

Supplementary Information for
Heat stress destabilizes symbiotic nutrient cycling in corals.

Nils Rådecker, Claudia Pogoreutz, Hagen M. Gegner, Anny Cardenas, Florian Roth, Jeremy Bougoure, Paul Guagliardo, Christian Wild, Mathieu Pernice, Jean-Baptiste Raina, Anders Meibom, Christian R. Voolstra

Nils Rådecker
Email: nils.radecker@kaust.edu.sa

This PDF file includes:

Figures S1 to S4
Tables S1 to S3
Legends for Datasets S1

Other supplementary materials for this manuscript include the following:

Datasets S1

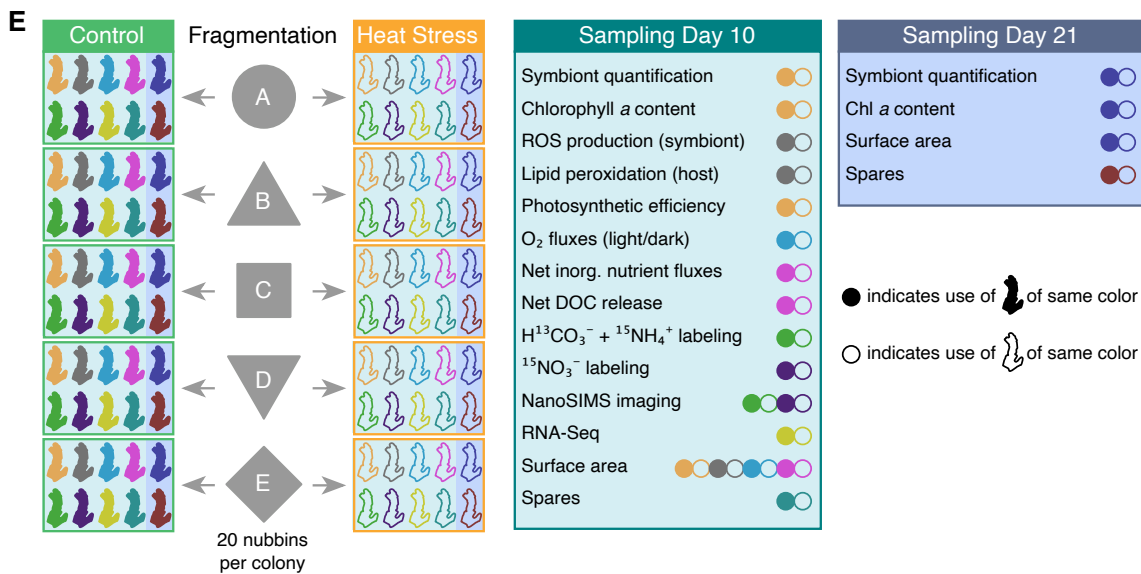
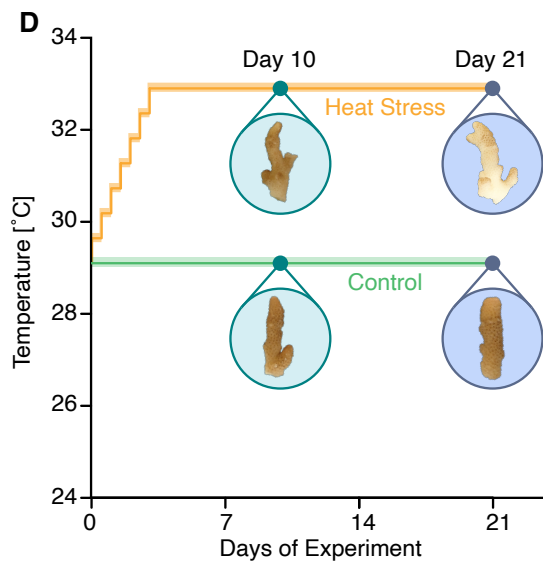
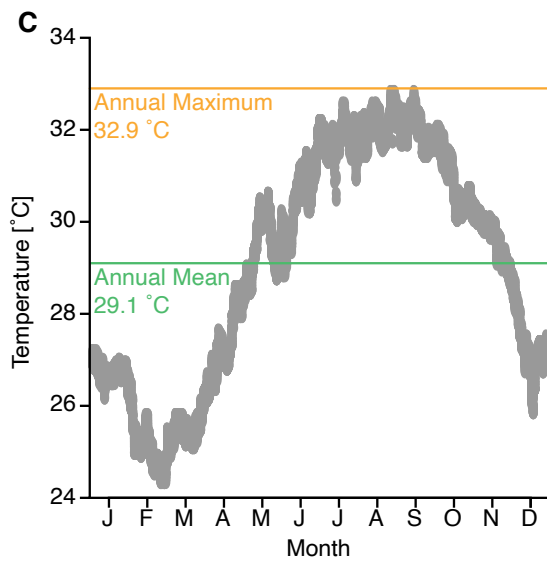


Fig. S1. Overview of the sampling site and its environmental conditions. **A,B** Coral colonies were collected at Abu Shosha reef (22°18'16.3"N; 39°02'57.7"E) in the central Red Sea, approximately 5 km off the Saudi Arabian coast. **C** The temperature profile for Abu Shosha reef at 5m water depth for the year 2017 was used to identify the annual mean and maximum temperature of a representative year without mass-bleaching. **D** Based on this information, corals were exposed to either annual mean temperatures (29.1 °C, control) or annual maximum temperatures (32.9 °C, heat stress) for a 21-day aquaria experiment. **E** Assignment of coral nubbins for experimental procedures from 5 colonies across aquaria and treatments. Corals nubbins were sampled on days 10 and 21 of the experiment. The respective color of the coral nubbins indicate their use in analysis of matching dot color.

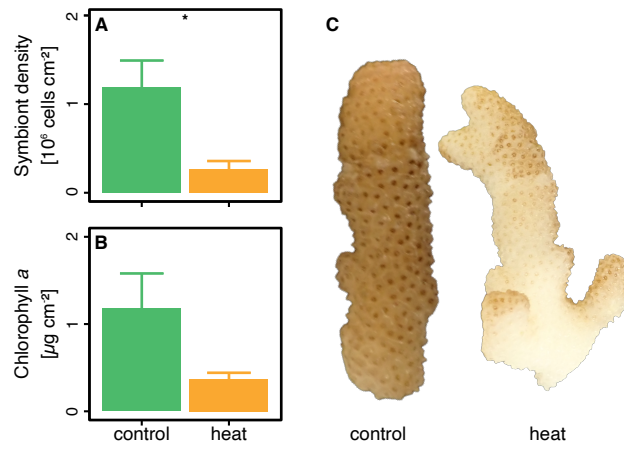


Fig. S2. State of symbiosis on day 21 of the experiment. **A-C** Corals showed clear visual signs of bleaching and a significant decline in symbiont densities as well as a reduction in chlorophyll a content. Bars indicate mean \pm SE. Asterisks indicated significant differences between treatments.

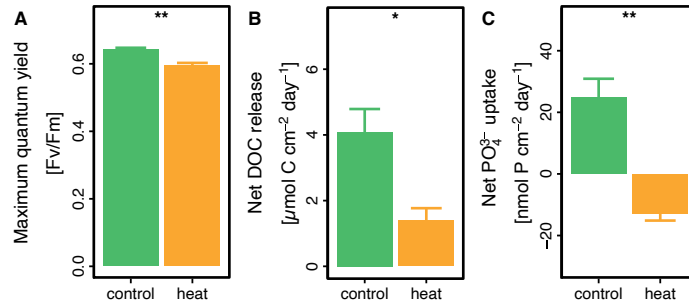
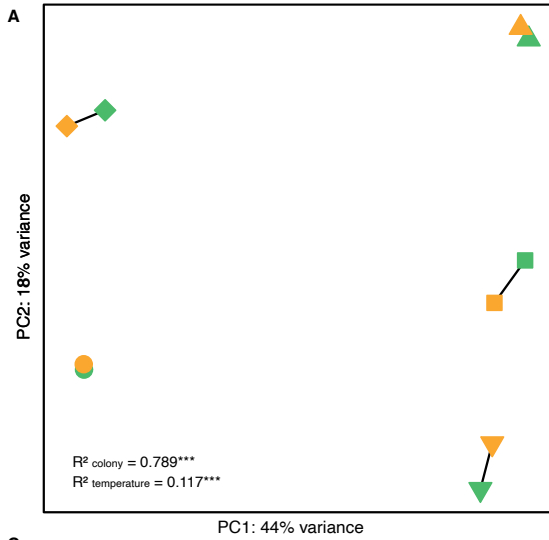


Fig. S3. Photophysiology and nutrient fluxes on day 10 of the experiment. **A** Maximum photosynthetic quantum yield of fragments after 30 min dark acclimation. **B** Net dissolved organic carbon (DOC) release from the coral holobiont. **C** Net phosphate (PO_4^{3-}) uptake from the coral holobiont. Negative bars indicate a net release of PO_4^{3-} . Bars indicate mean \pm SE. Asterisks indicated significant differences between treatments.

Stylophora pistillata



Symbiodinium microadriaticum

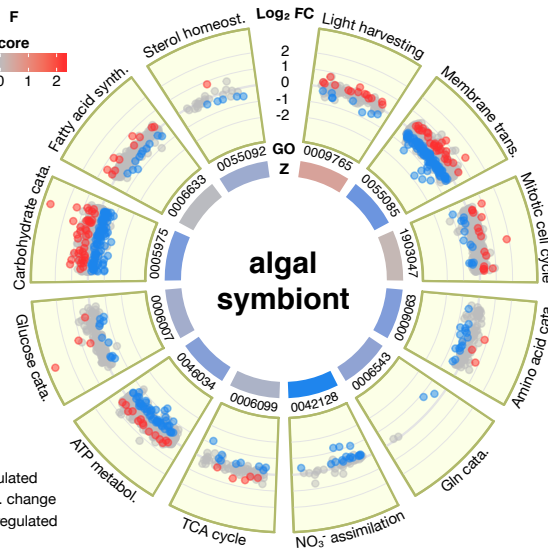
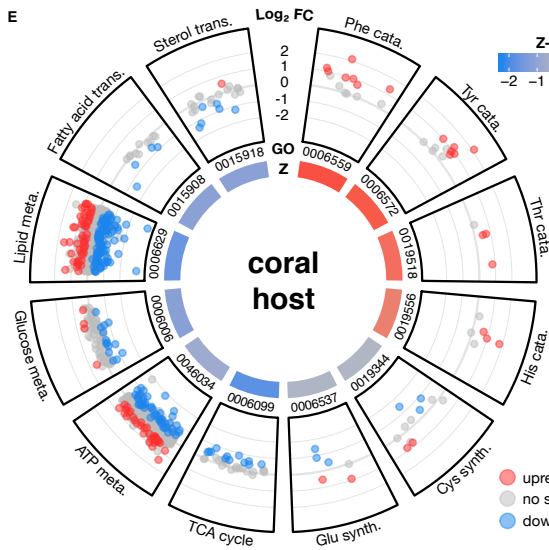
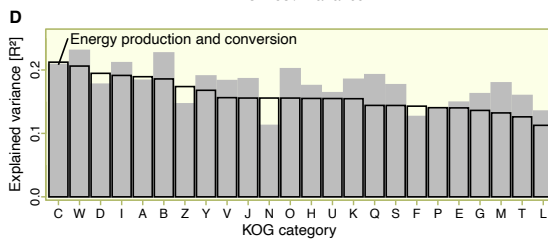
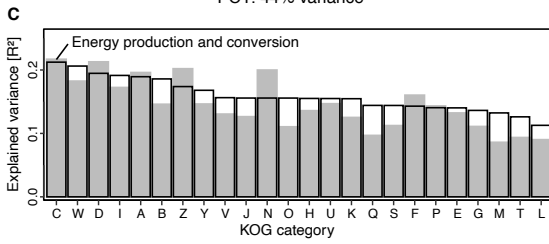
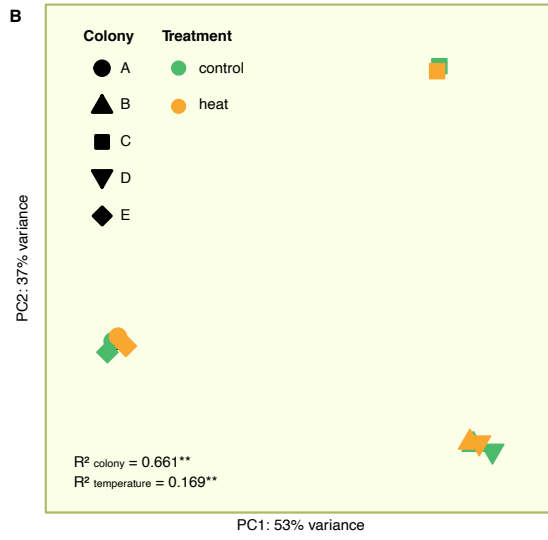


Fig. S4. Gene expression of **A,C,E** *Stylophora pistillata* and **B,D,F** *Symbiodinium microadriaticum* on day 10 of heat stress. **A,B** Principal component analysis of gene expression profiles. Lines connect control (green) and heat stress (orange) samples from the same colony of origin. **C,D** Permutational analysis of variance (Permanova) analyses of the proportion of variance in gene expression explained by the temperature treatment for each subset of genes related to individual EuKaryotic Orthologous Groups (KOGs). Grey bars indicate results for the respective symbiotic partner whereas empty black bars indicate mean responses of both symbiotic partners combined. **E,F** Selected significantly enriched Gene Ontology (GO) terms related to carbon and amino acid metabolism as well as inorganic nutrient transport. Points indicate mean \log_2 fold change of expression of genes associated to GO terms in heat stress compared to control conditions. Blue points indicate significant downregulation, whereas red points indicate significant upregulation. Boxes below individual GO terms indicate the overall direction of regulation (Z-score). ATP = adenosine triphosphate, cata. = catabolism, Cys = cysteine, Gln = glutamine, Glu = glutamate, His = histidine, homeost. = homeostasis, meta. = metabolism, Ser = serine, Phe = phenylalanine, synth. = biosynthesis, TCA = tricarboxylic acid, trans. = transport, Tyr = tyrosine. See Tab. S2, S3 for an overview of Permanova results. For a complete list of differentially expressed genes as well as significant GO terms see Dataset S1.

Table S1. Results of statistical analysis for physiological and NanoSIMS measurements. Physiological measurements were analyzed in a paired design based on the mother colony. Likewise, log-transformed NanoSIMS data were analyzed in linear mixed models using colony of origin as a random effect.

Day	Response parameter	Test	Replicates	Statistic	<i>p</i>
21	Symbiont density	Paired <i>t</i> -test	10	3.728	0.020
21	Chlorophyll <i>a</i> content	Paired Wilcoxon	10	15.000	0.063
10	Symbiont density	Paired <i>t</i> -test	10	0.347	0.746
10	Chlorophyll <i>a</i> content	Paired <i>t</i> -test	10	0.454	0.674
10	Photosynthetic efficiency	Paired <i>t</i> -test	10	6.778	0.002
10	Gross photosynthesis	Paired <i>t</i> -test	10	-3.469	0.026
10	Respiration	Paired <i>t</i> -test	10	-3.762	0.020
10	Dissolved organic carbon release	Paired <i>t</i> -test	10	3.841	0.018
10	NH ₄ ⁺ uptake	Paired <i>t</i> -test	10	-15.991	0.001
10	NO ₃ ⁻ uptake	Paired <i>t</i> -test	10	-3.041	0.038
10	PO ₄ ³⁻ uptake	Paired <i>t</i> -test	10	-6.328	0.003
10	ROS release (symbiont)	Paired <i>t</i> -test	10	-4.407	0.012
10	Lipid peroxidation (host)	Paired <i>t</i> -test	10	-5.735	0.005
10	Mitotic index	Paired <i>t</i> -test	10	-2.896	0.044
10	H ¹³ CO ₃ ⁻ assimilation (host)	Linear mixed model	250	8.000	0.005
10	H ¹³ CO ₃ ⁻ assimilation (symbiont)	Linear mixed model	250	52.88	0.001
10	¹⁵ NH ₄ ⁺ assimilation (host)	Linear mixed model	250	6.330	0.013
10	¹⁵ NH ₄ ⁺ assimilation (symbiont)	Linear mixed model	250	5.528	0.020
10	¹⁵ NO ₃ ⁻ assimilation (host)	Linear mixed model	140	40.715	0.001
10	¹⁵ NO ₃ ⁻ assimilation (host)	Linear mixed model	140	72.343	0.001

Table S2. Permutational multivariate analysis of variance (PERMANOVA) on the effects of mother colony and temperature on the transcriptomic response of *Stylophora pistillata*. Responses were assessed for the global transcriptome as well as for specific subsets of genes assigned to individual EuKaryotic Orthologous Groups (KOGs) categories.

Response category	Genes	Colony effect			Temperature effect		
		R ²	F	p	R ²	F	p
Global transcriptome	25769	0.789	8.362	0.001	0.117	4.938	0.001
KOG categories							
D - Cell cycle control, cell division, chromosome partitioning	406	0.692	7.218	0.001	0.212	8.867	0.001
M - Cell wall/membrane/envelope biogenesis	343	0.838	10.959	0.001	0.086	4.474	0.001
N - Cell motility	30	0.656	4.539	0.002	0.200	5.521	0.001
O - Post-translational modification, protein turnover, and chaperones	2125	0.788	7.690	0.001	0.110	4.296	0.001
T - Signal transduction mechanisms	3288	0.810	8.349	0.001	0.093	3.840	0.001
U - Intracellular trafficking, secretion, and vesicular transport	852	0.715	5.167	0.001	0.146	4.231	0.001
V - Defense mechanisms	128	0.736	5.473	0.001	0.130	3.871	0.002
W - Extracellular structures	120	0.666	4.393	0.001	0.182	4.801	0.001
Y - Nuclear structure	40	0.760	8.091	0.001	0.146	6.214	0.001
Z - Cytoskeleton	416	0.655	4.559	0.001	0.202	5.615	0.001
A - RNA processing and modification	500	0.712	7.694	0.001	0.196	8.455	0.001
B - Chromatin structure and dynamics	309	0.812	19.354	0.001	0.146	13.869	0.001
J - Translation, ribosomal structure and biogenesis	566	0.735	5.296	0.001	0.126	3.629	0.003
K - Transcription	1135	0.782	8.342	0.001	0.125	5.326	0.001
L - Replication, recombination and repair	1152	0.858	16.484	0.001	0.090	6.938	0.001
C - Energy production and conversion	365	0.636	4.306	0.001	0.217	5.865	0.001
E - Amino acid transport and metabolism	1072	0.767	7.561	0.001	0.132	5.200	0.001
F - Nucleotide transport and metabolism	184	0.744	7.753	0.001	0.160	6.662	0.001
G - Carbohydrate transport and metabolism	898	0.777	6.885	0.001	0.111	3.918	0.001
H - Coenzyme transport and metabolism	146	0.691	3.971	0.001	0.135	3.115	0.007
I - Lipid transport and metabolism	432	0.719	6.573	0.001	0.172	6.293	0.001
P - Inorganic ion transport and metabolism	595	0.720	5.269	0.001	0.143	4.179	0.001
Q - Secondary metabolites biosynthesis, transport, and catabolism	395	0.792	7.118	0.002	0.096	3.465	0.024
R - General function prediction only	0	NA	NA	NA	NA	NA	NA
S - Function unknown	4820	0.806	9.839	0.001	0.112	5.465	0.001

Table S3. Permutational multivariate analysis of variance (PERMANOVA) on the effects of mother colony and temperature on the transcriptomic response of *Symbiodinium microadriaticum*. Responses were assessed for the global transcriptome as well as for specific subsets of genes assigned to individual EuKaryotic Orthologous Groups (KOGs) categories.

Response category	Genes	Colony effect			Temperature effect		
		R ²	F	p	R ²	F	p
Global transcriptome	49109	0.661	3.877	0.002	0.169	3.959	0.002
KOG categories							
D - Cell cycle control, cell division, chromosome partitioning	269	0.646	3.641	0.001	0.177	3.994	0.001
M - Cell wall/membrane/envelope biogenesis	298	0.693	5.412	0.001	0.179	5.599	0.001
N - Cell motility	30	0.770	6.516	0.002	0.112	3.795	0.038
O - Post-translational modification, protein turnover, and chaperones	1240	0.640	4.049	0.001	0.201	5.096	0.001
T - Signal transduction mechanisms	1149	0.694	4.747	0.001	0.159	4.353	0.001
U - Intracellular trafficking, secretion, and vesicular transport	609	0.690	4.729	0.001	0.164	4.483	0.002
V - Defense mechanisms	157	0.693	5.553	0.001	0.183	5.861	0.002
W - Extracellular structures	29	0.586	3.197	0.001	0.230	5.025	0.001
Y - Nuclear structure	20	0.468	1.366	0.171	0.190	2.221	0.046
Z - Cytoskeleton	417	0.713	5.071	0.001	0.146	4.156	0.005
A - RNA processing and modification	404	0.624	3.242	0.001	0.183	3.807	0.001
B - Chromatin structure and dynamics	124	0.564	2.685	0.001	0.226	4.314	0.001
J - Translation, ribosomal structure and biogenesis	685	0.627	3.353	0.001	0.186	3.971	0.001
K - Transcription	332	0.655	4.100	0.001	0.185	4.623	0.001
L - Replication, recombination and repair	1497	0.693	4.023	0.001	0.135	3.141	0.006
C - Energy production and conversion	484	0.648	4.497	0.001	0.208	5.782	0.001
E - Amino acid transport and metabolism	620	0.712	5.118	0.001	0.149	4.274	0.004
F - Nucleotide transport and metabolism	204	0.763	6.860	0.001	0.126	4.537	0.002
G - Carbohydrate transport and metabolism	998	0.702	5.160	0.001	0.162	4.766	0.001
H - Coenzyme transport and metabolism	307	0.644	3.549	0.001	0.175	3.857	0.001
I - Lipid transport and metabolism	438	0.624	3.791	0.001	0.211	5.123	0.001
P - Inorganic ion transport and metabolism	641	0.770	8.358	0.001	0.138	6.008	0.001
Q - Secondary metabolites biosynthesis, transport, and catabolism	490	0.656	4.311	0.001	0.192	5.048	0.001
R - General function prediction only	0	NA	NA	NA	NA	NA	NA
S - Function unknown	2571	0.652	3.808	0.001	0.176	4.114	0.001

Dataset S1 (separate file). Overview of results of differential gene expression (DGE) and corresponding gene ontology (GO) term enrichment analysis for *Stylophora pistilata* (Spis) and *Symbiodinium microadriaticum* (Smic) during heat stress at day 10 of experiment.