Adaptive strategies of carbon transformation amongst coral symbionts (Symbiodiniaceae)

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PhD by Research

Submitted in fulfilment of the requirements for

The degree of Doctor of Philosophy

Climate Change Cluster

School of Life Sciences

University of Technology Sydney

July 2020

Certificate of original authorship

I, Mickael Ros declare that this thesis is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the Faculty of Science at the University of Technology Sydney.

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In addition, I certify that all information sources and literature used are indicated in the thesis.

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Date: 10th of July 2020

Acknowledgements

First and foremost, I would like to express my sincerest thanks and gratitude to my supervisor David Suggett, who with my co-supervisor Emma Camp agreed to accept me in his team for my PhD journey. I still remember our Skype interview! Thank you for your patience and your unfailing availability in preparing my arrival at UTS and overcome the multiple challenges inherent in moving to another country. Thanks to both of you for your support in all our weekly meetings to check if I was on track and continuously provide feedback on my progress.

I would then like to thank David Hughes, who was always patient and available in helping me lifting the veil on the arcane processes of photochemistry. You helped me go beyond my PhD duties and taught me how to be a (hopefully good!) aquarist, an activity that I truly enjoy and served as a means to get my mind off things when experiments or writing did not go according to the plan. Thank you for your support and your open-mindedness that are characteristic of a genuine friend.

Thank you to the past and present Future Reefs group members: Risa, Sam, Caitlin, Nerissa, Jen, Trent, Rachel, Sage, Amanda and Isabel in providing thoughtful insights regarding the questions I had and their help in the lab, and in organising well sought after lab dinners to get everyone together.

I want to address special thanks to the C3 technical staff, Paul Brooks who was always here to provide his assistance when everything was going wrong in the lab and helped me set my experiments up; and Susan Fenech for her patience and involvement in teaching me how to use and optimise the automated microscope and the TCN analyser.

Special thanks to my parents and family – for which it has been probably harder than for me to move overseas for these three and half years – for their emotional support and for being strong at all times and believing in me no matter what. I hope this document will make you proud!

Last but not least, I would like to thank Princes, my wife, who always has been there for the whole duration of this journey, who did her best to understand what it takes to do a PhD, and always cheered me up day after day. After this, a page will be turned, and I really look forward to starting the next one – our life together!

Table of contents

List of Figures

Chapter 1

Figure 1.6. Outline of the production and fate of reactive oxygen species (ROS) in a Symbiodiniaceae cell under ambient (**a**), and stressful (**b**) temperature and light conditions. Photosystem I (PSI), photosystem II (PSII), Calvin-Benson cycle (CBC), protein D1 (D1), superoxide dismutase (SOD), ascorbate peroxidase (APX), singlet oxygen ($^1O_2$), superoxide radical (O_2^-), hydroxyl radical (•OH), hydrogen peroxide (H_2O_2), ferrous ion (Fe^{2+}). Yellow flashes represent photoinhibition and damages to cellular pathways. They occur on D1 protein activity (Warner et al. 1999), thylakoid membranes (Tchernov et al. 2004), and RuBisCO (Venn et al. 2008). Modified from Weis et al. (2008).

Chapter 2

Figure 2.1. Schematic summarising carbon transfers and mechanisms occurring between the Symbiodiniaceae algae and their surrounding environment (here as free-living in culture). **(1)** Uptake. **(2)** Carboxylation. **(3)** Recycling of internal pools. **(4)** Respiration. **(5)** Active excretion or passive leakiness. **(6)** Heterotrophy. **(a)** Long-term storage for growth net carbon production (NPC) as the product of particulate organic carbon (POC) and growth rate (μ × POC). **(b)** Short-term storage for maintenance as gross carbon production (GPC, as ^{14}C fixed). POC: Particulate organic carbon. iDOC: Internal dissolved organic carbon. eDOC: External dissolved organic carbon. Red frames show the parameters measured in the study. Solid lines represent established pathways, and dashed lines represent hypothesised pathways.

Figure 2.2. Carbon fluxes (as ^{14}C) occurring within the 23 Symbiodiniaceae isolates after 1h of photosynthesis at 26°C. Data shown is the mean (n = 3) ± standard deviation for each Symbiodiniaceae isolate. Isolates are grouped by genus; *Symbiodinium* (blue), *Breviolum* (orange), *Cladocopium* (grey), *Durusdinium* (yellow), *Effrenium* (red), and *Fugacium* (green). **(a)** The gross carbon production (GPC) rates (pg C [cell h]$^{-1}$), where the upper bar is the rate of inorganic carbon fixation (C_{fix}) and the lower bar is the rate of organic carbon excretion in the media (as eDOC, C_{exc}). Relationships between the cell volume (μm^3) and **(b)** the percentage ($\%C_{fix}$) of GPC retained in the cell (i.e. C_{fix}/GPC × 100); and **(c)** the percentage ($\%C_{exc}$) of GPC excreted in the media (i.e. C_{exc}/GPC × 100).

Figure 2.3. Carbon productivity of the 23 Symbiodiniaceae isolates grown at 26°C. Data shown is the mean (n = 3) ± standard deviation for each Symbiodiniaceae isolate. Isolates are grouped by genus; *Symbiodinium* (blue), *Breviolum* (orange), *Cladocopium* (grey), *Durusdinium* (yellow), *Effrenium* (red), and *Fugacium* (green). **(a)** The net carbon production (NPC), as the product of particulate organic carbon (POC) and growth rate, normalised per illumination time (12h). **(b)** Relationship between gross carbon production (GPC) and NPC, where the black line represents the 1:1 ratio between the two variables. Each dot represents the mean (n = 3) data of one isolate. Error bars were not shown for clarity of the representation but can be found in the Supplementary Table S2.3.

Figure 2.4. Functional groupings based on measured factors associated with inorganic carbon uptake and utilisation (cell volume, C_{fix}, C_{exc} and NPC) across all 23 Symbiodiniaceae isolates grown at 26°C. Cluster analysis and principal component analysis (PCA) were performed on the average of each variable (n = 3) per isolate. Axes X and Y represent the first and second component of the PCA, respectively. Similarity at 95% level is shown at the 1.5 (red line) and 2 (dashed blue line) Euclidean distance levels, and vectors driving the clustering are shown in black. Isolates are grouped by genus for clarity; *Symbiodinium* (blue), *Breviolum* (orange), *Cladocopium* (grey), *Durusdinium* (yellow), *Effrenium* (red), and *Fugacium* (green).

Figure 2.5. Physiological parameters of the Symbiodiniaceae *Cladocopium* (C1-SCF124), *Durusdinium* (amur-D-MI) and *Effrenium* (E-421) isolates grown at 20°C, 26°C and 30°C. Data shown is the mean (n = 3) ± standard deviation for each Symbiodiniaceae isolate. Isolates and temperatures of growth are represented as: C1-SCF124 (grey), amur-D-MI (yellow), E-421 (red), 20°C (vertical hatches), 26°C (plain bars), and 30°C (dotted bars). **(a)** Growth rates (d^{-1}) of the three isolates. **(b)** Cell volumes (μm^3) of the three isolates. **(c)** Gross carbon production (GPC) rates (pg C [cell h]$^{-1}$), where the upper bar is the rate of inorganic carbon fixation (C_{fix}) and the lower bar is the rate of organic carbon excretion in the media (as eDOC, C_{exc}). **(d)** Percentage (%C_{exc}) of GPC excreted in the media (i.e. C_{exc}/GPC × 100). **(e)** Relationship between gross and net carbon production (respectively GPC and NPC; pg C [cell h]$^{-1}$), where the black line represents the 1:1 ratio between GPC and NPC. Each dot represents the mean (n = 3) data of one isolate grown at one temperature: 20°C (white-centre shapes), 26°C (regular shapes), and 30°C (black-bordered shapes). Error bars are not shown for clarity but are provided in Supplementary Table S2.10.

Figure 2.6. Functional groupings based on measured factors associated with inorganic carbon uptake and utilisation (cell volume, C_{fix}, C_{exc} and NPC) of the Symbiodiniaceae *Cladocopium* (C1-SCF124), *Durusdinium* (amur-D-MI) and *Effrenium* (E-421) isolates grown at 20°C, 26°C and 30°C. Cluster analysis and principal component analysis (PCA) were performed on the average of each variable (n = 3) per isolate. Axes X and Y represent the first and second components of the PCA, respectively. Similarity at 95% level is shown at the 1.5 (red line) and 2 (dashed blue line) Euclidean distance levels, and vectors driving the clustering are shown in black. Isolates and temperatures of growth are represented as: C1-SCF124 (grey), amur-D-MI (yellow), E-421 (red), 20°C (white-centre shapes), 26°C (regular shapes), and 30°C (black-bordered shapes).

Figure 2.7. Relationship between gross carbon production and net carbon production ratios (GPC:NPC) and O_2 consumption rates (fmol O_2 [cell h]$^{-1}$) for the Symbiodiniaceae *Symbiodinium* (A1-61 and A13-80) and *Breviolum* (B1-2) isolates grown at 26°C. Data shown is the mean (n = 3) ± standard deviation for each Symbiodiniaceae isolate. GPC:NPC ratios are calculated from the present study, whilst O_2 consumption rates are retrieved from Brading et al. (2011). The linear relationship between the two sets of variables is described by the equation GPC:NPC = (- 0.000461 × O_2 consumption rate) + 0.347. $R^2 = 0.996$, $P = 0.039$ (see Supplementary Table S2.14).

Chapter 3

Figure 3.1. Inorganic **c**arbon (Ci) uptake (bars; pg C [cell.h]$^{-1}$) of the three studied Symbiodiniaceae isolates (**a**) B1-UTS-B: *Breviolum minutum*, (**b**) C1-SCF124: *Cladocopium goreaui*, and (**c**) amur-D-MI: *Durusdinium trenchii* as labelled ^{14}C after 20 min of photosynthesis, matched with the photochemical efficiency of their PSII (lines; F_v/F_m) the day of the incubation. Control (white bars and dots ○) stayed at 26°C for the duration of the experiment. Treatment (grey bars and black dots ●) consisted of acclimation phase at 26°C (T0), ramping phase from 26°C to 32°C in two days (TI), and stress phase at 32°C for seven days (TE).

Figure 3.2. Venn diagrams illustrating the outcome of the analysis targeting 156 functional proteins of interest, returning **(a)** 76 total functions found in the extracted transcripts and **(b)** 54 functions encoded by differentially expressed genes (DEGs; log2 FC ≥1; FDR ≤ 0.05) of the three studied Symbiodiniaceae species (*Breviolum minutum*, *Cladocopium goreaui*, and *Durusdinium trenchii*) between the control (26°C) and treatment at the end of the experiment (TE, after seven days at 32°C). For both **(a)** and **(b)**, the number of gene isoforms is indicated between brackets (number of functions can be found in Table 3.1, list in Supplementary Table S3.1). Numbers over superposed circles correspond to the number of the conserved functions (and similar isoforms) expressed between the species that were allocated to the said circles. The list of commonly expressed transcripts associated to their functional groups can be found in Supplementary Table S3.9.

Figure 3.3. Number of functional gene isoforms of interest found to be differentially down- (purple) or up-regulated (yellow) in the three studied Symbiodiniaceae isolates (**a**) B1-UTS-B: *Breviolum minutum*, (**b**) C1-SCF124: *Cladocopium goreaui*, and (**c**) amur-D-MI: *Durusdinium trenchii* at the end of the experiment (TE, after seven days at 32°C), relative to their control (26°C). Transcripts are grouped by function, and the full list of genes can be found in Supplementary Table S3.10).

Chapter 4

Figure 4.1. Physiology of *Pocillopora acuta* (n = 5; mean ± SE) in the Low Isles reef site (blue) and Woody Isles mangrove lagoon (red). (**a**) respiration and gross photosynthesis (P_G) (as the sum of net photosynthesis and respiration) normalised per surface area, and (**b**) per endosymbiont cell , (**c**) photochemical quenching (1 – C) *versus* non-photochemical quenching (1 – Q).

Figure 4.2. Carbon enrichments at the bulk and single-cell levels. (**a**) Bulk (n = 4) $\delta^{13}C$ enrichment levels (normalised to natural abundances) for the different incubated fractions of the coral: ("Coral" and "In hosp. Sym." refer to the incubated holobiont, later separated between animal and algae fractions, respectively; "FIS" refer to freshly isolated Symbiodiniaceae). (**b**) Single cell $\delta^{13}C$ enrichment levels with NanoSIMS. The errors bars represent the standard error, the asterisk symbols denote statistical differences ($p < 0.05$) between (**c-d**; **g-h**) and mangrove (**e-f**; **i-j**) sites. Representative NanoSIMS images showing (**c-f**) the distribution of $^{12}C^{14}N^-$, indicative of the biological structure of the sample, and (**g-j**) the isotope ratio of $^{13}C/^{12}C^-$, with natural abundance in blue, changing to pink with increasing ^{13}C levels. Number of cells analysed: FIS (n = 18); in hospite Symbiodiniaceae (n = 19); and enriched areas in host tissue (n = 5).

Figure 4.3. Relative abundances (%) of recovered ITS2 sequences (upper section) and predicted major ITS type profiles (lower section) for *Pocillopora acuta* across the Low Isles reef and Woody Isles mangrove habitats on the Great Barrier Reef. Each stacked bar corresponds to a biological replicate of a different colony, and each replicate is plotted relative to each other between the upper and lower sections of the figure. Sequences with designated names (e.g. C1b, D4c, or D6) refer to sequences frequently found in the literature or already characterising ITS2 profiles previously ran through the SymPortal analytical framework (Hume et al. 2019). Other sequences designated by a unique database ID and their associated genus (e.g. 70776_D) refer to sequences that are less common and not previously used to characterise ITS type profiles.

Figure 4.4. Comparisons of (**a**) mean (± SE) gross photosynthesis (P_G, as the sum of net photosynthesis and respiration R) and respiration rates between reef (blue dots) and mangrove (red squares) sites. Species-specific metabolic shifts are represented by lines (black), and the dashed line indicates the 1:1 ratio. The table summarises the mean (± SE) P_G:R ratios for reef and mangrove sites. (**b**) Summary of key metrics shifts from reef and mangrove sites. Data for *Acropora millepora* and *Porites lutea* are retrieved from Camp et al. (2019) and for *Pocillopora acuta* come from the present study.

Figure 4.5. Comparisons of light-dependant dynamic quenching patterns between (**a**) *Pocillopora acuta* (present study) and (**b**) *Acropora millepora* (Camp et al. 2019) in the Low Isles reef (blue dashed

lines) and Woody Isles mangrove lagoon (red dashed lines). Values of [1 – C] stand for photochemical quenching (qP) and [1 – Q] for non-photochemical quenching (NPQ) (see Methods).

Chapter 5

Figure 5.1. Summary of the interplay between light reactions and dark reactions of photosynthesis and how strategies promoting survival affects Ci fixation. LR: light reactions, DR: dark reactions, PG: gross photosynthesis, qP: photochemical quenching, AEF: alternative electron flows, Ci fix: inorganic carbon fixation. Question marks stand for pathways that have been suggested to play a role in coral-Symbiodiniaceae fitness but are to be quantified.

Figure 5.2. Proposed roadmap of future studies over the short and mid-terms leading on the long term to a better understanding of the Symbiodiniaceae and their holobiont physiologies.

List of Tables

Chapter 1

Chapter 2

Chapter 3

Supplementary Table S3.1 (Abbreviations: CBC: Calvin-Benson cycle; PPP: pentose phosphate pathway; TCA: tricarboxylic acid – Krebs cycle).

Table 3.2. Summary of trends found across the studied Symbiodiniaceae isolates. Numbers between brackets represent the number of differentially expressed genes (DEGs) in a sequential order of isolate appearance in the table (*Breviolum minutum*, *Cladocopium goreaui*, *Durusdinium trenchii*). The percent of change of all DEGs indicate how are allocated the DEGs between down- and up-regulated genes. Arrows thickness represent the number of DEGs found in each functional group.

Chapter 4

Table 4.1. Summary of abiotic conditions characterising Woody Isles mangrove lagoon and the adjacent Low Isles reef in June 2017 and February-April 2018. Data were retrieved from Camp et al. (2019) and collected with a SeapHOx sensor set at 1 minute sampling frequency over 48 hours (2017 dataset) and at 5 minutes sampling frequency over 50 days (2018 dataset). Mean is provided with standard error (SE) and diel range of measurements with coefficient of variance (CV). Data for the reef in February-April 2018 is not available.

Chapter 5

Table 5.1. Summary of advantages and disadvantages of using Symbiodiniaceae in culture and in symbiosis with the holobiont. References: (**1**) Chapter 2; (**2**) Chapter 3; (**3**) Chapter 4; (**4**) Camp et al. 2019; (**5**) Hughes et al. 2018; (**6**) Gorbunov et al. 2001.

List of Supplementary Figures

Chapter 2

Supplementary Figure S2.1. Relative ^{14}C fixation (DPM) as (**a**) C_{fix} and (**b**) C_{exc} over time (5, 10, 20, 60, 120 and 360 minutes) of F1-156 and amur-D-MI, characterised as fastest and slowest growing isolates at the time of the assay, respectively.

Supplementary Figure S2.2. Carbon fluxes as (**a**) C_{fix} and (**b**) C_{exc} occurring within the 23 Symbiodiniaceae isolates grouped per genus after 1h of photosynthesis, as a function of their respective cell volumes.

Supplementary Figure S2.3. Summary of (**a**) C:N and (**b**) C:Chl*a* ratios for each of the 23 studied Symbiodiniaceae isolates.

Chapter 4

Supplementary Figure S4.1. Localisation of Low Isles on a map of Australia (**a**, denoted by a red rectangle) and relatively to Cairns (**b**, denoted by a red circle). Low Island reef (**c**) and Woody Island mangrove lagoon (**c**, **d**) were the collection sites of *Pocillopora acuta* colonies (**e**, in the mangrove).

Supplementary Figure S4.2. Mean (± SE) relative electron transport rates (rETR, mol e^{-} μm^{-3} s^{-1}) obtained using rapid light curves (see Methods) for *Pocillopora acuta* (n = 4) across the Woody Isles mangrove lagoon (red) and Low Isles reef (blue).

Supplementary Figure S4.3. Image of the stable isotope incubations set-up, where FIS are incubated (small vials) close to their respective holobiont fraction (large vessels).

Supplementary Figure S4.4. Mean (± SE) Symbiodiniaceae cell density (cells cm^{-2}) for *Pocillopora acuta* (n = 4) across the Woody Isles mangrove lagoon (red) and Low Isles reef (blue).

List of Supplementary Tables

Chapter 2

Supplementary Table S2.9. Summary of the regression analyses carried out between the mean (±SD) of cell volume (μm^3) and growth rate (μ, d^{-1}) of the three Symbiodiniaceae isolates grown at 20°C, 26°C and 30°C.

Supplementary Table S2.10. Carbon fluxes and productivity characteristics of the three studied Symbiodiniaceae isolates grown at 20°C, 26°C and 30°C: mean (±SD) (n = 3) of GPC (pg C [cell h]$^{-1}$), the detail of C_{fix} (pg C [cell h]$^{-1}$) and C_{exc} (pg C [cell h]$^{-1}$), and NPC (pg C [cell h]$^{-1}$).

Supplementary Table S2.11. Contribution of variables (in %) to each component of the PCA carried out on the dataset of isolates grown at sub-optimal temperatures.

Supplementary Table S2.12. Summary of significant non-parametric ANOVA (Kruskal-Wallis) with discrimination of the isolates specifically grown at the mentioned temperature, significantly apart from each other using Dunn-Bonferroni post-hoc tests, carried out on each dimension of the PCA carried out on the dataset of isolates grown at sub-optimal temperatures.

Supplementary Table S2.13. Mean (±SD) GPC:NPC ratios (present study) of specific isolates used in Brading et al. (2011) and their retrieved O_2 production rates (fmol O_2 [cell h]$^{-1}$). GPC:NPC ratios averaged per genus are from the present study.

Supplementary Table S2.14. Summary of the regression analyses carried out between the mean (±SD) GPC:NPC and O_2 production rates (fmol O_2 [cell h]$^{-1}$) of the three Symbiodiniaceae isolates retrieved from Brading et al. (2011), in light and dark conditions.

Supplementary Table S2.15. Photobiology characteristics of studied Symbiodiniaceae isolates: mean ± SD (n = 3) of photosystem II (PSII) maximum photochemical efficiency (F_v/F_m, dimensionless) and PSII absorption cross-section (σ_{PSII}, nm^2).

Supplementary Table S2.16. Photobiology characteristics of the three studied Symbiodiniaceae isolates grown at 20°C, 26°C and 30°C: mean ± SD (n = 3) of photosystem II (PSII) maximum photochemical efficiency (F_v/F_m, dimensionless) and PSII absorption cross-section (σ_{PSII}, nm^2).

Chapter 3

Supplementary Table S3.1 Full list of proteins and their associated genes and cellular functions used to target and refine the transcripts analysis in all the studied Symbiodiniaceae (*Breviolum minutum*, *Cladocopium goreaui,* and *Durusdinium trenchii*). Colours of cells represent the functional grouping proteins were associated with green: light reactions; blue: dark reactions; yellow: metabolism; orange: stress response; grey: transport. Information of protein function was retrieved from the UniProt knowledgebase (UniProtKB). "N/A" denotes a protein for which a gene name has not been identified.

Supplementary Table S3.2. Summary of DEGs for *Breviolum minutum* at the TI of the experiment (post-temperature ramping).

Supplementary Table S3.3. Summary of DEGs for *Cladocopium goreaui* at the TI of the experiment (post-temperature ramping).

Supplementary Table S3.4. Summary of DEGs for *Durusdinium trenchii* at the TI of the experiment (post-temperature ramping).

Supplementary Table S3.5. Summary of DEGs for *Breviolum minutum* at the TE of the experiment (after seven days at 32°C).

Supplementary Table S3.6. Summary of DEGs for *Cladocopium goreaui* at the TE of the experiment (after seven days at 32°C).

Supplementary Table S3.7. Summary of DEGs for *Durusdinium trenchii* at the TE of the experiment (after seven days at 32°C).

Supplementary Table S3.8. Summary of one-way ANOVAs carried out on the ^{14}C uptake data. Differences are between control and treatment for each time point. The star symbol denotes a significant difference at alpha = 0.05

Supplementary Table S3.9. Summary of genes (functional proteins between brackets) sharing a difference in expression between the studied Symbiodiniaceae isolates (B1: *Breviolum minutum*; C1: *C. goreaui*; D1a: *D. trenchii*) after seven days of thermal stress (32°C)

Supplementary Table S3.10. Heat-map summarising the differential gene expression at the end of the temperature ramping (TI) and after seven days of thermal stress at 32°C (TE) in our three Symbiodiniaceae isolates (B1: *B. minutum*; C1: *C. goreaui*; D1a: *D. trenchii*). Heat-map was populated first for the DEGs conforming to our threshold cut-off (log2 fold change [FC] ≥1 and false discovery rate [FDR] ≤ 0.05) for either TI and TE, and then the corresponding DEG for the same gene at the other time point was reported regardless of significance and log2 fold change. Blank cells denote the lack of DEGs according to our set cut-off for both TI and TE. The presence of a star in the column "Sig" denotes a FDR < 0.05. Shade of “Protein” column represent the functional grouping proteins were associated with green: light reactions; blue: dark reactions; yellow: metabolism; orange: stress response. Colour scale of heat-map varies from a gradient of purple (most down-regulated) to gold (most up-regulated).

Chapter 4

Supplementary Table S4.1. Summary of the *Pocillopora acuta* samples taken from both Low Isles reef and Woody Isles mangrove and their subsamples (for stable isotope incubations and genomic analyses). Abbreviations: HB, holobiont; FIS, freshly isolated symbionts; H, host; S, symbiont; EA, enrichment analysis; NA, natural abundance; NS, NanoSIMS; CC, cell counts; PAM, pulse-amplified fluorometry.

List of abbreviations

ADP	Adenosine diphosphate
AEF	Alternative electron flow
ANOVA	Analysis of variance
APX	Ascorbate peroxidase
ASW	Artificial seawater
ATP	Adenosine triphosphate
C	Carbon
CA	Carbonic anhydrase
$CaCl_2$	Calcium chloride
CAM	Crassulacean acid metabolism
CBC	Calvin-Benson-Bassham cycle
CCM	Carbon concentrating mechanism
CEF-PSI	Circular electron flow around PSI
C_{exc}	Rate of organic carbon excretion
$\%C_{exc}$	Fraction of GPC excreted in the media
C_{fix}	Rate of Ci fixation
$\%C_{fix}$	Fraction of GPC retained in the cell
$(CH_2O)_n$	Carbohydrate
Chl*a*	Chlorophyll-*a*
Ci	Inorganic carbon
CO_2	Carbon dioxide
COP	Conference of the Parties
CV	Coefficient of variance
Cyt b6f	Cytochrome b6f
DEG	Differentially expressed gene
DIC	Dissolved inorganic carbon
DIN	Dissolved inorganic nitrogen
DMSP	Dimethylsulfoniopropionate
DOC	Dissolved organic carbon
DOM	Dissolved organic matter

DPM	Disintegrations per minute
DR	Dark reactions
EA	Enrichment analysis
eDOC	External dissolved organic carbon
EDTA	Ethylenediaminetetraacetic acid
ENSO	El Niño-Southern Oscillation
FC	Fold change
Fd	Ferredoxin
FBP	Fructose-1,6-biphosphate
FDR	False discovery rate
Fe^{2+}	Ferrous ion
FIS	Freshly isolated symbionts
FNR	Ferredoxin-NADP reductase
FRRf	Fast repetition rate fluorometry
GAP	Glyceraldehyde-3-phosphate
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GBR	Great Barrier Reef
GBRMPA	Great Barrier Reef Marine Park Authority
GEB	Gene expression biomarker
GHG	Greenhouse gases
GPC	Gross carbon production
H_2O_2	Hydrogen peroxide
HB	Holobiont
HCl	Hydrochloric acid
HCO_3^-	Bicarbonate ion
$HgCl_2$	Mercuric chloride
HSI	Hue saturation intensity
HSP	Heat-shock protein
iDOC	Internal dissolved organic carbon
IPCC	Intergovernmental Panel on Climate Change
ITS2	Internal transcribed spacer 2
LED	Light-emitting diode

LEF	Linear electron flow
LHC	Light-harvesting complex
LIFT	Light-induced fluorescence transient
LR	Light reactions
MDS	Multidimensional scaling
MIMS	Membrane inlet mass spectrometry
MPP	Marine primary productivity
N	Nitrogen
N_2	Atmospheric nitrogen
NA	Natural abundance
NaCl	Sodium chloride
NADP(H)	Nicotinamide adenine dinucleotide phosphate
$NaH^{13/14}CO_3$	$^{13/14}$C-labelled sodium bicarbonate
NanoSIMS	Nanoscale secondary ion mass spectrometry
NH_4^+	Ammonium ion
NO_3^-	Nitrate ion
NPC	Net carbon production
NPQ	Nonphotochemical quenching
O2	Dioxygen
$^1O_2$	Singlet oxygen
O_2^-	Superoxide radical
OA	Ocean acidification
OAA	Oxaloacetic acid
•OH	Hydroxyl radical
ORF	Open reading frame
OsO_4	Osmium tetroxide
P	Phosphorus
PAM	Pulse amplitude modulation
PAR	Photosynthetically active radiation
PBS	Phosphate-buffered saline
PC	Plastocyanin
PCA	Principal components analysis

pCO_2	Partial pressure of CO_2
PCR	Polymerase chain reaction
PG	Gross photosynthesis
PGA	3-phosphoglyceric acid
Pi	Inorganic phosphorus
P-I	Photosynthesis response to irradiance
PN	Net photosynthesis
PO_4^{3-}	Phosphate ion
POC	Particulate organic carbon
POM	Particulate organic matter
PPP	Pentose phosphate pathway
PQ	Plastoquinone
PSI	Photosystem I
PSII	Photosystem II
Q_A	Electron transport chain
qP	Photochemical quenching
R	Respiration
RCF	Relative centrifuge force
RCII	Functional PSII reaction centre
RLC	Rapid light curve
ROS	Reactive oxygen species
RuBisCO	Ribulose-1,5-biphosphate carboxylase/oxygenase
SD	Standard deviation
SE	Standard error
SOD	Superoxide dismutase
SST	Sea surface temperature
TCA	Tricarboxylic acid
TEM	Transmission electron microscopy
TN	Total nitrogen
UNESCO	United Nations Educational, Scientific and Cultural Organization

List of symbols

[1 - C]	Photochemical quenching
[1 - Q]	Non photochemical quenching
$\delta^{13}C$	Difference in enrichment of ^{13}C from natural isotope abundance
E_k	Light saturation parameter
ETRmax	Maximal electron transport rate
ETR_{PSII}	Electron transport rate through PSII
F'	Fluorescence yield under actinic light
F_0	Minimum PSII fluorescence yield (dark acclimated state, RCII open)
F_0'	Minimum PSII fluorescence yield (light-acclimated state, RCII open)
F_m	Maximum PSII fluorescence yield (dark-acclimated state, RCII closed)
F_m'	Maximum PSII fluorescence yield (light-acclimated state, RCII closed)
F_v	Maximum variable PSII fluorescence yield (dark-acclimated state)
F_v/F_m	Maximum photochemical efficiency (dark-acclimated state)
μ	Growth rate
σ_{PSII}	Functional absorption cross-section of PSII
rETR	relative electron transport rate

Thesis abstract

Algal endosymbionts (family Symbiodiniaceae) fuel the metabolism of reef-forming corals through uptake and utilisation of inorganic carbon (Ci) from photosynthesis. Changes in photosynthetic performance both within, and between endosymbiont taxa influence the extent of organic carbon ultimately translocated to the host coral. However, how such changes are regulated by plasticity in light harvesting, *versus* Ci assimilation processes remains unknown. In this thesis, I therefore built on novel approaches to assess functional diversity of fitness traits across Symbiodiniaceae to identify the extent with which Ci-uptake and incorporation differed amongst taxa and the extent with which differences could be reconciled against evolutionary adaptation across the family to sustain reef functioning in response to climate change.

This thesis focused on direct assessment of Ci-uptake, and how it is linked to light harvesting and utilisation by Symbiodiniaceae both *ex hospite* (in culture) and *in hospite* (in symbiosis with their host). I first cultured a broad range of Symbiodiniaceae taxa to assess how Ci was invested into cellular uptake, excretion, and growth; and how these metrics changed when three isolates of different thermal tolerances were subjected to sub-optimal conditions of growth. I further examined how these different thermo-tolerant Symbiodiniaceae coped with a stress-inducing increase of temperature. In parallel with photophysiology and Ci-uptake rate measurements, transcriptomics were carried out to resolve the underlying molecular network driving physiological response to heat stress. Finally, I extended this laboratory-based approach to examine Ci-uptake performance of natural coral communities across complex environmental gradients (mangrove *vs*. reef corals) on the Great Barrier Reef to resolve the adaptations of symbionts linked to their survival to extreme environments.

My results revealed that environmental regulation outweighed evolutionary adaptation of Symbiodiniaceae in their capacity for Ci-uptake, suggesting that their ecological success

predominantly relies on plasticity of upstream photosynthetic processes (efficiency of light-harvesting and non-photochemical energy quenching) rather than those downstream (Ci-uptake, assimilation, and excretion). Despite exhibiting similar trends in functional gene expression, each studied Symbiodiniaceae isolate exhibited different photophysiology and Ci-uptake rates in response to thermal stress for both (previously well studied) light reactions and dark reactions of photosynthesis. When in symbiosis, flexibility in the major Symbiodiniaceae taxa between reef and mangrove corals was associated with a reduced Ci incorporation in mangrove corals compared to reef corals. Together, these results will serve as a stepping stone to future research on the long term, aiming to improve worldwide reef health in response to global climate change.

Declaration of the contribution to each Chapter

Chapter 2

This thesis is presented as the accepted final version for publication with Limnology & Oceanography following peer-review.

Ros M., Camp E.F., Hughes D.J., Crosswell J.R., Warner M.E., Leggat W.P., Suggett D.J. 2020. Unlocking the black-box of inorganic carbon-uptake and utilisation strategies amongst coral endosymbionts (Symbiodiniaceae). Limnol. Oceanogr. doi: 10.1002/lno.11416

MR, DJS and EFC designed, and MR conducted experiments at University of Technology Sydney; MR performed dissolved inorganic carbon measurements and analysis with the help of JRC at CSIRO; MR and DJH analysed the data and generated manuscript figures. MR wrote the manuscript with substantial critical contributions from DJS, EFC, DJH, MEW, JRC and WPL.

Chapter 3

MR, DJS and EFC designed the heat-stress experiment. MR, EFC, NF, LF, SG, and CAL oversaw culture maintenance and monitoring. MR, EFC, NF, LF, SG, DJS, and CAL were involved in sample collection. DV undertook the DNA extractions and photophysiology measurements. MR and DJH performed the ^{14}C incubations and DIC measurements. EFC performed the transcripts extractions, TK and DV oversaw the transcript analysis. MR created the figures. MR led the writing of the manuscript, with EFC, DJH and DJS providing significant editorials.

Chapter 4

MR, DJS, EFC and JE designed and conducted all fieldwork and MR conducted experiments; MR and DJH designed the dual fractions incubation chambers, EFC performed respirometry measurements and symbiont identity analysis; MR, DJS, EFC collected the coral samples; MR performed stable isotope incubations, cell density measurements, prepared samples for stable isotope analysis and analysed NanoSIMS images; TH performed host and symbiont DNA extraction for identity analysis; MK, JBR and MP prepared the samples for NanoSIMS analysis; PG and MRK performed the NanoSIMS image acquisition. MR led writing of the manuscript with substantial critical contributions from DJS, DJH and EFC.