Title: Convergent stress response repertoire to thermal challenges in a temperate aposymbiotic coral

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Abstract:
Corals are threatened worldwide due to rapidly warming oceans associated with anthropogenic climate change. A growing body of work has explored how corals respond to heat stress, however, responses of corals across the full range of their thermal breadth are rarely explored even though winter colds are also known to induce coral stress. Here, we leverage the temperate stony coral, *Astrangia poculata*, which naturally exhibits a facultative symbiosis with Symbiodiniaceae, to explicitly examine how thermal challenges influence coral hosts in isolation from their symbionts. Aposymbiotic *A. poculata* were collected from Woods Hole, MA, the northern range limit for this species. Corals were thermally challenged in two independent common garden experiments (Heat challenge: 31°C, 10 days; Cold challenge: 6°C, 16 days) to determine the effects of divergent thermal stressors. Behavioural responses to food stimuli were monitored throughout the thermal challenges and genome-wide gene expression profiling (TagSeq) was used to characterize molecular underpinnings of the coral’s response to stress in its aposymbiotic state. Behaviourally, both thermal challenges induced polyp retraction and colonies failed to respond to food stimuli. Surprisingly, seven times as many genes were differentially expressed under cold challenge heat challenge. Despite a greater magnitude of response under cold challenge, significant similarities in gene expression were detected across the two thermal challenge experiments, with many of the most responsive genes having been previously implicated in coral heat stress response. Given that our data were generated from aposymbiotic colonies, we hypothesize that these genes previously linked to heat stress are more likely indicative of a generalized stress response. Overall this work highlights the unique insights facultatively symbiotic corals offer for deciphering the stress response of the coral host in the absence of bleaching.
**Introduction**

Temperature is an important factor in determining species distribution patterns in ectothermic organisms [1]. As sea surface temperatures continue to rise, understanding how this will affect species distributions demands a broad understanding of an 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 [2,3]. Coral reefs are particularly sensitive to these thermal changes, which have been implicated in widespread reef declines [4]. Temperature anomalies are the primary driver of the breakdown of the obligate symbiotic relationship between corals and their endosymbiotic algae [5] in the family Symbiodiniaceae [6]. This breakdown results in the expulsion of algae from coral host tissue in a process commonly referred to as coral bleaching. 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 [7].

The majority of research on coral bleaching has focused on responses to elevated temperatures in tropical reef-building corals [8]. However, tropical corals are known to bleach in response to a variety of stressors, including high nutrients [9], ocean acidification [10], pathogens [11], low salinity [12] and chemical exposures [13]. Coral responses to cold stress remain understudied, even though these events can have substantial impacts on reefs. For example, in 2010 a cold water bleaching event decimated inshore coral populations along the Florida reef tract [14], and cold water has caused bleaching on the Great Barrier Reef [15]. While the main effect of climate change on marine systems is a net increase in mean global sea surface temperatures, these cold thermal
challenges will still occur [16] and may disproportionately affect subtropical and temperate coral species.

Tropical reef-building corals live in oligotrophic waters and cannot survive long-term without their symbionts. Because energy deprivation in these corals results from any mechanism of symbiont loss [17], uncoupling a thermal stress response from a general stress response (i.e. energy deficit) is challenging. Given that many tropical corals exhibit an obligate symbiotic relationship, it is difficult to uncouple the host’s stress response to extreme temperatures from that of the host’s response to algal by-products released under stress (i.e. reactive oxygen species (ROS) [18]) and the resulting energy deprivation from the loss their algal symbiont induced by a bleached state. However, there are several species of subtropical and temperate reef-building corals that exhibit facultative symbioses. The Northern Star Coral, *Astrangia poculata*, exhibits a facultatively symbiotic relationship with *Breviolum psygmophilum* [19] and can be found naturally in sympatry across two 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, aposymbiotic colonies of *A. poculata* appear white due to very low algal densities [20]. Unlike obligately symbiotic corals, *A. poculata* can thrive in its “bleached” state by feeding autonomously via heterotrophy. Additionally, *A. poculata* experiences large seasonal variation in temperature at its northern range, making these populations ideal models for investigating how corals might withstand wide thermal ranges. Taken together, *A. poculata* provides a unique opportunity to leverage its aposymbiotic state to determine how broad thermal anomalies influence the coral host in isolation from its symbionts.
Characterising changes in gene expression profiles provides a snapshot into the current physiological state of an organism, offering insights into the biological processes, molecular functions, and cellular components that corals engage to withstand various stressors. Modern transcriptomics have demonstrated that corals mount dynamic responses to pollutants [21,22], pH [23,24] and bacterial challenges [25,26] and considerable efforts have been made to understand how corals respond to heat challenges (for review see [8]). Interestingly, similar patterns of gene expression emerge from these different stressors. Barshis et al. [27] made the connection that corals demonstrate a widespread stress response across thousands of genes much like that of yeast in what is termed the environmental stress response (ESR). The ESR in yeast is characterized by a consistent response to diverse environmental stressors [28]. How corals respond to divergent stressors remains understudied, and testing single stressors does not indicate whether genes being expressed are a unique response to a particular stressor or a generalized ESR.

In order to disentangle how coral hosts respond to thermal challenges in isolation, we leverage the facultative symbiosis offered by A. poculata to assess how thermal challenges influence the host’s stress response. We present two experiments that independently assess the behavioural and molecular responses of aposymbiotic A. poculata to divergent thermal challenges. We then contrast these stress responses to better understand this coral’s ESR to a wide breadth of thermal challenges to explore how conserved these response repertoires might be.
Figure 1 | A) Map of the eastern seaboard of the United States with a native range of *Astrangia poculata* in green. Inset shows the Woods Hole collection site denoted with a yellow star. Distributions are based on [29]. B) Seasonal temperature profile at Woods Hole averaged over ten 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 mined from the National Oceanic and Atmospheric Administration weather buoy #BZBM3. C) Picture of an aposymbiotic *Astrangia poculata* colony fragment.

**Methods**

*Thermal challenge common garden experiments*
Aposymbiotic colonies of *Astrangia poculata* (n = 18; Figure 1C) 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 to 16°C for three weeks. On November 17, 2017, colonies were fragmented, and each nubbin was assigned a unique ID based on colony ID and glued to a labelled dish. Corals were then further acclimated at 16°C under a 12 L:12 D photoperiod with light levels ranging from 6-12 µmol m⁻² s⁻¹ and fed *Artemia* nauplii daily for 24 days.

**Thermal challenge I: Cold experiment**

Nine colonies were assigned to the cold challenge experiment. At least one nubbin (n = 43) from each colony was represented in one of three replicate tanks assigned to control conditions (22°C) and one of three to the cold challenge treatment (22°C that was incrementally lowered to 6°C over 16 days). It is worth noting that 6°C is above the minimum temperature *A. poculata* experience within their normal seasonal averages (Figure 1B), however, achieving lower temperatures was limited by the capacity of our chillers. In addition, the rate of chilling was much more rapid than what these corals experience naturally and given that these corals were collected in the summer, the rate of change and the temperature achieved is expected to represent a considerable thermal challenge.

**Thermal challenge II: Heat experiment**

An independent set of nine aposymbiotic colonies of *A. poculata* were fragmented (n = 43) and at least one nubbin from each colony were assigned to each treatment. There were three replicate tanks for control conditions (16°C) as well as the heat challenge treatment (23°C that was
incrementally ramped every three days until 31°C was achieved; t_{total} = 10 days) and at least one
nubbin of each colony was assigned to each treatment (Figure 3B). Due to insufficient RNA
quality, some samples were not successfully represented in library preparations. As a result, four
colonies are only represented in one of the treatments. It is also worth noting that the final heat
challenge temperature tested here was well above the maximum temperature these corals typically
experience at their source location (Figure 1B).

Coral polyp behaviour in response to food stimulus

In the cold challenge experiment, corals were fed daily and subsequent feeding behaviours were
recorded. In contrast, in the heat challenge experiment, corals were offered food every third day.
Coral polyp behaviour in response to the food stimulus was quantified as the total coral surface
area that had observable polyp extension relative to retracted polyps. This was scored on a scale
of 1 to 5 based on the estimated percentage of active polyps within a fragment (1 = 0%, 2 = 25%,
3 = 50%, 4 = 75%, 5 = 100%) and the same researcher conducted all behavioural assays to control
for observer biases. An ordered logistic regression was performed to establish if temperature
influenced polyp extension rates using the polr function as part of the MASS package (version 7.3-
51.1) in R.

Global gene expression profiling

Upon reaching maximum thermal differences between challenge and control treatments in both
experiments, several aposymbiotic 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 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 I (Ambion) digestion at 37°C for 45 minutes, then libraries were created from 1500 ng of total RNA following [30] and adapted for Illumina Hi-Seq sequencing [31,32]. In brief, RNA was heat-sheared and transcribed into first-strand cDNA using a template-switching oligo and SMARTScribe reverse transcriptase (Clontech). Complementary-DNA 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 (SE) 50 basepair (bp) at Tufts University Core Facility (TUCF). Of the 43 samples within each experiment, 23 and 27 libraries were prepared for the heat and cold challenge experiments, respectively.

**Gene Expression Analyses**

A preliminary *A. poculata* holobiont transcriptome [33] was first separated into the host and symbiont fractions (following [24]). In brief, the resulting host transcriptome was cleaned by removing all genes that had high-level matches to the SILVA ribosomal small (16s/18s) and large (34s/28s) subunit databases [34] using BLASTn [35]. Genes which had annotations associated with chloroplasts were also removed as they are indicative of symbiont or turf algae contamination. Raw reads across libraries ranged from 2.1-7.4 million SE 50 bp sequences. Illumina TruSeq adapters and poly-A tails were removed using the *FASTX-Toolkit* (v 0.0.14, http://hannonlab.cshl.edu/fastx_toolkit) and resulting sequences that were less than 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 mapped to the cleaned *A. poculata* transcriptome using Bowtie2 [36]. Raw count data for each challenge experiment were first tested for outliers using *arrayQualityMetrics* as part of DESeq [37] and no outliers were detected for either experiment. DESeq2 [38] was then used to identify differentially expressed genes (DEGs) associated with heat and cold thermal challenge relative to their respective controls using a Wald’s test. P-values were adjusted for multiple testing using the Benjamini and Hochberg method (FDR < 0.05; [39]). Lastly, expression data for each experiment were r-log normalized 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 [40].

Gene ontology (GO) enrichment analysis was performed using adaptive clustering of GO categories and Mann–Whitney U tests (GO-MWU) based on the ranking of signed log p-values [41], which is particularly suitable for non-model organisms [42]. Results were visualized in dendrograms tracing the level of gene sharing between significant categories and direction of change in treatment temperatures compared to their respective controls. All data for the two thermal challenge experiments were analyzed separately.

*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 for using a hypergeometric test. The DEGs at the intersection between
experiments (common DEGs) were visualized based on log fold change for each experiment and the most highly up- and down-regulated genes were highlighted and defined as convergently responding genes (CRG). GO categories that were independently identified as enriched (FDR < 0.05) in both experiments were visualized by their respective delta-ranks of enrichment to demonstrate the conservation of GO function across the thermal challenges (for details, see [42]).

Results

Astrangia poculata response to cold challenge

Throughout the experiment, Astrangia poculata behavioural responses to food stimulus under control conditions varied, however, nearly all colonies exhibited some polyp extension (Figure 2A). This contrasts with behaviours observed under cold challenge, where rapid declines in polyp activity were observed by day eight (12°C) and most polyps remained inactive across cooler temperatures (10°C-6°C, Figure 2A). Overall, A. poculata polyp activity was significantly reduced under cold challenge (p < 0.01). Transcriptomes of A. poculata were also significantly influenced by cold challenge and a strong treatment effect on overall gene expression was observed (Adonis p_{treatment} < 0.001, Figure 2C), with cold challenge resulting in 3,428 (7.8%) DEGs (FDR < 0.05; 1,468 (3.3%) up-regulated; 1,960 (4.5%) down-regulated). There were also many GO terms enriched between cold challenge and control conditions (FDR < 0.10; BP = 419, MF = 106, CC = 146; Supplemental Figure 1). Of these, notable GO terms include: SNAP receptor activity (GO:0005486), tumour necrosis factor-activated receptor (GO:0005031), apoptotic signalling (GO:0097190) and cell death (GO:0008219).
Figure 2 | *Astrangia poculata* behavioural and transcriptomic response to cold challenge. A) Frequency of behavioural scores (proportion of polyps extended per fragment, 1 = 0%, 2 = 25%, 3 = 50%, 4 = 75%, 5 = 100%) in response to food stimulus throughout the 16-day experiment (Left: Cold Control, Right: Cold Challenge, p < 0.01). B) Temperature profile of experimental tanks throughout the 16-day experiment, where blue represents cold challenge and grey represents control conditions. C) Principal component analysis of overall gene expression with blue symbols representing gene expression of coral fragments from cold challenge conditions and grey symbols representing gene expression of coral fragments under control conditions. Percentages represent the total amount of variance explained by each axis and shaded areas are 95% confidence ellipses. P-value indicates the significance of treatment using a permutational multivariate analysis of variance.
Astrangia poculata responses to heat challenge

Throughout the experiment, Astrangia poculata behavioural responses to food stimulus under control conditions were consistent and coral polyps remained fully extended (Figure 3A). 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 10), 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 3A). Overall, A. poculata polyp activity was significantly reduced under heat challenge (p < 0.01). Transcriptomes of A. poculata were also significantly influenced by heat challenge and a significant effect of treatment on overall gene expression was observed (Adonis \( p_{\text{treatment}} < 0.001 \), Figure 3C) with 485 (1.1%) DEGs (FDR < 0.05; 230 (0.53%) up-regulated; 255 (0.58%) down-regulated) observed. Many GO terms were found to be significantly enriched under heat challenge relative to control conditions (FDR < 0.10; BP = 179, MF = 91, CC = 93; Supplemental Figure 1) and notable GO terms include: NF-kappaB complex (GO:0033256), mitochondrial parts (GO:0005739), catabolic process (GO:0009894), and regulation of proteolysis (GO:0030162).
Figure 3 | *Astrangia poculata* behavioural and transcriptomic response to heat challenge. A) Frequency of behavioural scores (proportion of polyps extended per fragment, 1 = 0%, 2 = 25%, 3 = 50%, 4 = 75%, 5 = 100%) in response to food stimulus throughout the 10-day experiment (Left: Heat Control, Right: Heat Challenge, p < 0.01). B) Temperature profile of experimental tanks throughout the 16-day experiment, where red represents heat challenge and grey represents control conditions. C) Principal component analysis of overall gene expression with red symbols representing gene expression of coral fragments from heat challenge conditions and grey symbols representing gene expression of coral fragments under control conditions. Percentages represent the total amount of variance explained by each axis and shaded boxes are 95% confidence ellipses. P-value indicates the significance of treatment using a permutational multivariate analysis of variance.
Convergent response repertoires to heat and cold challenge in Astrangia poculata

Both sets of thermal challenges induced a reduction in polyp activity in response to food stimulus (Figure 2A, 3A), however, there was a more pronounced reduction in behaviour to cold challenge where nearly all polyps were retracted. There were seven times more genes that were differentially expressed under cold challenge compared to heat challenge (Figure 4). More than half (267 out of 485) of DEGs in the heat challenge experiment were also differentially expressed in the cold challenge experiment, which is significantly more genes shared between experiments than would be expected by chance (hypergeometric test, p < 0.05). Genes that were up- or down-regulated under both thermal challenges were visualized and the most notable highly up- or down-regulated genes are highlighted in Figure 4B. These genes include tumour necrosis receptor 3 (TRAF3), malignant in brain tumours 1 (DMBT1), universal stress protein A (USPA), trypsin, and oncoprotein-induced transcript 3 (OIT). GO terms enriched in both experiments were also plotted by heat and cold experimental delta-ranks of enrichment for each comparison (FDR < 0.10; BP = 113, CC = 49, MF = 24, supplemental figures 2-4) and the top three similarly enriched GO terms for each GO category have been highlighted (Table 1).

| Biological Process | Molecular function | Cellular Component |
|--------------------|--------------------|--------------------|
| Regulation of glucose import in response to insulin stimulus (GO:2001273) | Oxidoreductase activity, acting on a sulfur group of donors, quinone or similar compound as acceptor (GO:0016672) | Methylosome (GO:0034709) |
| Spliceosomal snRNP assembly (GO:0000387) | Death receptor activity (GO:0005035) | Azurophil granule membrane (GO:0035577) |
**Figure 4** | *Astrangia poculata* transcriptomic response to heat and cold thermal challenge. A) Venn diagram of differentially expressed genes that are shared (intersection) between cold (blue) and heat (red) thermal challenges. These genes were designated as convergently responding genes (CRG). B) CRG \((n = 267)\) under heat and cold thermal challenge with their respective log-2 fold changes. Annotated CRG of interest that are consistently up- or down-regulated are highlighted and labelled with gene symbols. Highlighted up-regulated CRG include *tumour necrosis receptor 3* (TRAF3), *deleted in malignant brain tumours 1* (DMBT1), *universal stress protein A* (USPA). Highlighted down-regulated CRG include *trypsin1*, and *oncoprotein-induced transcript 3* (OIT).
**Discussion**

Astrangia poculata exhibit strong response to cold challenge

*Astrangia poculata* from Woods Hole represents one of the most northern populations of the species and these corals experience a wide range of temperatures throughout the year (Figure 1B). Given this temperature range, it was surprising that such strong behavioural and transcriptomic responses were observed under cold challenge (Figure 2A; 2C). This reduction in polyp activity under cold temperatures is consistent with field observations during winter months, when corals fail to respond to stimulus in the field [43]. This dormant polyp behaviour is possibly a quiescent response to cope with long winter cold periods. However, very little is known about quiescence in coral, and even less is known about what a transcriptomic signature of quiescence might look like.

Previous gene expression work described larval transcriptomic profiles associated with diapause [44], which may have signatures similar to quiescence. Expression profiles associated with diapause included cell cycle arrest, reduced transcription, increased ribosome production and upregulation of oxidative stress defences [44]. Here, we observed strong overall transcriptomic modifications associated with the cold challenge (Figure 2C), however, these gene expression patterns were not consistent with diapause. Instead, we found GO enrichment that is consistent with stress signatures in previous coral studies. SNAP receptor activity (GO:0005486) was highly enriched under cold challenge, which has been implicated in stress from high solar irradiance and is thought to be involved in coral bleaching [45]. Tumour necrosis factor–activated receptor (GO:0005031), which is involved in regulating death receptor activity, was enriched and these receptors have been heavily implicated in responses to heat-related stressors [27,46,47]. This stress signature is further corroborated by enrichment of regulation of apoptotic signalling pathways (GO:0097190) and cell death (GO:0008219), which were enriched under cold challenge (Fig S1).
Apoptosis is a universal response of severely stressed cells [48] and its upregulation suggests that the cold challenge performed here elicited a strong stress response from *A. poculata*. This stress response is intriguing considering that the temperatures achieved in this experiment were well within the thermal range that these corals experience in their natural environment (Figure 1B). However, we also acknowledge that the rate of temperature change is important and that in their native environments these corals would experience these temperature shifts across seasons. We, therefore, suspect that this rate of change contributed to the strong stress signature observed here. We propose that future work would benefit from a gradual decrease in temperature to mirror the natural temporal progression of the field more closely. In addition, field-collected samples across seasons may be more likely to capture the gene expression signature of quiescence.

*Astrangia poculata* exhibit subtle responses to extreme heat challenge

Natural summer temperatures reached at Woods Hole over the last 10 years was much lower than the heat challenge reached in our experiment (Figure 1B). It is therefore surprising that *A. poculata* from this site did not exhibit extreme behavioural and transcriptomic responses under this heat challenge given that the temperature was considerably higher than temperatures they ever experience *in situ* (Figure 3A; 3C). Corals did exhibit reductions in polyp activity in response to food stimulus under heat challenge, however, unlike observed polyp inactivity during winter months [43], there is no prior evidence for a decrease in polyp activity due to warm temperatures in this species. In tropical corals, polyp contraction can occur as a response to heat stress and often precedes tissue necrosis [49]. We speculate that similar to the response to the cold challenge, this reduced behaviour was most likely due to stress.
A coral’s molecular response to heat stress has been meticulously explored in tropical reef-building corals (as reviewed in [8]), however, this is one of the first studies to explore the molecular response of an aposymbiotic reef-building coral to heat challenge in the absence of the algal symbiont. Much like we observed in response to cold challenge, heat challenge elicited a stress signature often observed in coral. For example, NF-kappaB complex (GO:0033256) which is important to coral innate immunity [50] was enriched under heat challenge (Figure S1) and this is consistent with other coral heat stress studies [51–53]. We also observed an enrichment of many terms related to mitochondria, suggesting that *A. poculata* were exhibiting increased respiration under heat challenge. Enrichment of respiration under high temperatures is not surprising given that these patterns have been demonstrated in heat-tolerant coral larvae [42]. Interestingly, enrichment of genes associated with respiration was also previously found under extreme $pCO_2$ stress in *Siderastrea siderea*, highlighting the potential for greater energetic demands associated with the putative coral ESR [24]. Lastly, we observed an enrichment of regulation of catabolic processes (GO:0009894), specifically the regulation of proteolysis (GO:0030162). Protein catabolism is typical during starvation [54], as it’s indicative of depleted stores of fats and carbohydrates following the cessation of heterotrophy (i.e. reduced polyp extension), which would be particularly detrimental to aposymbiotic corals. Overall, it appears that aposymbiotic *A. poculata* mirror the hallmarks of heat stress responses that have been previously described in their symbiotic tropical relatives. It would be interesting to pursue work contrasting how aposymbiotic and symbiotic *A. poculata* respond to heat stress to see how symbiotic state modulates the heat stress response.

*Cold challenge elicits a much stronger response than heat challenge in A. poculata*
Our data demonstrate that *A. poculata* exhibits greater behavioural and transcriptomic responses to the cold challenge applied here relative to heat challenge, which is surprising considering that the cold challenge temperatures achieved were within the ecological bounds of *A. poculata*'s normal environmental temperature range (Figure 1B). This contrasts with heat challenge temperatures, which far exceeded any temperature experienced within their native environment and very likely reached their thermal maximum in their southern range. While few studies have directly compared thermal extremes in corals, similar studies that have contrasted heat and cold challenges in marine invertebrates have demonstrated mixed results. For example, Roth & Deheyn found that acute cold stress was more detrimental to the tropical coral *Acropora yongei* than heat stress, however, they did suggest that heat stress may be more detrimental over long term timescales [55]. This contrasts with Nielsen *et al.* who found that *Acropora millepora* improved its physiological condition under cold temperatures compared with ambient or heat conditions [56]. Bellis & Denver found that heat stress caused greater bleaching in the sea anemone *Aiptasia* than did cold stress [57] and Zhu *et al.* observed similar transcriptional responses of oysters to both heat and cold stress [58]. While there is no clear consensus, it is widely accepted that the specific temperatures reached in each stress treatment and the rate at which those temperatures are reached are both important factors and these responses are dependent on the species-specific thermal niche breadth [59]. For example, the heat challenge exposure in our study may have elicited a more muted response because *A. poculata* were collected in the summer, so perhaps they were already acclimated to warmer conditions. Future experimental designs should, therefore, focus on assessing the influence of thermal challenges across seasons to better understand the influence of these divergent thermal stressors.
Convergent stress response repertoire to cold and heat challenge

Our behavioural results align with field observations of *A. poculata* where polyp extension diminishes during winter months [43]. This lack of polyp activity could be indicative of corals entering a quiescent state, where they lower metabolic activity as an adaptation to cooler temperatures. However, our behavioural data also suggest that this reduction in polyp activity is convergent across thermal challenges (Fig 2A, 3A). Interestingly, our transcriptomic responses under both thermal challenges show up-regulation of genes associated with catabolic activity, particularly protein catabolism (Fig S2-3). Large scale protein catabolism often occurs during starvation after an organism has utilized most of its carbohydrate and lipid stores [54], which is consistent with reductions in polyp activities observed here under both thermal challenges. Given that quiescence is associated with reduced metabolism, we suggest that even though our cold-challenged corals were exhibiting quiescence phenotypes, increases in catabolic-related genes point instead to high energetic demands associated with stress-related cell functions at both thermal extremes [48].

In addition to *A. poculata* exhibiting similar behavioural responses to both heat and cold thermal challenges, we also found a set of convergently responsive genes (CRG) that were consistently regulated across thermal challenge experiments (Figure 4B). These CRG included upregulation of *Deleted in malignant brain tumours 1* (DMBT1) under both thermal challenges. DMBT1 was previously found highly up-regulated in corals that survived bacterial [26] and lipopolysaccharide challenges [60]. Taken together, these data suggest that DMBT1 plays a role in both microbial and broad thermal stress responses. Furthermore, *Tumour necrosis factor receptor 3* (TRAF3) was also a highly up-regulated CRG under both thermal stressors. TRAF3 is an intracellular signalling
molecule that regulates mitogen-activated protein kinase activity and nuclear factor-κB (NF-κB) signalling [61], which has been shown to be upregulated during stress-induced bleaching in Aiptasia [50]. TRAF3 has also been found to be constitutively up-regulated or “front-loaded” in corals tolerant to heat stress [27,47,51] and has been found upregulated under low magnesium [62], white band disease [63], and high carbon dioxide treatments [64]. Our results provide evidence that TRAF3 may be a CRG that is consistently up-regulated in response to all stressors, not just high temperatures.

Universal stress protein A (USPA) was also an up-regulated CRG under heat and cold challenge (Fig 5B). USPA was previously found to be up-regulated under light stress [65] and, as the name would suggest, USPA is produced in response to a large number of different environmental stressors and is a highly conserved stress response gene in diverse taxa [66]. In addition to up-regulated CRGs, a number of CRGs are similarly down-regulated in A. poculata under both heat and cold challenge. These genes include oncprotein-induced transcript 3 (OIT), which - like TRAF3 - was previously found to be frontloaded in thermally tolerant corals [27]. OIT3 has been found to be up-regulated under toxic stressors from a crude oil treatment [22] and downregulated in response to low magnesium [62], providing evidence that this gene may serve a fundamental role in stress responses more generally. Trypsin was also found to be a CRG, however, it is not well established for its role in stress response in coral. Trypsin is involved in protein catabolism [67] and it is therefore consistent with our findings that protein catabolism is being regulated by both heat and cold challenges. Taken together, these CRG highlighted here appear to be regulated in response to a wide range of stressors and may represent a core set of genes comprising a generalized stress response.
In addition to CRG, we also found consistently enriched GO categories under both heat and cold challenges that corroborate the biological functions of the CRGs highlighted above. For example, regulation of glucose import in response to insulin stimulus (GO:2001273) was enriched under both challenges (Table 1). While there is no direct evidence of insulin in corals, insulin-like signalling has been reported to antagonize the transforming growth factor β pathway in sea sponges (TGFβ) [68]. TGFβ signalling has been widely implicated in coral immunity and stress responses including signalling apoptosis [69]. Apoptosis is a universal response of severely stressed cells [48] and is associated with death receptor activity (GO:0008219), which was also enriched under both heat and cold challenges. Corals that are sensitive to disease have also been observed to highly upregulate apoptotic pathways, which have been suggested as clear indications of stress [25]. Overall, the enrichment of apoptosis-associated GO terms observed here under thermal challenges likely indicate extreme stress and cell death and further confirm that the thermal challenges applied here were likely achieved at too rapid a pace to allow for acclimation.

Conclusions

While the stress response repertoire in tropical reef-building corals has been widely studied, especially in response to hot thermal extremes, this study represents the first to characterize the stress response of a naturally aposymbiotic coral to divergent thermal challenges. Our results demonstrate that divergent thermal challenges elicit a convergent stress response in aposymbiotic corals in the absence of symbiont-associated ROS. The CRG highlighted here will provide the foundation for future research into how the added complexity of symbiosis influences the stress response of coral. Overall, this work effectively 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.

Acknowledgements

This work was made possible through the Boston University Marine Program with special thanks to Justin Scace for husbandry of Astrangia poculata. The authors extend appreciation to K. Sharp, R. Rotjan, S. Grace and the annual Astrangia Workshop hosted by Roger Williams University and Southern Connecticut State University for fostering creative conversations and collaborations leading to this work. Also, we thank SJS Wuitchik and HE Aichelman for helpful edits and insights on this manuscript.

Funding

This work was partially funded by Boston University’s start-up package to S.W.D.

Data Availability

All sequences are available from the NCBI SRI under accession PRJNA595158. Code for all analyses are available by request or can be found at wuitchik.weebly.com/bioinformatics.

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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**Figure S1** | Gene ontology categories significantly enrichment (red: heat challenge, blue: cold challenge). Font identifies adjusted-value from Mann-Whitney U test. Dendrogram clustering similar GO categories.

**Figure S2** | Delta-ranks of enriched cellular components between cold and heat challenge experiments.
Figure S3| Delta-ranks of enriched molecular functions between cold and heat challenge experiments.
Figure S4| Delta-ranks of the enriched biological process between cold and heat challenge experiments.