Supplemental Information

Title: Deoxygenation lowers the thermal threshold of coral bleaching.

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Supplementary Figures

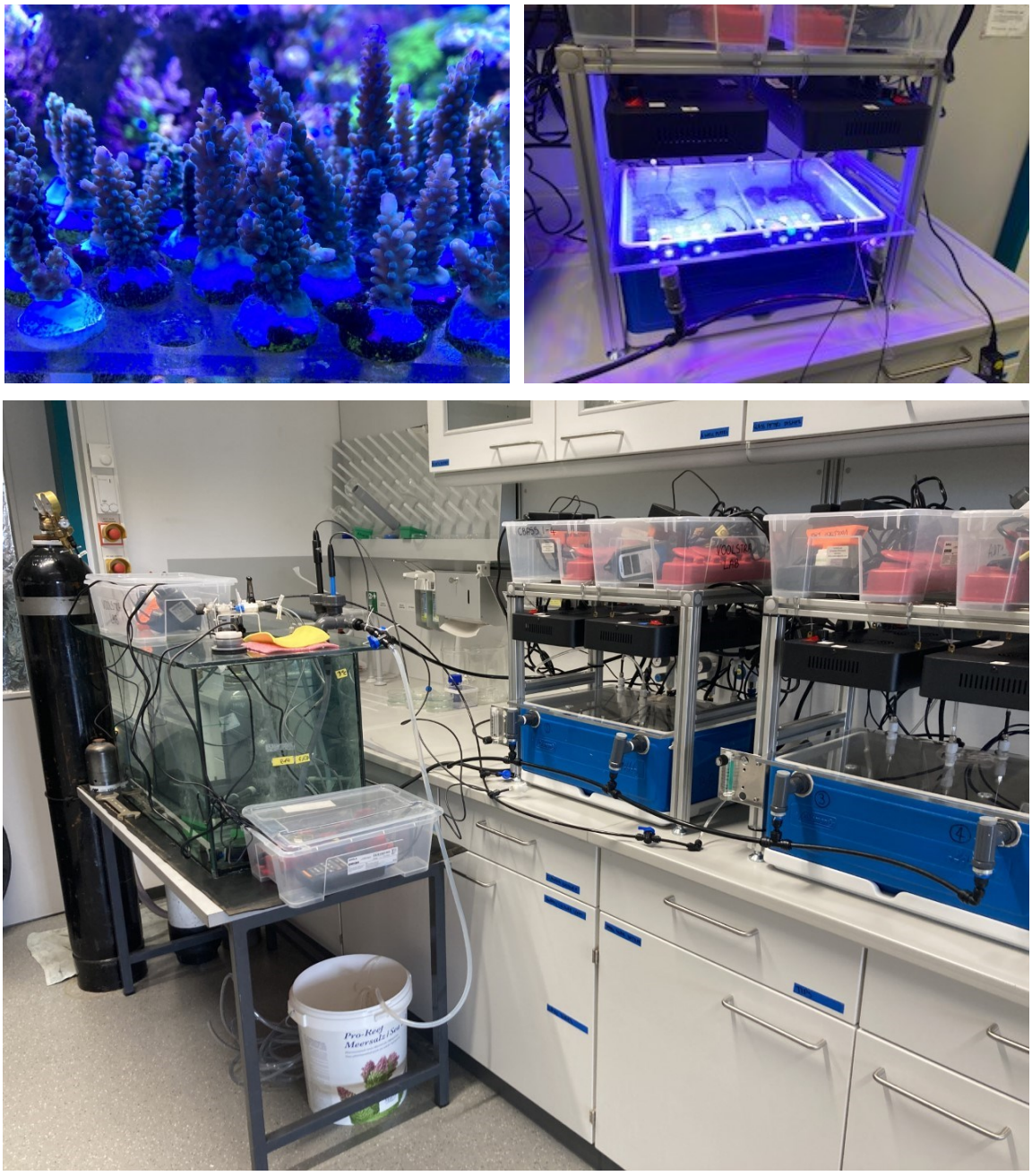

Figure S1. Images of the experimental setup. Shown are coral fragments positioned upright onto ceramic plugs (top left), the transparent lid used for sealing the CBASS tanks from exchange with ambient air (top right), and the overall setup including 4 CBASS tanks within 2 boxes and the reservoir tank.

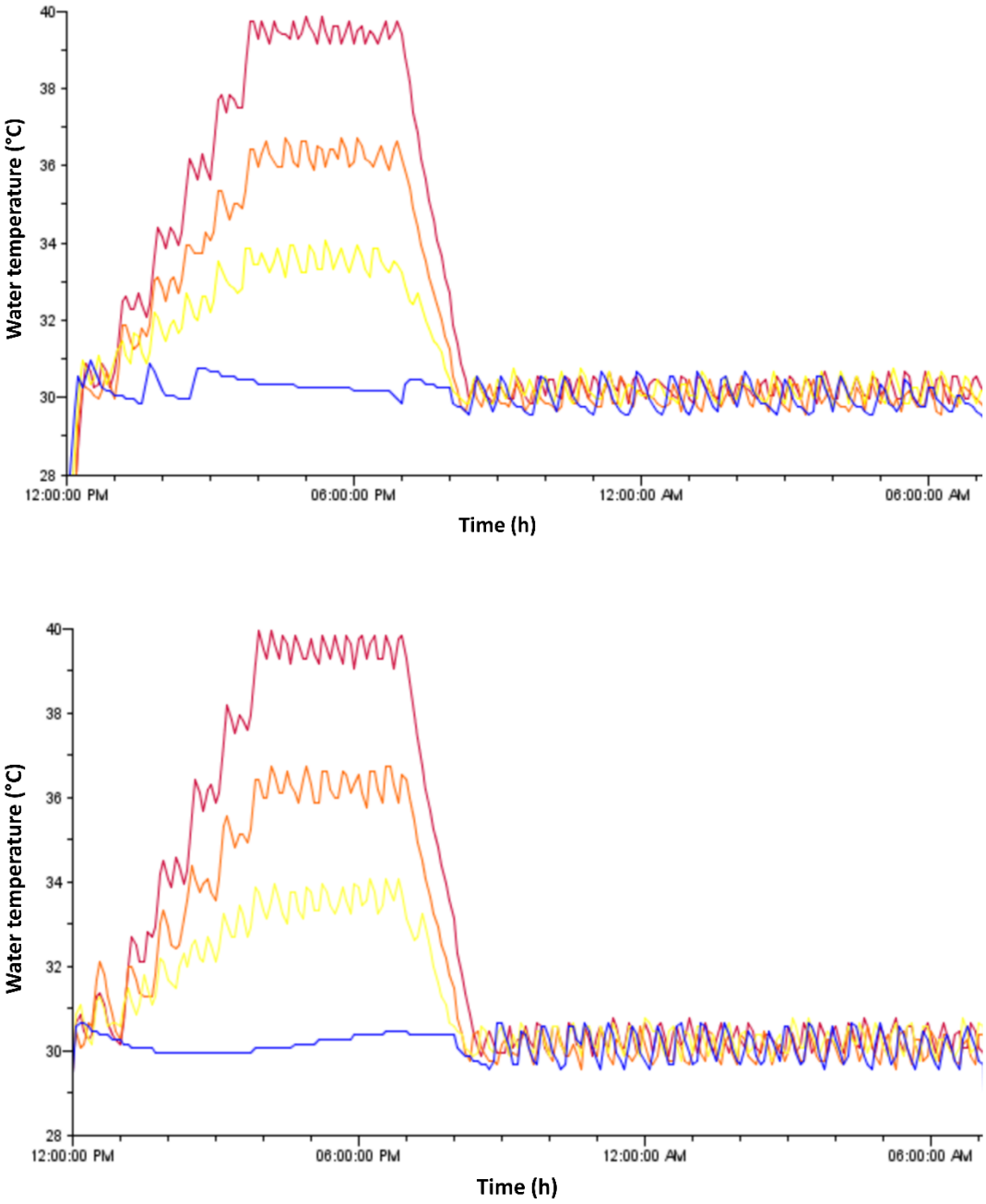

Figure S2. Thermal profiles of CBASS runs. HOBO logger data for each experimental tank of CBASS from 12pm until 7am the next day for the (i) heat only run on 31/05/2021 and (ii) the heat & deoxygenation run on 04/06/2021. Blue, yellow, orange, and red lines represent temperature data from tanks reaching 30°C, 33°C, 36°C, and 39°C, respectively.

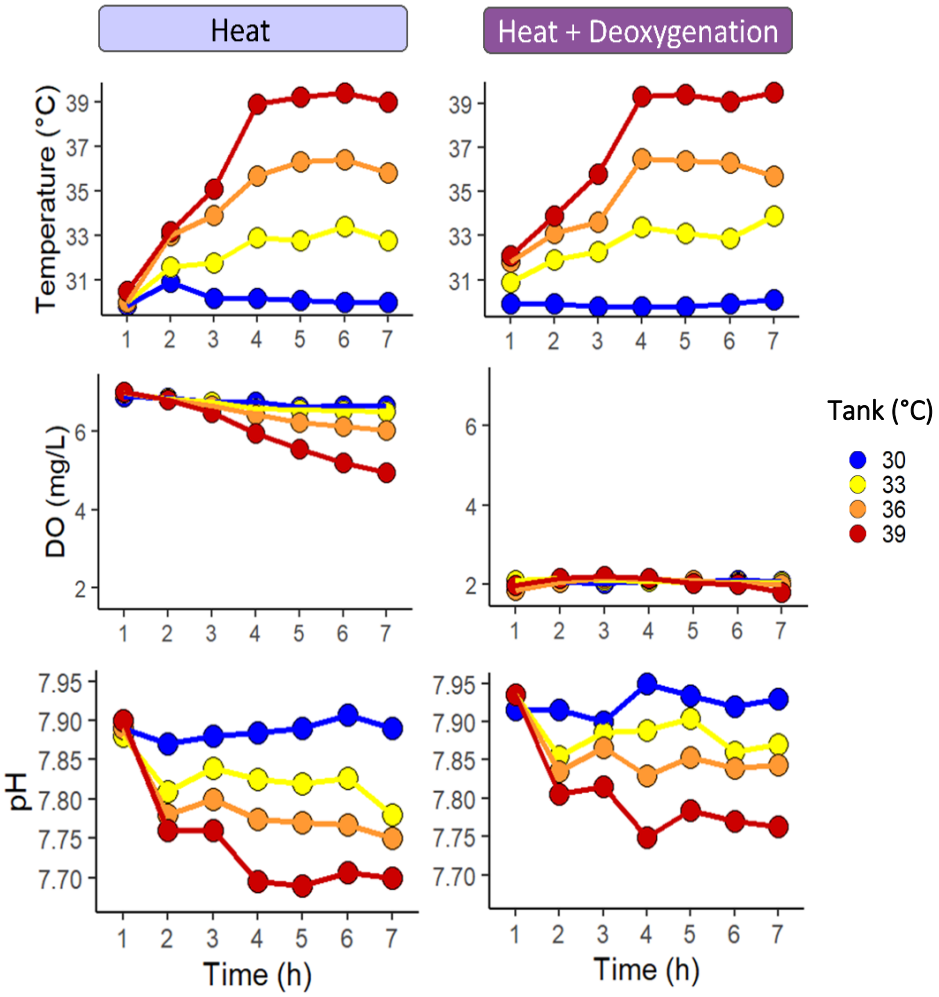

Figure S3. Hourly measurements of temperature (°C), dissolved oxygen (DO), and pH during 6 h heat stress phase. FireSting fibre-optic measurements using robust probes for each parameter during both CBASS runs, heat only on 31/05/2021 and heat & deoxygenation on 04/06/2021. Blue, yellow, orange, and red dot points represent temperature data from tanks reaching 30°C, 33°C, 36°C, and 39°C, respectively.

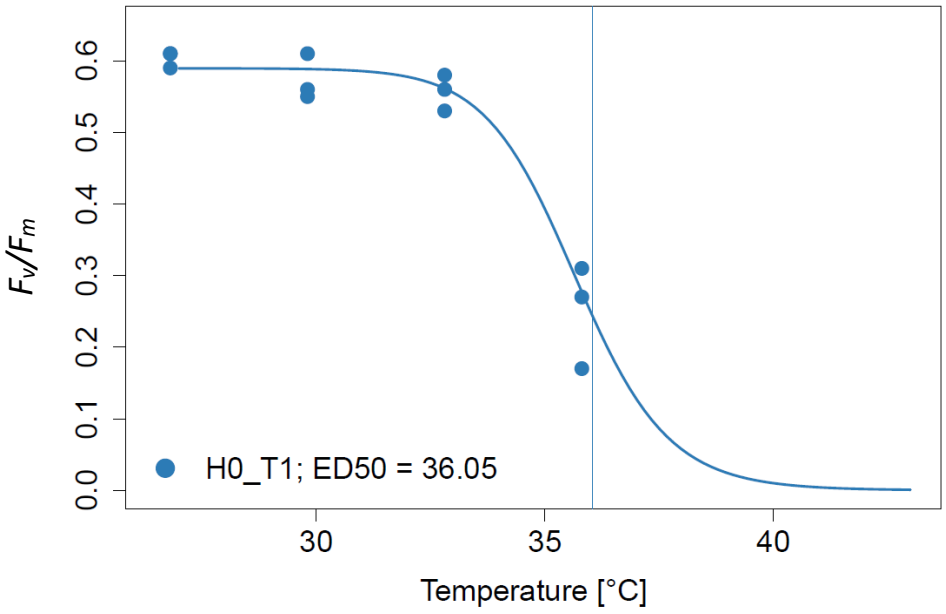

Figure S4. ED50 based on photosynthetic efficiency (F_v/F_m) over lower temperature range with a baseline of 27°C. The temperature at which the F_v/F_m is reduced by 50% is the effective dose 50, ED50, of corals, a standardized thermal tolerance diagnostic (sensu Evensen et al., 2021). Sample replicates n= 3 for this trial run to assess the optimal temperature range to model the ED50. The curve reflects the mean three parameter log-logistic model. H0= Heat only and T1= time point 1. The projected ED50 is higher than the highest treatment temperature, suboptimal for accurate modelling.

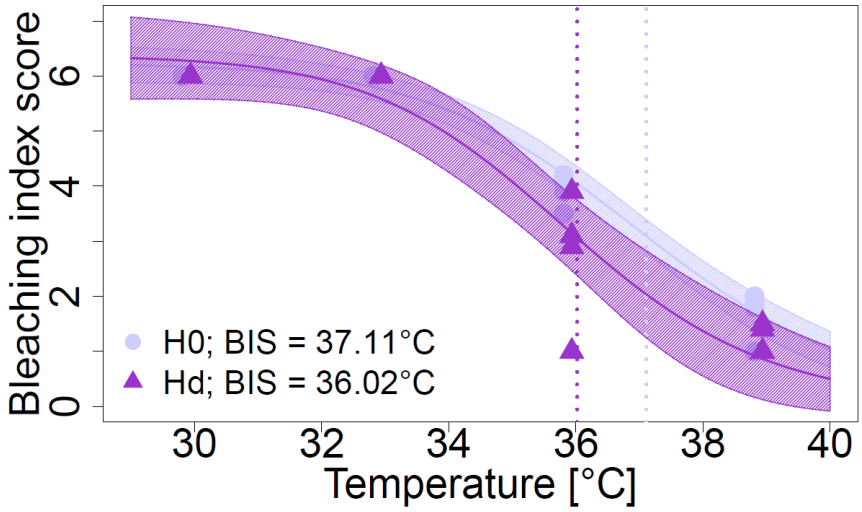

Figure S5. ED50 thermal threshold based on Bleaching Index Score (BIS). The temperature at which BIS is reduced by 50%, designates the effective dose 50, ED50 of corals, a standardized thermal tolerance diagnostic (sensu Evensen et al., 2021). Solid lines reflect the mean three parameter log-logistic model with 95% confidence intervals represented by the shaded areas. Hd= heat & deoxygenated conditions, H0= heat only indicated in dark and light purple respectively. n= 4 per heating tank.

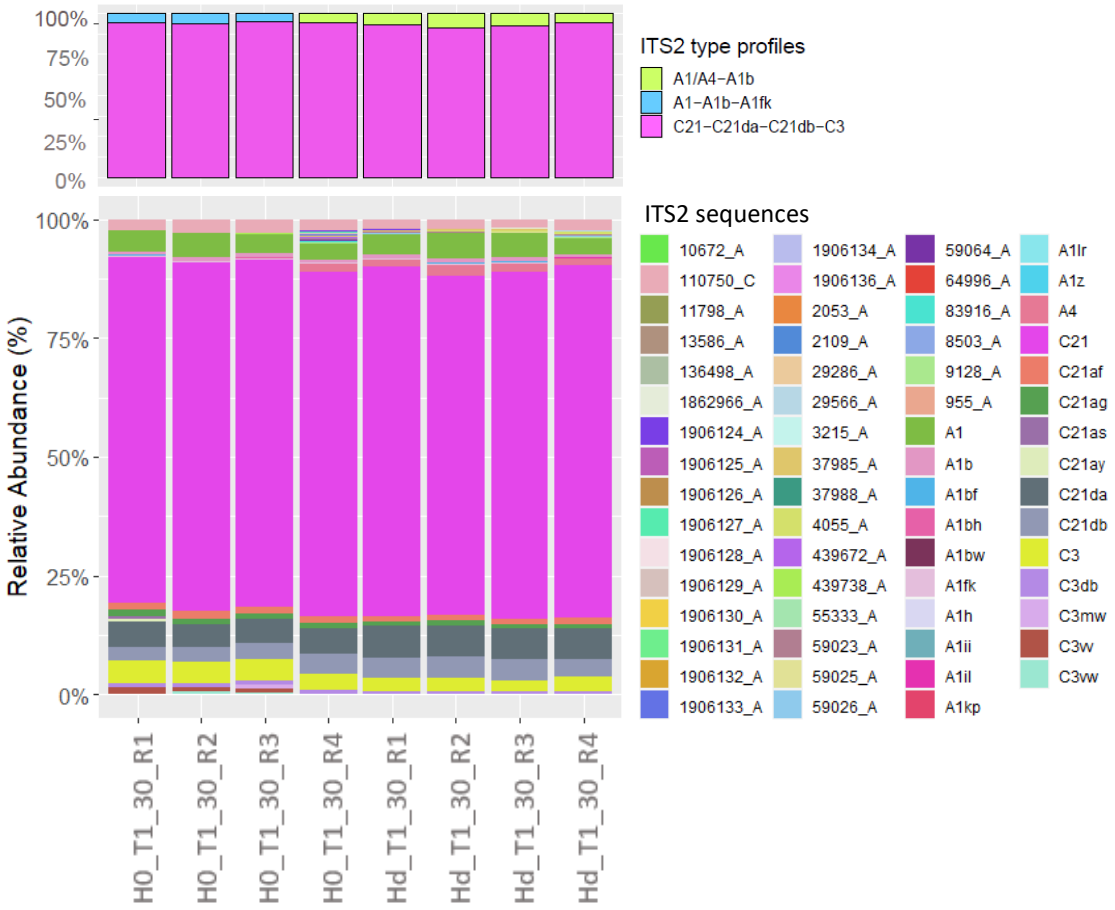

Figure S6. Symbiodiniaceae assemblage of experimental corals. Depicted are relative abundances of post-QC ITS2 sequences (lower panel) and predicted ITS2 type profiles (upper panel) processed by SymPortal for each coral sample at baseline temperature (30°C) from both heat only (H0) and heat & deoxygenation (Hd), n=4 per treatment.

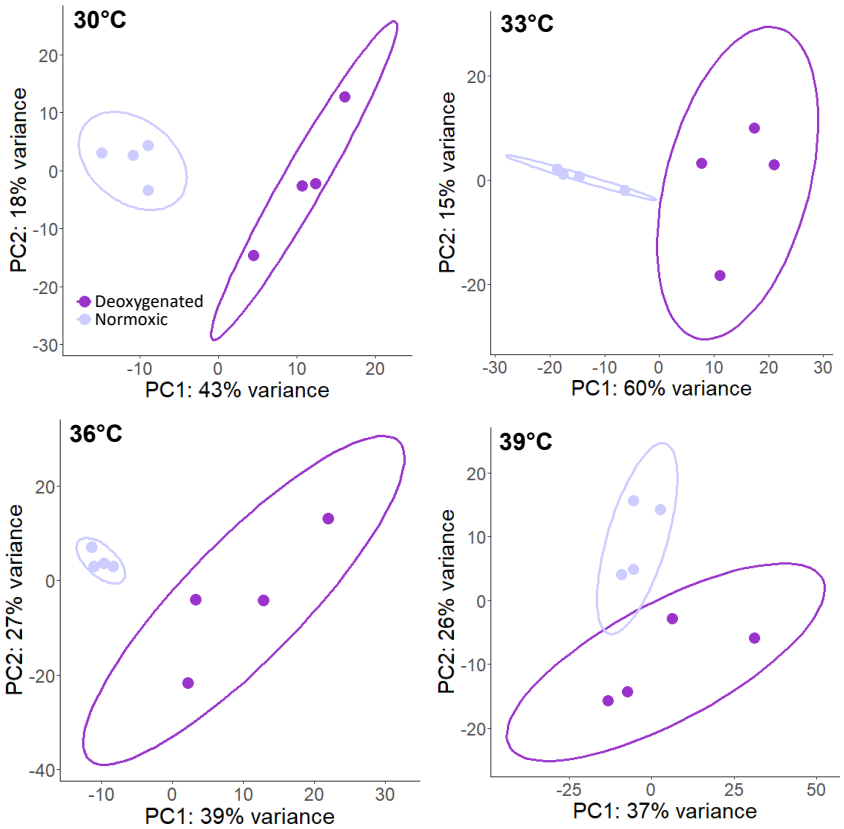

Figure S7. Transcriptome clustering in response to normoxic/deoxygenation heat stress. Similarity of *Acropora* sp. samples denoted by principal components analysis at four different temperatures (30°C, 33°C, 36°C, and 39°C). Dark and light purple represents deoxygenated and normoxic samples respectively. Circles denote 95% confidence level of dispersion estimates. Number of replicates per condition group n= 4.

Supplementary Tables

Table S1. Aquarium holding tank parameters recorded on 18/05/2021 and based on 10+ years of consistent aquarium-rearing conditions.

Parameters	Range
Dissolved oxygen	~6-7mg/L
Salinity	35 PSU
pH daily range	7.7-8.2
Temperature	26.5°C (winter months =25°C)
Light intensity range	95-240 µmol photons m^{-2} s^{-1}
Light spectrum	White & blue

Table S2. Read 1 count at different stages of the analysis pipeline. These stages included trimming sequenced reads of Illumina adaptors and low-quality regions, mapping them to the de novo transcriptome assembly of the *Acropora* sp. (with a total of 20115 contigs) and filtering out samples < 5 million read counts. Sample ID represents the following: H0 = heat only (control), HH= heat & deoxygenation (a.k.a Hd, treatment), T1= time point 1, 30, 33, 36, 39 = temperatures of heat assay, and 1-4 = replicate number.

Sample ID	Initial	Trimming		Mapping ≥ 500bp		Quality Check
	Read count	Paired end read count	% Counts	Paired end reads mapped	% Mapping rate	≥ 5 million counts
H0_T1_30_1	31429339	30464881	97	8510789	28	y
H0_T1_30_2	32238151	31167654	97	8264998	27	y
H0_T1_30_3	36506778	35219011	96	8819356	25	y
H0_T1_30_4	30612235	29567727	97	7102331	24	y
H0_T1_33_1	32145998	30958834	96	9052783	29	y
H0_T1_33_2	29420253	28405849	97	8404714	30	y
H0_T1_33_3	33421263	32271878	97	9794984	30	y
H0_T1_33_4	32641996	31619512	97	9056917	29	y
H0_T1_36_1	30269192	29290194	97	7466870	25	y
H0_T1_36_2	32553871	31509750	97	8210718	26	y
H0_T1_36_3	32448850	31461896	97	7560286	24	y
H0_T1_36_4	31213926	30276231	97	7930491	26	y
H0_T1_39_1	34280899	33089353	97	9083330	27	y
H0_T1_39_2	31649570	30599193	97	8322156	27	y
H0_T1_39_3	31163076	30183090	97	7907971	26	y
H0_T1_39_4	34728077	33669153	97	8942517	27	y
HH_T1_30_1	31344500	30280411	97	8549636	28	y
HH_T1_30_2	31782681	30724004	97	8820920	29	y
HH_T1_30_3	31607218	30533262	97	7558163	25	y
HH_T1_30_4	32836621	31761101	97	8725054	27	y
HH_T1_33_1	32141993	31022137	97	9797996	32	y
HH_T1_33_2	33817095	32661920	97	11486096	35	y
HH_T1_33_3	32908694	31822584	97	10350363	33	y
HH_T1_33_4	33312555	32144454	96	10345889	32	y
HH_T1_36_1	31863811	30752335	97	8388277	27	y
HH_T1_36_2	31135202	30329344	97	8160821	27	y
HH_T1_36_3	33050565	31954907	97	8687879	27	y
HH_T1_36_4	30672997	29621039	97	9444586	32	y
HH_T1_39_1	31566009	30418097	96	7099524	23	y
HH_T1_39_2	31871308	30707770	96	7557541	25	y
HH_T1_39_3	31108401	29931191	96	5378213	18	y
HH_T1_39_4	30272993	29133359	96	7770008	27	y
Av.	32125504	31048504	97	8517256	27	32/32

Table S3. Number of unique contigs per taxa group in de novo transcriptome assemblies. Coral holobiont taxa contributions of total contigs in percentage (%) based on BLAST query results of transcriptome assemblies when using all samples or only non-bleached samples (those at temperature 30°C and 33°C for both CBASS runs of heat only and heat with deoxygenation). Coral holobiont taxa groups of cnidaria, Dinophyceae, bacteria, fungi, virus and then other taxa were also considered. Number of contigs were also subset based on contig length. For this study the de novo transcriptome assembly used included non-bleached samples ≥ 500 bp from all taxa groups.

	Taxa group	≥0bp	% Taxa	≥500bp	% Taxa	% Removed
ALL samples	Cnidaria	101367	36	10861	69	89
	Dinophyceae	1090	0.38	170	1.08	84
	Bacteria	46802	16	1120	7	98
	Fungi	7010	2	263	2	96
	Virus	160	0.06	4	0.03	98
	sum	156429	55	12418	79	
	other taxa	128774	45	3264	21	
	Total contigs	285203	100	15682	100	95

	Taxa group	≥0bp	% Taxa	≥500bp	% Taxa	% Removed
non-bleached samples	Cnidaria	86210	74	17975	89	79
	Dinophyceae	974	0.83	441	2.19	55
	Bacteria	6362	5	284	1	96
	Fungi	2639	2	230	1	91
	Virus	47	0.04	6	0.03	87
	sum	96232	82	18921	94	
	other taxa	20837	18	1194	6	
	Total contigs	117069	100	20115	100	83

Table S4. De novo transcriptome assembly statistics when using all samples or only non-bleached samples. Statistics considering scaffolds and contigs based on SOAPdenovo-Trans statistic report and QUAST results. Non-B = non-bleached samples. Scaff. = scaffolds.

	Total scaff. ≥ 0bp	Total scaff. ≥500bp	% Scaff. ≥500bp	GC %	Median scaff. length	Av. scaff. length	Total bases	N50 ≥ 100bp	N50 ≥ 500bp
ALL	2096835	230724	11	48	209	321	674 M	368	1442
Non-B	844774	139488	17	45	183	413	349 M	854	1946

	Total contigs ≥0bp	Total contigs ≥500bp	% contigs ≥500bp	GC %	Median contig length	Av. contig length	Total bases	N50 ≥ 100bp	N50 ≥ 500bp
ALL	2508117	182540	7	45	252	158	634 M	390	1946
Non-B	1018615	121034	12	45	302	171	308 M	390	1946

Table S5. Number of differentially expressed transcripts between different temperatures. Differential expression based on Benjamini-Hochberg FDR correction (p adj. <0. 05). Comparisons show Hd = deoxygenated treatment and H0 = control samples for each temperature (33, 36, 39°C) versus control of 30°C (baseline temperature). n = number of samples in total for treatment (n=4) and control (n=4). Number of annotated differentially expressed transcripts based on 6556 annotations from EggNOG Orthology.

Comparison	**Total n**	**Total Upreg.**	**Total Downreg.**	**Annotated Upreg.**	**Annotated Downreg.**
33H0 vs 30H0	8	4408	4396	1655	2199
33Hd vs 30Hd	8	3841	3831	1333	1910
36H0 vs 30H0	8	5638	6715	2047	2980
36Hd vs 30Hd	8	5341	6322	2777	1956
39H0 vs 30H0	8	4995	5807	1768	2676
39Hd vs 30Hd	8	4562	5726	1551	2632

Table S6. Number of differentially expressed transcripts in pairwise comparisons between normoxia and deoxygenation for each temperature. Number of differentially expressed transcripts following Benjamini-Hochberg FDR correction (p adj. <0. 05). Comparisons show Hd = deoxygenated treatment and H0 = control samples for each temperature (temp.; °C). n = number of samples in total for treatment (n=4) and control (n=4). Number of annotated differentially expressed transcripts based on 6556 annotations from EggNOG Orthology.

Comparison	Temp. (°C)	Total n	Total Upreg.	Total Downreg.	Annotated Upreg.	Annotated Downreg.
Hd vs H0	30	8	382	491	152	209
Hd vs H0	33	8	1134	1137	399	594
Hd vs H0	36	8	187	356	81	154
Hd vs H0	39	8	130	73	52	31