2023

Host and symbiont-specific patterns of gene expression in response to cold stress in the temperate coral *Astrangia poculata*

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An Honors Paper for the Department of Biology

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Bowdoin College, 2023

ABSTRACT

The coral *Astrangia poculata* inhabits hard-bottom environments from the Gulf of Mexico to Massachusetts and withstands large seasonal variation in temperature (–2 to 26 °C). This thermal range and its ability to live in a facultative symbiosis makes this species an ideal model system for investigating stress responses to ocean temperature variation. Although it has been shown that aposymbiotic *A. poculata* upregulates more genes in response to cold stress than heat stress, the transcriptomic response of the holobiont (coral host and symbiotic algae) to stress is unknown. In this study, we characterize changes in gene expression in both the host and symbionts under cold stress (6ºC) and ambient (12ºC) seawater temperatures. We use RNAseq to visualize how patterns of global gene expression change in response to these temperatures within the transcriptomes of replicate corals (n=10, each temperature) and their symbiont partners. By filtering the holobiont assembly for known coral host and symbiont genes, we contrasted patterns of differential expression (DE) for each partner and the functional processes for each set of DE genes. Differential gene expression analyses revealed that the cnidarian coral host responds strongly to cold stress, while algal symbionts did not have a significant stress response. In the coral host, we found up-regulation of biological processes associated with DNA repair, immunity, and maintaining cellular homeostasis as well as downregulation of mechanisms associated with DNA repair and RNA splicing, indicating inhibition of necessary cellular processes due to environmental stress.

INTRODUCTION

Climate change is drastically altering the dynamics of marine populations including declines in marine animal biomass, changes in ecosystem structure, and effects on functional traits (Bryndum-Buchholz et al., 2019; Hewitt, Ellis, & Thrush, 2016). While different anthropogenic drivers threaten ecosystems worldwide, ocean systems are at greater risk than terrestrial habitats under climate change-driven environments due to two factors: increased thermal sensitivity and high rates of colonization that lead to greater rates of extirpations and species turnover in marine ecosystems (Pinsky, Eikeset, McCauley, Payne, & Sunday, 2019). Yet, in the face of naturally dynamic conditions, species worldwide have evolved resilience against these stressors (De Nadal, Ammerer, & Posas, 2011; Reusch & Wood, 2007). Within a population, the ability to withstand the physical and biological changes associated with climate change will ultimately depend on the amount of genetic variation in traits that reduce thermal stress (Hoffmann & Sgrò, 2011). At the individual level, surviving increasingly extreme daily or

seasonal anomalies will depend on behavior and physiological changes that buffers the impacts of extreme environmental variation on molecular and cellular processes (Somero, 2010).

The building blocks of coral reefs, the scleractinian (hard) corals are an important model for understanding the physiological response to climate change. Scleractinian corals are threatened by anthropogenic impacts, including overfishing, eutrophication, and impacts of climates that are historically unprecedented (Ove Hoegh-Guldberg et al., 2007; Hughes et al., 2003; Pandolfi & Kiessling, 2014). Tropical coral species are particularly vulnerable to climate change since they reside at the upper limit of their thermal range and are experiencing an increasing frequency of warming events associated with the El Nino Southern Oscillation climatic oscillation (Hughes et al., 2017; Hughes et al., 2018). These events result in the breakdown of an obligate symbiotic relationship with an endosymbiotic dinoflagellate algae in the family *Symbiodiniaceae* (formerly the genus *Symbiodinium*, now the family *Symbiodiniaceae* with seven named genera) (LaJeunesse et al., 2018) which provide critical carbon sugars to the coral animal and meet up to 95% of the host's energy needs (Muscatine & Porter, 1977). When this symbiosis breaks down in response to temperature stress, the host coral animal bleaches and ejects most of their symbionts and/or the symbionts reduce their compliments of chlorophylls, greatly increasing the probability of coral death (Grottoli et al., 2014; Stuart-Smith, Brown, Ceccarelli, & Edgar, 2018; Weis, 2008). Besides temperature, a variety of stressors can induce the bleaching response, including increased ultraviolet light, ocean acidification, increased nutrient loading, and synergy between these stressors (Baird, Bhagooli, Ralph, & Takahashi, 2009). Among species, populations, and among and within individual coral colonies, there is considerable variability in the temperature threshold at which the process of bleaching is initiated. This variability in temperature thresholds is partially explained by the total genetic

variation within the holobiont system, including the genomes of the host, algal symbionts, bacterial, and fungal communities. For example, a number of studies have shown that the temperature bleaching threshold depends on the specific combination of host and symbiont genomes, with the symbiont lineage playing a major role in this warm temperature threshold (Cooke et al., 2020; Fisher, Malme, & Dove, 2012; Hume et al., 2019; Parkinson et al., 2018; Silverstein, Correa, & Baker, 2012; Stat & Gates, 2011; Yuyama, Ishikawa, Nozawa, Yoshida, & Ikeo, 2018). These results suggest that the stress response in symbiotic corals is tightly controlled by a co-evolved interaction between symbiont and host genomes.

An important tool for understanding the physiological mechanisms of thermal tolerance in complicated symbioses such as scleractinian corals is the analysis of the entire suite of up- and down-regulated genes in response to environmental stress with transcriptomics (Alvarez, Schrey, & Richards, 2015). Thermal stressors greatly impact physiological processes such as those that mediate symbiotic relationships and an organism's ability to maintain positive energy budgets (Angilletta & Angilletta Jr, 2009; Dimitriadis, Gougoula, Anestis, Pörtner, & Michaelidis, 2012; Drury, 2020; Hammond & Hofmann, 2010; Hector, Hoang, Li, & King, 2022). When applied to corals, the transcriptomic approach has provided unique insights into the interaction between host and symbiont genomes. For example, two studies have shown that the genetic identity of the symbiont (in this case, different *Symbiodinium* species) modulates the specific response of the host transcriptome during heat stress (Cunning & Baker, 2020; M. K. DeSalvo, Sunagawa, Fisher, et al., 2010). Further, Savary et al. (2021) have simultaneously examined the host and symbiont response to high-temperature stress in the coral *Stylophora pistata* of the Red Sea and found that the coral host compliment of up-regulated genes exceeded that of the symbiont compliment, and had different dynamics in terms of transcriptome resilience, defined as the time

required to return to the ambient pattern of gene regulation. In addition to experimentally testing for these quantitative effects on host and symbiont gene regulation, transcriptomics is also revealing the functions of 100s and in some cases 1000s of genes (Ip, Zhang, Xie, Yeung, & Qiu, 2022; Zhu et al., 2022), providing new windows into the cellular environment when critical threshold temperatures are exceeded.

While much attention has been focused on the impacts of heat stress on scleractinian corals, more extreme weather is also driving unusually cool conditions in the tropics and elsewhere that can also result in host-symbiont stress and result in coral bleaching and coral mortality (N. P. Jones, Figueiredo, & Gilliam, 2020; Saxby, Dennison, & Hoegh-Guldberg, 2003; Yu, 2012). Cold water extremes resulting from climate change have similar or more severe impacts on coral physiology and mortality than heat stress (Hernández, 2010; O. Hoegh-guldberg & Fine, 2004; Liang et al., 2017; Nielsen et al., 2020; Parkinson et al., 2018). A few examples of cold-water bleaching events include (1) the 2008 temperature abnormality (1.8 °C lower than the average temperature in the last 25 years) in southwestern Gulf of California that led to over 90% of corals to bleach in some sites (Hernández, 2010), and (2) an event in year 2010 which devastated inshore coral populations along the Florida reef tract (Lirman et al., 2011). Yet physiological understanding of the molecular and cellular response to cold stress in scleractinian corals is still limited, particularly when considering host and symbiont gene expression dynamics. A particularly appropriate model to understand both warm and cold temperature stress is temperate corals that experience wide seasonal variation in temperature and therefore adapted to a much broader range of seasonal variation than their tropical counterparts. The northern star coral, *Astrangia poculata*, is one such example on the East Coast of the United States that inhabits hard-bottom environments from the Gulf of Mexico to Cape Cod, Massachusetts at

depths ranging between 0 and 263 m (Dimond & Carrington, 2008; Peters et al., 1988). The large seasonal variation in temperature throughout its range (–2 to 32 °C) coupled with the fact that symbiosis is facultative—colonies can host algal symbionts, *Brevolium psygmophilim*, or be aposymbiotic while living in the same habitat—makes this species a useful model organism for understanding how the host and symbiont system responds to cold stress.

Across *A. poculata's* range on the East Coast of the United States a handful of studies have focused on the physiological and genomic basis of its thermal stress tolerance in *A. poculata*. Aichelman, Zimmerman, and Barshis (2019) suggested that counter-gradient selection has resulted in higher respiration rates at warmer temperatures in northern populations of *A. poculata* compared to southern populations, suggesting that local adaptation to seasonal maxima and minima are driving local adaptation to seasonal temperature patterns within this broad geographic range. More recently, Wuitchik et al. (2021), used RNAseq to compare gene expression under hot and cold stress in an aposymbiotic Rhode Island population, and found that there were five times as many differentially expressed genes (5318 DEGs) under cold thermal challenge experiments compared to heat stress assays (1054 DEGs). At the same time, there was a significant overlap in identities of DEGs between hot and cold thermal stress experiments (657 genes) suggesting that a subset of the transcriptome is used for countering both types of thermal challenges. In terms of the cold stress response in the host animal, genes that are upregulated in corals include those involved in photosystems, motor function (myosin heavy chain 10 (MYH10; Hong et al., 2015) and immunity (e.g., proteasome core complex (GO:0005839) is indirectly linked to nuclear factor-κB which is part of a coral's innate immune response), while the genes that are downregulated are those involved in motor function (e.g., myosin complex (GO:0016459) (Dixon, Abbott, & Matz, 2020; Wuitchik et al., 2021). Further, Chan et al. (2021)

have found that symbiotic colonies have fewer DEGs than aposymbiotic colonies under heat stress, and that symbionts appear to be suppressing the host's inflammation response. Yet, to my knowledge, no study has documented the cold-water response at the transcriptomic level from the host and symbiont perspective in *A. poculata*.

In this study, I conducted a cold temperature stress experiment with *A. poculata* and quantified gene expression with RNAseq in annotated coral host and symbiotic algal genomes. *A. poculata* exhibits a facultatively symbiotic relationship with the dinoflagellate *Breviolum psygmophilum* (Thornhill, Kemp, Bruns, Fitt, & Schmidt, 2008), which is co-distributed with the coral host. Colonies with these symbiotic algae are a visually distinctive brown when corals have high levels of symbiosis while aposymbiotic white colonies have no or very few symbiont algal cells. Using brown symbiotic colonies collected near the northern range limit of *A. poculata* (Barnstable Harbor, Massachusetts), I conducted a cold-stress experiment by subjecting 10 replicate symbiotic colonies to a cold temperature of 6°C and 10 colonies to a control temperature of 12°C. After 24 hours of temperature exposure, I extracted holobiont RNA from each coral and measured patterns gene expression by sequencing cDNA libraries (n= 20) using RNAseq. With a new bioinformatic pipeline I could determine quantitative and qualitative patterns in gene expression in response to cold-stress response in each holobiont partner: the coral host and the algal symbionts.

METHODS

Thermal Stress Experiment

Figure 1. Bowdoin College Schiller Coastal Studies Center (SCSC) mean temperature from the first data collection on September 19, 2020, to the start of the acute temperature stress experiment on November 5, 2021. Seasonal temperature data was collected from the Bowdoin SCSC pier. The two dotted lines represent the experimental temperature of 6°C (blue) and the cold control temperature of 12°C (red). The solid lines depict the reported upper and lower thermal limit of 26°C (red) and -2°C (blue), respectively, of *A. poculata.*

Colonies of *Astrangia poculata* were collected from Barnstable Harbor near Woods Hole, Massachusetts by the Marine Resources Department at the Marine Biological Laboratory in the late summer of 2020. Colonies were shipped and maintained at the Bowdoin College Schiller Coastal Studies Center (SCSC) in Harpswell, Maine. Corals were maintained at ambient seawater temperatures in indoor flowing seawater tables (Seasonal range, 1-20°C, Fig. 1). Starting in the Fall of 2021, I acclimated a set of coral colonies to a 12h light/dark cycle with artificial LED lighting (Agrobrite, Hydrofarm, Pennsylvania, US) at levels of photosynthetic active radiation of 12 lum/ft². I divided colonies into smaller fragments (around 3 cm x 3 cm) on October 28 and 29 using a Dremel saw (Dremel Manufacturing Company, Racine, Wisconsin, USA) fitted with a 1 1/2-inch blade. One fragment was sampled from a single colony for my experiment, and all fragments were assumed to be genetically unique since reproduction in *A.*

poculata is thought to be strictly sexual (Fadlallah, 1983). All fragments were acclimated to 12°C for 7-8 days before starting the experiment. Temperature was controlled by a custom flowing seawater system built by Tenji Aquarium Design and Build (Newcastle, ME) that consists of an ethylene glycol loop, titanium heat exchangers, immersion heaters, and an Apex Fusion control system (Neptune Systems, Morgan Hill, CA). An ambient temperature of 12°C was chosen for acclimation and for the thermal stress experiment since it represents a typical ambient seawater temperature at the SCSC during mid-fall, while the cold stress temperature of 6°C is representative of early winter temperatures in Harpswell Sound and the Casco Bay region (Fig. 1). Each coral fragment was provided with a unique ID and placed into a labeled petri dish. During acclimation, all coral fragments were fed three days a week *ad libitum* with live brine shrimp nauplii (*Artemia spp.*).

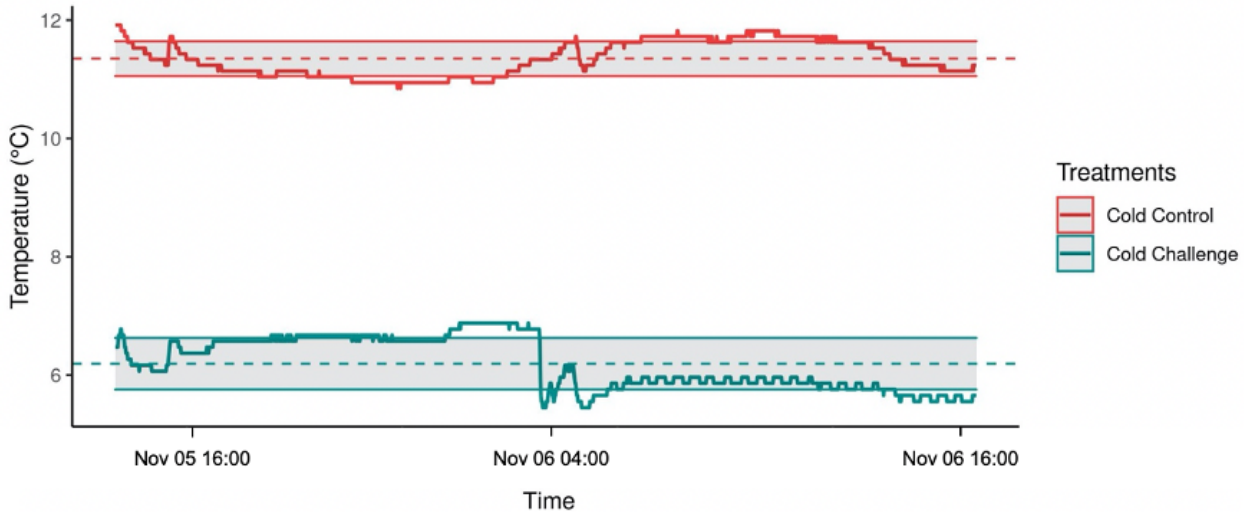

Figure 2. Temperature profiles for control and cold stress aquaria during the cold stress experiment. Dotted lines represent the mean temperature for both treatments. The solid lines and shading depict mean temperature +/- SD.

Following the acclimation period, each of the 20 fragments were randomly assigned to one of two, 20 L flowing seawater aquaria (n= 10 for each treatment). One aquarium was not

temperature-controlled and represented ambient conditions. The second aquarium was the cold stress treatment (6°C set point). Tank flow rates were between 0.8-0.9 L min^{-1}. The experiment included a ramp from ambient to 6°C over 6 hours, afterwards, starting on November 5, 2021, the coral fragments were maintained at ambient and cold temperature for 18 hours (Fig. 2). Tank temperatures for the duration of the experiment were monitored and recorded using Onset HOBO data loggers (Onset Computer Corporation, Bourne, MA). During experiments, ambient temperature ranged from 10.85°C-11.92°C (mean= 11.35°C) and the cold stress treatment temperature ranged from 5.45°C-6.87°C (mean=6.19°C) (Fig. 2). All coral fragments in the cold stress aquaria retracted their polyps for the duration of the 18 h experiment, while all coral fragments in the ambient aquaria extended their polyps. These behavioral differences suggested that the cold stress treatment induced significant physiological stress.

RNA Extraction and Sequencing

At the completion of the experiment on Nov. 6, 2021, I extracted whole RNA from each coral fragment. To do this, conical tissue samples were removed from three polyps from each fragment by inserting a triangular hobby knife blade into the center of the polyp, rotating, and removing the skeletal septa and attached tissue. These tissue samples were then placed in a 1.5 ml microcentrifuge tube containing 300 µl of RNAlater (Ambion, Thermo Fisher Scientific, Waltham, Massachusetts). The samples were stored at –80°C before RNA extractions the following week. I extracted RNA using the RNeasy Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's recommendations and used a TissueLyser (Qiagen, Hilden, Germany) for disruption and homogenization. Tissues were disrupted in Buffer RLT with 5% Beta-mercaptoethanol, using 1.5 ml tubes containing a single stainless-steel bead (3 mm mean diameter). The disruption cycle was 2 min at 20–30 Hz. Downstream extraction steps followed

the RNAlater kit instructions. The resulting RNA was sequenced by Novogene (Beijing, China) using a paired-end library preparation and a target sequencing depth of 3 Gigabases (Gb) per sample.

Bioinformatic Pipeline

Raw reads were cleaned of adapters and filtered for quality with Novogene's standard filters. To create a *de novo* assembly for the holobiont transcriptome I pooled all paired end reads from the 20 samples and assembled using Trinity (Broad Institute, Cambridge, Massachusetts) running on the Bowdoin High-Performance Computer (Command line arguments and R-scripts are available from the Carlon Lab at Bowdoin College by request). My pipeline flowed downstream from this holobiont transcriptome, ending in the differential gene expression analysis (DE) for each holobiont partner and enrichment analysis (Fig. 1). To separate assembled contigs in the holobiont transcriptome into coral host and algal symbiont transcriptomes, I conducted a BLAST search using the holobiont contigs against the NCBI nucleotide database (nt) and retaining the taxonomic terms for all the resulting subject sequences. These BLAST results were filtered using dplyr in R (Hadley Wickham, 2023) so that each query matched a single subject sequence that returned the highest identity match. Only queries that had matches of >80% and => 100 bp were retained in this list. To create sequence lists for each of two partners in the holobiont, I then filtered the list of Trinity sequence IDs with corresponding single BLAST matches by specific taxonomic keywords that were representative of each of two partners in the cnidarian – algal symbiosis. (Supplemental Table 1). This filtering step resulted in two lists of trinity sequence IDs, one for the cnidarian host, and one for the algal symbiont.

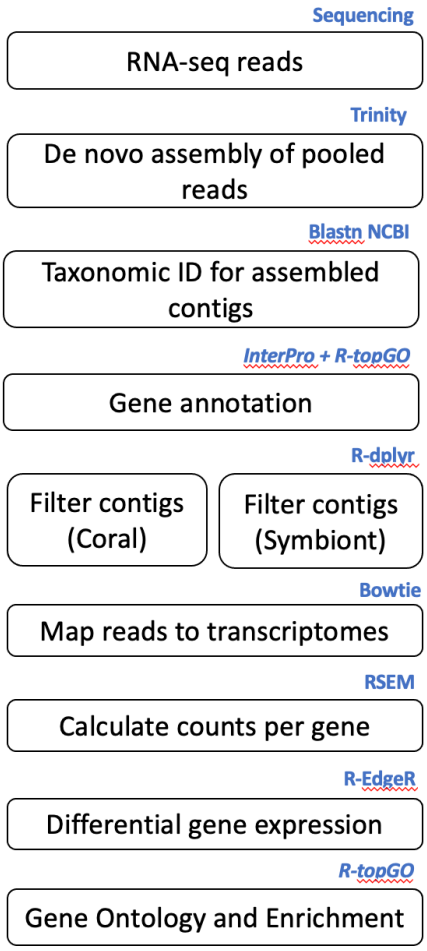

Figure 3. Bioinformatics pipeline including (1) Sequencing and quality control by Novogene, (2) Creating a de novo assembly of holobiont, (3) Retrieving taxonomic IDs for each assembled transcript using a nucleotide BLAST (4) Gene annotation using InterPro, (5) Filtering the holobiont data into putative coral host and symbiont transcriptomes, (6) Mapping the raw reads from each sample to each transcriptome with Bowtie, (7) Calculating read counts per gene using RSEM, (8) Estimating differential gene expression patterns with EdgeR, and (9) gene ontology and enrichment analysis with topGO. See text for software references.

The holobiont transcriptome was subsetted with the respective host and symbiont transcript IDS using the Trinity utility acc_list_to_fasta_entries.pl, resulting in putative coral host and algal symbiont transcriptomes. While I identified 17,559 putative coral host genes with the BLAST search and taxonomic filtering, I identified a relatively small number (only 752) *Symbiodinaceae* genes compared to the expectation of ~ 50,000 (Table 1), and therefore also used a published transcriptome of dinoflagellate *Breviolum psygmophilum* (formerly

Symbiodinium psygmophilum) from Parkinson et al. (2016); available at http://zoox.reefgenomics.org/) for downstream DE analysis. This assembly contains 50,745 contigs (Table 1) and is clearly more representative than our algal symbiont assembly created by filtering symbiont taxonomic terms from the NCBI nt database. Further, to determine the similarity between the contigs in this *Breviolum psygmophilum* reference and those in the holobiont transcriptome, I built a local BLAST nucleotide database from the *Breviolum psygmophilum* reference and ran a nucleotide BLAST with the entire holobiont data set against this reference. After filtering for matches with > 95% sequence identity, I identified 84,529 holobiont transcripts that matched at least one of 41,425 *Breviolum psygmophilum* transcripts. In fact, 68,448 of the holobiont transcripts had > 99% sequence identity. This result suggests that the published *Breviolum psygmophilum* transcriptome is a highly appropriate reference for the algal symbionts in my sampled corals, and that my holobiont sequencing has recovered a substantial component of the *Breviolum psygmophilum* transcriptome. I refer to the previously published transcriptome as the "*B. psygmophilum* transcriptome" in all downstream analysis.

My DGE analyses for coral host and symbiont transcriptomes are based on the methods outlined by Chen et al. (2016). We started with RSEM (Li & Dewey, 2011), which mapped the individual reads from each sample back to a specific reference to estimate read counts based on Trinity isoforms. I then used the expected counts from RSEM and the R-package edgeR (McCarthy, Chen, & Smyth, 2012; Tiberi & Robinson, 2020) to test for differences in gene expression between control and experimental treatments. The matrix of read counts was filtered so that genes were retained that had 0.5 cpm (counts per million) in at least eight libraries (= samples) for each experimental group. Genes were normalized using the calcNormFactors function and dispersion was estimated using a model statement that contrasted the two groups:

ambient and cold treatments. The quasi-likelihood model was fit using the glmQLFit function and genes with a False Discovery Rate (FDR) of < 0.05 were considered differentially expressed between the groups and plotted as volcano plots and heat maps. To increase the power of the model to detect coral-specific and symbiont-specific genes that were differentially expressed, I ran this DGE analysis using three different transcriptomes: the holobiont, the coral host, and the symbiont (*B. psygmophilum* transcriptome). I note that when corrected for FDR the power to detect DE genes will be inversely correlated to transcriptome size due to the number of possible comparisons.

To test for enrichment in gene function, I used the R package topGO (Alexa 2023) to compared gene ontology terms (GOs) for lists of significantly up- and down- regulated genes to those derived from entire transcriptomes. To extract gene annotation information including GO IDs, I used the InterProScan 5.61 package (Jones et al. 2014) that predicts protein families and the presence of functional domains and sites by combining several search applications. Since only the coral host showed differential expression in response to cold stress (see Results), tests for enrichment were only conducted on the coral host transcriptome with DE genes. To test for enrichment in the three GO annotation categories (biological processes, cellular compartment, and molecular function), I used the Kolmogorov-Smirnov test, *elim* algorithm, and a p-value cutoff of 0.05.

RESULTS

Transcriptome assembly

The assembled *Astrangia poculata* holobiont transcriptome consisted of 1,587,337 transcripts, of which there were 973,063 identified as Trinity genes (Table 1). Through BLAST and taxonomic filtering of this dataset, we identified > 17,000 coral host transcripts with a mean

contig length of 1,706 bp; and 853 algal symbionts transcripts with a mean contig length of 830 bp (Table 1). In contrast, the published *Breviolum psygmophilum* transcriptome contains > 50,000 transcripts. My coral host transcriptome assembly is an improvement over that of Wuitchik et al. (2021), which used single-end reads as the basis for assembly and samples from stress experiments with aposymbiotic *A. poculata*. They recovered fewer and shorter transcripts in this assembly (Table 1: 13,343 transcripts, mean contig length of 265).

Table 1. Transcriptome statistics based on the *A. poculata* holobiont, coral host, and symbionts. Also included are Holobiont W* and *B. psygmophilum*** statistics from previously published transcriptomes [Wuitchik et al. (2021)* and Parkinson et al. (2016)**]. I used the *B. psygmophilum* transcriptome for differential gene expression analysis and gene annotation.

Contig metric	Holobiont	Holobiont W*	Coral host	Symbiont	*B. psygmophilum***
Total assembled bases	629,060,278		29,963,600	737,173	57,231,880
Trinity genes	973,063		15,261	752	
Trinity transcripts	1,587,337	13,343	17,559	853	50,745
Mean contig length	588	265	1,706	830	1,128
Median contig length	350		1103	980.28	825
N50	775	258	2,913	1,216	1,618
N40	1,087		3,499	1,405	1,904
N30	1,536		4,262	1,587	2,272
N20	2,217		5,333	1,792	2,860
N10	3,543		7,137	2,415	4,222
GC content percent	42.52	42	42.26	50.50	51.37
% annotated		25.7%	40.6%		47.7%

Differential gene expression in responses to cold stress

Multidimensional scaling (MDS) of read counts mapped to the three transcriptomic data sets revealed that patterns in holobiont gene expression as a response to cold stress were obscuring patterns in differential gene expression under cold stress between the coral host and algal symbionts (Fig. 4). Samples grouped by experimental cluster in the MDS plot of coral host expression (Fig. 4B), but there was no such pattern in the MDS plot of holobiont expression (Fig. 4A) nor in the MDS plot the symbiont expression (Fig. 4C).

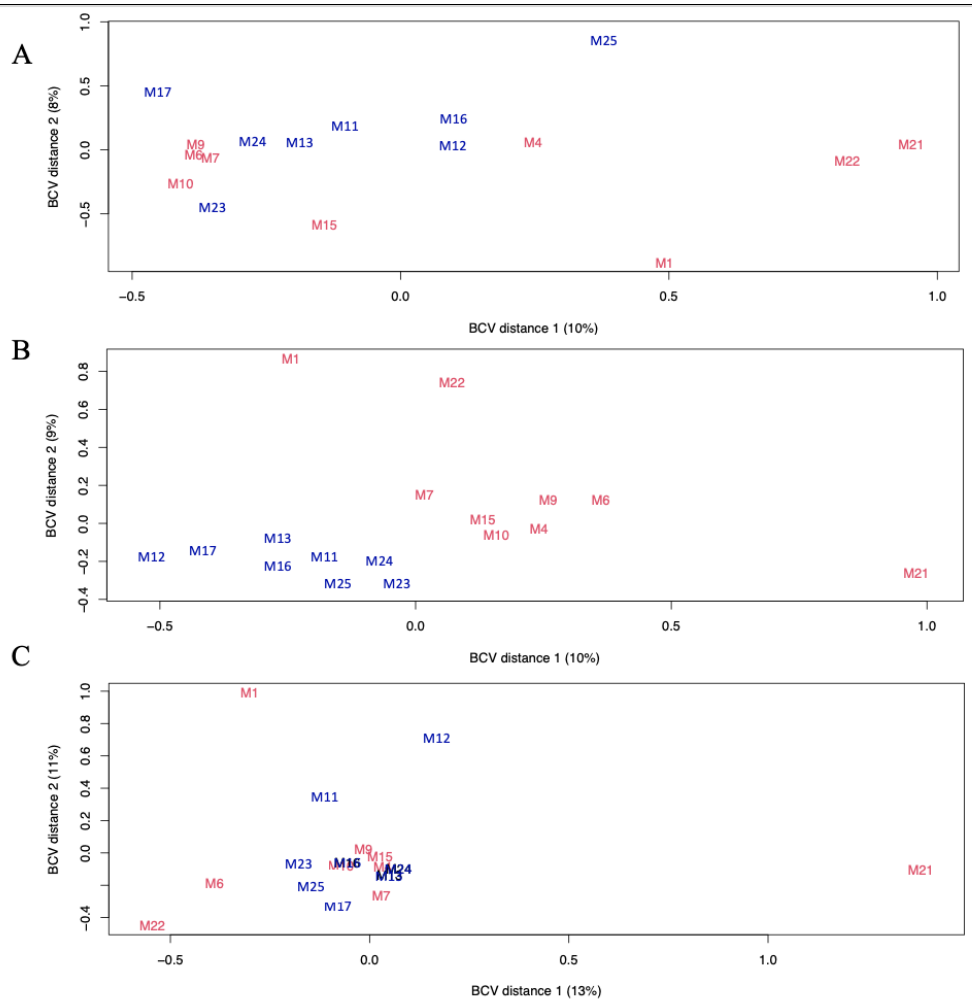

Figure 4. Multidimensional scaling analysis of gene expression under cold stress in *A. poculata* for the holobiont (A), coral host (B), and algal symbionts (C). Blue sample IDs indicate the cold-stress experimental group and red sampled IDs represent the ambient temperature group. The method for measuring the distance between samples is based on the biological coefficient of variation (BCV distance).

Patterns in differential gene expression (DGE) as determined by edgeR models were consistent with the MDS patterns (Fig. 5). At an FDR cutoff of 0.05, there were significant up- and down-regulated genes in response to cold stress in both the holobiont and coral host, however, the symbionts did not respond to cold stress in terms of DGE (contrast Figs. 5A, B, and C). In the differential expression gene analysis of the holobiont data, only 77 genes were significantly upregulated, and 7 genes were significantly downregulated (Fig. 5A). Power to detect DGE increased substantially when analyzing DGE based on the coral host, where there were 494 upregulated genes and 175 significantly downregulated genes observed (Fig. 5B). In contrast, symbiont DGE based on *Breviolum psygmophilum* transcriptome in response to the cold stress was not significant (Fig. 5C).

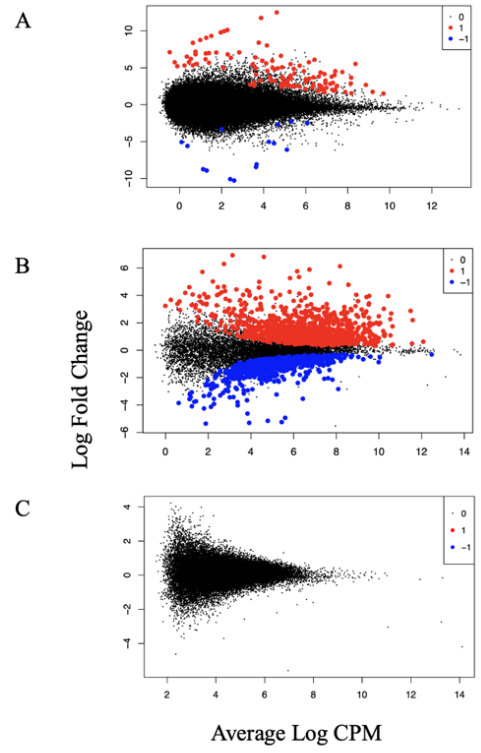

Figure 5. Volcano plot of DE genes for the holobiont (A), coral host (B), and symbiont (C). Red (upregulated) and blue (downregulated) symbols indicate significant genes after correcting for false discovery (FDR). The X axis is the average log counts per million reads (CPM), and the Y axis is the log-fold change relative to the null model.

Gene annotation and gene ontology/enrichment analyses

Annotation of the complete set of coral host transcripts revealed that several stress-related genes (e.g., Ubiquitin and E3 ligase) were strongly upregulated during cold stress (Fig. 7; Fig. 7A). The most highly up-regulated transcript was annotated to the mammalian suppressor Sec4 (Mss4), a protein known to regulate the stress response and cell death in mammals (Walter et al., 2012). Similarly, two chaperone proteins were also upregulated during cold stress (Ubiquitin-like domain, and E3 ubiquitin-protein ligase). In terms of downregulation, the most strongly down-regulated transcript was annotated to the U4/U6.U5 small nuclear ribonucleoprotein component Snu23 (Fig. 7B) which is a protein in the spliceosome complex, a RNA-protein complex involved in removing introns (Will & Lührmann, 2011).

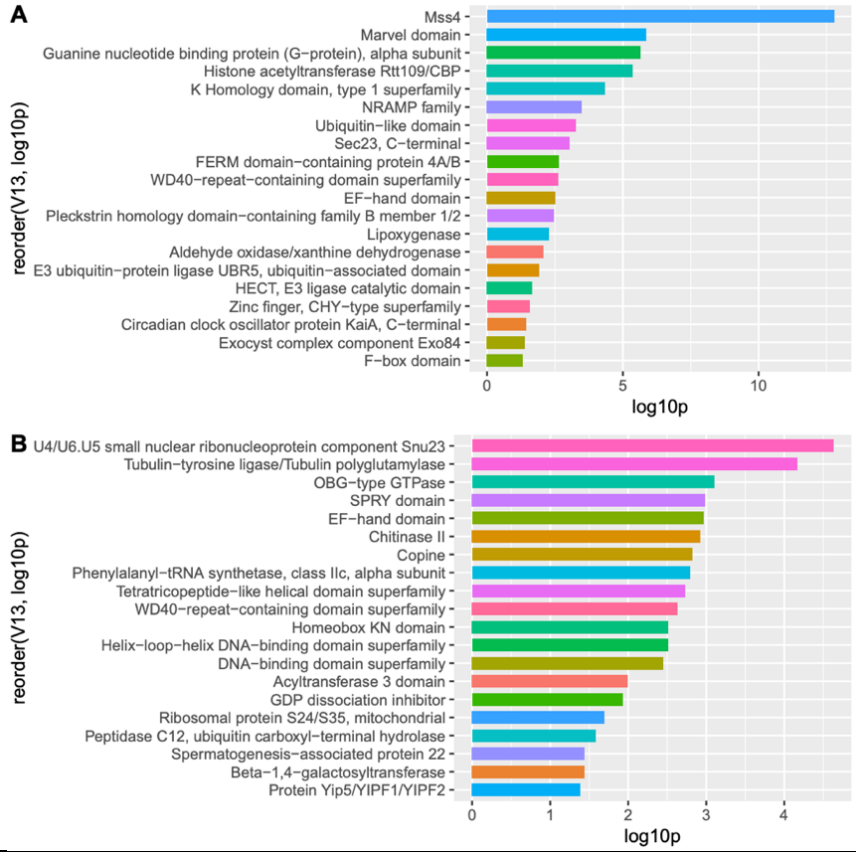

Figure 7. The top 25 annotated transcripts that were either up (A) or down (B) regulated in response to cold stress. Genes on the y-axis are ranked by the false discovery rate (FDR), and the x axis is the –log10 (FDR). FDR is inversely related to the x-axis scale.

The combined set of up- and down- regulated genes were significantly enriched in gene ontology terms (GO terms) among the three GO categories: biological processes, cellular components, and molecular functions (Fig. 8). The top-ten GO terms for each category revealed that there was considerable gene expression activity involving the mitochondria in response to cold stress (Fig. 8). In the cellular components category four of 43 GO terms were related to the mitochondria, including *mitochondrial matrix* (GO:0005759), *mitochondrial ribosome* (GO:0005761), *mitochondrial small ribosomal subunit* (GO:0005763), and *TIM23 mitochondrial import inner membrane* (GO:0005744). *Mitochondrial carrier proteins* tended to be up-regulated (GO:0055085), four different transcripts with this GO term were significantly upregulated under cold stress. In contrast, nine different mitochondrial transcripts were downregulated in response to cold stress, including ribosomal genes and a variety of different carrier proteins.

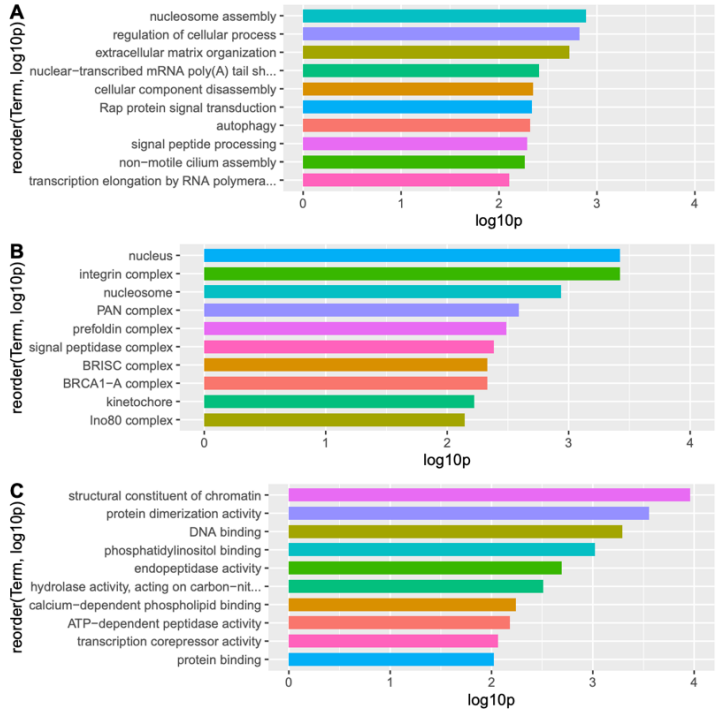

Figure 8. The top 10 functional annotations of differentially expressed genes in three gene annotation categories in the coral host under cold stress including (A) biological processes, (B) cellular component, and (C) molecular function. GO terms on the y-axis are ranked by p-values, and the x-axis is the –log10 (p-value). The p-value is inversely related to the x-axis scale.

A heat map visualization of all successfully annotated genes (187) in the coral host, revealed more up- regulation compared to and down- regulation in the cold treatment compared to the ambient control treatment (Fig. 9; FDR adjusted, $p<0.05$). In the control groups, there are consistent patterns of up- and down- regulation across all of the samples, however sample M21 demonstrates more variability across annotated genes which is consistent with the MDS plot for the coral host suggesting that there is genetic variation for gene expression among individuals (Fig. 9). The clustered trees (dendrograms) in the heat map also indicate that there is clustering across genes and samples based on significant differential gene expression patterns. Moreover, annotations associated with patterns of gene upregulation in this heat map were linked to processes of DNA repair (e.g., positive regulation of DNA repair) and returning to cellular homeostasis (e.g., Ubiquitin-dependent protein catabolic process) in the cold stress experiment (Fig. 9). On the other hand, there are also patterns of downregulation of DNA repair processes (e.g., DNA repair). Notable downregulation of gene ontology terms related to reproduction (e.g., spermine biosynthetic process) reveals that processes related to reproduction were limited in response to cold stress.

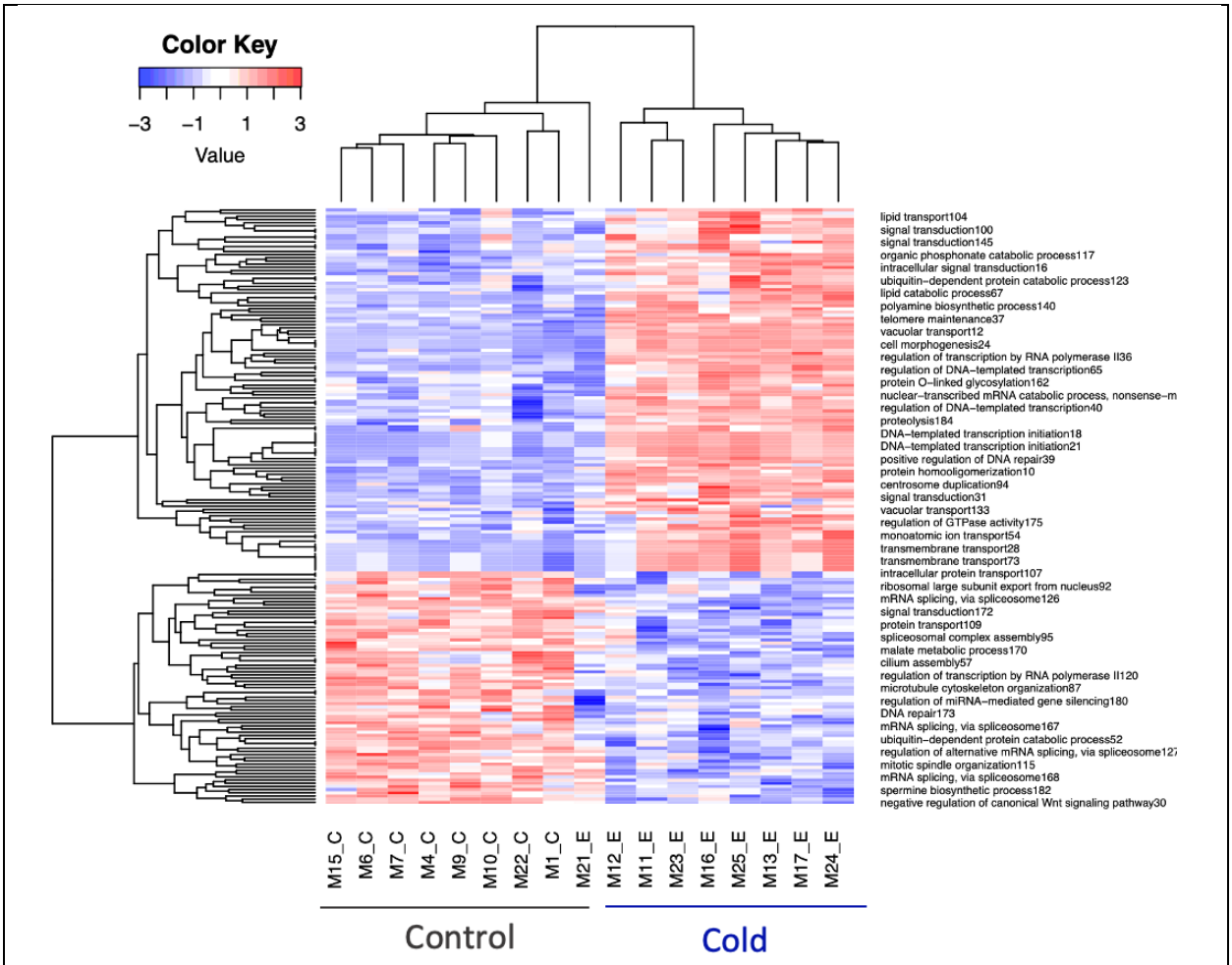

Figure 9. Clustered heat map of 187 differentially expressed genes (DEGs) that were also annotated in the coral host. Colors denote magnitude of response (blue, downregulated; red, upregulated) through a z-score of the difference in expression levels from the mean expression for each gene. Dendrograms indicate that genes have similar differential gene expression patterns (rows) or samples that with similar responses (columns). Row labels are the gene ontology terms related to selected transcripts and sample IDs grouped by experimental group are listed below the columns.

A heat map for the top 100 significant DE genes shows more up-regulation compared to down-regulation in response to cold stress (Fig. 10). As in the heatmap with all 187 annotated genes, genes involved in DNA repair were up-regulated in response to cold stress (e.g., positive regulation of DNA repair) (Fig. 10). However, other notable significant genes that we were able to pull out were those in the NF-κB family of transcription factors (e.g., I-KappaB kinase/NF-kappaB signaling) that regulate genes that control processes including tissue remodeling, cell death, immunity, and the cellular stress response (Perkins, 2007). Additionally, we observed up-

regulation of genes that were directly involved in cell death (e.g., regulation of apoptotic process) which is often an indicator of a strong stress response to an environmental condition.

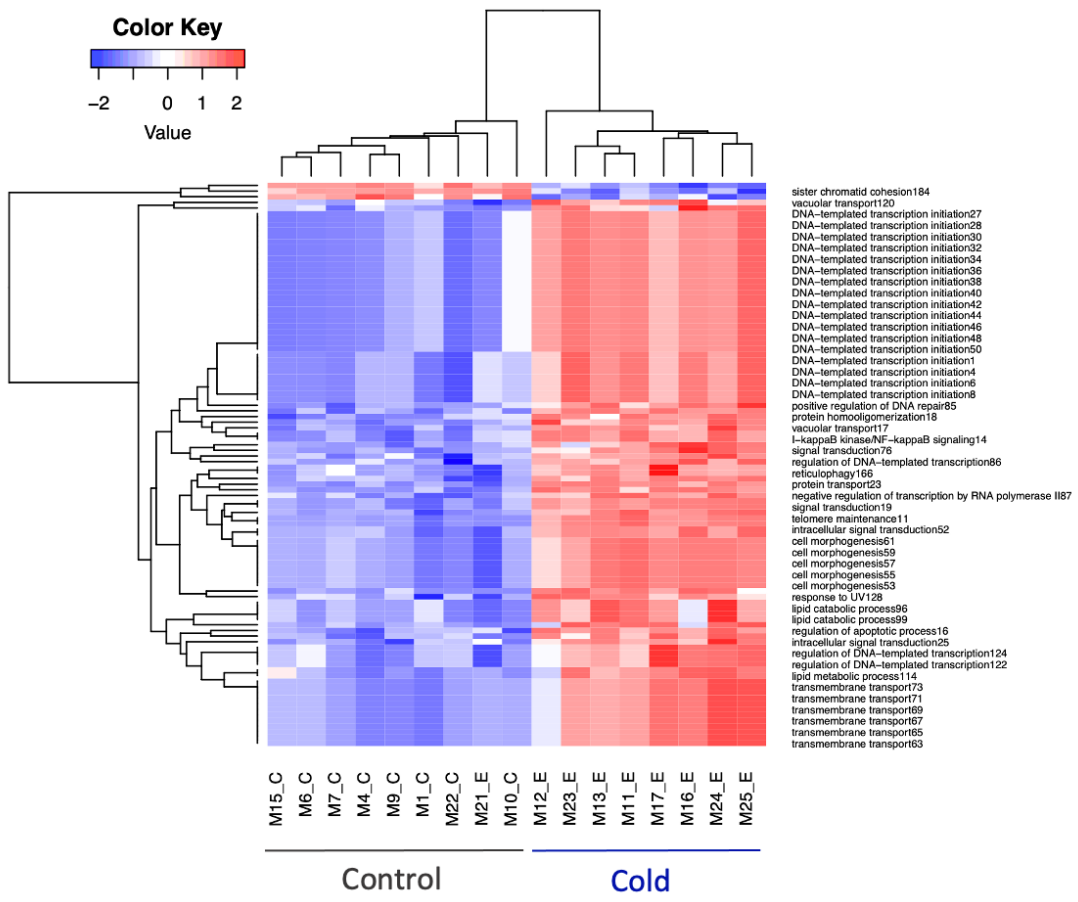

Figure 10. Clustered heat map of top 100 differentially expressed genes (DEGs) in the coral host. Colors denote magnitude of response (blue, downregulated; red, upregulated) through a z-score of the difference in expression levels from the mean expression for each gene. Dendrograms indicate that genes have similar differential gene expression patterns (rows) or samples that with similar responses (columns). Row labels are the gene ontology terms related to each transcript and sample IDs grouped by experimental group are listed below the columns.

DISCUSSION

In the symbiotic temperate coral *Astrangia poculata*, I found that the two primary partners in symbiosis: the coral (cnidarian) host and the symbiotic dinoflagellates; responded to cold stress in very different ways. The coral host was responsible for all detectable differences in gene expression, while I did not detect any changes in gene expression in the symbiotic

dinoflagellates. With regards to a general pattern emerging in the temperature stress response for both temperate and tropical hard corals that engage in symbioses with dinoflagellates, it appears that symbiotic dinoflagellates are less impacted by cold stress than their cnidarian hosts. For example, Huang et al. (2022) working with the tropical coral *Porites lutea* across the South China Sea, found that nearly 6000 DEGs in the coral host exposed to 12 d C compared to ambient temperatures of 19 d C, while only 0-12 DEGS (depending on population comparison) were detected in the *Symbiodinaceae* under these same treatments. Similar patterns in the number of possible up- and down regulated genes, have been found in tropical corals in response to heat stress when close to the thermal thresholds for bleaching. In the Indian Ocean and Red Sea, Voolstra et al. (2021) studied the response of the symbiotic partners in the coral *Stylophora pistillata* from two populations, the more southern (and cooler) Gulf of Aqaba and the northern (and much warmer) central Red Sea. They found that strong transcriptomic responses (significant genes) from each of the three holobiont partners (the coral host, symbiont, and the microbiome) in corals from the cooler environment of the Gulf of Aqaba, compared to more muted responses (fewer genes) among all symbiotic partners in corals from the warmer central Red Sea population which had higher overall levels of gene expression across transcriptomes.. Further, a companion study on corals from these same populations ((Savary et al., 2021), revealed that the large transcriptomic response from corals sampled from the cooler Gulf of Aqaba, was largely a coral host response, and found only a handful of heat stress genes that were up- or down- regulated in algal symbionts and these were at much lower log-fold changes. Other tropical species also have much muted symbiotic responses to heat stress, including analysis of the number of host and symbiont DEGs in the leaf coral *Pavona decussata* (Zhang et al., 2022); and from the comparative expression of heat shock proteins (HSPs), Leggat et al. (2011) found

10x less gene expression in HSPs originating from the host vs. symbionts in the reef coral *Acropora aspera*. While it remains largely unknown whether a similar differential exists between host and symbiont under heat stress for the temperate coral *A. poculata* (but see the pre-print Chan et al. (2021)), these collective results suggest that the dominant response to temperature stress in symbiotic corals comes from the coral host, perhaps signaling the beginning of the bleaching and end of symbioses in these partnerships.

Due to the link between transcriptomics and physiology, we can assume that the coral host is contributing most strongly to the physiological response to cold stress within the symbiotic relationship of *A. poculata* (Kaniewska et al., 2015). We found that the coral host was increasing its physiological capacity to mitigate its physical deterioration by minimizing cellular damage and increasing DNA repair. This indicates a strong transcriptomic response to cold stress in the host. Additionally, temperature stress and resulting oxidative damage (e.g., increased production of reactive oxygen species, a proxy of increased stress) lead to increased protein degradation in corals and we found this in our study with increased gene expression relating to processes of targeting proteins for degradation in the proteasome (M. K. DeSalvo, Sunagawa, Voolstra, & Medina, 2010; Downs et al., 2005). Post-transcriptional modifications using ubiquitin or ubiquitin-like proteins mark proteins for breakdown. Ubiquinated proteins are targeted by proteolytic enzymes that are called proteosomes and this allows for denatured proteins to be removed from cells while the proteins that are being removed are produced at higher rates to compensate for their loss in response to stress (Downs et al., 2005; Lan, Qiu, Xu, Liu, & Miao, 2022). In addition to these overrepresented biological processes, we also found sets of genes involved with immunity and cell death. We observed genes associated with apoptosis and the NF-κB signaling pathway in the cold stress response in the host. Apoptosis occurs when

cells are no longer able to maintain homeostasis and cell death pathways are induced in response to stress (Kültz, 2003). The gene that was significantly overrepresented in our dataset was I-KappaB (I- κB) kinase which is involved in regulating the NF-κB signaling pathway which is linked to coral immunity and regulates cell proliferation and death (Barshis et al., 2013; Lan et al., 2022; Mansfield et al., 2019). These genes dictate when cell repair, immunity, and apoptosis occur in the coral host in response to environmental stressors and are crucial for understanding the ability of individuals to have thermal resistance (Dixon et al., 2020). Further, if we consider that these same biological processes are typically upregulated by the coral host during the earliest phases of coral bleaching (Drury, 2020), the cellular processes are perhaps useful indicators that the symbiotic threshold tolerance has been crossed.

Biological processes related to mitochondria were highly enriched in our study, a finding that has been observed in other corals in response to temperature stress (M. K. DeSalvo, Sunagawa, Voolstra, et al., 2010; Dimos, Mahmud, Fuess, Mydlarz, & Pellegrino, 2019). Mitochondria are the primary site of ATP production and hold the key to aerobic energy turnover. Cold stress causes a general rise in aerobic capacity, reflected in rising mitochondrial densities, and/or increasing capacities of mitochondrial enzymes (Chung, Sparagna, Chicco, & Schulte, 2018; Guderley, 2004; Pörtner, 2002; Sanford & Kelly, 2011). Mitochondrial enlargement has also been attributed to elevated baseline metabolic rates (Angilletta & Angilletta Jr, 2009), a lower temperature minimum (Sommer & Pörtner, 2002), but also a lower upper thermal limit (Fangue, Podrabsky, Crawshaw, & Schulte, 2009). In *A. poculata*, similar to our study, links to mitochondrial function in response to cold stress have been observed as reflected by higher respiration rates in northern populations compared to southern populations that experience the coldest temperatures along its range (Aichelman et al., 2019). Since northern

populations experience a wider variation in temperature and have greater respiration rates, they are a useful species for examining physiological responses to cold stress. On the other hand, animal mitochondria are also sensitive to environmental stress which can impair mitochondrial integrity and function, and this affects performance and fitness. Mitochondrial degradation or inhibition of mitochondrial functions has also been attributed to the response of the coral host to temperature stress in corals and was inferred from patterns of gene regulation in our study (M. DeSalvo et al., 2008). Supporting this idea, (Dunn, Pernice, Green, Hoegh-Guldberg, & Dove, 2012) found that cold temperatures led to the degradation mitochondria in the coral host that was independent of the symbiont's reaction to environmental stress. Specifically, they observed that the primary site that deteriorated was downstream of complex III in the electron transport chain, which limits cytochrome c and ATP synthase production. This, in turn, prevents the coral host from dealing with reactive oxygen species, which are highly unstable chemicals that are produced in response to stress and damage cells, and decreases the amount of chemical energy available to maintain crucial cellular processes. The role of mitochondria in increasing temperature resistance and in the stress response to cold stress specifically in the cnidarian, as seen in our system and other corals, emphasizes the significance of the coral host response to temperature stress.

Our study emphasized the importance of the coral host in the response to environmental stress for corals and coral reefs and improves our understanding of how symbiotic corals will respond to climate change. The quantitative and qualitative physiological changes we have inferred from RNAseq-derived gene expression patterns are similar to tropical corals responding to both cold and heat stress. This is crucial to expand our knowledge of the physiological mechanisms of both coral host and its symbiotic community underlying this fundamental stress

response in a rapidly changing environment. The lack of response to cold stress from the symbiont can bring about a number of explanations including (1) symbiont-specific genetic variation is important to stress responses and *B. psygmophilim* is not a symbiont that reacts strongly to temperature stress and (2) the symbiont is more resilience to temperature stress—as seen by a weak transcriptomic response to the cold temperature—which can be the reason behind *B. psygmophilim* being the primary symbiont along *A. poculata's* range. Lastly, we found that the main differences in physiological responses to cold stress in our study compared to previous literature on heat stress effects in corals and coral reefs were mainly attributed to mitochondrial function. This is essential in improving our understanding of stress resilience to environmental changes because this is a mechanism of resistance that is observed across temperate and tropical corals.

Future studies should focus on unraveling the connection between increased stress resilience and increased mitochondrial capacity in cnidarian hosts through quantifying changes in aerobic capacity, mitochondrial densities, and capacities of mitochondrial enzymes as a result of heat and cold stress. Moreover, pairing the transcriptomic response with other morphological and genetic variables within and between populations can help us gain a better perspective on the underlying genetic variation in the holobiont partners that may hold the key for future adaptation. Lastly, the opportunity provided by our *A. poculata* holobiont transcriptome to delve into not only the role of the symbiotic partners (coral host and symbiont) but also the other microscopic organisms contributing to this thermal resilience in this system can also be leveraged. This is an opportunity to create a more nuanced understanding of *A. poculata's* stress response that is rapidly becoming a model to also understand more vulnerable tropical hard corals.

SUPPLEMENTAL MATERIALS

Supplemental Table 1. Keywords used in filtering BLAST results into coral host and symbiotic algae transcriptomes.

Taxonomic Keywords	
Coral Host	**Algal Symbiont**
Stylophora	*Symbiodinium*
Acropora	*Brevolium*
Pocillipora	
Nematostella	
Actinia	
Anthopleura	

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