Distribution, abundance and life cycle of free-living *Symbiodinium*

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PhD by Research

July 2018

A thesis submitted in fulfilment of the requirements for

the degree of Doctor of Philosophy in Science

Climate Change Cluster (C3),

School of Life Sciences,

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Certificate of Original Authorship

I, Risa Fujise declare that this thesis, submitted in fulfilment of the requirements for the award of the degree of Doctor of Philosophy in Science, in the School of Life Sciences, Faculty of Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise reference or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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Date: 2018.07.15

Acknowledgements

First and foremost, I would like to express my deepest gratitude to my supervisors; A/Prof. David Suggett and Prof. Peter Ralph, who consistently provided patient guidance and warm encouragement throughout the journey of my PhD. I am very fortunate to have Dave as my supervisor because he always encouraged me even when I hit those "brick walls" and he corrected the course of my PhD, keeping me positive all the time. I always looked forward to having a meeting with Peter because he always provided such a new interesting insight into my research which fuelled my energy to keep walking on the long journey of the PhD. I cannot tell how much I appreciate to my supervisors' huge help to bring me to the end of my PhD.

I would also like to thank my collaborators Dr. Michael Stat (Curtin University). It was a great opportunity for me to visit TrEnD lab and analyse the samples by myself in order to deepen my knowledge and understand the principal methodology of NGS techniques. And also to Dr. Jörg Frommlet (University of Aveiro) for valuable comments and suggestions on the cell cycle analysis of *Symbiodinium*, which was not an easy journey but finally became a big success. I also greatly appreciate Dr. Matthew Nitschke for his huge help and contribution; I could not have survived without him. To Dr. Tim Kahlke for building whole pipelines for bioinformatic analysis of *Symbiodinium* – it was simply not possible to make this research a success without his huge help. To Dr. Nahshon Siboni for helping me to build the qPCR assay and always made me laugh throughout the research. To Dr. Stephen Woodcock for checking whether I was on the right path or not for the various complex statistical analysis.

I would like to express my deepest appreciation to the staff at C3: Paul Brooks, Graeme

Poleweski, Gemma Armstrong, Susan Fenech, Kun Xiao, Lucia Bennar, John Moore, Mellisa Oey; Heron Island Research Station: Maureen Roberts, Elizabeth Perkins, Pauline Dusseau, Bec Tite; and TrEnD lab: Matthew Power, Nichole White, Megan Coghlan, for full support of the experiment, sampling and analyses required to make my PhD success. Especially to Paul who supported my long PhD journey and kept watch me as a child (or maybe a grandchild), keeping me on the straight and narrow.

I would like to offer my special thanks to all of the lab members in the Suggett Lab: David Hughes, Samantha Goyen, Steph Gardner, Caitlin Lawson, Emma Camp, Mickael Ros, Nerissa Fisher, Trent Haydon and Rachel Levin; TrEnD lab: Katrina West, Frederick Seersholm, Adam Koziol, Matt Heydenrych, Tiffany Simpson, Alicia Grealy, Miwa Takahashi, Daniel Werndly and Tina Berry, for such a wonderful time in the laboratory and warm encouragement for my entire PhD life, which has been fantastic. I really enjoyed the time in Australia and now I can say with confident that I made a great choice to come to Australia to do my PhD.

Of course, I also deeply appreciate for the fundings from the University of Technology Sydney (UTS International Research Scholarship (IRS) and UTS President's Scholarship (UTSP)), Climate Change Cluster (C3 PhD fund) and Yoshida Scholarship Foundation (Scholarship grants to Japanese students to study abroad) to be able to study, and undertake research using cutting edge technologies for contributing to the research field to advance knowledge.

Finally, special thanks to my family for the consistent and unconditional support and let me open my wings and fly towards the world.

Table of Contents

List of Figures

Chapter 1

Figure 1.4. Mechanisms for regulating *Symbiodinium* density *in hospite*. **(i)** Expulsion of either detached a whole host cell or a normal cell and/or a degraded cell by exocytosis. **(ii)** Degradation (digestion) of a cell by host phagocytosis. (i) and (ii) is termed the post-mitotic process. **(iii)** Inhibition of symbiont cell growth and division through controlling cell cycle progression which is termed the pre-mitotic process (adapted and modified from Davy et al. 2012).

Figure 1.5. Life cycle of *Symbiodinium microadriaticum*. **A.** Vegetative cell. **B.** Vegetative cell undergoing binary fission, producing two daughter cells. **C.** Vegetative cyst, differing from the vegetative cell mainly in cell wall thickness. **D.** Mature zoosporangium, containing a gymnodinioid zoospore. **E.** Gymnodinioid zoospore. **F.** Aplanospore. **G.** Cyst containing two autospores. **H.** Cyst containing developing isogametes. **I.** Liberated isogametes (taken from Freudenthal 1962).

Figure 1.6. Life cycle of *Symbiodinium* including asexual reproduction (right side) and possible sexual reproduction (left side). Solid lines denote observed transformations, while dashed lines are possible routes of sexual reproduction or meiosis. **1.** Vegetative coccoid cell. **2.** Dividing vegetative cell (doublet). **3**. Motile cells. **4.** Dividing vegetative cell (triplet). **5.** Isogametes. **6.** Diploid vegetative cell resulting from fusion of the gametes. **5.** Tetrad cell, formed from meiosis of the diploid vegetative cell (adapted and modified from Fitt and Trench 1983 and Stat et al. 2006).

Figure 1.7. *Symbiodinium* cell cycle progression. Irradiance (i.e. light period, denoted by yellow field) is required for cell growth and DNA synthesis (G_1 to S to G_2/M), while dark period (black field) is required for cytokinesis of mitotic division (G_2/M to G_1).

Motility of *Symbiodinium* are synchronized to the cell cycle, and increase at the onset of light which is correlated with the G_1 phase of gymnodinioid cells, decreasing when cells enter the S phase and the lowest at G_2/M phase with coccoid cell (adapted and modified from Wang et al. 2008).

Chapter 2

Figure 2.1. Location of sampling sites and coral species. **A-B.** Location of Heron Island in southern Great Barrier Reef in east coast of Australia. **C.** Location of sampling sites (1: *Acropora aspera* site, 2: *Montipora digitata* site, and 3: *Pocillopora damicornis* site). Collected host coral species: **D.** *A. aspera*, **E.** *M. digitata*, and **F.** *P. damicornis*. **G.** Example of coral community surrounded by reef environments including *Padina* sp. (collected macroalgae species).

Figure 2.2. Environmental data on Heron reef (2015-2016). Monthly averaged sea surface temperature (SST, °C) is shown as a black solid line (left Y axis) and monthly averaged photosynthetic active radiation (PAR, μmol photons m^{-2} s^{-1}) is shown as a red dashed line (right Y axis) from January 2015 to December 2016. Samplings were performed in October 2015 (spawning season) and March 2016 (summer season) and indicated by the grey areas.

Figure 2.3. Box plots for number of sequences, clades, ITS2 variants and OTUs in each site, habitat and season. Box plots for corals are shown as pink, water as blue, sediment as yellow and macroalgae as green.

Figure 2.4. Venn diagrams for *in hospite* (coral) and free-living *Symbiodinium* genetic types in environments. Number of **A.** ITS2 variants. **B.** OTUs, belong to clade C. Number of *Symbiodinium* ITS2 variants or OTUs *in hospite* are shown with orange circles and in environment (sum of water, sediment and macroalgae samples) with green circles for each site (coral species) and season (spawning vs. summer).

Figure 2.5. Relative abundance of *Symbiodinium* clades during the spawning and summer seasons obtained by DNA metabarcoding. 48 samples: 3 sites (coral species), 4 habitats (*in hospite*, water, sediment and macroalgae), 4 replicates (a, b, c, d) for 2 seasons (spawning vs. summer). Colour of bars for each clade are shown above the graphs.

Figure 2.6. nMDS plots of *Symbiodinium* community compositions. **A.** Clade. **B.** ITS2 variant. **C.** OTU. Non-metric multidimensional scaling (nMDS) was performed on each variable per variant using Bray-Curtis Similarity. CLUSTER analysis was performed for cladal composition (**A**); similarity is shown at the 60% (green solid lines), 70% (purple dashed lines) and 80% (pink dashed lines) levels and vectors driving the clustering are shown as blue lines. Corals are represented by pink, water by blue, sediment by yellow, and macroalgae by green with site 1 with circle, site 2 with rectangle, and site 3 with square markers. Plots for the spawning season are shown with closed markers and the summer season with open markers (all combinations of the markers are shown below the graphs).

Figure 2.7. Relative abundance of *Symbiodinium* ITS2 variants during the spawning (left panel) and summer seasons (right panel) obtained by DNA metabarcoding.

Relative abundance of ITS2 variants in each replicate (n = 4) were averaged and top three ITS2 variants in each clade are shown as bar graphs for each site, habitat and season.

Figure 2.8. Heatmap of *Symbiodinium* ITS2 variant compositions. Compositions of ITS2 variants in each replicate (n = 4) were averaged and top three ITS2 variants in each clade are displayed on the right. The colour (scale bar on the top of the graph) represents the proportion of each ITS2 variants in the sample (0-10% with blue gradient and 10-100% with red gradient), and white boxes indicate an absence of the ITS2 variant.

Figure 2.9. nMDS plot of *Symbiodinium* community compositions based on abundance of clades A, C and D obtained by qPCR. Non-metric multidimensional scaling (nMDS) and CLUSTER analysis were performed on each variable per variant using Bray-Curtis Similarity. Similarity is shown at the 50% (green solid lines) and 80% (pink dashed lines) levels. Corals are represented by pink, water by blue, sediment by yellow, and macroalgae by green with site 1 with circle, site 2 with rectangle, and site 3 with square markers. Plots for the spawning season are shown with closed markers and the summer season with open markers (all combinations of the markers are shown in the graph).

Figure 2.10. Cell density of free-living *Symbiodinium* in environmental habitats obtained by qPCR. Number of cells of free-living *Symbiodinium* belong to clades A, C and D were normalised per cm^3 for each sample type (habitat) in each site and season, and replicates (n = 2-4) were averaged. Cell densities are shown in both bubble charts

(upper panel) and bar graphs (lower panel).

Figure 2.11. nMDS plot for NGS vs. qPCR *Symbiodinium* community compositions of clades A, C and D in environmental habitats. Non-metric multidimensional scaling (nMDS) was performed on each variable per variant using Bray-Curtis Similarity. Water samples are represented by blue, sediment by yellow, and macroalgae by green markers. Plots for qPCR are shown with closed markers and NGS with open markers.

Figure 2.12. Relative abundance of *Symbiodinium* clades A, C and D during the spawning and summer seasons obtained by two techniques (NGS vs. qPCR). 36 samples per season per technique: 3 sites (coral species), 3 habitats (water, sediment and macroalgae), 4 replicates (a, b, c, d) for 2 seasons (spawning vs. summer) for 2 techniques (NGS vs. qPCR). Colour of bars for each clade are shown above the graphs.

Chapter 3

Figure 3.1. Location of sampling sites and host species. **A.** Location of sampling sites in Sydney Harbour (sites 1 and 2) and Botany Bay (site 3) together with host benthic coverage bar graphs. Benthic coverage of hard corals (*Plesiastrea versipora* and *Coscinaraea mcneilli*): pink bars, soft coral (*Capnella gaboensis*): orange bars, algae: green bars, and others (abiotic substrates): purple bars, were obtained by video transects. Mean percentage of coverages are shown above each bar and error bars indicate SD. Collected host species: **B.** *P. versipora* (hard coral) and **C.** *C. gaboensis* (soft coral). **D.** Bleached *P. versipora*. **E.** Tagged *P. versipora* during the bleaching (April 2016) and the recovery (July 2016).

Figure 3.2. Environmental data in Sydney Harbour (sites 1 and 2) and Botany Bay (site 3). Monthly averaged sea surface temperature (SST, °C) are shown as black solid lines (left Y axis) and monthly averaged photosynthetic active radiation (PAR, μmol photons m^{-2} s^{-1}) are shown as red dashed lines (right Y axis) from January 2015 to December 2016 for **A.** Sydney Harbour and **B.** Botany Bay. SST thermal anomalies from past 10-year (2007-2016) are shown as black dashed lines for **C.** Sydney Harbour and **D.** Botany Bay. Samplings were performed in December 2015 (pre-bleaching) and May 2016 (bleaching) and indicated by the grey areas.

Figure 3.3. Box plots for number of sequences, clades and OTUs based on the cp23S marker in each site, habitat and sampling time. Box plots for *P. versipora* (hard coral) are shown as pink, *C. gaboensis* (soft coral) as orange, water as blue, sediment as yellow and macroalgae as green.

Figure 3.4. Box plots for number of sequences, clades, ITS2 variants and OTUs based on the ITS2 marker in each site, habitat and season. Box plots for *P. versipora* (hard coral) are shown as pink, *C. gaboensis* (soft coral) as orange, water as blue, sediment as yellow and macroalgae as green.

Figure 3.5. Venn diagrams for *in hospite* (*P. versipora* and *C. gaboensis*) and free-living types in environment. Number of **A.** ITS2 variants. **B.** OTUs. Number of *Symbiodinium* ITS2 variants or OTUs in *P. versipora* are shown with pink circles, *C. gaboensis* with orange circles and in environment (sum of water, sediment and macroalgae samples) with green circles for each site and sampling time (pre-bleaching vs. bleaching).

Figure 3.6. Relative abundance of *Symbiodinium* clades during pre-bleaching and bleaching obtained by DNA metabarcoding based on the cp23S marker. 3 sites × 5 habitats (hard coral: *P. versipora*, soft coral: *C. gaboensis*, water, sediment and macroalgae) × 4 replicates (a, b, c, d) for 2 sampling time points (pre-bleaching vs. bleaching). Note that no *C. gaboensis* samples were collected at site 1 during bleaching, and no bars in sediment and macroalgae samples were due to no amplification/no sequences. Colour of bars for each clade are shown above the graphs.

Figure 3.7. nMDS plots of *Symbiodinium* community compositions based on the cp23S marker. **A.** Clade. **B.** OTU. Non-metric multidimensional scaling (nMDS) was performed on each variable per variant using Bray-Curtis Similarity. CLUSTER analysis was performed for cladal composition (**A**); similarity is shown at the 50% (green solid lines), 80% (pink dashed lines) levels and vectors driving the clustering are shown as blue lines. Hard corals are represented by pink, soft coral by orange, water by blue, sediment by yellow, and macroalgae by green with site 1 with circle, site 2 with rectangle, and site 3 with square markers. Plots for pre-bleaching samples are shown with closed markers and bleaching samples with open markers (all combinations of the markers are shown below the graphs).

Figure 3.8. Relative abundance of *Symbiodinium* clades during pre-bleaching and bleaching obtained by DNA metabarcoding based on the ITS2 marker. 3 sites × 5 habitats (hard coral: *P. versipora*, soft coral: *C. gaboensis*, water, sediment and macroalgae) × 4 replicates (a, b, c, d) for 2 sampling time points (pre-bleaching vs. bleaching). Note that no *C. gaboensis* samples were collected at site 1 during bleaching, and no bars in water, sediment and macroalgae samples were due to no

amplification/no sequences. Colour of bars for each clade are shown above the graphs.

Figure 3.9. nMDS plots of *Symbiodinium* community compositions based on the ITS2 marker. **A.** Clade. **B.** ITS2 variant. **C.** OTU. Non-metric multidimensional scaling (nMDS) was performed on each variable per variant using Bray-Curtis Similarity. CLUSTER analysis was performed for cladal composition (**A**); similarity is shown at the 50% (green solid lines), 80% (pink dashed lines) levels and vectors driving the clustering are shown as blue lines. Hard corals are represented by pink, soft coral by orange, water by blue, sediment by yellow, and macroalgae by green with site 1 with circle, site 2 with rectangle, and site 3 with square markers. Plots for pre-bleaching samples are shown with closed markers and bleaching samples with open markers (all combinations for the markers are shown below the graphs).

Figure 3.10. Relative abundance of *Symbiodinium* ITS2 variant during pre-bleaching (left panel) and bleaching (right panel) obtained by DNA metabarcoding based on the ITS2 marker. Relative abundance of ITS2 variants in each replicate (n = 2-4, depends on the samples) were averaged and top three ITS2 variants in each clade are shown as bar graphs for each site, habitat and sampling time point.

Figure 3.11. Heatmap of *Symbiodinium* ITS2 variant compositions. Compositions of ITS2 variants in each replicate (n = 2-4, depends on the samples) were averaged and top three ITS2 variants in each clade are displayed on the right. The colour (scale bar on the top of the graph) represents the proportion of each ITS2 variants in the sample (0-10% with blue gradient and 10-100% with red gradient), and white boxes indicate an absence of the ITS2 variant.

Figure 3.12. Box plots for number of clades, ITS2 variants and OTUs based on the ITS2 marker in each region and habitat. Box plots for hard corals are shown as pink, soft coral as orange, water as blue, sediment as yellow and macroalgae as green. Sites and seasons were pooled together.

Figure 3.13. Phylogenetic trees based on OTUs together with sequences in ITS2 reference database SymTyper. **A.** Clade A OTUs in Sydney Harbour with red letters plus clade A sequences in SymTyper with black letters. **B.** Clade B OTUs in Sydney Harbour with blue letters plus clade B sequences in SymTyper with black letters. **C.** Clade C OTUs in Heron Island reef with yellow letters plus clade C sequences in SymTyper with black letters. Phylogenetic trees were created using QIIME (*make_phylogeny.py*).

Figure 3.14. Environmental data in Sydney Harbour (temperate reef) and Heron Island (tropical reef). **A.** Monthly averaged sea surface temperature (SST, °C) from January 2015 to December 2016 including sampling periods for both Sydney Harbour (December 2015 and May 2016) and Heron Island (October 2015 and March 2016). Black solid line for Sydney Harbour SST and black dashed line for Heron Island SST. **B.** Monthly averaged photosynthetic active radiation (PAR, μmol photons m^{-2} s^{-1}) from January 2015 to December 2016. Red solid line for Sydney Harbour PAR (averaged PAR at depth 5-7 m where corals inhabit) and red dashed line for Heron PAR at sea surface.

Figure 3.15. Venn diagrams to show connectivity of *Symbiodinium* genetic types between Sydney Harbour (temperate region) and Heron Island (tropical region).

Number of **A.** ITS2 variants and **B.** OTUs. Number of *Symbiodinium* ITS2 variants or OTUs (combining across sites, habitats and seasons) in Sydney Harbour are shown with blue circles and Heron Island with pink circles.

Figure 3.16. nMDS plots of *Symbiodinium* community compositions from Sydney Harbour (temperate region) and Heron Island (tropical regions) based on the ITS2 marker. **A.** ITS2 variants. **B.** OTUs. Non-metric multidimensional scaling (nMDS) was performed on each variable per variant using Bray-Curtis Similarity. Hard corals are represented by pink, soft coral by orange, water by blue, sediment by yellow and macroalgae by green markers. Plots for Sydney Harbour samples are shown as closed markers and Heron Island samples as open markers (all combinations of the markers are shown at the right to the graph).

Chapter 4

Figure 4.1. DNA histograms of cell cycle progression through 24 h. Example cell cycle analysis of *Symbiodinium* type B1 under high light treatment. First distribution (blue) are cells in G_1 phase with 1n DNA content. Second distribution (green) is G_2/M phase, where cells have 2n DNA content (twice as much as G_1 phase cells). S phase cells (red) have intermediate DNA content between G_1 and G_2/M phase cells. White bar shows the light period (8:00-20:00) and black bar shows the dark period (20:00-8:00). All distributions were produced using ModFit LT.

Figure 4.2. Cell cycle progression of four *Symbiodinium* culture strains through 24 h under two light treatments (low light vs. high light). Y axis is percentage of G_1, S and G_2/M phase, and X axis is the sampling time points (T0-24). White and black bars on

the top of each graph show the light period (T0-12) and dark period (T12-24), respectively. G_1 phase is shown as dotted lines with black circles, S phase as dashed lines with open squares, and G_2/M phase as solid lines with black rectangles. Values represent mean ± SD (n = 3).

Figure 4.3. Multi-dimensional scaling (MDS) plots of four *Symbiodinium* culture strains with cell cycle proportions corresponding to the time of the G_2/M peak. **A.** Light treatment (low light (LL) vs. high light (HL)). **B.** Temperature treatment (control vs. heat). Cluster analysis and MDS were performed on the average of each variable per variant; similarity is shown at the 93% (solid lines) and 96% (dashed lines) levels and vectors driving the clustering are shown as blue lines. Low light and high light are represented by yellow squares and orange circles, respectively. Control temperatures and heat treatments are represented by blue squares and pink circles, respectively.

Figure 4.4. F_v/F_m of four *Symbiodinium* culture strains. Changes in photophysiology under light (low light (LL) vs. high light (HL), upper panels) and temperature (control vs. heat, lower panels) treatment. Low light and control temperatures are shown as solid lines with open circles and high light and heat treatments are shown as dashed lines with black circles. Values represent mean ± SD (n = 3). The temperature increase is shown on the top X axis of each graph for heat treatment (lower panel). The temperatures for heat treatments were; day 2: 27.7 ± 0.75°C, day 3: 29.7 ± 0.87°C, day 4: 31.9 ± 0.89°C, day 5: 32.0 ± 0.81°C, day 6: 32.1 ± 0.56°C, day 7: 32.3 ± 0.84°C (± SD for daily mean temperature measured using iButton® temperature logger).

Figure 4.5. Cell cycle progression of four *Symbiodinium* culture strains through 24 h under the temperature treatment (control vs. heat). Y axis is percentage of G_1, S and G_2/M phase, and X axis is the sampling time points (T0-24). White and black bars on the top of each graph show the light period (T0-12) and dark period (T12-24), respectively. G_1 phase is shown as dotted lines with black circles, S phase as dashed lines with open squares, and G_2/M phase as solid lines with black rectangles. Values represent mean ± SD (n = 3).

Chapter 5

Figure 5.1. Key findings from the thesis. **Chapter 2**: Transiently free-living *Symbiodinium* mainly existed in water and macroalgae habitats (seems to be a main source of symbionts for hosts), in contrast, exclusively free-living types dominated in sediment. **Chapter 3**: Unique free-living populations (temperate specific types) dominated in the high-latitude temperate coral communities and the populations would shift due to an impact of the heat wave. **Chapter 4**: Cell cycle progressions of *Symbiodinium* were conserved across species, but proportions differed by species. Heat stress arrested cells in G_1 phase and supressed the growth. Arrows indicate the linkages between the chapters: Chapter 1 → 2: **Community structure** of free-living *Symbiodinium* is shaped by reef structures and environmental conditions; Chapter 2 → 3: **Population dynamic** seems to contribute for regulating community structure of *Symbiodinium* via cell cycle; Chapter 3 → 1: **Biodiversity** of free-living *Symbiodinium* is maintained via individual population turnover.

Appendix: OTU analysis for *Symbiodinium* culture strains

Figure 6.1. Box plots for percent identity of sequences within each strain. Background colour were used to classify the clade of strains: red for clade A, blue for clade B, purple for clade D, green for clade E, and orange for clade F.

List of Tables

Chapter 1

Chapter 2

of the primer sets for qPCR assays. Number of cells per qPCR reaction (5 μL) used as the highest concentration of standard DNA are shown. DNA was diluted in 1/10, 1/100, 1/1,000, 1/10,000 for making five concentrations for the calibration curves for each strain.

Table 2.2. *Symbiodinium* culture strains used as quantification standards for the qPCR assays for assessing abundance of free-living *Symbiodinium*. One culture strain from each clade was selected for use as a quantification standard for the qPCR assay for each primer set (clades A-F, except E). Number of cells per qPCR reaction (5 μL) used for making calibration curves (five concentrations) and minimum detection limits are shown.

Table 2.3. Number of sequences, ITS2 variants and OTUs belong to clades A-I, except clade E.

Table 2.4. Cell density of free-living *Symbiodinium* obtained by qPCR. Cell densities of free-living *Symbiodinium* belong to clades A, C and D were detected within the quantification range based on the calibration curves using *Symbiodinium* culture strains as standards (list in Table 2.2); however, *Symbiodinium* belong to clades B and F were below the detection limits so not included in this table. Number of cells were normalised per mL for water and sediment samples and per wet weight (g) for macroalgae samples. In addition, number of cells in all sample types (habitats) were normalised per cm^3 for comparison between different sample types. Mean ± SD (n = 2-4) are shown for all samples. Cell density of clade which was only detected in one replicate per sample is shown with no SD.

Chapter 3

Table 3.1. *Symbiodinium* culture strains used as quantitative standards for qPCR assays for assessing abundance of free-living *Symbiodinium*. One culture strain from each clade was selected for use as a quantitative standard for qPCR assay for each primer set (clades A-F). Number of cells per qPCR reaction (5 μL) used for making calibration curves (five concentrations) and minimum detection limits are shown.

Table 3.2. Number of sequences and OTUs belong to clades A-C, E and F based on the cp23S marker.

Table 3.3. Number of sequences, ITS2 variants and OTUs belong to clades A-G based on the ITS2 marker.

Chapter 4

Table 4.1. Summary of *Symbiodinium* sp. type identifiers and source (geographic origin and host species) used for cell cycle analysis.

Table 4.2. Cell cycle and growth characteristics across four *Symbiodinium* culture strains under the light treatments (low light vs. high light). Cell cycle parameters: G_1 peak, S peak and G_2/M peak proportions (the maximum % of cells, for each phase, reached throughout a diel cycle) were obtained from analysis using cell cycle software: ModFit LT. Growth rates were calculated with equation 1 using cell densities. Mean ± SD (n = 3) are shown for all parameters except for cell volume (μm^3): median (upper–lower quartile ranges) are shown. Two-way ANOVA (strains × treatments) across

variants is also shown where superscript letters indicate *post-hoc* groupings of strain effects within each treatment.

Table 4.3. Cell cycle and growth characteristics across four *Symbiodinium* culture strains under the temperature treatments (control vs. heat). Cell cycle parameters: G_1 peak, S peak and G_2/M peak proportions (the maximum % of cells, for each phase, reached throughout a diel cycle) were obtained from analysis using cell cycle software: ModFit LT. Growth rates were calculated with equation 1 using cell densities. Mean ± SD (n = 3) are shown for all parameters except for cell volume (μm^3): median (upper–lower quartile ranges) are shown. Two-way ANOVA (strains × treatments) across variants is also shown where superscript letters indicate *post-hoc* groupings of strain effects within each treatment.

Appendix: OTU analysis for *Symbiodinium* culture strains

Table 6.1. *Symbiodinium* culture strains used for the NGS analysis. *****I included two identical strains of CCMP2548 (*S. natans*) (X and Y). ******SG_37 and SG_40 were isolated from *Plesiastrea versipora*, but the isolates were possibly surface contaminants from the environment, because *P. versipora* was dominated by B18-like types based on the NGS analysis of *in hospite* samples (see Chapter 3, section 3.4.7).

Table 6.2. OTU table (97% cut-off) for *Symbiodinium* culture strains. Top three OTUs (based on the number of sequences within each strain) are highlighted with colours and OTUs which contain ≥ 1% of the reads within each sample are shown as bold. Colour coding: red for clade A, blue for clade B, yellow for clade C, purple for clade D, green for clade E, and orange for clade F.

Table 6.3. OTU table (97% cut-off) for *Symbiodinium* culture strains after applying OTU abundance filtering. OTUs which contain < 1% of the reads within each sample were replaced by 0 and removed from the OTU table if those OTUs contain 0 sequence across all samples. OTUs contain sequences are highlighted with colours.

List of Supplementary Figures

Chapter 4

Figure S4.1. Example of manual gating for *Symbiodinium* populations in flow cytometry analysis. The gating for **A.** B1, **B.** C1, **C.** C1′, and **D.** D1a strains under high light treatment at sampling time point T0. X axis is forward scatter, and Y axis is side scatter in log scale for both.

Figure S4.2. Cell densities of four *Symbiodinium* culture strains under the light treatment (low light vs. high light). B1 was diluted into half concentration at day 3 both under low light and high light treatment to prevent the over growth. Cell cycle samples were collected through days 6-7. The days circled on the X axis and corresponding cell densities were used in calculating the growth rate (equation 1). Outliers which deviated from the exponential curve were treated as counting errors and not used for calculating the growth rate. Values represent mean ± SD (n = 3).

Figure S4.3. Correlation between cell cycle and growth parameters. Relationship between **A.** Growth rate and G_1 peak proportion, **B.** Growth rate and G_2/M peak proportion, **C.** Cell volume and G_1 peak proportion, **D.** Cell volume and growth rate. Values from low light and high light were plotted (n = 24).

Figure S4.4. Cell densities of four *Symbiodinium* culture strains under the temperature treatment (control vs. heat). Cell cycle samples were collected through days 6-7. The days circled on the X axis and corresponding cell densities were used in calculating

the growth rate (equation 1). Outliers which deviated from the exponential curve were treated as counting errors and not used for calculating the growth rate. Values represent mean ± SD (n = 3). The temperature increase is shown on the top X axis of each graph for heat treatment (lower panel). The temperatures were; day 1: 26.4 ± 0.72°C, day 2: 27.7 ± 0.75°C, day 3: 29.7 ± 0.87°C, day 4: 31.9 ± 0.89°C, day 5: 32.0 ± 0.81°C, day 6: 32.1 ± 0.56°C, day 7: 32.3 ± 0.84°C (± SD for daily mean temperature measured using iButton® temperature loggers).

List of Supplementary Tables

Chapter 2

Table S2.1. Number of sequences, clades, ITS2 variants and OTUs obtained by DNA metabarcoding. Total samples are 96 samples (2 seasons × 3 sites (coral species) × 4 habitats × 4 replicates).

Table S2.2. Summary of Kruskal-Wallis test for number of clades, ITS2 variants and OTUs. **A.** Main effects. **B.** *Post-hoc* pairwise comparison for sites × habitats × seasons. df: degrees of freedom, P value: * for $P < 0.05$, ** for $P < 0.01$.

Table S2.3. Summary of PERMANOVA main effects for *Symbiodinium* community compositions. **A.** Clades. **B.** ITS2 variants. **C.** OTUs. Relative abundance of community compositions was square-root transformed and PERMANOVA was performed with sites (coral species) (3 levels), habitats (4 levels) and seasons (2 levels) as fixed factors, using type III sum of squares and unrestricted permutation of raw data with 999 permutations. MS: mean square, df: degrees of freedom, *F*: Fisher statistic, P value: * for $P < 0.05$, ** for $P < 0.01$.

Table S2.4. Summary of SIMPER analysis for *Symbiodinium* cladal community compositions. Pairwise comparison within **A.** Spawning season and **B.** Summer season. Pairwise comparison **C.** Between spawning and summer seasons. Top two clades which contributed to dissimilarity of community compositions are shown with contribution percentages (%). Clades which were more abundant in variables in

columns are shown as bold letter and clades which were more abundant in variables in rows are shown as normal letter.

Table S2.5. List of ITS2 variants and relative abundance in each sample. **A.** Spawning season. **B.** Summer season. Compositions of ITS2 variants in each replicate (n = 4) were averaged and top three ITS2 variants in each clade are listed with relative abundance (%) in each sample.

Table S2.6. Summary of SIMPER analysis for *Symbiodinium* ITS2 variant community compositions. Pairwise comparison within **A.** Spawning season and **B.** Summer season. Pairwise comparison **C.** Between spawning and summer seasons. Top two ITS2 variants which contributed to dissimilarity of community compositions are shown with contribution percentages (%). ITS2 variants which were more abundant in variables in columns are shown as bold letter and ITS2 variants which were more abundant in variables in rows are shown as normal letter.

Table S2.7. Summary of PERMANOVA main effects for *Symbiodinium* community compositions based on the abundance of clades A, C and D obtained by qPCR. Cell densities of *Symbiodinium* belong to clades A, C and D in each environmental habitat, which were normalized by cm^3, were square-root transformed and PERMANOVA was performed with sites (coral species) (3 levels), habitats (4 levels), and seasons (2 levels) as fixed factors, using type III sum of squares and unrestricted permutation of raw data with 999 permutations. MS: mean square, df: degrees of freedom, F: Fisher statistic, P value: * for $P < 0.05$, ** for $P < 0.01$.

Chapter 3

Table S3.1. Number of sequences, clades and OTUs obtained by DNA metabarcoding using the cp23S primer set. Total samples which were successfully amplified and sequenced are 109/120 samples (4 replicates × 3 sites × 5 habitats × 2 sampling times).

Table S3.2. Summary of Kruskal-Wallis test for number of clades and OTUs based on the cp23S marker. **A.** Main effects. **B.** *Post-hoc* pairwise comparison for sites × habitats × seasons. df: degrees of freedom, P value: * for $P < 0.05$, ** for $P < 0.01$.

Table S3.3. Number of sequences, clades, ITS2 variants and OTUs obtained by DNA metabarcoding using the ITS2 primer set. Total samples which were successfully amplified and sequenced are 105/120 samples (4 replicates × 3 sites × 5 habitats × 2 sampling times).

Table S3.4. Summary of Kruskal-Wallis test for number of clades, ITS2 variants and OTUs based on the ITS2 marker. **A.** Main effects. **B.** *Post-hoc* pairwise comparison for sites × habitats × seasons. df: degrees of freedom, P value: * for $P < 0.05$, ** for $P < 0.01$.

Table S3.5. Summary of PERMANOVA main effects for *Symbiodinium* community compositions based on the cp23S marker. **A.** Clades. **B.** OTUs. Relative abundance of community compositions was square-root transformed and PERMANOVA was performed with sites (3 levels), habitats (5 levels), and seasons (2 levels) as fixed factors, using type III sum of squares and unrestricted permutation of raw data with 999 permutations. MS: mean square, df: degrees of freedom, *F*: Fisher statistic, P

value: * for $P < 0.05$, ** for $P < 0.01$.

Table S3.6. Summary of SIMPER analysis for *Symbiodinium* cladal community compositions based on the cp23S marker. Pairwise comparison within **A.** Pre-bleaching and **B.** Bleaching. Pairwise comparison **C.** Between pre-bleaching and bleaching sampling time points. Top two clades which contributed to dissimilarity of community compositions are shown with contribution percentages (%). Clades which were more abundant in variables in columns are shown as bold letter and clades which were more abundant in variables in rows are shown as normal letter.

Table S3.7. Summary of PERMANOVA main effects for *Symbiodinium* community compositions based on the ITS2 marker. **A.** Clades. **B.** ITS2 variants. **C.** OTUs. Relative abundance of community compositions was square-root transformed and PERMANOVA was performed with sites (3 levels), habitats (5 levels) and seasons (2 levels) as fixed factors, using type III sum of squares and unrestricted permutation of raw data with 999 permutations. MS: mean square, df: degrees of freedom, *F*: Fisher statistic, P value: * for $P < 0.05$, ** for $P < 0.01$.

Table S3.8. Summary of SIMPER analysis for *Symbiodinium* cladal community compositions based on the ITS2 marker. Pairwise comparison within **A.** Pre-bleaching and **B.** Bleaching. Pairwise comparison **C.** Between pre-bleaching and bleaching sampling time points. Top two clades which contributed to dissimilarity of community compositions are shown with contribution percentages (%). Clades which were more abundant in variables in columns are shown as bold letter and clades which were more abundant in variables in rows are shown as normal letter.

Table S3.9. List of ITS2 variants and relative abundance in each sample. **A.** Pre-bleaching. **B.** Bleaching. Compositions of ITS2 variants in each replicate (n = 2-4, depends on the samples) were averaged and top three ITS2 variants in each clade are listed with relative abundance (%) in each sample.

Table S3.10. Summary of SIMPER analysis for *Symbiodinium* ITS2 variants community compositions based on the ITS2 marker. Pairwise comparison within **A.** Pre-bleaching and **B.** Bleaching. Pairwise comparison **C.** Between pre-bleaching and bleaching sampling time points. Top two ITS2 variants which contributed to dissimilarity of community compositions are shown with contribution percentages (%). ITS2 variants which were more abundant in variables in columns are shown as bold letter and ITS2 variants which were more abundant in variables in rows are shown as normal letter.

Chapter 4

Table S4.1. Summary of two-way ANOVA for cell cycle and growth parameters under the light treatments. **A.** Main effects. *Post-hoc* pairwise comparison for **B.** strain effects and **C.** treatment effects. Factors: strain (B1, C1, C1′ and D1a), treatment (low light, high light). Cell cycle parameters (G_1 peak, S peak and G_2/M peak proportions) were arcsine transformed and cell volume was square root transformed. MS: mean square, df: degrees of freedom, F: Fisher statistic, P value: * for $P < 0.05$, ** for $P < 0.01$.

Table S4.2. Summary of two-way ANOVA for cell cycle and growth parameters under the temperature treatments. **A.** Main effects. *Post-hoc* pairwise comparison for **B.**

strain effects and **C.** treatment effects. Factors: strain (B1, C1, C1´ and D1a), treatment (control, heat). Cell cycle parameters (G_1 peak, S peak and G_2/M peak proportions) were arcsine transformed and cell volume was square root transformed. MS: mean square, df: degrees of freedom, *F*: Fisher statistic, P value: * for $P < 0.05$, ** for $P < 0.01$.

List of Electronic Files

Chapter 2

Appendix E2.1. Output file of PERMANOVA pairwise comparison (habitat, season) for *Symbiodinium* cladal compositions obtained by NGS analysis generated using PRIMER (version 6.1.16) and PERMANOVA+ (version 1.0.6) software. Pairwise comparison output files for habitat and season factors are included in this file which significant differences were detected for these factors from the main effects (Table S2.3A).

Appendix E2.2. Output file of PERMANOVA pairwise comparison (site, habitat, season) for *Symbiodinium* ITS2 variant compositions obtained by NGS analysis generated using PRIMER (version 6.1.16) and PERMANOVA+ (version 1.0.6) software. Pairwise comparison output files for site, habitat and season factors are included in this file which significant differences were detected for these factors from the main effects (Table S2.3B).

Appendix E2.3. Output file of PERMANOVA pairwise comparison (site, habitat, season) for *Symbiodinium* OTU compositions obtained by NGS analysis generated using PRIMER (version 6.1.16) and PERMANOVA+ (version 1.0.6) software. Pairwise comparison output files for site, habitat and season factors are included in this file which significant differences were detected for these factors from the main effects (Table S2.3C).

Appendix E2.4. Output file of PERMANOVA pairwise comparison (site, habitat) for *Symbiodinium* cladal compositions (only for clades A, C and D) obtained by qPCR analysis generated using PRIMER (version 6.1.16) and PERMANOVA+ (version 1.0.6) software. Pairwise comparison output files for site and habitat factors are included in this file which significant differences were detected for these factors from the main effects (Table S2.7).

Appendix E2.5. Output file of PERMANOVA pairwise comparison (technique) for *Symbiodinium* cladal compositions (only for clades A, C and D) obtained by both NGS and qPCR analysis generated using PRIMER (version 6.1.16) and PERMANOVA+ (version 1.0.6) software.

Chapter 3

Appendix E3.1. Output file of PERMANOVA pairwise comparison (site, habitat) for *Symbiodinium* cladal compositions obtained by NGS analysis based on the cp23S marker generated using PRIMER (version 6.1.16) and PERMANOVA+ (version 1.0.6) software. Pairwise comparison output files for site and habitat factors are included in this file which significant differences were detected for these factors from the main effects (Table S3.5A).

Appendix E3.2. Output file of PERMANOVA pairwise comparison (site, habitat) for *Symbiodinium* OTU compositions obtained by NGS analysis based on cp23S primer set generated using PRIMER (version 6.1.16) and PERMANOVA+ (version 1.0.6) software. Pairwise comparison output files for site and habitat factors are included in this file which significant differences were detected for the factors from the main

effects (Table S3.5B).

Appendix E3.3. Output file of PERMANOVA pairwise comparison (site, habitat) for *Symbiodinium* cladal compositions obtained by NGS analysis based on the ITS2 marker generated using PRIMER (version 6.1.16) and PERMANOVA+ (version 1.0.6) software. Pairwise comparison output files for site and habitat factors are included in this file which significant differences were detected for these factors from the main effects (Table S3.7A).

Appendix E3.4. Output file of PERMANOVA pairwise comparison (amplicon) for *Symbiodinium* cladal compositions obtained by NGS analysis based on both cp23S and ITS2 markers generated using PRIMER (version 6.1.16) and PERMANOVA+ (version 1.0.6) software.

Appendix E3.5. Output file of PERMANOVA pairwise comparison (site, habitat) for *Symbiodinium* ITS2 variant compositions obtained by NGS analysis based on the ITS2 marker generated using PRIMER (version 6.1.16) and PERMANOVA+ (version 1.0.6) software. Pairwise comparison output files for site and habitat factors are included in this file which significant differences were detected for these factors from the main effects (Table S3.7B).

Appendix E3.6. Output file of PERMANOVA pairwise comparison (site, habitat, season) for *Symbiodinium* OTU compositions obtained by NGS analysis based on the ITS2 marker generated using PRIMER (version 6.1.16) and PERMANOVA+ (version 1.0.6) software. Pairwise comparison output files for site, habitat, season factors are

included in this file which significant differences were detected for these factors from the main effects (Table S3.7C).

Chapter 4

Appendix E4.1. Image series of *Symbiodinium* DNA contents shifting through 24 h. Forward scatter (X axis, log scale) plotted against DNA fluorescent contents (Y axis, linear scale) of **A.** B1, **B.** C1, **C.** C1´ and **D.** D1a strain under high light treatment for one replicate.

Appendix: OTU analysis for *Symbiodinium* culture strains

Appendix E6.1. zOTU table obtained by NGS analysis based on the ITS2 marker for *Symbiodinium* culture strains. zOTU taxonomies were assigned against the *Symbiodinium* ITS2 reference database "SymTyper". OTUs observed in each sample were highlighted with colours. Colour coding: red for clade A, blue for clade B, purple for clade D, green for clade E, and orange for clade F.

Appendix E6.2. zOTU table for *Symbiodinium* culture strains after applying OTU abundance filtering. zOTUs which contain < 1% of the reads within each sample were replaced by 0 and removed from the OTU table if those zOTUs contain 0 sequence across all samples. zOTUs contain sequences were highlighted with colours. Taxonomy was assigned against SymTyper.

Abstract of Thesis

Symbiodinium are endosymbiotic microalgae of reef-building corals. Photosynthesis by these algae fuels the productivity of corals and ultimately the growth of entire reef systems. However, a critical phase of *Symbiodinium*'s life history is existence as "free-living" cells prior to acquisition by their host. Free-living populations are essential for establishing symbiosis for many corals that propagate larval generation without algal symbionts, but also for recombination of host-symbiont associations recovering from stress. Despite the importance of free-living populations, their underlying biodiversity and ecology remains a black box. For example, how they distribute spatially, temporally and regionally, and contribute to coral reef ecosystems as they currently face an era of "ecological crisis", are largely unknown.

To unlock the distribution, abundance and life cycle of free-living *Symbiodinium*, I applied novel dual NGS- (eDNA metabarcoding using next generation sequencing) and qPCR-based (using clade-specific primers) approaches to first explore the qualitative and quantitative distribution and abundance of free-living *Symbiodinium* for tropical (Heron Island) (**Chapter 2**) and temperate (Sydney Harbour) (**Chapter 3**) east coast Australian coral communities that are periodically connected by the Eastern Australian Current. To further evaluate how such diversity (which is only a snap shot of population dynamics over time) is sustained as a result of individual population turnover, I analysed the *Symbiodinium* cell cycle to establish a novel baseline for how population turnover is regulated by cell cycle dynamics across species and under alternate conditions (light and temperature) (**Chapter 4**).

This thesis highlights the importance of habitat variety in sustaining diverse free-living *Symbiodinium* communities, functional plasticity and hence resistance to disturbance (**Chapter 5**). For example, I provide new insight of macroalgae habitats as key reservoirs of symbiont availability to hosts via local supply but also wider dispersal. In the latter case, I discuss that dispersal of *Symbiodinium* is critical to support paradigms of high-latitude temperate reefs acting as refugia for tropical corals under climate change, since temperate and tropical *Symbiodinium* communities are currently geographically separated. Cell cycle dynamics differed between genetically different types (species), and results in specific types proliferating faster under certain environmental conditions thereby supporting shifts in community structure. As such, cell cycle dynamics comprises a key functional trait that is still overlooked but warrants further targeted investigation, not only amongst free-living populations, but also *in hospite* to clarify how functional equilibrium under steady-state symbioses is maintained as reef environments continue to be subjected to stressors into the future.

Declaration of the Contribution to Each Chapter

Chapters 2 and 3

The same format and approach was applied to deliver both Chapters 2 and 3: Experimental design was performed by myself (Lisa Fujise) supported by A/Prof. David Suggett (UTS) and Dr. Matthew Nitschke (University of Aveiro). I was primarily responsible for the all field sampling, laboratory work, data analysis and interpretation, and writing up of the manuscripts. Dr. Stephanie Gardner (UTS) provided assistance with sampling in Heron Island (Chapter 2) and Samantha Goyen (UTS) with sampling in both Heron Island (Chapter 2) and Sydney Harbour (Chapter 3). Dr. Michael Stat and Prof. Michael Bunce (Curtin University) provided support to analyse samples using next generation sequencing (NGS) in their ultra-clean lab facility. Dr. Tim Kahlke (UTS) provided assistance for building bioinformatic pipelines for *Symbiodinium* sequence analysis, Dr. Nahshon Siboni (UTS) for qPCR assay design, and Dr. Stephen Woodcock (UTS) for statistical analysis. A/Prof. David Suggett, Prof. Peter Ralph (UTS), Dr. Matthew Nitschke provided detailed feedback on both manuscripts at various stages.

Chapter 4

This chapter has been published in *Journal of Eukaryotic Microbiology* (in January 2018):

Fujise, L., Nitschke, M. R., Frommlet, J. C., Serôdio, J., Woodcock, S., Ralph, P. J., & Suggett, D. J. 2018. Cell Cycle Dynamics of Cultured Coral Endosymbiotic Microalgae (*Symbiodinium*) Across Different Types (Species) Under Alternate Light

and Temperature Conditions. J. Eukaryot. Microbiol. doi:10.1111/jeu.12497.

Experimental design was performed by myself (Lisa Fujise) supported by A/Prof. David Suggett. Laboratory experiment, sample analysis, data analysis and writing up of the manuscript were all performed by myself with helps. Dr. Stephen Woodcock (UTS) provided assistance for statistical analysis. A/Prof. David Suggett, Dr. Jörg C. Frommlet (University of Aveiro), Dr. João Serôdio (University of Aveiro), Dr. Matthew Nitschke (University of Aveiro), and Prof. Peter Ralph (UTS) provided comprehensive feedback on the manuscript at various stages.