Supplementary Information

Title: Loss of coral thermotolerance following year-long in situ nursery propagation with a consecutively high summer heat-load

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Supplementary Figures

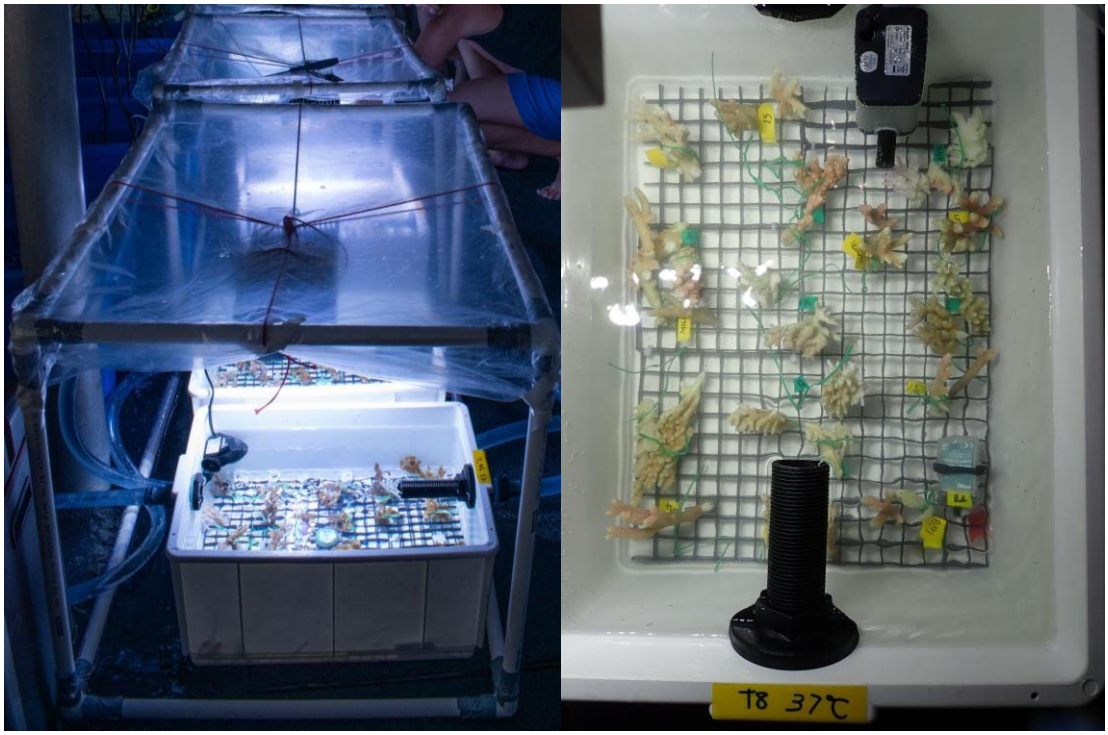

Fig. S1 Images of the experimental setup. Portable experimental temperature tanks with flow-through system set up on the boat deck (left-side). An example of coral fragments (mostly bleached) in the 37°C tank (right-side) along with the water circulation powerhead (top right corner) and hobo temperature logger (bottom right corner). Note, there are also *Acropora millepora* fragments in this photo that were not part of this manuscript.

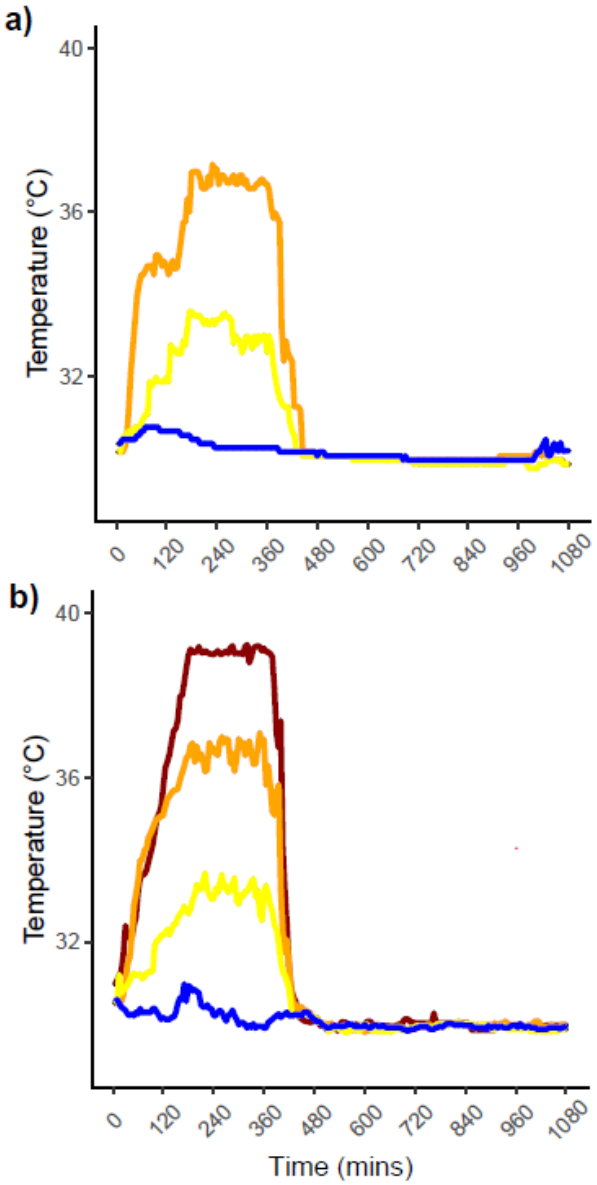

Fig. S2 Thermal profiles of CBASS runs. HOBO logger data for each experimental tank of CBASS from noon until 6 am the next day for a) donor colonies and b) nursery-propagated corals runs. Blue, yellow, orange, and red lines represent temperature data from tanks reaching 30°C, 33°C, 37°C, and 39°C, respectively. Note that temperature 39°C is missing for donor corals CBASS run due to overload of power supply on the boat. For ED50-based thermal threshold modelling, F_v/F_m values from the 39°C nursery-propagated corals were used for the donor corals.

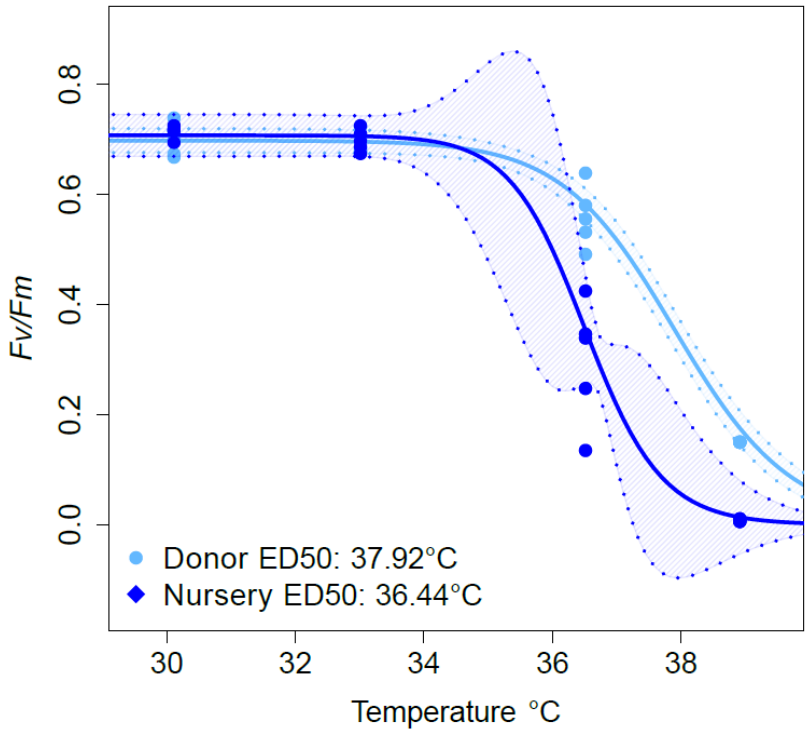

Fig. S3 ED50 based on photosynthetic efficiency (F_v/F_m) modelled using higher F_v/F_m values for the highest temperature treatment (39°C) of the donor colonies. The temperature at which the F_v/F_m is reduced by 50% is the effective dose 50, ED50, a standardised coral thermotolerance diagnostic (sensu Evensen et al., 2021). Highest temperature F_v/F_m for donor colonies was set to 0.15 rather than < 0.02 (nursery-propagated) across colonies to assess how conservative the modelling is depending on the highest temperature values used. Sample replicates n = 5 per colony source (donor versus nursery). The curve reflects the mean three parameter log-logistic model. The ED50 is higher than when using values closer to zero (i.e. values from corresponding nursery-propagated corals), highlighting this approach to be a less conservative option.

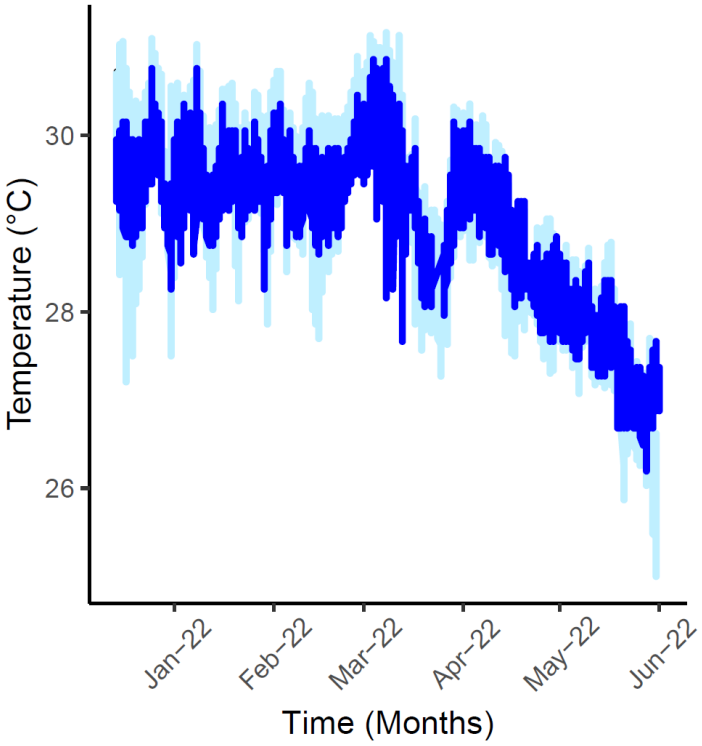

Fig. S4 Ambient seawater temperature time series. HOBO logger data for the donor (light blue) and nursery (dark blue) site across 7 months (December 2021 - June 2022). Note, that the nursery HOBO logger was 80 m away from the nursery frame and the donor data is the average of 3 HOBO loggers within the reef collection site.

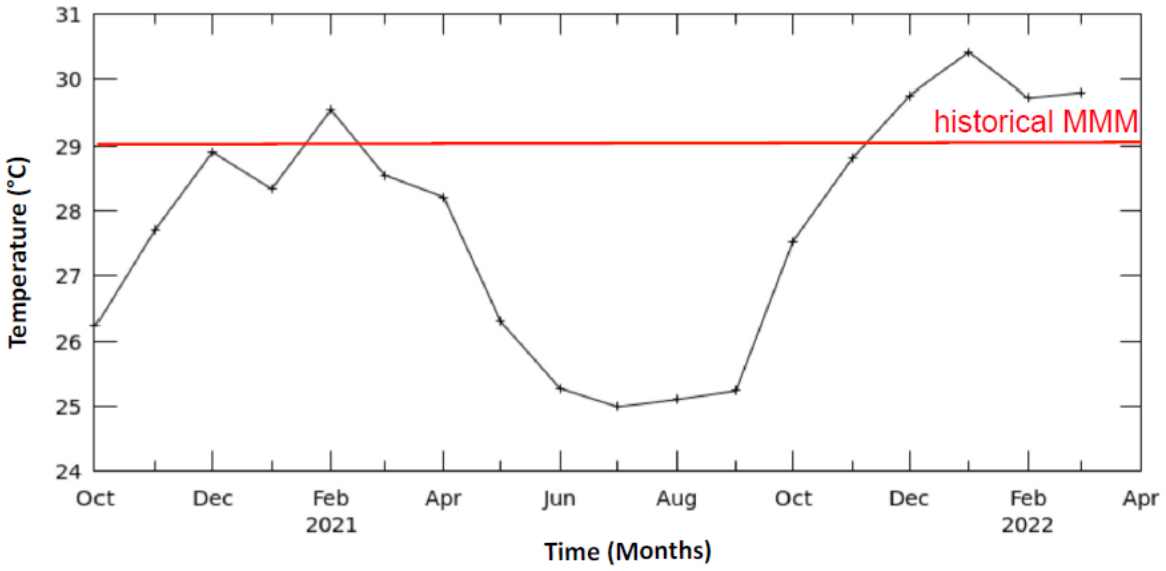

Fig. S5 Time series of area-averaged sea surface temperature (daytime measurements per month) using Giovanni v4.38 on NASA observational satellite data. Region covers a rectangular boundary of 145.918E, 16.3614S, 145.9647E, 16.3229S between October 2020 to April 2022 which captures data for the study site of Opal reef. Red line represents the historical average maximum monthly mean temperature determined from the NOAA Coral Reef Watch 5 km database which covers 1985-2012 (Liu et al., 2014).

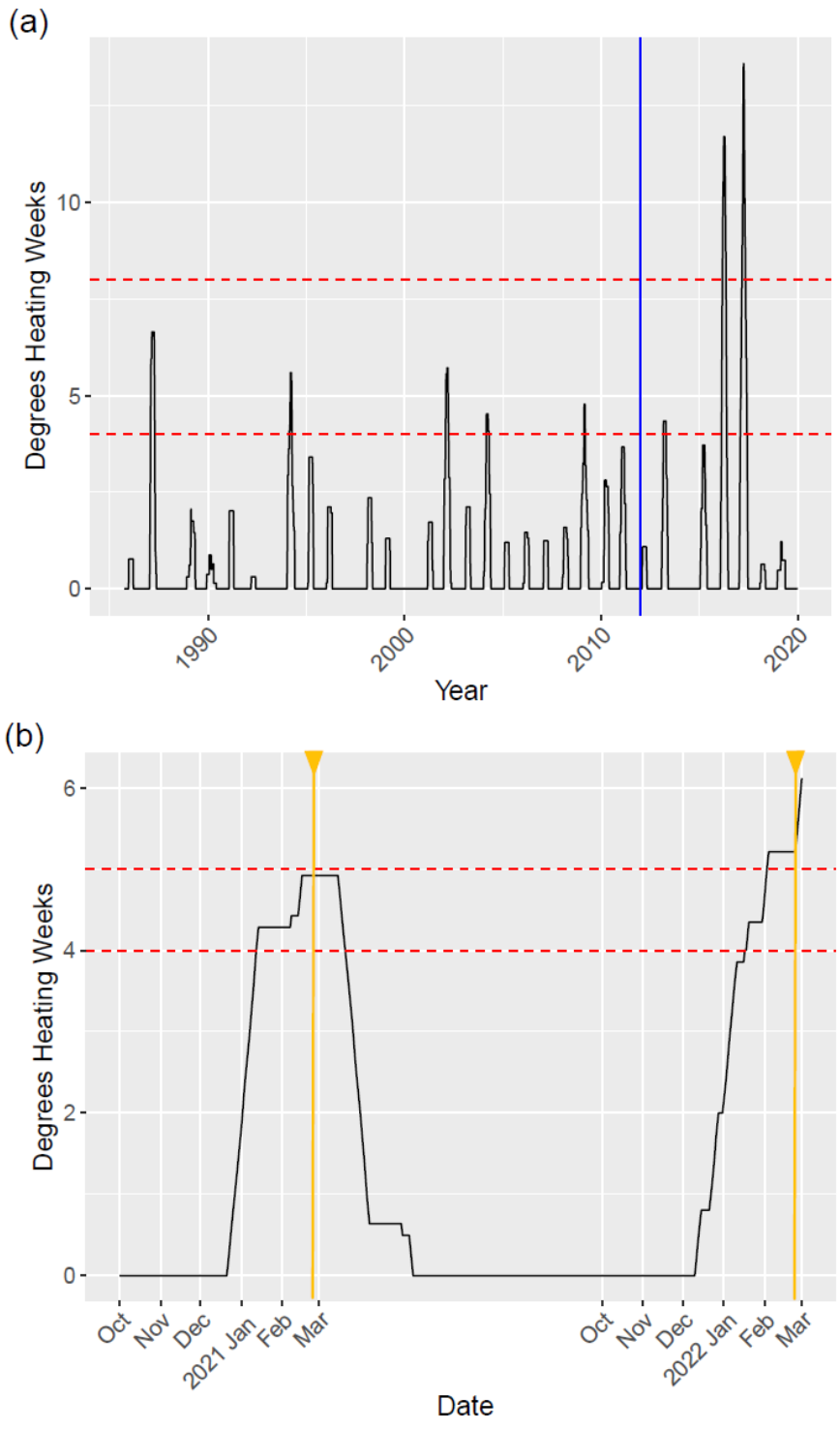

Fig. S6 Degrees Heating Weeks (DHW) (a) prior to conducting the experiment highlighting how DHW is much higher after 2012 (indicated by blue vertical line) which is not considered in the determining the historical average maximum monthly mean temperature (b) before CBASS testing for donor (25th February 2021) and nursery-propagated (25th February 2022) corals. DHW is determined from the NOAA Coral Reef Watch daily global 5km satellite database as a product of accumulating Coral Bleaching HotSpots greater than 1°C over a 12-week window (Liu et al., 2014). Red horizontal dashed lines represent 4 and 8 DHW in (a) and 4 and 5 DHW in (b). Yellow vertical lines represent the CBASS sampling on 25th February 2021 and 2022, for donor and nursery-propagated corals, respectively.

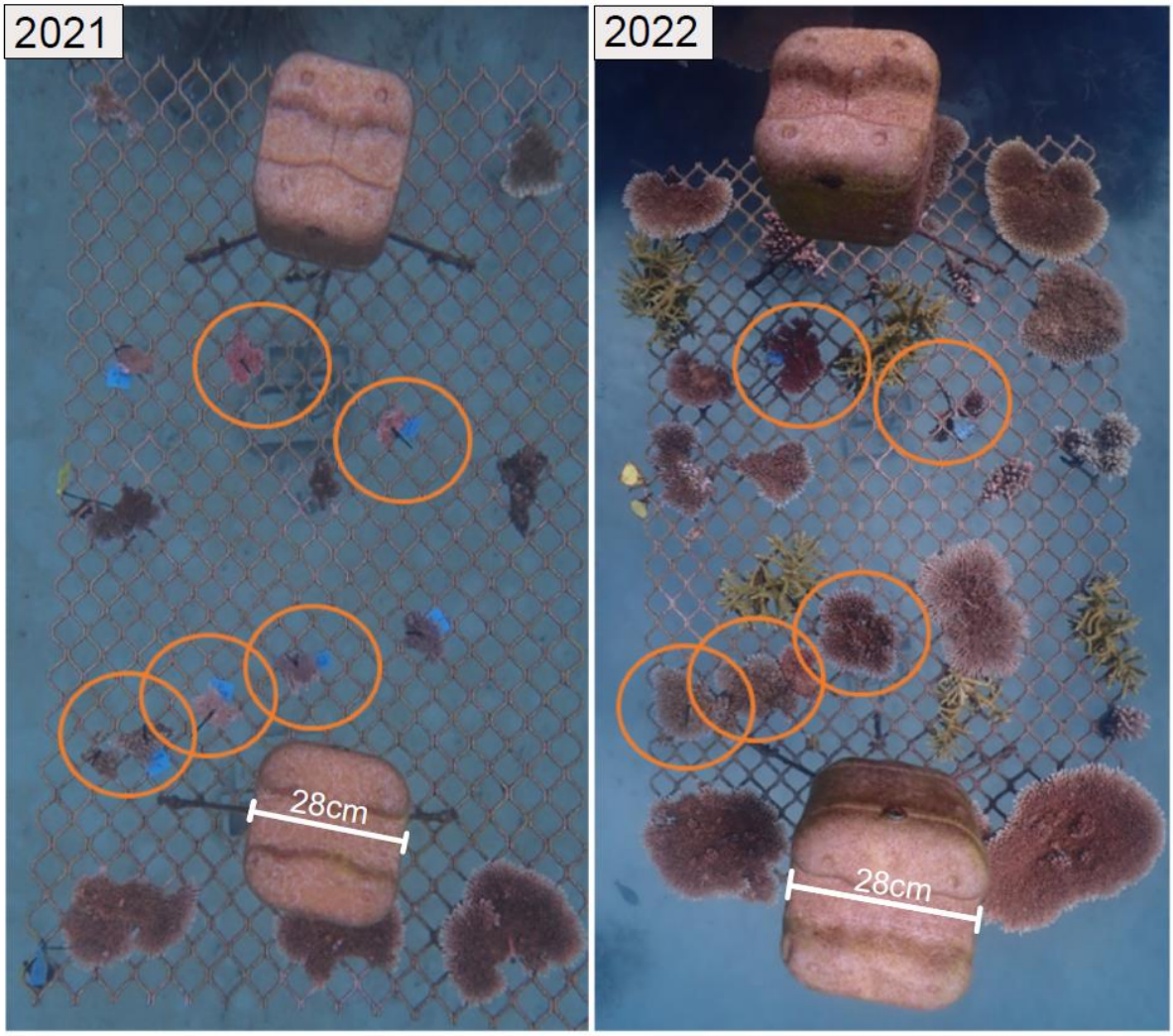

Fig. S7 No visual paling (i.e. bleaching) of nursery-propagated coral after one year. Photographs of nursery frames at the beginning and after one year of propagation. Note multiple coral species are present on this nursery frame which were not included in this experiment. Corals circled in orange were included. Plastic buoys are used as a size reference.

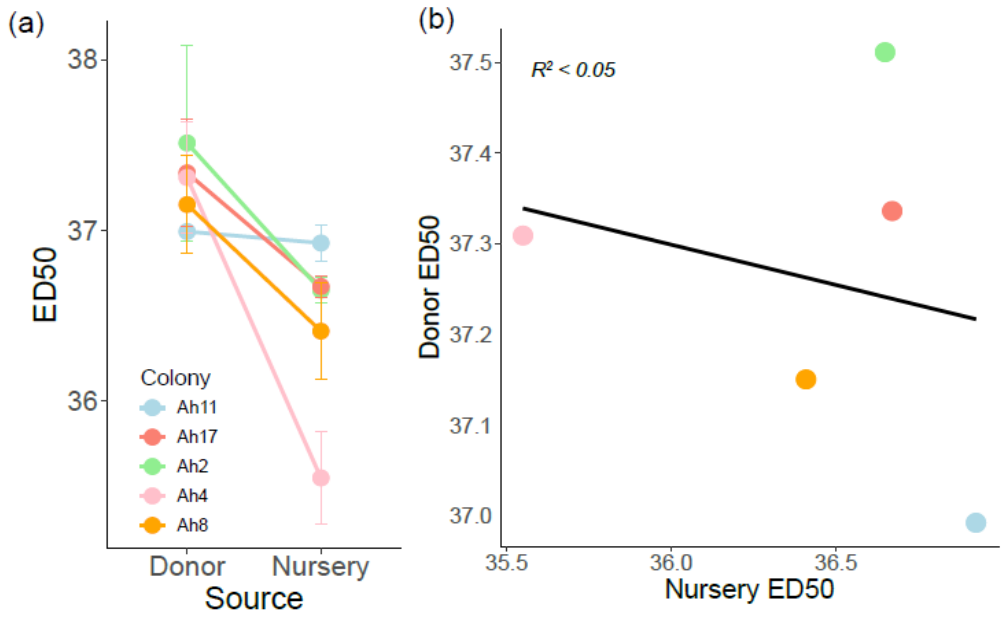

Fig. S8 Ranking of ED50's across donor and nursery corals. Mean colony-level ED50s with standard error bars (left side) and linear regression of colony-level ED50s (right side) for nursery-propagated and corresponding donor colonies with a weak R^2 value of <0.05.

Supplementary Tables

Table S1 Total read count of paired-end libraries at different stages of the analysis pipeline. These stages included trimming reads of Illumina adaptors and low-quality regions using Trimmomatic, STAR alignment to the reference genomic gene set of *Acropora hyacinthus* (*n* = 23,148), and filtering out samples < 5 million mapped read counts. Note, number of reads mapped to genes is relative to the total of read 1 and 2 rather than the initial STAR summary output which treats paired-end reads as ends of 1 read pair i.e. half the total count (Dobin et al., 2013). Sample ID represents the following: Ah = *Acropora hyacinthus*; 2, 4, 8, 11, 17 = colony number; 30, 33 & 37 = temperatures of heat assay; and D/N = donor or nursery-propagated colonies.

		Trimmomatic		STAR alignment		Quality Check
Sample ID	**Initial**	**Paired Post-trim**	**%**	**Mapped to Genes**	**%**	**≥ 5 million Reads**
Ah11_30_D	62,228,642	58,004,268	93	25,924,182	45	y
Ah11_33_D	53,692,430	50,356,726	94	7,674,184	15	y
Ah11_37_D	40,063,042	37,378,392	93	23,579,360	63	y
Ah17_30_D	52,772,150	47,043,964	89	19,959,644	42	y
Ah17_33_D	42,826,958	39,781,610	93	19,447,360	49	y
Ah17_37_D	44,816,322	41,988,436	94	459,796	1	n
Ah2_30_D	58,301,694	54,894,750	94	11,150,954	20	y
Ah2_33_D	47,304,252	43,884,244	93	17,703,056	40	y
Ah2_37_D	61,040,034	57,097,028	94	6,549,546	11	y
Ah4_30_D	49,635,792	46,352,022	93	27,907,060	60	y
Ah4_33_D	45,486,890	42,595,186	94	10,705,880	25	y
Ah4_37_D	45,786,320	43,023,658	94	13,548,518	31	y
Ah8_30_D	45,177,518	42,142,378	93	12,649,906	30	y
Ah8_33_D	61,106,872	57,256,130	94	3,850,526	7	n
Ah8_37_D	47,050,238	44,016,416	94	10,598,468	24	y
Ah11_30_N	31,193,254	27,836,440	89	10,746,392	39	y
Ah11_33_N	49,432,054	44,395,212	90	25,478,404	57	y
Ah11_37_N	37,082,896	33,375,780	90	443,828	1	n
Ah17_30_N	46,096,488	41,318,934	90	20,836,036	50	y
Ah17_33_N	45,336,210	40,310,988	89	21,207,234	53	y
Ah17_37_N	44,858,464	39,648,962	88	257,064	1	n
Ah2_30_N	45,499,876	41,208,216	91	20,458,144	50	y
Ah2_33_N	44,898,124	40,284,856	90	20,047,188	50	y
Ah2_37_N	48,027,440	43,104,872	90	1,624,254	4	n
Ah4_30_N	42,443,598	37,655,884	89	19,494,480	52	y
Ah4_33_N	47,851,568	42,833,666	90	27,584,504	64	y
Ah4_37_N	69,238,136	59,536,238	86	1,205,876	2	n

Ah8_30_N	38,774,726	34,924,962	90	14,684,864	42	y
Ah8_33_N	34,940,004	30,933,476	89	18,090,130	58	y
Ah8_37_N	29,665,532	26,594,546	90	192,776	1	n
Av. of used samples	47,249,955		91		42	19/30

Table S2 Number of differentially expressed genes. Differential expression based on Benjamini-Hochberg FDR correction (p adj. < 0.05) with a Log_2 fold change > 0. Comparisons show *Acropora hyacinthus* samples at 30°C or 33°C from donor (D) and nursery-propagated (N; one year in nursery) colonies. *n* = number of biological replicates in total for baseline D (*n* = 5) and N (*n* = 5). Note, a sample was removed from analysis due to an insufficient number of sequenced reads; donor colony #8 at 33°C (See Table S1). Number of annotated differentially expressed genes based on 9,883 annotations from EggNOG (Evolutionary Genealogy of Genes: Non-supervised Orthologous Groups) Orthology (Huerta-Cepas et al. 2019) of *Acropora hyacinthus* reference protein coding sequences.

Comparison	Total *n*	Total Upreg.	Total Downreg.	Annotated Upreg.	Annotated Downreg.
30°C D vs 30°C N	10	1997	1567	1719	1050
33°C D vs 33°C N	9	1357	1352	1115	941
30°C D vs 33°C D	9	27	15	14	13
30°C N vs 33°C N	10	438	480	302	399

Table S3 Statistical results for mean Effective Dose (ED) 50 values between donor and nursery colonies. Testing for normal distribution using Shapiro-wilk, homogeneity of variance using Bartlett, and difference in mean ED50s using Welch t-test. Tests performed also for the 'dummy' run using 0.15 as the F_v/F_m value for corals at the highest temperature of heat assay (39°C).

Comparison	Total *n*	Shapiro-wilk (D, N)	Bartlett	Welch t-test
Donor vs Nursery	20	0.93, 0.19	K^2=3.0, df= 1, p = 0.08	t= 3.2, df= 5.08, p = 0.02
Donor vs Nursery dummy	20	0.79, 0.19	K^2=1.7, df= 1, p =0.19	t=5.60, df =5.81, p= 0.00

Table S4 List of overlapping differentially expressed genes from venn diagram comparing donor and nursery corals baseline temperature (30 D vs 30 N) against heat stress (34 D vs 34 N). Gene annotations based on EggNOG (Huerta-Cepas et al., 2019) functional inference of the *Acropora hyacinthus* reference genome (Shinzato et al., 2021). Log2 fold change (FC) values extracted from DESeq2 output files. Note that log_2 fold change value will be positive or negative depending on the order of DESeq2 contrast comparison e.g. HDAC2 gene will be -0.85 or 0.85 for '30°C D vs 30°C N' or '30°C N vs 30°C D', respectively. Values for '30°C N vs 30°C D' and '34°C N vs 34°C D', respectively, have been provided here, meaning that these genes were in most cases upregulated in the nursery compared to donor corals.

Gene ID	Description	Gene name	Log_2 FC
ahya_s0001.g98	Belongs to the histone deacetylase family. HD Type 1 subfamily	HDAC2	0.85, 1.34
ahya_s0003.g126	DNA helicase that acts as a chromatin remodelling factor	CHD7	1.51, 1.57
ahya_s0038.g122	positive regulation of histone deubiquitination	Rnp4F	0.59, 0.59
ahya_s0074.g43	histone monoubiquitination	RNF20	0.92, 1.02
ahya_s0204.g8	Involved in remodelling chromatin and mediating histone acetylation and deubiquitination.	ENY2	-0.97, -1.15
ahya_s0397.g7	histone H3-K23 acetylation	BRPF1	1.04, 0.95
ahya_s0067.g29	telomerase activity	DUF4062	0.86, 0.67
ahya_s0006.g178	chromatin remodelling	SMARCE1	-0.62, -0.94
ahya_s0026.g58	chromatin remodelling	athp-2	2.52, 2.46
ahya_s0040.g53	chromatin remodelling	athp-2	1.09, 1.23

Table S5 Differentially expressed genes associated with the gene enrichment (GO) terms of interest reported in results. Gene annotations based on EggNOG (Huerta-Cepas et al., 2019) functional inference of the *Acropora hyacinthus* reference genome (Shinzato et al., 2021). Note that log_2 fold change value will be positive or negative depending on the order of DESeq2 contrast comparison e.g. DCK1 gene will be -1.13 or 1.13 for '30°C N vs 33°C N' or '33°C N vs 30°C N', respectively.

Gene ID	Description	Gene name	Log_2 FC
30°C D vs 30°C N (GO: response to starvation)			
ahya_s0012.g62	Glucokinase (GO: glucose sensing/starvation)	GCK	1.09
ahya_s0072.g112	Phosphoenolpyruvate carboxykinase (GO:	PCK1	1.13
ahya_s0140.g15	Gamma-aminobutyric acid A receptors	GABARAP	1.36
34°C D vs 34°C N (GO: response to reactive oxygen species/ hydrogen peroxide)			
ahya_s0020.g108	Superoxide dismutase	SOD1	1.30
ahya_s0034.g78	Glutathione peroxidase	GSHPX	1.66
ahya_s0078.g13	Peroxiredoxin	PRDX5	1.93
ahya_s0135.g51	Bcl-2 adenovirus E1B	BNIP3	1.96
33°C N vs 30°C N (GO: telomere maintenance/protein folding chaperoning)			
ahya_s0001.g873	box H/ACA snoRNA 3'-end processing	DCK1	1.13
ahya_s0026.g137	belonging to the small heat shock protein 20 family	CRYAB	1.71

Supplementary Data

Data S1 Results from topGO Gene Ontology enrichment of differentially expressed genes (see Table S2 for comparison groups).

Data S2 SymPortal ITS2 type profile results for donor corals of *Acropora hyacinthus*.

References

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