

Micro-scale measurements of marine microbial interactions with global scale consequences

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Certificate of Original Authorship

I, Marco Giardina, declare that this thesis is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Life Sciences at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise reference or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

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I have changed a lot throughout the course of my PhD, but one aspect of my personality that hasn't changed (and at this point I guess it never will) is that I do not go straight to the point when I speak/write because I like to start from very...VERY FAR.

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Declaration of the contribution to each chapter

Chapter 2

MG, JRS, MP and JBR conceived and designed the study; MG, PG and PLC carried out the NanoSIMS data acquisition; SC and MG carried out the ToF-SIMS data acquisition; CM: carried out the peak deconvolution; MG and RP carried out the EA-IRMS data acquisition. MG and JBR drafted the manuscript. All authors read and approved the final manuscript.

Chapter 3

MG, JRS, MP and JBR conceived and designed the study; MG performed the experiments; MG, PG, MK and PLC carried out the NanoSIMS data acquisition; MG analysed the data and did the statistics. MG drafted the manuscript. All authors read and approved the final manuscript.

Chapter 4

MG, JRS, JBR, SS and RS conceived and designed the study; MG performed the laboratory experiments; MP and SS provided support in setting up the experiment; MG, PG and PLC carried out the NanoSIMS data acquisition; ES and MU developed and provided the *M. adhaerens* strains; DRB developed the theoretical model; UK performed the IRMS analysis; MG analysed the data and tested them statistically. MG, JBR, DRB and JRS drafted the manuscript. All authors read and approved the final manuscript.

Summary

Interactions between marine phytoplankton and heterotrophic bacteria are emerging as key ecological processes that control marine biogeochemical cycles and ecosystem productivity. While these interactions have large-scale implications, they are generally played out across very small spatiotemporal scales and often involve intimate ecological relationships involving the exchange of a diverse suite of metabolites and infochemicals. Previous studies have focussed on the ecological relationships between heterotrophic bacteria and large phytoplankton cells, such as diatoms and dinoflagellates, however, the photosynthetic biomass across much of the global ocean is dominated by picocyanobacteria, mainly comprising two genera, *Prochlorococcus* and *Synechococcus*. It has recently been suggested that the nitrogen-rich exudates of *Synechococcus* may be consumed by heterotrophic bacteria, potentially establishing metabolic, and eventually physical interactions. Yet, due to extremely small size of both partners (0.8-2 μm), it is extremely challenging to observe and quantify their metabolic exchanges at the single-cell level using conventional methods. This means that some of the ecological and biogeochemical consequences of these interactions have potentially been overlooked until now. Recently, technological breakthroughs in high-resolution single-cell imaging techniques, such as Secondary Ion Mass Spectrometry (SIMS), have opened the door for studying microbial associations at relevant scales, allowing for more accurate quantification of their impact on nutrient cycling and oceanic productivity.

This thesis focused on the associations between the picocyanobacteria *Synechococcus* and heterotrophic bacteria, I applied a combination of stable isotope labelling approaches and SIMS to study the metabolic exchanges and the behavioural mechanisms underpinning the onset of the interaction between these two partners, at the single-cell level. First, I compared bulk-scale mass spectrometry with two SIMS techniques (NanoSIMS and ToF-SIMS) to define their advantages and limitations in measuring nutrient uptake at both community and single-cell level. After determining that NanoSIMS was the most suitable tool to investigate *Synechococcus*-heterotrophic bacteria interactions, I applied this technique to determine if nutrient exchanges between *Synechococcus* and two of its culture-associated bacterial isolates were reciprocal. Finally, I determined the role that bacterial behaviour may have on the exploitation of *Synechococcus*-derived nutrients.

This thesis demonstrates the single-cell variability and heterogeneity of the nutrient uptake and cycling between these small and ubiquitous marine microbes, this observed heterogeneity would have been completely missed by large-scale approaches. The associations between *Synechococcus* and different bacterial species lead to species-specific differences in nutrient exchanges. Cells can access significantly more *Synechococcus* derived nutrients by means of physical attachment and despite the small size of *Synechococcus* cells, this association is likely mediated by bacterial behaviour such as chemotaxis. The dynamics that determine these single-cell microbial interactions can have vast implications for global-scale processes.