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Symbiosis modulates gene expression of symbionts, but not coral hosts, under thermal challenge

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Abstract

Increasing ocean temperatures are causing dysbiosis between coral hosts and their symbionts. Previous work suggests that coral host gene expression responds more strongly to environmental stress compared to their intracellular symbionts; however, the causes and consequences of this phenomenon remain untested. We hypothesized that symbionts are less responsive because hosts modulate symbiont environments to buffer stress. To test this hypothesis, we leveraged the facultative symbiosis between the scleractinian coral *Oculina arbuscula* and its symbiont *Breviolum psygmophilum* to characterize gene expression responses of both symbiotic partners in and ex hospite under thermal challenges. To characterize host and in hospite symbiont responses, symbiotic and aposymbiotic *O. arbuscula* were exposed to three treatments: (1) control (18°C), (2) heat (32°C), and (3) cold (6°C). This experiment was replicated with *B. psygmophilum* cultured from *O. arbuscula* to characterize ex hospite symbiont responses. Both thermal challenges elicited classic environmental stress responses (ESRs) in *O. arbuscula* regardless of symbiotic state, with hosts responding more strongly to cold challenge. Hosts also exhibited stronger responses than in hospite symbionts. In and ex hospite *B. psygmophilum* both down-regulated gene ontology pathways associated with photosynthesis under thermal challenge; however, ex hospite symbionts exhibited greater gene expression plasticity and differential expression of genes associated with ESRs. Taken together, these findings suggest that *O. arbuscula* hosts may buffer environments of *B. psygmophilum* symbionts; however, we outline the future work needed to confirm this hypothesis.

KEYWORDS
coral, gene expression, symbiosis, thermal challenge

1 | INTRODUCTION

Endosymbioses—associations where one organism lives within cells of its host (Sagan, 1967)—have driven evolutionary innovations and allowed species to access resources and environments that would otherwise be unavailable (Melo Clavijo et al., 2018; Wernegreen, 2012). Endosymbioses span the tree of life and comprise exemplary innovations including deep-sea hydrothermal vent tubeworms (*Riftia pachyptila*) that rely on chemosynthetic bacterial endosymbionts (e.g., Robidart et al., 2008) and salamanders (*Ambystoma maculatum*) benefiting from photosynthetic endosymbionts (*Oophila amblystomatis*) as embryos (e.g., Burns et al., 2017).

Endosymbionts often live within a host compartment, such as a vacuole or membrane, which facilitates the exchange of nutrients and metabolites, serving as the backbone for these symbioses (Dean et al., 2016; Wernegreen, 2012).

Corals are one of the most iconic examples of endosymbiosis, and their symbiosis with single celled algal symbionts [dinoflagellate algae in the family Symbiodiniaceae, hereafter 'symbiont' (LaJeunesse et al., 2018)] enables diverse tropical reef ecosystems to thrive in oligotrophic waters (Melo Clavijo et al., 2018). Symbiodiniaceae live in coral gastrodermal cells in specialized vacuoles called symbiosomes (Davy et al., 2012; Wernegreen, 2012). This endosymbiosis facilitates the transfer of materials between host and symbiont, where symbionts share photosynthetically derived carbon sugars and in return receive inorganic compounds from coral metabolic waste in addition to protection (Muscatine, 1990; Muscatine et al., 1981). Once symbiosis is established, hosts can actively modulate symbiont physiology by manipulating the symbiosome environment. For example, symbiont photosynthesis is dependent on nitrogen availability, and host-mediated nitrogen limitation enables maintenance of primary production and control of symbiont growth (Falkowski et al., 1993; Rädecker et al., 2015). Additionally, coral hosts acidify the symbiosome via expression of V-type proton ATPases, which facilitates increased photosynthesis (Barott et al., 2015).

Tropical corals live near their upper thermal limits, making them particularly susceptible to temperature changes (Baker et al., 2008; Berkelmans & Willis, 1999). Increases in anthropogenic carbon dioxide levels are elevating ocean temperatures and leading to marine heatwaves (i.e., Smale et al., 2019), which threaten corals globally (Frieler et al., 2013). Specifically, temperature increases lead to a breakdown of coral-algal symbioses in a process called 'coral bleaching', and extended periods of dysbiosis can lead to coral starvation and eventual mortality (Brown, 1997). While bleaching in response to elevated temperatures is well documented, cold temperatures can also induce coral bleaching (e.g., Rich et al., 2022). The mechanisms that cause coral bleaching in response to both heat and cold stress appear similar (Marangoni et al., 2021), and it is theorized that reactive oxygen species (ROS) generated by algal symbionts under temperature stress can damage cellular components, cause photoinhibition, and trigger coral bleaching [reviewed in (Szabó et al., 2020)]. However, even though both symbiotic partners exhibit a wide array of stress responses, symbionts are assumed to initiate dysbiosis due to ROS production (e.g., Berkelmans & van Oppen, 2006; Stat et al., 2006; Stat & Gates, 2010). Additionally, several lines of evidence demonstrate that hosts exhibit strong gene expression responses to stress (e.g., Davies et al., 2016; DeSalvo et al., 2010; Meyer et al., 2011), reviewed in (Dixon et al., 2020), while the symbiont's response is muted (e.g., Barshis et al., 2014; Baumgarten et al., 2013; Leggat et al., 2011). This paucity of an algal response suggests that corals may regulate their symbiont's environment to buffer algae from stress; however, alternative explanations include that the symbiont's transcriptomic machinery is less responsive to stress regardless of symbiotic state.

Understanding the independent and interactive roles of coral hosts and Symbiodiniaceae algae in holobiont (i.e., assemblage of coral host and associated algal and microbial symbionts) resilience is difficult in a tropical coral system [reviewed in (Bove et al., 2022)] because it is impossible to disentangle the host's aposymbiotic state from stress and nutrient limitation given tropical coral reliance on Symbiodiniaceae-derived carbon. To address these difficulties, facultative symbioses have emerged as tractable systems for answering fundamental questions about coral symbiosis (Puntin et al., 2022). Here, we used gene expression profiling in the facultatively symbiotic coral *O. arbuscula* and its symbiont *Breviolum psygmophilum* to address two questions: (1) what is the consequence of symbiosis for coral hosts under thermal challenge? and (2) how does symbiosis modulate symbiont responses to thermal challenges? We hypothesized that, compared to aposymbiotic corals, symbiotic corals under thermal challenge would exhibit gene expression patterns consistent with environmental stress responses (ESRs) of tropical corals because of symbiont-derived ROS produced under thermal stress. Based on previous work documenting minimal in hospite symbiont gene expression responses, we predicted greater responses of symbiotic hosts compared to symbionts in hospite and muted responses of symbionts in hospite compared to ex hospite, consistent with coral hosts modulating symbiont environments. To answer these questions, we conducted temperature challenge assays and characterized host and symbiont responses via gene expression profiling.

2 | MATERIALS AND METHODS

2.1 | Experiment 1: *Oculina arbuscula* and *Breviolum psygmophilum* holobiont responses to temperature challenges in symbiosis

2.1.1 | Coral collection and experimental design

In June 2018, 16 *O. arbuscula* colonies ($n = 8$ symbiotic, $n = 8$ aposymbiotic) were collected from approximately the same depth (~3 m) at Radio Island, North Carolina (NC) (34.712590° N, −76.684308° W) under NC Division of Marine Fisheries permits #706481 and #1627488 (Figure 1a). Colonies were shipped overnight to Boston University, fragmented, attached to Petri dishes using cyanoacrylate glue, and maintained in a common garden aquarium at ambient conditions (18°C, 33–35 PSU, 40–50 µmol photons $m^{-2} s^{-1}$ PAR on a 12:12 h light:dark schedule) for approximately 5 months. Experimental temperatures were informed by 2017 (January 1–December 31, 2017) in situ temperature data recorded by the NOAA buoy closest to the collection site (Station BFTN7; minimum temperature recorded = 3°C, maximum temperature recorded = 28.5°C; Figure 1b). One fragment from each colony ($N = 48$ fragments) was placed in one of three treatments: (1) control: 18°C, (2) heat challenge: temperature increased 1°C day^{-1} from 18°C to 32°C, and (3) cold challenge: temperature decreased 1°C day^{-1} from 18°C to 6°C. Treatments were maintained for 15 days using Aqua Logic digital

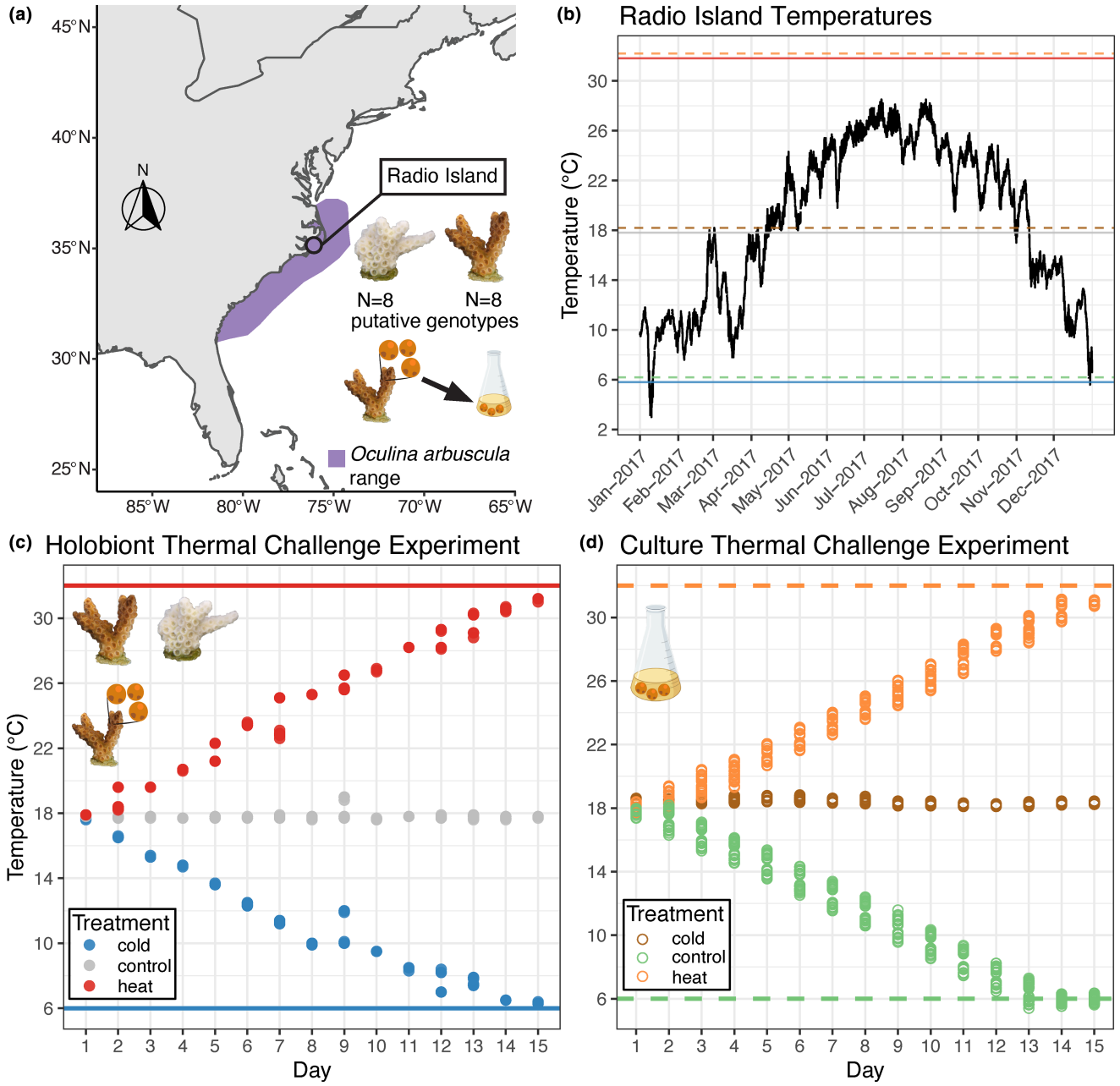

FIGURE 1 Experimental overview. (a) Map of collection site (Radio Island, North Carolina) of *Oculina arbuscula* used in the holobiont thermal challenge experiment ($n = 8$/symbiotic state). Purple shading indicates *O. arbuscula* range. (b) Water temperatures (black line) in 2017 recorded at the NOAA buoy near Radio Island with thermal challenge treatments (heat = 32°C, control = 18°C, cold = 6°C) overlaid. (c) Temperatures recorded during holobiont thermal challenge experiment. (d) Temperatures recorded during culture thermal challenge experiment. Culture icons were created with BioRender.com.

temperature controllers calibrated with a NIST-certified glass thermometer and were recorded using HOBO loggers (Figure 1c).

2.1.2 | In hospite symbiont physiology

Pulse Amplitude Modulation (PAM) fluorometry measured dark-acclimated photochemical efficiency of photosystem II (*Fv/Fm*) of symbiotic corals using a Junior PAM approximately every 3 days throughout the experiment. Corals received 8 h of dark acclimation before *Fv/Fm* was measured in triplicate. The effect of temperature challenge on symbiont *Fv/Fm* over time was analysed using a linear mixed effects model (*lmer*), with interactions of fixed effects of treatment and day plus a random effect of genotype. Pairwise model outputs were compared using *emmeans* (Lenth 2022). All analyses were performed in the R v4.2.0 statistical environment (R Core Team & R: A language and environment for statistical computing, 2022), and all code and raw data for the analyses detailed here are hosted on Github: https://github.com/hannahaichelman/Oculina_Host_Sym_GE.

2.1.3 | *Oculina arbuscula* holobiont gene expression profiling

On day 15, tissue from all fragments ($N = 48$) was sampled using sterilized bone cutters, immediately preserved in 200 proof ethanol, and maintained at −80°C. Samples were homogenized with lysis buffer and glass beads and total RNA was extracted using an RNAqueous kit (ThermoFisher Scientific) following manufacturer's instructions. DNA contamination was removed via DNAse1 and TagSeq libraries were prepared using 1.5 μg of RNA [following (Meyer et al., 2011), (Lohman et al., 2016)]. Successful libraries ($N = 47$) were sequenced on Illumina HiSeq 2500 (single-end 50 bp) at Tufts University Core Facility. TagSeq analyses followed https://github.com/z0on/tag-based_RNAseq. Raw reads were quality filtered to remove Illumina adapters, poly-A sequences, PCR duplicates, reads less than 20 bp long, and reads with a quality score less than 33 (number of reads summarized in Table S1).

2.1.4 | Putative coral clone identification

We tested for the presence of clones in our data set following methods presented in Bove et al. (2023). Quality filtered reads were mapped to concatenated *O. arbuscula* and *B. psygmophilum* transcriptomes [presented in (Rivera & Davies, 2021)] using Bowtie2 (Langmead & Salzberg, 2012). Symbiont reads were removed from the data set, genotyping and identification of host single nucleotide polymorphisms (SNPs) was performed using ANGSD (Korneliussen et al., 2014), and putative clones were distinguished using a hierarchical clustering tree (*hclust*) based on pairwise identity by state (IBS) distances calculated in ANGSD (Figure S1). After removing clones, 33 samples ($N = 7$ putative symbiotic genotypes, $N = 4$ putative aposymbiotic genotypes) were used in downstream analyses. See Appendix S1.

2.1.5 | *Oculina arbuscula* and in hospite *B. psygmophilum* gene expression analyses

Quality-filtered reads were mapped to the same concatenated transcriptomes described above using Bowtie2 (Langmead & Salzberg, 2012), but with different parameters (*-k* mode, $k = 5$, --no-hd, --no-sq) with an average mapping efficiency across all samples of 29.7% (Table S1). Host and symbiont reads were separated to produce two separate files of counts per gene (counts summarized in Table S1), and independent runs of DESeq2 (Love et al., 2014) identified differentially expressed genes (DEGs) in response to heat and cold thermal challenge relative to control using Wald's tests. The proportion of *B. psygmophilum* counts was calculated as a general proxy for symbiotic state as the number of symbiont counts divided by the total number of host and symbiont counts. Symbiotic and aposymbiotic hosts were confirmed to host significantly different proportions of *B. psygmophilum* counts ($p = .02$; Figure S2).

Rlog-transformed host and symbiont gene expression (GE) data were used as input for separate principal component analyses (PCAs) using *plotPCA* (package = DESeq2) to determine the effect of temperature on GE profiles using PERMANOVAs via the *adonis2* function [package = vegan; (Oksanen et al., 2022)]. GE plasticity, defined as the distance in PC space between an individual's GE profile and the average GE of all samples in the control, was calculated from host and symbiont PCAs following Bove et al. (2023). Differences in GE plasticity between treatments were tested using an ANOVA followed by Tukey's HSD post hoc tests. Model assumptions were assessed using *check_model* [package = performance; (Lüdecke et al., 2021)].

Gene ontology (GO) enrichment analyses were performed using Mann–Whitney *U* tests based on the ranking of signed log *p*-values (Wright et al., 2015) for both host and symbiont datasets. Results were visualized in dendrograms, which indicate the amount of gene sharing between significant GO categories and the direction of change relative to the control treatment. Results from the GO enrichment analyses were used for two functional analyses, detailed below.

First, GO delta ranks, which quantify the tendency of genes assigned to a GO category toward up- or down-regulation in treatment versus controls, were used to compare *O. arbuscula* host response under thermal challenges relative to a meta-analysis from Dixon et al. (2020) that characterized the GE signatures of stress in *Acropora* corals. This meta-analysis identified two classes of coral stress responses: 'type A', which was positively correlated across projects and consistent with the coral environmental stress response (ESR), and 'type B', which was negatively correlated and indicated lower intensity stress. Host delta-ranks for Biological Processes (BP) GO terms were plotted against BP delta-ranks of all 'type A' studies identified by Dixon et al. (2020). While not a formal statistical test, this analysis indicates whether *O. arbuscula* responses were functionally similar to a 'type A' ESR. Second, *B. psygmophilum* symbiont GO results identified underrepresentation of photosynthesis-related GO terms (two biological processes [BP] terms, two cellular components [CC] terms, and two molecular function [MF] terms) under cold challenge. Genes associated with these GO categories (unadjusted *p*-value <.10) were explored by constructing a heatmap using *pheatmap*.

Symbiont species identity of all symbiotic coral samples was confirmed using metabarcoding of the ITS2 locus [forward primer = *ITS-DINO* (Pochon et al., 2001), reverse primer = *ITS2Rev2* (Stat et al., 2009)]. Raw ITS2 data were submitted to SymPortal (Hume et al., 2019) to identify ITS2 defining intragenomic variant (DIV) profiles and relative abundances of DIVs across *O. arbuscula* fragments were compared using a bar plot constructed with *phyloseq* (McMurdie & Holmes, 2013). $N = 39/48$ samples were successfully sequenced, and all of these samples were confirmed to host *B. psygmophilum* (Figure S3). See Appendix S1 for additional details on library preparation and sequencing.

2.1.6 | Comparing orthologous genes in *Oculina arbuscula* and *Breviolum psygmophilum* in symbiosis

To compare *O. arbuscula* (symbiotic and aposymbiotic) and *B. psygmophilum* in hospite responses to temperature challenges, independent GE analyses were completed on orthologous genes. This analysis allowed us to test two predictions: (1) symbiotic hosts would respond more to temperature challenge than aposymbiotic hosts, and (2) symbiotic hosts would respond more than their symbionts in hospite. Orthologous genes were identified following Dixon and Kenkel (2019) with additional specifics for Symbiodiniaceae described here: https://github.com/grovesdixon/symbiodinium_orthologs (details in Appendix S1). Briefly, Transdecoder v5.5.0 (Haas et al., 2013) predicted protein coding sequences, FastOrtho assigned predicted coding sequences to orthologous groups (Li et al., 2003), and approximately maximum-likelihood phylogenetic trees of these protein sequences were built using MAFFT (Katoh & Standley, 2013) and FastTree (Price et al., 2009), which resulted in a total of 1962 single-copy orthologs.

Single-copy orthologs were extracted from host and symbiont counts files and orthologs with mean count >2 in at least 80% of samples were retained, leaving 152 nonzero read count orthologs. To directly compare responses to thermal challenge of symbiotic hosts, aposymbiotic hosts, and symbionts in hospite, *O. arbuscula* and *B. psygmophilum* count data (summarized in Table S2) for these orthologs were analysed in DESeq2, which modelled aggregate factors of temperature treatment and sample type (symbiotic host, aposymbiotic host, or symbiont in hospite). Responses of sample types to heat and cold challenge relative to control were quantified as the number of differentially expressed orthologs (DEOs, adjusted p-value <.1). Differences in the proportion of DEOs across sample types were tested with a two-proportions z-test.

2.2 | Experiment 2: *B. psygmophilum* response in culture—ex hospite

2.2.1 | Symbiont cell culture maintenance

To isolate *B. psygmophilum* responses to temperature challenge ex hospite, *O. arbuscula* holobiont thermal challenges were replicated on cultured *B. psygmophilum*. *Breviolum psygmophilum* cells were isolated from *O. arbuscula* from Radio Island, NC by serially diluting airbrushed host tissue into sterile F/2 media (Bigelow NCMA, East Boothbay, ME, USA). The "ancestral culture" was maintained in F/2 media based on artificial seawater (Instant Ocean), with monthly transfers to fresh media, in a Percival incubator (model AL-30L2) at 26°C and irradiance of 30 μmol photons $m^{-2} sec^{-1}$ on a 14:10 h light:dark cycle. After 4 months, this ancestral culture was split into three flasks, each with 100 mL of F/2 media and 0.5 mL of dense cells. These "daughter cultures" were acclimated to 18°C by decreasing temperatures at a rate of 1°C day^{-1} over a span of 9 days. A preliminary experiment established semi-continuous culture methodology (Figure S4A; Appendix S1). Cultured symbiont species identity was confirmed prior to thermal challenge experiments using Sanger sequencing (details in Appendix S1).

2.2.2 | Thermal challenge experiment

Breviolum psygmophilum cultures were exposed to thermal challenges that mirrored treatments used in holobiont experiments detailed in Part I. Ex hospite symbiont thermal challenges began after a 51-week acclimation at 18°C. Experimental cultures ($n = 4$ flasks per treatment) were established from long-term acclimated daughter flasks, with initial cell densities of 200,000 cells mL^{-1} in 100 mL of F/2 media. Heat and cold challenge flasks were placed in separate Percival incubators (model AL-30L2), and control flasks were maintained in a temperature-controlled room (Harris Environmental Systems, Andover, MA). All treatments began at 18°C and followed a 14:10 h light:dark cycle at ~50 μmol photons $m^{-2} s^{-1}$. Experimental cultures were grown semi-continuously, with the timing of transfers determined using the preliminary experiment (Figure S4).

2.2.3 | Ex hospite *B. psygmophilum* gene expression profiling

On day 15, all cultures were thoroughly mixed, concentrated via centrifugation (5000 RPM, 7 min), flash frozen in liquid nitrogen and stored at −80°C. To obtain sufficient RNA for TagSeq, replicate cultures in cold challenge treatments were pooled, such that there were four pooled replicates extracted separately. Lower cell densities in cold challenge were due to reduced growth (Figure S4), which was not the case for heat challenge and control flasks. Flash frozen pellets were ground for 3 min in a mortar and pestle that was pre-chilled with liquid nitrogen. Additional liquid nitrogen was added to keep the cell pellet frozen and RNA was extracted using RNAqueous-micro kits (ThermoFisher Scientific) following manufacturer's instructions, except elution volume was 15 μL. DNA was removed via DNA-free DNA Removal Kit (ThermoFisher Scientific). RNA was normalized using concentrations from a Quant-iT PicoGreen dsDNA Assay (ThermoFisher Scientific) and total RNA was sent to the University of Texas at Austin Genome Sequencing and Analysis Facility (GSAF), where it was prepared for TagSeq following Meyer et al. (2011) and sequenced on a NovaSeq 6000 machine (single-end 100 bp).

2.2.4 | Gene expression analyses on ex hospite *B. psygmophilum*

Raw read processing of ex hospite *B. psygmophilum* TagSeq data followed methods detailed in Part I except samples were only mapped to the *B. psygmophilum* reference transcriptome (average mapping efficiency = 36.1%; raw and filtered reads and count information

summarized in Table S3). Principal component analysis (PCAs), GE plasticity, and GO enrichment analyses were conducted as detailed in Part I. A heat-map of significantly differentially expressed genes (DEGs) with GO annotations related to photosynthesis (four biological processes [BP] terms, two cellular components [CC] terms, and two molecular function [MF] terms) was generated on the culture dataset with an adjusted *p*-value <.05 to restrict the number of DEGs included. In addition, a heatmap of genes with GO annotations related to oxidative stress (five biological processes [BP] terms and three molecular function [MF] terms) was constructed with the same cut-off (adjusted *p*-value <.05), as these terms were consistently enriched in ex hospite GO analyses.

2.3 | Comparing *B. psygmophilum* response in and ex hospite

To test the prediction that symbionts would respond more to temperature challenge ex hospite compared to in hospite, *B. psygmophilum* GE data sets from Experiments I and II were analysed together. A batch effect correction was conducted on combined raw count data using ComBat-seq (Zhang et al., 2020), with a specified batch of experiment type (in hospite or ex hospite) and temperature treatment (heat challenge, cold challenge or control) as the treatment of interest. Batch-corrected data were included in the same DESeq2 (Love et al., 2014) model, which modelled a main effect of the aggregate factor of treatment (cold challenge, heat challenge or control) and sample type (in hospite or ex hospite). Genes were only retained when present in at least 80% of samples (33/41 samples) at a mean count of 2 or higher, which left 1885 genes for downstream analyses.

Following PCAs detailed in Experiment I, combined symbiont count data were *rlog*-transformed and used as input for a PCA to test the effect of the aggregate factor of temperature treatment and sample type on GE. Significance was assessed with PERMANOVA, using the *adonis2* function [package = vegan; (Oksanen et al., 2022)]. GE plasticity was also calculated following methods detailed above.

3 | RESULTS

3.1 | Independent responses of *Oculina arbuscula* and *Breviolum psygmophilum* to temperature challenges in symbiosis

3.1.1 | *Oculina arbuscula* hosts exhibit stronger gene expression responses to cold challenge than heat challenge regardless of symbiotic state

The aggregate factor of temperature treatment and symbiotic state had a significant effect on *O. arbuscula* host gene expression patterns (Figure 2a; *ADONIS* $p = .001$) with cold challenge eliciting higher gene expression plasticity in both symbiotic (*Tukey HSD* $p < .001$) and aposymbiotic (*Tukey's HSD* $p = .013$) hosts compared to heat challenge (Figure 2b; $p < .001$). Symbiotic state did not influence gene expression plasticity within temperature treatments (cold challenge, *Tukey's HSD* $p = .22$; heat challenge, *Tukey HSD* $p = .99$; Figure 2b).

When comparing GO delta ranks of the 'type A' module from Dixon et al. (2020) to symbiotic and aposymbiotic *O. arbuscula* host delta-ranks from the thermal challenges, positive relationships were observed for biological processes GO terms for all comparisons (Figure 2c).

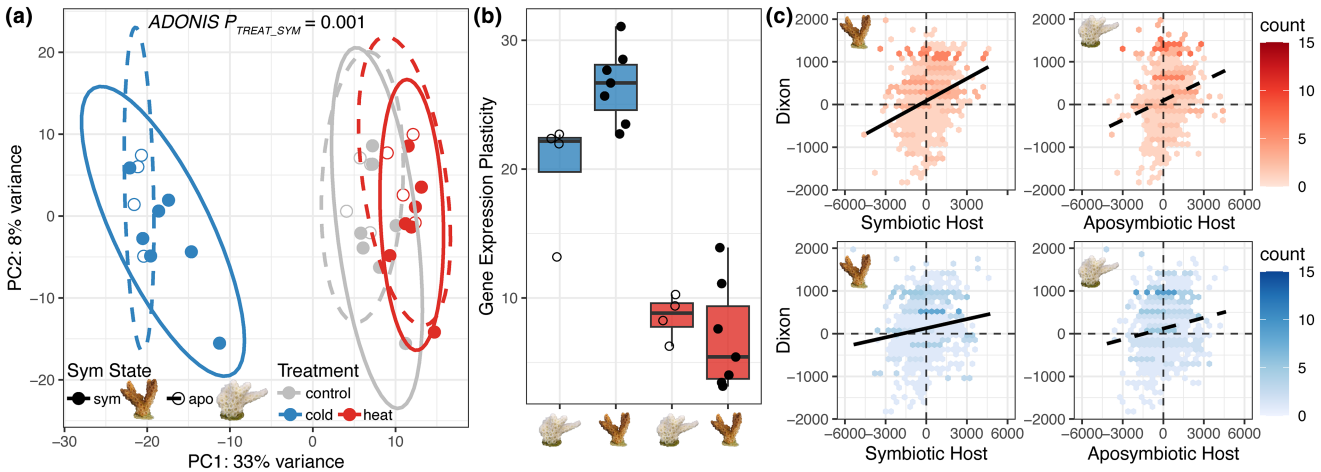

FIGURE 2 Symbiotic and aposymbiotic *Oculina arbuscula* host gene expression responses to temperature challenges. (a) Principal component analysis (PCA) of gene expression of symbiotic (solid point and line) and aposymbiotic (open points, dashed line) coral hosts under control (grey), cold (blue), and heat (red) challenge. The x- and y-axes represent the % variance explained by the first and second PC respectively. (b) Gene expression plasticity of symbiotic and aposymbiotic hosts under cold and heat challenges. (c) Relationship between biological processes (BP) gene ontology (GO) delta ranks from symbiotic (left) and aposymbiotic (right) hosts under heat (top) and cold (bottom) challenge with BP delta-ranks of all "type A" studies from (Dixon et al., 2020). Positive slopes represent "type A" environmental stress responses.

3.1.2 | Negative effects of cold challenge on *Breviolum psygmophilum* photosynthetic function

ITS2 metabarcoding confirmed all *O.arbuscula* genotypes hosted a majority of *B.psygmophilum* defining intragenomic variants (DIVs) (Figure S3). All but one individual hosted 100% *B.psygmophilum*, and all symbiotic *O.arbuscula* fragments hosted the same DIV of *B.psygmophilum* (Figure S3). Photosynthetic efficiency (*Fv/Fm*) of in hospite *B.psygmophilum* was reduced by temperature challenges through time (Figure 3a; $p < .001$). By day 8 (cold challenge = 11°C, heat challenge = 25°C), *Fv/Fm* had significantly declined in the cold challenge relative to controls ($p = .02$), but not in heat challenge ($p = .23$). On day 14, *Fv/Fm* was significantly reduced in both cold and heat challenge relative to the control (Figure 3a; $p < .001$ for all comparisons). *Fv/Fm* in cold challenge corals was more dramatically reduced than under heat challenge, with fixed effect parameter estimates on day 14 of 0.082 in heat challenge and −0.24 in cold challenge relative to control (Figure 3a).

Temperature challenges significantly affected gene expression profiles of in hospite *B.psygmophilum* (Figure 3b; *ADONIS* $p = .001$). However, no differences in gene expression plasticity between symbionts in cold and heat challenge were observed (Figure 3c; *Tukey's HSD* $p = .62$). Corroborating negative effects of cold challenge on *B.psygmophilum Fv/Fm* (Figure 3a), six GO terms related to photosynthetic processes were underrepresented in cold challenge relative to control conditions (photosystem [CC; GO:0009521], photosynthesis, light harvesting [BP; GO:0009765], chlorophyll binding [MF; GO:0016168], protein-chromophore linkage [BP; GO:0018298], thylakoid membrane [CC; GO:0042651] and tetrapyrrole binding [MF; GO:0046906]). Six annotated genes within these GO terms were down-regulated under cold challenge (unadjusted *p*-value <.10) relative to control conditions and included light-harvesting complex (LHC) and chloroplast genes (Figure 3d). It is important to note that filtering based on unadjusted *p*-values here means the genes are not significantly differentially expressed, but this method is used to explore patterns of genes within the significantly enriched GO categories.

3.2 | Comparing response of *Oculina arbuscula and Breviolum psygmophilum* in symbiosis using orthologous genes

More differentially expressed orthologs (DEOs) were observed in symbiotic hosts compared to aposymbiotic hosts under cold

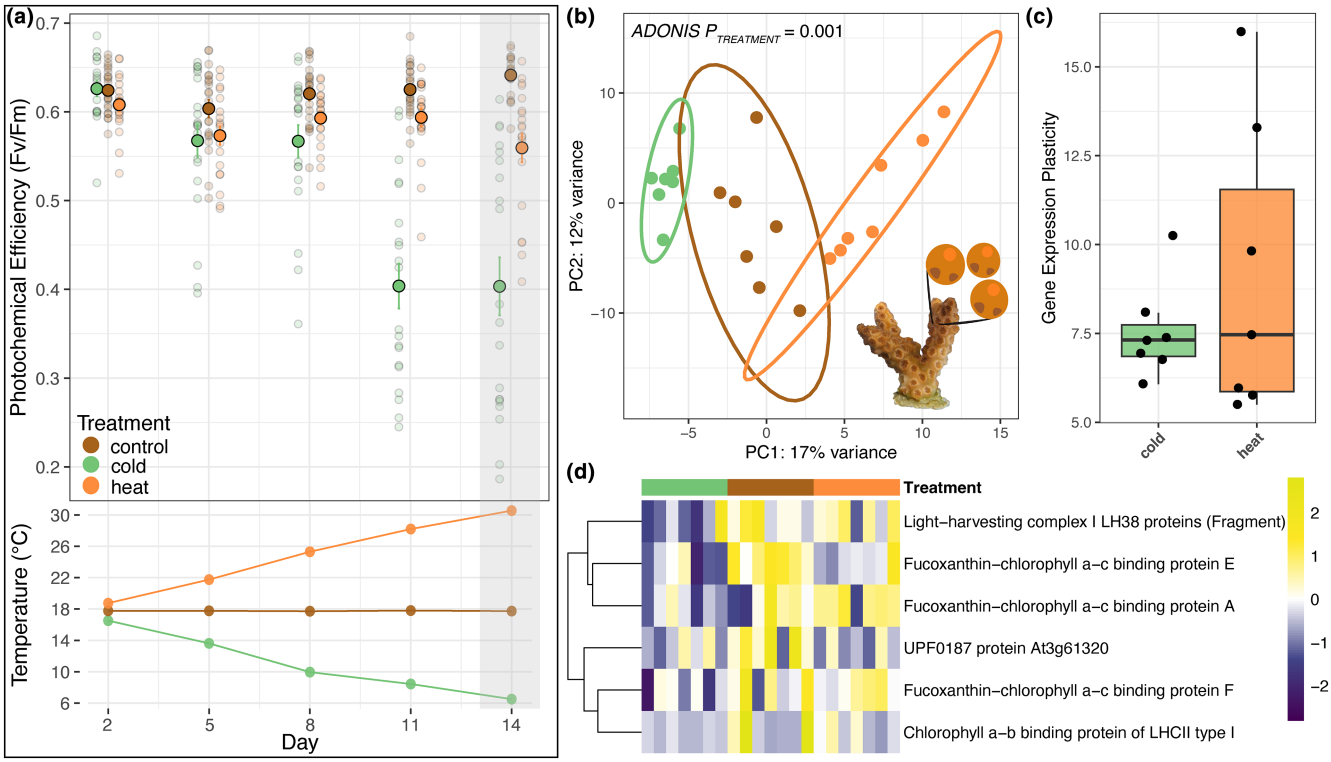

FIGURE 3 In hospite symbiont physiology and gene expression responses to temperature challenges. (a) Photochemical efficiency (*Fv/Fm*, top panel) of in hospite symbionts through time as temperatures diverged (bottom panel). Top: Large points represent mean *Fv/Fm* ± standard error across treatments and transparent points represent a fragment's *Fv/Fm* at each time point. The grey bar indicates the time point immediately prior to sampling for gene expression. (b). Principal component analysis (PCA) of gene expression of in hospite symbionts from A on day 15. (c) Gene expression plasticity of in hospite symbionts under cold (green) and heat (orange) challenge was not significantly different (*Tukey's HSD* $p = .62$). (d) Heatmap showing differentially expressed genes (DEGs; unadjusted *p*-value <.1) belonging to photosynthesis gene ontology (GO) terms, where each row is a gene and each column is a sample. The colour scale represents log2 fold change relative to the gene's mean, where yellow represents up-regulation and purple represents down-regulation.

challenge ($X^2=4.76$; $p=.015$), but not heat challenge (Figure 4; $X^2=0.482$; $p=.24$). Similarly, aposymbiotic hosts exhibited more DEOs compared to in hospite *B. psygmophilum* under cold challenge ($X^2=37.7$; $p<.0001$), but not heat challenge (Figure 4; $X^2=0.781$; $p=.19$). The number of DEOs was greater in symbiotic hosts compared to in hospite symbionts under both cold challenge ($X^2=64.3$; $p<.0001$) and heat challenge (Figure 4; $X^2=3.23$; $p=.036$).

3.3 | *Breviolum psygmophilum* response to temperature challenge out of symbiosis—ex hospite

Sanger sequencing confirmed that all parent cultures matched *B. psygmophilum* (GenBank Accession ID LK934671.1) with 100% identity and 53%–87% query coverage. *Breviolum psygmophilum* cultures in all treatments were maintained in exponential growth phase throughout the experiment (Figure S4B,C). Thermal challenges significantly affected gene expression patterns of ex hospite *B. psygmophilum* (Figure 5a; *ADONIS* $p=.001$). Gene expression plasticity was greater in ex hospite *B. psygmophilum* under cold challenge relative to heat challenge (Figure 5b; *Tukey HSD* $p=.008$), whereas no significant effect was observed in hospite (Figure 3c).

Eight photosynthesis-related GO terms were underrepresented in ex hospite *B. psygmophilum* under cold challenge (photosystem [CC; GO:0009521], photosynthesis, light harvesting [BP; GO:0009765], chloroplast-nucleus signalling pathway [BP; GO:0010019], photosynthesis [BP; GO:0015979], chlorophyll binding [MF; GO:0016168], protein-chromophore linkage [BP; GO:0018298], thylakoid membrane [CC; GO:0042651], and tetrapyrrole binding [MF; GO:0046906]). A heat map of 63 DEGs (adjusted *p*-value <.05) under cold challenge belonging to these GO terms showcased a small group of up-regulated genes and a larger group of down-regulated genes under cold challenge (Figure 6a). Up-regulated photosynthesis-related genes included "Pentatricopeptide repeat-containing proteins", which are involved in RNA editing events in chloroplasts (Barkan & Small, 2014). Similar to *B. psygmophilum* in symbiosis (Figure 3c), genes involved in the LHC were down-regulated under cold challenge (Figure 6a).

Five GO terms commonly associated with stress were differentially enriched in ex hospite *B. psygmophilum* under cold challenge relative to control conditions (protein folding [BP; GO:0006457], cellular response to oxidative stress [BP; GO:0034599], hydrogen peroxide metabolic process [BP; GO:0042743], unfolded protein binding [MF; GO:0051082], cellular response to chemical stress [BP; GO:0062197]). A heat map of 70 DEGs under cold challenge (adjusted *p*-value <.05) assigned to these GO terms and revealed two groups of genes, one down-regulated and one up-regulated under cold challenge relative to control and heat challenge cultures (Figure 6b).

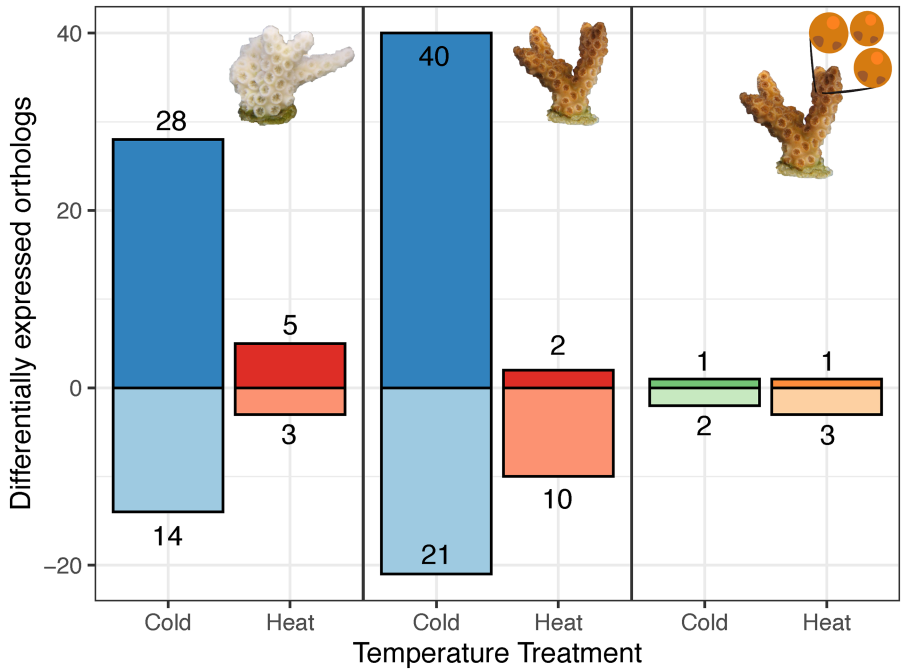

FIGURE 4 Coral hosts exhibit more differentially expressed orthologs (DEOs) than in hospite symbionts under thermal challenges. Bar plots representing the number of DEOs (positive values = up-regulated, negative values = down-regulated) in response to temperature challenges in aposymbiotic hosts (left), symbiotic hosts (centre) and in hospite symbionts (right). Symbiotic *Oculina arbuscula* had significantly more DEOs than in hospite *Breviolum psygmophilum* under cold challenge ($p<.0001$) and heat challenge ($p=.036$). Aposymbiotic *O. arbuscula* had significantly more DEOs than in hospite *B. psygmophilum* under cold challenge ($p<.0001$), but not heat challenge ($p=.19$). Symbiotic *O. arbuscula* had significantly more DEOs than aposymbiotic *O. arbuscula* under cold challenge ($p=.015$), but not heat challenge ($p=.24$).

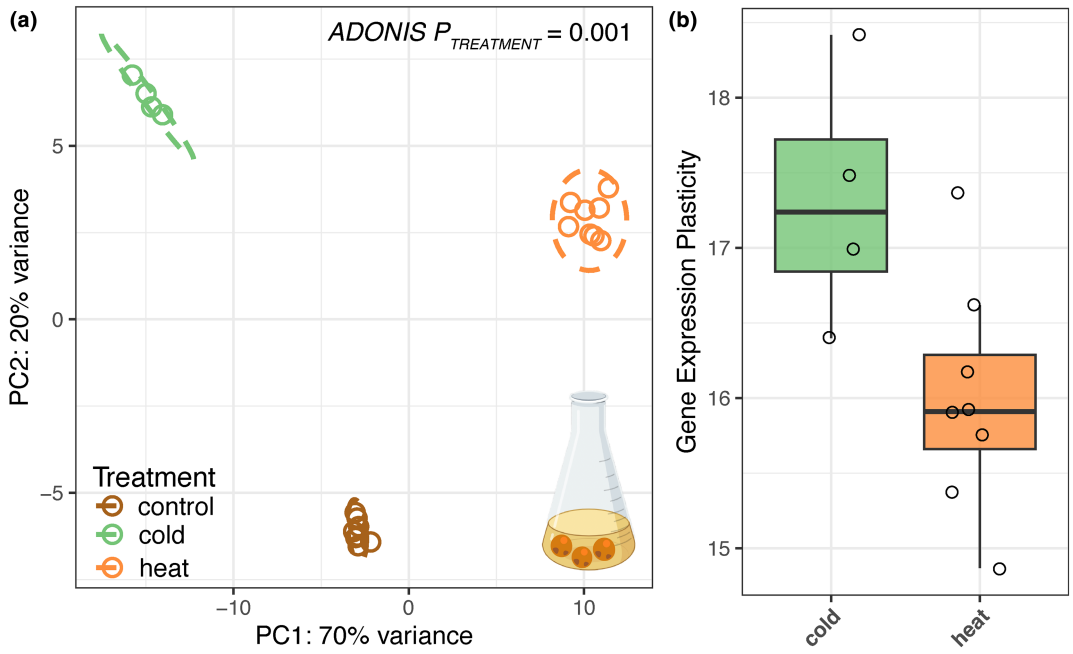

FIGURE 5 Ex hospite symbiont gene expression responses to temperature challenges. (a) Principal component analysis (PCA) of gene expression of ex hospite symbionts under temperature challenges. (b) Gene expression plasticity of symbionts ex hospite under cold (green) and heat (orange) challenge. Gene expression plasticity was significantly greater under cold challenge compared to heat challenge (*Tukey's HSD* $p = .008$). Culture icon created with BioRender.com.

3.4 | Comparing responses of in *and* ex hospite *B. psygmophilum*

When analysing in hospite and ex hospite *B. psygmophilum* in the same DESeq2 model, a significant effect of the aggregate factor of temperature treatment and symbiotic state was observed (Figure 7a; *ADONIS* $p = .001$). Temperature and symbiotic state also had significant effects on gene expression plasticity (Figure 7b; $p < .0001$), and ex hospite *B. psygmophilum* had significantly higher gene expression plasticity compared to in hospite *B. psygmophilum*, both under cold challenge (*Tukey's HSD* $p < .0001$) and heat challenge (Figure 7b; *Tukey's HSD* $p = .0001$).

4 | DISCUSSION

4.1 | Both aposymbiotic and symbiotic coral hosts exhibit classic environmental stress responses to temperature challenges

We leveraged genome-wide gene expression profiling of in and ex hospite facultative coral hosts (*Oculina arbuscula*) and their algal symbionts (*Breviolum psygmophilum*) to disentangle the independent responses of hosts and symbionts to divergent thermal challenges across symbiotic states. In contrast to our prediction that symbiosis would alter the response of corals to thermal challenge, we found that both heat and cold challenges elicited general ESRs ['type A'; Dixon et al., 2020] regardless of symbiotic state. Additionally, both symbiotic and aposymbiotic hosts exhibited greater gene expression plasticity in response to cold challenge compared to heat, aligning with previous work on the facultatively symbiotic coral *Astrangia poculata* when exposed to similar temperature challenges (Wuitchik et al., 2021). Wuitchik et al. (2021) found that aposymbiotic *A. poculata* in cold challenge (6°C) exhibited five times more differentially expressed genes (DEGs) than in heat (31°C), corroborating the higher gene expression plasticity and number of differentially expressed orthologs (DEOs) observed in *O. arbuscula* under cold challenge. However, Wuitchik et al. (2021) found cold challenge elicited a more severe ESR response ['type A'; Dixon et al., 2020] than heat challenge ['type B'; Dixon et al., 2020], contrasting our findings that both thermal challenges elicited type A responses across symbiotic states. It is possible that this discrepancy is due to differences in the distribution of these two species, as *O. arbuscula* is found primarily in subtropical environments but *A. poculata* is largely restricted to temperate environments (Thornhill et al., 2008). Regardless, this pattern suggests that even though *O. arbuscula* exhibited higher gene expression plasticity under cold challenge, corals in both temperature challenges were exhibiting stress responses consistent with a tropical coral's ESR, highlighting the utility of *O. arbuscula* as a calcifying model for symbiosis (Rivera & Davies, 2021).

The type A response presented in Dixon et al. (2020) is characterized by functional enrichment of the coral ESR, including down-regulation of cell division and up-regulation of cell death, response to ROS, protein degradation, NF-κB signalling, immune response and protein folding. Specifically, type A data sets in tropical *Acropora* showcased up-regulation of ROS and protein folding

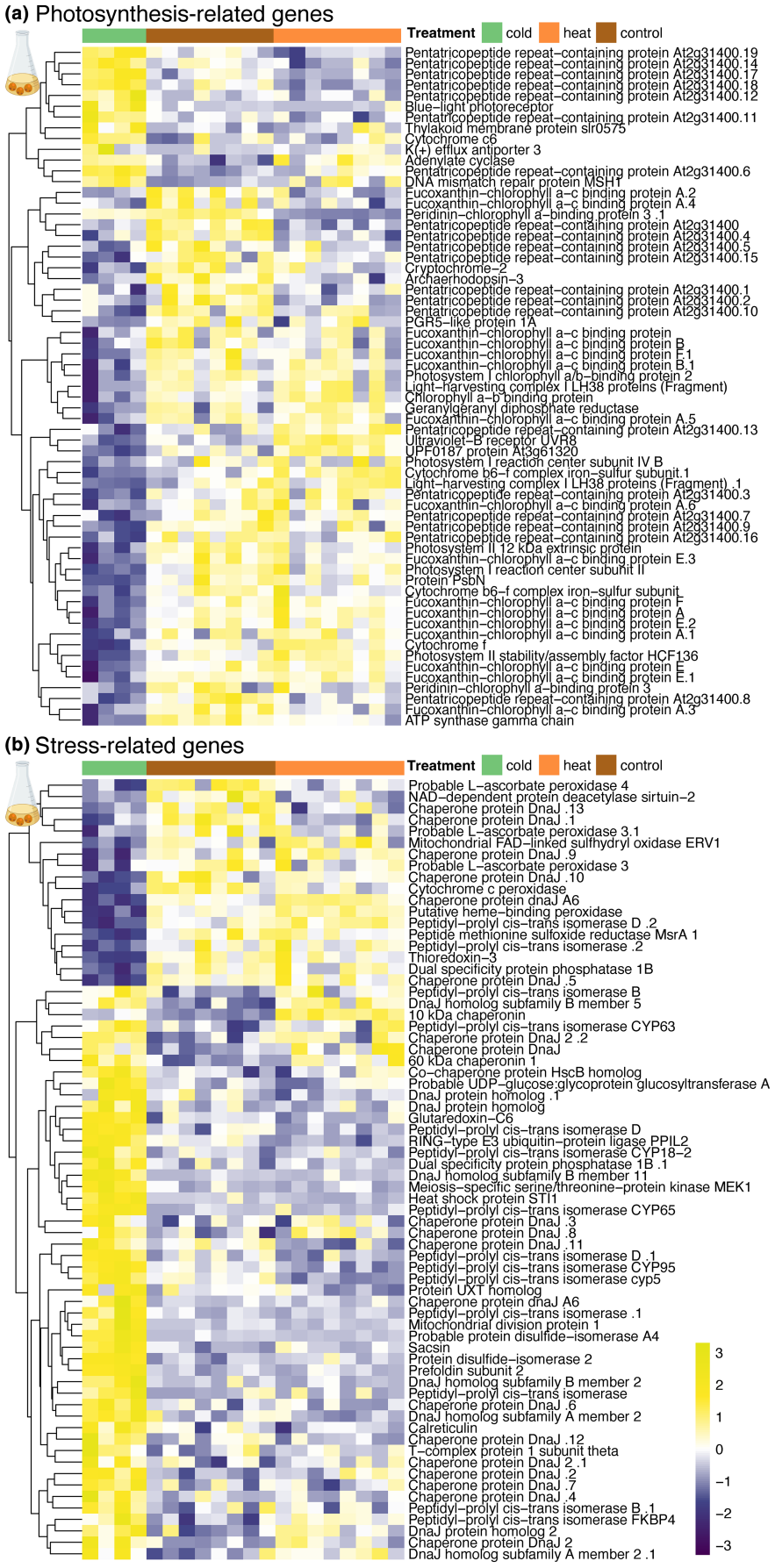

FIGURE 6 Ex hospite symbionts exhibit differential expression of photosynthesis and stress-related genes under cold challenge. Heatmap showing differentially expressed genes (DEGs; adjusted *p*-value <.05) belonging to photosynthesis (a) and stress (b) gene ontology (GO) terms, where each row is a gene and each column is a sample. The colour scale represents log2 fold change relative to the gene's mean, where yellow represents up-regulation and purple represents down-regulation. Culture icons created with BioRender.com.

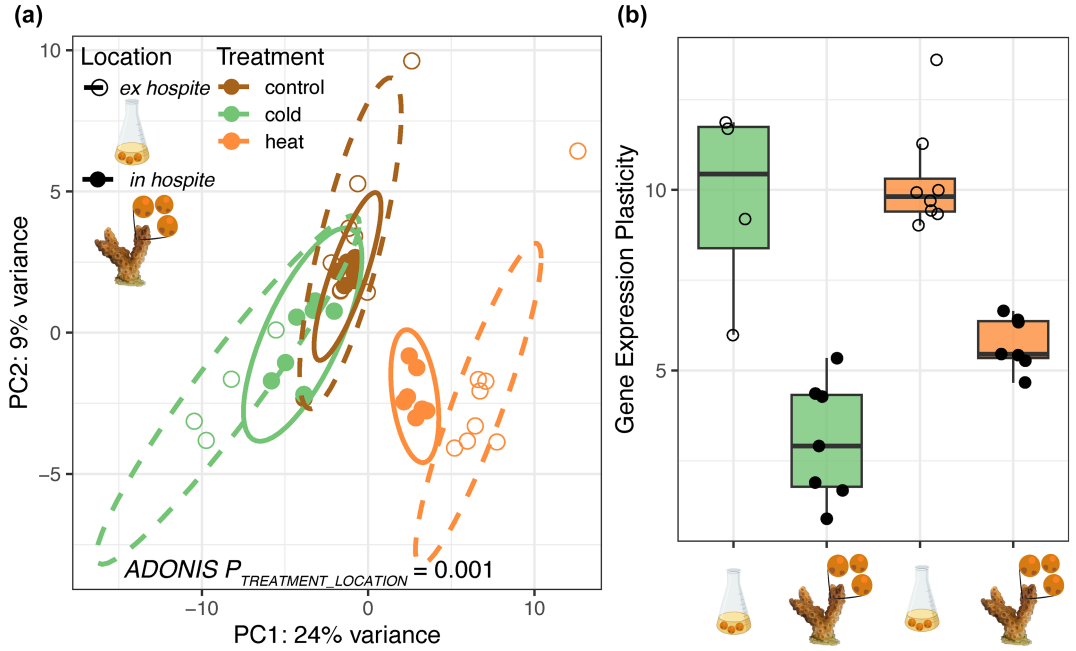

FIGURE 7 Ex hospite symbionts respond more strongly to thermal challenges than in hospite symbionts. (a) Principal component analysis (PCA) of gene expression of ex hospite (open circles, dashed lines) and in hospite (solid points and lines) symbionts under control, cold and heat temperatures. (b) Gene expression plasticity of ex hospite and in hospite symbionts under cold (green) and heat (orange) challenge. Gene expression plasticity was significantly greater in ex hospite symbionts compared to in hospite symbionts under both cold challenge (*Tukey HSD* $p < .0001$) and heat challenge (*Tukey HSD* $p = .0001$). Culture icons created with BioRender.com.

(Dixon et al., 2020). This informed our hypothesis that temperature challenge would result in an ESR-like response, akin to a type A response, in symbiotic *O. arbuscula* but not in aposymbiotic individuals. Instead, we observed that both symbiotic and aposymbiotic *O. arbuscula* exhibited type A responses under heat and cold challenge. This pattern could be the result of background symbionts in aposymbiotic corals [as previously observed in aposymbiotic *A. poculata*, e.g., (Sharp et al., 2017)] producing ROS and leading to a type A response. Alternatively, aposymbiotic corals may be light-stressed as they lack shading from symbionts (e.g., Scheufen et al., 2017); however, all corals were collected from approximately the same depth, which suggests they were acclimated to similar light environments. Additionally, the temperature challenges applied here were relatively short, and it is possible that symbiotic and aposymbiotic *O. arbuscula* would have exhibited differential responses if the challenges had been more extreme in temperature or duration (McLachlan et al., 2020). In general, facultatively symbiotic corals are understudied, and future work should explore the responses of symbiotic and aposymbiotic corals under different stressors (i.e. light, nutrients) and for longer durations (as in Aichelman et al., 2021).

4.2 | Evidence of host buffering in *O. arbuscula* holobionts

We present three forms of evidence suggesting that *O. arbuscula* hosts are buffering their algal symbionts under thermal extremes: 1. The coral host exhibited more differentially expressed orthologs (DEOs) than its symbiont under cold challenge, 2. Stress-related genes were differentially expressed in symbionts ex hospite but not in hospite, and 3. Ex hospite symbionts exhibited higher gene expression plasticity in response to temperature challenges than in hospite symbionts. Higher gene expression plasticity in coral hosts compared to symbionts in symbiosis aligns with previous evidence suggesting that cnidarian hosts and their algal symbionts exhibit strong differences in the magnitude of gene expression responses under environmental challenges. For example, Davies et al. (2018) reported that the tropical coral *Siderastrea siderea* exposed to 95-day temperature and ocean acidification challenges resulted in hosts consistently exhibiting greater differential expression of highly conserved genes compared to their symbiont *Cladocopium goreaui*. Barshis et al. (2014) also found no changes in gene expression in either heat-susceptible *Cladocopium* (type C3K) or heat-tolerant *Durusdinium* (type D2) in symbiosis with *Acropora hyacinthus* following 3 days of high temperature exposure, which contrasted strong gene expression responses in the host (Barshis et al., 2013). Corroborating these patterns, Leggat et al. (2011) observed that algae (*Cladocopium* C3) exhibited little change in expression of six stress and metabolic genes compared to their hosts (*Acropora aspera*). Finally, Parkinson et al. (2015) found that *Acropora palmata* hosts with more dynamic gene expression patterns (i.e., more DEGs) maintained symbiont photosynthetic performance compared to hosts with fewer DEGs under cold challenge. The authors suggest a "phenotypic buffering effect" could be responsible and propose that the ability of the host to manage its redox state and iron availability influences the symbiont's response to cold challenge.

Symbiosis itself has also been observed to alter gene expression patterns and physiology in Symbiodiniaceae algae. Here, we observed differential regulation of stress-related GO terms under cold challenge ex hospite, and genes within those terms included up-regulation of a heat shock protein (heat shock protein STI1) and a ubiquitin-related gene (RING-type E3 ubiquitin-protein ligase PPIL2). These genes are both classic ESR genes in tropical corals (Dixon et al., 2020) and their differential regulation ex hospite highlights the potential benefits of a symbiotic lifestyle for Symbiodiniaceae. Symbiosis mitigating Symbiodiniaceae stress responses have been previously shown. For example, gene expression of ex hospite *Durusdinium trenchii* maintained at 28°C exhibited enrichment of the GO term "response to temperature stimulus" relative to in hospite *D.trenchii* in *Exaiptasia pallida*, which authors attributed to the protective microenvironment of the symbiosome (Bellantuono et al., 2019). Additionally, Maor-Landaw et al. (2020) compared gene expression of *Breviolum minutum* in culture to *B.minutum* freshly isolated from *Exaiptasia diaphana* and observed down-regulation of genes indicative of the protected and stress-reduced environment of the symbiosome. Specifically, pentatricopeptide repeats (PPR), which have been previously associated with Symbiodiniaceae RNA processing in response to environmental stress and were included in the repertoire of "stress responsive genes" in *Fugacium kawagutii* (Lin et al., 2019), were down-regulated in freshly isolated *B.minutum* (Maor-Landaw et al., 2020). These findings support our third piece of evidence for host buffering. Together, these data suggest that in hospite symbionts respond less at the level of gene expression to cope with temperature challenges compared to ex hospite symbionts, providing further evidence that cnidarian hosts exert control over the symbiont's micro-environment under environmental stress.

4.3 | Cold challenge elicited negative effects on photosynthesis of ex hospite and in hospite *B.psygmophilum*

Although responses of *B.psygmophilum* in hospite were muted (i.e., fewer DEGs and DEOs) under temperature challenges compared to responses ex hospite, negative effects on photosynthesis were observed at the level of phenotype (in hospite *Fv/Fm*) and gene expression (both in hospite and ex hospite), particularly under cold challenge. Ex hospite *B.psygmophilum* exhibited differential expression of GO pathways related to photosynthesis and stress, which aligns with previous work investigating how symbiosis affects Symbiodiniaceae photosynthesis. For example, Bellantuono et al. (2019) found that photosynthetic processes were modified in *D.trenchii* living in hospite compared to ex hospite. Specifically, GO terms related to photosynthesis (i.e., photosynthesis, photosystem II repair and light harvesting) were enriched in hospite compared to ex hospite, which may result from host carbon concentrating mechanisms increasing the availability of CO_2 in hospite. In addition, we observed reduced *Fv/Fm* of in hospite *B.psygmophilum*, aligning with previous work demonstrating reduced *Fv/Fm* in cultured *B.psygmophilum* exposed to simulated seasonal temperature declines (cooled from 26°C to 10°C and maintained at 10°C for 2 weeks before returning to 26°C) (Thornhill et al., 2008). In that study, *B.psygmophilum Fv/Fm* recovered to pre-challenge values once temperatures were returned to baseline, while other Symbiodiniaceae species that typically associate with tropical corals failed to regain *Fv/Fm* following cold challenge (Thornhill et al., 2008). This difference was attributed to *B.psygmophilum's* symbiosis with corals in temperate/subtropical areas where they experience larger annual temperature variation, aligning with a recent report of its wide thermal breadth (16.15°C) compared to six other Symbiodiniaceae isolates (Dilernia et al., 2023). Therefore, *Fv/Fm* declines coupled with down-regulation of photosynthesis genes could represent *B.psygmophilum*'s seasonal response to low temperatures, and if the cultures were returned to control conditions, they may have recovered.

While we only observed strong phenotypic and gene expression responses of *B.psygmophilum* under cold challenge and not heat challenge, these links between photosynthetic disruption and transcriptional regulation of photosynthetic machinery align closely with previous work on Symbiodiniaceae under heat stress (Takahashi et al., 2008; Tchernov et al., 2004). Additionally, while Symbiodiniaceae gene expression in response to cold challenge remains largely unexplored, evidence suggests that cold challenge induces similar photophysiology responses as heat challenge in Symbiodiniaceae [e.g., (Marangoni et al., 2021; Thornhill et al., 2008; Kemp et al., 2011; Roth et al., 2012; Saxby et al., 2003)]. It is therefore possible that the heat challenge explored here was not sufficient to elicit a similarly strong response as observed under cold challenge, and indeed in hospite *B.psygmophilum Fv/Fm* under heat challenge remained significantly higher than in cold challenge. This pattern aligns with Roth et al. (2012), who found that cold challenge was more immediately harmful for *Acropora yongei* symbiosis, but heat stress was more harmful in the long term. Altogether, future work would benefit from longer and more extreme temperature challenges to ensure that the entire thermal niche is investigated. Lastly, studies quantifying additional phenotypes in both in and ex hospite *B.psygmophilum* to determine if such convergent responses to temperature challenge exist are warranted.

4.4 | Alternative hypotheses for "host buffering" and proposed future experiments

Transcriptional regulation is only one molecular process involved in responding to thermal stress. Mounting evidence suggests that a lack of transcriptional response under environmental challenges could be the result of multiple post-transcriptional and/or post-translational mechanisms in Symbiodiniaceae, which we detail in Table 1 along with other alternative hypotheses and corresponding evidence from the literature. Below we also highlight future studies and data needed to further test the host buffering hypothesis.

Our work supports a scenario in which coral hosts modulate the environment of in hospite Symbiodiniaceae algae to buffer their

TABLE 1 Alternative hypotheses for "host buffering" and supporting literature. Lack of in hospite symbiont responses to thermal challenge found here may not be host buffering, but instead other post-transcriptional or post-translational mechanisms previously found in Symbiodiniaceae that are not tested here. This table presents limitations to the current study and alternative hypotheses that may also explain the observed patterns.

Alternative hypothesis	Supporting literature
MicroRNA (miRNA)-based gene regulatory mechanisms, including smRNAs, could occur over transcriptional regulation in hospite *Breviolum psygmophilum*	Lin et al. (2015) and Baumgarten et al. (2013)
Gene duplication could act as a mechanism that increases transcript and protein levels of genes over transcriptional regulation	Aranda et al. (2016)
Spliced leader RNA *trans*-splicing could act as a mechanism that regulates gene expression	Bayer et al. (2012) and Zhang et al. (2007)
Nutritional status could complicate comparing differential expression across in and ex hospite *B. psygmophilum*, as algae in culture exist in nutrient replete conditions	Maruyama and Weis (2021)
The eurythermal nature of *B. psygmophilum* could limit its transcriptional response in hospite, resulting in the predominant host response	Thornhill et al. (2008) and Dilernia et al. (2023)
Lower depth of coverage of in hospite *B. psygmophilum* sequencing data captured here could have limited our ability to detect transcriptional responses	Stupnikov et al. (2021)

responses to temperature challenges; however, additional experiments are needed to validate this hypothesis. First, replicating the temperature challenges performed here but leveraging proteomic and gene expression profiling in parallel (e.g., Camp et al., 2022) will be critical in establishing whether lack of gene expression of in hospite symbionts translates to a lack of proteomic response under stress. Including nutrient controls, namely ex hospite Symbiodiniaceae in nutrient-depleted media that replicate the nutritional environment of the symbiosome, are necessary to address the confounding variable of nutritional status. Additionally, this proposed experiment should implement RNA extraction methods that prioritize obtaining and sequencing equal amounts of genetic material from host and symbiont.

While understanding the response of subtropical corals to thermal extremes is valuable in its own right, the facultative symbiosis, calcifying nature and available genomic resources of *O. arbuscula* make it a unique model for linking results to tropical coral responses as climate change progresses (Rivera & Davies, 2021). If coral hosts are able to regulate the environments of their symbionts, and this regulation in turn can serve to limit stress in the holobiont and ultimately reduce coral bleaching, then this host buffering phenotype may allow for the identification of coral–algal pairings that will be more resilient under future global change conditions.

AUTHOR CONTRIBUTIONS

HEA and SWD designed research; HEA, AKH, DMW, KFA, ES, NH and RMW performed research; HEA and GD analysed data; HEA and SWD wrote the paper. All co-authors provided final feedback on the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Raw sequencing data are deposited in NCBI's SRA (including RNAseq and ITS2 metabarcoding data; BioProject ID=PRJNA1077922). All other data, code and materials used in the analyses can be found in the Github repository associated with this project (https://github.com/hannahaichelman/Oculina_Host_Sym_GE) and is additionally hosted on Zenodo (DOI: doi.org/10.5281/zenodo.10697818).

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REFERENCES

Aichelman, H. E., Bove, C. B., Castillo, K. D., Boulton, J. M., Knowlton, A. C., Nieves, O. C., Ries, J. B., & Davies, S. W. (2021). Exposure duration modulates the response of Caribbean corals to global change stressors. *Limnology and Oceanography*, *66*, 3100–3115.

Aranda, M., Li, Y., Liew, Y. J., Baumgarten, S., Simakov, O., Wilson, M. C., Piel, J., Ashoor, H., Bougouffa, S., Bajic, V. B., Ryu, T., Ravasi, T., Bayer, T., Micklem, G., Kim, H., Bhak, J., LaJeunesse, T. C., & Voolstra, C. R. (2016). Genomes of coral dinoflagellate symbionts highlight evolutionary adaptations conducive to a symbiotic lifestyle. *Scientific Reports*, *6*, 39734.

Baker, A. C., Glynn, P. W., & Riegl, B. (2008). Climate change and coral reef bleaching: An ecological assessment of long-term impacts, recovery trends and future outlook. *Estuarine, Coastal and Shelf Science*, *80*, 435–471.

Barkan, A., & Small, I. (2014). Pentatricopeptide repeat proteins in plants. *Annual Review of Plant Biology*, *65*, 415–442.

Barott, K. L., Venn, A. A., Perez, S. O., Tambutté, S., & Tresguerres, M. (2015). Coral host cells acidify symbiotic algal microenvironment to promote photosynthesis. *Proceedings of the National Academy of Sciences*, *112*, 607–612.

Barshis, D. J., Ladner, J. T., Oliver, T. A., & Palumbi, S. R. (2014). Lineage-specific transcriptional profiles of Symbiodinium spp. unaltered by heat stress in a coral host. *Molecular Biology and Evolution*, *31*, 1343–1352.

Barshis, D. J., Ladner, J. T., Oliver, T. A., Seneca, F. O., Traylor-Knowles, N., & Palumbi, S. R. (2013). Genomic basis for coral resilience to climate change. *Proceedings of the National Academy of Sciences*, *110*, 1387–1392.

Baumgarten, S., Bayer, T., Aranda, M., Liew, Y. J., Carr, A., Micklem, G., & Voolstra, C. R. (2013). Integrating microRNA and mRNA expression profiling in Symbiodinium microadriaticum, a dinoflagellate symbiont of reef-building corals. *BMC Genomics*, *14*, 704.

Bayer, T., Aranda, M., Sunagawa, S., Yum, L. K., DeSalvo, M. K., Lindquist, E., Coffroth, M. A., Voolstra, C. R., & Medina, M. (2012). Symbiodinium transcriptomes: Genome insights into the dinoflagellate symbionts of reef-building corals. *PLoS One*, *7*, e35269.

Bellantuono, A. J., Dougan, K. E., Granados-Cifuentes, C., & Rodriguez-Lanetty, M. (2019). Free-living and symbiotic lifestyles of a thermotolerant coral endosymbiont display profoundly distinct transcriptomes under both stable and heat stress conditions. *Molecular Ecology*, *28*, 5265–5281.

Berkelmans, R., & van Oppen, M. J. H. (2006). The role of zooxanthellae in the thermal tolerance of corals: A 'nugget of hope' for coral reefs in an era of climate change. *Proceedings of the Royal Society B: Biological Sciences*, *273*, 2305–2312.

Berkelmans, R., & Willis, B. L. (1999). Seasonal and local spatial patterns in the upper thermal limits of corals on the inshore central great barrier reef. *Coral Reefs*, *18*, 219–228.

Bove, C. B., Greene, K., Sugierski, S., Kriefall, N., Huzar, A., Hughes, A., Sharp, K., Fogarty, N. D., & Davies, S. W. (2023). Exposure to global change and microplastics elicits an immune response in an endangered coral. *Frontiers in Marine Science*, *9*, 1037130.

Bove, C. B., Ingersoll, M. V., & Davies, S. W. (2022). Help Me, Symbionts, You're my only Hope: Approaches to accelerate our understanding of coral Holobiont interactions. *Integrative and Comparative Biology*, *62*, icac141.

Brown, B. E. (1997). Coral bleaching: Causes and consequences. *Coral Reefs*, *16*, S129–S138.

Burns, J. A., Zhang, H., Hill, E., Kim, E., & Kerney, R. (2017). Transcriptome analysis illuminates the nature of the intracellular interaction in a vertebrate-algal symbiosis. *eLife*, *6*, e22054.

Camp, E. F., Kahlke, T., Signal, B., Oakley, C. A., Lutz, A., Davy, S. K., Suggett, D. J., & Leggat, W. P. (2022). Proteome metabolome and transcriptome data for three Symbiodiniaceae under ambient and heat stress conditions. *Scientific Data*, *9*, 153.

Davies, S. W., Marchetti, A., Ries, J. B., & Castillo, K. D. (2016). Thermal and pCO2 stress elicit divergent transcriptomic responses in a resilient coral. *Frontiers in Marine Science*, *3*, 112.

Davies, S. W., Ries, J. B., Marchetti, A., & Castillo, K. D. (2018). Symbiodinium functional diversity in the coral *Siderastrea siderea* is influenced by thermal stress and reef environment, but not ocean acidification. *Frontiers in Marine Science*, *5*, 150.

Davy, S. K., Allemand, D., & Weis, V. M. (2012). Cell biology of cnidarian-dinoflagellate Symbiosis. *Microbiology and Molecular Biology Reviews*, *76*, 229–261.

Dean, A. D., Minter, E. J. A., Sørensen, M. E. S., Lowe, C. D., Cameron, D. D., Brockhurst, M. A., & Jamie Wood, A. (2016). Host control and nutrient trading in a photosynthetic symbiosis. *Journal of Theoretical Biology*, *405*, 82–93.

DeSalvo, M. K., Sunagawa, S., Voolstra, C. R., & Medina, M. (2010). Transcriptomic responses to heat stress and bleaching in the elkhorn coral *Acropora palmata*. *Marine Ecology Progress Series*, *402*, 97–113.

Dilernia, N. J., Camp, E. F., Bartels, N., & Suggett, D. J. (2023). Contrasting the thermal performance of cultured coral endosymbiont photophysiology. *Journal of Experimental Marine Biology and Ecology*, *561*, 151865.

Dixon, G. B., Abbott, E., & Matz, M. (2020). Meta-analysis of the coral environmental stress response: Acropora corals show opposing responses depending on stress intensity. *Molecular Ecology*, *29*, 2855–2870.

Dixon, G. B., & Kenkel, C. D. (2019). Molecular convergence and positive selection associated with the evolution of symbiont transmission mode in stony corals. *Proceedings of the Royal Society B: Biological Sciences*, *286*, 20190111.

Falkowski, P. G., Dubinsky, Z., Muscatine, L., & McCloskey, L. (1993). Population control in symbiotic corals: Ammonium ions and organic materials maintain the density of zooxanthellae. *Bioscience*, *43*, 606–611.

Frieler, K., Meinshausen, M., Golly, A., Mengel, M., Lebek, K., Donner, S. D., & Hoegh-Guldberg, O. (2013). Limiting global warming to 2°C is unlikely to save most coral reefs. *Nature Climate Change*, *3*, 165–170.

Haas, B. J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P. D., Bowden, J., Couger, M. B., Eccles, D., Li, B., Lieber, M., MacManes, M. D., Ott, M., Orvis, J., Pochet, N., Strozzi, F., Weeks, N., Westerman, R., William, T., Dewey, C. N., … Regev, A. (2013). De novo transcript sequence reconstruction from RNA-seq using the trinity platform for reference generation and analysis. *Nature Protocols*, *8*, 1494–1512.

Hume, B. C. C., Smith, E. G., Ziegler, M., Warrington, H. J. M., Burt, J. A., LaJeunesse, T. C., Wiedenmann, J., & Voolstra, C. R. (2019). SymPortal: A novel analytical framework and platform for coral algal symbiont next-generation sequencing ITS2 profiling. *Molecular Ecology Resources*, *19*, 1063–1080.

Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, *30*, 772–780.

Kemp, D. W., Oakley, C. A., Thornhill, D. J., Newcomb, L. A., Schmidt, G. W., & Fitt, W. K. (2011). Catastrophic mortality on inshore coral reefs of the Florida keys due to severe low-temperature stress. *Global Change Biology*, *17*, 3468–3477.

Korneliussen, T. S., Albrechtsen, A., & Nielsen, R. (2014). ANGSD: Analysis of next generation sequencing data. *BMC Bioinformatics*, *15*, 356.

LaJeunesse, T. C., Parkinson, J. E., Gabrielson, P. W., Jeong, H. J., Reimer, J. D., Voolstra, C. R., & Santos, S. R. (2018). Systematic revision of Symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. *Current Biology*, *28*, 2570–2580.e6.

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with bowtie 2. *Nature Methods*, *9*, 357–359.

Leggat, W., Seneca, F., Wasmund, K., Ukani, L., Yellowlees, D., & Ainsworth, T. D. (2011). Differential responses of the coral host and their algal symbiont to thermal stress. *PLoS One*, *6*, e26687.

Lenth, R. (2022). *emmeans: Estimated Marginal Means, aka Least-Squares Means*. R package version 1.8.2.

Li, L., Stoeckert, C. J., & Roos, D. S. (2003). OrthoMCL: Identification of Ortholog groups for eukaryotic genomes. *Genome Research*, *13*, 2178–2189.

Lin, S., Cheng, S., Song, B., Zhong, X., Lin, X., Li, W., Li, L., Zhang, Y., Zhang, H., Ji, Z., Cai, M., Zhuang, Y., Shi, X., Lin, L., Wang, L., Wang, Z., Liu, X., Yu, S., Zeng, P., … Morse, D. (2015). The *Symbiodinium kawagutii* genome illuminates dinoflagellate gene expression and coral symbiosis. *Science*, *350*, 691–694.

Lin, S., Yu, L., & Zhang, H. (2019). Transcriptomic responses to thermal stress and varied phosphorus conditions in *Fugacium kawagutii*. *Microorganisms*, *7*, 96.

Lohman, B. K., Weber, J. N., & Bolnick, D. I. (2016). Evaluation of TagSeq, a reliable low-cost alternative for RNAseq. *Molecular Ecology Resources*, *16*, 1315–1321.

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*, 550.

Lüdecke, D., Ben-Shachar, M. S., Patil, I., Waggoner, P., & Makowski, D. (2021). Performance: An R package for assessment, comparison and testing of statistical models. *J Open Source Softw*, *6*, 3139.

Maor-Landaw, K., van Oppen, M. J. H., & McFadden, G. I. (2020). Symbiotic lifestyle triggers drastic changes in the gene expression of the algal endosymbiont *Breviolum minutum* (Symbiodiniaceae). *Ecology and Evolution*, *10*, 451–466.

Marangoni, L. F. B., Rottier, C., & Ferrier-Pagès, C. (2021). Symbiont regulation in Stylophora pistillata during cold stress: An acclimation mechanism against oxidative stress and severe bleaching. *The Journal of Experimental Biology*, *224*, jeb235275.

Maruyama, S., & Weis, V. M. (2021). Limitations of using cultured algae to study cnidarian-algal symbioses and suggestions for future studies. *Journal of Phycology*, *57*, 30–38.

McLachlan, R. H., Price, J. T., Solomon, S. L., & Grottoli, A. G. (2020). Thirty years of coral heat-stress experiments: A review of methods. *Coral Reefs*, *39*, 885–902.

McMurdie, P. J., & Holmes, S. (2013). Phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*, *8*, e61217.

Melo Clavijo, J., Donath, A., Serôdio, J., & Christa, G. (2018). Polymorphic adaptations in metazoans to establish and maintain photosymbioses. *Biological Reviews*, *93*, 2006–2020.

Meyer, E., Aglyamova, G. V., & Matz, M. V. (2011). Profiling gene expression responses of coral larvae (Acropora millepora) to elevated temperature and settlement inducers using a novel RNA-Seq procedure. *Molecular Ecology*, *20*, 3599–3616.

Muscatine, L. (1990). *The role of symbiotic algae in carbon and energy flux in reef corals*. Ecosyst World.

Muscatine, L., McCloskey, L. R., & Marian, R. E. (1981). Estimating the daily contribution of carbon from zooxanthellae to coral animal respiration1. *Limnology and Oceanography*, *26*, 601–611.

Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., O'Hara, R., Solymos, P., Stevens, M., Szoecs, E, Wagner, H, Barbour, M, Bedward, M, Bolker, B, Borcard, D, Carvalho, G, Chirico, M, De Caceres, M, Durand, S, … Weedon, J (2022). *vegan: Community Ecology Package*. R package version 2.6-4.

Parkinson, J. E., Banaszak, A. T., Altman, N. S., LaJeunesse, T. C., & Baums, I. B. (2015). Intraspecific diversity among partners drives functional variation in coral symbioses. *Scientific Reports*, *5*, 15667.

Pochon, X., Pawlowski, J., Zaninetti, L., & Rowan, R. (2001). High genetic diversity and relative specificity among Symbiodinium-like endosymbiotic dinoflagellates in soritid foraminiferans. *Marine Biology*, *139*, 1069–1078.

Price, M. N., Dehal, P. S., & Arkin, A. P. (2009). FastTree: Computing large minimum evolution trees with profiles instead of a distance matrix. *Molecular Biology and Evolution*, *26*, 1641–1650.

Puntin, G., Sweet, M., Fraune, S., Medina, M., Sharp, K., Weis, V. M., & Ziegler, M. (2022). Harnessing the power of model organisms to unravel microbial functions in the coral Holobiont. *Microbiology and Molecular Biology Reviews*, *86*, e0005322.

R Core Team, & R: A language and environment for statistical computing. (2022). *R foundation for statistical computing*. Austria.

Rädecker, N., Pogoreutz, C., Voolstra, C. R., Wiedenmann, J., & Wild, C. (2015). Nitrogen cycling in corals: The key to understanding holobiont functioning? *Trends in Microbiology*, *23*, 490–497.

Rich, W. A., Carvalho, S., & Berumen, M. L. (2022). Coral bleaching due to cold stress on a central Red Sea reef flat. *Ecology and Evolution*, *12*, e9450.

Rivera, H. E., & Davies, S. W. (2021). Symbiosis maintenance in the facultative coral, *Oculina arbuscula*, relies on nitrogen cycling, cell cycle modulation, and immunity. *Scientific Reports*, *11*, 21226.

Robidart, J. C., Bench, S. R., Feldman, R. A., Novoradovsky, A., Podell, S. B., Gaasterland, T., Allen, E. E., & Felbeck, H. (2008). Metabolic versatility of the *Riftia pachyptila* endosymbiont revealed through metagenomics. *Environmental Microbiology*, *10*, 727–737.

Roth, M. S., Goericke, R., & Deheyn, D. D. (2012). Cold induces acute stress but heat is ultimately more deleterious for the reef-building coral Acropora yongei. *Scientific Reports*, *2*, 240.

Sagan, L. (1967). On the origin of mitosing cells. *Journal of Theoretical Biology*, *14*, 255.

Saxby, T., Dennison, W. C., & Hoegh-Guldberg, O. (2003). Photosynthetic responses of the coral Montipora digitata to cold temperature stress. *Marine Ecology Progress Series*, *248*, 85–97.

Scheufen, T., Iglesias-Prieto, R., & Enríquez, S. (2017). Changes in the number of symbionts and Symbiodinium cell pigmentation modulate differentially coral light absorption and photosynthetic performance. *Frontiers in Marine Science*, *4*, 4.

Sharp, K. H., Pratte, Z. A., Kerwin, A. H., Rotjan, R. D., & Stewart, F. J. (2017). Season, but not symbiont state, drives microbiome structure in the temperate coral *Astrangia poculata*. *Microbiome*, *5*, 120.

Smale, D. A., Wernberg, T., Oliver, E. C. J., Thomsen, M., Harvey, B. P., Straub, S. C., Burrows, M. T., Alexander, L. V., Benthuysen, J. A., Donat, M. G., Feng, M., Hobday, A. J., Holbrook, N. J., Perkins-Kirkpatrick, S. E., Scannell, H. A., Sen Gupta, A., Payne, B. L., & Moore, P. J. (2019). Marine heatwaves threaten global biodiversity and the provision of ecosystem services. *Nature Climate Change*, *9*, 306–312.

Stat, M., Carter, D., & Hoegh-Guldberg, O. (2006). The evolutionary history of Symbiodinium and scleractinian hosts—Symbiosis, diversity, and the effect of climate change. *Perspectives in Plant Ecology, Evolution and Systematics*, *8*, 23–43.

Stat, M., & Gates, R. D. (2010). Clade D *Symbiodinium* in Scleractinian corals: A "nugget" of Hope, a selfish opportunist, an ominous sign, or all of the above? *Journal of Marine Science*, *2011*, e730715.

Stat, M., Pochon, X., Cowie, R. O. M., & Gates, R. D. (2009). Specificity in communities of Symbiodinium in corals from Johnston atoll. *Marine Ecology Progress Series*, *386*, 83–96.

Stupnikov, A., McInerney, C. E., Savage, K. I., McIntosh, S. A., Emmert-Streib, F., Kennedy, R., Salto-Tellez, M., Prise, K. M., & McArt, D. G. (2021). Robustness of differential gene expression analysis of RNA-seq. *Computational and Structural Biotechnology Journal*, *19*, 3470–3481.

Szabó, M., Larkum, A. W. D., & Vass, I. (2020). A review: The role of reactive oxygen species in mass coral bleaching. In A. W. D. Larkum, A. R. Grossman, & J. A. Raven (Eds.), (Eds.) *Photosynthesis in algae: Biochemical and physiological mechanisms* (pp. 459–488). Springer International Publishing.

Takahashi, S., Whitney, S., Itoh, S., Maruyama, T., & Badger, M. (2008). Heat stress causes inhibition of the de novo synthesis of antenna proteins and photobleaching in cultured Symbiodinium. *Proceedings of the National Academy of Sciences*, *105*, 4203–4208.

Tchernov, D., Gorbunov, M. Y., de Vargas, C., Narayan Yadav, S., Milligan, A. J., Häggblom, M., & Falkowski, P. G. (2004). Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. *Proceedings of the National Academy of Sciences*, *101*, 13531–13535.

Thornhill, D. J., Kemp, D. W., Bruns, B. U., Fitt, W. K., & Schmidt, G. W. (2008). Correspondence between cold tolerance and temperate biogeography in a Western Atlantic Symbiodinium (dinophyta) Lineage1. *Journal of Phycology*, *44*, 1126–1135.

Wernegreen, J. J. (2012). Endosymbiosis. *Current Biology*, *22*, R555–R561.

Wright, R. M., Aglyamova, G. V., Meyer, E., & Matz, M. V. (2015). Gene expression associated with white syndromes in a reef building coral, *Acropora hyacinthus*. *BMC Genomics*, *16*, 371.

Wuitchik, D. M., Almanzar, A., Benson, B. E., Brennan, S., Chavez, J. D., Liesegang, M. B., Reavis, J. L., Reyes, C. L., Schniedewind, M. K., Trumble, I. F., & Davies, S. W. (2021). Characterizing environmental stress responses of aposymbiotic *Astrangia poculata* to divergent thermal challenges. *Molecular Ecology*, *30*, 5064–5079.

Zhang, H., Hou, Y., Miranda, L., Campbell, D. A., Sturm, N. R., Gaasterland, T., & Lin, S. (2007). Spliced leader RNA trans-splicing in dinoflagellates. *Proceedings of the National Academy of Sciences*, *104*, 4618–4623.

Zhang, Y., Parmigiani, G., & Johnson, W. E. (2020). ComBat-seq: Batch effect adjustment for RNA-seq count data. *NAR Genomics and Bioinformatics*, *2*, lqaa078.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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