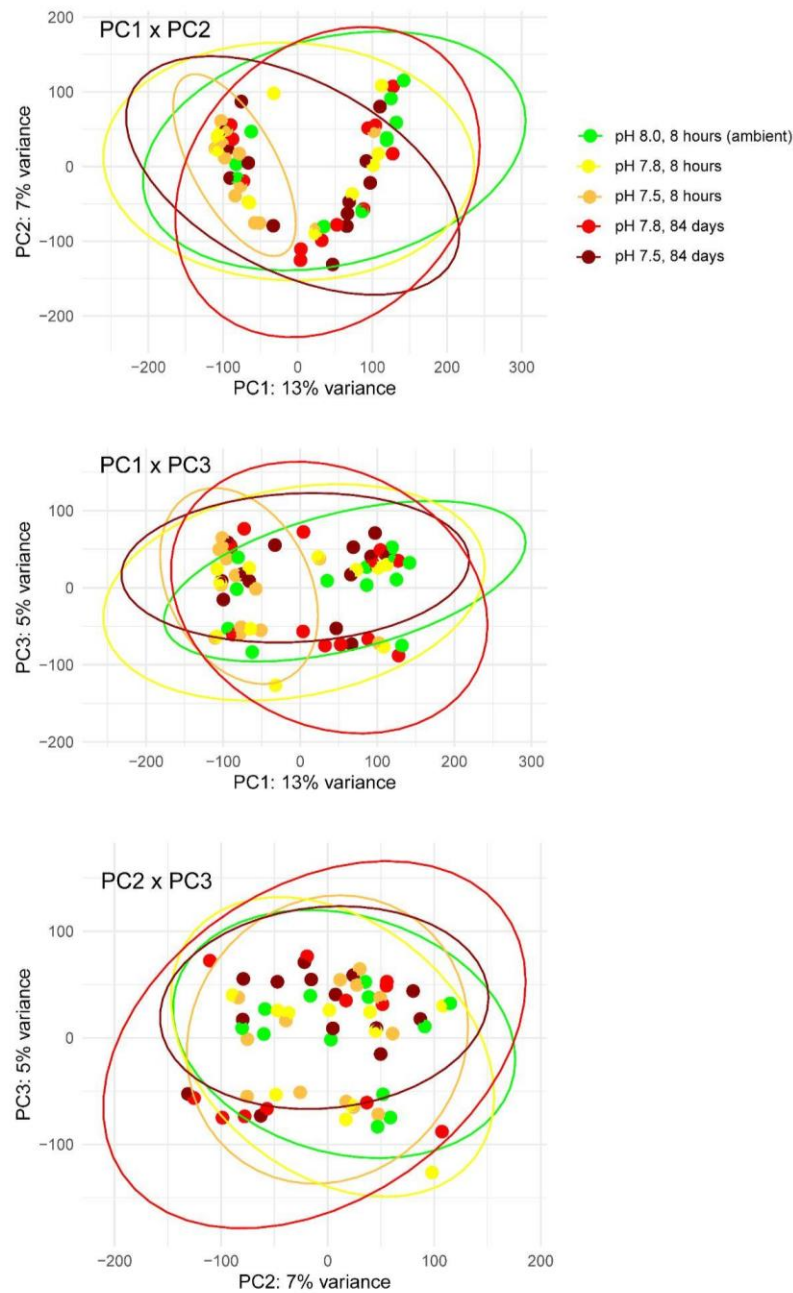
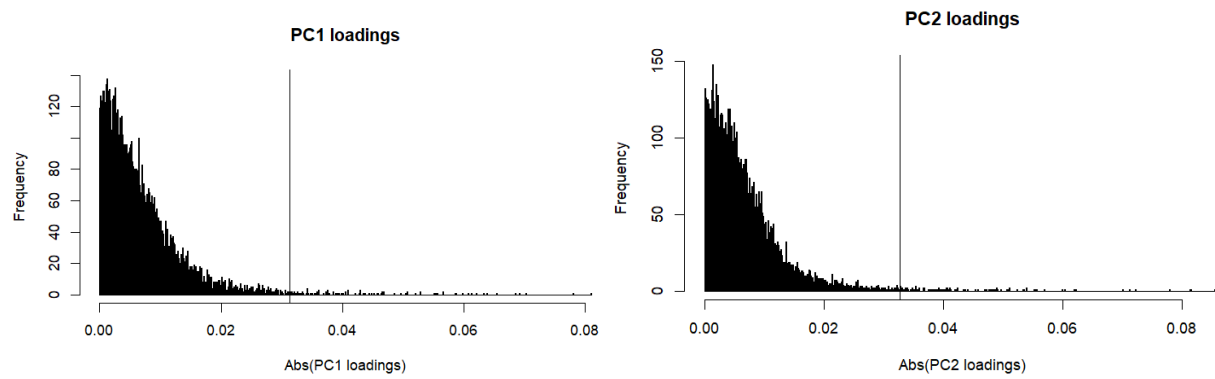


- 1 Supplemental Materials for *Short-term mechanisms, long-term*
- 2 *consequences: molecular effects of ocean acidification on juvenile snow*
- 3 *crab*
- 4 Laura H Spencer^{1,3}, Ingrid Spies³, Jennifer L. Gardner², Steven Roberts¹, W. Christopher Long²



5 Figure S1: Principal component analysis biplots (PC1-PC3) without including surrogate variable
6 analysis to control for unknown latent effects. The horseshoe pattern in PC1xPC2 and clustering
7 by factors not related to treatment in biplots along PC1 and PC3 demonstrate the expression
8 heterogeneity of technical or biological variables that obfuscates acclimatory response to
9 acidification.



10 **Figure S2:** Histograms of PC1 & PC2 loadings (absolute values) from the VST- and SVA-
 11 controlled count matrix, the top 1% of which were >0.032 (to the right of vertical lines).

12

13

14 **Table S1:** Highlighted list of annotated genes that were differentially expressed in both
15 moderate (pH 7.8) and severe (pH 7.5) OA after 8-hr exposure. Log₂ Fold Change (L₂FC) and -
16 Log(P-adjusted) are compared to control crab held in ambient conditions (pH 8.0) at time-0. For
17 all genes, the relative expression compared to control (higher, lower) was consistent in both OA
18 treatments. **Indicates genes that were also differentially expressed after 88-days in both
19 treatments.

Gene Name	Gene ID	Mod. OA Δ%	Mod. OA -Log(P)	Sev. OA Δ%	Sev. OA -Log(P)	Protein	Biological Processes
Higher Expression in both OA treatments at 8 h							
Ndufs2	GWK47_042301	22%	1.45	25%	2.34	NADH dehydrogenase [ubiquinone] iron-sulfur protein 2, mitochondrial	Mitochondrial respiration, energy metabolism
Ndufs3	GWK47_035555	19%	1.42	28%	3.15	NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial	Mitochondrial respiration, energy metabolism, redox
NDUBA	GWK47_048997	20%	1.36	27%	2.61	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 10	Mitochondrial respiration, energy metabolism
MRPS22	GWK47_024976	24%	1.98	21%	2.32	28S ribosomal protein S22, mitochondrial	Mitochondrial translation, energy metabolism
mRpl35	GWK47_003027	20%	1.43	20%	1.96	39S ribosomal protein L35, mitochondrial	Mitochondrial translation, energy metabolism
MRPL30	GWK47_027395	18%	1.36	27%	2.97	39S ribosomal protein L30, mitochondrial	Mitochondrial translation, energy metabolism
UGT2B15_3	GWK47_007253	117%	1.52	7%	1.31	UDP-glucuronosyltransferase 2B15	Detoxification, xenobiotic metabolism
4CL_0	GWK47_015047	110%	1.87	52%	1.36	4-coumarate--CoA ligase	Energy metabolism
Echs1	GWK47_021840	21%	1.60	17%	1.65	Enoyl-CoA hydratase, mitochondrial	Lipid metabolism, β-oxidation
miox	GWK47_050003	40%	1.31	44%	2.07	Inositol oxygenase	Carbohydrate metabolism
emc2-b	GWK47_005824	23%	1.93	13%	1.32	ER membrane protein complex subunit 2-B	Protein processing, ER membrane insertion
Xpnpep1_2	GWK47_044723	11%	1.41	9%	1.38	Xaa-Pro aminopeptidase 1	Protein turnover, stress response
MED27	GWK47_029699	17%	1.36	16%	1.79	Mediator of RNA polymerase II transcription subunit 27	Transcriptional regulation (RNA polymerase II)
prmt6	GWK47_007501	24%	1.87	26%	2.79	Protein arginine N-methyltransferase 6	Epigenetic and transcriptional regulation
Srsf7	GWK47_020456	16%	1.36	16%	2.06	Serine/arginine-rich splicing factor 7	RNA splicing, mRNA processing
U2af50	GWK47_001080	15%	1.49	13%	1.56	Splicing factor U2AF subunit	RNA splicing, mRNA processing
Tsnax	GWK47_041448	14%	1.41	16%	2.22	Translin-associated protein X	RNA silencing, reproductive process
Myo5a_0	GWK47_011520	61%	2.46	27%	1.44	Unconventional myosin-Va	Cytoskeletal transport, vesicle trafficking
**NUP43	GWK47_010369	27%	1.41	21%	1.45	Nucleoporin Nup43	Nucleocytoplasmic transport, cell cycle regulation
Lower Expression in both OA treatments at 8 h							
oct-1_1	GWK47_003819	-71%	1.93	-61%	1.90	Organic cation transporter 1	Ion transport, acid–base homeostasis
Gstt4	GWK47_034834	-41%	2.38	-23%	1.32	Glutathione S-transferase theta-4	Detoxification, oxidative stress response
RSPRY1_1	GWK47_007365	-17%	1.34	-16%	1.62	RING finger and SPRY domain-containing protein 1	Protein degradation, turnover
USP19	GWK47_030223	-15%	1.38	-14%	1.55	Ubiquitin carboxyl-terminal hydrolase 19	Protein quality control, stress response
Phm	GWK47_018134	-46%	1.84	-34%	1.63	Peptidylglycine alpha-hydroxylating monooxygenase	Peptide processing, reproduction

PCE_0	GWK47_027739	-43%	1.63	-41%	2.17	Proclotting enzyme	Immune, coagulation response
PCE_2	GWK47_051844	-35%	1.37	-43%	2.64	Proclotting enzyme	Immune, coagulation response
VPS13C_1	GWK47_005657	-27%	1.31	-30%	2.10	Vacuolar protein sorting-associated protein 13C	Lipid transport, organelle organization (mitochondrial)
PDXK	GWK47_032265	-38%	1.86	-24%	1.33	Pyridoxal kinase	Cofactor metabolism, cellular metabolism
TGFB1	GWK47_026454	-37%	1.60	-31%	1.68	Transforming growth factor-beta-induced protein ig-h3	Cell adhesion, structural organization
**Cad87A	GWK47_018958	-23%	1.86	-19%	2.02	Cadherin-87A	Cell adhesion, signaling
sas_3	GWK47_043552	-30%	1.31	-36%	2.34	putative epidermal cell surface receptor	Cell signaling, membrane receptor activity
rhpn2	GWK47_028156	-22%	1.58	-17%	1.44	Rhopilin-2	Cytoskeletal signaling
MYO18A	GWK47_044957	-18%	1.52	-15%	1.45	Unconventional myosin-XVIIIa	Cytoskeletal transport, cell organization
CycG	GWK47_003032	-23%	1.41	-24%	2.11	Cyclin G	Cell cycle regulation, growth control
A1CF	GWK47_037491	-21%	1.36	-20%	1.78	APOBEC1 complementation factor	Reproduction, RNA editing
DOP1A	GWK47_009521	-15%	1.61	-15%	2.27	Protein dopey-1	Vesicle trafficking, protein transport

20

21 Descriptions of Tables S2-S6

22 *Please also see [Google Spreadsheet](#) for Tables S2-S6 (in addition to .csv files).*

23

24 **Table S2:** Genes with top loadings along PC1 and PC2.

25 **Table S3:** Full list of DEGs after 8 hr OA exposure.

26 **Table S4:** Persistent DEGs, those from 8 hr that were also DEGs at 88 days.

27 **Table S5:** Lists of DEGs among OA treatments at 88 days.

28 **Table S6:** Enriched biological processes.

29 Short term acclimation mechanisms that persisted at day 88

30 We did not generate expression data for the ambient treatment at day 88, so cannot fully
31 separate OA effects from other potential influences of long-term laboratory exposure (e.g., diet,
32 tank environment). To focus on genes most consistently associated with OA, we identified
33 differentially expressed genes (DEGs) at 8 h that remained differentially expressed at 88 days
34 (Figure S3, Table S4).

35 In moderate OA, 18 DEGs identified at 8-hr persisted at 88 d (Table S4), which were
36 enriched for RNA binding (GO:0003723) and included several involved in transcription and

splicing, proteolysis, retinol metabolism, cell adhesion/structure, and possibly circadian rhythm (Mandibular organ-inhibiting hormone, see Fig 3).

In the severe OA treatment, 59 DEGs identified at 8-hr persisted at 88 d (Table S4), which were not significant enriched for any processes, but broadly reflect genes involved in mitochondrial energy metabolism, detoxification, and defense against oxidative or hypoxic stress (HSP22, CYP2L1, SIRT4, CBS, Gstt4), cell division and transcriptional control (e.g. cdk1, Mafg, Usp21), cell adhesion and cytoskeleton dynamics (TTN, Actr3, Cad87), and hormone and growth factor signaling (FLT4, LHCGR, Tdrd9). These “persistent” DEGs suggest that severe OA induces long-lasting shifts in stress response, signaling, and cellular regulation (Table X).

Two genes were found to persist in both moderate and severe OA treatments: cad87A which codes for a cadherin, and nup43 which codes for a nucleoporin, and which were expressed at lower and higher levels, respectively, in OA treatments compared to control (Figure S4).

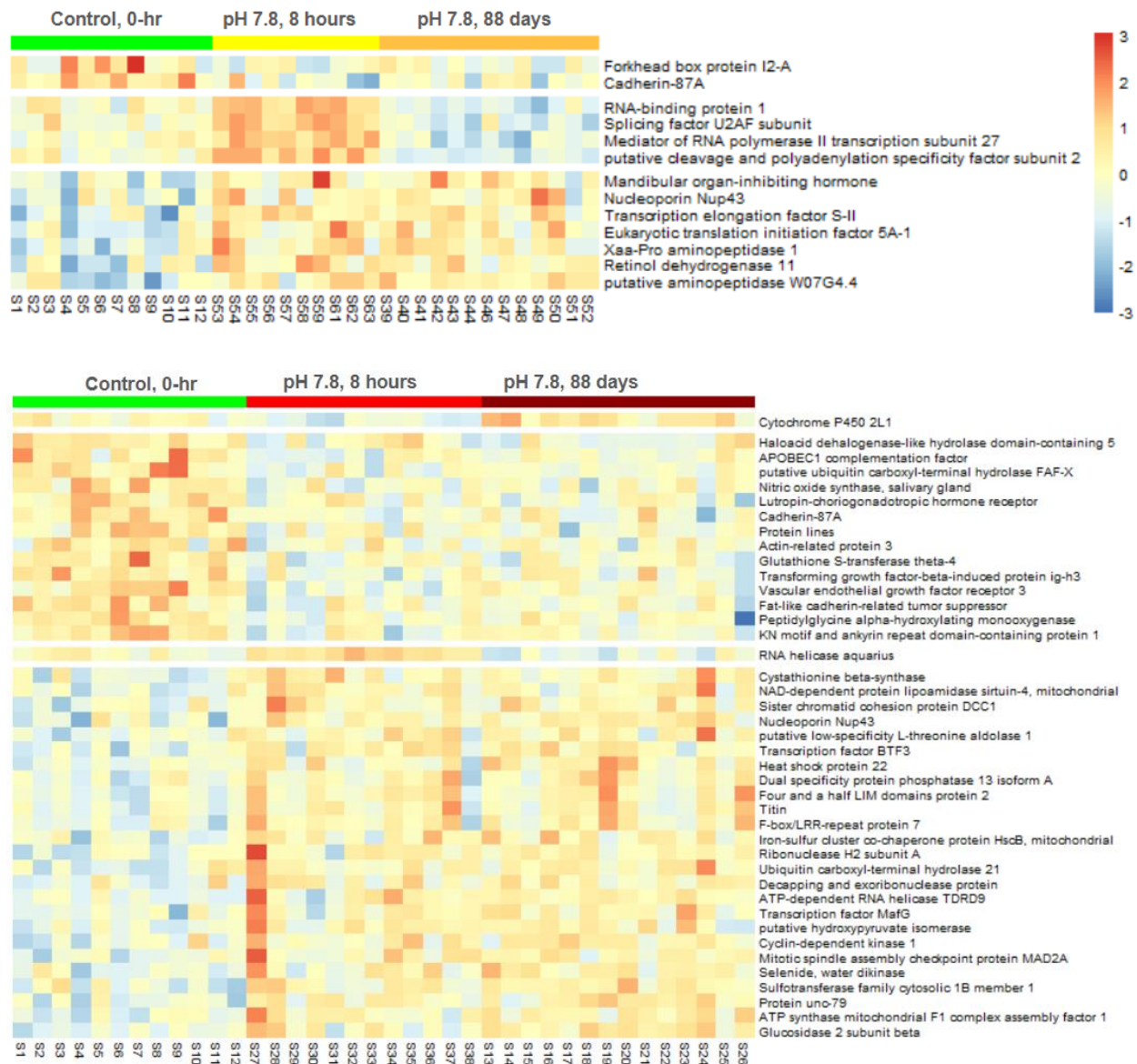


Figure S3: Heatmaps of persistent DEGs, defined as genes differentially expressed at both 8 h and 88 d. Results are shown for moderate OA (pH 7.8, top) and severe OA (pH 7.5, bottom) relative to control conditions measured at time 0. Colors indicate relative expression (z-scores), with red = higher and blue = lower expression, columns represent individual crabs, and rows represent individual genes, which are grouped by their expression pattern. Two genes were persistent DEGs in both OA treatments: cadherin-87A (*cad87a*) and nucleoporin (*nup43*), which were expressed at lower and higher levels, respectively, in OA compared to control.