

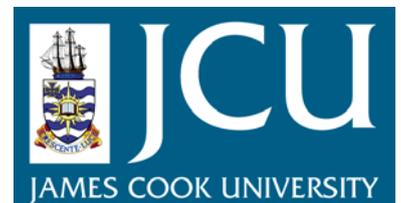
JCU ePrints

This file is part of the following reference:

Abrego, David (2008) *Temporal and environmental influences on the early establishment and maintenance of coral-Symbiodinium symbioses*. PhD thesis, James Cook University.

Access to this file is available from:

<http://eprints.jcu.edu.au/5226>



Temporal and environmental influences on the early establishment and
maintenance of coral-*Symbiodinium* symbioses

Thesis submitted by

David Abrego

M. Appl. Sci. JCU

December 2008

for the degree of Doctor of Philosophy
in the School of Marine and Tropical Biology
James Cook University

STATEMENT ON SOURCES

Declaration

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

.....

(Signature)

.....

(Date)

STATEMENT OF ACCESS

I, the undersigned author of this work, understand that James Cook University will make this thesis available for use within the University Library and, via the Australian Digital Theses network, for use elsewhere.

I understand that, as an unpublished work, a thesis has significant protection under the Copyright Act and;

I do not wish to place any further restriction on access to this work

Or

I wish this work to be embargoed until

Or

I wish the following restrictions to be placed on this work:

Signature

Date

ELECTRONIC COPY

I, the undersigned, the author of this work, declare that the electronic copy of this thesis provided to the James Cook University Library, is an accurate copy of the print thesis submitted, within the limits of the technology available.

Signature

Date

STATEMENT ON THE CONTRIBUTION OF OTHERS

Funding for the research within this thesis was obtained from the Australian Research Council, James Cook University and the Australian Institute of Marine Science. Stipend support was provided by the National Council for Science and Technology (CONACYT Mexico) and the State of Jalisco/Brockmann Scholarship Foundation (Mexico).

Intellectual and editorial support was provided by the supervisory team consisting of Professor Bette Willis (James Cook University) and Dr. Madeleine van Oppen (Australian Institute of Marine Science).

Dr. Karin Ulstrup collected and analysed the data involving oxygen microelectrode characterization of photosynthesis and respiration in coral juveniles presented in Chapter 5.

Dr. Andrew Negri provided intellectual support in the development of the HPLC protocol used for analysis of pigments presented in Chapter 5.

Acknowledgments

It has been a very interesting journey and there are many people to thank and blame (in a good way) for accompanying me along some or most of it. First of all there is my wonderful supervisory team, Bette and Madeleine. I will always think of you as the ying and yang of postgrad students. You make a great complimentary team of supervisors. Bette, thank you for your support in all aspects of my academic and professional development. Your enthusiasm in the field is truly inspiring and your clever suggestions to my writing helped me focus my arguments in ways that I often found startling. Madeleine, thank you for your patience, for always getting me to think about alternative explanations and most of all, for your amazing ability to provide input with almost incomprehensible expediency. You are the embodiment of the highly productive, cool and collected researcher that many of us hope to become when we grow up.

Along with my team of supervisors, several people contributed with intellectual discussions, help with development of protocols, and advice during statistical panic attacks. Ray Berkelmans, Andrew Negri, Sean Connolly, Marc McCormick, François Seneca, and Luke O'Donnell are in this group and I thank you all. I hope to continue to work with you in the future.

In the past few years, a portion of my life that is larger than I care to admit has been spent inside laboratories. The following people have made this portion enjoyable with their help, patience, and the occasional complicity in creative use of lab and field gear: Lesa Peplow, Andy Muirhead, Claudia McGrath, John Morrison, Peter Wruck, Rob Gegg, François Seneca, Jos Mieog, Eneour Puill-Stephan, Karin Ulstrup. A special thanks goes to Phil Osmond for always allowing me to carefully conduct field work even when fierce winds, waves and crocodiles conspired to keep me out of the water. Thanks are also due to Anna, Ollie, Pete, Rob, and Kylie at Orpheus Island Research Station, where I spent many weeks not diving. Gordon Bailey and Vince Püllella in BioSci IT helped me through several near-fatal hard-drive crashes.

The best time of my research was spent in the field and this was in large part thanks to the many volunteers that came out to help during coral spawning time or during sampling time. Claudia McGrath, Jeremy Downs, Brian Kesner, Pia Rheinlander, James Moore, James Kealey, Mikael Dahl, Emily Howells, Eneour Puill-Stephan, Patricia Warner, Vivian Cumbo, Allison Paley, Matthew Reeves, Marnie Freckelton, Naomi Gardiner, Blanche Danastas, Ian Tuart, Elise Brown, Roger Beeden, Jeremy Goldberg,

Caroline Palmer and several others whose names escaped me but their faces and great memories of fun in the field have not. Go the pool noodles!

Finally, the biggest thank you goes to my family and friends who have seen me through this journey and never flinched in their support, confidence and love. Thank you Silvia, Adriana, Francisca, Emily, Jimmy, En, and Murph. A special thanks to Emily for her love and support and for the many wonderful and fun times in the lab, the field, everywhere really. I am looking forward to many more journeys together.

This thesis is dedicated to my parents Silvia, and David, and my aunt Adriana.

Publications resulting from the research in this thesis

Abrego, D., Ulstrup, K.E., Willis, B.L., van Oppen, M.J.H. **2008** Species-specific interactions between algal endosymbionts and coral hosts define their bleaching response to heat and light stress. *Proc. R. Soc. B.* **275**, 2273-2282. DOI 10.1098/rspb.2008.0180.

Abrego, D, van Oppen, MJH, Willis, BL. **2009** Highly infectious symbiont dominates initial uptake in coral juveniles. *Molecular Ecology* (accepted).

Abrego, D, van Oppen, MJH, Willis, BL. **2009** Algal endosymbiont specificity varies among closely related species of *Acropora* corals. *Molecular Ecology* (accepted).

ABSTRACT

Understanding mechanisms underlying the formation and maintenance of coral-*Symbiodinium* symbioses as well as factors affecting the integrity of these symbioses is critical to predicting how coral holobionts might change in response to warming oceans predicted by climate change. Research reported in this thesis aims to enhance current knowledge of coral-*Symbiodinium* symbioses by: (1) examining temporal variation in *Symbiodinium* uptake by coral juveniles, (2) exploring the role of parental effects and ontogenetic stage in determining *Symbiodinium* associations, (3) assessing the impact of environmental parameters in the establishment of symbioses, and (4) evaluating whether some host-symbiont combinations are more resilient to environmental stress than others.

I found that newly settled juveniles of the corals *Acropora millepora* and *A. tenuis* do not necessarily take up the *Symbiodinium* type present in parental colonies, and that a potentially opportunist type D *Symbiodinium* quickly dominates symbioses in juveniles of both species at three sites in the central Great Barrier Reef. I also found that adult patterns of association may not become established for up to 2.5-3.5 years, suggesting a delay in the expression of symbiont specificity. In *A. tenuis*, continuing changes in *Symbiodinium* communities over the first 3.5 years are interpreted as fine-tuning of specificity mechanisms leading to establishment of the homologous algal symbiont characteristic of adult populations. Algal endosymbioses were much more stable over the same time period in juveniles of *A. millepora*, although further research is required to distinguish between absence of specificity and delayed expression of specificity. Changes in *Symbiodinium* communities in *A. tenuis* juveniles are not linked to the onset of reproductive maturity but may be linked to changes in micro-environmental conditions (possibly light intensity or access to nutrients) associated with growth of the colony.

Field studies investigating the role of environmental parameters in the establishment of symbioses revealed that light has little effect on the type of *Symbiodinium* initially acquired by both *A. millepora* and *A. tenuis*. This result was confirmed by experimental manipulations in aquaria where equal amounts of *Symbiodinium* types C1 and D were offered to newly settled juveniles maintained in two light levels by three temperature treatments. In contrast, I found that temperature has a significant effect on algal symbioses by affecting the type of *Symbiodinium* acquired by both coral species and by slowing and potentially stopping *Symbiodinium* uptake and the onset of symbioses at elevated temperatures. Type D *Symbiodinium* was found in larger proportions in juveniles at elevated temperatures (30 and 31°C), providing further evidence of the infective and potentially opportunistic nature of this *Symbiodinium* type. The benefits of type D to the host require further investigation as these juveniles had low levels of infection and it is unclear if their survival would depend on other mechanisms, such as a shift towards heterotrophy.

Comparisons of the resilience of corals hosting type C1 or D *Symbiodinium* to environmental stress indicate that *A. tenuis* juveniles have lower metabolic costs and enhanced physiological tolerance when hosting type C1 *Symbiodinium*. In other studies, the same D-type has been shown to confer higher thermal tolerance than both C2 in adults and C1 in juveniles of the closely related coral *A. millepora*. My results challenge speculations that associations with type D are universally most robust to thermal stress and highlight a potential role of host factors in determining the physiological performance of the holobiont. They also show that although the heat tolerance of corals may be contingent on the *Symbiodinium* strain *in hospite*, their response to heat and light stress is determined by species-specific interactions between both partners in the association.

CONTENTS

Abstract	i
Contents	iii
List of Tables	vi
List of Figures	vii
Chapter 1.0 Background and General Introduction	1
1.1 Background	2
1.2 Patterns of <i>Symbiodinium</i> association, acquisition, and regulation mechanisms	3
1.3 Physiological diversity of <i>Symbiodinium</i> and implications for climate change	6
1.4 Aims of thesis	7
Chapter 2.0 Temporal and geographical variation in natural symbiont uptake by juvenile <i>Acropora tenuis</i> and <i>A. millepora</i>	10
2.1 Introduction	11
2.2 Materials and methods	13
2.2.1 Study sites and experimental design	13
2.2.2 Collection of gametes, culture and settlement of juveniles, and reciprocal explants	15
2.2.3 Determination of <i>Symbiodinium</i> genotype at initial uptake	16
2.2.4 Effect of light environment on symbiont uptake	18
2.2.5 <i>Symbiodinium</i> diversity and relative abundance on the reef	19
2.2.6 Statistical analysis	21
2.3 Results	22
2.3.1 Uptake in <i>A. tenuis</i> juveniles	22
2.3.2 Uptake in <i>A. millepora</i> juveniles	23
2.3.3 Effect of light environment on <i>Symbiodinium</i> uptake	26
2.3.4 <i>Symbiodinium</i> diversity and relative abundance on the reef	27
2.4 Discussion	32
2.4.1 Non-specific uptake of <i>Symbiodinium</i> in coral juveniles is dominated by highly infectious/opportunistic types	32
2.4.2 No effect of light on <i>Symbiodinium</i> selection	37
Chapter 3.0 Impact of light and temperature on the uptake of algal symbionts by juveniles of <i>Acropora tenuis</i> and <i>A. millepora</i>	40
3.1 Introduction	41
3.2 Materials and methods	43
3.2.1 Experimental corals, <i>Symbiodinium</i> inoculation, and genetic identification	43
3.2.2 Experimental design	43

3.2.3	Effects of temperature and light on <i>Symbiodinium</i> uptake	44
3.2.4	Effects of temperature and light on the type of <i>Symbiodinium</i> acquired and maintained by coral juveniles	46
3.2.5	Data analysis	47
3.3	Results	48
3.3.1	Effects of temperature and light on the onset of the symbiosis	48
3.3.2	Effects of temperature and light on the type of symbiont acquired and maintained	51
3.4	Discussion	53
Chapter 4.0 Long term patterns in succession of <i>Symbiodinium</i> types in juveniles of <i>Acropora tenuis</i> and <i>A. millepora</i>		59
4.1	Introduction	60
4.2	Materials and methods	62
4.2.1	Specificity and succession of <i>Symbiodinium</i> types in coral juveniles	63
4.2.2	Monitoring of algal types over time	64
4.2.3	Onset of reproductive maturity	65
4.2.4	Statistical analysis	66
4.3	Results	66
4.3.1	Symbiont succession in <i>Acropora tenuis</i> juveniles	66
4.3.2	Symbiont succession in <i>A. millepora</i> juveniles	70
4.3.3	Onset of reproductive maturity and <i>Symbiodinium</i> community <i>in hospite</i>	72
4.4	Discussion	73
4.4.1	Delayed onset of specificity in <i>Acropora tenuis</i> juveniles	74
4.4.2	Unresolved specificity in <i>A. millepora</i> juveniles	79
4.4.3	No link between onset of sexual maturity and symbiont composition	81
4.4.4	Conclusion	81
Chapter 5.0 Physiological contributions of different <i>Symbiodinium</i> types to thermal tolerance of <i>Acropora tenuis</i> juveniles		83
5.1	Introduction	84
5.2	Materials and methods	87
5.2.1	Experimental corals, <i>Symbiodinium</i> inoculation and genetic identification	87
5.2.2	Experimental design	88
5.2.3	Experimental setup	89
5.2.4	Bleaching condition of corals – Pilot study and Experiment 1	91
5.2.5	Photochemistry of heat stressed corals	91
5.2.6	Oxygen microelectrode characterization of photosynthesis and respiration	92
5.2.7	Chlorophyll <i>a</i> content and xanthophyll pigments	94
5.2.8	Reflectance spectra of corals and calculation of chlorophyll <i>a</i> specific absorption coefficient ($a^*_{\text{Chl } a}$)	97
5.2.9	Statistical analysis	98
5.3	Results	98
5.3.1	Bleaching condition of corals	98
5.3.2	Photochemistry of heat-stressed coral juveniles	100
5.3.3	Oxygen microelectrode characterization of photosynthesis and respiration	105
5.3.4	Chlorophyll <i>a</i> content, absorption coefficient ($a^*_{\text{Chl } a}$), and xanthophyll pigments	107
5.4	Discussion	110

5.4.1	Photochemical confirmation of enhanced thermal tolerance of C1-juveniles	111
5.4.2	The role of light in the bleaching response of heat-stressed corals	112
5.4.3	Contribution of symbionts to metabolic costs incurred during heat stress	113
5.4.4	Potential role of host factors in the heat stress response	114
Chapter 6.0	General discussion, major findings, and future research	117
6.1	General Discussion	118
6.2	Major findings of this thesis	123
6.3	The future	123
References		126

LIST OF TABLES

Table 2.1. Summary of juveniles available for reciprocal grow-out experiments.	16
Table 2.2. Comparisons of <i>Symbiodinium</i> distributions in <i>A. tenuis</i> and <i>A. millepora</i> juveniles.	26
Table 2.3. <i>Symbiodinium</i> diversity in cnidarian hosts at Magnetic and Orpheus Islands (GBR).	30
Table 3.1. Summary of total number of juveniles counted at each temperature by light treatment during the mid-experiment census (mid) and for the census at the end of the experiment (end).	50
Table 3.2. Repeated measures ANOVA results comparing changes in D:C cell ratios in <i>Acropora tenuis</i> (a) and <i>A. millepora</i> (b) juveniles kept at three temperatures (28, 30, or 31°C) by two light levels (390 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ or 180 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$).	53
Table 4.1. Comparisons of <i>Symbiodinium</i> type distributions in four cohorts (2003-2006) of <i>A. tenuis</i> juveniles raised at Magnetic Island.	69
Table 5.1. Summary of physiological assays, experimental setup and number of colonies for each heat stress experiment.	91
Table 5.2. HPLC analytical gradient protocol. Flow rate was maintained at 1 ml min ⁻¹ for the duration of the analysis.	96

LIST OF FIGURES

Fig. 2.1. Study sites.	14
Fig. 2.2. Schematic representation of two tile arrangements deployed on the reef.	19
Fig. 2.3. Uptake of <i>Symbiodinium</i> in <i>Acropora tenuis</i> juveniles.	23
Fig. 2.4. Uptake of <i>Symbiodinium</i> in <i>Acropora millepora</i> juveniles.	25
Fig. 2.5. Uptake of <i>Symbiodinium</i> in different light environments.	27
Fig. 2.6. <i>Symbiodinium</i> diversity and distribution in cnidarian hosts on the reefs at Magnetic and Orpheus Islands.	29
Fig. 3.1. Visual assessment of <i>Symbiodinium</i> uptake.	45
Fig. 3.2. Pigmentation ratios of <i>A. tenuis</i> juveniles kept at 28, 30, or 31°C and under high light (390 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) or low light (180 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) levels.	49
Fig. 3.3. Pigmentation ratio of <i>A. millepora</i> juveniles kept at 28, 30, or 31°C and under high light (390 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) or low light (180 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) levels.	49
Fig. 3.4. Relative survival of juveniles in the 28, 30, or 31°C treatments and under high light (390 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) or low light (180 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) levels for <i>Acropora tenuis</i> after 20 days and <i>A. millepora</i> after 30 days.	50
Fig. 3.5. Change in <i>Symbiodinium</i> D:C cell ratios over time in <i>Acropora tenuis</i> juveniles at 28, 30, or 31°C in high light (390 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and low light levels (180 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$).	51
Fig. 3.6. Change in <i>Symbiodinium</i> D:C cell ratios over time in <i>Acropora millepora</i> juveniles at 28, 30, or 31°C in high light (390 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and low light levels (180 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$).	52
Fig. 4.1. Succession of <i>Symbiodinium</i> types in <i>Acropora tenuis</i> juveniles raised at Magnetic Island after four spawning events (2003- 2006).	68
Fig. 4.2. Succession of <i>Symbiodinium</i> types in <i>Acropora millepora</i> juveniles raised at Magnetic Island after four spawning events (2003-2006).	72
Fig. 4.3. a) Symbiont communities in naturally recruited juveniles of <i>A. tenuis</i> at Magnetic Island. Pie charts show the proportion of juveniles hosting: <i>Symbiodinium</i> C1 (blue), or D (red). b) Percentage of the same colonies with mature eggs (pink) or with no mature eggs (white).	73
Fig. 4.4. Changes in growth form of <i>Acropora tenuis</i> juveniles over time.	77
Fig. 5.1. Visual scoring of colonies hosting <i>Symbiodinium</i> type C1 or D. <i>Symbiodinium</i> type, temperature treatment (28°C or 32°C) and sample size (n) are shown for each graph.	99
Fig. 5.2. Visual scoring of colonies hosting <i>Symbiodinium</i> type C1 or D. <i>Symbiodinium</i> type, temperature treatment (30°C or 31°C) and sample size (n) are shown for each graph.	100
Fig. 5.3. Maximum quantum yield (F_v/F_m) of corals hosting either <i>Symbiodinium</i> C1 (●) or D (○) at 28°C (a) or 32°C (b) during the Pilot Study.	101
Fig. 5.4. Maximum quantum yield (F_v/F_m) of corals hosting either <i>Symbiodinium</i> C1 (●) or D (○) at 30°C (a) or 31°C (b) during the Pilot Study.	102
Fig. 5.5. Maximum quantum yield (F_v/F_m) of corals hosting either <i>Symbiodinium</i> C1 (●) or D (○).	103
Fig. 5.6. a) Maximum excitation pressure over PSII (Q_m) of C1 (●) or D-corals (○) at 26°C, 29°C, and 32 °C. b) Maximum quantum yield (F_v/F_m) of the same corals.	105

Fig. 5.7. O₂ microelectrode measurement of photosynthesis in C1 (black columns) or D-corals (grey columns). 106

Fig. 5.8. **a)** Chl *a* content in sub samples of C1 (black bars) or D-corals (grey bars). **b)** Specific absorption coefficient of Chl *a* ($a^*_{\text{chl } a}$) in the same samples as in **a)**. 108

Fig. 5.9. Changes in xanthophyll ratio (ratio of diatoxanthin to the sum of diatoxanthin and diadinoxanthin) of C1 (black bars) or D-corals (grey bars). 109