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

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February 2019

A thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy in Science

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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.

This research was supported by an Australian Government Research Training Program and an Australian Research Council Discovery Grant DP140101045

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Date: 19/02/2019

Acknowledgements

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.

In fact, I would like to start my acknowledgements thanking **Manuela Coci** and **Gianluca Corno,** my two Master's supervisors, who five years ago introduced me to aquatic microbial ecology and encouraged me, at the end of my Master's degree, to think about doing a PhD. More importantly, they showed me, for the first time, that science does not have frontiers and that mainly young scientist must be involved to the scientific community. In addition, it is thank to their letter of reference that today I am writing these words, close to the submission of my PhD thesis.

Now I go straight to the point, promised.

I am very grateful to my two PhD supervisors, **Justin Seymour** and **Jean-Baptiste Raina,** for having done such a great job with me. They had always time for me despite their very busy schedule. I thank them for having made me feel always comfortable and positive, without never letting me down even when problems rose up. I also thank both of them for always encouraging me to aim high and take all the good opportunities I encountered during my PhD, and I will encounter in my career.

Particularly, I thank **Justin** for taking me on board despite that 250 words "proposal" I presented him when I started the application process for my PhD. Since I joined the Ocean Microbiology Group, he has been a guide for me.

I am also thankful to **JB**, for the huge patience in teaching me all I know about working in the lab and for the late nights spent helping me out with writing, preparing posters and experiments.

Other people have contributed to my PhD thesis.

In particular, I would like to thank greatly **Mathieu Pernice** for his invaluable scientific support and for his positive attitude in approaching science and life that was essential for me in time of troubles.

I am also thankful to **Peta Clode** who made me feel at home every time I was in Perth. In particular, for having facilitated my work while I was there and to provide always new solution when the experiments were not successful. I also thank her for the beers we had after full days of work.

I would like also to thank **Paul Guagliardo** for the great support provided with the NanoSIMS analysis and for gradually teaching me how to do the analysis by myself.

From the Centre of Microscopy, Characterization and Analysis I would also like to thank **Matt Kilburn, Jeremy Shaw, Lyn Kirilak, John Murphy.**

I am also grateful to **Doug Brumley** for his great enthusiasm and effort put in our collaboration.

It was a pleasure to do my PhD at the Climate Change Cluster C3. It was the perfect environment for me to develop as scientist and personally. The team is very strong and I would like to acknowledge in particular the technical staff that made the impossible possible: **Paul Brooks**, **Graeme Polewesky, Gemma Armstrong**, **Lucia Bennar**, **Sue Fennech.** Also thanks to the director of C3, **Peter Ralph**, for having made of C3 a very dynamic and productive work environment.

I also thank **Anita Giraldo, Stefano Aragone, Zouzou** and **Kate Eiloart** for giving me hospitality in Perth and the great time spent together.

A special thanks to all the people that supported me in editing and formatting my thesis: **Caitlin Lawson**, **Sammy Goyen**, **Kirsty Milner**, **Dave Hughes**, **Deepa Varkey, Mahrita Harahap and Steven Woodcock**.

I also want to thank all of my friends in Australia, my old friends in Italy and all those spread around the World for the support and the good times (I would need other 121 pages to acknowledge you all).

Voglio ringraziare mio zio **Fulvio** per avermi consigliato saggiamente quando mi sono trovato di fronte a delle scelte professionali: e' grazie a lui che sono venuto in Australia nel momento giusto. Ringrazio anche mio fratello **Stefano** e mia zia **Gabriella** per essere sempre presenti nei momenti importanti della mia vita.

Ringrazio **Oriana** per avermi sostenuto ed avermi aiutato ad alleviare lo stress in questi ultimi due anni di dottorato.

Infine, il piu' grosso ringraziamento va' ai miei genitori: dedico a loro questa tesi.

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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.