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

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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.

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Abstract

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, A*cropora tenuis* (broadcast spawner), *Pocillopora damicornis* (brooder) and *Tubastrea faulkneri* (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 *α-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}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.

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