Spatial and temporal dynamics of photosynthesis regulation of genetically defined coral/algal symbiosis associations

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy by

Karin Elizabeth Ulstrup

(B.Sc., M.Sc. Biology, University of Copenhagen)

Institute of Water and Environmental Resource Management Department of Environmental Sciences University of Technology, Sydney

June 2007

Certificate

The work presented in this thesis, and the research to which it pertains, are the product of my own work and, to the best of my knowledge and belief, original. Any ideas or quotations from the work of others, published or otherwise, are fully acknowledged in accordance with the standard referencing practices of the discipline. Co-authors of published, submitted articles and articles in preparation were involved in two or more stages of a study including **a**) planning of the study, **b**) essential expertise and support with field and laboratory work, **c**) critical evaluation of the material and literature and **d**) final interpretation and writing up of the results. Material has not been submitted, either in whole or in part, for a degree at this or any other university.

Production Note: Signed Signature removed prior to publication.

Karin E. Ulstrup (PhD Candidate)



Acknowledgements

Facilities for my PhD studies were provided by University of Technology, Sydney (UTS), Australian Institute of Marine Science (AIMS), University of Sydney (USYD) and the University of Copenhagen, Denmark (KU). These places all provided expertise and infrastructure for my project.

I would like to thank my principal supervisor, Assoc. Prof. Peter Ralph (UTS) for his support and know-how throughout the duration of my candidature. Dr. Madeleine van Oppen (AIMS) provided invaluable guidance on the molecular part of my project and personal encouragement. I wish to thank Prof. Michael Kühl (KU) for inspiration and for teaching me the principles of working with microsensors. Prof. Tony Larkum (USYD) and Dr. Ray Berkelmans (AIMS) were also instrumental in the success of parts of my thesis for which I am sincerely grateful. All of the above provided stimulating discussions and assisted in clarifying and refining my thoughts on physiological and genetic aspects of coral science.

The field work in this study was undertaken at Heron Island Research Station, Lizard Island Research Station and on vessels belonging to the AIMS fleet. My time spent in the field was both productive and enjoyable as a result of the thoughtfulness, advice and efforts of the staff at these facilities. I would like to thank the following for their hands-on assistance in the field: Jeremy Downs, Steve Hornabrook, Martin Macnaughton, Tim Cooper and Jens Ulstrup who all volunteered their time and assistance. In the laboratory I was assisted by Martine Goldner, Alex Griffin, Lesa Peplow and Jeremy Downs. I also thank all members of the Aquatic Photosynthesis Lab (UTS). Special thanks to my family who provided me with personal support and encouragement.

Funding from a number of organisations made this project possible and I am grateful for their contribution. University of Technology, Sydney awarded me an International Postgraduate Research Scholarship and a Science Faculty stipend. Research support was generously provided by the National Geographic Society, PADI Foundation, Great Barrier Reef Marine Park Authority, HRH Crown Prince Frederiks Foundation, Denmark, Winifred Violet Scott Foundation, Sigma Xi and a Graduate Fellowship from the International Society for Reef Studies. The Australian Coral Reef Society, Knud Højgaards Fond, Denmark, as well as internal research allowances at University of Technology, Sydney provided me with financial support to attend scientific conferences.

Publications

Peer-reviewed journal articles arising directly from this thesis

Ulstrup KE, Berkelmans R, Ralph PJ, van Oppen MJH (2006) Variation in bleaching sensitivity of two coral species across a latitudinal gradient on the Great Barrier Reef: the role of zooxanthellae. *Marine Ecology Progress Series* 314:135-148.

Ulstrup KE, Ralph PJ, Larkum AWD, Kühl M (2006) Intra-colonial variability in light acclimation of zooxanthellae in coral tissues of *Pocillopora damicornis*. *Marine Biology* 149:1325-1335.

Peer-reviewed journal articles relevant to the thesis but not forming part of it

Ulstrup KE, Kühl M, Bourne D (2007) Zooxanthellae harvested by ciliates associated with brown band syndrome of corals remain photosynthetically competent *Applied Environmental Microbiology* 73:1968-1975

Ulstrup KE, Hill R, Ralph PJ (2005) Photosynthetic impact of hypoxia on *in hospite* zooxanthellae in the scleractinian coral *Pocillopora damicornis*. *Marine Ecology Progress Series* 286:125-132.

Conference abstracts (* poster)

Ulstrup KE, Ralph PJ, Larkum AWD, Kühl M (2006) Intra-colonial variability in light acclimation of zooxanthellae in coral tissues. European Meeting for International Society for Reef Studies, Bremen.

***Ulstrup KE**, Ralph PJ, Larkum AWD, Kühl M (2006) Intra-colonial variability in light acclimation of zooxanthellae in coral tissues. American Society for Limnology and Oceanography, 6th Ocean Sciences Meeting, Honolulu, USA.

Ulstrup KE, Ralph PJ, Larkum AWD, Kühl M (2005) Intra-colonial variability in light acclimation of zooxanthellae in coral tissues. Australian Coral Reef Society 81st Annual Conference, Heron Island, Australia.

Hill R, **Ulstrup KE** (2005) Imaging-PAM: operation and possibilities. Coral Reef Targeted Research and Capacity Building for Management Workshop, Puerto Morelos, Mexico.

Ulstrup KE (2004) Coral Bleaching - Integrating physiology with genetics. Scandinavian Protozoological Meeting, Helsingør, Denmark.

Ulstrup KE, Berkelmans R, Ralph P, van Oppen M (2004) Bleaching sensitivity of two conspecific corals on a latitudinal gradient. International Society for Reef Studies, 10th International Coral Reef Symposium, Okinawa, Japan.

Table of contents

Certificate Acknowledgements Publications Table of contents Table of figures Table of tables Abstract	II IV VI IX XIII
Chapter 1 Background, concepts and thesis outline	1
 1.0 Background 1.1 Thermal sensitivity of corals and global warming 1.2 The role of zooxanthellae 1.2.1 Diversity, occurrence and distribution of <i>Symbiodinium</i> 1.2.2 Physiological diversity of zooxanthellae	1 3 4 6 8
Chapter 2 Intra-colonial variability in light acclimation of zooxanthellae in co tissues of <i>Pocillopora damicornis</i>	
 2.0 Introduction	13 14 14 15 16 16 16 23 24 25 rETR
2.4 Conclusion	
Chapter 3 Inter-polyp genetic and physiological characterisation of <i>Symbiodin</i> in an <i>Acropora valida</i> colony	
 3.0 Introduction	31 32 32 33 34 34

3.2.1 Genetic composition and distribution of Symbiodinium	35
3.2.2 Photo-physiology of individual polyps with contrasting branch orientation and	
symbiont composition	36
3.3 Discussion	38
3.3.1 Relative occurrence of clade A	39
3.3.2 Photo-physiology of individual polyps with contrasting orientation and symbiont	t
composition	40
3.3.2.1 Oxygen dynamics	40
3.3.2.3 Influence of light history on capacity for photo-acclimation	

4.0 Introduction	43
4.1 Materials and methods	45
4.1.1 Annual temperature	45
4.1.2 Molecular methods	45
4.1.3 Symbiont cell determination	46
4.1.4 Zooxanthellar xanthophyll determination	46
4.1.5 PAM fluorescence measurements of PSII	47
4.1.6 Fast induction kinetics of PSII	48
4.1.7 Statistical analyses	49
4.2 Results	49
4.2.1 Measurements of temperature	49
4.2.2 Genotype variability	50
4.2.3 Symbiont cell density	51
4.2.4 Xanthophyll cycling and NPQ _{RLC}	52
4.2.5 Quantum yields	53
4.2.6 Descriptive parameters of fitted rETR-curves	55
4.2.7 Fast induction kinetics	56
4.3 Discussion	57
4.3.1 Genetic diversity of Symbiodinium	58
4.3.2 Symbiont cell density	59
4.3.3 Photo-protection	59
4.3.4 Photochemical efficiency and photo-acclimation	60
4.4 Conclusions	61

5.2.2.3 Effects of location	.72
5.2.2.4 Interactions among temperatures and between locations	.72
5.2.3 Genetic characterisation of zooxanthellae	.77
5.3 Discussion	.77
5.3.1 Effects of sampling time	.77
5.3.2 Temperature and location	.78
5.3.3 Summary	.79

6.0 Introduction	80
6.1 Materials and methods	82
6.1.1 Sampling	82
6.1.2 Sea temperature measurements	84
6.1.3 Experimental procedure	85
6.1.4 Genetic identification of zooxanthellae in field colonies	
6.1.5 Statistical analyses	
6.2 Results	
6.2.1 Temperature range	
6.2.2 Mortality	
6.2.3 Zooxanthella density	
$6.2.4$ Photochemical efficiency (F_v/F_m)	94
6.2.4.1 Pocillopora damicornis	
6.2.4.2 T. reniformis	
6.2.5 Genotyping of zooxanthellae	98
6.3 Discussion	98
6.3.1 Local adaptation and phenotypic plasticity	99
6.3.2 Mortality	100
6.3.3 Zooxanthella density	101
6.3.4 Photochemical efficiency	101
6.3.5 Symbiont selection	102
6.4 Conclusions	103
Chapter 7 Key findings, implications and future research	104
7.0 General discussion	
7.0.1 Technical points on photo-physiological measurements at the microscale	105
7.0.2 Specificity of coral/algal associations	106

7.0.2 Specificity of coral/algal associations	106
7.0.3 Mixed coral/algal associations	107
7.0.4 Large-scale studies of coral photobiology in time and space	108
7.1 Summary of key findings	109
7.2 Future research	110

References	
Appendix A	
Appendix B	
Appendix C	
Appendix D	
Appendix E	
11	

Table of figures

Figure 2.1. Spectral scalar irradiance measurements of sun- and shade-adapted polyp tissues of *P. damicornis*. Data are normalised to the incident downwelling scalar irradiance (E_d). Arrow indicates the absorption wavelength of chlorophyll *a* (675 nm).

Figure 4.2a-b. Xanthophyll ratio (ratio of diatoxanthin to the sum of diatoxanthin and diadinoxanthin) in sun- (white bars) and shade-adapted surfaces (hatched bars) and non-

photochemical quenching in sun- (O) and shade-adapted surfaces (\bullet) of individual colonies of a) *P. damicornis* and b) *A. valida*. Averages ± SE are shown (n =10)......53

Figure 5.3. Annual variation in day length (h) at Lizard Island (\bullet) and Broadhurst Reef (O), also representing Davies Reef between May 2005 and April 2006. The data is derived from the Geoscience Australia and represent monthly averages of daily recording ± SE. Arrows indicate twice yearly sampling time at Lizard Island (upward-pointing arrows) and Broadhurst Reef and Davies Reef (downward-pointing arrows)..66

Figure 5.5. Descriptive parameters (P_{max} [nmol cm⁻³ s⁻¹], α , and E_k [µmol photons m⁻² s⁻¹]) of fitted PI-curves of *T. reniformis* from Lizard Island (black bars) and Davies Reef (grey bars) as a function of temperature treatment (n =4 ± SE). Significant differences (p <0.05) are denoted with asterisks (*) for 2-way ANOVA and with + for 1-way ANOVA.

Figure 5.6. Measurements of P_{gmax} (nmol cm⁻³ s⁻¹), P_{nmax} (nmol cm⁻² s⁻¹), R_D (nmol cm⁻² s⁻¹), P_{nmax} :R and O_{2max} (µmol l⁻¹) for *P. damicornis* in winter at Lizard Island (black bars) and Broadhurst Reef (grey bars) and in summer at Lizard Island (black bars) and Davies Reef (grey bars) (n =4 ± SE). All maximum measurements are obtained at 900

 μ mol photons m⁻² s⁻¹. Significant differences (p <0.05) are denoted with asterisks (*) for

Figure 5.7. Measurements of P_{gmax} (nmol cm⁻³ s⁻¹), P_{nmax} (nmol cm⁻² s⁻¹), R_D (nmol cm⁻² s⁻¹), P_{nmax} :R and O_{2max} (µmol l⁻¹) for *T. reniformis* in winter and summer at Lizard Island (black bars) and Davies Reef (grey bars) (n =4 \pm SE). All maximum measurements are obtained at 900 μ mol photons m⁻² s⁻¹. Significant differences (p <0.05) are denoted with

Figure 6.1. Map of the Australian eastern sea border showing collection sites: ^aLocations of sea temperature measurements; ^blocations of experimental bleaching

Figure 6.2. Average daily temperatures for the reef slope (6-9 m depth) for each day (48 measurements per day) at Lizard Island, Davis Reef, Hardy Reef, East Cay and Heron Island. A 10 day smoothing function is applied to indicate the general trend in yearly

Figure 6.3. Accumulated mortality (% of n = 27) of explants of *P. damicornis* from Lizard Island (black bars), Big Broadhurst Reef (light grey bars) and Wistari Reef (dark grey bars) over the course of the experiment. Elevated temperature exposure is represented in the first 2 weeks (1-13 days, grey top bar) and recovery (26°C) (15-27

Figure 6.4. Accumulated mortality (% of n = 27) of explants of T. reniformis from Lizard Island (black bars), Big Broadhurst Reef (light grey bars) and Heralds Prong Reef (dark grey bars) over the course of the experiment. Exposure to 33°C is represented in the first 2 weeks (1-13 days, grey top bar) and recovery (26°C) is

Figure 6.5. Relative change (%) of zooxanthella density (10^6 cm^{-2}) in relation to controls of explants of P. damicornis from Lizard Island (black bars), Big Broadhurst Reef (light grey bars) and Wistari Reef (dark grey bars) after 2 weeks of exposure to elevated temperatures (29°C and 31°C) and 2 weeks of recovery. Significant differences were calculated by testing between sites at specific temperatures. Averages are shown (n = 3) including SE bars. Significant differences (p < 0.05) are shown with an asterisk.

Figure 6.6. Relative change (%) of zooxanthella density (10^6 cm^{-2}) in relation to controls of explants of T. reniformis from Lizard Island (black bars), Big Broadhurst Reef (light grey bars) and Heralds Prong Reef (dark grey bars) after 2 weeks of exposure to elevated temperatures (29°C and 31°C) and 2 weeks of recovery (n = $3 \pm$ SE). Significant differences were calculated by testing between sites at specific

Figure 6.7a-d. Relative change (%) of photochemical efficiency (F_v/F_m) in relation to pre-bleaching responses (n =27 \pm SE) of explants of *P. damicornis* from Lizard Island (\bullet), Big Broadhurst Reef (O) and Wistari Reef ($\mathbf{\nabla}$). Two weeks of control (a:26°C) and bleaching treatments (b:29°C, c:31°C, d:33°C) are shown (1-13 days, grey top bars) followed by 2 weeks (15-27 days, white top bars) of recovery at 26°C......96

Figure 6.8a-d. Relative change (%) of photochemical efficiency (F_v/F_m) in relation to pre-bleaching responses (n =27 ± SE) of explants of *T. reniformis* from Lizard Island (•), Big Broadhurst Reef (O) and Heralds Prong Reef ($\mathbf{\nabla}$). Two weeks of control (a:26°C) and bleaching treatments (b:29°C, c:31°C, d:33°C) are shown (1-13 days, grey top bars) followed by 2 weeks (15-27 days, white top bars) of recovery at 26°C........97

Appendix A, Figure 1. Scheme of experimental setup for combined microsensor measurements of oxygen and variable chlorophyll *a* fluorescence. The combined microsensor is mounted on a micromanipulator (MM). The O_2 microelectrode part of the sensor is connected to a picoamperemeter (pA) and the sensor signal is recorded on a strip chart recorder (REC). The fibre-optic microprobe part of the sensor is connected to a photomultiplier detector unit (PM), which is connected to a PC-interfaced controlling unit (PAM control). The photomultiplier detector unit and the LED ring, which served as an actinic light source, were also connected to the controlling unit...129

Table of tables

Table 2.1. Differences in F_v/F_m , and Φ_{PSII} and NPQ of sun- and shade-adapted coenosarc and polyp tissues of *P. damicornis* determined at 358 and 328 µmol photons m⁻² s⁻¹ for sun- and shade-adapted coenosarc tissues, respectively, and at 406 and 290 µmol photons m⁻² s⁻¹ for sun- and shade-adapted polyp tissues, respectively (n =5 ± SE). Significant differences (p <0.05) between sun- and shade-adapted regions of coenosarc and polyp tissue are denoted with superscript letters. Significant p-value is bolded......18

Table 4.1a-b. Observed frequencies of *Symbiodinium* type(s) within (sun- and shadeadapted surfaces [abbreviated as Sun and Shade, respectively]) and among colonies of a) *P. damicornis* and b) *A. valida* from Heron Island. Sample numbers (n), observed SSCP genotype frequencies (f) and proportion (%) of clade A (f_A) and C (f_{CI} , f_{C2}). Where multiple *Symbiodinium* types were present the dominant frequency is bolded...50

Appendix B, Table 1a-b. Quantitative parameters derived from fitted steady-state relative electron transport rates (rETR) and gross photosynthesis rate curves of

Appendix C, Table 6a-b. Maximum quantum yield, F_v/F_m for sun-adapted fragments Summary of repeated measures ANOVAs comparing responses of maximum quantum yield, F_v/F_m , to differences among seasons and between measurements at dusk and dawn of a) *P. damicornis* and b) *A. valida*. Year (2004, 2005, 2006; within-subject factor), season (summer, winter; between-subject factor) and time (dusk, dawn; between-subject factor). Significant differences (p < 0.05) are in bold......139

Appendix C, Table 7a-b. Maximum quantum yield, F_v/F_m for shade-adapted fragments Summary of repeated measures ANOVAs comparing responses of maximum quantum yield, F_v/F_m , to differences among seasons and between measurements at dusk and dawn of a) *P. damicornis* and b) *A. valida*. Year (2004, 2005, 2006; within-subject factor), season (summer, winter; between-subject factor) and time (dusk, dawn; between-subject factor). Significant differences (p <0.05) are in bold......140

Appendix C, Table 14a-b. Fluorescence rise step I. Univariate analyses of a) *P. damicornis* and b) *A. valida* of the fluorescence rise step I. Year (2004, 2005, 2006; within-subject factor), season (summer, winter; between-subject factor) and position

Appendix E, Table 1. Univariate analyses of a) heating and, b) recovery of *P. damicornis* testing the hypothesis that changes in F_v/F_m following a change in heating temperature are independent of sampling origin. Day (d0, d1, d3, d5, d7, d9, d11, d13, d15, d17, d19, d21, d23, d25, d27; within-subject factor), temperature (26°C, 29°C, 31°C, 33°C; between-subject factor) and location (LI, Lizard Island; BBR, Big Broadhurst Reef; WR, Wistari Reef; between-subject factor).

Appendix E, Table 2. Univariate analyses of a) heating and b) recovery of *T. reniformis* testing the hypothesis that changes in rate of F_v/F_m recovery following the heating temperature are independent of sampling origin. Day (d0, d1, d3, d5, d7, d9, d11 13,

d15, d17, d19, d21, d23, d25, d27; within-subject factor), temperature (26°C, 29°C, 31°C, 33°C; between-subject factor) and location (LI, Lizard Island; BBR, Big Broadhurst Reef; HPR, Heralds Prong Reef; between-subject factor)......157

Abstract

Photosynthetic capacity of scleractinian corals relies predominantly on the productivity of single-celled endosymbiotic dinoflagellates of the genus *Symbiodinium*, known as zooxanthellae, residing intracellularly within coral endoderm tissue. The regulation of photosynthesis of zooxanthellae is in turn dependent on light and temperature. This thesis explores the genetic basis for variation in photosynthesis capacity of zooxanthellae by examining the photo-physiology of genetically characterised *Symbiodinium* communities at a range of spatial and temporal scales. *In situ* and manipulative experiments were conducted to improve our understanding of metabolic responses of zooxanthellae under climate change scenarios.

Fine scale measurements of irradiance and photosynthesis allowed the assessment of photo-physiological changes across individual colonies of *Pocillopora damicornis* and *Acropora valida*. *Pocillopora damicornis* generally contain genetically homogeneous populations of *Symbiodinium*, whilst genetically diverse *Symbiodinium* communities exist within *Acropora valida*. Measurements of light absorption in *P. damicornis* were conducted using a scalar irradiance microprobe and it was found that light absorption was greatest in shade-adapted polyp tissue and smallest in sun-adapted coenosarc tissue. Genetic heterogeneities, found at the scale of individual polyps in *A. valida*, correlated with O₂ concentration at the surface of the colony which was greater in polyps that harboured the two clades (A + C) than in polyps that only harboured clade C. In both corals, measurements using an O₂ microelectrode and a fibre-optic microprobe yielded dissimilar results when used at moderate to high irradiances.

Seasonal changes in photosynthetic capacity suggested that *P. damicornis* is more sensitive to combined effects of relatively higher temperature and irradiance in summer than *A. valida* suggesting that the symbiont community of *A. valida* may not be physiologically compromised possibly due to phylogenetic changes of *Symbiodinium*. Furthermore, thermal tolerances of conspecific corals were examined at narrow and wide spatial scales across the length of the Great Barrier Reef. *Pocillopora damicornis*, which harboured *Symbiodinium* type C1, thus bleached in correlation with latitude, whereas *Turbinaria reniformis* bleached in correlation with the presence and absence of the known thermo-tolerant *Symbiodinium* clade D.

The results, integrating over spatial and temporal scales suggest that the acclimatisation capacity of corals to light and temperature is determined by i) history of

light and temperature exposure and in cases where corals associate with multiple *Symbiodinium* types ii) the distribution of *Symbiodinium*.