The Biochemical Energy Balance of the Coral Symbiosis

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Certificate of Original Authorship

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Date:

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Abbreviations

ANOVA	Analysis of variance
ATP	Adenosine triphosphate
ATR	Attenuated total reflectance
BSA	Bovine serum albumin
CD	Circular dichroism
Chl *a*	Chlorophyll a
DNA	Deoxyribonucleic acid
EMSC	Extended multiplicative scatter correction
FPA	Focal plane array
FTIR	Fourier transform infrared
GBR	Great Barrier Reef
GC-MS	Gas chromatography – mass spectrometry
HCA	Hierarchical clustering analysis
Hsp	Heat shock protein
IR	Infrared
IRENI	Infrared environmental imaging
LHC	Light harvesting complex
MS	Mass spectrometry

NMR	Nuclear magnetic resonance
OEC	Oxygen evolving complex
PAM	Pulse amplitude modulated
PCA	Principal component analysis
PCP	Peridinin-chlorophyll-protein
PLS	Partial least squares
PLSDA	Partial least squares discriminant analysis
PSI	Photosystem I
PSII	Photosystem II
rmANOVA	Repeated-measures analysis of variance
RMSEP	Root mean square error of prediction
SEM	Standard error of the mean
sp.	Species
SRC	Synchrotron Radiation Center
SST	Sea surface temperature
TEM	Transmission electron microscopy
XRD	X-Ray diffraction

Abstract

Over the last three decades, coral reefs around the world have declined by an estimated 51%. This has largely been caused by anthropogenic climate change resulting in increases in ocean acidification and sea surface temperatures. At their core, corals are a symbiotic relationship between a microscopic algae of the genus *Symbiodinium* known as “zooxanthellae”, the cnidarian coral host and associated bacterial communities. Under severe environmental stress, the coral will expel the algae. This results in the host losing its major source of organic carbon.

Extensive research into tolerance of the algae, have revealed a large genetic diversity within the genus *Symbiodinium* and it is thought that macromolecular content (carbohydrates, proteins, lipids and phosphorylated compounds) have an effect on biochemical processes responsible for energy acquisition and repair of photosynthetic membranes within the cells. Metabolomics, the study of macromolecular compounds within a biological system, has been applied in various forms, to describe individual compounds such as fatty acids or sterols, contained within different clades of *Symbiodinium*.

In this study, two clades of *Symbiodinium* sp. were chosen based on their differing tolerance to environmental stress, and analysed to investigate macromolecular changes in the face of fluctuations in light and temperature. Under normal growth conditions, clades of *Symbiodinium* sp. differed in protein and lipid structure. This is the first time this has been reported to date.

In order to further explore these differences in macromolecular content and structure, the cells were subjected to sub-lethal light and temperature treatments. Under these conditions, it was found that both clades increased their β-sheet protein secondary

structure. When exposed to elevated light, lipid was stored and carbohydrate consumed whereas the opposite was found under elevated temperature. This has further implications for nutrient exchange *in hospite*.

Clades of *Symbiodinium* sp. were then exposed elevated temperature to simulate bleaching conditions. Under these high temperatures, clade A was found to exhibit the largest decline in maximum quantum yield of PSII indicating photodamage. This decline in F_v/F_m was linked to changes in lipid and protein secondary structure indicating a change in thylakoid membrane structure occurred under extreme stress. It was also proposed that the change in protein secondary structure was related to protein subunits associated with the oxygen evolving complex, and subsequently photodamage and PSII repair mechanisms.

Synchrotron FTIR spectroscopic chemical imaging was also used to further analyse these changes in *Symbiodinium* sp. from a single-cell perspective. Macromolecular compound groups (protein, lipid, carbohydrate and phosphorylated compounds) were shown to be distributed differently across the cells. Further to this, there appeared to be a difference in the regions in which α-helix and β-sheet protein structures clustered across the individual cells.