

Supplementary information

**Ion transporter gene expression is linked to the thermal sensitivity
of calcification in the reef coral *Stylophora pistillata***

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Supplementary Table 1. List of *S. pistillata* ion transport genes with RT-PCR primers and product size. Further information can be obtained from the Cnidarian Database www.data.centrescientifique.mc (Karako Lampert et al. 2014).

Target gene	Reference	Real-time PCR primers		Product size	
Ca ²⁺ channel	Zoccola <i>et al.</i> (1999)	F	TGCTACAAGATCGCCACCAACAC	82 pb	
		R	TCAGCCGCCAGCAGAATACTACTC		
NHE	Capasso <i>et al.</i> (in prep)	F	TCATTCTCCTTGGCTGTTTTACTG	138 pb	
		R	CATGAAGAATCCGCACAAGAGGTT		
PMCA1	Zoccola <i>et al.</i> (2004)	F	CCAAAACCTCCCAAAACCTTCCT	131 pb	
		R	GACCCCTCGCAATCCTCTGG		
PMCA2	Wang <i>et al.</i> (in prep)	F	GTACTGGCAAGAGCAACACC	125 pb	
		R	AAGACTGGCCATCATTAGC		
PMCA3		F	TCACTGGAGATCCGCGTTTTTACA	140 pb	
		R	ACCGGTTTGCTTTGGGAGACATT		
SpisSLC4β	Zoccola <i>et al.</i> (2015)	F	CCAATGTTGCGTTCGGTAGTCTTT	143 pb	
		R	GCCCTGTAGCGCCAATAATAATCA		
SpisSLC4γ		F	CTTGGAGGGCTGTTTCTTGAT	152 pb	
		R	TATTTTCCGCTCCCTACCCTGTTC		
SpisSLC4δ		F	TCTTGCTGGTGAATTGGTGGAGT	150 pb	
		R	TAAACGGAATGGCATGTAGTCAAG		
SpisSLC4ε		F	GGCTGCCTACCCACCAAACCA	113 pb	
		R	GGGGCAAATCCAAATCCACATAG		
SpisSLC26α		F	GCGAAATTTCTGCCATCTA	133 pb	
		R	TCGCCACGGTTATCCCTCCTGCTA		
SpisSLC26β		F	CATCGCGGCACTTGCTGTAG	159 pb	
		R	GTCCATAACGCGGTGCCTTCTGTC		
SpisSLC26γ		F	GATTGCCGCGTCATTTCACTG	131 pb	
		R	ATTCCCTGCATTTCCCCTGTAA		
SpisCA1		Moya <i>et al.</i> (2008)	F	GGAGGGACCCGACACGTGGAA	110 pb
			R	AGTCGGCAAGTCCCGGTTTCG	
SpisCA2	Bertucci <i>et al.</i> (2011)	F	TGCGGCCGAGTGACTGGAGA	91 pb	
		R	CGCCACTGAGTTTAGCGCCTCC		
SpisCA3	Del Prete <i>et al.</i> (2018)	F	TGGCTCTGGTGGCCCAAATGGT	136 pb	
		R	TGCTGGGCACGGTGACAGAGGT		
SpisCA4	Le Goff <i>et al.</i> 2016	F	GGTGCCGCTTGTTTTAGCGCA	87 pb	
		R	TCCGTTGCGCATGTCCCTGT		
V-ATPase	Capasso <i>et al.</i> (in prep)	F	GAGGAGTTTGATTTGGGGAAGTA	97 pb	
		R	AGTCGCAAATAAGAGGCTGTGTT		
L22	Karako Lampert <i>et al.</i> 2014	F	TGATGTGCCATTGATCGTC	137 pb	
		R	CATAGGTAGCTTGTGCAGATG		
36B4	Moya <i>et al.</i> , 2008	F	AACAAGGTGGCAGCCCCAGC	91 pb	
		R	GTCTTCTCGGGACCCAGGCCA		

Supplementary Table 2. Results of two-way ANOVA tests on the effect of temperature and day/night on calcification and respiration rates (Figure 1A and B) and the results of one-way ANOVA tests on the effect of temperature on photosynthetic rate (Figure 1C) and symbiont density (Figure 1D).

Variable	Effect	df	F	P	Post-hoc
Calcification rate	Temperature	9, 118	70.203	< 0.00001	See table S3
	Time	1, 118	285.72	< 0.00001	See table S3
	Temperature x Time	9, 118	7.386	< 0.00001	See table S3
Respiration rate	Temperature	9, 124	36.381	< 0.00001	See table S4
	Time	1, 124	115.537	< 0.00001	See table S4
	Temperature x Time	9, 124	2.292	< 0.05	See table S4
Photosynthetic rate	Temperature	9, 62	18.72	< 0.00001	21,23,25,27,29,31 > 21,27,19,32 > 17,33
Symbiont density	Temperature	4, 10	11.14	< 0.001	25°C ≥ 31°C ≥ 19°C > 17°C, 32°C

Supplementary Table 3. Results of post-hoc pairwise t tests on calcification rate. Letters (x and y) indicate statistical differences ($p < 0.05$) with respect to 25 °C, and between night and day.

Temperature	Time	25°C		Difference between night / day
		Night	Day	
17°C	Night	x		-
	Day		x	
19°C	Night	-		-
	Day		x	
21°C	Night	-		y
	Day		x	
23°C	Night	-		y
	Day		x	
27°C	Night	-		y
	Day		-	
29°C	Night	-		y
	Day		x	
31°C	Night	-		y
	Day		-	
32°C	Night	x		y

	Day		x	
33°C	Night	x		y
	Day		x	

Supplementary Table 4. Results of post-hoc pairwise t tests on respiration rate. Letters (x and y) indicate statistical differences ($p < 0.05$) with respect to 25 °C, and between night and day.

Temperature	Time	25°C		Difference between night / day
		Night	Day	
17°C	Night	x		-
	Day		x	
19°C	Night	x		-
	Day		x	
21°C	Night	x		y
	Day		x	
23°C	Night	x		y
	Day		-	
27°C	Night	-		y
	Day		-	
29°C	Night	-		y
	Day		-	
31°C	Night	-		y
	Day		-	
32°C	Night	x		y
	Day		x	
33°C	Night	x		y
	Day		x	

Supplementary Table 5. Eigen values from descriptive discriminant analysis of transcellular transporter genes

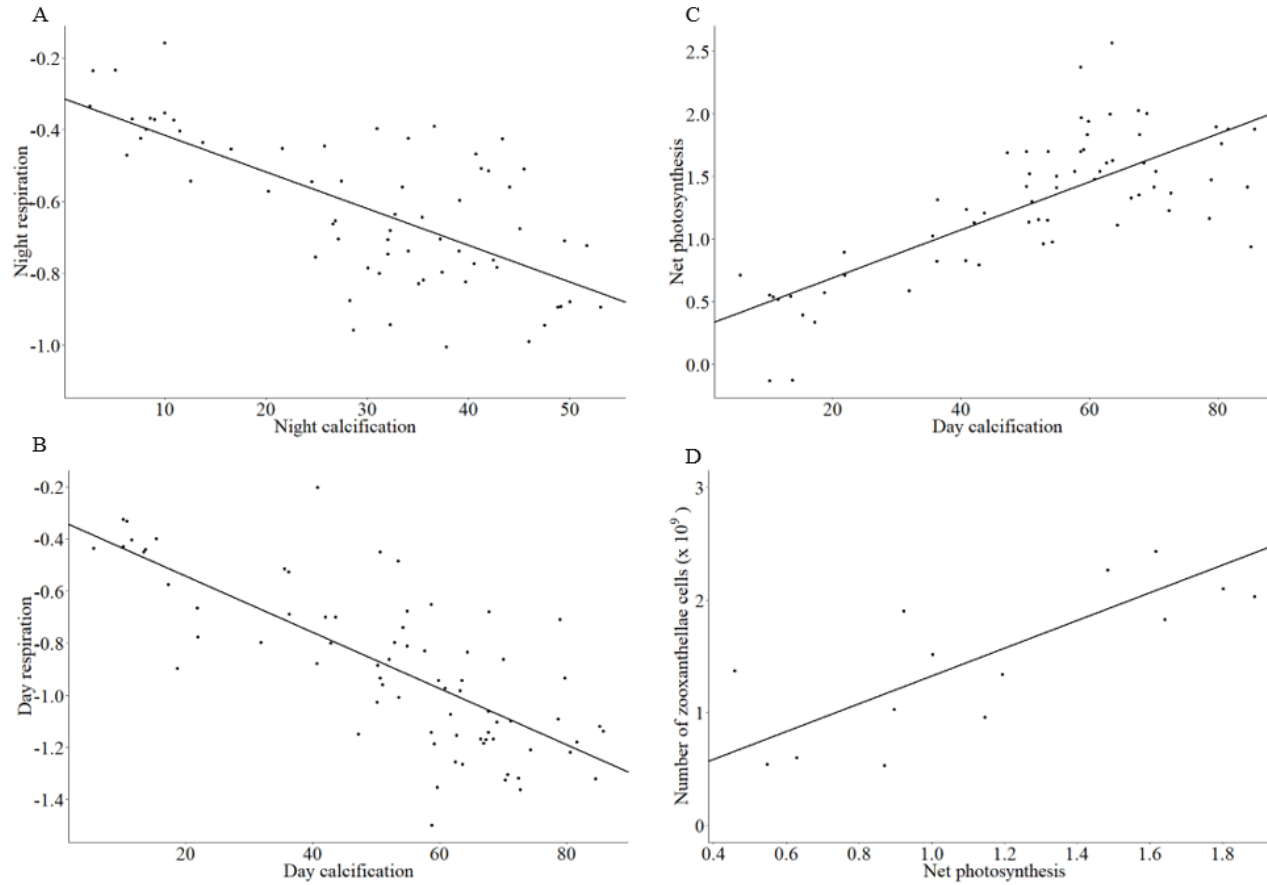
	DF1	DF2	DF3	DF4	DF5
Eigen values	0.967	0.95	0.905	0.776	0.419
Proportion	24.081	23.638	22.536	19.313	10.434
Cumulative Proportion	24.081	47.718	70.254	89.566	100

Supplementary Table 6. Discriminant variables from descriptive discriminant analysis of transcellular transporter genes

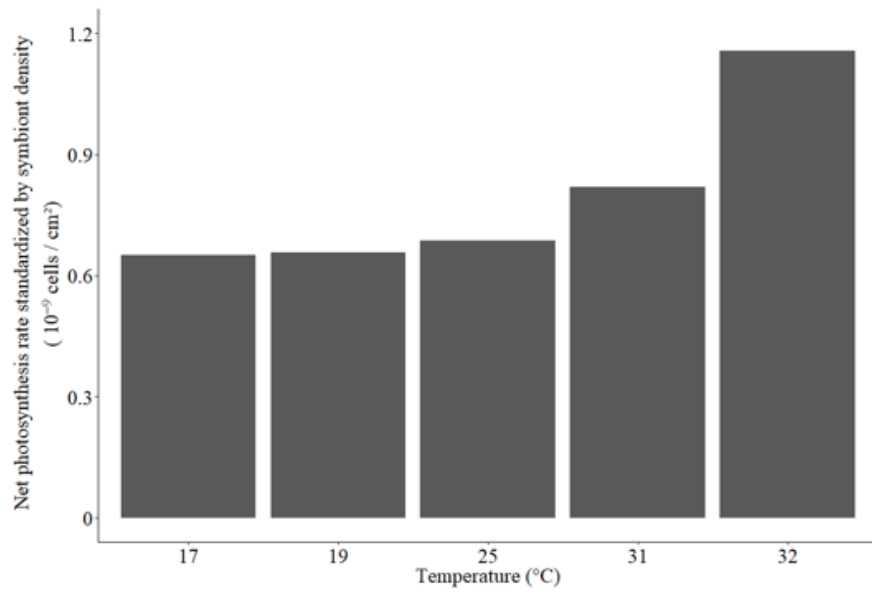
	DF1	DF2	DF3	DF4	DF5
Ca ²⁺ channel	-1.25174	-0.50275	0.82883	0.92	1.50667
PMCA1	1.49177	2.30532	-2.37027	0.7289	-3.0821
PMCA2	0.84803	0.53783	2.229	-2.0158	-4.70668
PMCA3	-0.412	0.39785	1.07867	-0.6077	1.01399
NHE	-2.04806	-1.61509	-0.80468	-1.4671	-0.78038
SpisSLC4 β	0.13748	0.39	-0.57168	1.2192	-3.02595
SpisSLC4g	-0.1545	-0.51738	0.27089	3.0924	1.27347
SpisSLC4 γ	0.55733	0.26041	0.22834	-1.1867	-1.45829
SpisSLC4e	0.61186	-0.91709	0.16733	-1.0479	4.7902
SpisSLC4 δ	0.14967	-0.02118	-1.00771	1.0861	-1.40355
SpisSLC26b	-0.02647	0.06913	-0.23149	0.1728	0.12823
SpisSLC4 ϵ	-0.41848	-0.03035	0.69804	0.9642	-0.71527
SpisCA1	-0.58458	-0.50437	1.3165	-2.7138	-0.08673
SpisCA2	0.19726	0.62296	-0.3189	0.2463	-1.87507
SpisCA3	0.77479	0.93835	-1.31863	1.9029	0.03303
SpisCA4	-1.0053	-0.57662	0.2773	-1.0983	1.76961
V-ATPase	0.23082	-1.08646	0.07618	-1.0984	6.54858

Supplementary Table 7. Results of three-way ANOVA tests on the effect of temperature (17, 25 and 32°C), time of day (day/nighttime) and gene identity on relative gene expressions (Figures 3 and 4 in main manuscript). Results of posthoc tests are given in main manuscript in Tables 1 and 2.

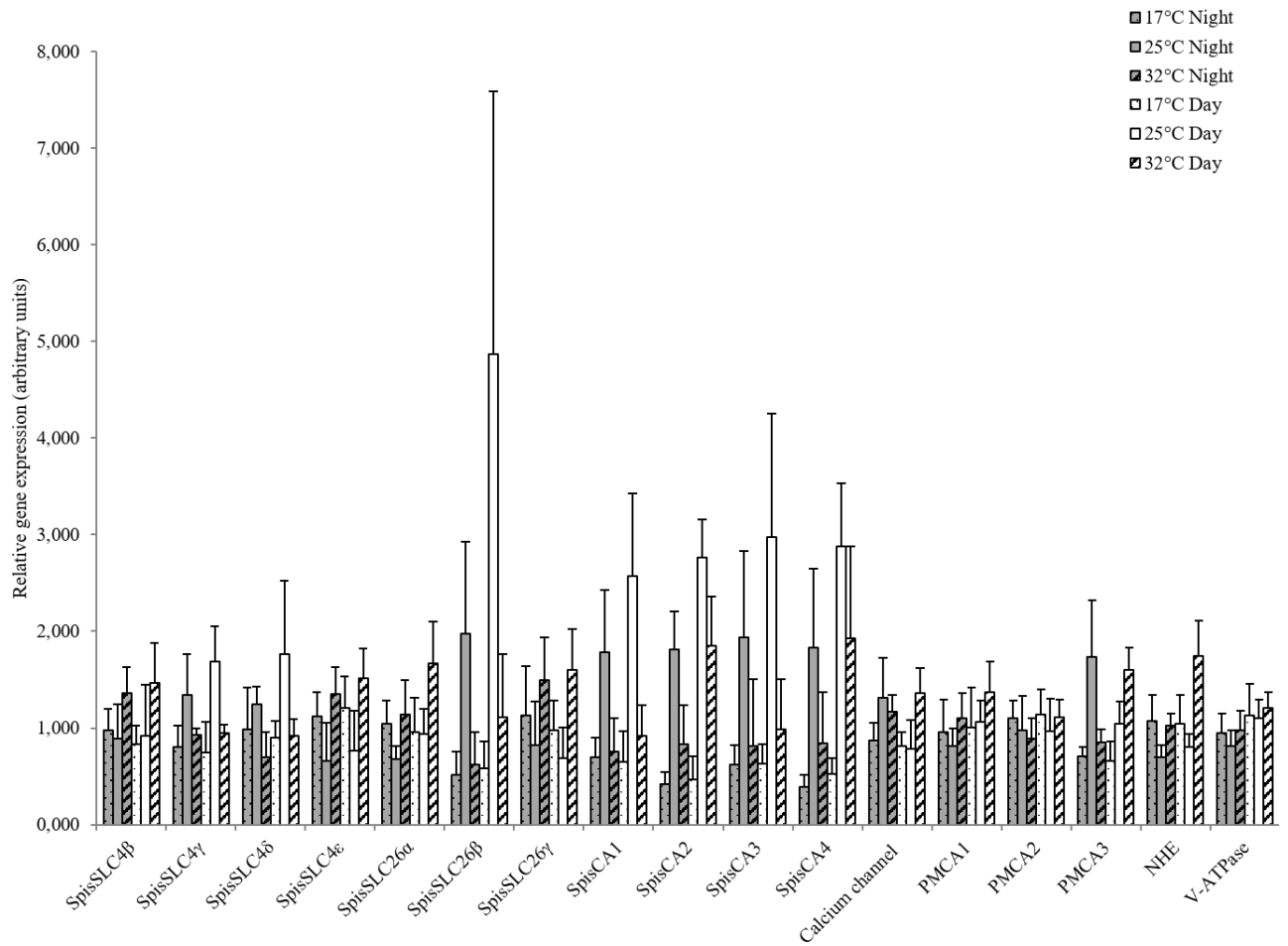
Variable	Effect	df	F	P
gene expression	Temperature	2, 510	85.8017	< 0,00001
	Time	1, 510	46.8169	< 0,00001
	Genes	16, 510	4.1862	< 0,00001
	Temperature*Time	2, 510	12.3359	< 0,00001
	Temperature*Genes	32, 510	16.9948	< 0,00001
	Time*Genes	16, 510	4.0744	< 0,00001
	Temperature*Time*Genes	32, 510	2.8592	< 0,00001



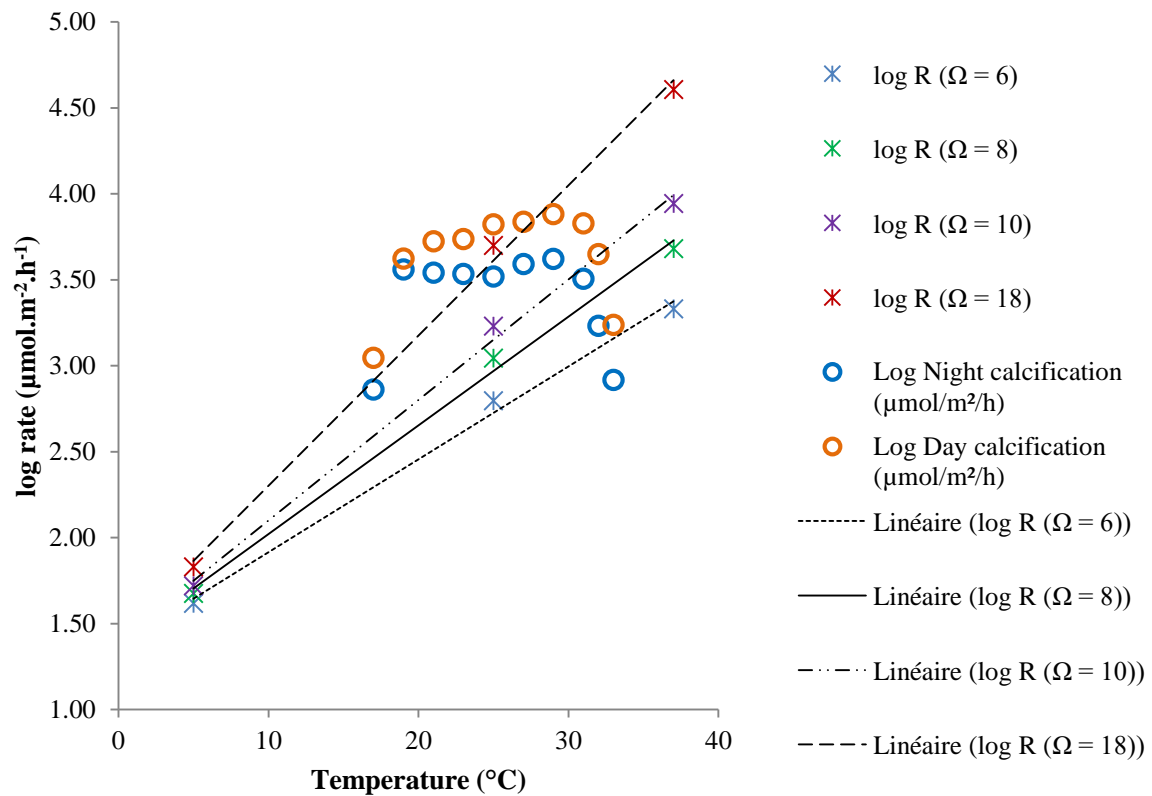
Supplementary Figure 1. Correlation of (A) night respiration to night calcification ($p = 1.6 \cdot 10^{-10}$ and $R^2 = 0.47$), (B) day respiration to day calcification ($p = 3.1 \cdot 10^{-14}$ and $R^2 = 0.56$), (C) net photosynthesis to day calcification ($p = 3.1 \cdot 10^{-13}$ and $R^2 = 0.56$), (D) symbiont density to net photosynthesis ($p = 4.4 \cdot 10^{-4}$ and $R^2 = 0.60$).



Supplementary Figure 2. Photosynthetic rate normalized to symbiont density.



Supplementary Figure 3. Gene expression (mean \pm SD) of ion transport genes relative to geometric mean of expression of the reference genes L22 and 36B4. The same data is presented in the main manuscript as log₁₀ transformation plots in Figure 3 and 4 relative to 25°C and nighttime.



Supplementary Figure 4. Abiotic precipitation rate of aragonite as a function of aragonite saturation state at 5°C, 25°C and 37°C in seawater (modified from Burton and Walter, 1987), and precipitation rate of aragonite in *S. pistillata* from 17°C to 33°C during night (blue circles) and day (orange circles).

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