the temporary high freshwater input. Furthermore, it could also be extended to other water-bodies of the Coorong region to follow changes in primary production and salinity over time.

Elevated levels of biotic stress in a marine Middle to Late Devonian palaeoenvironment

An integrated analytical study, including use of biomarkers, elemental and stable isotope values, Rock Eval pyrolysis and palynological data, is undertaken on a depositional setting from the Lennard Shelf, Canning Basin, WA associated with reef-systems close to or at the Givetian-Frasnian (G-F) boundary and later in the Frasnian to investigate the palaeoenvironmental conditions and changes possibly related to events associated with the Late Devonian extinctions **(Chapter 5)**. As part of this investigation, a novel approach for reconstructions of freshwater incursions in marine palaeoenvironments is also developed (**Chapter 4**; see following section).

Most research of the Late Devonian extinctions, which are amongst the biggest mass extinction events in the Phanerozoic, have focussed on the most pronounced biodiversity loss at the Frasnian-Famennian (F-F) boundary, and investigations of potential events in the Givetian and Early to Middle Frasnian have been comparatively rare. Altogether, only little is known about causes or the exact timing and amount of associated extinction pulses.

The corresponding analyses of the Canning Basin sediments provided evidence of a persistently stratified water-column with freshwater incursions overlying more saline bottom waters as well as prevailing anoxia and photic zone euxinia (PZE) in the marine palaeoenvironment close to or at the G-F boundary. These conditions are all consistent with increased levels of biotic stress for reef-builders and associated organisms, which were amongst the most severely affected life-forms during the Late Devonian extinctions. Later in the Frasnian, the analytical data are indicative of more oxic conditions and a vertically mixed water-column without any evidence of PZE (**Chapter 5**). Distributions and abundances of algal and bacterial biomarkers also showed significant variations between the Late Givetian/Early Frasnian and later in the Frasnian, possibly reflecting riverine incursions in the older sediments. The dataset presented in **Chapter 5** indicates a distinct time period of biotic crisis in the Devonian Canning Basin close to the G-F boundary, which adds to the knowledge about conditions in palaeoenvironments associated with the Late Devonian extinctions and might be useful to reconstruct global events if correlated with other datasets. **Chapter 4** and **5** also provide a further indication that PZE and enhanced terrigenous nutrient input (likely related to the expansion of vascular plants) may have significantly contributed to mass extinctions in the Middle to Late Devonian, also prior to the F-F boundary, which is consistent with previous studies. In the near future the present study will be complemented with detailed palynological analyses in the same core. Similar biomarker and stable isotope studies should also be used to explore palaeoenvironmental changes in other regions of the Canning Basin, particularly since this study has furthermore shown an extremely good preservation of biomarkers and OM in the Gogo Formation.

Organic matter (OM) preservation in the Canning Basin, WA

The Canning Basin, WA is famous for its extensive and well-exposed Middle to Late Devonian reef systems showing only minor geological alteration in most areas. The Gogo Formation, which represents Givetian to Frasnian basin facies, was laid down predominantly under suboxic and anoxic conditions and is of particular interest for many scientists. It forms the source rocks for the high-quality oils in the Canning Basin and contains exceptionally well-preserved macrofossils. A few studies also indicated a very unusual preservation of molecular fossils (i.e. biomarkers) in some sections, which showed very immature profiles despite their Palaeozoic age. The data collected in **Chapter 5** also confirm this very high level of OM and biomarker preservation in the Gogo formation. A high proportion of hopanes and steranes still exhibited their less thermally stable biological configuration and even some unsaturated precursors were present. Furthermore, the presumably oldest preservation of original C/N was reported in the samples analysed in **Chapter 5**. A possibility to further extend this study would be the analysis of more polar compounds, which might be well preserved as indicated by the extremely low thermal maturity of the OM.

Significance of MTTCs in palaeoenvironmental reconstructions

Origin of MTTCs in geological samples

The “chroman ratio” between different MTTC isomers is a frequently used palaeosalinity indicator, despite uncertainties about the origin and formation pathway of these compounds. Condensation reactions between chlorophyll-derived phytol and presumably higher plant-derived alkylphenols during early stages of diagenesis represent a likely formation pathway, however direct biosynthesis of MTTCs (or structurally similar precursors) from phytoplankton has also been suggested. A clarification of their source(s) would help to more accurately utilize chroman ratios to infer palaeosalinities (especially in a stratified water-column) and it may also broaden the field of MTTC applications in palaeoenvironmental reconstructions, particularly regarding a potential relation to terrigenous input or freshwater incursions.

Chapter 3 presents a new and relatively simple flash pyrolysis-gas chromatography-isotope ratio mass spectrometry (Py-GC-irMS) method to support the investigation of MTTC origins. This analytical technique enables the separate $\delta^{13}C$ measurement in the isoprenoid and alkylphenol subunits of a 5,7,8-trimethyl-MTTC standard (often the most abundant natural isomer) with high accuracy and reproducibility. The formation of MTTCs by condensation reactions of higher plant-derived alkylphenols with predominantly phytoplankton-derived phytol (typically main source of chlorophyll in marine environments), should be reflected by the different $^{12}C/^{13}C$ composition of the respective MTTC subunits. However, matrix effects prevented the formation of 2,3,5-trimethylphenol (alkylphenol subunit of 5,7,8-trimethyl-MTTC) when isolates of this chroman from sediments were analysed. Further optimisation of sample preparation or pyrolysis conditions will therefore be necessary before this method can be effectively applied to geological sediments. It might also be useful to use this method for the analysis of tocopherols, which show strong structural similarities to MTTCs but are biosynthesised, to detect potential isotopic differences in the subunits resulting from biosythestic processes and compare these to chromans in natural samples.

Novel conceptual biomarker-model for freshwater incursions in marine palaeoenvironments

Chapter 4 provides valuable information for the investigation of origin and formation pathway of sedimentary MTTCs, which point towards an origin from the previously described early diagenetic condensation reactions. Furthermore, a novel conceptual model using MTTCs as indicators for freshwater incursions in a marine depositional environment was introduced. This hypothesis was based on the analysis of MTTCs in sediments corresponding to a Middle to Late Devonian marine palaeoenvironment in which the disposition of reef systems restricted water exchange with the open ocean, leading to a stagnant and persistently stratified water-column (also see "Elevated levels of biotic stress in a marine Middle to Late Devonian palaeoenvironment" page 202). The relation of chroman ratios and MTTC abundances to other molecular and stable isotopic indicators for water-column stratification, anoxia, salinity and PZE indicate that the chroman ratio is strongly influenced by the persistency of water-column stratification and generally reflected low salinities in the overlying freshwater lens. Furthermore, the strong positive correlation of sedimentary abundances of MTTCs and perylene indicate a potential relation to terrigenous input.

In the future, similar studies should also be performed in sediments from other stratified marine palaeoenvirmonments as well as from freshwater settings with high chroman abundances to validate this study and further explore the MTTC formation pathway and the relation of the chroman ratio to other molecular parameters. Furthermore, it should still be considered to keep searching for potential MTTC precursors in extent organisms, although this study strongly indicates a diagenetic origin of these compounds.

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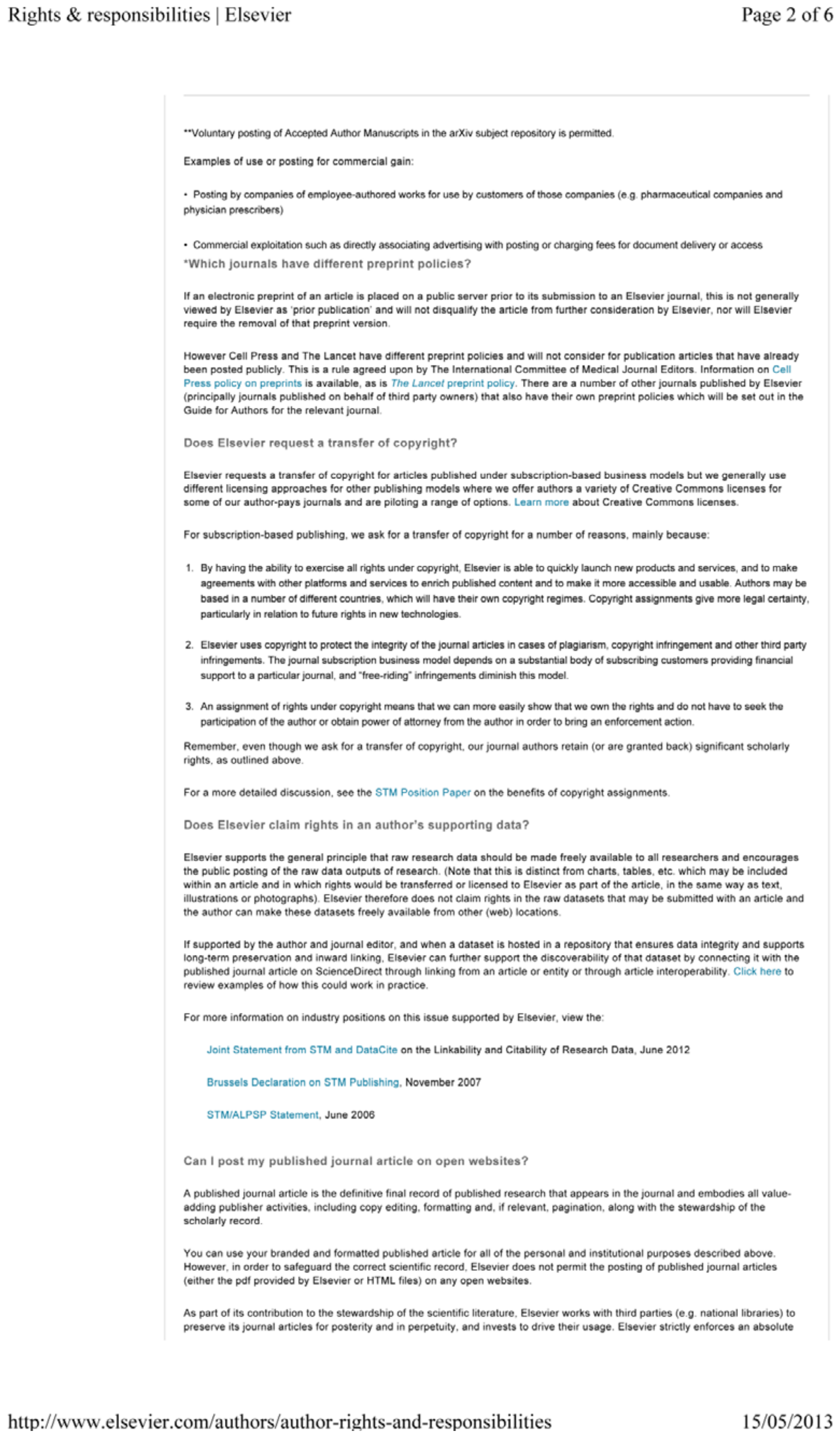

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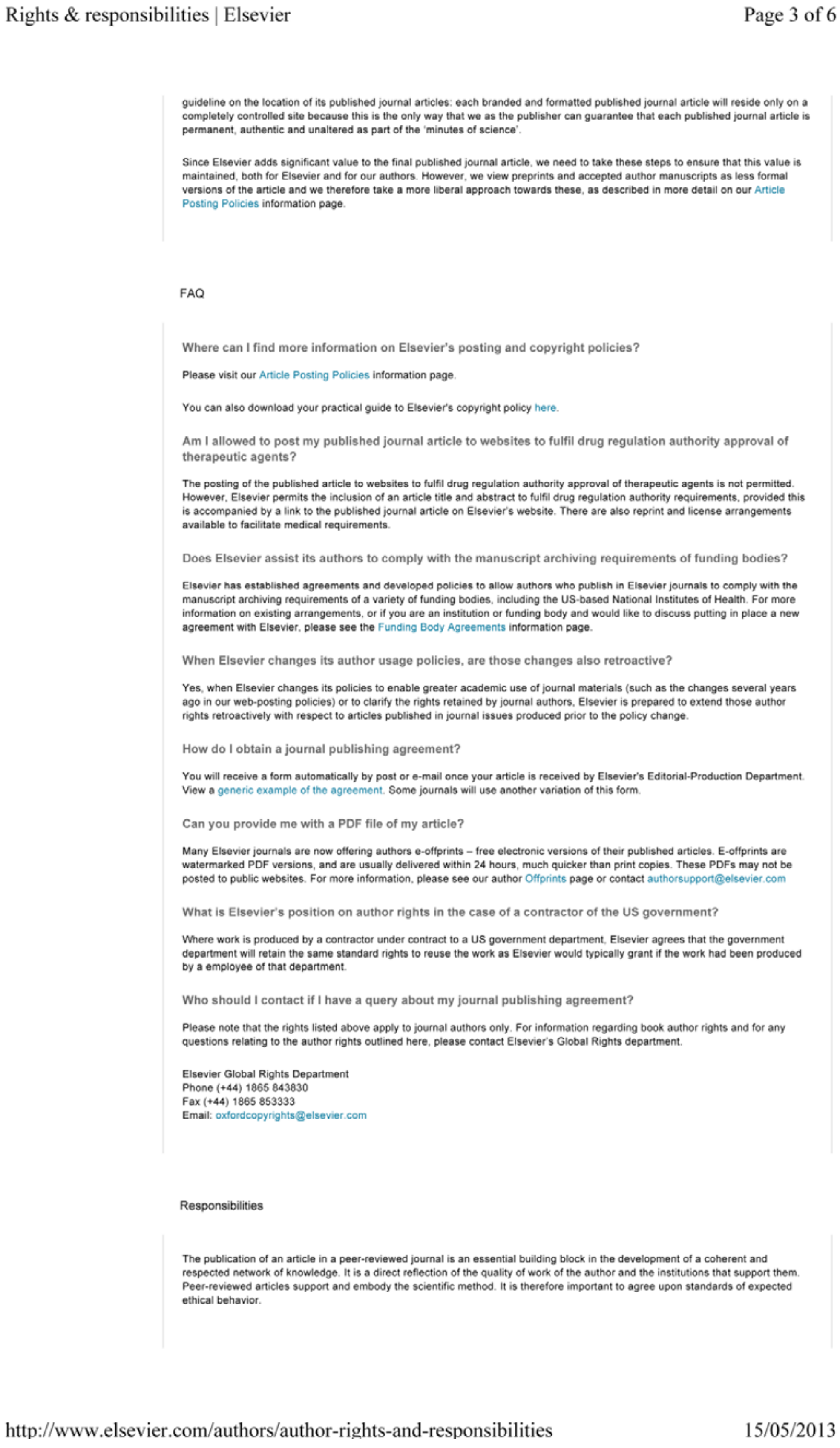

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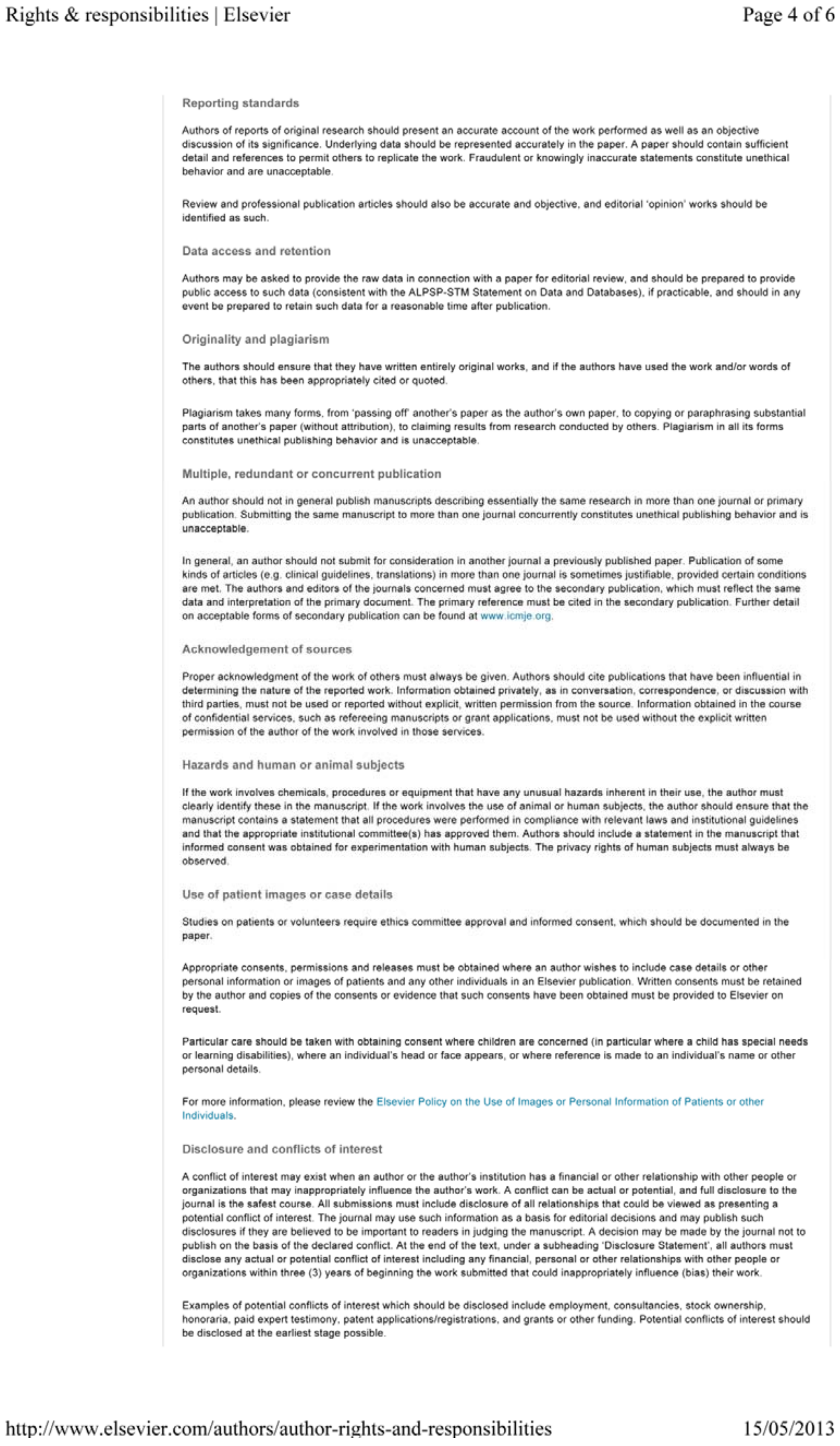

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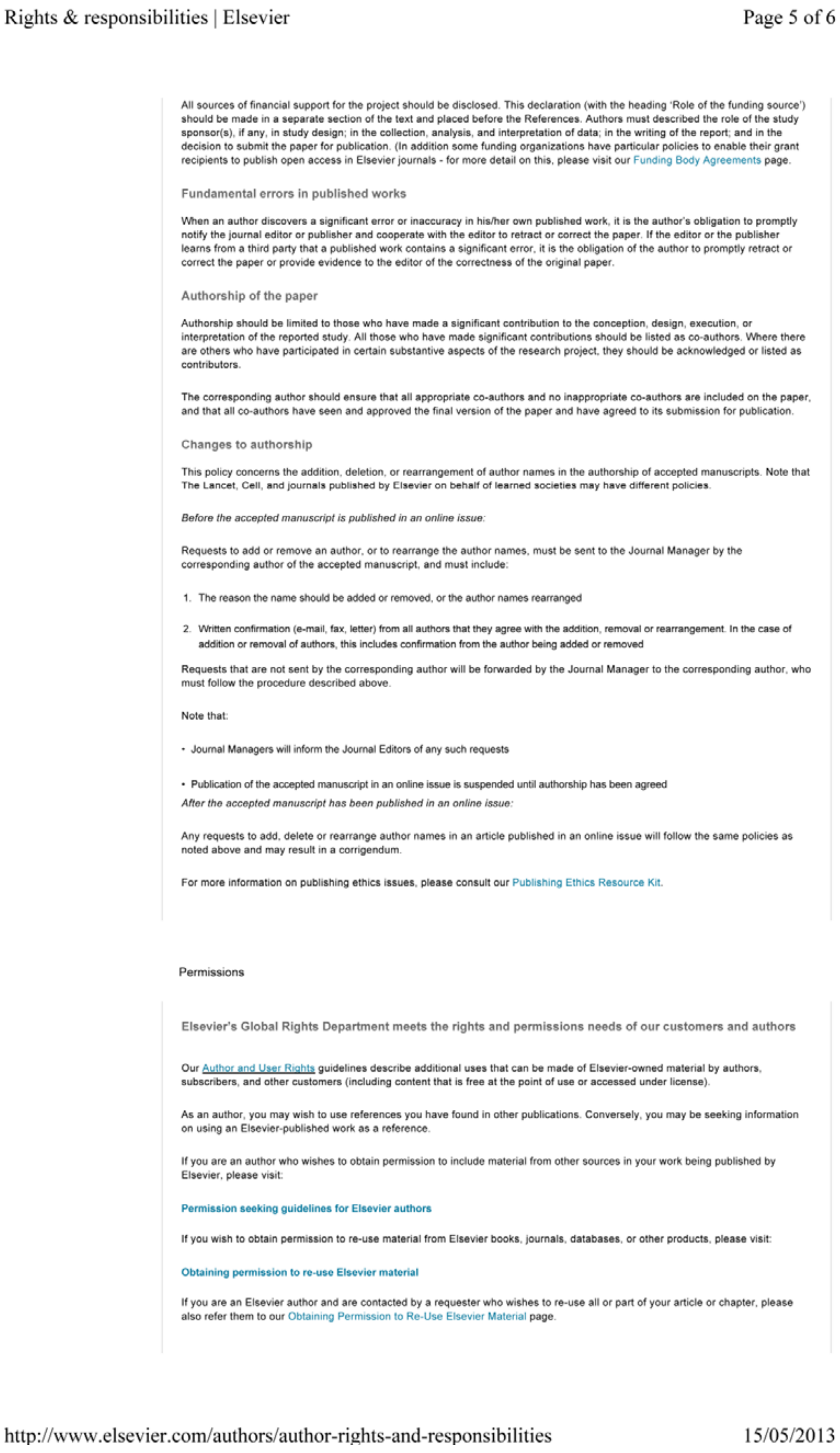

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