

RESEARCH ARTICLE

Host Coenzyme Q Redox State Is an Early Biomarker of Thermal Stress in the Coral *Acropora millepora*

Adrian Lutz^{1,2,3,4,6}*, Jean-Baptiste Raina^{1,2,4,5,6}, Cherie A. Motti², David J. Miller^{3,4}, Madeleine J. H. van Oppen^{2,4,6}

1 AIMS@JCU, James Cook University, Townsville, Queensland, Australia, **2** Australian Institute of Marine Science, Townsville, Queensland, Australia, **3** Comparative Genomics Centre and Department of Molecular and Cell Biology, James Cook University, Townsville, Queensland, Australia, **4** ARC Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, Queensland, Australia, **5** College of Marine and Environmental Sciences, James Cook University, Townsville, Queensland, Australia, **6** School of BioSciences, The University of Melbourne, Parkville, Melbourne, Victoria, Australia

✉ These authors contributed equally to this work.

✉a Current address: Metabolomics Australia, School of Biosciences, The University of Melbourne, Parkville, Victoria, Australia

✉b Current address: Plant Functional Biology and Climate Change Cluster (C3), University of Technology Sydney, Sydney, NSW, Australia

* adrian.lutz@my.jcu.edu.au



CrossMark
click for updates

OPEN ACCESS

Citation: Lutz A, Raina J-B, Motti CA, Miller DJ, van Oppen MJH (2015) Host Coenzyme Q Redox State Is an Early Biomarker of Thermal Stress in the Coral *Acropora millepora*. PLoS ONE 10(10): e0139290. doi:10.1371/journal.pone.0139290

Editor: Mónica Medina, Pennsylvania State University, UNITED STATES

Received: May 21, 2015

Accepted: September 9, 2015

Published: October 1, 2015

Copyright: © 2015 Lutz et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This work has been funded by the Australian Institute of Marine Science (www.aims.gov.au) and AIMS@JCU (aims.jcu.edu.au), a collaborative scheme between the Australian Institute for Marine Science and James Cook University. A.L. and J-B. R. were supported by AIMS@JCU PhD Scholarships. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abstract

Bleaching episodes caused by increasing seawater temperatures may induce mass coral mortality and are regarded as one of the biggest threats to coral reef ecosystems worldwide. The current consensus is that this phenomenon results from enhanced production of harmful reactive oxygen species (ROS) that disrupt the symbiosis between corals and their endosymbiotic dinoflagellates, *Symbiodinium*. Here, the responses of two important antioxidant defence components, the host coenzyme Q (CoQ) and symbiont plastoquinone (PQ) pools, are investigated for the first time in colonies of the scleractinian coral, *Acropora millepora*, during experimentally-induced bleaching under ecologically relevant conditions. Liquid chromatography-mass spectrometry (LC-MS) was used to quantify the states of these two pools, together with physiological parameters assessing the general state of the symbiosis (including photosystem II photochemical efficiency, chlorophyll concentration and *Symbiodinium* cell densities). The results show that the responses of the two antioxidant systems occur on different timescales: (i) the redox state of the *Symbiodinium* PQ pool remained stable until twelve days into the experiment, after which there was an abrupt oxidative shift; (ii) by contrast, an oxidative shift of approximately 10% had occurred in the host CoQ pool after 6 days of thermal stress, prior to significant changes in any other physiological parameter measured. Host CoQ pool oxidation is thus an early biomarker of thermal stress in corals, and this antioxidant pool is likely to play a key role in quenching thermally-induced ROS in the coral-algal symbiosis. This study adds to a growing body of work that indicates host cellular responses may precede the bleaching process and symbiont dysfunction.

Competing Interests: The authors have declared that no competing interests exist.

Introduction

Elevated seawater temperatures in conjunction with high solar irradiance disrupt the relationship between reef-building corals (Cnidaria: Scleractinia) and their dinoflagellate symbionts (*Symbiodinium* sp.) [1] and have been implicated in causing mass coral bleaching events [2–4]. Although the molecular events underlying the loss of *Symbiodinium* cells via exocytosis [5] and apoptosis [6] remain unclear, it is broadly accepted that coral bleaching is preceded by oxidative stress: the excessive formation of reactive oxygen species (ROS) which eventually overwhelm the antioxidant defence capacity of the symbiosis [7–9]. Initial impairment of photosynthesis is thought to increase ROS formation in the symbionts, leading to oxidative damage in the host, which then initiates bleaching [7]. Several potential primary damage sites have been identified in *Symbiodinium* during thermal stress, including photosystem II (PSII) reaction centres [10–12], antenna pigments [13], the Calvin cycle [14], and the thylakoid membranes [15]. Other evidence suggests that the primary site of thermal damage in *Symbiodinium* varies among coral species and symbiont types [16] which may explain some of the apparent contradictory results to date. In addition, there is increasing evidence suggesting that the cnidarian host plays a more significant role in the bleaching cascade than previously thought because thermal stress can compromise host cells prior to damaging the symbiont [17–20] and because bleaching can occur in darkness, independent of photosynthetically produced ROS [21]. Nonetheless, it is clear that the coral host has substantial antioxidant potential, indicating ROS scavenging during exposure to thermal and irradiance stress is essential in both symbiotic partners in order to prevent bleaching [22–26]. Hence, oxidative stress is likely to reflect an imbalance between the antioxidant capacity of both partners and the performance of the electron transport chains (ETC) of coral mitochondria and *Symbiodinium* chloroplasts [7].

As components of both antioxidant defence systems and the electron transport chains that generate ROS, the prenylquinones coenzyme Q (CoQ; ubiquinone) and plastoquinone (PQ) and their respective reduced (antioxidant) forms ubiquinol (CoQH₂) and plastoquinol (PQH₂) may play key roles in the bleaching response. These redox carriers play an integral role in electron transport (CoQ/CoQH₂ in the mitochondrial ETC and PQ/PQH₂ in the photosynthetic ETC) but also have important antioxidant functions within mitochondrial [27], cellular [28] and thylakoid [29] membranes. The reduced forms of these prenylquinones are highly effective lipid peroxidation chain breakers, and are involved in the regeneration of other antioxidants such as ascorbate and α -tocopherol [28, 30–32]. In addition, PQH₂ is an effective singlet oxygen (¹O₂) quencher in chloroplasts [33, 34]. Consequently, shifts in the proportion of reduced to oxidised prenylquinones (%CoQH₂; %PQH₂) have been used to infer oxidative stress and ROS scavenging activity in plant models [30, 33, 35, 36].

Little is known about how the coral CoQ and symbiont PQ pools respond to hyperthermal stress. In a proof of concept study, Lutz et al. [37] demonstrated that the *Acropora millepora* CoQ and the *Symbiodinium* PQ pool redox states are maintained predominantly in their reduced forms (a prerequisite for antioxidant action), and acute heat-stress causes increased oxidation of the coral CoQ pool consistent with evidence that oxidative stress occurs in both host and symbiont [23, 24, 38]. However, due to the acute nature of the stress applied, it is unclear whether the observed oxidative shift was a consequence of metabolic failure, or whether the CoQ pool is sensitive to prolonged elevated temperature stress under more ecologically relevant conditions.

Here, quantitative liquid chromatography-mass spectrometry (LC-MS) was used to estimate the redox states of host CoQ and *Symbiodinium* PQ pools in colonies of the scleractinian coral *A. millepora* during experimentally-induced bleaching under ecologically relevant temperature conditions. The data on CoQ and PQ pool redox status, in combination with PSII

photochemical efficiency, chlorophyll concentration and *Symbiodinium* density estimates were used to follow the effects of thermal stress on the state of the symbiosis over time.

Materials and Methods

Ethics Statement

All necessary permits were obtained for the described field studies. Specimens for this study were collected under permit number G09/30237.1, issued by the Australian Government's Great Barrier Reef Marine Park Authority. The locations of sample collection are not privately owned, and no endangered or protected species were collected.

Reagents

All reagents, and the standards ubiquinone-9 (CoQ₉) and ubiquinone-10 (CoQ₁₀), were purchased from Sigma Aldrich (USA). Plastoquinone-9 (PQ₉) was a kind gift from Professor Ewa Swiezewska from the Polish Academy of Sciences, Poland. All solvents used were HPLC grade (Mallinckrodt, Australia).

Experimental design

Twelve *A. millepora* colonies roughly 50 cm in diameter containing type C2 *Symbiodinium* (ITS1 terminology, see below) were collected from Pelorus Island, Great Barrier Reef, Australia (18°33' S/146°29' E) in May 2010. Colonies were transferred to the Australian Institute of Marine Science (Townsville) and divided into a total of 24 fragments, each comprising approximately 25 branches. Fragments were arranged in eight indoor tanks in a balanced randomised block design, resulting in the allocation of twelve coral fragments (three per tank) to each of the control and thermal stress temperature treatments (27°C and 32°C, respectively). All tanks were continuously supplied with fresh, 1 µm filtered seawater at a rate of 1.5 L min⁻¹ from 500 L reservoirs in a temperature-controlled room maintained at 27 ± 0.5°C (two reservoirs per treatment). Each reservoir was heated with two titanium heaters (3 kW) controlled by a CR1000 datalogger (Campbell Scientific) and a temperature sensor in the treatment tanks. All tanks were fitted with a small power head pump to maintain water movement and an air stone and pump to provide aeration. UV-filtered 400 W metal halide lights (BLV, Germany) were mounted above each tank and provided an average underwater light intensity of 350 µmol photons m⁻² s⁻¹ (12:12 h light:dark cycle). The UV-filters were used to minimise UV-radiation-induced bleaching [39].

The colony fragments were acclimated for two weeks prior to starting the experiment, then seawater temperatures in four tanks were ramped at a constant rate (0.7°C d⁻¹) to 32 ± 0.5°C over seven days; the remaining four control tanks were maintained at 27°C for the entire duration of the experiment (Fig 1). The heat stress temperature was chosen to represent an ecologically relevant 1°C above the estimated local bleaching threshold of approximately 31°C for nearby Orpheus Island, Great Barrier Reef (18°35' S/146°29' E; ~31°C: [40]). Coral branches were sampled at four time points during the experiment: at the end of the acclimation period ($t = 0$ d), upon reaching the 32°C target temperature in the hyperthermal stress treatment ($t = 7$ d), and after five ($t = 12$ d) and ten days ($t = 17$ d) at 32°C. At each time point, coral nubbins (approximately 50 mm in length) were collected from each coral fragment after six hours of light ($n = 12$ in control and heat treatment, respectively) and from a subset of fragments after six hours of darkness ($n = 9$ in control and heat treatment, respectively). Samples were immediately snap-frozen in liquid nitrogen at time of collection to quench the PQ and CoQ

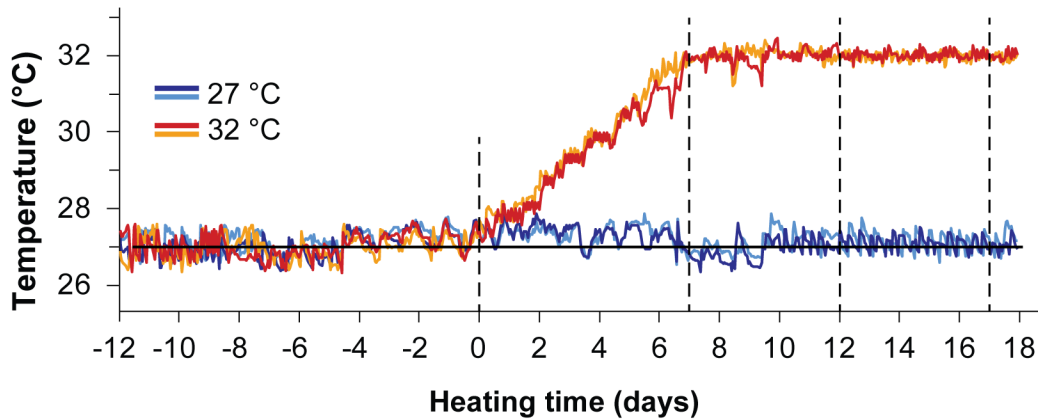


Fig 1. Temperature logger data for the experimental period. Thermal log of the four temperature sensors placed in heated (32°C) and control (27°C) seawater aquarium tanks for the duration of the experimental period. Two temperature sensors were used per treatment. Dashed lines indicate sampling time points.

doi:10.1371/journal.pone.0139290.g001

pool redox states. Total sample size was optimised to ensure all samples could be processed in less than four weeks after sampling to ascertain the redox stability of the extracts.

Photosystem II photochemical efficiency

Photosystem II (PSII) photochemical efficiency, expressed as maximum quantum yield $((F_M - F_O)/F_M = F_V/F_M)$ was measured daily with a Diving-PAM (Walz GmbH, Germany) from three randomly chosen branches per coral fragment two hours before the start of the light cycle [41]. A 6 mm fibre optic probe was placed perpendicular to the surface at least 20 mm from the tip of the branch and 3 mm from the coral tissue surface (controlled via a rubber spacer) to obtain the measurements. Minimum fluorescence (F_O) was measured using a weak pulsed measuring light ($< 0.15 \mu\text{mol photons m}^{-2} \text{s}^{-1}$; gain = 3) and maximum fluorescence (F_M) was measured upon application of a saturating pulse of light ($> 4000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$).

Prenylquinone quantification

Coral nubbins for prenylquinone extraction were stored in liquid nitrogen for a maximum of 48 hours. Nubbins were extracted using a 1:1 mixture of isopropanol and ethyl acetate containing $0.1 \mu\text{M CoQ}_9$ (internal standard). Coral CoQ_{10} and *Symbiodinium* PQ_9 pools were quantified by LC-MS using a slightly modified method of Lutz et al. [25]. In brief: prenylquinones were resolved using a Phenomenex Kinetex C18 column ($150 \text{ mm} \times 4.6 \text{ mm}$, $2.6 \mu\text{m}$ particle size) on an Agilent 1100 series HPLC (Agilent, USA) coupled to a Bruker Esquire 3000 (Bruker Daltonics, USA). Absolute quantities of the prenylquinones were calculated from calibration plots obtained from standard compounds containing $0.1 \mu\text{M CoQ}_9$ (internal standard). CoQ and PQ redox states ($\% \text{PQH}_2$ and $\% \text{CoQH}_2$) were expressed as the proportion of reduced to total (oxidised + reduced) prenylquinone. Coral CoQ data could potentially be biased by CoQ of *Symbiodinium*; however, symbiont CoQ was not detected with the method applied here, either because *Symbiodinium* type C2 contains a different isoform than the host CoQ_{10} or because concentrations are below the detection limit [37].

Symbiodinium densities

For *Symbiodinium* densities, one coral nubbin per coral fragment and time point was immediately processed at midday ($n = 12$ in control and heat treatment, respectively). Coral nubbins

were airbrushed in individual plastic bags in 4 mL of 0.2 μm filtered seawater. The slurry was homogenised using a Turrax disperser (IKA, Germany) to break down aggregates and was centrifuged at 3000 g. The supernatant was removed and the pellet resuspended in 1 mL of 10% formalin in phosphate buffer saline (PBS). *Symbiodinium* cells were counted under a light microscope (eight technical replicates per sample) using a haemocytometer (depth 0.1 mm).

Calculation of surface area and chlorophyll concentrations

Tissue remaining on the coral nubbins was removed by soaking in diluted commercial bleach (0.5% NaClO) overnight. Surface areas of the coral nubbins were determined using a wax dipping technique [42]. Chlorophyll concentrations (a and c_2) were measured from aliquots of the prenylquinone extracts on a microplate reader (Powerwave, Bio-Tek Instruments, USA) [43] and determined using the equations presented in Ritchie et al. [44].

Symbiodinium genotyping

The *Symbiodinium* genotypes were identified based on sequence differences in the nuclear ribosomal DNA internal transcribed spacer 1 (ITS1) region using single-strand conformation polymorphism (SSCP) analysis as described by van Oppen et al. [45]. Total coral and *Symbiodinium* DNA was extracted using a modified protocol [46] and the *Symbiodinium* ITS1 region amplified with fluorescently labelled Sym ITS1 PCR primers for SSCP analysis on non-denaturing polyacrylamide gels. The symbiont genotype was determined by comparison of manually scored gel images of known reference standards run in parallel with the samples [47]. SSCP profiles from all colonies were single bands identical to type C2 *Symbiodinium* (GenBank Accession AF380552) *sensu* van Oppen et al. [45].

Transmission electron microscopy (TEM)

At each time point, one coral branch was sampled in four coral fragments per treatment, transferred directly into fixative (1.25% glutaraldehyde + 0.5% paraformaldehyde in 0.2 μm filtered seawater) and stored at 4°C until required. Fixed coral nubbins were decalcified in a formic acid:fixative mixture (1:3), with the solution changed every 12 h until complete dissolution of the skeleton. Three individual polyps per sample were postfixed in osmium and subsequently dehydrated with increasing concentrations of ethanol followed by dry acetone. Dehydrated samples were infiltrated in increasing concentrations of Araldite resin before being cured for 24 h at 60°C. Longitudinal sections, 90-nm thick, were collected on copper grids and imaged at 120 kV in a JEOL 2100 TEM.

BLAST analysis

The *A. millepora* transcriptome [48], the *Acropora digitifera* genome [49] and the cnidarian protein and nucleotide database at NCBI were searched for enzymes involved in CoQ redox reactions. Homologue proteins and gene sequences were identified using BLAST (blastp, blastx, tblastx, tblastn) at <http://blast.ncbi.nlm.nih.gov> and <http://marinegenomics.oist.jp>, and the *A. digitifera* annotation available at <http://bioserv7.bioinfo.pbf.hr/Zoophyte/index.jsp> [50]. All identified sequences were assessed against the SwissProt database (<http://www.uniprot.org/>).

Statistical analysis

Linear mixed models [51, 52] were applied to assess treatment effects using time (sampling day), treatment (control vs. heated) and the interaction as fixed effects and a random intercept for each coral fragment to account for repeated measures of the same colonies. F_V/F_M , %PQH₂

and %CoQH₂ data were power transformed; PQ concentration was log transformed. Model comparison was conducted using Akaike's information criterion (AIC). Tank effects were non-significant (fixed) and redundant (random), and thus discarded to avoid overfitting in all models. First order autocorrelation covariate structure was determined as best model fit in all models. Multiple pairwise comparisons were corrected using the false discovery rate following Hochberg and Benjamini [53]. All statistical analyses were conducted using SPSS version 17.0.

Results

A. millepora colony fragments exposed to hyperthermal stress (32°C) showed clear symptoms of bleaching, when compared to ambient (27°C) treatment controls (Fig 2A–2D). In the thermal stress treatment group, *Symbiodinium* cell densities were reduced by 25.7% after five days and by 82.4% at the end of the experiment (Fig 2E). No significant changes were observed in cellular chlorophyll concentrations (*a* and *c*₂) during the experiment (mean = 29.6 ± 4.5 pg cell⁻¹; *p* = 0.46). Mortality was low; of the 24 colony fragments used, only two of the 12 exposed to thermal stress showed signs of necrosis, patchy tissue sloughing and algal overgrowth. No further data were collected for these fragments after day 13 and 15, respectively, when symptoms of mortality were first observed. PSII photochemical efficiency ($F_v/F_M \pm 95\%$ confidence interval (CI)) remained stable in control colonies (mean = 0.68 ± 0.1) but declined markedly in the 32°C treatment group concomitant with the loss of *Symbiodinium* cells after day 9 (Fig 2F; *p* < 0.001; Table 1). The declining trend of F_v/F_M was observable from the third day after heating commenced; however, F_v/F_M did not differ significantly from control samples until day five (t-test; $F_{1,278} = 10.683$; *p* = 0.0012). TEM images showed no impact on the *Symbiodinium* thylakoid membrane or cell wall structure in the first seven days of the experiment; however, disintegrated internal organelles were observed in 7% of the cells examined (total 1502). After five days exposure to 32°C (*t* = 12 d), all of the remaining *Symbiodinium* cells exhibited both structurally compromised thylakoid membranes and widespread disintegration of organelles in the cytoplasm (Fig 3). While damage to internal structures was apparent, cell walls appeared intact and no fragmented symbiont cells were observed.

The *Symbiodinium* PQ pool was predominantly reduced at the start of the experiment, and remained approximately constant during the first twelve days of heat stress (from *t* = 0 h to 12 d: mean = 90.8 ± 1.4% in light and 87.3 ± 4.2 in dark); note that the PQ redox state (%PQH₂ ± 95% CI) did not differ significantly between the light and dark periods (Fig 2G; Table 1). However, the heat stressed colonies exhibited a highly significant 12% (light) and 11% (dark) decline in PQH₂ at the end of the experiment (at *t* = 17 d, mean = 78.9 ± 3.5% and 76.2 ± 5.1%; respectively; *p* < 0.002). When normalised per *Symbiodinium* cell, this decline in PQH₂ coincided with an apparent five-fold increase in total PQ concentration (PQ + PQH₂) from 1.49 ± 0.23 pmol cell⁻¹ (mean, *t* = 0 d to 12 d) to 5.11 ± 2.06 pmol cell⁻¹ at *t* = 17 d (Fig 2I; *p* = 0.008) in the heat treatment.

Whereas the redox state of the PQ pool remained essentially stable for 12 days, the coral CoQ pool (%CoQH₂ ± 95% CI) became oxidised more rapidly in response to the hyperthermal stress. CoQH₂ declined from 89.4 ± 1.0% to 80.9 ± 2.6 in the light, and from 89.7 ± 1.1% to 82.3 ± 3.3% in the dark, within the first seven days of the experiment (i.e. at the end of the heating phase from 27°C to 32°C; Fig 2H; *p* < 0.002). In the 32°C treatment group, %CoQH₂ continued to decline steadily over the next ten days to 77.3 ± 2.9% (light) and 75.4 ± 7.9% (dark). As in the case of the plastoquinone pool, light and dark estimates of %CoQH₂ did not differ significantly. Likewise, total CoQ (CoQ + CoQH₂; normalised per coral surface area) did not differ between control and heat stress, remaining stable throughout the experiment (Fig 2J; Light: mean = 0.41 ± 0.03 nmol cm⁻²; *p* = 0.36; Dark: mean = 0.40 ± 0.03 nmol cm⁻²; *p* = 0.21).

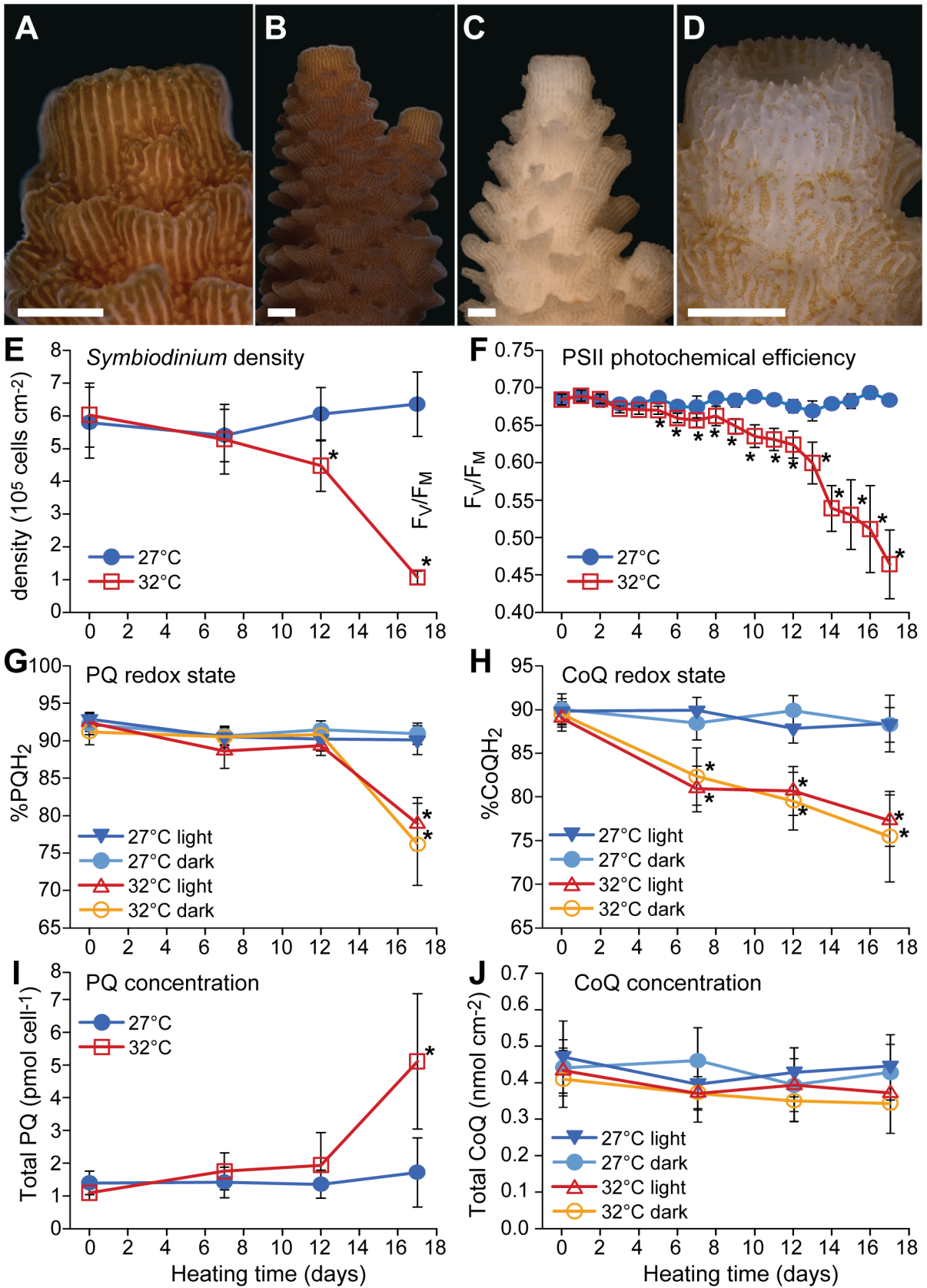


Fig 2. Effects of thermal stress on physiological parameters of the scleractinian coral *Acropora millepora*. Images of representative coral nubbins demonstrating the visual difference in *Symbiodinium* cell densities within *A. millepora* tissues under control (27°C) (A—B) and thermal stress (32°C) (C—D) conditions at day 17 (end of experiment). Scale bars = 1 mm. Thermal stress effects on (E) *Symbiodinium* density; (F) photosystem II photochemical efficiency; (G) plastoquinone (%PQH₂) and (H) coenzyme Q (%CoQH₂) pool redox states; (I) total plastoquinone concentration (PQ + PQH₂) per *Symbiodinium* cell and (J) total coenzyme Q concentration (CoQ + CoQH₂) per coral surface area over the course of the experiment. All data points are means ± 95% CI; * indicate significant differences between control and treatment at *p* < 0.05; *n* = 6–12 (see Table 1 for details).

doi:10.1371/journal.pone.0139290.g002

Discussion

Coenzyme Q pool redox state

The results presented here demonstrate that the *A. millepora* host CoQ redox state is sensitive to hyperthermal stress, exhibiting an overall 13% decline of CoQH₂ in response to the stressor (Fig 2H). This oxidative shift was not caused by an increase of *de novo* synthesised CoQ, as the total CoQ concentration (CoQ + CoQH₂) did not increase during the heat stress. Oxidation of the CoQ pool occurred early in the thermal stress treatment, upon reaching 32°C (after seven days with a daily temperature increase of approximately 0.7°C per day) and prior to any measurable loss of *Symbiodinium* cells from the host tissue. Oxidation of the CoQ pool occurred before a major decline in PSII photochemical efficiency was observed, i.e., while the effects of the hyperthermal stress on the *Symbiodinium* photosynthesis apparatus were still limited ($F_v/F_M > 0.65$). Moderate irradiance levels (350 μmol photons m⁻² s⁻¹) were used in order to avoid major light stress concomitantly with the applied hyperthermal stress [2, 54, 55]. Maximum daily irradiance at 1–3 m depths regularly exceeds 1000 μmol photons m⁻² s⁻¹ for nearby (< 25 km), equally turbid Great Palm Island waters [56]. The results therefore indicate that the *A. millepora* CoQ pool is oxidised significantly in response to hyperthermal stress in the absence of strong light exacerbating ROS leakage from the symbiont [57, 58]. Although a contribution of photosynthetically derived ROS to the observed CoQ pool oxidation cannot be discounted, the results presented here add to a growing body of work that indicates host cellular responses may precede the bleaching process and symbiont dysfunction [5, 17–20, 59]. In

Table 1. Linear mixed model testing for differences in temperature treatments (27°C = control; 32°C = stress) during a hyperthermal bleaching experiment of *Acropora millepora* containing *Symbiodinium* type C2.

	<i>n</i>	Treatment				Time ^a			Treatment × time		
		df	<i>F</i>	<i>p</i>	df	<i>F</i>	<i>p</i>	df	<i>F</i>	<i>p</i>	
F_v/F_M	12 ^b	22.0	104.2	<0.001	201.8	15.8	<0.001	201.8	13.8	<0.001	
%PQH ₂ (light)	12 ^b	22.1	12.0	0.002	48.8	21.6	<0.001	48.8	6.3	0.001	
%PQH ₂ (dark)	9 ^c	19.6	10.0	0.005	41.1	9.6	<0.001	41.1	5.7	0.002	
Total PQ cell ⁻¹	6	9.0	5.5	0.043	17.9	7.7	0.002	17.9	5.5	0.008	
Total chlorophyll cell ⁻¹	6	10.4	0.6	0.46	20.8	0.2	0.88	n/a			
%CoQH ₂ (light)	12 ^b	22.1	24.5	<0.001	41.2	17.0	<0.001	41.2	14.8	<0.001	
%CoQH ₂ (dark)	9 ^c	15.9	22.5	<0.001	33.6	12.8	<0.001	33.6	6.3	0.002	
Total CoQ cm ⁻² (light)	12 ^b	22.3	0.9	0.36	42.0	3.5	0.023	42.0	0.2	0.91	
Total CoQ cm ⁻² (dark)	9 ^c	15.9	1.7	0.21	34.4	2.0	0.135	n/a			

CoQ, coenzyme Q; %CoQH₂, coenzyme Q pool redox state; F_v/F_M , maximum quantum yield; PQ, plastoquinone; %PQH₂, plastoquinone pool redox state.

^a F_v/F_M was measured daily (18 time points), other measurements at four time points.

^{b,c} replication number given is for the full set. Due to dropouts, for the last time point *n* = 10 (^b) and *n* = 8 (^c).

p-values significant at $\alpha < 0.05$ are highlighted in boldface.

doi:10.1371/journal.pone.0139290.t001

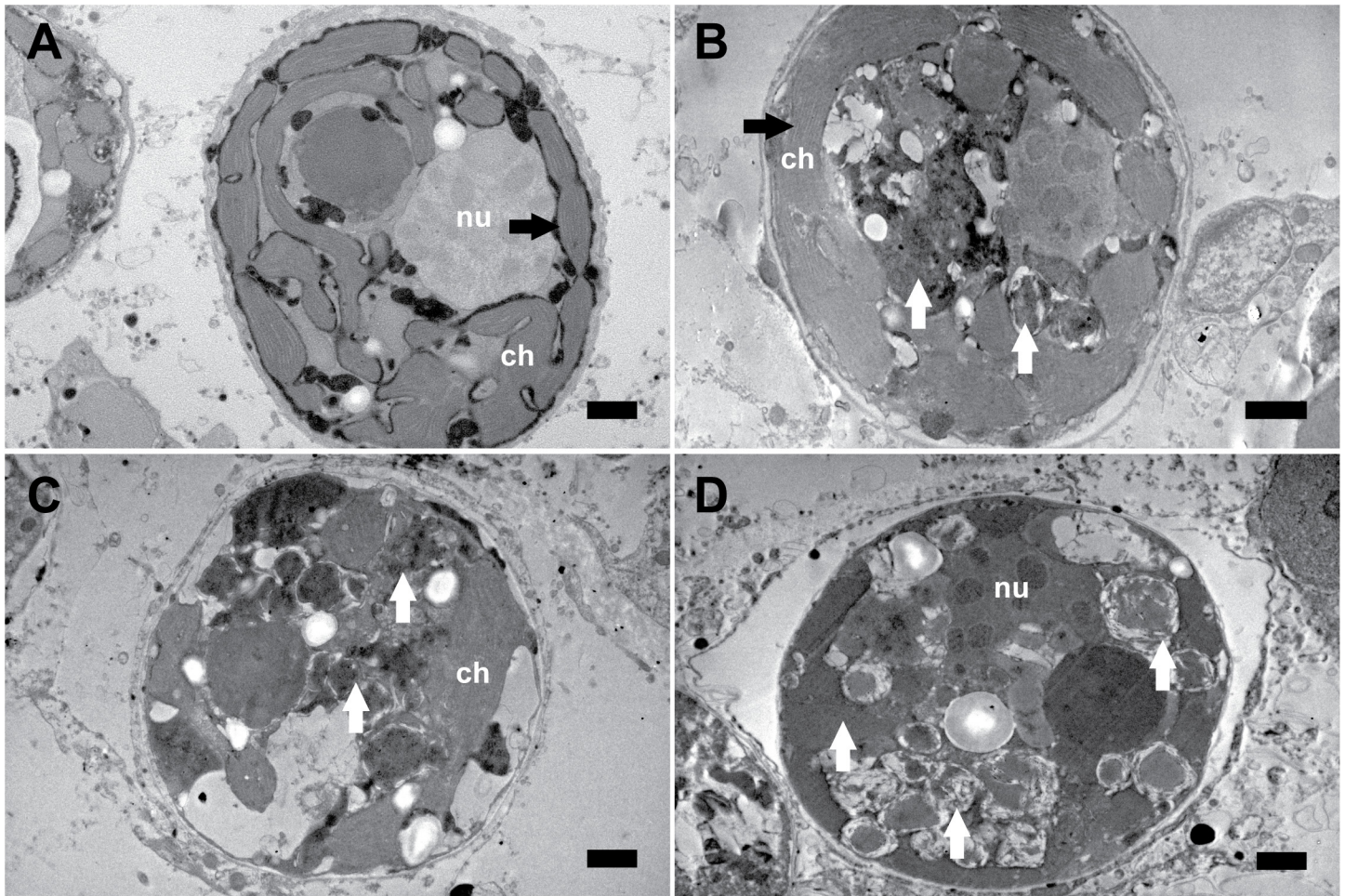
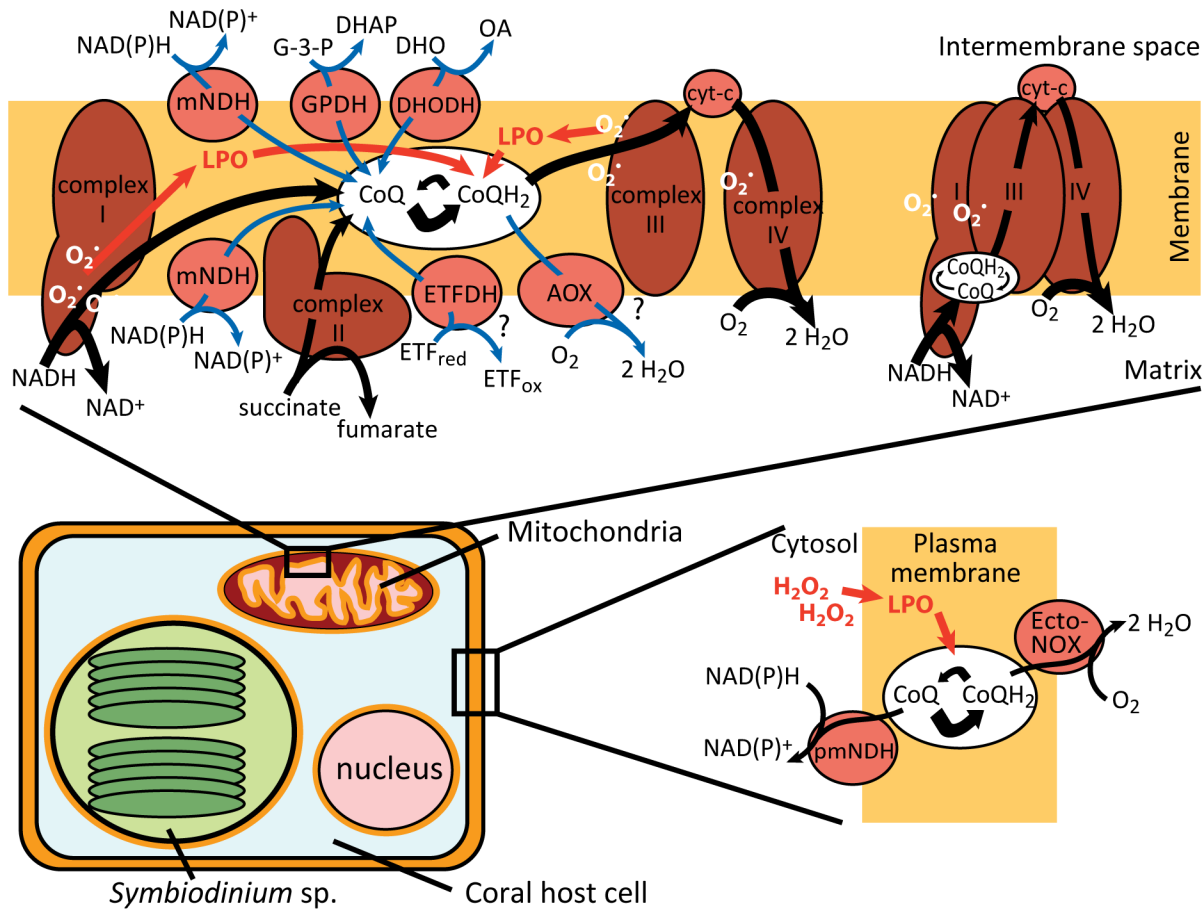


Fig 3. Representative transmission electron micrographs documenting the effects of thermal stress on the internal structure of endosymbiotic *Symbiodinium* cells within tissue of *Acropora millepora*. (A) *Symbiodinium* exposed to 27°C showing intact organelles and thylakoid membranes (black arrow). (B) First signs of degraded internal structures in some *Symbiodinium* cells after 7 days of heat stress (white arrows). Note the intact structure of the thylakoid membranes (black arrow). (C and D) *Symbiodinium* exposed to 32°C revealing degraded internal structures (white arrows). Scale bars, 1 μ m; ch, chloroplast; nu, nucleus.

doi:10.1371/journal.pone.0139290.g003

addition, other thermal stress-related responses such as transcriptional and physiological changes that were not measured here are expected to occur in both coral symbiosis partners prior to host CoQ pool oxidation. For example, other reported early changes include a reduction in epithelial tissue, signs of increased apoptosis in the gastrodermis, and changes to the transcriptome, which have been associated with an upregulation of chaperone and antioxidant defence genes alongside transcriptional changes that, by analogy to vertebrate models, are assumed to be linked to apoptosis [18, 25, 26, 60–63]. It should also be noted that the heat/light sensitivity of the photosynthetic apparatus varies among different symbiont types and that this affects host sensitivity to bleaching [64–66]; however, bleaching susceptibility differs widely among different coral genera despite often hosting the same *Symbiodinium* types [67–69]. Considering this, the results presented here require confirmation in other symbiont-host associations prior to postulation of a generalized physiological response during the bleaching cascade. Nonetheless, the oxidative shift in the CoQ redox state observed here is among the earliest known metabolic changes in the coral partner in response to a realistic temperature level.



Note: orange highlighted membranes are expected to contain CoQ/CoQH₂

Fig 4. Schematic diagram of electron transfer reactions using the coenzyme Q (CoQ) pool in the coral mitochondrial and plasma membrane electron transport. Respiratory “linear” electron flows (black arrows) proceed from NADH in the mitochondrial matrix to H₂O via the CoQ pool and the enzyme complexes I, II, III, and IV, forming ubiquinol (CoQH₂) as an intermediary product. The electron flows via complexes I, III and IV occur (mostly) via tunnelling or micro-diffusion of CoQ/CoQH₂ in I-II-IV supercomplexes rather than via the larger mobile CoQ pool [72]. “Non-linear” electron flows (dark blue arrows) proceed from electron donors (e.g. NAD(P)H) via several quinone dehydrogenases to the CoQ pool, and to H₂O from CoQH₂ via AOX. Plasma membrane electron transport occurs from NAD(P)H to H₂O via one or more type of NAD(P)H-CoQ reductases, the plasma membrane CoQ pool and Ecto-NOX. CoQH₂ ROS scavenging occurs continuously in O₂ metabolism primarily via chain breaking of lipid peroxidation (LPO) caused by O₂^{•-} and H₂O₂. Abbreviations: AOX, alternative oxidase; cyt-c, cytochrome c; DHAP, dihydroxyacetone phosphate; DHO, dihydroorotate; DHODH, dihydroorotate dehydrogenase; Ecto-NOX, external quinone oxidase; ETF_{red/ox}, reduced/oxidised electron-transferring-flavoprotein; ETFDH, electron-transferring-flavoprotein dehydrogenase reduced/oxidised; Ecto-NOX, external quinone oxidase; GPDH, glycerol-3-phosphate dehydrogenase; G-3-P, glycerol-3-phosphate; H₂O₂, hydrogen peroxide; LPO, lipid peroxidation; pmNDH/mNDH, plasma membrane/mitochondrial NAD(P)H dehydrogenases; OA, orotate; O₂^{•-}, superoxide.

doi:10.1371/journal.pone.0139290.g004

Coenzyme Q pool redox state regulation. CoQ/CoQH₂ is present (in varying quantities) in all intracellular membranes of every animal with the highest concentrations found in the mitochondrial membranes at the primary site of ROS production [32, 70]. In eukaryotes, CoQ redox processes are relatively complex (Fig 4; for relevant enzymes identified in *Acropora* sp. see S1 Table). Within mitochondrial membranes, CoQH₂ is continuously regenerated by the respiratory chain (complex I, II and alternative NAD(P)H dehydrogenases) [71] and other mitochondrial enzymes (glycerol-3-phosphate dehydrogenase, electron-transferring flavoprotein dehydrogenase, dihydroorotate dehydrogenase; [72]). In other membranes, several enzymes catalyse CoQ reduction including a NADH-cytochrome b₅ reductase [73] and a distinct, unresolved NADPH-CoQ reductase [74]. Interestingly, a cytosolic NAD(P)H:quinone

reductase (NQO1; formerly DT-diaphorase) [75]—the most studied CoQ reducing enzyme—appears to be absent in cnidarians along with other NQO genes [76].

In contrast to the reduction of the CoQ pool, CoQH₂ is oxidised by direct interaction with ROS, in particular lipid peroxy radicals and the lipid peroxidation initiating peroxyl radicals (Fe₃O₂^{•-}) found in all membranes [28], by complex III and alternative oxidases (AOX) of the mitochondrial ETC [77], and by the external oxidases of the plasma membrane electron transport (Ecto-NOX; [78]). Considering the known CoQ pool redox mechanisms of other animals, the oxidative shift in the CoQ redox state in *A. millepora* can therefore be attributed to: (1) an increase in ROS scavenging by CoQH₂; (2) a decline in net CoQ reduction by the mitochondrial ETC; (3) extra-mitochondrial pathways; or 4) any combination of these processes.

Coenzyme Q pool reactive oxygen species scavenging in corals. Attributing the thermal stress-induced oxidative shift in CoQ redox state of *A. millepora* to a specific physiological mechanism is difficult, primarily because current understanding of CoQ functions in the coral-*Symbiodinium* symbiosis is very limited and existing methods cannot distinguish between functionally and spatially different CoQ pools present in different organelles [79]. Theoretically, a net decline in CoQ reduction caused by the mitochondrial ETC or extra-mitochondrial pathways are conceivable by postulating a decline in CoQ reducing or an increase in CoQH₂ oxidising enzyme activities; however, no such direct impact of thermal stress on the CoQ pool has been demonstrated so far. In particular, the emerging consensus that the complexes I-III-IV occur mostly as supercomplexes further complicates attributing shifts in the CoQ redox state to a specific location in the mitochondrial ETC as electron transfer in these supercomplexes appears to occur via tunnelling or microdiffusion of CoQ/CoQH₂ rather than via a mobile CoQ pool in mitochondrial membranes [72].

Short term heat stress in the bleaching model *Aiptasia* has been reported to cause the degradation of host mitochondria prior to symbiont impairment and to lead to the downregulation of genes associated with ATP production and electron transport at the site of, and downstream from, cytochrome c [19]. However, the report did not include any genes upstream of complex III (Fig 4), thus there is no indication that the CoQ pool reducing side of the mitochondrial ETC was affected. A recent transcriptional analysis provided further evidence of the thermal stability of complex III gene expression in *A. millepora* [80] but analyses at the enzyme activity level in cnidarians remain outstanding. The visible, pre-bleaching mitochondrial damage in *Aiptasia* [19] would be expected to incapacitate the mitochondrial ETC and potentially lead to CoQ oxidation due to a decline in electron flux to the pool; however, there is currently no data available to lend support to such a model. On the other hand, an inefficient mitochondrial ETC is likely leading to the increased formation of ROS [8, 59, 81], in particular during daytime hyperoxia [24, 82]. Thus, even though it is possible that any components of the CoQ pool redox mechanisms are affected by thermal stress, attributing the oxidative shift in the CoQ redox state to increased CoQH₂ ROS scavenging in response to hyperthermal stress currently provides the most parsimonious explanation.

In mammals, oxidative shifts in the CoQ pool redox state have been observed in a variety of pathological conditions that are associated with oxidative stress [35, 36, 83]. These oxidative shifts are understood to result from an increasingly challenged antioxidant defence [28]. In cnidarian-*Symbiodinium* symbioses, it has been repeatedly demonstrated that the cnidarian host reacts to thermal stress and high light by increasing its antioxidant activities, which indicates an increased requirement to detoxify ROS in the host tissues [7, 38, 54, 84–86]. ROS formation also occurs in aposymbiotic cnidarians upon exposure to light and elevated temperatures, although in symbiosis, the hyperoxia caused by algal photosynthesis aggravates the coral's innate ROS formation because it increases relative to oxygen concentration [24, 59, 87–91]. Bleaching in symbiotic cnidarians can also be triggered in the absence of photosynthetically

produced ROS by thermal stress in darkness [21]. It is not yet understood what role non-photosynthetically produced ROS play in this dark-bleaching; however, mitochondria would appear to be the most likely origin for these ROS. Mitochondria (the primary source of ROS in animals) are the location of the highest cellular CoQ/CoQH₂ concentration in eukaryotes [28, 78]. Here, superoxide (O₂^{•-}) and other ROS are generated by enzymes involved in the ETC, particularly the NADH dehydrogenase of complex I, and the interface between the CoQ pool and complex III [91, 92]. The co-localisation of the ROS producing respiratory ETC and the CoQ pool within mitochondrial membranes is likely an important factor in the high antioxidant effectiveness of CoQH₂ [28, 93]. In addition to ROS generation by the coral, ROS leakage from *Symbiodinium* probably exceeds the host's innate ROS generation [24, 57, 58]. Moreover, impaired or damaged photosynthetic ETC may further increase ROS formation and, ultimately, ROS leaking into the host [7, 8, 94]. The expulsion of *Symbiodinium* cells by the coral host has therefore been regarded as a protective mechanism: the coral prevents further ROS leakage from *Symbiodinium* into host cells by removing the primary source of ROS production and also by reducing tissue hyperoxia during daylight exposure [95, 96]. The CoQ pool likely provides an early line of antioxidant defence because ROS leaking from *Symbiodinium* cells would need to cross the host-derived symbiosomal membrane, which like all animal membranes is expected to contain CoQ/CoQH₂ [32, 70]. Nonetheless, it would be expected that an increase in *Symbiodinium* cellular ROS concentrations to a point where leakage into the host occurs would manifest as a distinct decline in PSII photochemical efficiency. However, a major decline in F_v/F_M was only observed here after significant CoQ pool oxidation had already occurred (Fig 2F and 2H). This suggests that ROS leakage is unlikely to be a major contributing factor to the initial oxidation of the CoQ pool, although this cannot be ruled out in the later stages where a major decline in F_v/F_M was observed.

Plastoquinone pool redox state

In contrast to the host CoQ pool redox state, the *Symbiodinium* PQ redox state remained stable until the point at which PSII photochemical efficiency was severely impaired and coral nubbins were distinctly bleached (Fig 2C and 2D). The observed initial stability of the PQ redox state, despite hyperthermal stress, is consistent with short-term acute heat stress results [37]. By analogy with high light stress [29, 97], the oxidative shift due to hyperthermal stress could be caused by increased ROS scavenging of PQH₂ within *Symbiodinium* chloroplasts or changes in photosynthetic ETC such as increased plastid terminal oxidase activity. PQH₂ is a highly effective quencher of ¹O₂ [33, 34] and, like CoQ, acts as a lipid peroxidation chain breaker either directly or via the regeneration of α -tocopherol [30, 31]. Even though irradiance was maintained at a moderate level during the experiment described here, the applied temperature stress caused chronic photoinhibition of PSII, which is commonly reported in coral bleaching experiments (e.g. [14, 15, 98]) and a known indicator of ROS formation within the photosynthetic ETC [99].

A five-fold increase in PQ pool concentrations was recorded concomitantly with the observed PQ pool oxidation (Fig 2I). Newly synthesized PQ is predominantly in the reduced form (PQH₂, not PQ) [97], thus the observed oxidative shift in the PQ pool at this stage should be the result of increased non-enzymatic formation of PQ from PQH₂ after its interaction with ROS, which are increasingly generated by a thermally damaged photosynthetic ETC [7, 14, 81, 94]. In plants and algae, a considerable proportion of the PQ pool is associated with the chloroplast plastoglobuli which are thought to act as PQH₂ reservoirs [100, 101]. Consequently, the size of the PQ pool increases when plants and algae are exposed to conditions that induce the formation of ¹O₂, such as high light exposure [29, 33, 97]. Accordingly, the increase in the total

PQ pool observed here could be seen as a cellular protective mechanism against the oxidative stress caused by the increasingly impaired photosynthetic ETC. The lack of compromised cell walls in the TEM images (Fig 3) implies that this increase is unlikely to be the result of a normalisation artefact: i.e. loss of structurally compromised cells during the extraction procedure, thus underestimating cell counts. Nonetheless, the concomitant loss of internal cellular structure and the highly compromised state of thylakoid membranes at this time point indicate a need for further experimental work before a definitive protective mechanism can be attributed to *de novo* synthesised (reduced) PQ during bleaching.

Conclusions

This study demonstrated that hyperthermal stress in *A. millepora* was associated with oxidation of the coral host CoQ pool redox state. This oxidation occurred prior to any measurable loss of *Symbiodinium* cells from the host and major decline in PSII photochemical efficiency. Thus the oxidation of CoQ pool redox state is among the earliest known impacts of hyperthermal stress on the cellular chemistry of the coral host and adds to a growing body of work that indicates host cellular responses may precede the bleaching process and symbiont dysfunction. Furthermore, the *Symbiodinium* PQ pool redox state remained unaffected by hyperthermal stress until PSII photochemical efficiency was severely impaired. At this stage, the PQ pool exhibited a five-fold increase in concentration and a distinct oxidative shift.

Supporting Information

S1 Table. Enzymes involved in coenzyme Q pool redox reactions identified in the *Acropora digitifera* genome^a, the *Acropora millepora* transcriptome^b and the EST sequences deposited at GenBank^c.

(PDF)

Acknowledgments

The authors want to thank and acknowledge Walter Dunlap for his contribution to this manuscript. We also thank F. Seneca, S. Schmidt-Roach and A. Negri for their support during the experiment.

Author Contributions

Conceived and designed the experiments: AL JBR DJM MJHvO. Performed the experiments: AL JBR. Analyzed the data: AL JBR. Contributed reagents/materials/analysis tools: AL JBR CAM DJM MJHvO. Wrote the paper: AL JBR CAM DJM MJHvO.

References

1. Hoegh-Guldberg O, Bruno JF. The impact of climate change on the world's marine ecosystems. *Science*. 2010; 328(5985):1523–8. doi: [10.1126/science.1189930](https://doi.org/10.1126/science.1189930) PMID: [20558709](https://pubmed.ncbi.nlm.nih.gov/20558709/)
2. Hoegh-Guldberg O, Smith GJ. The effect of sudden changes in temperature, light and salinity on the population density and export of zooxanthellae from the reef corals *Stylophora pistillata* Esper and *Seriatopora hystrix* Dana. *J Exp Mar Biol Ecol*. 1989; 129(3):279–303. doi: [10.1016/0022-0981\(89\)90109-3](https://doi.org/10.1016/0022-0981(89)90109-3)
3. Szmant AM, Gassman NJ. The effects of prolonged “bleaching” on the tissue biomass and reproduction of the reef coral *Montastrea annularis*. *Coral Reefs*. 1990; 8(4):217–24. doi: [10.1007/BF00265014](https://doi.org/10.1007/BF00265014)
4. Eakin CM, Lough JM, Heron SF. Climate variability and change: monitoring data and evidence for increased coral bleaching stress. In: van Oppen MJH, Lough JM, editors. *Coral Bleaching: Patterns, Processes, Causes and Consequences*. Berlin Heidelberg: Springer; 2009. p. 41–67.

5. Gates RD, Baghdasarian G, Muscatine L. Temperature stress causes host cell detachment in symbiotic cnidarians: Implications for coral bleaching. *Biol Bull.* 1992; 182(3):324–32.
6. Dunn SR, Schnitzler CE, Weis VM. Apoptosis and autophagy as mechanisms of dinoflagellate symbiont release during cnidarian bleaching: every which way you lose. *Proc R Soc B.* 2007; 274(1629):3079–85. doi: [10.1098/rspb.2007.0711](https://doi.org/10.1098/rspb.2007.0711) PMID: [17925275](https://pubmed.ncbi.nlm.nih.gov/17925275/)
7. Lesser MP. Coral bleaching: causes and mechanisms. In: Dubinsky Z, Stambler N, editors. *Coral Reefs: An Ecosystem in Transition*. Springer Netherlands; 2011. p. 405–20.
8. Weis VM. Cellular mechanisms of cnidarian bleaching: stress causes the collapse of symbiosis. *J Exp Biol.* 2008; 211(19):3059–66. doi: [10.1242/jeb.009597](https://doi.org/10.1242/jeb.009597)
9. Venn AA, Loram JE, Douglas AE. Photosynthetic symbioses in animals. *J Exp Bot.* 2008; 59(5):1069–80. doi: [10.1093/jxb/erm328](https://doi.org/10.1093/jxb/erm328) PMID: [18267943](https://pubmed.ncbi.nlm.nih.gov/18267943/)
10. Warner ME, Fitt WK, Schmidt GW. Damage to photosystem II in symbiotic dinoflagellates: A determinant of coral bleaching. *Proc Natl Acad Sci U S A.* 1999; 96(14):8007–12. doi: [10.1073/pnas.96.14.8007](https://doi.org/10.1073/pnas.96.14.8007) PMID: [10393938](https://pubmed.ncbi.nlm.nih.gov/10393938/)
11. Takahashi S, Whitney SM, Badger MR. Different thermal sensitivity of the repair of photodamaged photosynthetic machinery in cultured *Symbiodinium* species. *Proc Natl Acad Sci U S A.* 2009; 106(9):3237. doi: [10.1073/pnas.0808363106](https://doi.org/10.1073/pnas.0808363106) PMID: [19202067](https://pubmed.ncbi.nlm.nih.gov/19202067/)
12. Hill R, Brown CM, DeZeeuw K, Campbell DA, Ralph PJ. Increased rate of D1 repair in coral symbionts during bleaching is insufficient to counter accelerated photo-inactivation. *Limnol Oceanogr.* 2011; 56(1):139–46. doi: [10.4319/lo.2011.56.1.0139](https://doi.org/10.4319/lo.2011.56.1.0139)
13. Takahashi S, Murata N. How do environmental stresses accelerate photoinhibition? *Trends Plant Sci.* 2008; 13(4):178–82. doi: [10.1016/j.tplants.2008.01.005](https://doi.org/10.1016/j.tplants.2008.01.005) PMID: [18328775](https://pubmed.ncbi.nlm.nih.gov/18328775/)
14. Jones RJ, Hoegh-Guldberg O, Larkum AWD, Schreiber U. Temperature-induced bleaching of corals begins with impairment of the CO₂ fixation mechanism in zooxanthellae. *Plant Cell Environ.* 1998; 21:1219–30. doi: [10.1046/j.1365-3040.1998.00345.x](https://doi.org/10.1046/j.1365-3040.1998.00345.x)
15. Tchernov D, Gorbunov MY, de Vargas C, Narayan Yadav S, Milligan AJ, Hagglom M, et al. Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. *PNAS.* 2004; 101(37):13531–5. doi: [10.1073/pnas.0402907101](https://doi.org/10.1073/pnas.0402907101) PMID: [15340154](https://pubmed.ncbi.nlm.nih.gov/15340154/)
16. Buxton L, Takahashi S, Hill R, Ralph PJ. Variability in the primary site of photosynthetic damage in *Symbiodinium* sp. (dinophyceae) exposed to thermal stress. *J Phycol.* 2012; 48:117–26. doi: [10.1111/j.1529-8817.2011.01099.x](https://doi.org/10.1111/j.1529-8817.2011.01099.x)
17. Paxton CW, Davy SK, Weis VM. Stress and death of cnidarian host cells play a role in cnidarian bleaching. *The Journal of Experimental Biology.* 2013; 216(15):2813–20. doi: [10.1242/jeb.087858](https://doi.org/10.1242/jeb.087858)
18. Ainsworth TD, Hoegh-Guldberg O, Heron SF, Skirving WJ, Leggat W. Early cellular changes are indicators of pre-bleaching thermal stress in the coral host. *J Exp Mar Biol Ecol.* 2008; 364(2):63–71. doi: [10.1016/j.jembe.2008.06.032](https://doi.org/10.1016/j.jembe.2008.06.032)
19. Dunn SR, Pernice M, Green K, Hoegh-Guldberg O, Dove SG. Thermal stress promotes host mitochondrial degradation in symbiotic cnidarians: Are the batteries of the reef going to run out? *PLoS ONE.* 2012; 7(7):e39024. doi: [10.1371/journal.pone.0039024](https://doi.org/10.1371/journal.pone.0039024) PMID: [22815696](https://pubmed.ncbi.nlm.nih.gov/22815696/)
20. Hawkins TD, Krueger T, Becker S, Fisher PL, Davy SK. Differential nitric oxide synthesis and host apoptotic events correlate with bleaching susceptibility in reef corals. *Coral Reefs.* 2014; 33(1):141–53. doi: [10.1007/s00338-013-1103-4](https://doi.org/10.1007/s00338-013-1103-4)
21. Tolleter D, Seneca François O, DeNofrio Jan C, Krediet Cory J, Palumbi Stephen R, Pringle John R, et al. Coral bleaching independent of photosynthetic activity. *Curr Biol.* 2013; 23(18):1782–6. doi: [10.1016/j.cub.2013.07.041](https://doi.org/10.1016/j.cub.2013.07.041) PMID: [24012312](https://pubmed.ncbi.nlm.nih.gov/24012312/)
22. Lesser MP. Oxidative stress causes coral bleaching during exposure to elevated temperatures. *Coral Reefs.* 1997; 16(3):187–92. doi: [10.1007/s003380050073](https://doi.org/10.1007/s003380050073)
23. Lesser MP, Farrell JH. Exposure to solar radiation increases damage to both host tissues and algal symbionts of corals during thermal stress. *Coral Reefs.* 2004; 23(3):367–77. doi: [10.1007/s00338-004-0392-z](https://doi.org/10.1007/s00338-004-0392-z)
24. Dykens JA, Shick JM, Benoit C, Buettner GR, Winston GW. Oxygen radical production in the sea anemone *Anthopleura elegantissima* and its endosymbiotic algae. *J Exp Biol.* 1992; 168(1):219–41.
25. Bellantuono AJ, Granados-Cifuentes C, Miller DJ, Hoegh-Guldberg O, Rodriguez-Lanetty M. Coral thermal tolerance: Tuning gene expression to resist thermal stress. *PLoS ONE.* 2012; 7(11):e50685. doi: [10.1371/journal.pone.0050685](https://doi.org/10.1371/journal.pone.0050685) PMID: [23226355](https://pubmed.ncbi.nlm.nih.gov/23226355/)
26. DeSalvo MK, Estrada A, Sunagawa S, Medina M. Transcriptomic responses to darkness stress point to common coral bleaching mechanisms. *Coral Reefs.* 2012; 31(1):215–28. doi: [10.1007/s00338-011-0833-4](https://doi.org/10.1007/s00338-011-0833-4)

27. Ernster L, Forsmark-Andrée P. Ubiquinol: An endogenous antioxidant in aerobic organisms. *Clin Investig.* 1993; 71(8):S60–S5. doi: [10.1007/bf00226842](https://doi.org/10.1007/bf00226842) PMID: [8241707](https://pubmed.ncbi.nlm.nih.gov/8241707/)
28. Bentinger M, Brismar K, Dallner G. The antioxidant role of coenzyme Q. *Mitochondrion.* 2007; 7(S): S41–50. doi: [10.1016/j.mito.2007.02.006](https://doi.org/10.1016/j.mito.2007.02.006) PMID: [17482888](https://pubmed.ncbi.nlm.nih.gov/17482888/)
29. Nowicka B, Kruk J. Plastoquinol is more active than α -tocopherol in singlet oxygen scavenging during high light stress of *Chlamydomonas reinhardtii*. *BBA-Bioenergetics.* 2012; 1817(3):389–94. doi: [10.1016/j.bbabi.2011.12.002](https://doi.org/10.1016/j.bbabi.2011.12.002) PMID: [22192719](https://pubmed.ncbi.nlm.nih.gov/22192719/)
30. Hundal T, Forsmark-Andree P, Ernster L, Andersson B. Antioxidant activity of reduced plastoquinone in chloroplast thylakoid membranes. *Arch Biochem Biophys.* 1995; 324(1):117–22. doi: [10.1006/abbi.1995.9920](https://doi.org/10.1006/abbi.1995.9920) PMID: [7503545](https://pubmed.ncbi.nlm.nih.gov/7503545/)
31. Mukai K, Itoh S, Morimoto H. Stopped-flow kinetic study of vitamin E regeneration reaction with biological hydroquinones (reduced forms of ubiquinone, vitamin K, and tocopherolquinone) in solution. *J Biol Chem.* 1992; 267(31):22277–81. PMID: [1429580](https://pubmed.ncbi.nlm.nih.gov/1429580/)
32. Turunen M, Olsson J, Dallner G. Metabolism and function of coenzyme Q. *BBA-Biomembranes.* 2004; 1660(1–2):171–99. doi: [10.1016/j.bbamem.2003.11.012](https://doi.org/10.1016/j.bbamem.2003.11.012) PMID: [14757233](https://pubmed.ncbi.nlm.nih.gov/14757233/)
33. Kruk J, Trebst A. Plastoquinol as a singlet oxygen scavenger in photosystem II. *BBA-Bioenergetics.* 2008; 1777(2):154–62. doi: [10.1016/j.bbabi.2007.10.008](https://doi.org/10.1016/j.bbabi.2007.10.008) PMID: [18005659](https://pubmed.ncbi.nlm.nih.gov/18005659/)
34. Yadav DK, Kruk J, Sinha RK, Pospisil P. Singlet oxygen scavenging activity of plastoquinol in photosystem II of higher plants: Electron paramagnetic resonance spin-trapping study. *BBA-Bioenergetics.* 2010; 1797:1807–11. doi: [10.1016/j.bbabi.2010.07.003](https://doi.org/10.1016/j.bbabi.2010.07.003) PMID: [20637718](https://pubmed.ncbi.nlm.nih.gov/20637718/)
35. Yamamoto Y, Yamashita S. Plasma ratio of ubiquinol and ubiquinone as a marker of oxidative stress. *Mol Aspects Med.* 1997; 18:79–84. doi: [10.1016/S0098-2997\(97\)00007-1](https://doi.org/10.1016/S0098-2997(97)00007-1)
36. Miles L, Miles MV, Tang PH, Horn PS, Quinlan JG, Wong B, et al. Ubiquinol: A potential biomarker for tissue energy requirements and oxidative stress. *Clin Chim Acta.* 2005; 360(1):87–96. doi: [10.1016/j.cccn.2005.04.009](https://doi.org/10.1016/j.cccn.2005.04.009)
37. Lutz A, Motti CA, Freckelton ML, van Oppen MJH, Miller DJ, Dunlap WC. Simultaneous determination of coenzyme Q and plastoquinone redox states in the coral–*Symbiodinium* symbiosis during thermally induced bleaching. *J Exp Mar Biol Ecol.* 2014; 455(0):1–6. doi: [10.1016/j.jembe.2014.02.006](https://doi.org/10.1016/j.jembe.2014.02.006)
38. Levy O, Achituv Y, Yacobi YZ, Dubinsky Z, Stambler N. Diel 'tuning' of coral metabolism: physiological responses to light cues. *J Exp Biol.* 2006; 209(2):273–83. doi: [10.1242/jeb.01983](https://doi.org/10.1242/jeb.01983)
39. Gleason DF, Wellington GM. Ultraviolet radiation and coral bleaching. *Nature.* 1993; 365:836–8. doi: [10.1038/365836a0](https://doi.org/10.1038/365836a0)
40. Berkelmans R. Bleaching and mortality thresholds: How much is too much? In: Oppen MJH, Lough JM, editors. *Coral Bleaching: Patterns, Processes, Causes and Consequences.* Ecological Studies. 205: Springer Berlin Heidelberg; 2009. p. 103–19.
41. Schreiber U. Pulse-amplitude-modulation (PAM) fluorometry and saturation pulse method: An overview. In: Papageorgiou GC, Govindjee, editors. *Chlorophyll Fluorescence: A signature of photosynthesis.* Advances in Photosynthesis and Respiration. 19: Springer Netherlands; 2004. p. 279–319.
42. Veal CJ, Carmi M, Fine M, Hoegh-Guldberg O. Increasing the accuracy of surface area estimation using single wax dipping of coral fragments. *Coral Reefs.* 2010; 29(4):893–7. doi: [10.1007/s00338-010-0647-9](https://doi.org/10.1007/s00338-010-0647-9)
43. Warren CR. Rapid measurement of chlorophylls with a microplate reader. *Journal of Plant Nutrition.* 2008; 31(7):1321–32. doi: [10.1080/01904160802135092](https://doi.org/10.1080/01904160802135092)
44. Ritchie RJ. Consistent sets of spectrophotometric chlorophyll equations for acetone, methanol and ethanol solvents. *Photosynth Res.* 2006; 89(1):27–41. doi: [10.1007/s11120-006-9065-9](https://doi.org/10.1007/s11120-006-9065-9) PMID: [16763878](https://pubmed.ncbi.nlm.nih.gov/16763878/)
45. van Oppen MJH, Palstra FP, Piquet AM-T, Miller DJ. Patterns of coral-dinoflagellate associations in *Acropora*: Significance of local availability and physiology of *Symbiodinium* strains and host-symbiont selectivity. *Proc R Soc B.* 2001; 268(1478):1759–67. doi: [10.1098/rspb.2001.1733](https://doi.org/10.1098/rspb.2001.1733) PMID: [11522193](https://pubmed.ncbi.nlm.nih.gov/11522193/)
46. Wilson K, Li Y, Whan V, Lehnert S, Byrne K, Moore S, et al. Genetic mapping of the black tiger shrimp *Penaeus monodon* with amplified fragment length polymorphism. *Aquaculture.* 2002; 204(3):297–309. doi: [10.1016/S0044-8486\(01\)00842-0](https://doi.org/10.1016/S0044-8486(01)00842-0)
47. Fabricius KE, Mieog JC, Colin PL, Idip D, van Oppen MJH. Identity and diversity of coral endosymbionts (zooxanthellae) from three Palauan reefs with contrasting bleaching, temperature and shading histories. *Mol Ecol.* 2004; 13(8):2445–58. doi: [10.1111/j.1365-294X.2004.02230.x](https://doi.org/10.1111/j.1365-294X.2004.02230.x) PMID: [15245416](https://pubmed.ncbi.nlm.nih.gov/15245416/)
48. Moya A, Huisman L, Ball EE, Hayward DC, Grasso LC, Chua CM, et al. Whole transcriptome analysis of the coral *Acropora millepora* reveals complex responses to CO₂-driven acidification during the initiation of calcification. *Mol Ecol.* 2012; 21(10):2440–54. doi: [10.1111/j.1365-294X.2012.05554.x](https://doi.org/10.1111/j.1365-294X.2012.05554.x) PMID: [22490231](https://pubmed.ncbi.nlm.nih.gov/22490231/)

49. Shinzato C, Shoguchi E, Kawashima T, Hamada M, Hisata K, Tanaka M, et al. Using the *Acropora digitifera* genome to understand coral responses to environmental change. *Nature*. 2011; 476(7360):320–3. doi: [10.1038/nature10249](https://doi.org/10.1038/nature10249) PMID: [21785439](https://pubmed.ncbi.nlm.nih.gov/21785439/)
50. Dunlap WC, Starcevic A, Baranasic D, Diminic J, Zucko J, Gacesa R, et al. KEGG orthology-based annotation of the predicted proteome of *Acropora digitifera*: ZoophyteBase—an open access and searchable database of a coral genome. *BMC Genomics*. 2013; 14(1):1–59. doi: [10.1186/1471-2164-14-509](https://doi.org/10.1186/1471-2164-14-509)
51. Pinheiro JC, Bates DM. Mixed-effects models in S and S-PLUS. Chambers J, Eddy W, Härdle W, Sheather S, Tierney L, editors. New York: Springer Verlag; 2000.
52. Zuur AF, Ieno EN, Walker N, Saveliev AA, Smith GM. Mixed effects models and extensions in ecology with R. Gail M, Krickeberg K, Samet J, Tsiatis A, Wong W, editors. New York: Springer; 2009.
53. Hochberg Y, Benjamini Y. More powerful procedures for multiple significance testing. *Stat Med*. 1990; 9(7):811–8. doi: [10.1002/sim.4780090710](https://doi.org/10.1002/sim.4780090710) PMID: [2218183](https://pubmed.ncbi.nlm.nih.gov/2218183/)
54. Lesser MP, Stochaj WR, Tapley DW, Shick JM. Bleaching in coral reef anthozoans: effects of irradiance, ultraviolet radiation, and temperature on the activities of protective enzymes against active oxygen. *Coral Reefs*. 1990; 8(4):225–32. doi: [10.1007/BF00265015](https://doi.org/10.1007/BF00265015)
55. Dunne R, Brown B. The influence of solar radiation on bleaching of shallow water reef corals in the Andaman Sea, 1993–1998. *Coral Reefs*. 2001; 20(3):201–10. doi: [10.1007/s003380100160](https://doi.org/10.1007/s003380100160)
56. Uthicke S, Klumpp DW. Microphytobenthos community production at a near-shore coral reef: Seasonal variation and response to ammonium recycled by holothurians. *Mar Ecol Prog Ser*. 1998; 169:1–11. doi: [10.3354/meps169001](https://doi.org/10.3354/meps169001)
57. Suggett DJ, Warner ME, Smith DJ, Davey P, Hennige S, Baker NR. Photosynthesis and production of hydrogen peroxide by *Symbiodinium* (Pyrrophyta) phylotypes with different thermal tolerances. *J Phycol*. 2008; 44(4):948–56. doi: [10.1111/j.1529-8817.2008.00537.x](https://doi.org/10.1111/j.1529-8817.2008.00537.x)
58. McGinty ES, Pieczonka J, Mydlarz LD. Variations in reactive oxygen release and antioxidant activity in multiple *Symbiodinium* types in response to elevated temperature. *Microb Ecol*. 2012; 64(4):1000–7. doi: [10.1007/s00248-012-0085-z](https://doi.org/10.1007/s00248-012-0085-z) PMID: [22767124](https://pubmed.ncbi.nlm.nih.gov/22767124/)
59. Nii CM, Muscatine L. Oxidative stress in the symbiotic sea anemone *Aiptasia pulchella* (Carlgren, 1943): Contribution of the animal to superoxide ion production at elevated temperature. *Biol Bull*. 1997; 192(3):444–56.
60. Ainsworth TD, Wasmund K, Ukani L, Seneca F, Yellowlees D, Miller D, et al. Defining the tipping point. A complex cellular life/death balance in corals in response to stress. *Scientific Reports*. 2011; 1:160. doi: [10.1038/srep00160](https://doi.org/10.1038/srep00160) PMID: [22355675](https://pubmed.ncbi.nlm.nih.gov/22355675/)
61. Desalvo MK, Voolstra CR, Sunagawa S, Schwarz JA, Stillman JH, Coffroth MA, et al. Differential gene expression during thermal stress and bleaching in the Caribbean coral *Montastraea faveolata*. *Mol Ecol*. 2008; 17(17):3952–71. doi: [10.1111/j.1365-294X.2008.03879.x](https://doi.org/10.1111/j.1365-294X.2008.03879.x) PMID: [18662230](https://pubmed.ncbi.nlm.nih.gov/18662230/)
62. Rodriguez-Lanetty M, Harii S, Hoegh-Guldberg O. Early molecular responses of coral larvae to hyperthermal stress. *Mol Ecol*. 2009; 18(24):5101–14. doi: [10.1111/j.1365-294X.2009.04419.x](https://doi.org/10.1111/j.1365-294X.2009.04419.x) PMID: [19900172](https://pubmed.ncbi.nlm.nih.gov/19900172/)
63. DeSalvo MK, Sunagawa S, Voolstra CR, Medina M. Transcriptomic responses to heat stress and bleaching in the elkhorn coral *Acropora palmata*. *Mar Ecol Prog Ser*. 2010; 402:97–113. doi: [10.3354/meps08372](https://doi.org/10.3354/meps08372)
64. Fitt WK, Warner ME. Bleaching patterns of four species of Caribbean reef corals. *Biol Bull*. 1995; 189(3):298–307. doi: [10.2307/1542147](https://doi.org/10.2307/1542147)
65. Warner ME, Fitt WK, Schmidt GW. The effects of elevated temperature on the photosynthetic efficiency of zooxanthellae in hospite from four different species of reef coral: A novel approach. *Plant Cell Environ*. 1996; 19:291–9. doi: [10.1111/j.1365-3040.1996.tb00251.x](https://doi.org/10.1111/j.1365-3040.1996.tb00251.x)
66. Howells EJ, Beltran VH, Larsen NW, Bay LK, Willis BL, van Oppen MJH. Coral thermal tolerance shaped by local adaptation of photosymbionts. *Nature Climate Change*. 2011; 2(2):116–20. doi: [10.1038/nclimate1330](https://doi.org/10.1038/nclimate1330)
67. Baird AH, Bhagooli R, Ralph PJ, Takahashi S. Coral bleaching: the role of the host. *Trends in Ecology & Evolution*. 2009; 24(1):16–20. doi: [10.1016/j.tree.2008.09.005](https://doi.org/10.1016/j.tree.2008.09.005)
68. Loya Y, Sakai K, Yamazato K, Nakano Y, Sambali H, van Woesik R. Coral bleaching: the winners and the losers. *Ecology Letters*. 2001; 4(2):122–31. doi: [10.1046/j.1461-0248.2001.00203.x](https://doi.org/10.1046/j.1461-0248.2001.00203.x)
69. McClanahan TR, Baird AH, Marshall PA, Toscano MA. Comparing bleaching and mortality responses of hard corals between southern Kenya and the Great Barrier Reef, Australia. *Marine Pollution Bulletin*. 2004; 48(3–4):327–35. <http://dx.doi.org/10.1016/j.marpolbul.2003.08.024>. PMID: [14972585](https://pubmed.ncbi.nlm.nih.gov/14972585/)

70. Swiezewska E, Dallner G, Andersson B, Ernster L. Biosynthesis of ubiquinone and plastoquinone in the endoplasmic reticulum—Golgi membranes of spinach leaves. *J Biol Chem*. 1993; 268(2):1494–9. PMID: [8419349](#)
71. Matus-Ortega MG, Salmerón-Santiago KG, Flores-Herrera O, Guerra-Sánchez G, Martínez F, Rendón JL, et al. The alternative NADH dehydrogenase is present in mitochondria of some animal taxa. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*. 2011; 6(3):256–63. doi: [10.1016/j.cbd.2011.05.002](#)
72. Genova ML, Lenaz G. New developments on the functions of coenzyme Q in mitochondria. *Biofactors*. 2011; 37(5):330–54. doi: [10.1002/biof.168](#) PMID: [21989973](#)
73. Arroyo A, Navarro F, Navas P, Villalba JM. Ubiquinol regeneration by plasma membrane ubiquinone reductase. *Protoplasma*. 1998; 205(1–4):107–13. doi: [10.1007/bf01279300](#)
74. Takahashi T, Yamaguchi T, Shitashige M, Okamoto T, Kishi T. Reduction of ubiquinone in membrane lipids by rat liver cytosol and its involvement in the cellular defence system against lipid peroxidation. *Biochem J*. 1995; 309(3):883. doi: [10.1042/bj3090883](#)
75. Beyer RE, Segura-Aguilar J, Di Bernardo S, Cavazzoni M, Fato R, Fiorentini D, et al. The role of DT-diaphorase in the maintenance of the reduced antioxidant form of coenzyme Q in membrane systems. *Proc Natl Acad Sci U S A*. 1996; 93(6):2528–32. doi: [10.1073/pnas.93.6.2528](#) PMID: [8637908](#)
76. Goldstone J. Environmental sensing and response genes in cnidaria: The chemical defensome in the sea anemone *Nematostella vectensis*. *Cell Biol Toxicol*. 2008; 24(6):483–502. doi: [10.1007/s10565-008-9107-5](#) PMID: [18956243](#)
77. McDonald AE, Vanlerberghe GC, Staples JF. Alternative oxidase in animals: unique characteristics and taxonomic distribution. *J Exp Biol*. 2009; 212(16):2627–34. doi: [10.1242/jeb.032151](#)
78. Morré DJ, Morré DM. Non-mitochondrial coenzyme Q. *Biofactors*. 2011; 37(5):355–60. doi: [10.1002/biof.156](#) PMID: [21674641](#)
79. Bentinger M, Tekle M, Dallner G. Coenzyme Q—biosynthesis and functions. *Biochem Biophys Res Commun*. 2010; 396(1):74–9. doi: [10.1016/j.bbrc.2010.02.147](#) PMID: [20494114](#)
80. Rocker MM, Noonan S, Humphrey C, Moya A, Willis BL, Bay LK. Expression of calcification and metabolism-related genes in response to elevated pCO₂ and temperature in the reef-building coral *Acropora millepora*. *Marine Genomics*. 2015. doi: [10.1016/j.margen.2015.08.001](#)
81. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Oxford (fourth edition), UK: Oxford University Press; 2007.
82. Furla P, Allemand D, Shick JM, Ferrier-Pagès C, Richier S, Plantivaux A, et al. The symbiotic anthozoan: A physiological chimera between alga and animal. *Integrative and Comparative Biology*. 2005; 45(4):595–604. doi: [10.1093/icb/45.4.595](#) PMID: [21676806](#)
83. Yamashita S, Yamamoto Y. Simultaneous detection of ubiquinol and ubiquinone in human plasma as a marker of oxidative stress. *Anal Biochem*. 1997; 250(1):66–73. PMID: [9234900](#)
84. Flores-Ramírez LA, Liñán-Cabello MA. Relationships among thermal stress, bleaching and oxidative damage in the hermatypic coral, *Pocillopora capitata*. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*. 2007; 146(1–2):194–202. doi: [10.1016/j.cbpc.2006.09.008](#)
85. Richier S, Sabourault C, Courtiade J, Zucchini N, Allemand D, Furla P. Oxidative stress and apoptotic events during thermal stress in the symbiotic sea anemone, *Anemonia viridis*. *FEBS Journal*. 2006; 273(18):4186–98. doi: [10.1111/j.1742-4658.2006.05414.x](#) PMID: [16907933](#)
86. Császár NBM, Seneca FO, van Oppen MJH. Variation in antioxidant gene expression in the scleractinian coral *Acropora millepora* under laboratory thermal stress. *Mar Ecol Prog Ser*. 2009; 392:93–102. doi: [10.3354/meps08194](#)
87. Richier S, Furla P, Plantivaux A, Merle P-L, Allemand D. Symbiosis-induced adaptation to oxidative stress. *J Exp Biol*. 2005; 208(2):277–85. doi: [10.1242/jeb.01368](#)
88. Dykens JA, Shick JM. Oxygen production by endosymbiotic algae controls superoxide dismutase activity in their animal host. *Nature*. 1982; 297(5867):579–80. doi: [10.1038/297579a0](#)
89. Kühl M, Cohen Y, Dalsgaard T, Jorgensen BB, Revsbech NP. Microenvironment and photosynthesis of zooxanthellae in scleractinian corals studied with microsensors for O₂, pH and light. *Mar Ecol Prog Ser*. 1995; 117:159–72.
90. Richier S, Merle PL, Furla P, Pigozzi D, Sola F, Allemand D. Characterization of superoxide dismutases in anoxia- and hyperoxia-tolerant symbiotic cnidarians. *BBA-Gen Subjects*. 2003; 1621(1):84–91. doi: [10.1016/S0304-4165\(03\)00049-7](#)
91. Turrens JF. Mitochondrial formation of reactive oxygen species. *J Physiol (Lond)*. 2003; 552(2):335–44. doi: [10.1113/jphysiol.2003.049478](#)

92. Brookes PS. Mitochondrial H⁺ leak and ROS generation: an odd couple. *Free Radic Biol Med.* 2005; 38(1):12–23. doi: [10.1016/j.freeradbiomed.2004.10.016](https://doi.org/10.1016/j.freeradbiomed.2004.10.016) PMID: [15589367](https://pubmed.ncbi.nlm.nih.gov/15589367/)
93. Lenaz G, Genova ML. Mobility and function of Coenzyme Q (ubiquinone) in the mitochondrial respiratory chain. *BBA-Bioenergetics.* 2009; 1787(6):563–73. doi: [10.1016/j.bbabi.2009.02.019](https://doi.org/10.1016/j.bbabi.2009.02.019) PMID: [19268424](https://pubmed.ncbi.nlm.nih.gov/19268424/)
94. Smith DJ, Suggett DJ, Baker NR. Is photoinhibition of zooxanthellae photosynthesis the primary cause of thermal bleaching in corals? *Global Change Biology.* 2005; 11(1):1–11. doi: [10.1111/j.1529-8817.2003.00895.x](https://doi.org/10.1111/j.1529-8817.2003.00895.x)
95. Downs CA, Fauth JE, Halas JC, Dustan P, Bemiss J, Woodley CM. Oxidative stress and seasonal coral bleaching. *Free Radic Biol Med.* 2002; 33(4):533–43. doi: [10.1016/S0891-5849\(02\)00907-3](https://doi.org/10.1016/S0891-5849(02)00907-3) PMID: [12160935](https://pubmed.ncbi.nlm.nih.gov/12160935/)
96. Lesser MP. Oxidative stress in marine environments: biochemistry and physiological ecology. *Annu Rev Physiol.* 2006; 68:253–78. doi: [10.1146/annurev.physiol.68.040104.110001](https://doi.org/10.1146/annurev.physiol.68.040104.110001) PMID: [16460273](https://pubmed.ncbi.nlm.nih.gov/16460273/)
97. Szymańska R, Kruk J. Plastoquinol is the main prenyl lipid synthesized during acclimation to high light conditions in *Arabidopsis* and is converted to plastocholesterol by tocopherol cyclase. *Plant Cell Physiol.* 2010; 51(4):537–45. doi: [10.1093/pcp/pcq017](https://doi.org/10.1093/pcp/pcq017) PMID: [20164151](https://pubmed.ncbi.nlm.nih.gov/20164151/)
98. Iglesias-Prieto R, Matta JL, Robins WA, Trench RK. Photosynthetic response to elevated temperature in the symbiotic dinoflagellate *Symbiodinium microadriaticum* in culture. *Proc Natl Acad Sci U S A.* 1992; 89(21):10302–5. PMID: [11607337](https://pubmed.ncbi.nlm.nih.gov/11607337/)
99. Murata N, Allakhverdiev SI, Nishiyama Y. The mechanism of photoinhibition *in vivo*: Re-evaluation of the roles of catalase, α -tocopherol, non-photochemical quenching, and electron transport. *BBA-Bioenergetics.* 2012; 1817(8):1127–33. doi: [10.1016/j.bbabi.2012.02.020](https://doi.org/10.1016/j.bbabi.2012.02.020) PMID: [22387427](https://pubmed.ncbi.nlm.nih.gov/22387427/)
100. Lichtenthaler HK. Biosynthesis, accumulation and emission of carotenoids, α -tocopherol, plastoquinone, and isoprene in leaves under high photosynthetic irradiance. *Photosynth Res.* 2007; 92(2):163–79. doi: [10.1007/s11120-007-9204-y](https://doi.org/10.1007/s11120-007-9204-y) PMID: [17634750](https://pubmed.ncbi.nlm.nih.gov/17634750/)
101. Eugeni Piller L, Abraham M, Dörmann P, Kessler F, Besagni C. Plastid lipid droplets at the crossroads of prenylquinone metabolism. *J Exp Bot.* 2012; 63(4):1609–18. doi: [10.1093/jxb/ers016](https://doi.org/10.1093/jxb/ers016) PMID: [22371323](https://pubmed.ncbi.nlm.nih.gov/22371323/)