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*Biogeography of photoheterotrophic
microbes in coastal waters of Australia*

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TABLE OF CONTENTS

Certificate of original authorship.....	IV
Acknowledgments.....	V
Abstract.....	VI
Thesis format.....	IX
List of abbreviations.....	X

Chapter 1 – General introduction of the study

Introduction.....	2
Microbes in the ocean.....	2
Microbial Metabolism in the ocean.....	3
Photoheterotrophy.....	4
Proteorhodopsin containing marine bacteria.....	5
Aerobic anoxygenic phototrophic bacteria.....	9
Ecological theory towards understanding microbial oceanography.....	14
Australian marine ecosystems and climate change.....	16
Aims.....	19

Chapter 2 - Spatial and temporal variability of aerobic anoxygenic photoheterotrophic bacteria along the east coast of Australia

Abstract.....	22
Introduction.....	23
Results.....	27
Discussion.....	41
Conclusion.....	47
Methods.....	48
Supplementary information.....	56

Chapter 3 - Regional heterogeneity and seasonal succession in proteorhodopsin containing microbial assemblages

Abstract.....	64
Introduction.....	65
Results and Discussion.....	68
Conclusion.....	80
Methods.....	83
Supplementary information.....	88

Chapter 4 - Shifts in the abundance and diversity of photoheterotrophic microbes across two oceanographic provinces in tropical northern Australia

Abstract.....	102
Introduction.....	103
Methods.....	106
Results.....	115
Discussion.....	126
Conclusion.....	132
Supplementary information.....	134

Chapter 5 – General Discussion

Discussion.....	148
Future directions.....	164
Conclusions and Perspectives.....	168
Bibliography.....	171

Appendix I.....	182
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Appendix II.....	186
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Appendix III	190
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Appendix IV.....	214
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CERTIFICATE OF ORIGINAL AUTHORSHIP

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I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as part of the collaborative doctoral degree and/or fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Signature of Student:

Date: 19/07/2017

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ABSTRACT (general)

Marine microbes control the flux of energy and chemicals in the ocean and therefore mediate ocean productivity and biogeochemistry, which ultimately sustains marine life and controls global climate. A numerically and functionally important group of marine microbes, for which there is currently a significant gap in knowledge regarding their ecology and biogeography, are the photoheterotrophic bacteria. These microbes, including both Aerobic Anoxygenic Phototrophic Bacteria (AAnPB) and proteorhodopsin based phototrophic bacteria and Archaea (PRBA) comprise up to 70% of microbial communities within surface waters of the ocean. Capable of gaining energy from light-induced proton translocation and from the oxidation of organic material, these often highly abundant bacteria have been proposed to significantly influence marine biogeochemistry and trophodynamics. However, the environmental (i.e. physicochemical, seasonal, oceanographic) factors influencing the abundance and diversity of these organisms are currently poorly defined, particularly within the often highly productive waters of the Southern Hemisphere. The aim of this thesis was to examine changes in photoheterotrophic community abundance and composition in coastal waters of Australia and identify the key environmental influences on the ecology of these microbes. Samples collected from a combination of oceanographic research voyages and time-series sampling regimes were analysed using both amplicon sequencing approaches and quantitative PCR targeting *pufM* and proteorhodopsin genes to characterise AAnPB and PRBA populations respectively.

High temporal resolution analysis of photoheterotrophic bacterial population dynamics in the coastal waters of Australia indicated seasonality in the abundance and diversity of these groups. Along the eastern coast of Australia, AAnPB abundance was highly variable, with *pufM* gene copies ranging from 1.1×10^2 to 1.4×10^5 mL⁻¹, and positively correlated with day length and solar radiation. *pufM* gene amplicon sequencing revealed that the majority of sequences were closely related to those obtained previously in other environments, suggesting that key AAnPB groups are widely distributed across similar environments globally. Temperature was a major structuring factor for AAnPB assemblages across large spatial scales, correlating positively with richness and *Gammaproteobacteria* (phylogroup K) abundance, but negatively with Roseobacter-clade (phylogroup E) abundance, with temperatures between 16-18 °C identified as a potential transition zone between these groups. Coastal PRBA assemblages were also dynamic in both space and time, with diversity correlated to indicators of waterbody oligotrophy including positively to temperature, Secchi depth and *Prochlorococcus* cell abundance and negatively to phosphate concentration. Shifts in taxonomic composition were best explained by temperature and day length. Seasonality in taxonomic structure was accompanied by temporal variability in proteorhodopsin spectral tuning. Finally, we provide evidence for spatial transitions in the abundance and diversity of key photoheterotrophic bacterial groups between different oceanographic provinces within northern Australian waters, indicating that shifts in physical and biotic characteristics can lead to sharp changes in the importance of these important marine microbial assemblages across

water masses. Total bacterial abundance was higher in ATS waters, which was reflected by higher AAnPB abundance in this region, whereas the major proteorhodopsin containing clade, SAR11, displayed the opposite trend, with higher abundances in the Coral Sea. Among the AAnPB, the Gammaproteobacterial phylogroup K dominated the community in both regions, with relatively stable composition of AAnPB across regions. Conversely, the PRBA community displayed clear differences between the two regions, with the Arafura Timor Sea (ATS) dominated by SAR11-like and Betaproteobacterial sequences, while the Coral Sea was dominated by Archaea group IIb and members of SAR11 clade A and B. In conclusion, this thesis has demonstrated how dynamic these populations are and that each of these two groups should not be treated as single population, but one that is comprised of many interacting and potentially competing units, that are each governed by different environmental factors and thereby fill discrete niches.

Key words: microbial oceanography, next-generation sequencing, photoheterotrophs, community composition, diversity, seasonality, biogeography, proteorhodopsin bacteria, aerobic anoxygenic phototrophic bacteria, East Australian current

THESIS FORMAT

This thesis has been formatted according to publication styles, with each of the three experimental chapters corresponding to manuscripts submitted, or in final preparation for submission, to international research journals. The research chapters correspond to the following manuscripts.

- I. **Bibiloni-Isaksson J**, Seymour JR, Ingleton T, van de Kamp J, Levente Bodrossy L, and Brown MV. (2016) Spatial and temporal variability of aerobic anoxygenic photoheterotrophic bacterial along the east coast of Australia. (*Accepted to Environmental Microbiology on the 28-Jun-2016*)
- II. **Bibiloni-Isaksson J**, Seymour JR, , Ingleton T, van de Kamp J, Levente Bodrossy L, and Brown MV (2016) Diversity patterns of proteorhodopsin containing bacteria along the east coast of Australia. (*Submitted to Environmental Microbiology Reports*)
- III. **Bibiloni-Isaksson J**, Brown MV, Seymour JR (2016) Photoheterotrophic microbial community structure along the North coast of Australia. (In preparation for submission)

LIST OF ABBREVIATIONS

AAnPB	Aerobic Anoxygenic Photosynthetic bacteria
PRBA	Proteorhodopsin-containing Bacteria and Archaea
BCP	Biological carbon pump
bp	Base pairs
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
EAC	East Australian Current
ENSO	El Niño-Southern Oscillation
IMOS	Integrated Marine Observing System
OM	Organic matter
OTU	Operational taxonomic unit
PCR	Polymerase chain reaction
perMANOVA	Permutation-based analysis of variance
PR	Protein Proteorhodopsin
qPCR	Quantitative Polymerase chain reaction
rRNA	Ribosomal ribonucleic acid
SAR11	A lineage of bacteria that was original discovery in the Sargasso Sea

“
There is only one corner of the universe you can be certain
of improving, and that's your own self”

Aldous Huxley

To my family