CORAL BLEACHING: PHOTOSYNTHETIC IMPACTS ON SYMBIOTIC DINOFLAGELLATES

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A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN SCIENCE

DEPARTMENT OF ENVIRONMENTAL SCIENCES
INSTITUTE FOR WATER AND ENVIRONMENTAL RESOURCE MANAGEMENT
UNIVERSITY OF TECHNOLOGY, SYDNEY
CERTIFICATE OF AUTHORSHIP/ORIGINALLITY

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

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

Ross Hill
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PUBLICATIONS

PEER REVIEWED JOURNAL ARTICLES ARISING DIRECTLY FROM THIS THESIS:

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**Figure 6.5:** $F_v/F_m$ (open squares), $T_c$ (closed circles) and $T_p$ (closed triangles) of expelled zooxanthellae from *P. damicornis* exposed to the bleaching conditions of 400 μmol photons m$^{-2}$ s$^{-1}$ and 32°C. Measurements were taken at the time intervals of 0-1 h, 1-2 h, 2-4 h and 4-8 h. The temperature (°C) is indicated on the left y-axis and $F_v/F_m$ on the right y-axis. Averages ± standard error of mean shown ($n = 4$).
Figure 7.1: Maximum or effective quantum yield of *in hospite* (bars) and expelled (circles) zooxanthellae of *Pocillopora damicornis* during experimental treatment. Effective quantum yield measurements were taken prior to temperature ramping, once the temperature reached 32°C (0 h) and at 6, 12 and 36 h. Maximum quantum yield measurements were taken at 24 h following overnight darkness. The top bar indicates the temperature regime (white = 28°C, grey = ramp, black = 32°C) and the second bar indicates the light regime (white = light, black = dark) over the experiment. Light intensity = 400 μmol photons m⁻² s⁻¹. Averages ± S.E. of mean shown (n = 4). Letters indicate statistically distinct groups of *in hospite* zooxanthellae measurements from Tukey’s post hoc comparison tests.

Figure 7.2: Maximum or effective quantum yield of expelled zooxanthellae in each of the four populations: 0-6 h (a), 6-12 h (b), 12-24 h (c) and 24-36 h (d). The effective quantum yield was measured at 6, 12, 36, 60 and 84 h, while maximum quantum yield was measured at 24, 48, 72 and 96 h. Therefore the first data points in (a), (b) and (d) are effective quantum yield, while the first data point in (c) is maximum quantum yield. Measurements on zooxanthellae from the 28°C (●), 30°C (⊙) and 32°C (▲) treatments are shown from the time of expulsion up until 96 h. The bar indicates the light regime over the experiment (white = light, black = dark). Light intensity = 400 μmol photons m⁻² s⁻¹ during initial expulsion and 100 μmol photons m⁻² s⁻¹ at other time periods. Averages ± S.E. of mean shown (n = 4).
Figure 7.3: Percentage of healthy-looking zooxanthellae in the four expelled zooxanthellae populations: 0-6 h (a), 6-12 h (b), 12-24 h (c) and 24-36 h (d). The percentage of healthy-looking zooxanthellae from the 28°C (○), 30°C (□) and 32°C (▲) treatments are shown from the time of expulsion up until 96 h. The bar indicates the light regime over the experiment (white = light, black = dark). Light intensity = 100 μmol photons m⁻² s⁻¹. Averages ± S.E. of mean shown (n = 4).

Figure 8.1: Chlorophyll fluorescence induction kinetics measured in a control sample. The NPQ components are indicated. SP = saturating pulse; AL = actinic light.

Figure 8.2: Maximum quantum yield for the controls (○; 225 μmol photons m⁻² s⁻¹ and 25°C), the high-light (▼; 475 μmol photons m⁻² s⁻¹ and 25°C), the elevated temperature (■; 225 μmol photons m⁻² s⁻¹ and 32°C) and high-light plus elevated temperature (▲; 475 μmol photons m⁻² s⁻¹ and 32°C) treatments. The 0 h control and 1-8 h time periods are plotted. Averages ± standard error of mean are shown (n = 4).

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Figure 9.1: The relative intensity of light between the wavelengths of 180-880 nm for light sources used throughout the experiments. a) wavelengths from the PSI double modulation fluorometer’s blue, red and far-red LEDs. b) the wavelengths of light from the sun at 06:00 (solid line) and 12:00 (dashed line) hrs. c) the difference between the 12:00 and 06:00 hrs solar spectra. d) the spectra of the halogen lights used in Experiment 3.

Figure 9.2: Fast induction curves at (a) 04:00 hrs, and (b) 06:00 following darkness (●), 10 s exposure to far-red light then 0.1 s of darkness (△), and 10 s exposure to blue and red light then 0.1 s darkness (■) for Cyphastrea serailia. Average curves are shown (n = 4). The corresponding F\(_{v}/F_{m}\) value for each light treatment is shown for (c) 04:00 hrs, and (d) 06:00 hrs. Averages ± standard error of mean shown (n = 4). The letters above the columns in c) and d) are the result from Tukey’s post hoc comparisons test.
**Figure 9.3:** Effect of length of dark-adaptation (DA) on fast induction curves and $F_v/F_m$ values. Fast induction curves for (a) *Pocillopora damicornis*, (b) *Acropora nobilis*, and (c) *Cyphastrea serailia* following 5 min of DA, then 10 s far-red light and 0.1 s darkness (●), 5 min DA (☐), 10 min DA (○), 20 min DA (▼), 30 min DA (Δ), and 60 min DA (■). Average curves are shown ($n = 4$). The corresponding $F_v/F_m$ value for each light treatment is shown for (d) *P. damicornis*, (e) *A. nobilis*, and (f) *C. serailia*. Averages ± standard error of mean shown ($n = 4$). Asterisks (*) indicate where the far-red light treatment had a significantly higher $F_v/F_m$ than the darkness treatment.

**Figure 9.4:** Fast induction curves for (a) *Pocillopora damicornis*, (b) *Acropora nobilis*, and (c) *Cyphastrea serailia* during control conditions, and for (d) *P. damicornis*, (e) *A. nobilis*, and (f) *C. serailia* following 5 h under bleaching conditions. Corals were given 10 min dark-adaptation (DA) (●), 10 min DA, 10 s far-red light and 0.1 s darkness (☐), 10 min DA, 10 s far-red light and 1 s darkness (○), 10 min DA, 10 s far-red light and 10 s darkness (▼), and 10 min DA, 10 s far-red light and 200 s darkness (Δ). Average curves are shown ($n = 4$).

**Figure 10.1:** Conceptual model of impacts to the light reactions of photosynthesis under (a) optimal and (b) bleaching conditions. Under optimal conditions electrons are donated by the OEC to the PSII electron acceptors of $Q_A$ (on the D2 protein) and $Q_B$ (on the D1 protein), then transported to PSI (P700). Excess light energy absorbed by PSII is dissipated by NPQ. 60% is dissipated via qE, 20% via qT and 20% via qI pathways. Under these conditions, the OEC is thermally stable up to 35°C and the thylakoid
membrane is stable up to 37°C. Under bleaching conditions, a rise in the abundance of
QB non-reducing centres (PSII_X) on the D1 protein occurs, resulting in reduced electron
flow. A greater amount of absorbed light energy is dissipated by NPQ, with a rise in the
contribution of qT to total NPQ. Under these conditions 40% is dissipated via qE, 40%
via qT and 20% via qI pathways. Furthermore, OEC thermostability increases to 39°C
and thylakoid membrane thermostability increases to 42°C under bleaching conditions.

All photographs were taken by the author, unless otherwise stated in the Figure caption.
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**Table 4.1:** Zooxanthellae density (cm$^{-2} \times 10^6$) and chl $a$ and $c_2$ concentration per cm$^{-2}$ (μg) in *P. damicornis*, *A. nobilis* and *C. serailia*. Measurements were taken from the end of the control treatment (control), the first measurement prior to exposure to bleaching conditions (pre-treatment) and the end of the exposure period (exposure). Averages ± S.E. of mean shown ($n = 4$). Asterisk (*) indicates significant differences between treatments (where, $\alpha = 0.05$) and superscript letters indicate where these differences lie.

**Table 5.1:** Zooxanthellae density (cm$^{-2} \times 10^6$) and chlorophyll $a$ and $c_2$ concentrations (μg cm$^{-2}$) in *P. damicornis* before (pre) and after (post) exposure to the control and bleaching treatments for the 12 h and 5 d experiments. Averages ± S.E. of mean shown ($n = 4$). Asterisk (*) indicates significant differences between treatments (where, $\alpha = 0.05$) and superscript letters indicate where these differences lie.
**Table 5.2:** Effective quantum yield ($\Phi_{\text{PSII}}$) of expelled zooxanthellae from the 5 d experiment before and after DPC addition. Measurements were taken 1 h prior to the lights turning off each evening at the end of each of the 5 days. Averages ± S.E. of mean shown ($n = 4$). $P$ values indicate whether any significant differences exist between the $\Phi_{\text{PSII}}$ values taken before and after DPC addition (right-hand column) or between the values measured on each day (bottom row). Asterisk (*) indicates significant differences between treatments (where, $\alpha = 0.05$) and superscript letters indicate where differences lie between $\Phi_{\text{PSII}}$ values on each day.

**Table 6.1:** Effect of heating rate and presence/absence of far-red light on initial $F_{\text{r}}/F_{\text{m}}$, $T_c$ and $T_p$ of F-T curves performed on cultured *Symbiodinium* sp. (CS-156). Averages ± standard error of mean shown ($n = 6$). $P$ values and Tukey’s post hoc comparison tests are shown as superscript letters.

**Table 6.2:** Light intensity (μmol photons m$^{-2}$ s$^{-1}$, provided by halogen lights, Portable Floodlight, FL200, Arlec Lighting) and temperature (°C) of the four experimental treatments (control (low light and low temperature), high light + low temperature, low light + elevated temperature and high light + elevated temperate) for the cultured and freshly isolated zooxanthellae samples.

**Table 6.3 (see page 151):** The $F_{\text{r}}/F_{\text{m}}$, $T_c$, $T_p$, $F_{\text{initial}}/F_{\text{maximum}}$, $T_{50}$ and $T_0$ parameters for each of the 10 coral species studied during summer and winter, as well as for cultured *Symbiodinium* sp. and *A. carterae*. Coral species are grouped by zooxanthellae genotype.
Superscript letters indicate variation between species and show the groups into which they fall within each season for each parameter (determined by Tukey’s post hoc test where $\alpha = 0.05$. The $P$ value is shown at the bottom of each list of species). Asterisk (*) indicates differences between summer and winter for each species and each parameter and is shown on the significantly higher value ($\alpha = 0.05$). Averages ± standard error of mean shown ($n = 8$).

**Table 6.4**: Coral species, sample number ($n$) and observed SSCP genotype frequency ($f$) of clade A, C (C1, C2 and C•) and D ($f_A$, $f_{C1}$, $f_{C2}$, $f_{C•}$ and $f_D$, respectively). Dominance is given where multiple types were harboured simultaneously.
ABBREVIATIONS

A  Absorbance
A_0  Primary electron acceptor of PSI
A_1  Secondary electron acceptor of PSI
ANOVA  Analysis of variance
chl  Chlorophyll
CSIRO  Commonwealth Scientific and Industrial Research Organisation
DA  Dark-adaptation
DCMU  3-(3,4-dichlorophenyl)-1,1-dimethylurea
DPC  Diphenyl carbazide
Dz  Degraded zooxanthellae
e^-  Electron
F_E  Steady fluorescence after DCMU addition
FIC  Fast induction curve
F_{initial}/F_{maximum}  Ratio of the initial F_o to the maximum F_o
FIZ  Freshly isolated zooxanthellae
F_m  Dark-adapted maximum fluorescence
F_m'  Light-adapted maximum fluorescence
F_o  Dark-adapted minimum fluorescence
F_T  Steady maximum fluorescence after DCMU addition
F_t  Steady-state fluorescence
F-T  Fluorescence-temperature
\( F_v \) Variable fluorescence
\( F_{v/F_m} \) Maximum quantum yield of Photosystem II
\( H^+ \) Proton/hydrogen ion
\( Hz \) Healthy-looking zooxanthellae
ITS1 Internal transcribed spacer 1
LED Light emitting diode
LHC Light harvesting complex
NADP\(^+\) Nicotinamide adenine dinucleotide phosphate
NADPH Reduced form of NADP\(^+\)
NPQ Non-photochemical quenching
OEC Oxygen evolving complex
O-I\(_1\)-I\(_2\)-P Peak nomenclature along a fast polyphasic fluorescence rise kinetic transient from Fo to Fm (Neubauer and Schreiber 1987)
O-J-I-P Step nomenclature along a fast polyphasic fluorescence rise kinetic transient from F\(_o\) to F\(_m\)
P680 Photosystem II reaction centre
P700 Photosystem I reaction centre
PAM Pulse amplitude modulated
PCR Polymerase chain reaction
PEA Plant efficiency analyser
Phaeo Phaeophytin
PQ Plastoquinone
PSI Photosystem I
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<td>SSCP</td>
<td>Single stranded conformational polymorphism</td>
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<td>T_0</td>
<td>Temperature at which F_v/F_m reaches zero</td>
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<td>T_50</td>
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<td>T_p</td>
<td>Temperature of peak fluorescence</td>
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<td>Tris</td>
<td>Tris (hydroxymethyl)aminomethane</td>
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<td>UV</td>
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Global climate change is leading to the rise of ocean temperatures and is triggering mass coral bleaching events on reefs around the world. This involves the expulsion of the symbiotic dinoflagellate algae, known as zooxanthellae, from the coral host. Coral bleaching is believed to occur as a result of damage to the photosynthetic apparatus of these symbionts, although the specific site of initial impact is yet to be conclusively resolved. This thesis examined a number of sites within the light reactions of photosynthesis and evaluated the efficiency of photoprotective heat dissipating pathways. Upon expulsion, the capacity for long-term survivorship of expelled zooxanthellae in the water column was also assessed.

A reduction in photosystem II (PSII) photochemical efficiency during exposure to elevated temperature and high light (bleaching conditions) was found to be highly dependent upon the increase in abundance of Q_b non-reducing PSII centres (inactive PSII centres), indicating damage to the site of the secondary electron acceptor, Q_B, resulting in a limited capacity for its reduction. Therefore, this reduced the rate of the reoxidation of the primary electron acceptor, Q_A-. Fast induction curve (FIC) analysis of the rise from minimum fluorescence to maximum fluorescence revealed a lower amplitude in the J step along this curve, which was consistent with a reduction in the rate of Q_A- reoxidation. This photoinhibition of PSII was found to occur once the effectiveness of excess energy dissipation through energy-dependent quenching and state-transition quenching was exceeded, suggesting that these mechanisms were incapable of preventing photodamage.
Antenna size heterogeneity showed little change under bleaching conditions with a significant increase in PSIIβ only apparent in one species of coral.

The thermostability of the oxygen evolving complex (OEC) and thylakoid membrane were found to increase during exposure to bleaching conditions and exceeded bleaching thresholds of corals. This rapid rise in temperature-dependent thermostability also occurred over seasons, where variation in ocean temperatures was matched by gradual shifts in OEC and thylakoid membrane thermostolerance. Variation in thermostability between species was not found to be linked to zooxanthellae genotype, and instead was related to the bleaching susceptibility of the host. Despite this capacity for resilience to bleaching conditions, the PSII reaction centres did not exhibit such a mechanism for rapid acclimatisation. Corals can only be as tolerant to bleaching conditions as their most sensitive component allows. The formation of nonfunctional PSII centres is therefore suggested to be involved in the initial photochemical damage to zooxanthellae which leads to a bleaching response.

Zooxanthellae were found to be expelled irrespective of OEC function and thylakoid membrane integrity, as these sites of the photosynthetic apparatus were still intact when cells were collected from the water column. Although zooxanthellae were photosynthetically competent and morphologically intact upon expulsion, their longevity in the water column was dependent on the time of expulsion following the onset of bleaching and the ambient water temperatures. The survivorship of these zooxanthellae was restricted to a maximum of 5 days in the water column which suggests that unless
expelled zooxanthellae inhabit other environs of coral reefs which may be more favourable for survival, their capacity for persistence in the environment is extremely limited.

Chlorophyll a fluorescence measurements are a common tool for investigating photosynthetic impacts to in hospite zooxanthellae of corals. Pathways causing dark-reduction of the plastoquinone pool are shown to be active in corals and affect measurements which require dark-adaptation. Pre-exposure to far-red light was found to be an effective procedure to oxidise the inter-system electron transport chain and ensure determination of the true maximum quantum yield of PSII and accurate FICs.

It is concluded that the trigger for coral bleaching lies in the photosynthetic apparatus of zooxanthellae and evidence is presented in support of this impact site not being the OEC or thylakoid membrane.