

# **Coral-associated microbial communities in reef-building corals of Ningaloo Reef Western Australia**

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**This thesis is submitted to Murdoch University  
for the degree of Doctor of Philosophy**

**School of Biology & Biotechnology  
Murdoch University**

**Submitted May 2011**

# **To Coral Reefs**

I declare this thesis to be a record of my own research, which has not been presented for the award of a degree at any other university.

Janja Ceh

# Abstract

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Coral reefs are at risk and human-induced environmental stressors in synergism with microorganisms have been shown to be the key players for their deterioration. Little is known about the dynamics of coral-microbial associations through different life stages of the coral holobiont and virtually nothing is known about coral-microbial partners in Western Australian coral reef systems. This project intended to investigate the presence, diversity, community structure and role of coral-associated microbes in Ningaloo Reef spawning and brooding corals. Different coral life stages were assessed.

To determine ‘normal ranges’ of coral-associated microbes, three coral species (*Acropora tenuis*, *Pocillopora damicornis* and *Favites abdita*) were tagged and examined over a period of one year, with sampling deployed every three months. One coral species was additionally sampled on Rottnest Island, 1200km south of Ningaloo Reef, to provide comparisons between coral-associated microbes in different geographical areas. The community structure of the coral-associated microorganisms was analysed by phylogenetic analysis of 16S rRNA gene clone libraries. Principal component analysis (PCA) revealed that samples grouped according to time and not species, indicating that coral-microbial associations may be a result of environmental drivers such as oceanographic characteristics, benthic community structure and temperature. Tissue samples from Rottnest Island corals revealed similarities in bacteria to the samples at Ningaloo Reef. This study highlights that coral-associated microbial communities are highly diverse; however, the complex interactions that determine the stability of these associations are not necessarily dependant on coral host specificity.

Reproduction plays a crucial role in the survival of species, therefore, data was acquired from three adult coral colonies, *Acropora tenuis* (broadcast spawner), *Pocillopora damicornis* (brooder) and *Tubastrea falkneri* (ahermatypic), before and after coral mass spawning to determine if and through which drivers coral microbial communities changed through this event. A contemporary 454 sequencing approach was implemented and results revealed distinct bacterial shifts through coral mass spawning for all corals, independently of reproductive activity. Clear changes in bacterial assemblages were also detected for brooders after planulation. This infers that coral-associated microbial communities change through a coral mass spawning event and are likely driven by environmental factors and the respective bacterial community in the seawater, as well as by actual coral reproduction. Differences in coral-microbial communities reflected different life styles between brooding and spawning corals. Most  $\alpha$ -*Proteobacteria* increased in abundance after spawning as well as after planulation, suggesting that specific bacteria are involved in coral reproduction irrespective of reproductive strategies; particularly bacteria affiliated with the *Roseobacter* clade followed this pattern.

The assessment of seawater collected from the broadcast spawning coral *A. tenuis* and *P. damicornis* after spawning and planulation, respectively revealed that adult corals, irrespective of their reproductive strategy release bacteria with their offspring which likely increases the fitness in the following processes involved in settlement and survival. Species affiliated with the genera *Roseobacter* and *Alteromonas* appear to play important roles in coral reproduction and early life history in corals.

Isolates from *P. damicornis* planulae were mainly affiliated with the genera *Vibrio* and *Alteromonas* and were found to be similar to bacteria released by the mother colony during planulation.

Finally the establishment of coral-microbial partnerships in coral larval stages and the potential role of these symbiotic relationships were studied. The early onset of bacterial associations

in brooding and broadcast spawning corals was visualized, exploring bacterial presence and their location in the coral organism, determining when and how bacteria enter coral tissues and their cycling of nutrients towards the coral-symbiotic algal partners. Nano-scale Second Ion Mass Spectrometry (SIMS) was applied to detect, image and map the uptake and translocation of  $^{15}\text{N}$  from bacteria into coral larvae on a sub-cellular level. The study also combined Fluorescent *In Situ* Hybridisation (FISH) to co-localize the labelled substrate with bacteria and Transmission Electron Microscopy (TEM) to allow for ultra-structural resolution images to provide high resolution images. This study for the first time demonstrated the beneficial role of specific bacteria in translocating nitrogen into the coral holobiont, which is particularly important in the nutrient-poor environments corals live in.

# Acknowledgements

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I'm feeling privileged to have met and worked with some amazing people and it is my pleasure to express my gratitude to everyone who made this PhD-adventure possible and so enjoyable!

I'm incredibly grateful to my wonderful supervisors and friends Mike van Keulen and David Bourne. Their unconventional ways and 'nothing is impossible attitude' opened a lot of space for creativity and 'out of the box thinking'. Thanks for sharing your knowledge, resources and great times.

Thanks beyond words go to my friend Jean-Baptiste Raina for exposing me to his generosity, humor, honest opinions, statistics wizardry, excellent taste of music and for sharing my passion for science and coral reefs. I loved sharing a lab bench with you – even though you constantly 'displaced' my lab equipment.

I also want to thank people at Murdoch University for their patient assistance, Graham O'Hara, Gordon Thompson, and Michael Taylor. Special thanks go to Leila Eshraghi for sharing her lab space when I was in desperate need of some and for being such a nice person.

I'm happy to express my appreciation for all my volunteers in the field: Joetta Perrett, Sam Wells, Kim Mars, Swaantje Bennecke and Bernard O'Reilly. Thanks to Bruce Portman for letting me transform his beautiful yacht Pi Phrontis into a research vessel for a while. I specially want to thank Frazer McGregor, who not only provided his incredible knowledge and time to take me to suitable sampling sites, but also showed me amazing snorkeling spots and introduced me to some of his wondrous manta ray friends. Thanks Frazer – you're awesome!

I also want to thank my colleagues at the Australian Institute of Marine Science: Sebastian Schmidt and Andrew Negri & team for providing coral larvae, Rochelle Soo and Andy Miurhead for help in the lab, Beth Ballment for making me wear my lab coat, Nicole Koestner who volunteered counting thousands of coral larvae and Kim Lema and Eneour Puill-Stephan for introducing me to the wonderful world of kite surfing.

Thank you to everyone at the Centre of Microscopy and Characterization (University of Western Australia): Lyn Kirilak for her patience and sharing little microscopy secrets, John Murphy for helping with sections and keeping me entertained with interesting conversations about literature, cinematography and traveling, Peter Duncan for his constant good mood, Jeremy Shaw and Peta Clode for scientific advice, and the great NanoSIMS team: Matt Kilburn for his kindness, wit and intelligence and John Cliff for sharing his broad scientific knowledge and providing the needed cynicism to foresee reviewers' criticism. I truly enjoyed working with you.

I would also like to express my gratitude to people at the Wagner lab (Department of Microbial Ecology) for inviting me to conduct my FISH work in Vienna; special thanks to Markus Schmidt, Christoph Boehm and Faris Behnam for help in the lab and to Michi Wagner for fruitful advice and conversation.

The inspiration for this research was sparked by the work of Eugene Rosenberg, and Yossi Loya who exposed me to it. Thanks for that!

This research was made possible by the support of Murdoch University, the Australian Institute of Marine Science, the Western Australian Marine Science Institution and the Australian Microscopy & Microanalysis Research Facility.



Thanks for providing cozy work space, delicious food, great coffee and suitable entertainment through the last weeks of my thesis writing go to cafes in Fremantle: the X-Wray, the Wild Poppy and the Ootong and Lincoln; and thanks to my former housemate David Robertson for serenading through the chimney in the endless hours when I was blasting my sequences, and to an anonymous neighbour for so generously sharing their wireless internet.

Much appreciation goes to my parents Franc & Milka Ceh who planted the seeds of curiosity in me and to my brother Michael Ceh for all the laughter.

Finally I would like to thank Bernie O'Reilly – without you and all our little adventures life wouldn't be as much fun!

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