Title: Divergent thermal challenges elicit convergent stress signatures in aposymbiotic *Astrangia poculata*

Running head: Thermal challenges in a temperate coral

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Abstract

Anthropogenic climate change threatens corals globally and both high and low temperatures are known to induce coral bleaching. However, coral stress responses across wide thermal breadths are rarely explored. In addition, it is difficult to disentangle the role of symbiosis on the stress response of obligately symbiotic coral hosts. Here, we leverage aposymbiotic colonies of the facultatively symbiotic coral, *Astrangia poculata,* which lives naturally with and without its algal symbiont, to examine how broad thermal challenges influence coral hosts. *A. poculata* were collected from their northern range limit and thermally challenged in two independent 16-day common garden experiments (heat and cold challenge) and behavioral responses to food stimuli and genome-wide gene expression profiling (TagSeq) were performed. Both thermal challenges elicited significant reductions in polyp extension. However, five times as many genes were differentially expressed under cold challenge compared to heat challenge. Despite more genes responding to cold challenge, there was significant overlap in which genes were differentially expressed across thermal challenges. These convergently responding genes (CRGs) were associated with downregulation of motor functions and nematocysts while others were consistent with stress responses previously identified in tropical corals. The fact that these responses were observed in aposymbiotic colonies highlights that many genes previously implicated in stress responses in symbiotic species may simply represent the coral's stress response in or out of symbiosis.

Introduction

Temperature is an important factor in determining species distribution patterns in ectothermic organisms (Angilleta 2009). As sea surface temperatures continue to rise, understanding how these changes will affect species distributions demands a broad understanding of organisms' physiological sensitivities to temperature across their native range. There is overwhelming evidence that temperature increases associated with anthropogenic climate change are having widespread ecological consequences on marine species distributions (Hoegh-Guldberg *et al.* 2008; Pinsky *et al.* 2019). Coral reefs are particularly sensitive to these thermal changes, which have been implicated in widespread reef declines (Hughes *et al.* 2017). Temperature anomalies are the primary driver of the breakdown in the obligate symbiotic relationship between tropical corals and their endosymbiotic algae (family Symbiodiniaceae; LaJeunesse *et al.* 2018). This breakdown results in the expulsion of algae from coral host tissue in a process known as coral bleaching (Gates

55 *et al.* 1992; Venn *et al.* 2008). Because symbiotic algae translocate carbon sugars to the coral host,
56 losing these symbionts results in significant energy loss and many corals are unable to survive
57 extended periods in a bleached state (Weis 2008).

58 The majority of research on coral bleaching has focused on responses to elevated
59 temperatures in tropical reef-building corals (Cziesielski *et al.* 2019). However, tropical corals can
60 bleach in response to a variety of stressors, including high nutrients (Wiedenmann *et al.* 2013),
61 ocean acidification (Anthony *et al.* 2008), pathogens (Ben-Haim & Rosenberg 2002), low salinity
62 (Goreau 1964), chemical exposures (Cervino *et al.* 2003), and cold stress (Saxby *et al.* 2003).
63 Coral responses to cold stress remain understudied, even though these events can have substantial
64 impacts on reefs. For example, a cold-water bleaching event in 2010 decimated inshore coral
65 populations along the Florida reef tract (Lirman *et al.* 2011), and cold water has caused bleaching
66 on the Great Barrier Reef (Hoegh-Guldberg & Fine 2004). While the main effect of climate change
67 on marine systems is a net increase in mean global sea surface temperatures, these cold thermal
68 challenges may be exacerbated by the pace of Arctic warming (twice as fast as the global average),
69 which may influence upper-level atmospheric activity and storm tracks resulting in more frequent
70 extreme cold outbreaks at northern mid-latitudes (Cohen *et al.* 2014). These cold extremes are
71 therefore relevant thermal challenges to subtropical and temperate coral species.

72 One way to monitor responses to stress is to characterize changes in gene expression
73 profiles, which provides a snapshot into the physiological state of an organism and offers insights
74 into the biological processes, molecular functions, and cellular components that corals engage to
75 tolerate various stressors. Modern transcriptomics have demonstrated that corals mount dynamic
76 responses to pollutants (Gust *et al.* 2014; Ruiz-Ramos *et al.* 2017), pH (Moya *et al.* 2012; Davies
77 *et al.* 2016) and bacterial challenges (Fuess *et al.* 2017; Wright *et al.* 2017) and considerable efforts

78 have been made to understand how corals respond to heat challenges (for review see Cziesielski
79 *et al.* 2019). Interestingly, similar patterns of gene expression emerge from these different
80 stressors. Barshis *et al.* (2013) demonstrates that corals exhibit a widespread stress response across
81 thousands of genes, and this environmental stress response (ESR) is consistent with the conserved
82 response to diverse environmental stressors in yeast (Gasch *et al.* 2000). A meta-analysis
83 comparing the transcriptomic responses of coral from the genus *Acropora* to various stressors
84 found these coral exhibit a stereotyped ESR (Dixon *et al.* 2020). There, it was found that there
85 is consistent upregulation of genes involved in cell death, response to reactive oxygen species, NF-
86 kB signaling, immune response, protein folding, and protein degradation to a variety of acute stress
87 exposures. This research highlights that testing a single stressor cannot elucidate whether genes
88 being expressed are unique to the stressor or emerge from a more generalized ESR.

89 Most work exploring the stress responses of corals have focused on tropical reef-building
90 corals that live in oligotrophic waters and cannot survive long-term without their algal symbionts.
91 Because energy deprivation in coral hosts results from any mechanism of symbiont loss (Baena-
92 González & Sheen 2008), uncoupling a thermal stress response from an energy deprivation
93 response is challenging. Furthermore, given that many tropical corals exhibit an obligate symbiotic
94 relationship, it is difficult to disentangle the host's stress response to extreme temperatures from
95 the host's response to stress-induced algal by-products (i.e. reactive oxygen species (ROS);
96 McGinty *et al.* 2012) and the resulting energy deprivation from dysbiosis. However, there are
97 several species of subtropical and temperate reef-building corals that exhibit facultative symbioses
98 and offer promising avenues to better understand stress responses.

99 The Northern Star Coral (*Astrangia poculata*) exhibits a facultatively symbiotic
100 relationship with *Breviolum psygmophilum* (LaJeunesse *et al.* 2012) and can be found in sympatry

101 in varying symbiotic states that are visually distinguishable by colour. Symbiotic colonies appear
102 brown due to high densities of *B. psygmophilum*, and much like a bleached coral, some *A. poculata*
103 appear white (Figure 1C) due to very low algal densities (Dimond & Carrington 2007). This white
104 phenotype is commonly referred to as "aposymbiotic" (Grace 2017; Sharp *et al.* 2017; Burmester
105 *et al.* 2018) due to the paucity of algal symbionts. Unlike obligate symbiotic corals, *A. poculata*
106 can thrive in its aposymbiotic state relying only on heterotrophy (Dimond & Carrington 2007).
107 Additionally, *A. poculata* experiences large seasonal variation in temperature at its northern range,
108 making these populations ideal models for investigating how corals might withstand wide thermal
109 challenges. Taken together, aposymbiotic *A. poculata* provide a unique opportunity to disentangle
110 how broad thermal challenges influence the coral host in isolation from its algal symbiont. Here,
111 we present two thermal challenge experiments that independently assess the behavioural and
112 molecular responses of aposymbiotic *A. poculata* to divergent thermal challenges.
113
114 ***Methods***
115
116 *Thermal challenge common garden experiments*
117 Eighteen unique aposymbiotic colonies of *Astrangia poculata* were collected in Woods Hole,
118 Massachusetts (41.54°N, 70.64°W; Figure 1A) in October, 2017 and transported to the Marine
119 Invertebrate Research Facility at Boston University. Colonies were acclimated at 16°C for three
120 weeks. On November 17, 2017, colonies were fragmented, each coral nubbin was assigned a
121 unique ID and glued to a labelled dish (Figure 1C). Nubbins were allowed to recover from
122 fragmentation and further acclimated at 16°C under a 12 L:12 D photoperiod with light levels
123 ranging from 6-12 µmol $m^{-2}s^{-1}$ and fed *Artemia spp.* nauplii daily for 24 days.
124

125 *Thermal challenge I: cold challenge experiment*

126 Nine unique aposymbiotic colonies were assigned to the cold challenge experiment (Table 1). At
127 least one nubbin from each colony was represented in one of three replicate tanks assigned to
128 control conditions (maintained at 22˚C) and one of three replicate tanks assigned to the cold
129 challenge treatment (incrementally lowered from 23˚C by approximately 1˚C/day to a final
130 temperature of 6ºC; Figure 2A). When additional fragments remained from a colony, they were
131 randomly stratified into different tanks (n=43 nubbins total). Several aspects of this experimental
132 design are noteworthy. The first is that our control treatment (22˚C) was 6˚C higher than the coral
133 acclimation temperature, which may have caused an initial stress response in the first few days.
134 The second aspect is that 6ºC is warmer than the minimum temperature *A. poculata* experience
135 within their seasonal averages (Figure 1B); however, achieving lower temperatures was limited by
136 the capacity of our aquarium chillers. In addition, these colonies were collected in October so this
137 thermal minimum and the rate at which this minimum was achieved likely represents a
138 considerable thermal challenge for these corals.

139

140 *Thermal challenge II: heat challenge experiment*

141 An independent set of nine unique aposymbiotic *A. poculata* colonies were fragmented and at least
142 one nubbin from each colony was assigned to each treatment. There were three tank replicates for
143 control conditions (maintained at 16ºC) and three replicate tanks for the heat challenge treatment.
144 At least one nubbin of each colony was assigned to each treatment, and when additional colony
145 fragments remained, they were randomly stratified into different tanks (Table 1). At the beginning
146 of the 16 day heat challenge experiment, all tanks were maintained at 16˚C. Heat challenge tanks
147 were ramped from 16˚C to 23˚C over 6 days (approximately 1˚C/day) but no phenotypic data were

148 recorded during this time. Phenotype observations were conducted on days 7-16 during which heat
149 challenge tanks were incrementally ramped 2-3˚C in one day followed by a 2-day recovery period.
150 This ramping protocol continued until 31ºC was achieved (Figure 2A). It is worth noting several
151 aspects of this experimental design: the final heat challenge temperature was well above the
152 maximum temperature these corals experience at their source location (Figure 1B) and the heat
153 challenge experiment was conducted independently from the cold challenge experiment described
154 above (Figure 2A).
155
156 *Coral polyp behaviour in response to food stimulus*
157 In the cold challenge experiment, corals were fed daily and feeding behaviours were recorded 30
158 minutes after feeding. In contrast, in the heat challenge experiment, phenotypic measurements
159 were not conducted in the first 6 days. Heat challenge phenotypic measurements began on day 7
160 and continued after corals were offered food every third day for the duration of the experiment (16
161 days). Coral polyp behaviour in response to food stimulus was quantified by the total coral surface
162 area that had observable polyp extension relative to retracted polyps. This score was on a scale of
163 1 to 5 based on the estimated percentage of active polyps within a fragment (1 = 0%, 2 = 25%, 3
164 = 50%, 4 = 75%, 5 = 100%, similar to Burmester *et al.* 2018) and the same researcher conducted
165 all behavioural assays within each thermal challenge experiment to limit observer biases. An
166 ordered logistic regression was performed to establish if temperature influenced polyp extension
167 rates using the *polr* function as part of the *MASS* package (version 7.3-51.1; Venables & Ripley
168 2002) in R.
169
170 *Global gene expression profiling*

171 Upon reaching maximum thermal differences between challenge and control treatments in both
172 experiments (Day 16), several white polyps from all colonies were sampled using sterilized bone
173 cutters, immediately placed in 200 proof ethanol and stored at -80ºC. Total RNA was extracted
174 using an RNAqueous kit (Ambion by LifeTechnologies) following the manufacturer's
175 recommendations. An additional step was implemented using 0.5 mm glass beads (BioSpec),
176 which were added to the vial of lysis buffer and samples were homogenized using a bead beater
177 for 1 min. RNA quantity and integrity were determined using a DeNovix DS-11+
178 spectrophotometer and ribosomal RNA bands were confirmed on 1% agarose gels. Trace DNA
179 contamination was removed using a DNase 1 (Ambion) digestion at 37ºC for 45 minutes. Libraries
180 were created from 1500 ng of total RNA (following Meyer *et al.* 2011) and adapted for Illumina
181 Hi-Seq sequencing (Dixon *et al.* 2015; Lohman *et al.* 2016). In brief, RNA was heat-sheared and
182 transcribed into first-strand cDNA using a template-switching oligo and SMARTScribe reverse
183 transcriptase (Clontech). cDNA was then PCR-amplified, individual libraries were normalized,
184 and Illumina barcodes were incorporated using a secondary PCR. Samples were pooled and size-
185 selected prior to sequencing on Illumina Hiseq 2500 single-end (SE) 50 basepair (bp) at Tufts
186 University Core Facility (TUCF). Due to insufficient RNA yield, some samples were not
187 successfully represented in library preparations. Of the 42 samples within each of the cold and heat
188 challenge experiments, 26 and 22 libraries were prepared, respectively (Table 1).

189

190 *Transcriptome assembly and gene expression analyses*

191 Illumina TruSeq adapters and poly-A tails were first removed using the *FASTX-Toolkit* (v 0.0.14,
192 Hannon, G.J. (2010) FASTX-Toolkit. http://hannonlab.cshl.edu/fastx_toolkit.) and resulting
193 sequences that were less than 20 bp in length were removed. In addition, only those sequences
194 with > 90% of bases having a quality score > 20 were retained. PCR duplicates were removed and

195 resulting quality-filtered reads were concatenated and used to assemble a novel transcriptome
196 using Trinity (Grabherr *et al.* 2013). Contigs were then annotated using BLAST (Altschul *et al.*
197 1990) searches against UniProt and Swiss-Prot NCBI NR protein databases. This newly assembled
198 transcriptome along with its annotation files are included as Supplementary Files 1-4 (1:
199 transcriptome fasta file, 2: seq2iso file, 3: iso2gene, 4: iso2GO and are also available at
200 http://sites.bu.edu/davieslab/data-code/).

201 Quality-filtered reads were then mapped to the newly assembled transcriptome using
202 *Bowtie2* (Langmead & Salzberg 2012). There were an average 520,662 mapped reads across both
203 experiments with mapping efficiencies ranging from 36%-57% (Supplementary File 5). Raw count
204 files for each experiment are available in Supplemental Files 6 (cold challenge) and 7 (heat
205 challenge). Data from each challenge experiment were analyzed independently. First, data were
206 tested for outliers using *arrayQualityMetrics* as part of DESeq (Anders & Huber 2010) and no
207 outliers were detected for either experiment. DESeq2 (Love *et al.* 2014) was then used to identify
208 differentially expressed genes (DEGs, Supplemental Files 8-9) associated with cold and heat
209 thermal challenge relative to their respective controls using a Wald's test. P-values were adjusted
210 for multiple testing using the Benjamini and Hochberg method (FDR < 0.05; Benjamini &
211 Hochberg 1995). Lastly, expression data for each experiment were *r-log* transformed and these
212 data were used as input for a principal component analysis. A permutational multivariate analysis
213 of variance was then used to determine if overall gene expression patterns between thermal
214 challenge treatments differed significantly from their controls using the *adonis* function in vegan
215 v2.5-4 (Oksanen *et al.* 2019).

216 Gene ontology (GO) enrichment analyses were performed using adaptive clustering of GO
217 categories and Mann–Whitney U tests (GO-MWU) based on the ranking of signed log p-values

218 (Voolstra *et al.* 2011), which is particularly suitable for non-model organisms (Dixon *et al.* 2015).
219 Results were visualized in dendrograms tracing the level of gene sharing between significant
220 categories and direction of change in treatment temperatures compared to their respective controls.
221
222 *Testing for a convergent response to thermal challenge*
223 Lists of DEGs (FDR < 0.05) between the two thermal challenge experiments were compared and
224 visualized using a Venn Diagram; and, significant enrichment of genes at the intersection between
225 experiments was tested for using a hypergeometric test. The DEGs at the intersection between
226 experiments (common DEGs) were visualized based on $\log_2$ fold change for each experiment; and,
227 the most highly up- and downregulated genes were highlighted and defined as convergently
228 responding genes (CRGs). GO categories that were independently identified as enriched (FDR <
229 0.05) in both experiments were visualized by their respective delta-ranks of enrichment to
230 demonstrate the conservation of GO function across the thermal challenges (for details, see Dixon
231 *et al.* 2015).
232
233 ***Results***
234 Astrangia poculata *response to cold challenge*
235 Although behavioural responses of *A. poculata* to a food stimulus under control conditions varied,
236 nearly all colonies exhibited some polyp extension (Figure 2B). This contrasts with behaviours
237 observed under cold challenge, where rapid declines in polyp activity were observed by day eight
238 (12˚C) and most polyps remained inactive as cooler temperatures were reached (10˚C - 6˚C, Figure
239 2B). Overall, *A. poculata* polyp activity was significantly reduced under cold challenge ($p < 0.01$).
240 *A. poculata* gene expression was also significantly influenced by cold challenge: a strong treatment
241 effect on overall gene expression was observed (*Adonis* $p_{treatment} < 0.001$, Figure 2C), with cold

242 challenge resulting in 5318 (40%) DEGs (FDR < 0.05; 2244 (17%) upregulated; 1, 3074 (23%)
243 downregulated). Many GO terms were also enriched between cold challenge and control
244 conditions (FDR < 0.10; CC = 77, MF =50, BP = 78; Figure 3). Of these, notable GO terms include:
245 *myosin complex* (GO:0016459), *proteasome core complex* (GO:0005839), *translation regulator*
246 *activity, nucleic acid binding* (GO:0008135; GO:0090079), *extracellular matrix structural*
247 *constituent* (GO:0005201), *muscle system process* (GO:0006936; GO:0003012) and *proteolysis*
248 (GO:0006508).
249
250 Astrangia poculata *response to heat challenge*
251 Behavioural responses of *A. poculata* to a food stimulus under control conditions were stable and
252 coral polyps remained fully extended throughout the experiment (Day 7 - 14; Figure 2B). This
253 contrasts with behavioural responses under heat challenge, where corals exhibited less polyp
254 activity in response to food stimulus as temperatures increased. By the end of the experiment (day
255 16), only one colony under heat challenge was observed to have 100% polyp extension and half
256 of the colonies had less than 25% of their polyps extended (Figure 2B). Overall, *A. poculata* polyp
257 activity was significantly reduced under heat challenge ($p < 0.01$). *A. poculata* gene expression
258 was also significantly influenced by heat challenge: a significant effect of treatment on overall
259 gene expression was observed (*Adonis* $p_{treatment} < 0.001$, Figure 2C) with 1,054 (7.9%) DEGs (FDR
260 < 0.05; 410 (3.1%) upregulated; 644 (4.9%) downregulated. Many GO terms were significantly
261 enriched under heat challenge relative to control conditions (FDR < 0.10; CC = 34, MF = 47, BP
262 = 22; Figure 3). Notable GO terms include: *nematocyst* (GO:0042151), *proteasome core complex*
263 (GO:0005839), *threonine-type endopeptidase activity* (GO:0004298; GO:0070003), *unfolded*
264 *protein binding* (GO:0051082), *protein folding* (GO:0006457) and *response to cold*
265 (GO:0009409).

266

267 *Convergent response repertoires to heat and cold challenge in* Astrangia poculata

268 Both cold and heat thermal challenges induced a reduction in polyp activity in response to food
269 stimulus (Figure 2B). However, this reduction was more pronounced under cold challenge where
270 nearly all polyps were retracted by day 16. In addition, five times as many genes were differentially
271 expressed under cold challenge compared to heat challenge (Figure 4A). More than half (657 out
272 of 1054) of DEGs in the heat challenge experiment were also differentially expressed under cold
273 challenge, which is significantly more genes shared between experiments than would be expected
274 by chance (hypergeometric test, $p < 0.01$). Genes that were highly upregulated under both thermal
275 challenges include: *tumour necrosis receptor 3* (TRAF3), *Lon protease 2, peroxisomal* (LONP2),
276 and *increased sodium tolerance 1* (ITS1). Genes that were highly downregulated under both
277 thermal challenge treatments include: *DELTA-thalatoxin-AVl2a* (AVL2A), *myosin regulatory*
278 *light polypeptide 9* (MYL9), and *Protein-glucosylgalactosylhydroxylysine glucosidase* (PGGHG).
279 GO terms consistently enriched in both experiments were also visualized using experimental delta-
280 ranks of enrichment for each thermal challenge (FDR < 0.10; MF = 11, BP = 4, CC = 14, Figure
281 5A-C). These terms included *response to mechanical stimulus* (GO:0009612) and *locomotion*
282 (GO:0040011) as well as GO terms associated with the proteasome (GO:0004298, GO:0006515,
283 GO:0008540, GO:0022624, and GO:0005839).

284

285 ***Discussion***

286 *Modulation of genes associated with motor function and stress response in* Astrangia poculata
287 *under cold challenge*

288 *Astrangia poculata* from Woods Hole represent the most northern range for this species with corals
289 experiencing a wide range of temperatures throughout the year (Figure 1B). Given this temperature

290 range, it was surprising that such strong behavioural and transcriptomic responses were observed
291 under cold challenge (Figure 2B; 2C). This reduction in polyp activity under cold temperatures is
292 consistent with field observations during winter months, when corals fail to respond to stimuli (e.g.
293 quiescence, Grace 2017). The dormant polyp behaviour observed here might be interpreted as
294 quiescence. However, very little is known about coral quiescence.

295 In mammalian cells, quiescent cells increase expression of certain myosin genes, notably
296 *myosin heavy chain 10* (MYH10; Hong *et al.* 2015), which is the opposite pattern observed here
297 under cold challenge (MYOH10 downregulated; Figure 3A). However, cold challenge did cause
298 downregulation of other muscle responses, including *muscle system process* (MSP; GO:0006936;
299 GO:0003012) and *myosin complex* (GO:0016459; Figure 3C), which corresponds with decreased
300 *A. poculata* polyp activity under cold challenge. In contrast, *myosin-Ie* (MYO1E), which is an
301 important gene for clathrin-mediated endocytosis and immunity was significantly upregulated
302 under cold challenge. This result is consistent with previous work exploring how mice respond to
303 cold stress (Wenzel *et al.* 2015) and myosins as a whole are often upregulated in bleached corals
304 under heat stress (Desalvo *et al.* 2008) so it is possible that regulation of this gene is more likely
305 related to immunity rather than muscle movement directly. The transcriptional responses match
306 behavioral data under cold challenge, putting forth the hypothesis that reduced polyp activity under
307 cold challenge may be regulated by downregulation of key MSP genes.

308

309 Ultimately, stress may reorganize the actin cytoskeleton in *A. poculata* under cold
310 challenge, given the downregulation of *extracellular matrix structural constituent* (EMSC;
311 GO:0005201). Many collagen genes are categorized within the EMSC GO category and collagen
312 plays an integral role in forming the structure of the extracellular matrix (Kielty & Grant 2003).

313 Collagen genes have been previously shown to be highly reactive to a variety of stressors in corals
314 (Traylor-Knowles 2019), which, in conjunction with these data, suggests that cold challenges lead
315 to decreases in muscular movement and overall motor processes in aposymbiotic *A. poculata*, and
316 potentially other corals.

317 Genes associated with *translation regulator activity nucleic acid binding* (GO:0008135;
318 GO:0090079) were upregulated in *A. poculata* under cold challenge. Genes in this category are
319 mostly composed of *eukaryotic translation initiation factor 4 gamma* (EIF4G) genes, which are
320 consistently upregulated under a wide range of stressors, including temperature, osmotic stress and
321 nutrient deprivation (Jones *et al.* 2013). Interestingly, EIF4G genes may play important roles for
322 higher latitude species, like *A. poculata* here, as only northern populations of the porcelain crab
323 *Petrolisthes cinctipes* upregulated EIF4G genes in response to cold stress (Stillman & Tagmount
324 2009). Given that it has already been shown that different *A. poculata* populations exhibit broadly
325 different thermal responses (Aichelman *et al.* 2019), future work contrasting gene expression
326 responses to stress across populations would be worthwhile.

327 Another GO category that demonstrated strong upregulation in *A. poculata* under cold
328 challenge was *proteasome core complex* (PCC; GO:0005839). PCC upregulation has been
329 previously observed in tropical corals under heat stress (Seneca & Palumbi 2015), however, this
330 is the first to associate PCC upregulation in response to cold challenge. The majority of PCC genes
331 are involved in the functioning of the 20S core proteasome, which is responsible for degradation
332 of oxidized proteins (Davies 2001). Additionally, proteasomes are required for internal proteolysis
333 of p105 into p50 to activate nuclear factor-κB (NF-κB) (Rape & Jentsch 2002). NF-κB is a key
334 pathway in coral innate immunity and is upregulated during stress-induced bleaching in sea
335 anemones (Mansfield *et al.* 2017).

336 Overall, cold challenge elicited strong effects on both behavior and transcriptomic profiles
337 of *A. poculata* (Figure 2C); however, these patterns do not align with quiescence. Instead, these
338 signatures are consistent with stress responses described in previous cnidarian studies and
339 emphasize that consistent results between obligate tropical corals and aposymbiotic corals serve
340 to highlight the host's response to thermal challenges even in the absence of symbionts.
341
342 *Modulation of genes associated with heterotrophy and stress response in* Astrangia poculata *under*
343 *heat challenge*
344 Even though summer temperatures at Woods Hole over the last 10 years were much lower
345 than temperatures achieved during the experimental heat challenge here (Figure 1B), we observed
346 that *A. poculata* exhibited more muted behavioural and transcriptomic responses when compared
347 to responses to cold challenge (Figure 2B; 2C). While *A. poculata* significantly reduced polyp
348 activity in response to food stimulus under heat challenge, the majority of corals maintained some
349 extension even at warm extremes. Unlike naturally observed polyp inactivity during winter months
350 (Grace 2017), this is the first observation of decreased polyp activity due to warm temperatures in
351 *A. poculata*.
352 Interestingly, we observed downregulation of genes associated with *nematocyst*
353 (GO:0042151) under heat challenge, which are cnidarian stinging cells used to capture food
354 (Holstein & Tardent 1984). Tropical corals have also been observed to reduce feeding rates under
355 heat stress (Ferrier-Pagès *et al.* 2010). Taken together, the decreased polyp extension and
356 downregulation of genes associated with nematocysts (Fig. 2B, 3A), suggest reduced opportunity
357 for heterotrophy in *A. poculata.* Given that heterotrophy has been shown to mitigate coral
358 bleaching in another facultatively symbiotic coral (*Oculina arbuscula;* Aichelman *et al.* 2016),

359 this reduction in heterotrophy, in addition to stress associated with increased temperatures, would
360 be interesting to explore in aposymbiotic and symbiotic colonies.

361 Consistent with previous work in heat challenged cnidarians, we observed upregulation of
362 many mitochondria-related GO terms in heat challenged *A. poculata* (Figure 3). Mitochondria are
363 fundamental in the regulation of cellular stress and have a dedicated unfolded protein response,
364 which influences free radical detoxification and innate immunity in tropical corals (Dimos *et al.*
365 2019). We observed enrichment in both *protein folding* (GO:0006457) and *unfolded protein*
366 *binding* (GO:0051082) under heat challenge, which is consistent with a variety of coral stress
367 studies (i.e. Dixon et al. 2020). Genes within these GO categories are largely associated with heat
368 shock protein production, which have been consistently implicated in coral gene expression studies
369 (reviewed in Cziesielski *et al.* 2019) and heat stress experiments across a wide range of taxa
370 (reviewed in Chen *et al.* 2018). Unexpectedly, we observed upregulation of *response to cold*
371 (GO:0009409), which is a salient example of how expression of some genes are often associated
372 with a specific stressor, when in reality their expression is more likely a universal environmental
373 stress response (ESR).

374 *Cold challenge elicits a much stronger response than heat challenge in* A. poculata

375 Our data demonstrate that *A. poculata* exhibit greater behavioural and transcriptomic
376 responses to the cold challenge applied here when compared to heat challenge, which is surprising
377 considering that cold challenge temperatures were within *A. poculata's* thermal range, while heat
378 challenge temperatures were not (Figure 1B). In fact, the heat challenge exceeded any temperature
379 experienced within their native environment over the last decade. Few studies have directly
380 contrasted a coral's response to thermal extremes in parallel, and studies that have demonstrated
381 mixed results. In a tropical coral (*Acropora millepora*), Nielsen *et al.* (2020) observed improved

382 coral condition under cold temperatures relative to ambient or heated conditions. In contrast to our
383 results, heat stress causes a larger bleaching response than cold stress in *Aiptasia* (Bellis & Denver
384 2017). Conversely, Roth & Deheyn (2013) found that acute cold stress was more detrimental to
385 the tropical coral *Acropora yongei* than heat stress, but did suggest that heat stress may be more
386 detrimental over longer temporal scales. In a study investigating the responses of oysters
387 (*Crassostrea gigas*) to heat and cold stress, Zhu *et al.* (2016) observed similar transcriptional
388 responses to both stressors. While there is no clear consensus among studies, it is widely accepted
389 that the specific temperatures reached in each stress treatment and the rate at which those
390 temperatures are reached are both important factors (McLachlan *et al.* 2020). Heat challenge in
391 our study may have elicited a more muted response, because *A. poculata* were collected in the
392 summer, so these colonies were likely acclimated to warmer conditions, which would have made
393 the cold challenge more stressful.
394
395 Astrangia poculata *exhibits a convergent stress response repertoire to cold and heat challenge*
396 Despite highly divergent temperatures reached between temperature challenge
397 experiments, we observed convergent behavioral and transcriptomic responses in *Astrangia*
398 *poculata*. First, we observed reductions in feeding behaviour under both thermal challenges
399 (Figure 2B), which were corroborated with convergent downregulation of genes associated with
400 *locomotion* (GO: 0040011) and *response to mechanical stimulus* (GO: 0009612). *DELTA-*
401 *thalatoxin-Avl2a* (AVL2A) was downregulated under both challenges; thalatoxin and other toxins
402 are used while feeding in cnidarians (Schmidt *et al.* 2019) and are categorized under the *nematocyst*
403 (GO:0042151) GO category. Furthermore, *myosin regulatory light polypeptide 9* (MYL9) was
404 downregulated under both thermal challenges (Figure 4B) and this gene plays an important role in

405 cell contractile activity via phosphorylation (Kumar *et al.* 1989) and may be instrumental for coral
406 heterotrophy. Reduced polyp activity under thermal challenges may be due to temperatures
407 exceeding local high and low temperatures or corals could be entering quiescent states, where
408 lowered metabolic activity acts as an adaptation to extreme temperatures (Stuart & Brown 2006).
409 Our transcriptomic results do not support quiescence and instead suggest large scale protein
410 catabolism, which often occurs during starvation after an organism has metabolized most of its
411 carbohydrate and lipid stores (Kaur & Debnath 2015; Davies *et al.* 2016). Increases in catabolic-
412 related pathways point instead to high energetic demands associated with stress-related cell
413 functions at both thermal extremes (Kültz 2005).

414 The other major convergent response observed under both thermal challenges was a
415 generalized stress response. For example, *glutathione transferase activity* (GO: 0004364) was
416 upregulated under both temperature extremes and this GO term is associated with detoxification
417 of environmental pollutants and oxidative stress response in tropical corals (Downs *et al.* 2005).
418 In addition, most enriched GO categories observed in both thermally-challenged *A. poculata* were
419 involved in maintenance of the proteasome (Figure 5A-C). The role of the proteasome (discussed
420 above) is integral to degradation and catabolism of oxidized proteins (Davies 2001) and may be
421 important for the activation of NF-kB under stress (Rape & Jentsch 2002). These enriched GO
422 terms across wide thermal challenges highlight conserved ESR pathways under both heat and cold
423 thermal challenges.

424 In addition to convergently enriched GO terms, a number of individual genes were
425 differentially expressed under both challenges. *Lon protease homolog 2, peroxisomal* (LONP2)
426 was highly up-regulated in both experiments and this gene is involved in degradation of
427 oxidatively damaged mitochondrial genes (Yang *et al.* 2018). LONP2 has been shown to be

428 upregulated under high temperatures and under heavy metal stress in oysters (Sanni *et al* 2008).
429 Additionally, *protein-glucosylgalactosylhydroxylysine glucosidase* (PGGHG) was downregulated
430 under both thermal challenges (Figure 4B). PGGHG is a catalyst for the hydrolysis of glucose in
431 hydroxylysine-linked residues of collagen (and collagen-like) proteins (Hamazaki & Hamazaki
432 2016). It is also a major component of isolated collagens from other marine invertebrates (e.g. sea
433 anemones, Katzman *et al.* 1972), which are very reactive to a range of stressors (Traylor-Knowles
434 2019). Taken together, LONP2 and PGGHG play important roles in aposymbiotic *A. poculata*'s
435 stress response.

436 The mitogen-activated protein kinase (MAPK) signaling pathway is key for mediating cell
437 differentiation and apoptosis (Whitmarsh 2010) and has been previously implicated in a coral's
438 response to environmental stimuli (Sun *et al.* 2013). *A. poculata* consistently upregulated
439 *increased sodium tolerance 1* (IST1) under both thermal challenges, which is also known as
440 *putative MAPK-activating protein* (PM28; Figure 4B). In addition to IST1, *Tumour necrosis*
441 *factor receptor 3* (TRAF3) was also highly upregulated under both thermal stressors (Figure 4B).
442 TRAF3 is an intracellular signaling molecule that regulates MAPK activity and nuclear factor-κB
443 (Nf-κB) signaling (Häcker 2011), which has been shown to be upregulated during stress-induced
444 bleaching in *Aiptasia* (Mansfield *et al.* 2017). TRAF3 is constitutively upregulated or "front-
445 loaded" in corals that are tolerant to heat stress (DeSalvo *et al.* 2010; Barshis *et al.* 2013; Seneca
446 & Palumbi 2015) and is upregulated under low magnesium (Yuyama & Higuchi 2019), white band
447 disease (Libro *et al.* 2013), and high carbon dioxide treatments (Kaniewska *et al.* 2012) in various
448 coral species. Our results provide supporting evidence that TRAF3, along with IST1 and LONP2,
449 may be part of the coral ESR and demonstrate consistent upregulation in response to various
450 stressors, not just high temperatures.

451

452 ***Conclusions***

453 While stress response repertoires of tropical reef-building corals have been widely studied,
454 especially in response to upper thermal extremes, this study represents the first to characterize the
455 stress response of a naturally aposymbiotic coral to divergent thermal challenges. Our results
456 demonstrate a strong response to cold challenge and a comparatively muted response to heat
457 challenge. In addition, we provide evidence for a convergent stress response to divergent thermal
458 challenges in *A. poculata* that is consistent with responses observed for tropical obligate coral
459 species, which is surprising given the absence of symbiont-associated reactive oxygen species.
460 The repertoire of convergent responses to thermal challenges highlighted here will provide the
461 foundation for future research to investigate how symbiosis influences the coral stress response.
462 We identified a number of genes that are differentially regulated under both thermal challenges,
463 suggesting a universal stress response in a core set of CRGs. This work highlights the benefits to
464 studying facultatively symbiotic corals to disentangle stress responses of the coral host from their
465 algal symbionts, and future work leveraging this facultative relationship may lead to a stronger
466 mechanistic understanding of why coral dysbiosis is increasing in frequency in corals worldwide.

467

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Data Availability

All sequences are available from the NCBI SRI under accession PRJNA595158. Code for all analyses are attached in supplementary materials, and are also available at https://github.com/wuitchik along with transcriptome files.

Author Contributions

S.W.D designed the experiment. A.A., S.A.B., J.D.C., M.B.L., J.L.R., M.K.S., and I.F.T. conducted the experiment. B.E.B. and C.L.R. completed all molecular work and TagSeq library preparations. D.M.W. performed all statistical and bioinformatic analyses and drafted the manuscript. S.W.D. supervised the experiment, analyses and co-authored the manuscript. All authors edited and approved the manuscript.

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Zhu Q, Zhang L, Li L, Que H, Zhang G (2016) Expression Characterization of Stress Genes Under High and Low Temperature Stresses in the Pacific Oyster , Crassostrea gigas. *Marine Biotechnology*, **18**, 176–188.

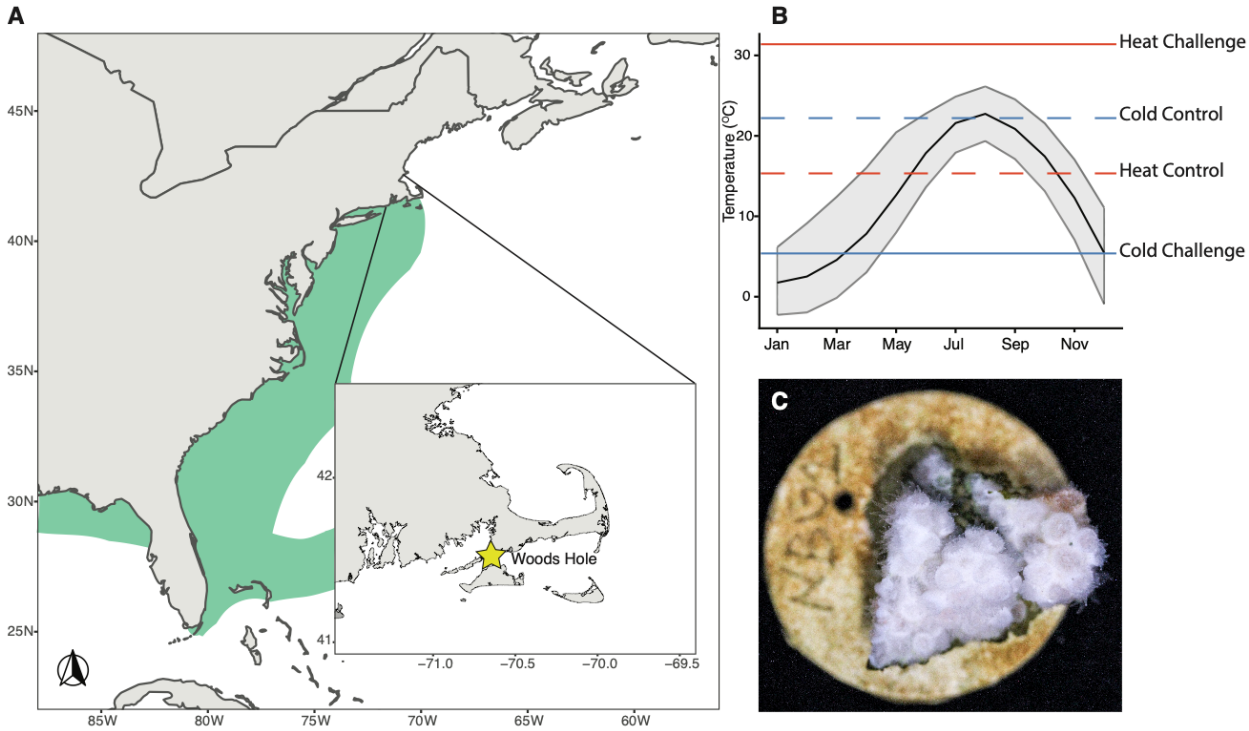


Figure 1 | A) Map of the eastern seaboard of the United States with the *Astrangia poculata* range in green. Inset shows the Woods Hole collection site denoted with a yellow star (distributions

695 based on Thornhill *et al.* 2008). **B)** Seasonal temperature profile at Woods Hole averaged over ten
696 years (2008-2018). The black solid line indicates mean monthly temperatures with mean monthly
697 maximum and minimum temperatures in grey. Temperatures (˚C) of thermal challenge
698 experimental controls (dashed lines) and treatments (solid lines) are superimposed with cold
699 challenge treatments in blue and heat challenge treatments in red. Seasonal temperatures were
700 obtained from the National Oceanic and Atmospheric Administration weather buoy #BZBM3. **C)**
701 Picture of an aposymbiotic *A. poculata* colony fragment.
702
703
704

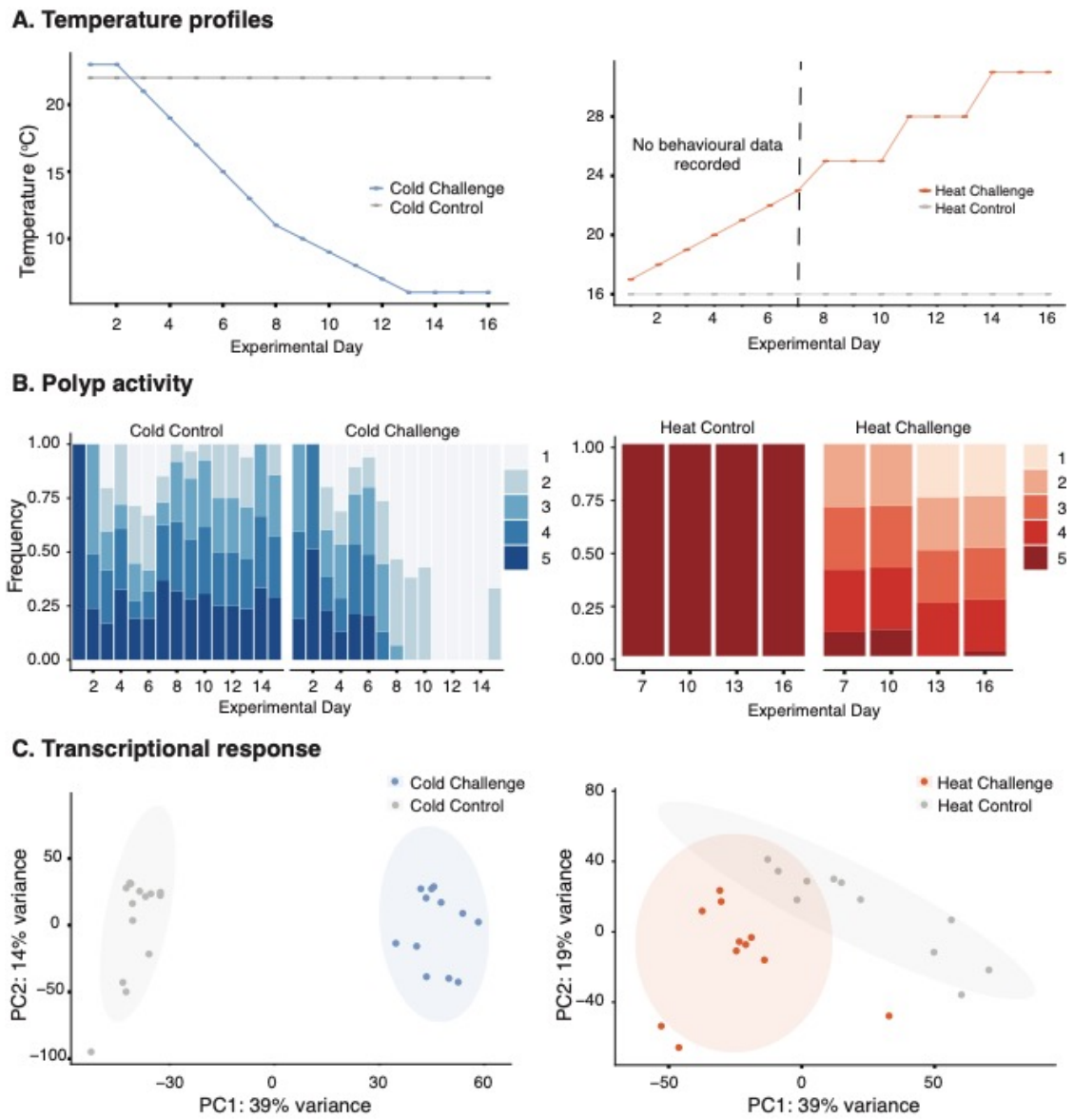
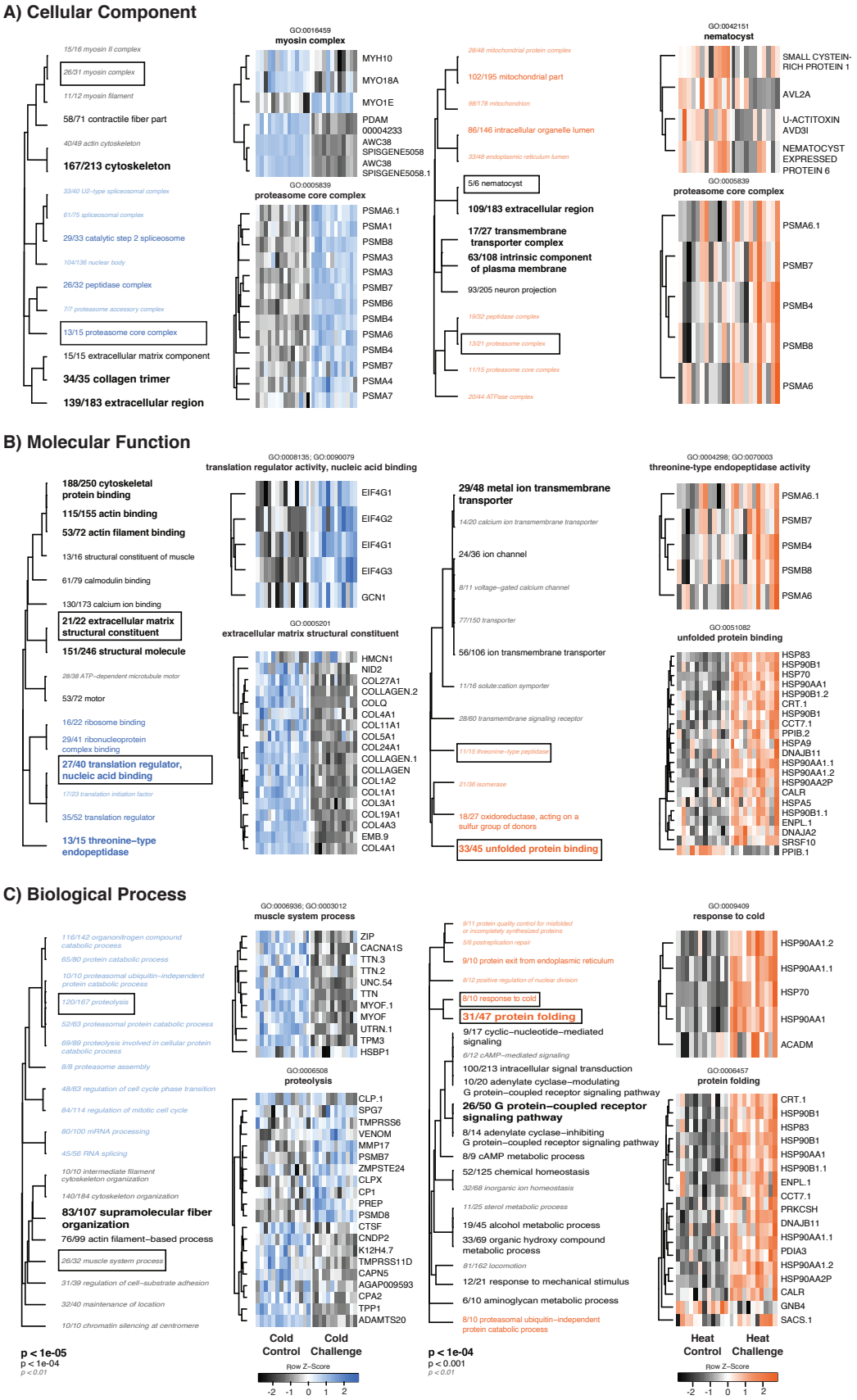
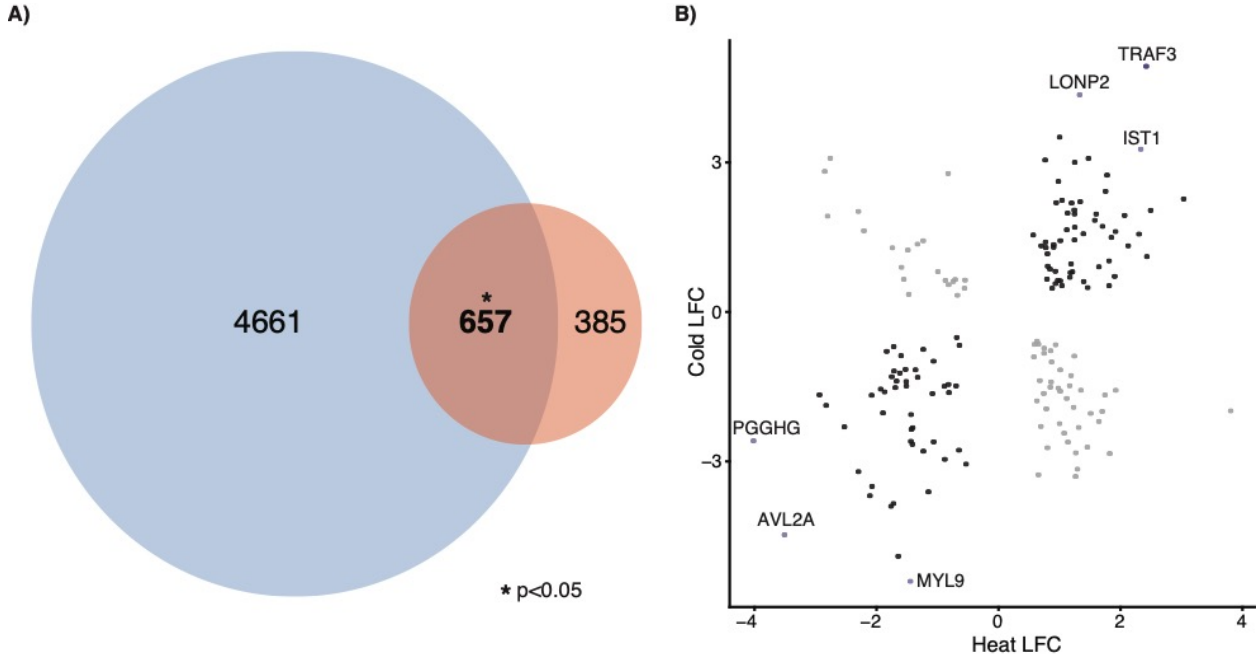


Figure 2 | Thermal challenge experiments on *Astrangia poculata.* Left: cold challenge, Right: heat challenge. A) 16-day temperature ramp. B) Polyp activity scored based on the proportion of polyps extended per fragment (1 = 0%, 2 = 25%, 3 = 50%, 4 = 75%, 5 = 100%) in response to food stimuli across the 16-day experiments. Note that behavioral data collection in the heat challenge experiment did not commence until day 7. C) Principal component analysis of overall gene expression of samples under control and thermal challenge at day 16. Percentages represent the

712 total variance explained by each axis and shaded areas are 95% confidence ellipses. P-value
713 indicates significance of treatment using a permutational multivariate analysis of variance.
714


A) Cellular Component
15/16 myosin II complex
26/31 myosin complex
11/12 myosin filament
58/71 contractile fiber part
40/49 actin cytoskeleton
167/213 cytoskeleton
33/40 U2-type spliceosomal complex
61/75 spliceosomal complex
29/33 catalytic step 2 spliceosome
104/136 nuclear body
26/32 peptidase complex
7/7 proteasome accessory complex
13/15 proteasome core complex
15/15 extracellular matrix component
34/35 collagen trimer
139/183 extracellular region
GO:0016459
myosin complex
MYH10
MYO18A
MYO1E
PDAM
00004233
AWC38
SPISGENE5058
AWC38
SPISGENE5058.1
GO:0005839
proteasome core complex
PSMA6.1
PSMA1
PSMB8
PSMA3
PSMA3
PSMB7
PSMB6
PSMB4
PSMA6
PSMB4
PSMB7
PSMA4
PSMA7
28/48 mitochondrial protein complex
102/195 mitochondrial part
98/178 mitochondrion
86/146 intracellular organelle lumen
33/48 endoplasmic reticulum lumen
5/6 nematocyst
109/183 extracellular region
17/27 transmembrane transporter complex
63/108 intrinsic component of plasma membrane
93/205 neuron projection
19/32 peptidase complex
13/21 proteasome complex
11/15 proteasome core complex
20/44 ATPase complex
GO:0042151
nematocyst
SMALL CYSTEIN-RICH PROTEIN 1
AVL2A
U-ACTITOXIN AVD3I
NEMATOCYST EXPRESSED PROTEIN 6
GO:0005839
proteasome core complex
PSMA6.1
PSMB7
PSMB4
PSMB8
PSMA6
B) Molecular Function
188/250 cytoskeletal protein binding
115/155 actin binding
53/72 actin filament binding
13/16 structural constituent of muscle
61/79 calmodulin binding
130/173 calcium ion binding
21/22 extracellular matrix structural constituent
151/246 structural molecule
28/38 ATP-dependent microtubule motor
53/72 motor
16/22 ribosome binding
29/41 ribonucleoprotein complex binding
27/40 translation regulator, nucleic acid binding
17/23 translation initiation factor
35/52 translation regulator
13/15 threonine-type endopeptidase
GO:0008135; GO:0090079
translation regulator activity, nucleic acid binding
EIF4G1
EIF4G2
EIF4G1
EIF4G3
GCN1
GO:0005201
extracellular matrix structural constituent
HMCN1
NID2
COL27A1
COLLAGEN.2
COLQ
COL4A1
COL11A1
COL5A1
COL24A1
COLLAGEN.1
COLLAGEN
COL1A2
COL1A1
COL3A1
COL19A1
COL4A3
EMB.9
COL4A1
29/48 metal ion transmembrane transporter
14/20 calcium ion transmembrane transporter
24/36 ion channel
8/11 voltage-gated calcium channel
77/150 transporter
56/106 ion transmembrane transporter
11/16 solute:cation symporter
28/60 transmembrane signaling receptor
11/15 threonine-type peptidase
21/36 isomerase
18/27 oxidoreductase, acting on a sulfur group of donors
33/45 unfolded protein binding
GO:0004298; GO:0070003
threonine-type endopeptidase activity
PSMA6.1
PSMB7
PSMB4
PSMB8
PSMA6
GO:0051082
unfolded protein binding
HSP83
HSP90B1
HSP70
HSP90AA1
HSP90B1.2
CRT.1
HSP90B1
CCT7.1
PPIB.2
HSPA9
DNAJB11
HSP90AA1.1
HSP90AA1.2
HSP90AA2P
CALR
HSPA5
HSP90B1.1
ENPL.1
DNAJA2
SRSF10
PPIB.1
C) Biological Process
116/142 organonitrogen compound catabolic process
65/80 protein catabolic process
10/10 proteasomal ubiquitin-independent protein catabolic process
120/167 proteolysis
52/63 proteasomal protein catabolic process
69/89 proteolysis involved in cellular protein catabolic process
8/8 proteasome assembly
48/63 regulation of cell cycle phase transition
84/114 regulation of mitotic cell cycle
80/100 mRNA processing
45/56 RNA splicing
10/10 intermediate filament cytoskeleton organization
140/184 cytoskeleton organization
83/107 supramolecular fiber organization
76/99 actin filament-based process
26/32 muscle system process
31/39 regulation of cell-substrate adhesion
32/40 maintenance of location
10/10 chromatin silencing at centromere
p < 1e-05
p < 1e-04
p < 0.01
GO:0006936; GO:0003012
muscle system process
ZIP
CACNA1S
TTN.3
TTN.2
UNC.54
TTN
MYOF.1
MYOF
UTRN.1
TPM3
HSBP1
GO:0006508
proteolysis
CLP.1
SPG7
TMPRSS6
VENOM
MMP17
PSMB7
ZMPSTE24
CLPX
CP1
PREP
PSMD8
CTSF
CNDP2
K12H4.7
TMPRSS11D
CAPN5
AGAP009593
CPA2
TPP1
ADAMTS20
Cold Control
Cold Challenge
Row Z-Score
-2 -1 0 1 2
9/11 protein quality control for misfolded or incompletely synthesized proteins
5/6 postreplication repair
9/10 protein exit from endoplasmic reticulum
8/12 positive regulation of nuclear division
8/10 response to cold
31/47 protein folding
9/17 cyclic-nucleotide-mediated signaling
6/12 cAMP-mediated signaling
100/213 intracellular signal transduction
10/20 adenylate cyclase-modulating G protein-coupled receptor signaling pathway
26/50 G protein-coupled receptor signaling pathway
8/14 adenylate cyclase-inhibiting G protein-coupled receptor signaling pathway
8/9 cAMP metabolic process
52/125 chemical homeostasis
32/68 inorganic ion homeostasis
11/25 sterol metabolic process
19/45 alcohol metabolic process
33/69 organic hydroxy compound metabolic process
81/162 locomotion
12/21 response to mechanical stimulus
6/10 aminoglycan metabolic process
8/10 proteasomal ubiquitin-independent protein catabolic process
p < 1e-04
p < 0.001
p < 0.01
GO:0009409
response to cold
HSP90AA1.2
HSP90AA1.1
HSP70
HSP90AA1
ACADM
GO:0006457
protein folding
CRT.1
HSP90B1
HSP83
HSP90B1
HSP90AA1
HSP90B1.1
ENPL.1
CCT7.1
PRKCSH
DNAJB11
HSP90AA1.1
PDIA3
HSP90AA1.2
HSP90AA2P
CALR
GNB4
SACS.1
Heat Control
Heat Challenge
Row Z-Score
-2 -1 0 1 2

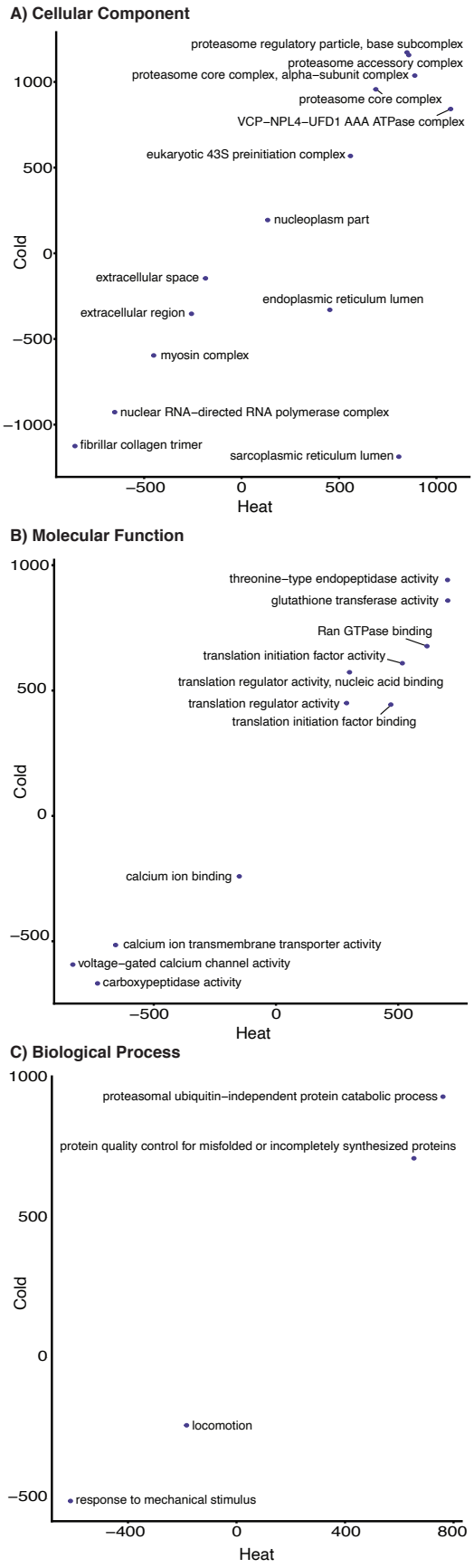
716 **Figure 3 |** Gene ontology (GO) enrichment under thermal challenges: Left: cold challenge, Right:
717 heat challenge. Enriched GO terms of A) Cellular Components B) Molecular Functions, and C)
718 Biological Processes were determined via Mann-Whitney U tests. Font size and boldness of text
719 corresponds to p-values with colour designating directionality of enrichment (blue: cold challenge,
720 red: heat challenge, black: controls). GO terms are clustered based on the number of shared genes
721 between categories. Hierarchical clustered heatmaps were generated from annotated DEGs with a
722 highlighted GO term (black box) and each row was labelled with its gene symbol. Colors denote
723 magnitude of response (blue: upregulated in cold challenge, red: upregulated in heat challenge)
724 through z-score of the difference in expression levels from that of mean expression for each gene.
725



726
727

728 **Figure 4 |** Convergent transcriptomic response of *Astrangia poculata* to thermal challenges. A)
729 Venn diagram of differentially expressed genes shared (intersection) between cold (blue) and heat
730 (red) challenge experiments. B) Of these 657 shared DEGs, those with annotations are visualized

731 by their respective Log_2fold change (LFC) in each experiment. Genes with consistent direction in
732 their respective LFC are designated as convergently responsive genes (CRGs) depicted as black
733 circles and key CRGs are highlighted in purple and labeled by gene symbol. Grey circles are
734 divergent in response to thermal challenges.
735


A) Cellular Component
proteasome regulatory particle, base subcomplex
proteasome accessory complex
proteasome core complex, alpha−subunit complex
proteasome core complex
VCP−NPL4−UFD1 AAA ATPase complex
eukaryotic 43S preinitiation complex
nucleoplasm part
extracellular space
endoplasmic reticulum lumen
extracellular region
myosin complex
nuclear RNA−directed RNA polymerase complex
fibrillar collagen trimer
sarcoplasmic reticulum lumen
Cold
Heat
B) Molecular Function
threonine−type endopeptidase activity
glutathione transferase activity
Ran GTPase binding
translation initiation factor activity
translation regulator activity, nucleic acid binding
translation regulator activity
translation initiation factor binding
calcium ion binding
calcium ion transmembrane transporter activity
voltage−gated calcium channel activity
carboxypeptidase activity
Cold
Heat
C) Biological Process
proteasomal ubiquitin−independent protein catabolic process
protein quality control for misfolded or incompletely synthesized proteins
locomotion
response to mechanical stimulus
Cold
Heat

737 **Figure 5 |** Correlation of GO delta-ranks which is the difference between mean rank of genes
738 belonging to the GO category A) Molecular Function B) Biological Process, and C) Cellular
739 Component, and mean rank of all other genes. Positive values indicate enrichment with up-
740 regulated genes.

741

742 **Table 1 |** Summary of distribution of coral genotypes among treatments for the A) cold challenge
743 and B) heat challenge experiment. Numbers in cells represent the number of fragments of each
744 genotype in each treatment; numbers in parentheses represent the number of fragments that were
745 successfully sequenced via TagSeq.
746

A) Cold Challenge Experiment			B) Heat Challenge Experiment		
Genotype	Control (TagSeq)	Challenge (TagSeq)	Genotype	Control (TagSeq)	Challenge (TagSeq)
D	1 (1)	1 (0)	A	1 (1)	3 (1)
E	3 (2)	3 (1)	B	3 (2)	3 (2)
F	3 (2)	3 (2)	C	3 (2)	3 (0)
G	2 (0)	2 (1)	D	3 (2)	3 (1)
H	3 (2)	1 (1)	F	1 (1)	1 (0)
I	2 (2)	2 (0)	K	2 (0)	2 (1)
J	3 (2)	3 (2)	N	3 (0)	1 (1)
P	1 (1)	3 (2)	O	2 (1)	2 (2)
T	3 (2)	3 (3)	Q	3 (3)	3 (2)
Total	21 (14)	21 (12)		21 (12)	21 (10)

747

748
749