

# 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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March 2020

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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.

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## Acknowledgements

I'd like to thank my supervisors: Peter J. Ralph, John A. Raven and Mathieu Pernice for their guidance, support, patience and criticisms. I would also like to thank my co-authors: Kasper Elgetti Brodersen, Paul Guagliardo, Matt R. Kilburn, Anthony W. D. Larkum, Alexander Watson-Lazowski, and Milán Szabó for their contributions, without which the published work contained in this thesis would not have been possible. I'd also like to thank Paul Brooks and UTS technical staff for helping me with the various training and inductions, your help in preparing for each experiment has not been taken for granted. To all the people I worked with along the way, thank you all for your help throughout my PhD. Finally, thank you to my friends and family, the completion of this thesis was only possible as a result of your continued support.

## 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 4<sup>th</sup> 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  $^{13}\text{C}$  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 6<sup>th</sup> 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 15<sup>th</sup> 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.

## Table of Contents

CERTIFICATE OF ORIGINAL AUTHORSHIP .....	i
Acknowledgements.....	ii
Preface .....	iii
List of publications included in the thesis:.....	iii
List of Figures and Tables.....	ix
Thesis abstract .....	xvi
Chapter 1: Thesis introduction .....	1
Seagrass adaptations to the marine environment .....	1
Ecological roles and ecosystem services provided by seagrass.....	2
Seagrass decline.....	3
Molecular biology of seagrasses.....	5
Photosynthetic biochemistry of seagrass .....	6
CO <sub>2</sub> concentrating mechanisms in seagrasses.....	7
C <sub>3</sub> and C <sub>4</sub> photosynthesis .....	11
Molecular investigation of photosynthesis in seagrass .....	12
<i>Zostera muelleri</i> subsp. <i>capricorni</i> ( <i>Z. muelleri</i> ) as an Australian model organism .....	13
Aims of the thesis.....	15
References .....	16
Chapter 2: Low oxygen affects photophysiology and the level of expression of two carbon metabolism genes in the seagrass <i>Zostera muelleri</i> .....	30
Abstract.....	32

Introduction .....	33
Material and methods .....	39
Results.....	48
Discussion.....	54
Conclusion.....	60
References .....	61
Chapter 3: Effect of reduced irradiance on $^{13}\text{C}$ uptake, gene expression and protein activity of the seagrass <i>Zostera muelleri</i> .....	
	72
Abstract.....	74
Introduction .....	75
Material and methods .....	78
Results.....	86
Discussion.....	93
Conclusion.....	102
References .....	104
Chapter 4: Effect of carbonic anhydrase inhibitors on photosynthesis, $^{13}\text{C}$ enrichment and gene expression in the seagrass <i>Zostera muelleri</i> .....	
	115
Abstract.....	116
Introduction .....	117
Materials and methods.....	120
Results.....	126
Discussion.....	130



Conclusion.....	136
References .....	138
Chapter 5: Synthesis, limitations, perspective research and concluding remarks .....	148
Synthesis .....	149
Limitations .....	154
Prospective research and concluding remarks .....	161
References .....	163
Appendix 1: Low oxygen affects photosynthesis and the level of expression of two carbon metabolism genes in the seagrass <i>Zostera muelleri</i> .....	168
Supplementary Figures and Tables 1 .....	170
Appendix 2: Effect of reduced irradiance on <sup>13</sup> C uptake, gene expression and protein activity of the seagrass <i>Zostera muelleri</i> .....	182
Supplementary Figures and Tables 2 .....	183
Appendix 3: Effect of carbonic anhydrase inhibitors on photosynthesis, <sup>13</sup> C enrichment and gene expression in the seagrass <i>Zostera muelleri</i> .....	187
Supplementary Figures and Tables 3 .....	187
Appendix 4: Synthesis, limitations, perspective research and concluding remarks.....	190
Supplementary Figures and Tables 4 .....	190
Appendix 5: Further contributions.....	198
Development of an efficient protein extraction method compatible with LC-MS/MS for proteome mapping in two Australian seagrasses <i>Zostera muelleri</i> and <i>Posidonia australis</i> .....	198

## List of Figures and Tables

Figure 1. 1. Global distribution of seagrasses (blue points) relative to geographic bioregions 1; Temperate North Atlantic, 2; Tropical Atlantic, 3; Mediterranean, 4; Temperate North Pacific, 5; Tropical Indo-Pacific and 6; Temperate Southern Oceans (Short et al. 2007). .....	1
Figure 1. 2. Conceptual diagram of the photosynthesis light reactions. Photosynthetically active radiation (PAR) of wavelengths 680 nm excites the protein complex P680 in photosystem II producing a strong oxidant that oxidises water, exciting electrons. These electrons are released and transported down the electron transport chain. PAR of wavelengths greater than 680 nm excites the protein complex P700 in photosystem I producing a strong reductant which reduces NADP <sup>+</sup> to produce NADPH. This system forms the basis of photosynthetic electron transport (Taiz and Zeiger 2003). .....	7
Figure 1. 3. Conceptual diagram illustrating a potential CCM located on the outer surface of the epidermal cell wall or cuticle. P1 represents a CA dependent CO <sub>2</sub> acquisition mechanism, P2 represents an H <sup>+</sup> /HCO <sub>3</sub> <sup>-</sup> symport mechanism and P3 represents a CA linked HCO <sub>3</sub> <sup>-</sup> mechanism carrying CO <sub>2</sub> into the cytoplasm. CA-1, CA-2, CA-3 and CA-4 are carbonic anhydrases (Larkum et al. 2006). .....	9
Table 1. 1. Studies on seagrass photosynthesis in response to cell impermeable/cell permeable CA inhibitors: AZ and EZ respectively and TRIS buffer. Presence or absence of AZ, EZ or TRIS use in references cited are indicated by Y or – respectively. ....	9
Figure 1. 4. Geographical distribution of <i>Z. muelleri</i> . Extant populations (indicated in orange) have been located in Papua New Guinea, New Zealand and Australia. Image taken from <a href="https://www.iucnredlist.org/">https://www.iucnredlist.org/</a> .....	15
Table 2. 1. Reference genes and target genes investigated in <i>Zostera muelleri</i> by using RT-qPCR. Accession numbers of the closest sequence matches available online in the data repository for <i>Zostera marina</i> EST ( <a href="http://drzompo.uni-muenster.de/">http://drzompo.uni-muenster.de/</a> ) primers sequences, amplicon length, melting	

temperature, geometric mean of cycle threshold (CT) and RT-qPCR efficiency are indicated. GADPH: Glyceraldehyde 3-phosphate dehydrogenase; EloF: Translation initiation factor 1 subunit beta; Calmodulin; TubB: Tubulin beta-1 chain; Actin; PolyA: Poly(A) RNA polymerase; S4: 30S ribosomal protein S4; PEPC-1: Phosphoenolpyruvate carboxylase – isoform 1; PEPC-2: Phosphoenolpyruvate carboxylase – isoform 2 and  $\gamma$ -CA:  $\gamma$  Carbonic anhydrase. .... 45

Figure 2. 1. Vertical  $O_2$  concentration micro-profiles measured at the surface of *Zostera muelleri* leaves. Filled circles and solid lines represent data from the control leaves (i.e. leaves exposed to a water-column  $O_2$  concentration of  $\sim 231 \mu\text{mol } O_2 \text{ L}^{-1}$ ), while open circles and dashed lines represent data from leaves exposed to low  $O_2$  conditions (i.e.  $\sim 8 \mu\text{mol } O_2 \text{ L}^{-1}$ ). Incident photon irradiances are indicated by the figure legend (i.e. 0, 25, 50, 100, 200, 500 and  $700 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ). Error bars are  $\pm$  standard error of the mean (SEM). Y = 0 indicate the leaf tissue surface. n=3. .... 49

Figure 2. 2. Net photosynthesis of *Zostera muelleri* leaves at an incident photon irradiance of 0, 25, 50, 100, 200, 500 and  $700 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . Data points were fitted with an exponential saturation function (Webb et al., 1974) with an added respiration term, R, to account for respiration (Spilling et al., 2010). Black squares and line represent data of leaves kept in  $\sim 231 \mu\text{mol } O_2 \text{ L}^{-1}$  (i.e. control plants), while open triangles and dashed line represent data of leaves kept in  $\sim 8 \mu\text{mol } O_2 \text{ L}^{-1}$  (i.e. low  $O_2$  treatment). Error bars are  $\pm$ SEM; while statistically different values are indicated by \* (Permutation t-test,  $P < 0.05$ ). n=3. .... 50

Table 2. 2. The initial slope of the P-I curve in the light-limiting phase ( $\alpha$ ), maximum net photosynthetic rate ( $P_{\text{max}}$ ), dark respiration rate (R), compensation irradiance ( $E_c$ ) and minimum saturating irradiance ( $E_k$ ) in *Zostera muelleri* leaves exposed to water-column  $O_2$  levels of  $\sim 231 \mu\text{mol } O_2 \text{ L}^{-1}$  (control) and  $\sim 8 \mu\text{mol } O_2 \text{ L}^{-1}$  (low  $O_2$ ). Values are given as a mean  $\pm$ SEM (n=3); with their corresponding P values (Permutation t-test), where \* indicate significant difference between treatments on a 5% level. .... 51

Figure 2. 3. Normalized relative quantity (NRQ) of Phosphoenolpyruvate carboxylase (PEPC-1 isoform 1 and PEPC-2 isoform 2) and  $\gamma$  Carbonic anhydrase ( $\gamma$ -CA) in *Zostera muelleri* under control (solid

bars) and low O<sub>2</sub> conditions (open bars) relative to the two most stable reference genes: S4 and GADPH. Statistical differences in the mean are indicated with \* (Permutation t-test, P< 0.05) and error bars are ±SEM. n=4. .... 53

Figure 3. 1. <sup>13</sup>C enrichment. A: Enrichment of <sup>13</sup>C in control (solid bars) and LL (open bars) treated samples expressed in VPDB (Vienna Pee Dee Belemnite), error bars are ±1 SE, n = 4. NanoSIMS image depicting <sup>13</sup>C assimilation within the epidermal cell layer of *Z. muelleri* leaves: greyscale <sup>12</sup>C<sup>14</sup>N images (B and D) clearly display the chloroplasts (Ch) within the epidermal cells (Ep) and the corresponding <sup>13</sup>C : <sup>12</sup>C (C and E) images reflect the <sup>13</sup>C enrichment as shown via hue-saturation intensity (HSI) images. The HSI colour scale for <sup>13</sup>C : <sup>12</sup>C ranges from the natural abundance in blue (200) to enriched in pink (350). Scale bars are 10 µm in all images. .... 88

Figure 3. 2. Heatmap. Agglomerative clustered heatmap representing the z-score of the significantly DR genes: putative phosphate, pyruvate dikinase regulatory protein (PDRP), gamma carbonic anhydrase (γCA), alanine aminotransferase (ALT), serine glyoxylate aminotransferase (SGAT), glycine dehydrogenase decarboxylating (GLDC), serine hydroxymethyltransferase (SHMT), photosystem I P700 apoprotein A2 (psaA2), phosphoenol pyruvate carboxylase (PEPC) and Rubisco large subunit (RBL) across replicates in the control (C 1 – 4) and low light (LL 1- 4) treatment groups. .... 89

Figure 3. 3. Regulation of target genes. Normalized average counts of genes: putative phosphate, pyruvate dikinase regulatory protein (PDRP), gamma carbonic anhydrase (γCA), alanine aminotransferase (ALT), serine glyoxylate aminotransferase (SGAT), glycine dehydrogenase decarboxylating (GLDC), serine hydroxymethyltransferase (SHMT), photosystem I P700 apoprotein A2 (psaA2), phosphoenol pyruvate carboxylase (PEPC) and rubisco large subunit (RBL) in control (solid bars) and LL (open bars) treated samples. Error bars are ±1 standard error (SE), \*\*\* indicates P < 0.001, \*\* indicates P ≤ 0.005, \* indicates P < 0.05, n = 4. .... 91

Figure 3. 4. Enzyme content and activity. Content of Ribulose bisphosphate carboxylase/oxygenase shown as maximum rate of carboxylation (V<sub>cm<sub>max</sub></sub>) (A), and activity of phosphoenolpyruvate

carboxylase in  $\mu\text{mol HCO}_3^- \text{ m}^{-2} \text{ s}^{-1}$  (B) in Control (solid bars) and LL (open bars) treated samples. Error bars are  $\pm 1$  SE, n=3..... 92

Figure 3. 5. Conceptual diagram of gene expression. Simplified diagram of the cellular processes altered by light limitation based on our data. Reduced irradiance decreased C assimilation and down regulated gene expression, predominately those involved in photorespiration: gamma carbonic anhydrase ( $\gamma\text{CA}$ ), alanine aminotransferase (ALT), serine glyoxylate aminotransferase (SGAT), glycine dehydrogenase decarboxylating (GLDC), serine hydroxymethyltransferase (SHMT), photosystem I P700 apoprotein A2 (psaA2) and rubisco large subunit (RBL)..... 102

Figure 4. 1. Rate of photosynthesis expressed as a percentage of the control (striped bar) in response to AZ (solid bar) and EZ (open bar). Error bars are SE, \* indicates significant difference between the control,  $p < 0.05$  (n = 4)..... 126

Figure 4. 2. Content of  $^{13}\text{C}$  in control (striped bar), AZ treated (solid bar) and EZ treated (open bar) samples expressed in VPDB (Vienna Pee Dee Belemnite). Error bars are SE (n = 4)..... 127

Figure 4. 3. Normalised mean counts of differentially expressed genes: alanine aminotransferase (ALT), glycine dehydrogenase decarboxylating (GLDC), photosystem I P700 apoprotein A2 (psaA2) and serine glyoxylate aminotransferase (SGAT) in control (solid bars) and AZ (open bars) treated samples. Error bars are SE, \* indicates significant difference between the control,  $p < 0.05$  (n = 4).128

Figure 4. 4. Normalised mean counts of differentially expressed genes: light-harvesting complex II chlorophyll a/b binding protein (LHCB), glutathione synthase reductase (GSR) and putative pyruvate, phosphate dikinase regulatory protein (PDRP) in control (solid bars) and EZ (open bars) treated samples. Error bars are SE, \* indicates significant difference between the control,  $p < 0.05$  (n = 4).129

Figure 5. 1. Conceptual diagram of an epidermal cell illustrating the potential mechanisms for  $\text{HCO}_3^-$  use in *Z. muelleri*. System A represents the extracellular dehydration of  $\text{HCO}_3^-$  by a membrane bound CA; System B represents a  $\text{H}^+/\text{HCO}_3^-$  co-transporter that transports  $\text{HCO}_3^-$  into the cell where it is then dehydrated to  $\text{CO}_2$  via an intracellular CA and System C represents the localised reduction in pH

within the DBL, enhancing the dissociation of $\text{HCO}_3^-$ to $\text{CO}_2$ . Main metabolic pathways investigated in this thesis with specific genes targeted are indicated in red.....	153
Figure 5. 2. 2.0 % agarose gel depicting multiple PCR products from beta-carbonic anhydrase ( $\beta\text{CA}$ ) primers (A) and single PCR products from gamma-carbonic anhydrase ( $\gamma\text{CA}$ ) primers (B). Both primer products were expected to be 121 bp and 110 bp for $\beta\text{CA}$ and $\gamma\text{CA}$ respectively. DNA ladders (L) and negative controls (N) were added to the remaining lanes. ....	155
Figure 5. 3. Western blot detection of RbcL. Expected molecular weight was 52.7 kDa. Lanes contained: A) 1/1 dilution, B) 1/5 dilution control group and L) molecular weight ladder (kDa). ....	158
Figure 5. 4. Western blot detection of $\gamma\text{CA}$ . Expected molecular weights of 3 isoforms of $\gamma\text{CA}$ were: 9.97 kDa, 30 kDa and 27.83 kDa. Lanes contained: A) control group, B) treatment group and L) molecular weight ladder (kDa).....	158
Figure 5. 5. Fluorescence image of a <i>Z. muelleri</i> leaf containing: epidermal cells (Ep), mesophyll cells (M) and vascular bundle (V). Red autofluorescence (A), green autofluorescence (B), purple fluorescent secondary antibody specific to PEPC (C), and combined fluorescence image with amber fluorescence indicating combined autofluorescence and PEPC (D). Red bar indicates 10 $\mu\text{m}$ . ....	160
Figure 5. 6. Fluorescence image of a <i>Z. muelleri</i> leaf containing: epidermal cells (Ep), mesophyll cells (M) and vascular bundle (V). Red autofluorescence (A), green autofluorescence (B), purple fluorescent secondary antibody specific to RbcL (C), and combined fluorescence image with purple fluorescence indicating location of RbcL (D). Red bar indicates 10 $\mu\text{m}$ . ....	160
Supplementary Figure S1. 1. Experimental set up and tank replication used for low $\text{O}_2$ treatment. Control tank set up was the same (just bubbling air instead of $\text{N}_2$ and $\text{CO}_2$ ). ....	170
Supplementary Figure S1. 2. Concentration of $\text{O}_2$ in $\mu\text{mol/L}$ over the experimental period in control (crosses) and treatment (open diamonds) tanks.....	171
Supplementary Figure S1. 3. pH measurements over the experimental period in control (crosses) and treatment (open diamonds) tanks.....	172

Supplementary Figure S1. 4. Standard curves, efficiency and R <sup>2</sup> for all the reference and target genes investigated in this study: (A) SRp4: 30S ribosomal protein S4; (B) Actin; (C) PolyA: Poly(A) RNA polymerase; (D) GADPH: Glyceraldehyde 3-phosphate dehydrogenase; (E) TubB: Tubulin beta-1 chain; (F) Calmo: Calmodulin; (G) EloF1: Translation initiation factor 1 subunit beta (H) PEPC-2: Phosphoenolpyruvate carboxylase – isoform 2; (I) PEPC-1: Phosphoenolpyruvate carboxylase – isoform 1; (J) $\gamma$ -CA: $\gamma$ -Carbonic anhydrase .....	179
Supplementary Figure S1. 5. Analysis of expression stability of 7 potential reference genes using Normfinder software in <i>Zostera muelleri</i> exposed to low O <sub>2</sub> level. Average expression stability value (lower M values corresponding to more stable gene expression, as defined by Normfinder) for each candidate gene. TubB: Tubulin beta-1 chain; Calmodulin; PolyA: Poly(A) RNA polymerase; EloF: Translation initiation factor 1 subunit beta; RpS4: 30S ribosomal protein S4; Actin; GADPH: Glyceraldehyde 3-phosphate dehydrogenase .....	180
Supplementary Figure S1. 6. Maximum quantum yield of photosystem II (F <sub>v</sub> /F <sub>m</sub> ) of leaves: 1 (diamonds), 2 (squares) and 3 (triangles) over the microsensor experimental period of 48 hours as measured by PAM-fluorometry. ....	181
Supplementary Figure S2. 1. Rapid light curves. Relative electron transport rate measured immediately prior to low light treatment (A) and after 48 h of low light treatment (B). Black squares represent the control group and red circles represent low light treatment group. Error bars are SE, n=4. ....	184
Supplementary Table S2. 1. Details of target genes. NanoSTRING Reporter and Capture probes were labelled using their Gene identifiers and Accession number, and designed to target a specific sequence. ....	184
Supplementary Table S3. 1. Details of reference genes and target genes. NanoSTRING Reporter and Capture probes were labelled using their Gene identifiers and Accession number and designed to target a specific sequence. ....	187

Supplementary Table S4. 1. Details of all 42 genes included in all NanoString analyses. Reporter and Capture probes were labelled using their Gene identifier and Accession number and designed to target the specified sequence at the specified position. Reference genes are indicated by \*..... 190



## 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<sub>2</sub> 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<sub>2</sub> 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, <sup>13</sup>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.