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Characterizing environmental stress responses of aposymbiotic *Astrangia poculata* to divergent thermal challenges

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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 remain understudied. Disentangling the role of symbiosis on the stress response in obligately symbiotic corals is challenging because this response is inherently coupled with nutritional stress. Here, we leverage aposymbiotic colonies of the facultatively symbiotic coral, *Astrangia poculata*, which lives naturally with and without its algal symbionts, to examine how broad thermal challenges influence coral hosts in the absence of symbiosis. *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 behavioural responses to food stimuli and genome-wide gene expression profiling (TagSeq) were performed. Both thermal challenges elicited significant reductions in polyp extension. However, there were five times as many differentially expressed genes (DEGs) under cold challenge compared to heat challenge. Despite an overall stronger response to cold challenge, there was significant overlap in DEGs between thermal challenges. We contrasted these responses to a previously identified module of genes associated with the environmental stress response (ESR) in tropical reef-building corals. Cold challenged corals exhibited a pattern consistent with more severe stressors while the heat challenge response was consistent with lower intensity stressors. Given that these responses were observed in aposymbiotic colonies, many genes previously implicated in ESRs in tropical symbiotic species may represent the coral host's stress response in or out of symbiosis.

KEYWORDS
astrangia poculata, coral, gene expression, reef, temperature stress, transcriptomics

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

Research on coral bleaching has often focused on responses to elevated temperatures in tropical reef-building corals (for review, see Cziesielski et al., 2019). However, tropical corals can bleach in response to a variety of stressors, including high nutrients (Wiedenmann et al., 2013), ocean acidification (Anthony et al., 2008), pathogens (Ben-Haim & Rosenberg, 2002), low salinity (Goreau, 1964), chemical exposures (Cervino et al., 2003), and cold stress (Saxby et al., 2003). Coral responses to cold stress remain understudied, even though these events can have substantial impacts on reefs. For example, a cold-water bleaching event in 2010 decimated inshore coral populations along the Florida reef tract (Lirman et al., 2011), and cold water has caused bleaching on the Great Barrier Reef (Hoegh-Guldberg & Fine, 2004). While increasing temperatures are the most imminent threat to coral reefs, cold water extremes also represent relevant thermal challenges to coral species and are rarely investigated in parallel (but see Nielsen et al., 2020; Parkinson et al., 2018; Roth & Deheyn, 2013).

One way to monitor responses to stress is to characterize changes in gene expression profiles, which provide a snapshot into the physiological state of an organism and offer insights into the biological processes, molecular functions, and cellular components that corals engage to tolerate various stressors. Modern transcriptomics have demonstrated that corals mount dynamic responses to pollutants (Gust et al., 2014; Ruiz-Ramos et al., 2017), pH (Davies et al., 2016; Moya et al., 2012) and bacterial challenges (Fuess et al., 2017; Wright et al., 2017) and considerable efforts have been made to understand how corals respond to heat challenges (for review see Cziesielski et al., 2019). Interestingly, core patterns of gene expression consistently emerge from these different stressors and have been termed the "environmental stress response (ESR)" (Barshis et al., 2013; Dixon et al., 2020). For example, (DeSalvo et al., 2012) demonstrate that exposing corals to darkness elicited transcriptomic responses that mirrored the thermal stress response, suggesting that different stressors elicit a core transcriptomic ESR. Barshis et al. (2013) demonstrated that corals exhibited a core stress response across thousands of genes, and this ESR was consistent with the conserved response of yeast under diverse environmental stressors (Gasch et al., 2000). More recently, a meta-analysis comparing the transcriptomic responses of coral from the genus *Acropora* to various stressors found that these coral exhibited stereotyped ESRs (Dixon et al., 2020) with consistent upregulation of genes involved in cell death, response to reactive oxygen species, NF-kB signalling, immune response, protein folding, and protein degradation in response to a variety of stress exposures. This research highlights that testing a single stressor cannot elucidate whether genes being expressed are unique to the stressor or emerge from a more generalized ESR.

Most work exploring the stress responses of corals has focused on tropical reef-building corals. For example, a recent review (Cziesielski et al., 2019) on coral heat stress studies contained no examples of subtropical or temperate coral responses to stress. Tropical corals largely live in oligotrophic waters where they receive most of their energetic requirements from their algal symbionts (Muscatine et al., 1984). Because energy deprivation in coral hosts results from any mechanism of symbiont loss (Baena-González & Sheen, 2008), uncoupling a thermal stress response from an energy deprivation response is challenging. Furthermore, given that many tropical corals exhibit an obligate symbiotic relationship, it is difficult to disentangle the host's stress response to extreme temperatures from the host's response to stress-induced algal byproducts (i.e., reactive oxygen species (ROS); McGinty et al., 2012) and the resulting energy deprivation from this dysbiosis (the breakdown of symbiosis). However, this focus on tropical species has left several species of subtropical and temperate reef-building corals that exhibit facultative symbioses relatively understudied and they represent promising avenues for better understanding environmental stress responses in corals.

The Northern Star Coral (*Astrangia poculata*) exhibits a facultatively symbiotic relationship with *Breviolum psygmophilum* (LaJeunesse et al., 2012) and can be found in sympatry in varying symbiotic states that are visually distinguishable by colour. Symbiotic colonies appear brown due to high densities of *B. psygmophilum*, and much like a bleached coral, some *A. poculata* appear white (Figure 1) due to very low algal densities (Dimond & Carrington, 2007). This white phenotype is commonly referred to as "aposymbiotic" (Burmester et al., 2018; Grace, 2017; Sharp et al., 2017), however its white coloration does not guarantee that these colonies are truly symbiont free, as aposymbiotic *A. poculata* are known to harbour background populations of algal symbionts (approximately 1×10^5/cm^2; Dimond & Carrington, 2008). However, these corals are effectively nonsymbiotic given that the algal symbiont population is too small to have a significant physiological effect on the coral holobiont (see Jacques et al., 1983).

Unlike obligate symbiotic corals, *A. poculata* can thrive in its aposymbiotic state relying only on heterotrophy (Dimond & Carrington, 2007). Cnidarian hosts that are not associated with algae in the family Symbiodiniacaea must rely on heterotrophy and the tentacles of *A. poculata* are studded with nematocysts, which aid in the capture of planktonic prey (for review of coral feeding, see Goldberg, 2018). The extension and foraging behaviours of these tentacles can be visually scored (see Burmester et al., 2018) to assess the environmental influence on polyp activity, which can be used as a proxy for heterotrophy. Additionally, *A. poculata* experience large seasonal variation in temperature at its northern range (–2 to 26°C; Figure 1), making these coral populations ideal models for investigating how

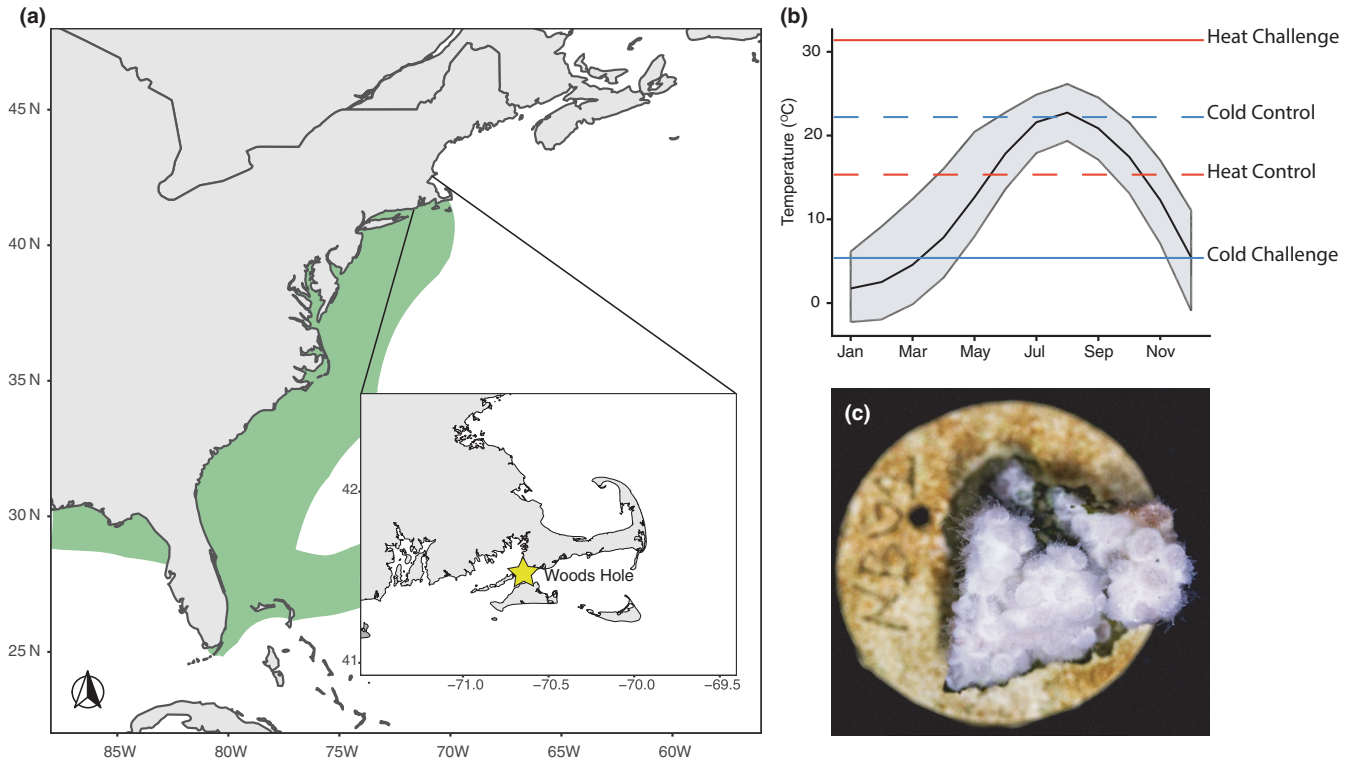

FIGURE 1 (a) Map of the eastern seaboard of the United States with the *Astrangia poculata* range in green. Inset shows the Woods Hole collection site denoted with a yellow star (distributions based on Thornhill et al., 2008). (b) Seasonal temperature profile at Woods Hole averaged over 10 years (2008–2018). The black solid line indicates mean monthly temperatures with mean monthly maximum and minimum temperatures in grey. Temperatures (°C) of thermal challenge experimental controls (dashed lines) and treatments (solid lines) are superimposed with cold challenge treatments in blue and heat challenge treatments in red. Seasonal temperatures were obtained from the National Oceanic and Atmospheric Administration weather buoy no. BZBM3. (c) Picture of a typical fragment of aposymbiotic *A. poculata* colony fragment used in both experiments

corals might withstand wide thermal challenges. Taken together, aposymbiotic *A. poculata* provide a unique opportunity to disentangle how thermal challenges influence the coral host with limited influence from its algal symbiont. Here, we present two thermal challenge experiments that independently assess the behavioural and molecular responses of aposymbiotic *A. poculata* to divergent thermal challenges.

2 | MATERIALS AND METHODS

2.1 | Thermal challenge common garden experiments

Eighteen unique aposymbiotic colonies of *Astrangia poculata* were collected in Woods Hole, Massachusetts (41.54°N, 70.64°W; Figure 1a) in October, 2017 and transported to the Marine Invertebrate Research Facility at Boston University. Colonies were acclimated at 16°C for three weeks in aquariums with artificial seawater (instant ocean brand) and recirculated through 200 micron felt filters. On November 17, 2017, colonies were fragmented, each coral nubbin was assigned a unique ID and glued to a labelled dish (Figure 1c). Light illumination (12 h light: 12 h dark) in all tanks was maintained at 6–12 μmol m^{-2} s^{-1} throughout the study in the two independent thermal challenge experiments. Light levels were quantified using an MQ-500 Full-Spectrum Quantum Flux Meter. Both the cold and heat challenge experiments were run independently as student-led classroom term projects as part of Boston University's Marine Program. While these experiments are complementary and were run at the same time, they were conducted as separate research projects (e.g., different coral genets and different experimental designs) and therefore all behavioural and gene expression data analyses were conducted independently within each project and then results were contrasted between experiments to investigate common responses to thermal challenges in *A. poculata*. Seasonal temperatures profiles from the collection site were created from data obtained by the National Oceanic and Atmospheric Administration weather buoy no. BZBM3.

2.2 | Thermal challenge I: Cold challenge experiment

Nine unique aposymbiotic colonies were removed from their holding tanks (16°C) and assigned to various aquariums as part of the cold challenge experiment (Table S1). At least one nubbin from each

colony was represented in one of three replicate tanks assigned to control conditions (maintained at 22°C) and one of three replicate tanks assigned to the cold challenge treatment (incrementally lowered from 23°C by approximately 1°C/day to a final temperature of 6°C; Figure 2a). When additional fragments remained from a colony, they were randomly stratified into different tanks (n = 42 nubbins total). Several aspects of this experimental design are noteworthy. The first is that our control treatment (22°C) was 6°C higher than the coral acclimation temperature, which may have elicited an initial stress response in the first few days of the experiment. The second aspect is that 6°C is warmer than the minimum temperature *A. poculata* experience within their seasonal averages (Figure 1b); however, achieving lower temperatures was limited by the capacity of our aquarium chillers. In addition, these colonies were collected in October so this thermal minimum and the rate at which this minimum was achieved probably represent a considerable thermal challenge for these corals.

2.3 | Thermal challenge II: Heat challenge experiment

An independent set aposymbiotic colonies (n = 9) were fragmented and at least one nubbin from each colony was assigned to each treatment. There were three tank replicates for control conditions (maintained at 16°C) and three replicate tanks for the heat challenge treatment. At least one nubbin of each colony was assigned to each treatment, and when additional colony fragments remained, they were randomly stratified into different tanks (Table S1). At the beginning of the 16-day heat challenge experiment, all tanks were maintained at 16°C. Heat challenge tanks were ramped from 16 to 23°C over six days (approximately 1°C/day) but no phenotypic data were recorded during this time. Phenotype observations were conducted on days 7–16 during which heat challenge tanks were incrementally ramped 2–3°C in one day followed by a 2-day recovery period. This ramping protocol continued until 31°C was achieved (Figure 2a). It is worth noting several aspects of the heat challenge experimental design which differ from the cold challenge experiment described above: the final heat challenge temperature was well above the maximum temperature these corals experience at their source location (Figure 2a; October range = 11.5–21°C, mean = 16.6°C) and the heat challenge experiment was conducted independently from the cold challenge experiment described above with different rates of thermal changes in the challenge treatment.

2.4 | Coral polyp behaviour in response to food stimulus

In both thermal challenge experiments, the polyp activity (ranked 1–5) of each coral fragment was recorded 30 min after feeding. Corals often extend their polyps after a food stimulus, so waiting 30 min after feeding allowed for consistent behavioural evaluations. In the cold challenge experiment, corals were fed daily whereas in the heat challenge experiment, neither feeding nor phenotypic measurements were conducted during the first 6 days and then observations were taken every three days for the remainder of the experiment (Figure 1: 16 days). Coral polyp behaviour was quantified by the total coral surface area that had observable polyp extension relative to retracted polyps. This score was on a scale of 1 to 5 based on the approximate percentage of active polyps within a fragment (1 = 0%, 2 = 25%, 3 = 50%, 4 = 75%, 5 = 100%, similar to Burmester et al., 2018) and the same researcher conducted all behavioural assays within each thermal challenge experiment to limit observer biases. To determine whether the experimental treatments had an effect on polyp activity, we ran a Bayesian mixed effects ordinal regression model treating coral genotype and the specific aquarium system as crossed random effects using the brms package in R (Bürkner, 2017). All population-level fixed effects (e.g., treatment) had weakly informative flat priors.

2.5 | Global gene expression profiling

Upon reaching maximum thermal differences between challenge and control treatments in both experiments (Day 16), several white polyps from all colonies were sampled using sterilized bone cutters, immediately placed in 200 proof ethanol, and stored at −80°C. Total RNA was extracted using an RNAqueous kit (Ambion by LifeTechnologies) following the manufacturer's recommendations. An additional step was implemented using 0.5 mm glass beads (BioSpec), which were added to the vial of lysis buffer and samples were homogenized using a bead beater for 1 min. RNA quantity and integrity were determined using a DeNovix DS-11+ spectrophotometer and ribosomal RNA bands were confirmed on 1% agarose gels. Trace DNA contamination was removed using a DNase 1 (Ambion) digestion at 37°C for 45 min. Libraries were created from 1500 ng of total RNA (following (Meyer et al., 2011) and adapted for Illumina Hi-Seq sequencing (Dixon et al., 2015; Lohman et al., 2016). In brief, RNA was heat-sheared and transcribed into first-strand cDNA using a template-switching oligo and SMARTScribe reverse transcriptase (Clontech). cDNA was then PCR-amplified, individual libraries were normalized, and Illumina barcodes were incorporated using a secondary PCR. Samples were pooled and size-selected prior to sequencing on Illumina Hiseq 2500 single-end 50 base pair at Tufts University Core Facility. Due to insufficient RNA yield, some samples were not successfully represented in library preparations. Of the 42 samples within each of the cold and heat challenge experiments, 26 and 22 libraries were prepared, respectively (Table S1).

2.6 | Transcriptome assembly and gene expression analyses

Illumina TruSeq adapters and poly-A tails were first removed using the FASTX-Toolkit (v 0.0.14, Gordon & Hannon, (2010) FASTX-Toolkit.

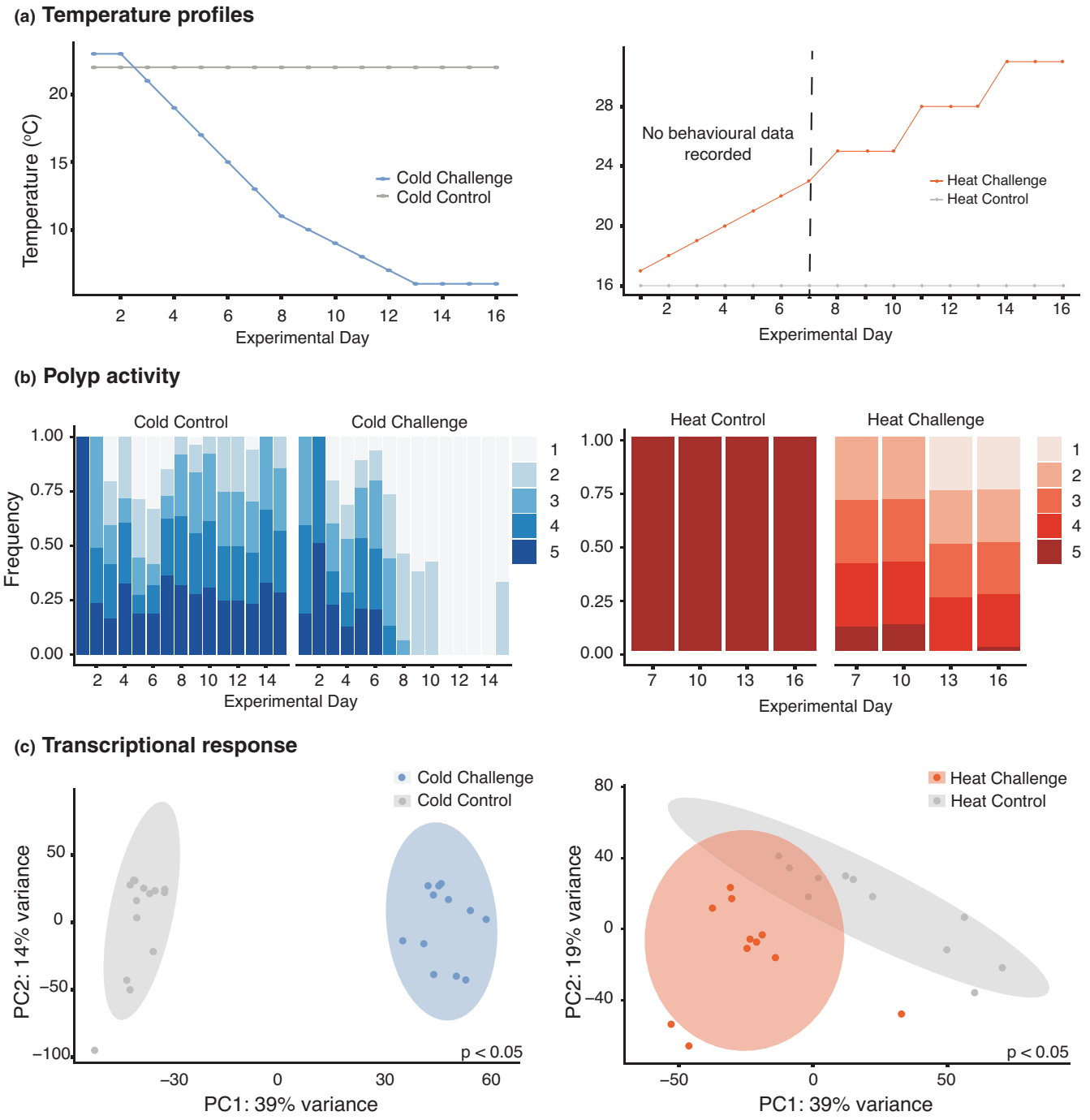

FIGURE 2 *Astrangia poculata* thermal challenge experiments. Left: cold challenge, Right: heat challenge. (a) 16-day temperature ramp. (b) Polyp activity scored based on the proportion of polyps extended per fragment (1 = 0%, 2 = 25%, 3 = 50%, 4 = 75%, 5 = 100%) in response to food stimuli across the 16-day experiments. Note that behavioural data collection in the heat challenge experiment did not commence until day 7. (c) Principal component (PC) analysis of overall gene expression of samples under control and thermal challenge at day 16. Percentages represent the total variance explained by each axis and shaded areas are 95% confidence ellipses. *p*-value indicates significance of treatment using a permutational multivariate analysis of variance

http://hannonlab.cshl.edu/fastx_toolkit.) and resulting sequences that were <20 bp in length were removed. In addition, only those sequences with >90% of bases having a quality score >20 were retained. PCR duplicates were removed and resulting quality-filtered reads were concatenated and used to assemble a novel transcriptome using TRINITY (Grabherr et al., 2013) with default parameters. Contigs were then annotated using BLAST (Altschul et al.,1990) with an e-value cutoff of e^{-04} against UniProt and Swiss-Prot NCBI NR protein databases. TANSRATE (v 1.0.3; Smith-Unna et al., 2016) was used to determine a number of quality metrics in addition to comparing it to the transcriptome of another facultatively symbiotic temperate coral, *Oculina arbuscula* (http://sites.bu.edu/davieslab/

data-code/). While we recognize that this transcriptomic resource will potentially be of little use for the larger coral community, there is some precedent in the literature for assembling a transcriptome from single-end short read data (i.e., Aichelman & Barshis, 2020) when no superior resource is available, which was the case for *A. poculata* at the time of analysis. Additionally, while this transcriptome lacks the resolution of a traditional transcriptome, this would only serve to reduce our ability to successfully annotate genes and thereby limit our power to detect differential enrichment in gene ontology (GO) analyses.

Quality-filtered reads were then mapped to the newly assembled transcriptome using Bowtie2 (Langmead & Salzberg, 2012). There were an average 520,662 mapped reads across both experiments with mapping efficiencies ranging from 36% to 57% (File S5). Raw count files for each experiment are available in File S6 (cold challenge) and 7 (heat challenge). Data from each challenge experiment were analysed independently. First, data were tested for outliers using *arrayQualityMetrics* as part of DESeq (Anders & Huber, 2010) and no outliers were detected for either experiment. DESeq2 (Love et al., 2014) was then used to identify differentially expressed genes (DEGs; Files S8–S9) associated with cold and heat thermal challenge relative to their respective controls using Wald's tests. *p*-values were adjusted for multiple testing using the Benjamini and Hochberg method (FDR < 0.05; (Benjamini & Hochberg, 1995). Expression data for each experiment were *r-log* transformed and these data were used as input for a principal component analysis. A permutational multivariate analysis of variance was then used to determine if overall gene expression patterns between thermal challenge treatments differed significantly from their controls using the adonis function in vegan v2.5-4 (Oksanen et al., 2019). Lastly, we assessed the differing magnitude of responses between experiments by comparing the absolute value of the $\log_2$ fold changes across (1) all genes and (2) DEGs using a one-way ANOVA followed by a Tukey's honest significant differences post-hoc test.

GO enrichment analyses were performed using adaptive clustering of GO categories and Mann–Whitney *U* tests (GO-MWU) based on the ranking of signed log *p*-values (Voolstra et al., 2011), which is particularly suitable for non-model organisms (Dixon et al., 2015). Results were visualized in dendrograms tracing the level of gene sharing between significant categories with direction of change in treatment temperatures compared to their respective controls being noted via text colour.

2.7 | Testing for a convergent response to thermal challenge

Lists of DEGs (FDR < 0.05) between the two thermal challenge experiments were compared and visualized using a Venn Diagram and significant enrichment of genes at the intersection between experiments was tested using a hypergeometric test. DEGs at the intersection between experiments (common DEGs) were visualized based on $\log_2$ fold change for each experiment; and, the most highly up- and downregulated genes were highlighted and defined as convergently responding genes (CRGs). GO categories that were independently identified as enriched (FDR < 0.05) in both experiments were visualized by their delta-ranks (the difference in the mean rank for the GO term and the mean rank for all other GO terms).

2.8 | Comparing *A. poculata* functional responses to heat and cold challenges to the environmental stress response of tropical corals

The functional enrichment from both the heat and cold challenge experiments were contrasted with results of a meta-analysis from Dixon et al. (2020) that characterized transcriptomic profiles of corals from the genus *Acropora* across 18 different stress experiments using a weighted gene correlation network analysis (WGCNA). This analysis isolated a cluster of co-regulated genes that responded to environmental stressors, which was termed the "red module" and was associated with the coral's environmental stress response (ESR). Two types of stress responses were observed: the type "A" stress response which is associated with high severity stressors and considered a general stress response which contrasts with the type "B" stress response where typical stress-response processes tended to be downregulated. We explored the similarity of functional responses between our thermal challenge experiments and this stress module by plotting the delta-ranks of the GO-MWU tests described above. While not a formal statistical analysis, a positive relationship within a GO category would suggest that results are consistent with a type "A" stress response and a negative relationship would suggest a type "B" stress response. This allows us to broadly compare functional responses of *A. poculata* under thermal challenges to those responses of tropical reef-building corals.

3 | RESULTS

3.1 | *Astrangia poculata* transcriptome assembly and annotation

Our assembled *A. poculata* transcriptome consists of 13,343 contigs (mean size 265 bases, N50 of 258, GC content 42%); however, only 25.7% of these contigs were successfully annotated (Table S4). When comparing with the transcriptome of *Oculina arbuscula*, 6420 contigs had a conditional reciprocal best (CRB) BLAST hit, which was proportional to being 0.48 of all contigs (Table S5). Overall, while this transcriptome was effective for mapping TagSeq reads in this study, caution should be taken if using this transcriptome for other data sets.

3.2 | *Astrangia poculata* response to cold challenge

Although behavioural responses of *A. poculata* to a food stimulus under control conditions varied, nearly all colonies exhibited some

polyp extension (Figure 2b). This contrasts with behaviours observed under cold challenge, where rapid declines in polyp activity were observed by day 8 (12°C) and most polyps remained inactive as cooler temperatures were reached (10–6°C, Figure 2b). With >99.9% confidence, we see that cold challenge reduced polyp activity levels (Table S2).

Astrangia poculata gene expression was also significantly influenced by cold challenge: a strong treatment effect on overall gene expression was observed (*Adonis* $p_{treatment}$ <.001, Figure 2c), with cold challenge resulting in 5318 (40%) DEGs (FDR < 0.05; 2244 (17%) upregulated; 3074 (23%) downregulated). Many GO terms were also enriched between cold challenge and control conditions (FDR < 0.10; CC = 77, MF = 50, BP = 78; Figure 3). Individual gene responses within notable GO terms were visualized in heatmaps (Figure 3) and include: *myosin complex* (GO:0016459), *proteasome core complex* (GO:0005839), *translation regulator activity, nucleic acid binding* (GO:0008135; GO:0090079), *extracellular matrix structural constituent* (GO:0005201), *muscle system process* (GO:0006936; GO:0003012) and *proteolysis* (GO:0006508).

3.3 | *Astrangia poculata* response to heat challenge

Behavioural responses of *A. poculata* to a food stimulus under control conditions were stable and coral polyps remained fully extended throughout the experiment (Day 7–14; Figure 2b). This contrasts with behavioural responses under heat challenge, where corals exhibited less polyp activity in response to food stimulus as temperatures increased. By the end of the experiment (day 16), only one colony under heat challenge was observed to have 100% polyp extension and half of the colonies had less than 25% of their polyps extended (Figure 2b). With >99.9% confidence, we assess that the heat challenge negatively influenced polyp activity levels (Table S3).

Astrangia poculata gene expression was also significantly influenced by heat challenge with significant overall gene expression differences observed by treatment (*Adonis* $p_{treatment}$ < .001, Figure 2c) with 1054 (7.9%) DEGs (FDR < 0.05; 410 (3.1%) upregulated; 644 (4.9%) downregulated. Many GO terms were significantly enriched under heat challenge relative to control conditions (FDR < 0.10; CC = 34, MF = 47, BP = 22; Figure 3). Individual gene responses within notable GO terms were visualized in heatmaps (Figure 3) and include: *nematocyst* (GO:0042151), *proteasome core complex* (GO:0005839), *threonine-type endopeptidase activity* (GO:0004298; GO:0070003), *unfolded protein binding* (GO:0051082), *protein folding* (GO:0006457) and *response to cold* (GO:0009409).

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

Both cold and heat thermal challenges induced a reduction in *A. poculata* polyp activity in response to food stimulus (Figure 2b). However, this reduction was more pronounced under cold challenge where nearly all polyps were retracted by day 16. Furthermore, there was a greater magnitude of gene expression response (i.e., mean $\log_2$ fold change) to treatment conditions in the cold challenge experiment than the heat challenge experiment (Figure S1). Five times as many genes were differentially expressed under cold challenge compared to heat challenge (Figure 4a). More than half (657 out of 1,054) of DEGs in the heat challenge experiment were also differentially expressed under cold challenge, which is significantly more DEGs shared between experiments than would be expected by chance (hypergeometric test, $p < .01$). Genes that were highly upregulated under both thermal challenges include: *tumour necrosis receptor 3* (TRAF3), *Lon protease 2, peroxisomal* (LONP2), and *increased sodium tolerance 1* (ITS1). Genes that were highly downregulated under both thermal challenge treatments include: *DELTA-thalatoxin-AVl2a* (AVL2A), *myosin regulatory light polypeptide 9* (MYL9), and *Protein-gl ucosylgalactosylhydroxylysine glucosidase* (PGGHG). GO terms consistently enriched in both experiments were also visualized using experimental delta-ranks of enrichment for each thermal challenge (FDR < 0.10; MF = 11, BP = 4, CC = 14, Figure S2a–c). These terms included *response to mechanical stimulus* (GO:0009612) and *locomotion* (GO:0040011) as well as GO terms associated with the proteasome (GO:0004298, GO:0006515, GO:0008540, GO:0022624, and GO:0005839).

3.5 | Relationship between thermal challenges in *A. poculata* and the environmental stress response of tropical corals

Contrasting the delta-ranks of the red ESR module from Dixon et al. (2020) with delta-ranks from our cold challenge experiment resulted in a weakly positive relationship for GO categories belonging to biological processes (Figure 5a) and cellular components (Figure 5e), but not molecular functions (Figure 5c). This contrasts with delta-ranks from the heat challenge experiment where negative relationships between the red ESR module across all three GO categories were observed (Figure 5b,d, f). The positive relationships with the red ESR module observed for the cold challenged corals are consistent with a type "A" stress response in *Acropora* corals and the negative relationships observed for the heat challenged corals are consistent with a type "B" stress response.

4 | DISCUSSION

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

Astrangia poculata from Woods Hole represent the northernmost limit of this species' distribution and corals from this location experience a wide range of temperatures throughout the year (Figure 1b). Given that the cold challenge temperature (6°C) was well within the

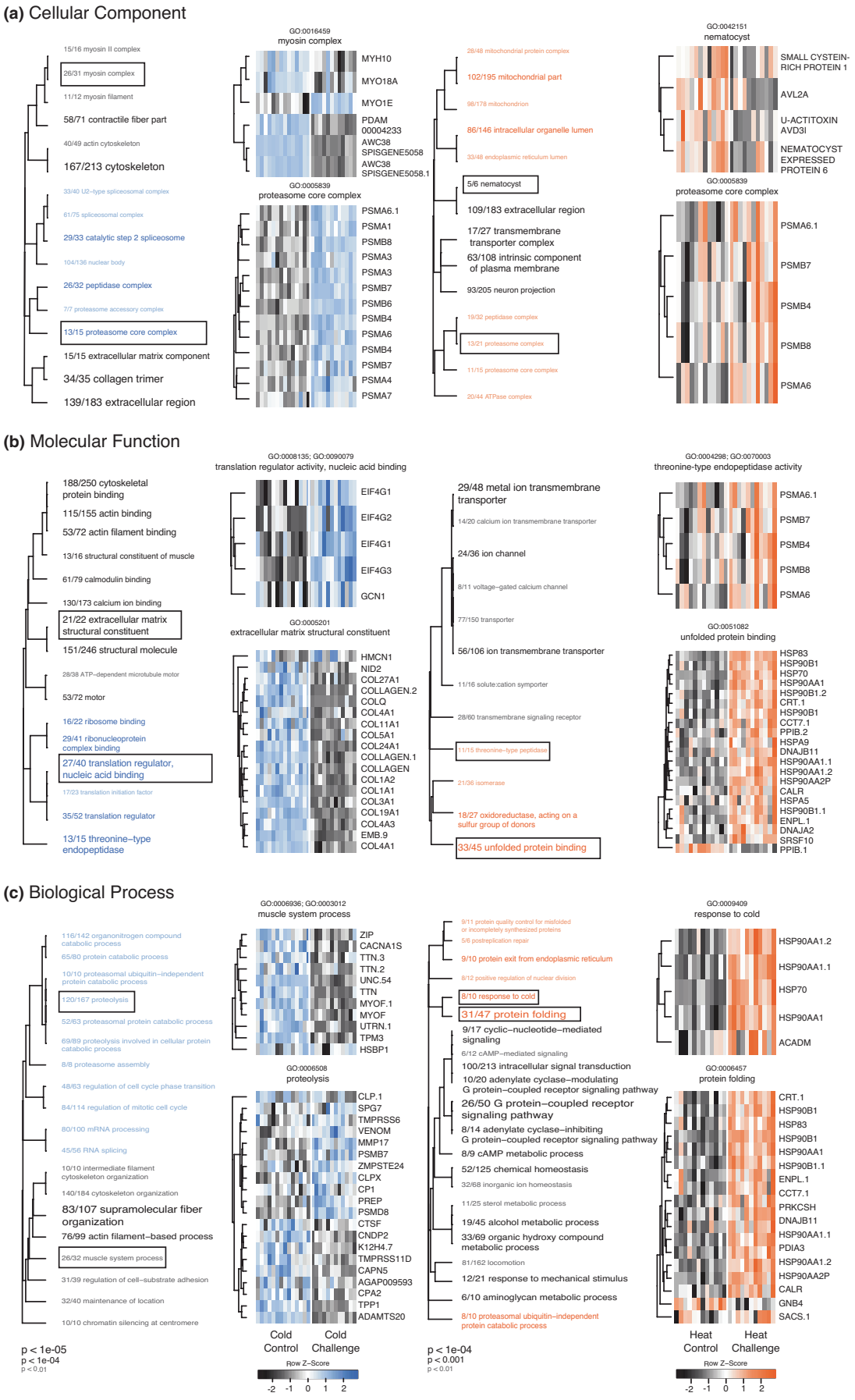
(a) Cellular Component
15/16 myosin II complex
26/31 myosin complex
11/12 myosin filament
58/71 contractile fiber part
40/49 actin cytoskeleton
167/213 cytoskeleton
33/40 U2−type spliceosomal complex
61/75 spliceosomal complex
29/33 catalytic step 2 spliceosome
104/136 nuclear body
26/32 peptidase complex
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

FIGURE 3 Gene ontology (GO) enrichment under thermal challenges: Left: cold challenge, Right: heat challenge. Enriched GO terms of (a) cellular components (b) molecular functions, and (c) biological processes were determined via Mann-Whitney U tests. Font size and boldness of text corresponds to *p*-values with colour designating directionality of enrichment (blue: enriched in cold challenge, orange: enriched in heat challenge, black: enriched in controls). GO terms are clustered based on the number of shared genes between categories. Hierarchical clustered heatmaps were generated from annotated differentially expressed genes (DEGs) within a highlighted GO term (black box) and each row was labelled with its gene symbol. Colours denote magnitude of response (blue, upregulated in cold challenge; orange, upregulated in heat challenge) through z-score of the difference in expression levels from the mean expression for each gene

boundary of what these corals experience in the field, it was surprising that such strong behavioural and transcriptomic responses were observed under cold challenge (Figure 2b, c). This reduction in polyp activity under cold temperatures is consistent with field observations during winter months, when corals fail to respond to physical stimuli (e.g., quiescence; Grace, 2017). Without their polyps extended, *A. poculata* are presumably no longer actively feeding, which may explain the negative growth rates previously observed under cold temperatures (Jacques et al., 1983). Given that our reduced polyp behaviours observed under cold challenge mirror field observations, it stands to reason that these corals may have entered a dormant state, although responses to tactile stimuli were not explicitly conducted here. Dormant polyp behaviours have been observed to limit coral growth and determine population ranges in this species (Dimond et al., 2013). However, very little is known about coral quiescence and its gene expression signatures in corals. Given that seasonal temperature changes are coupled with changes in light and many other environmental parameters, in situ sampling of *A. poculata* across seasons are more likely to reflect gene expression signatures of quiescence and represents a promising area of future research.

The transcriptomic signatures observed here are much more consistent with the classic coral ESR. For example, in mammalian cells, quiescence increases expression of certain myosin genes, notably *myosin heavy chain 10* (MYH10; Hong et al., 2015), which demonstrates the opposite pattern observed here under cold challenge (MYOH10 downregulated; Figure 3a). However, cold challenge did cause underrepresentation of other GO associated with muscle responses, including *muscle system process* (MSP; GO:0006936; GO:0003012) and *myosin complex* (GO:0016459; Figure 3c), which corresponds with decreased *A. poculata* polyp activity under cold challenge. In contrast, *myosin-Ie* (MYO1E), which is an important gene for clathrin-mediated endocytosis and immunity was significantly upregulated under cold challenge. This result is consistent with previous work exploring how mice respond to cold stress (Wenzel et al., 2015) and myosins as a whole are often upregulated in bleached corals under heat stress (DeSalvo et al., 2010) so it is possible that regulation of this gene is more likely related to immunity rather than muscle movement directly. Overall, the transcriptional responses here put forth the hypothesis that reduced polyp activity under cold challenge may be mediated by downregulation of key MSP genes.

Genes associated with *translation regulator activity nucleic acid binding* (GO:0008135; GO:0090079) were upregulated in *A. poculata* under cold challenge. Genes in this category are largely composed of *eukaryotic translation initiation factor 4 gamma* (EIF4G) genes, and have been found to be consistently upregulated under a wide range of stressors, including temperature, osmotic stress and nutrient deprivation in rotifers (Jones et al., 2013). Interestingly, EIF4G genes may play an important role in higher latitude species. For example, the porcelain crab *Petrolisthes cinctipes*, which shares similar temperature environments to *A. poculata*, has been shown to upregulate EIF4G genes in response to cold stress (Stillman & Tagmount, 2009). Given that it has already been shown that different *A. poculata* populations exhibit broadly different thermal responses (Aichelman et al., 2019), future work contrasting gene expression responses to stress across populations at different latitudes would be worthwhile.

Another GO category that demonstrated strong upregulation in *A. poculata* under cold challenge was *proteasome core complex* (PCC; GO:0005839). PCC upregulation has been previously observed in tropical corals under heat stress (Seneca & Palumbi, 2015), however, this is the first to associate PCC upregulation in response to cold challenge. The majority of PCC genes are involved in the functioning of the 20S core proteasome, which is responsible for degradation of oxidized proteins (Davies, 2001). Additionally, proteasomes are known to be required for internal proteolysis of p105 into p50 to activate nuclear factor-κB (NF-κB; Rape & Jentsch, 2002) a key pathway in coral innate immunity that is upregulated during stress-induced bleaching in sea anemones (Mansfield et al., 2017). However, proteasome-mediated processing has not been directly demonstrated to induce NF-κB in cnidarians (Williams & Gilmore, 2020).

Overall, cold challenge elicited strong effects on both behaviour and transcriptomic profiles of *A. poculata* (Figure 2c); however, these patterns do not align with quiescence. Instead, these signatures are consistent with stress responses described in previous cnidarian studies and emphasize that results between obligate tropical corals and aposymbiotic corals serve to highlight the hosts response to thermal challenges even in the relative absence of algal symbionts. Future research should aim to leverage both symbiotic states in *A. poculata* to more effectively resolve the influence of algal symbionts on the coral thermal response.

4.2 | *Astrangia poculata* modulate genes associated with heterotrophy and stress response under heat challenge

Even though summer temperatures at Woods Hole over the last 10 years were much lower than temperatures achieved during the

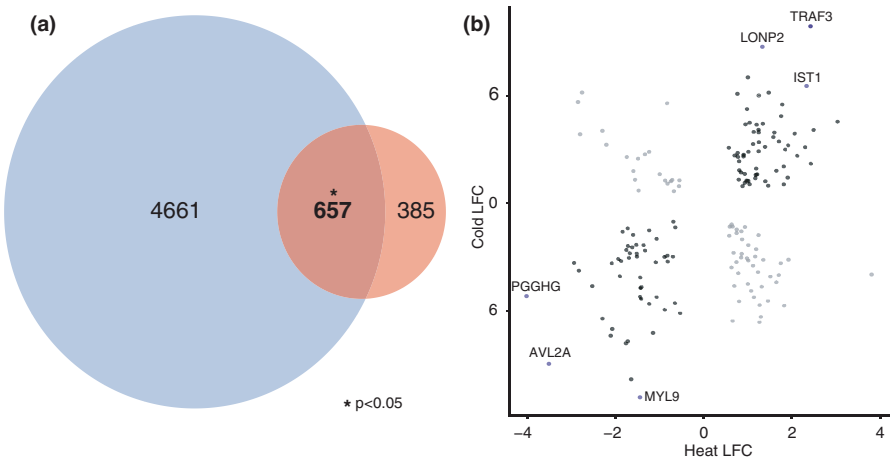

FIGURE 4 Convergent transcriptomic response of *Astrangia poculata* to thermal challenges. (a) Venn diagram of differentially expressed genes shared (intersection) between cold (blue) and heat (red) challenge experiments. (b) Of these 657 shared DEGs, those with annotations are visualized by their respective Log_2fold change (LFC) in each experiment. Genes with consistent direction in their respective LFC are designated as convergently responsive genes (CRGs) depicted as black circles and key CRGs are highlighted in purple and labelled by gene symbol. Grey circles are divergent in response to thermal challenges

experimental heat challenge here (Figure 1b), we observed that *A. poculata* exhibited more muted behavioural and transcriptomic responses when compared to responses to cold challenge (Figure 2b, c). While *A. poculata* significantly reduced polyp activity in response to food stimulus under heat challenge, the majority of corals maintained some extension even at warm extremes. It is possible that reduced feeding responses of corals under cold challenge may be due to those corals being offered food daily, whereas heat challenged corals were offered food only every three days. Future experiments should control for these differences across experiments to more comprehensively contrast *A. poculata's* response to thermal challenges. Nevertheless, unlike naturally observed polyp inactivity during winter months (Grace, 2017), this is the first observation of decreased polyp activity due to heat challenge in *A. poculata*.

Interestingly, we observed downregulation of genes associated with *nematocyst* (GO:0042151) under heat challenge, which are cnidarian stinging cells used to capture food (Holstein & Tardent, 1984). Tropical corals have also been observed to reduce feeding rates under heat stress (Ferrier-Pagès et al., 2010). Taken together, decreased polyp extension and downregulation of genes associated with nematocysts (Figures 2b, 3a), suggest reduced opportunity for heterotrophy in *A. poculata*. Given that heterotrophy has been shown to mitigate coral bleaching in another facultatively symbiotic coral (*Oculina arbuscula*; (Aichelman et al., 2016), this reduction in heterotrophy, in addition to stress associated with increased temperatures, would be interesting to explore in aposymbiotic and symbiotic fragments.

Consistent with previous work in heat challenged cnidarians, we observed enrichment of many mitochondria-related GO terms in heat challenged *A. poculata* (Figure 3). This enrichment is probably due to increases in metabolic compensation, which is consistent with previous work demonstrating *A. poculata* exhibited increased respiration rates under warmer temperatures (Jacques et al., 1983). Furthermore, mitochondria are fundamental in the regulation of cellular stress and have a dedicated unfolded protein response, which influences free radical detoxification and innate immunity in tropical corals (Dimos et al., 2019; Dixon et al., 2020). We observed enrichment in both *protein folding* (GO:0006457) and *unfolded protein binding* (GO:0051082) under heat challenge, which is consistent with a variety of coral stress studies (i.e., Dixon et al., 2020). Genes within these GO categories are largely associated with heat shock protein production, which have been consistently implicated in coral gene expression studies (reviewed in Cziesielski et al., 2019) and heat stress experiments across a wide range of taxa (reviewed in Chen et al., 2018). Unexpectedly, we observed upregulation of *response to cold* (GO:0009409), which is a salient example of how expression of some genes are often associated with a specific stressor, when in reality their expression is more likely a more broad ESR.

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

Our data demonstrate that *A. poculata* exhibit greater behavioural and transcriptomic responses to the cold challenge applied here when compared to heat challenge, which is surprising considering that cold challenge temperatures were within *A. poculata's* thermal range, while heat challenge temperatures were not (Figure 1b). In fact, the heat challenge temperature exceeded any temperature experienced within their native environment over the last decade. Few studies have directly contrasted a coral's response to thermal extremes in parallel, and studies that have demonstrated mixed results. In an RNAseq experiment, there were four times as many DEGs when fragments of *Acropora cervicornis* were exposed to a heat shock compared to that of a cold shock (Parkinson et al., 2018). In another tropical coral (*Acropora millepora*), Nielsen et al. (2020)

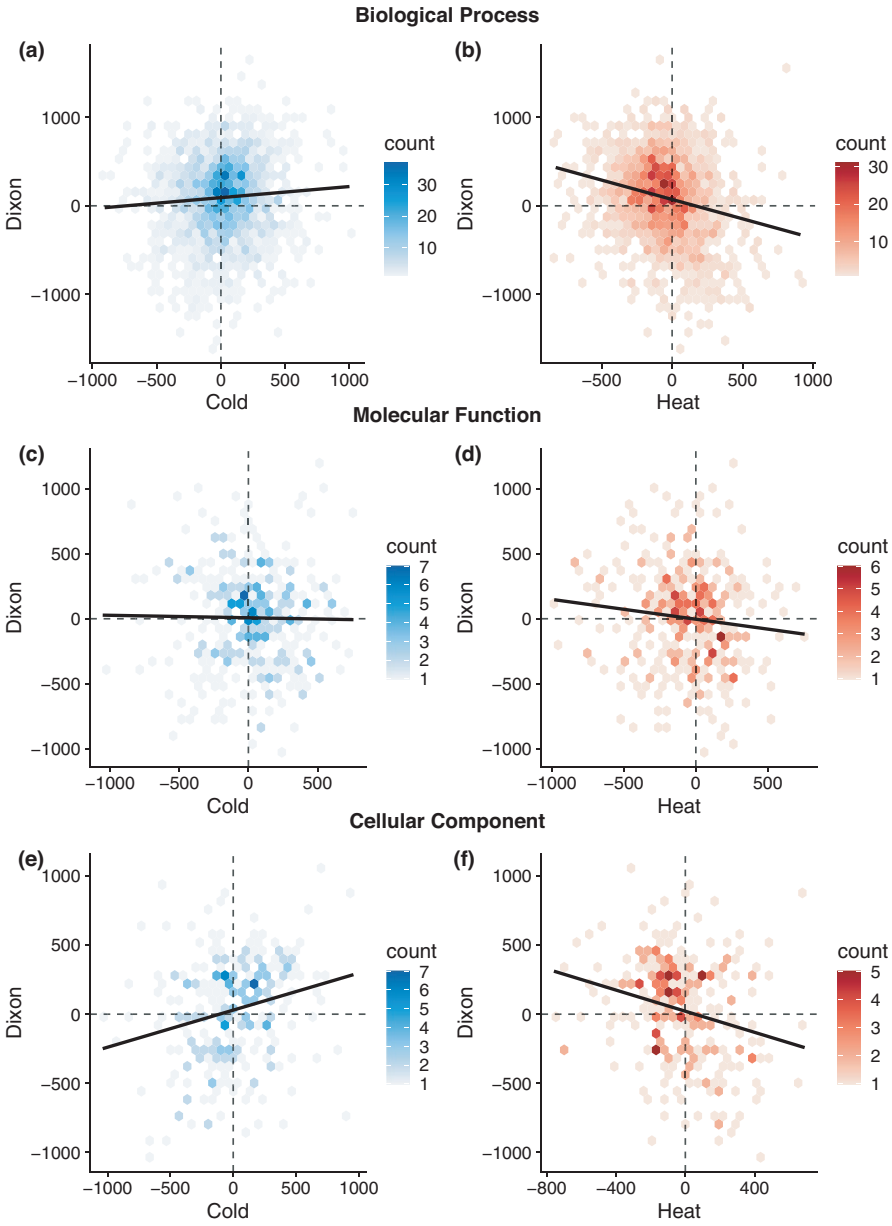

FIGURE 5 Relationship of gene ontology (GO) delta ranks from the "red ESR module" from a meta-analysis of stress responses in tropical reef-building *Acropora* corals (Dixon et al., 2020) relative to GO delta-ranks of those same terms in response to cold (a, c, e) or heat (b, d, f) challenge experiments in *Astrangia poculata*. Correlations are shown for all three GO categories: molecular function, biological process, and cellular component. A positive slope indicates a type A stress response and a negative slope indicates a type B stress response

observed improved coral condition (i.e., symbiont cell density, increased protein content) under cold temperatures relative to ambient or heated conditions. Conversely, Roth and Deheyn (2013) found that acute cold stress was more detrimental to the tropical coral *Acropora yongei* than heat stress, but did suggest that heat stress may be more detrimental over longer temporal scales.

The rate of cooling and warming in our experiments differ from comparable work in other cnidarians and this rate of temperature change probably influenced the responses we observed. It is not uncommon for coral thermal stress experiments to subject corals to thermal "pulses" or short duration acute exposures. For example, Bellis and Denver (2017) recorded the bleaching response of *Aiptasia* anemones to a 5 h cold exposure versus 10 h heat exposure and they found that the heat shock elicited a larger stress response (bleaching) in certain strains of anemone compared to cold shock. Parkinson et al. (2018) also found a greater transcriptomic response to a 1 h heat exposure versus a 1 h cold exposure in a tropical coral (*Acropora cervicornis*). While there is no clear consensus among studies, it is widely accepted that the specific temperatures reached in each stress treatment and the rate at which those temperatures are achieved are both important factors (McLachlan et al., 2020). In addition to rate of temperature change, host genetic background is known to play a role in the stress response of corals (Fuller et al., 2020; Parkinson et al., 2018) and since different genotypes were used in each of the two thermal challenge experiments it is possible that genetic variation may be influencing our comparisons between hot and cold challenges. However, these differences across individuals would probably accentuate differences in responses to thermal challenge and therefore we posit that results presented here are probably an underestimate of the convergence in the stress response across these thermal challenges. Furthermore, the heat challenge temperature in our study may have elicited a more muted response

because *A. poculata* were collected in October, after the corals were acclimated to warmer summer and early fall temperatures (Figure 1). This collection period may explain why corals under cold challenge appeared more stressed; however, future work on winter collected corals would need to be performed to test this hypothesis.

4.4 | *Astrangia poculata* exhibits a convergent stress response repertoire to cold and heat challenge

Despite highly divergent temperatures reached between temperature challenge experiments, we observed convergent behavioural and transcriptomic responses in *A. poculata*. First, we observed reductions in feeding behaviour under both thermal challenges (Figure 2b), which were corroborated with convergent downregulation of genes associated with *locomotion* (GO: 0040011) and *response to mechanical stimulus* (GO: 0009612). Furthermore, *DELTA-thalatoxin-Avl2a* (AVL2A) was downregulated under both challenges; thalatoxin and other toxins are used while feeding in cnidarians (Schmidt et al., 2019) and are categorized under the *nematocyst* (GO:0042151) GO term. In addition, *myosin regulatory light polypeptide 9* (MYL9) was downregulated under both thermal challenges (Figure 4b) and this gene plays an important role in cell contractile activity via phosphorylation (Kumar et al., 1989) and may be instrumental for coral heterotrophy. Our behavioural data showing reduced polyp activity under thermal challenges coupled with enrichment of genes associated with nematocyst production suggest reduced heterotrophic consumption, however this phenotype was not directly measured and future work quantifying heterotrophy via stable isotopes or directly quantifying feeding rates is warranted.

Reduced polyp activity under thermal challenges may be due to temperatures exceeding their critical thermal minima and maxima or corals could be entering quiescent states, where lowered metabolic activity acts as an adaptation to extreme temperatures (Stuart & Brown, 2006). Our gene expression results do not support quiescence and instead suggest large scale protein catabolism, which often occurs during starvation after an organism has metabolized most of its carbohydrate and lipid stores (Davies et al., 2016; Kaur & Debnath, 2015). This result is perhaps unsurprising given that the rate in which temperatures were reduced in this experiment was much faster than the steady decline in temperatures associated with seasonality. This enrichment of catabolic-associated pathways point instead to high energetic demands associated with stress-related cell functions at both thermal extremes (Kültz, 2005).

The other major convergent response observed under both thermal challenges was a generalized stress response. For example, *glutathione transferase activity* (GO: 0004364) was enriched under both temperature extremes and this GO term is associated with detoxification of environmental pollutants and oxidative stress response in tropical corals (Downs et al., 2005). In addition, most enriched GO categories observed in both thermally-challenged *A. poculata* were involved in maintenance of the proteasome (Figures S2a–c). The role of the proteasome (discussed above) is integral to degradation and catabolism of oxidized proteins (Davies, 2001) and may be important for the activation of NF-kB under stress (Rape & Jentsch, 2002). These enriched GO terms across wide thermal challenges highlight conserved ESR pathways under both heat and cold thermal challenges.

In addition to convergently enriched GO terms, a number of individual genes were differentially expressed under both challenges. *Lon protease homologue 2, peroxisomal* (LONP2) was highly upregulated in both experiments and this gene is involved in degradation of oxidatively damaged mitochondrial genes (Yang et al., 2018). LONP2 has been shown to be upregulated under high temperatures and under heavy metal stress in oysters (Sanni et al., 2008). Additionally, *protein-glucosylgalactosylhydroxylysine glucosidase* (PGGHG) was downregulated under both thermal challenges (Figure 4b). PGGHG is a catalyst for the hydrolysis of glucose in hydroxylysine-linked residues of collagen (and collagen-like) proteins (Hamazaki & Hamazaki, 2016). It is also a major component of isolated collagens from other marine invertebrates (e.g., sea anemones; Katzman et al., 1972). Therefore, these convergently responding genes (e.g., LONP2, PGGHG) probably play important roles in *A. poculata's* ESR.

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

4.5 | Contextualizing the stress response of *Astrangia poculata* to stress responses of tropical corals

We compared the functional enrichment of GO terms from our thermal challenge experiments with a module of genes characterized by their involvement in the stress response from the genus *Acropora* (Dixon et al., 2020). This module highlighted two types of stress responses – the type A response, which occurs under severe stressors and the type B response which occurs under milder stressors.

Contrasting results from our two experiments with the findings of Dixon et al. (2020) serves two purposes. First, it provides insights into which experiment was more stressful for *A. poculata*. Second, it synthesizes how the transcriptomic responses of *A. poculata* under temperature challenges relate to the stress response of tropical corals. The results of the heat challenge experiment are consistent with the type B response (Figure 5), whereas the cold challenge treatment has functional enrichment consistent with a type A response. These results suggest that the cold challenge experiment was more stressful for *A. poculata* than the heat challenge experiment, which is consistent with the magnitude of transcriptomic response both in terms of number of DEGs (Figure 4a), principal component analysis (Figure 2c), and mean $\log_2$ fold change (Figure S1).

5 | CONCLUSIONS

While stress response repertoires of tropical reef-building corals have been widely studied, especially in response to upper thermal extremes, this study represents the first to characterize the stress response of a naturally aposymbiotic coral to divergent thermal challenges. Our results demonstrate a strong response to cold challenge and a comparatively muted response to heat challenge. In addition, we provide evidence for a convergent stress response to divergent thermal challenges in *A. poculata* that is consistent with responses observed for tropical obligate coral species, which is surprising given the relative absence of symbiont-associated reactive oxygen species. Lastly, by leveraging a module of genes previously associated with the *Acropora* ESR, we determined that *A. poculata's* ESR is consistent with responses of tropical reef-building corals, although cold challenge exhibited gene expression profiles consistent with severe stress (type A) and heat challenge exhibited profiles consistent with milder stress (type B). This work highlights the benefits to studying facultatively symbiotic corals to disentangle stress responses of the coral host from their algal symbionts, and future work leveraging this facultative relationship may lead to a stronger mechanistic understanding of why coral dysbiosis is increasing in frequency in corals worldwide.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

Sarah W. Davies designed the experiment. Adeline Almanzar, Sara A. Brennan, Juan D. Chavaz, Mary B. Lesegang, Jennifer L. Reavis, Mikeala K. Schniedewind, and Isabela F. Trumble conducted the experiment. Brooke E. Benson and Christopher L. Reyes completed all molecular work and TagSeq library preparations. Daniel M. Wuitchik performed all statistical and bioinformatic analyses and drafted the manuscript. Sarah W. Davies supervised the experiment, analyses and coauthored the manuscript. All authors edited and approved the manuscript.

DATA AVAILABILITY STATEMENT

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

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