

Adaptive strategies of carbon transformation amongst coral symbionts (Symbiodiniaceae)

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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.

This thesis is wholly my own work unless otherwise reference or acknowledged.

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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.

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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 C _i fixation
%C _{fix}	Fraction of GPC retained in the cell
(CH ₂ O) _n	Carbohydrate
Chl <i>a</i>	Chlorophyll- <i>a</i>
C _i	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 ₂ O 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
O ₂	Dioxygen
¹ O 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
$\delta^{13}\text{C}$	Difference in enrichment of ^{13}C from natural isotope abundance
E_k	Light saturation parameter
ETR_{max}	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 (C_i-uptake, assimilation, and excretion). Despite exhibiting similar trends in functional gene expression, each studied Symbiodiniaceae isolate exhibited different photophysiology and C_i-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 C_i 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.

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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.

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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 ¹⁴C 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.