CORAL BLEACHING: PHOTOSYNTHETIC IMPACTS ON SYMBIOTIC DINOFLAGELLATES

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Ross Hill

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PUBLICATIONS

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Chapter 4:

Hill R, Ralph PJ (2006) Photosystem II heterogeneity of *in hospite* zooxanthellae in scleractinian corals exposed to bleaching conditions. Photochemistry and Photobiology 82: 1577-1585

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Chapter 5:

Hill R, Ralph PJ (in press) Impact of bleaching stress on the function of the oxygen evolving complex of zooxanthellae from scleractinian corals. Journal of Phycology

Chapter 6:

Hill R, Ulstrup KE, Ralph PJ (in review) Temperature induced changes in thylakoid membrane thermostability of cultured, freshly isolated and expelled zooxanthellae from scleractinian corals. Journal of Experimental Marine Biology and Ecology

Chapter 7:

Hill R, Ralph PJ (2007) Post-bleaching viability of expelled zooxanthellae from the scleractinian coral *Pocillopora damicornis*. Marine Ecology Progress Series 352: 137-144

See Appendix 3

Chapter 8:

Hill R, Frankart C, Ralph PJ (2005) Impact of bleaching conditions on the components of non-photochemical quenching in the zooxanthellae of a coral. Journal of Experimental Marine Biology and Ecology 322: 83-92

See Appendix 4

Chapter 9:

Hill R, Ralph PJ (in press) Dark-induced reduction of the plastoquinone pool in zooxanthellae of scleractinian corals and implications for measurements of chlorophyll *a* fluorescence. Symbiosis

PEER REVIEWED JOURNAL ARTICLES RELEVANT TO THE THESIS, BUT NOT CONTRIBUTING TO IT:

Hill R, Larkum AWD, Frankart C, Kühl M, Ralph PJ (2004) Loss of functional Photosystem II reaction centres in zooxanthellae of corals exposed to bleaching conditions: using fluorescence rise kinetics. Photosynthesis Research 82: 59-72

Hill R, Schreiber U, Gademann R, Larkum AWD, Kühl M, Ralph PJ (2004) Spatial heterogeneity of photosynthesis and the effect of temperature-induced bleaching conditions in three species of corals. Marine Biology 144: 633-640

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

Ulstrup KE, **Hill R**, van Oppen MJH, Larkum AWD, Ralph PJ (in review) Seasonal variation in photo-physiological functions of homogenous and mixed *Symbiodinium* communities in two scleractinian corals. Marine Ecology Progress Series

TABLE OF CONTENTS

LIST OF FIGURES

Figure 3.3: Representative fast induction kinetic transients from the first day of each two-day study in each season. The time periods of 0700, 0900, 1200, 1500 and 0000 are shown to indicate the general diel trend seen in each of three coral species, *Pocillopora damicornis* (a, b), *Acropora nobilis* (c, d) and *Cyphastrea serailia* (e, f), in both winter and summer. Average curves are plotted on a $\log_{10}$ time scale ($n = 4$).

Figure 3.4: Amplitude of the O, J, I and P steps along the fast induction curves at each sampling period. Measurements of *Pocillopora damicornis* (a, b), *Acropora nobilis* (c, d) and *Cyphastrea serailia* (e, f), in both winter and summer are shown. Averages ± standard error of mean are shown ($n = 4$). The black and white bars indicate night and day, respectively.

Figure 4.1: Maximum quantum yield (F_v/F_m) of control (closed circles) and treatment (open squares) in *P. damicornis* (a), *A. nobilis* (b) and *C. serailia* (c). The pre-exposure measurement is indicated by the white bar, the bleaching treatment as the black bar (from 0-12 h) and the recovery treatment as the grey bar (12-36 h). Averages ± standard error of mean shown ($n = 4$).

Figure 4.2: Fast induction curves of the pre-treatment and bleaching treatment (0, 4, 8, 12 h) in *P. damicornis* (a), *A. nobilis* (b) and *C. serailia* (c) and the recovery treatment (24, 36 h; indicated by R) in *P. damicornis* (d), *A. nobilis* (e) and *C. serailia* (f). Curves are plotted on a $\log_{10}$ time scale. The O, J, I and P steps are indicated. Average curves are shown ($n = 4$).

Figure 4.3: Q_A reoxidation curves following a single turnover flash in *P. damicornis* (a), *A. nobilis* (b) and *C. serailia* (c). The pre-treatment, 2, 6 and 12 h bleaching treatment and 24 and 36 h recovery treatment (indicated by R) are shown. A break in the y-axis data is used to demonstrate the maximum fluorescence and the biphasic Q_A reoxidation kinetics. The dashed line at 0.1 s represents the division between $PSII_A$ (< 0.1 s) and $PSII_X$ (> 0.1 s). Curves are plotted on a $\log_{10}$ time scale. Average curves are shown (n = 4).

Figure 4.4: The proportion of $PSII_X$ centres in the control (closed circles) and treatment (open squares) in *P. damicornis* (a), *A. nobilis* (b) and *C. serailia* (c). The pre-exposure measurement is indicated by the white bar, the bleaching treatment as the black bar (from 0-12 h) and the recovery treatment as the grey bar (12-36 h). Averages ± standard error of mean shown (n = 4).

Figure 4.5: The proportion of PSIIα (closed circles) and PSIIβ (open squares) centres in the controls of *P. damicornis* (a), *A. nobilis* (b) and *C. serailia* (c), and in the exposure treatment of *P. damicornis* (d), *A. nobilis* (e) and *C. serailia* (f). The pre-exposure measurement is indicated by the white bar, the bleaching treatment as the black bar (from 0-12 h) and the recovery treatment as the grey bar (12-36 h). Averages ± standard error of mean shown (n = 4).

Figure 5.1: Representative fluorescence traces (of n = 4) for culture CS-156 upon (a) addition of tris and DPC and (b) upon addition of filtered seawater (FSW). Following 5 mins of dark-adaptation, a saturating pulse was applied and the maximum quantum yield was determined. Actinic light was turned on (40 µmol photons m^{-2} s^{-1}) and effective quantum yield measurements taken every 60 s. The left y-axis indicates the fluorescence intensity of the trace (relative units) and the right y-axis indicates the quantum yield values (●) over time.

Figure 5.2: Representative trace of oxygen production (% saturation) of cultured zooxanthellae. Arrows show time of tris and DPC addition. Black bars indicate darkness and white bar indicates light.

Figure 5.3: FIC traces of the culture CS-156 under control conditions, following exposure to tris and exposure to tris and DPC. (a) FICs normalised to O (F_t/F_o) and (b) relative variable fluorescence of the FICs $(F_t-F_o)/(F_m-F_o)$. The O, K, J, I and P steps are labels on (a). The control curve (●) was taken following 5 mins DA. Tris was then added to the solution (0.6 M) and 20 mins later a second FIC was measured (▲). DPC was then added (0.5 mM) and 20 mins later the third FIC was measured (□). Average curves are shown (n = 4).

Figure 5.4: Effective quantum yield values before (black columns) and after (white columns) DPC addition on cultured zooxanthellae (CS-156) and freshly isolated zooxanthellae (from *P. damicornis*). (a) shows the control (25°C and 40 µmol photons m^{-}

^{2} s^{-1}) and (b) shows the bleaching (32°C and 425 µmol photons m^{-2} s^{-1}) treatment for the cultured zooxanthellae. (c) shows the control (25°C and 100 µmol photons m^{-2} s^{-1}) and (d) shows the bleaching (32°C and 425 µmol photons m^{-2} s^{-1}) treatment for the freshly isolated zooxanthellae. During this 12 h experiment, measurements were taken prior to exposure to experimental treatments (pre) and then at 0, 1 (only for cultured zooxanthellae), 2, 4, 6, 8, 10 and 12 h. The letters above the columns in (b) and (d) indicate the result from Tukey's post hoc comparisons test. Averages ± standard error of mean shown (n = 4).

Figure 5.5: Effective quantum yield values before (black columns) and after (white columns) DPC addition on freshly isolated zooxanthellae from *P. damicornis* under (a) control (25°C and 100 µmol photons m^{-2} s^{-1}) and (b) bleaching (32°C and 425 µmol photons m^{-2} s^{-1}) conditions over a 5 d period. The 12:12 h light cycle is indicated by the white (light) and black (dark) bars. The letters above the columns in (b) indicate the result from Tukey's post hoc comparisons test. Averages ± standard error of mean shown (n = 4).

Figure 5.6: Relative variable fluorescence of FICs $(F_t-F_o)/(F_m-F_o)$ showing the evolution of the K step for *P. damicornis* (a), *A. nobilis* (b) and *C. serailia* (c) during winter and of *P. damicornis* (d), *A. nobilis* (e) and *C. serailia* (f) during summer. Curves for the i) control (○; 21°C in winter and 28°C in summer), ii) the highest temperature where the K step was not present (▲), iii) the temperature where the K step was first observed (◊)

and, iv) one degree above this temperature (■) are shown in each graph. Average curves are shown (n = 4).

Figure 5.7: Representative traces of oxygen production (% saturation) in nubbins of *P. damicornis* after 8 h exposure to (a) control and (b) bleaching conditions. Following 30 mins of exposure to light (white bar), the sample was placed in darkness (black bar). At this time, in the controls, the temperature either remained at 25°C (solid line), was immediately increased to 34°C (long dashed line), or was increased to 35°C (short dashed line). In the bleaching treatment, when the sample was placed in darkness, the temperature either remained at 32°C (solid line), was immediately increased to 38°C (long dashed line), or was increased to 39°C (short dashed line). Following 5 mins of darkness, the samples were re-illuminated (white bar) for a further 30 mins.

Figure 6.1: Representative F-T curve, where the temperature was increased at a speed of 1°C min^{-1}. The location of F_o, F_m, F_v/F_m T_c, T_p, $F_{initial}$ and $F_{maximum}$, are shown. The temperature (°C) is indicated above the x-axis and time (min) indicated below. This example is of *A. millepora* during summer.

Figure 6.2: Representative scanning transmission electron micrographs of *Symbiodinium* sp. cells from CS-156 at 25°C (A), 30°C (B), 35°C (C), 36°C (D), 37°C (E), 38°C (F), 39°C (G), 40°C (H) and 45°C (I) during a temperature ramp of 1°C min^{-1}.

Figure 6.3: F_v/F_m (open squares), T_c (closed circles) and T_p (closed triangles) of cultured zooxanthellae exposed to control conditions (A) of low light (40 µmol photons m^{-2} s^{-1}) and low temperature (25°C), high light and low temperature (B; 400 µmol photons m^{-2} s^{-1} and 25°C), low light and elevated temperature (C; 40 µmol photons m^{-2} s^{-1} and 32°C), and high light and elevated temperature (D; 400 µmol photons m^{-2} s^{-1} and 32°C) from 0-9 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 6.4: F_v/F_m (open squares), T_c (closed circles) and T_p (closed triangles) of freshly isolated zooxanthellae from *P. damicornis* exposed to control conditions (A) of low light (100 µmol photons m^{-2} s^{-1}) and low temperature (22°C), high light and low temperature (B; 400 µmol photons m^{-2} s^{-1} and 22°C), low light and elevated temperature (C; 100 µmol photons m^{-2} s^{-1} and 32°C), and high light and elevated temperature (D; 400 µmol photons m^{-2} s^{-1} and 32°C) at time intervals of 0, 1, 2, 4 and 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 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).

Figure 8.3: Total non-photochemical quenching (NPQ) for controls (●; 225 µmol photons m^{-2} s^{-1} and 25˚C), the high-light (▼; 475 µmol photons m^{-2} s^{-1} and 25˚C), 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).

Figure 8.4: The contribution of the three components of non-photochemical quenching (qE = black columns; qT = light grey columns; qI = dark grey columns) to the total non-photochemical quenching for the controls (a; 225 µmol photons m^{-2} s^{-1} and 25˚C), high-light treatment (b; 475 µmol photons m^{-2} s^{-1} and 25˚C), the elevated temperature treatment (c; 225 µmol photons m^{-2} s^{-1} and 32˚C) and the high-light plus elevated temperature treatment (d; 475 µmol photons m^{-2} s^{-1} and 32˚C). The 0 h control and 1-8 h time periods are plotted. Averages ± standard error of mean are shown (n = 4).

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 (Δ), 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 Q_B 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.

LIST OF TABLES

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}, $f_{C\bullet}$ 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

PSII	Photosystem II
$PSII_A$	Active PSII centres/Q_B reducing centres
$PSII_X$	Inactive PSII centres/Q_B non-reducing centres
PSIIα	PSII centres with both inner and peripheral LHCs
PSIIβ	PSII centres with only inner LHC
Q_A	Oxidised primary electron acceptor of PSII
Q_A^-	Reduced primary electron acceptor of PSII
Q_B	Oxidised secondary electron acceptor of PSII
Q_B^-	Reduced secondary electron acceptor of PSII
qE	Energy dependent quenching
qI	Photoinhibitory quenching
qP	Photochemical quenching
qT	State transition quenching
RC	Reaction centre
rmANOVA	Repeat measures analysis of variance
ROS	Reactive oxygen species
SSCP	Single stranded conformational polymorphism
T_0	Temperature at which F_v/F_m reaches zero
T_{50}	Temperature at which F_v/F_m reaches 50% of its initial
T_c	Critical temperature
t_{Fmax}	Time to reach maximum fluorescence
T_p	Temperature of peak fluorescence
Tris	Tris (hydroxtmethyl)aminomethane

UV	Ultra violet
V	Volts
ΔpH	pH gradient
Φ_{PSII}	Effective quantum yield of Photosystem II

ABSTRACT

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 thermotolerance. 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.