

Molecular response of the brain to cross-generational warming in a coral reef fish

Supplementary Materials

Table S1. Sex of the F1 and F2 individuals used in the analyses of gene expression.

Generation	Sample ID	Treatment	Sex
F1	A B O103 124B	Control	Male
F1	A B O118B	Control	Male
F1	A B O33	Control	Male
F1	A B O43	Control	Female
F1	A B O64	Control	Male
F1	A B O98 35	Control	Male
F1	B B O11	1.5C	Female
F1	B B O36 13	1.5C	male
F1	B B O41	1.5C	male
F1	B B O61 82	1.5C	male
F1	B B O7 8	1.5C	male
F1	B B O91 90	1.5C	male
F1	C B 074 75	3.0C	male
F1	C B O18 23	3.0C	male
F1	C B O28 29B	3.0C	female
F1	C B O50	3.0C	male
F1	C B O65 64	3.0C	male
F1	C B O72 73	3.0C	male
F2	A1 B	Control	Female
F2	A2 B	Control	Male
F2	A3 B	Control	Male
F2	A4 B	Control	Male
F2	A5 B	Control	female
F2	A6 B	Control	female
F2	B1 B	1.5C	female
F2	B2 B	1.5C	male
F2	B3 B	1.5C	male
F2	B4 B B	1.5C	male
F2	B5 B B	1.5C	male
F2	C1 B B	3.0C	male
F2	C2 B	3.0C	female
F2	C3 B B	3.0C	female
F2	C4 B B	3.0C	male

F2	C5_B	3.0C	male
F2	C6_B	3.0C	female
F2	sC1_B_B	1.5C/3.0C	female
F2	sC3_B_B	1.5C/3.0C	male
F2	sC4_B	1.5C/3.0C	female
F2	sC6_B	1.5C/3.0C	male
F2	tB1_B	1.5C	female
F2	tB2_B	1.5C	male
F2	tB3_B	1.5C	female
F2	tB4_B	1.5C	female
F2	tC1_B_B	3.0C	male
F2	tC2_B_B	3.0C	male
F2	tC3_B_B	3.0C	male
F2	tC4_B_B	3.0C	female
F2	tC5_B_B	3.0C	female

Table S2. Genetic relationships of F1 samples studied for the analyses of gene expression. Details of breeding pair (F0) IDs are provided for the different treatments.

Generation	Sample ID	Treatment	Parents F0
F1	A_B_O103_124B	Control	6
F1	A_B_O118B	Control	37
F1	A_B_O33	Control	48
F1	A_B_O43	Control	41
F1	A_B_O64	Control	48
F1	A_B_O98_35	Control	69
F1	B_B_O11	1.5C	48
F1	B_B_O36_13	1.5C	69
F1	B_B_O41	1.5C	48
F1	B_B_O61_82	1.5C	14
F1	B_B_O7_8	1.5C	68
F1	B_B_O91_90	1.5C	41
F1	C_B_074_75	3.0C	41
F1	C_B_O18_23	3.0C	6
F1	C_B_O28_29B	3.0C	48
F1	C_B_O50	3.0C	14
F1	C_B_O65_64	3.0C	6
F1	C_B_O72_73	3.0C	68

Table S3. Genetic relationships of F2 samples studied for the analyses of gene expression. Details of both individual parent (F1) and grandparent pair (F0) IDs are provided for the different treatments.

Sample	Treatment	Parent ID (F1)		Grandparent ID (F0)	
		Father	Mother	Father	Mother
A1_B	Control	86	104	6	41
A2_B	Control	31	127	68	48
A3_B	Control	118	32	37	6
A4_B	Control	102	77	6	14
A5_B	Control	107	79	69	14
A6_B	Control	107	79	69	14
B1_B	1.5C	118	32	37	6
B2_B	1.5C	107	79	69	14
B3_B	1.5C	104	86	6	41
B4_B_B	1.5C	33	34	48	37
B5_B_B	1.5C	76	106	48	6
C1_B_B	3.0C	102	77	6	14
C2_B	3.0C	33	34	48	37
C3_B_B	3.0C	107	79	69	14
C4_B_B	3.0C	76	106	48	6
C5_B	3.0C	33	34	48	37
C6_B	3.0C	33	34	48	37
sC1_B_B	1.5C/3.0C	16	17	41	3
sC3_B_B	1.5C/3.0C	16	17	41	3
sC4_B	1.5C/3.0C	16	17	41	3
sC6_B	1.5C/3.0C	14	15	68	41
tB1_B	1.5C	6	40	48	37
tB2_B	1.5C	12	11	41	48
tB3_B	1.5C	6	40	48	37
tB4_B	1.5C	6	40	48	37
tC1_B_B	3.0C	75	74	41	3
tC2_B_B	3.0C	56	55	41	48
tC3_B_B	3.0C	56	55	41	48
tC4_B_B	3.0C	75	74	41	3
tC5_B_B	3.0C	25	24	68	41

Table S4. Number of significant, non-significant and total genes analyzed for each of the comparisons, using the Likelihood ratio test (LRT), as well as the pairwise-contrast ($p < 0.05$ after Benjamini-Hochberg correction).

Comparison	Method	Non-significant	Significant DEGs	Total
All variables	LRT	18,591	9,344	27,935
Temperature (minus effect generation + sex + time of exposure)	LRT	21,359	362	27,721
Sex (minus effect generation + temperature Grandparents + time of exposure)	LRT	27,343	8	27,351
Grandparents (minus effect generation + sex + Temperature + time of exposure)	LRT	28,336	268	28,604
Generation (minus effect temperature + sex + Grandparents + time of exposure)	LRT	16,918	11,060	27,978
Time of exposure (minus effect temperature + sex + Grandparents + generation)	LRT	27,971	7	27,978
Control F1 vs. Control F2	Pairwise Contrast	19,140	7,550	26,690
Control vs Dev +1.5C (F1)	Pairwise Contrast	21,585	122	21,707
Control vs Dev +3.0C (F1)	Pairwise Contrast	25,819	249	26,068
Dev +1.5C vs Dev +3.0C (F1)	Pairwise Contrast	26,049	19	26,068
Control vs Dev +1.5C (F2)	Pairwise Contrast	20,431	27	20,458
Control vs Dev +3.0C (F2)	Pairwise Contrast	24,014	187	24,201
Dev +1.5C vs Dev +3.0C (F2)	Pairwise Contrast	27,301	10	27,311
Control vs Tran +1.5C (F2)	Pairwise Contrast	26,640	50	26,690
Control vs Tran +3.0C (F2)	Pairwise Contrast	27,199	112	27,311
Dev +1.5C vs Tran +1.5C (F2)	Pairwise Contrast	26,030	38	26,068
Dev +3.0C vs Tran +3.0C (F2)	Pairwise Contrast	26,037	31	26,068
Control vs. Step +3.0C (F2)	Pairwise Contrast	26642	48	26,690
Dev +1.5C vs Step +3.0C (F2)	Pairwise Contrast	32317	2	32,319
Dev +3.0C vs Step +3.0C (F2)	Pairwise Contrast	25427	17	25,444
Tran +1.5C vs Step +3.0C (F2)	Pairwise Contrast	32,317	149	32,466
Tran +3.0C vs Step +3.0C (F2)	Pairwise Contrast	26,688	2	26,690
Tran +1.5C vs Tran +3.0C (F2)	Pairwise Contrast	24,461	361	24,822

Supplementary Figures

Supplementary Figure 1. Overlap of differentially expressed genes among pairwise comparisons of developmental treatments vs control in F1 and F2 (A-C) and between developmental and transgenerational treatments in the F2 (D and E). Figure F represents the overlap between Control vs Transgenerational +1.5°C and Control vs Transgenerational +3.0°C in the F2.

