Molecular and cellular investigations of photosynthesis driven carbon fixation in the seagrass *Zostera muelleri*

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PhD by Research

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CERTIFICATE OF ORIGINAL AUTHORSHIP

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

This thesis is wholly my own work unless otherwise referenced 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 is supported by an Australian Government Research Training Program.

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Preface

This thesis has been prepared for submission as a thesis by compilation, whereby the thesis contains a combination of published and publishable work. As a result, there is a degree of repetition across chapters, particularly within the introductions and materials and methods sections of Chapter 2, 3 and 4. Published works have been incorporated into this thesis and appear as they were presented to the journal immediately prior to publication with the following alterations: i) the font and format was changed so as to maintain consistency across the thesis, ii) figures and tables were re-numbered to reflect the chapter numbering and iii) supplementary information for each chapter appear in the appendix and have been re-numbered accordingly. The referencing format used throughout this thesis conforms to the requirements of the journal; Photosynthesis Research.

List of publications included in the thesis:

Chapter 2:

Low oxygen affects photophysiology and the level of expression of two-carbon metabolism genes in the seagrass *Zostera muelleri*

Mikael Kim, Kasper Elgetti Brodersen, Milán Szabó, Anthony W.D. Larkum, John A. Raven, Peter J. Ralph and Mathieu Pernice

Published on the 4th of October, 2017 in the journal 'Photosynthesis Research', volume: 136, issue 2, page: 147-160

Contributions

MK, KEB, MS, AWDL, JAR, PJR and MP designed the experiment; MK and MP carried out the experiment; MK, KEB and MS performed the microsensor analysis; MK and MP performed the RTqPCR analysis; MK wrote the manuscript with contributions from all authors.

Chapter 3:

Effect of reduced irradiance on ^{13}C uptake, gene expression and protein activity of the seagrass *Zostera muelleri*

Mikael Kim, Mathieu Pernice, Alexander Watson-Lazowski, Paul Guagliardo, Matt R. Killburn, Anthony W.D. Larkum, John A. Raven and Peter J. Ralph

Published on the 6th of June, 2019 in the journal 'Marine Environmental Research', volume: 149, page: 80-89

Contributions

MK, MP, AWDL, JAR and PJR designed the experiment; MK and MP carried out the experiment; MK and MP performed the nanoSTRING analysis; MK and AWL performed the enzyme assays; PG and MRK performed the nanoSIMS analysis; MK wrote the manuscript with contributions from all authors.

Another article was published in association with my PhD, however it does not form a part of this thesis. The title page of this publication is included in the appendix of this thesis:

Development of an efficient protein extraction method compatible with LC-MS/MS for proteome mapping in two Australian seagrasses *Zostera muelleri* and *Posidonia australis*

Zhijian Jiang, Manoj Kumar, Matthew P. Padula, Mathieu Pernice, Tim Kahlke, Mikael Kim and Peter J. Ralph

Published on the 15th of August, 2017 in the journal 'Frontiers in Plant Science', volume: 8, article no. 1416

Contributions

ZJ, MaK, MP, and PR conceived and designed research. ZJ, MaK, and MPP performed 2D-IEF and protein identification using LC-MS/MS and analyzed the data. PD and MiK performed Western Blot analysis while kindly providing the primary and secondary antibodies, and standard for PEPC enzyme. MaK, ZJ, and MPP, wrote manuscript. TK assisted in bioinformatics analysis. PR revised and edit the manuscript. All authors read and approved the manuscript.

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Thesis abstract

Seagrasses are marine foundation species that have evolved to live in shallow coastal waters, an environment regularly subjected to changes through tidal patterns and anthropogenic activity. Global seagrass coverage is declining worldwide, mainly driven by decreasing water quality due to anthropogenic activity. Therefore, there is an urgent need to better understand seagrass biology, particularly the way their growth will be impacted by changing environments in order to assist their preservation. Through the use of emerging techniques such as gene expression analyses, the underlying mechanism driving photosynthesis can now be explored at the molecular level and is an emerging field in seagrass research.

The overarching aim of my thesis was to identify the molecular mechanisms driving carbon metabolism in the seagrass *Zostera muelleri.* To accomplish this aim, a combination of physiological and molecular analytical tools were used to measure changes in photosynthesis in response to decreased O_2 concentrations in the water column, decreased irradiance and chemical inhibition of carbonic anhydrase (CA).

The results presented in this thesis describe the ways in which genes associated with carbon metabolism were regulated in response to the different environmental factors as listed above. Specifically, we found that in low O_2 conditions, photosynthetic efficiency was enhanced while genes involved in the photorespiratory and recycling of TCA cycle intermediates were down-regulated. In addition, we found that under reduced irradiance conditions, ^{13}C uptake was reduced and this correlated with a down-regulation of genes involved in photosynthetic, photorespiratory and recycling of TCA cycle intermediates pathways. Finally, when exposed to inhibition of CA, *Z. muelleri* plants displayed a decrease in photosynthetic rate concomitant with up-regulation of photosynthetic and photorespiratory genes, suggesting the presence of a compensatory mechanism in this seagrass specie in order to overcome CA inhibition. In view of these results, we provide further details to the

carbon metabolism pathways in *Z. muelleri* and presents new techniques in molecular analysis that can be applied to different areas of seagrass research.