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1 **Title:** Symbiosis modulates gene expression of symbionts, but not hosts, under thermal challenge

2 **Running Head:** Coral holobiont gene expression

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10 **Abstract**

11 Increasing ocean temperatures are causing dysbiosis between coral hosts and their symbionts.
12 Previous work suggests that coral host gene expression responds more strongly to environmental
13 stress compared to their intracellular symbionts; however, the causes and consequences of this
14 phenomenon remain untested. We hypothesized that symbionts are less responsive because hosts
15 modulate symbiont environments to buffer stress. To test this hypothesis, we leveraged the
16 facultative symbiosis between the scleractinian coral *Oculina arbuscula* and its symbiont
17 *Breviolum psygmophilum* to characterize gene expression responses of both symbiotic partners *in*
18 and *ex hospite* under thermal challenges. To characterize host and *in hospite* symbiont responses,
19 symbiotic and aposymbiotic *O. arbuscula* were exposed to three treatments: 1) control (18°C), 2)
20 heat (32°C), and 3) cold (6°C). This experiment was replicated with *B. psygmophilum* cultured
21 from *O. arbuscula* to characterize *ex hospite* symbiont responses. Both thermal challenges elicited
22 classic environmental stress responses (ESRs) in *O. arbuscula* regardless of symbiotic state, with
23 hosts responding more strongly to cold challenge. Hosts also exhibited stronger responses than *in*
24 *hospite* symbionts. *In* and *ex hospite B. psygmophilum* both downregulated genes associated with
25 photosynthesis under thermal challenge; however, *ex hospite* symbionts exhibited greater gene
26 expression plasticity and differential expression of genes associated with ESRs. Taken together,
27 these findings suggest that *O. arbuscula* hosts may buffer environments of *B. psygmophilum*
28 symbionts; however, we outline the future work needed to confirm this hypothesis.
29

30 **Keywords:** thermal challenge, symbiosis, gene expression, coral

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34 **Introduction**

35 Endosymbioses—associations where one organism lives within cells of its host [1]—have
36 driven evolutionary innovations and allowed species to access resources and environments that
37 would otherwise be unavailable [2, 3]. Endosymbioses span the tree of life and comprise
38 exemplary innovations including deep-sea hydrothermal vent tubeworms (*Riftia pachyptila*) that
39 rely on chemosynthetic bacterial endosymbionts [*e.g.*, 4] and salamanders (*Ambystoma*
40 *maculatum*) benefiting from photosynthetic endosymbionts (*Oophila amblystomatis*) as embryos
41 [*e.g.,* 5]. Endosymbionts often live within a host compartment, such as a vacuole or membrane,
42 which facilitates the exchange of nutrients and metabolites, serving as the backbone for these
43 symbioses [2, 6].

44 Corals are one of the most iconic examples of endosymbiosis, and their symbiosis with
45 single celled algal symbionts [dinoflagellate algae in the family Symbiodiniaceae, hereafter
46 'symbiont', 7] enables diverse tropical reef ecosystems to thrive in oligotrophic waters [3].
47 Symbiodiniaceae live in coral gastrodermal cells in specialized vacuoles called symbiosomes [2,
48 8]. This endosymbiosis facilitates the transfer of materials between host and symbiont, where
49 Symbiodiniaceae share photosynthetically-derived carbon sugars and in return receive inorganic
50 compounds from coral metabolic waste in addition to protection [9, 10]. Once symbiosis is
51 established, hosts can actively modulate symbiont physiology by manipulating the symbiosome
52 environment. For example, symbiont photosynthesis is dependent on nitrogen availability, and
53 host-mediated nitrogen limitation enables maintenance of primary production and control of
54 symbiont growth [11, 12]. Additionally, coral hosts acidify the symbiosome *via* expression of V-
55 type proton ATPases, which facilitates increased photosynthesis [13].

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56 Tropical corals live near their upper thermal limits, making them particularly susceptible
57 to temperature changes [14, 15]. Increases in anthropogenic carbon dioxide levels are elevating
58 ocean temperatures and leading to marine heatwaves [*i.e.*, 16], which threaten corals globally [17].
59 Specifically, temperature increases lead to a breakdown of coral-algal symbioses in a process
60 called 'coral bleaching', and extended periods of dysbiosis can lead to coral starvation and eventual
61 mortality [18]. It is theorized that reactive oxygen species (ROS) generated by algal symbionts
62 under temperature stress can damage cellular components, cause photoinhibition, and trigger coral
63 bleaching [reviewed in 19]. However, even though both symbiotic partners exhibit a wide array of
64 stress responses, symbionts are assumed to initiate dysbiosis due to ROS production [*e.g.,* 20–22].
65 Additionally, several lines of evidence demonstrate that hosts exhibit strong gene expression
66 responses to stress [*e.g.,* 23–25, reviewed in 26], while the symbiont's response is muted [*e.g.,* 27–
67 29]. This paucity of an algal response suggests that corals may regulate their symbiont's
68 environment to buffer algae from stress; however, alternative explanations include that the
69 symbiont's transcriptomic machinery is less responsive to stress regardless of symbiotic state.

70 Understanding the independent and interactive roles of coral hosts and Symbiodiniaceae
71 algae in holobiont (*i.e.,* assemblage of coral host and associated algal and microbial symbionts)
72 resilience is difficult in a tropical coral system [reviewed in 30] because it is impossible to
73 disentangle the host's aposymbiotic state from stress and nutrient limitation given tropical coral
74 reliance on Symbiodiniaceae-derived carbon. To address these difficulties, facultative symbioses
75 have emerged as tractable systems for answering fundamental questions about coral symbiosis
76 [31]. Here, we used gene expression profiling in the facultatively symbiotic coral *Oculina*
77 *arbuscula* and its symbiont *Breviolum psygmophilum* to address two questions: 1) what is the
78 consequence of symbiosis for coral hosts under thermal challenge? and 2) how does symbiosis

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79 modulate symbiont responses to thermal challenges? We hypothesized that, compared to
80 aposymbiotic corals, symbiotic corals under thermal challenge would exhibit gene expression
81 patterns consistent with environmental stress responses (ESRs) of tropical corals because of
82 symbiont-derived ROS produced under thermal stress. Based on previous work documenting
83 minimal *in hospite* symbiont gene expression responses, we predicted greater responses of
84 symbiotic hosts compared to symbionts *in hospite* and muted responses of symbionts *in hospite*
85 compared to *ex hospite,* consistent with coral hosts modulating symbiont environments. To answer
86 these questions, we conducted temperature challenge assays and characterized host and symbiont
87 responses *via* gene expression profiling.
88

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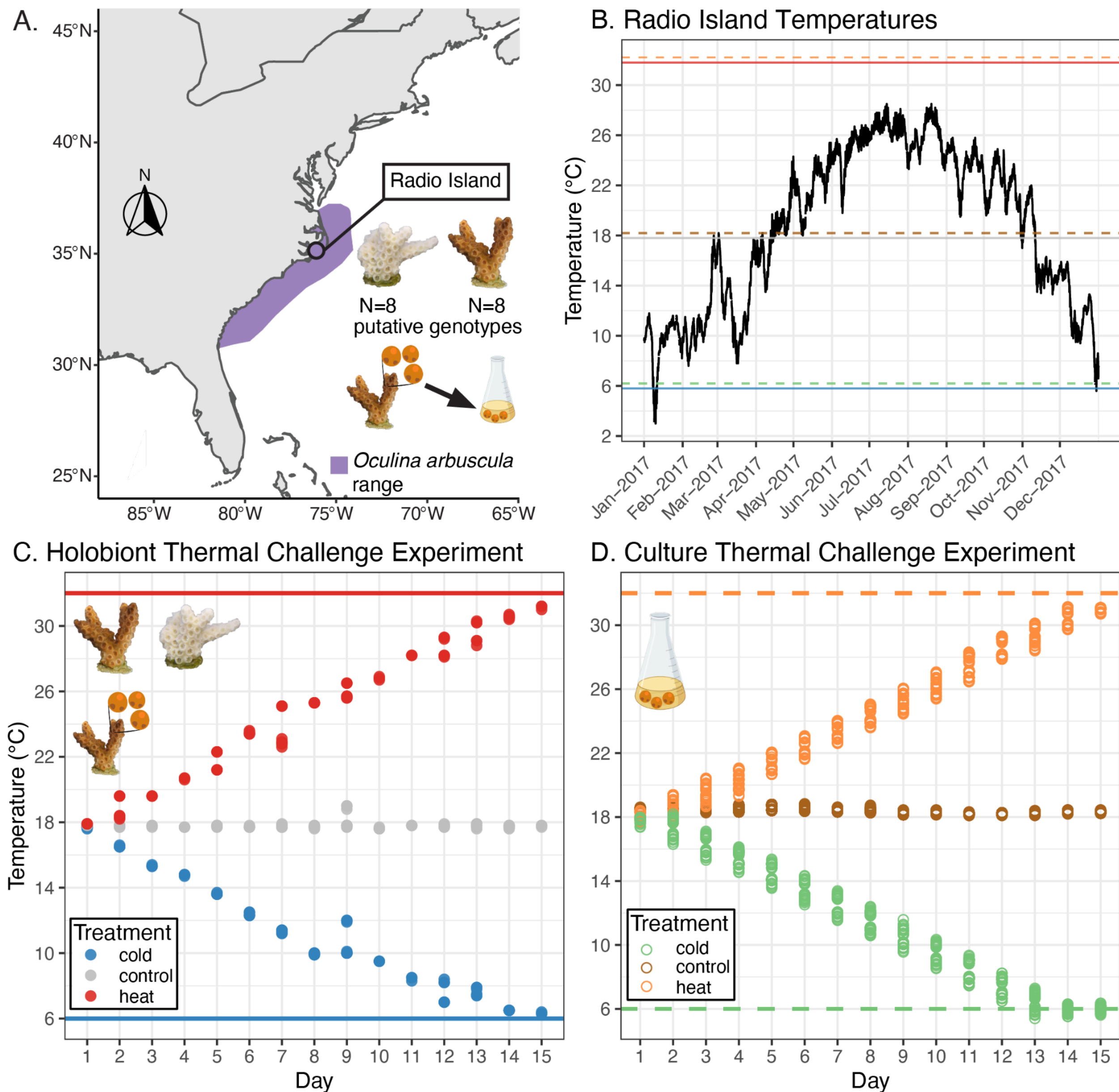

89

90 **Figure 1. Experimental overview. A.** Map of collection site (Radio Island, North Carolina) of
91 *Oculina arbuscula* used in the holobiont thermal challenge experiment (N=8/symbiotic state).
92 Purple shading indicates *O. arbuscula* range. **B.** Water temperatures (black line) in 2017 recorded
93 at the NOAA buoy near Radio Island with thermal challenge treatments (heat=32°C,
94 control=18°C, cold=6°C) overlaid. **C.** Temperatures recorded during holobiont thermal challenge
95 experiment. **D.** Temperatures recorded during culture thermal challenge experiment. Culture icons
96 were created with BioRender.com.
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102 **Materials and Methods**

103 ***I. Experiment 1:* Oculina arbuscula *and* Breviolum psygmophilum *holobiont responses to***

104 ***temperature challenges in symbiosis***

105 *Coral Collection & Experimental Design*

106 In June 2018, 16 *O. arbuscula* colonies (N=8 symbiotic, N=8 aposymbiotic) were collected
107 from Radio Island, North Carolina (NC) (34.712590°N, -76.684308°W) under NC Division of
108 Marine Fisheries permits #706481 and #1627488 (Figure 1A). Colonies were shipped overnight
109 to Boston University, fragmented, attached to petri dishes using cyanoacrylate glue, and
110 maintained at ambient conditions (18°C, 33-35 PSU) for approximately 5 months. Experimental
111 temperatures were informed by 2017 (January 1 - December 31, 2017) *in situ* temperature data
112 recorded by the NOAA buoy closest to the collection site (Station BFTN7; minimum temperature
113 recorded=3°C, maximum temperature recorded=28.5°C; Figure 1B). One fragment from each
114 colony (N=48 fragments) was placed in one of three treatments: 1) control: 18°C, 2) heat
115 challenge: temperature increased 1°C day^{-1} from 18°C to 32°C, and 3) cold challenge: temperature
116 decreased 1°C day^{-1} from 18°C to 6°C. Treatments were maintained for 15 days using Aqua Logic
117 digital temperature controllers calibrated with a NIST-certified glass thermometer and were
118 recorded using HOBO loggers (Figure 1C).

119

120 In hospite *symbiont physiology*

121 Pulse Amplitude Modulation (PAM) fluorometry measured dark-acclimated
122 photochemical efficiency of photosystem II (Fv/Fm) of symbiotic corals using a Junior PAM
123 approximately every three days throughout the experiment. Corals received 8 hours of dark
124 acclimation before Fv/Fm was measured in triplicate. The effect of temperature challenge on
125 symbiont Fv/Fm over time was analyzed using a linear mixed effects model (*lmer*), with

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126 interactions of fixed effects of treatment and day plus a random effect of genotype. Pairwise model
127 outputs were compared using *emmeans* [32]. All analyses were performed in the R v4.2.0 statistical
128 environment [33], and all code and raw data for the analyses detailed here can be found on Github:
129 https://github.com/hannahaichelman/Oculina_Host_Sym_GE.
130

131 Oculina arbuscula *holobiont gene expression profiling*

132 On day 15, tissue from all fragments (N=48) was sampled using sterilized bone cutters,
133 immediately preserved in 200 proof ethanol, and maintained at -80°C. Samples were homogenized
134 with lysis buffer and glass beads and total RNA was extracted using an RNAqueous kit
135 (ThermoFisher Scientific) following manufacturer's instructions. DNA contamination was
136 removed *via* DNAse1 and TagSeq libraries were prepared using 1.5 μg of RNA [following 25,
137 34]. Successful libraries (N=47) were sequenced on Illumina HiSeq 2500 (single-end 50 bp) at
138 Tufts University Core Facility. TagSeq analyses followed
139 https://github.com/z0on/tag-based_RNAseq. Raw reads were quality filtered to remove Illumina
140 adapters, poly-A sequences, PCR duplicates, reads less than 20 bp long, and reads with a quality
141 score less than 33.
142

143 *Putative coral clone identification*

144 We tested for the presence of clones in our dataset following methods presented in Bove
145 et al. [35]. Quality filtered reads were mapped to concatenated *O. arbuscula* and *B. psygmophilum*
146 transcriptomes [presented in 36] using Bowtie2 [37]. Symbiont reads were removed from the
147 dataset, genotyping and identification of host single nucleotide polymorphisms (SNPs) was
148 performed using ANGSD [38], and putative clones were distinguished using a hierarchical

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149 clustering tree (*hclust*) based on pairwise identity by state (IBS) distances calculated in ANGSD
150 (Figure S1). After removing clones, 33 samples (N=7 putative symbiotic genotypes, N=4 putative
151 aposymbiotic genotypes) were used in downstream analyses. See supplementary methods for
152 additional details.
153
154 *Oculina arbuscula* and *in hospite B. psygmophilum* gene expression analyses
155 Quality filtered reads were mapped to the same concatenated transcriptomes described
156 above using Bowtie2 [37], but with different parameters (-k mode, k=5, --no-hd, --no-sq). Host
157 and symbiont reads were separated to produce two separate files of counts per gene, and
158 independent runs of DESeq2 [39] identified differentially expressed genes (DEGs) in response to
159 heat and cold thermal challenge relative to control using Wald's tests.
160 *Rlog*-transformed host and symbiont gene expression (GE) data were used as input for
161 separate principal component analyses (PCAs) using *plotPCA* (package=DESeq2) to determine
162 the effect of temperature on GE profiles using PERMANOVAs *via* the *adonis2* function
163 [package=vegan; 40]. GE plasticity, defined as the distance in PC space between an individual's
164 GE profile and the average GE of all samples in the control, was calculated from host and symbiont
165 PCAs following Bove et al. [35]. Differences in GE plasticity between treatments were tested using
166 an ANOVA followed by Tukey's HSD post-hoc tests. Model assumptions were assessed using
167 *check_model* [package=performance; 41].
168 Gene ontology (GO) enrichment analyses were performed using Mann-Whitney *U* tests
169 based on the ranking of signed log p-values [42] for both host and symbiont datasets. Results were
170 visualized in dendrograms, which indicate the amount of gene sharing between significant GO

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171 categories and the direction of change relative to the control treatment. Results from the GO
172 enrichment analyses were used for two functional analyses, detailed below.

173 First, GO delta-ranks, which quantify the tendency of genes assigned to a GO category
174 toward up- or downregulation in treatment versus controls, were used to compare *O. arbuscula*
175 host response under thermal challenges relative to a meta-analysis from Dixon et al. [26] that
176 characterized the GE signatures of stress in *Acropora* corals. This meta-analysis identified two
177 classes of coral stress responses: "type A", which was positively correlated across projects and
178 consistent with the coral environmental stress response (ESR), and "type B", which was negatively
179 correlated and indicated lower intensity stress. Host delta-ranks for Biological Processes (BP) GO
180 terms were plotted against BP delta-ranks of all 'type A' studies identified by Dixon et al. [26].
181 While not a formal statistical test, this analysis indicates whether *O. arbuscula* responses were
182 functionally similar to a 'type A' ESR. Second, *B. psygmophilum* symbiont GO results identified
183 underrepresentation of photosynthesis-related GO terms under cold challenge. Genes associated
184 with these GO categories (unadjusted p-value <0.10) were explored by constructing a heatmap
185 using *pheatmap*.

186 Symbiont species identity of all symbiotic coral samples was confirmed using
187 metabarcoding of the ITS2 locus (forward primer=*ITS-DINO* [43], reverse primer=*ITS2Rev2*
188 [44]). Raw ITS2 data were submitted to SymPortal [45] to identify ITS2 defining intragenomic
189 variant (DIV) profiles and relative abundances of DIVs across *O. arbuscula* fragments were
190 compared using a bar plot constructed with *phyloseq* [46]. N=39/48 samples were successfully
191 sequenced and confirmed to host *B. psygmophilum* (Figure S2). See supplementary methods for
192 additional details on library preparation and sequencing.

193

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194 *Comparing orthologous genes in* Oculina arbuscula *and* Breviolum psygmophilum *in symbiosis*

195 To compare *O. arbuscula* (symbiotic and aposymbiotic) and *B. psygmophilum in hospite*
196 responses to temperature challenges, independent GE analyses were completed on orthologous
197 genes. This analysis allowed us to test two predictions: 1) symbiotic hosts would respond more to
198 temperature challenge than aposymbiotic hosts, and 2) symbiotic hosts would respond more than
199 their symbionts *in hospite*. Orthologous genes were identified following Dixon and Kenkel [47]
200 with additional specifics for Symbiodiniaceae described here:
201 https://github.com/grovesdixon/symbiodinium_orthologs (details in supplemental methods).
202 Briefly, Transdecoder v5.5.0 [48] predicted protein coding sequences, FastOrtho assigned
203 predicted coding sequences to orthologous groups [49], and approximately-maximum-likelihood
204 phylogenetic trees of these protein sequences were built using MAFFT [50] and FastTree [51],
205 which resulted in a total of 1962 single-copy orthologs.

206 Single-copy orthologs (N=1962) were extracted from host and symbiont counts files and
207 orthologs with mean count >2 in at least 80% of samples were retained, leaving 152 nonzero read
208 count orthologs. To directly compare responses to thermal challenge of symbiotic hosts,
209 aposymbiotic hosts, and symbionts *in hospite*, *O. arbuscula* and *B. psygmophilum* count data for
210 these orthologs were analyzed in DESeq2, which modeled aggregate factors of temperature
211 treatment and sample type (symbiotic host, aposymbiotic host, or symbiont *in hospite*). Responses
212 of sample types to heat and cold challenge relative to control were quantified as the number of
213 differentially expressed orthologs (DEOs, adjusted p-value <0.1). Differences in the proportion of
214 DEOs across sample types were tested with a two-proportions z-test.

215

216 ***II. Experiment 2:* Breviolum psygmophilum *response in culture* - ex hospite**

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217 *Symbiont cell culture maintenance*

218 To isolate *B. psygmophilum* responses to temperature challenge *ex hospite*, *O. arbuscula*
219 holobiont thermal challenges were replicated on cultured *B. psygmophilum*. *Breviolum*
220 *psygmophilum* cells were isolated from *O. arbuscula* from Radio Island, NC by serially diluting
221 airbrushed host tissue into sterile F/2 media (Bigelow NCMA, East Boothbay, ME, USA). The
222 "ancestral culture" was maintained in F/2 media based on artificial seawater (Instant Ocean), with
223 monthly transfers to fresh media, in a Percival incubator (model AL-30L2) at 26°C and irradiance
224 of 30 μmol photons m^{-2} sec^{-1} on a 14:10 hour light:dark cycle. After four months, this ancestral
225 culture was split into three flasks, each with 100 mL of F/2 media and 0.5 mL of dense cells. These
226 "daughter cultures" were acclimated to 18°C by decreasing temperatures at a rate of 1°C day^{-1} over
227 a span of nine days. A preliminary experiment established semi-continuous culture methodology
228 (Figure S3A; supplementary methods). Cultured symbiont species identity was confirmed prior to
229 thermal challenge experiments using Sanger sequencing (details in supplementary methods).
230

231 *Thermal challenge experiment*

232 *Breviolum psygmophilum* cultures were exposed to thermal challenges that mirrored
233 treatments used in holobiont experiments detailed in Part I. *Ex hospite* symbiont thermal
234 challenges began after a 51-week acclimation at 18°C. Experimental cultures (N=4 flasks per
235 treatment) were established from long-term acclimated daughter flasks, with initial cell densities
236 of 200,000 cells mL^{-1} in 100 mL of F/2 media. Heat and cold challenge flasks were placed in
237 separate Percival incubators (model AL-30L2), and control flasks were maintained in a
238 temperature-controlled room (Harris Environmental Systems, Andover, MA). All treatments
239 began at 18°C and followed a 14:10 hour light:dark cycle at ~50 μmol photons m^{-2} sec^{-1}.

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240 Experimental cultures were grown semi-continuously, with the timing of transfers determined
241 using the preliminary experiment (Figure S3).
242
243 Ex hospite Breviolum psygmophilum *gene expression profiling*
244 On day 15, all cultures were thoroughly mixed, concentrated *via* centrifugation (5000
245 RPM, 7 minutes), flash frozen in liquid nitrogen, and stored at -80°C. To obtain sufficient RNA
246 for TagSeq, replicate cultures in cold challenge treatments were pooled, such that there were four
247 pooled replicates extracted separately. Lower cell densities in cold challenge were due to reduced
248 growth (Figure S3), which was not the case for heat challenge and control flasks. Flash frozen
249 pellets were ground for three minutes in a mortar and pestle that was pre-chilled with liquid
250 nitrogen. Additional liquid nitrogen was added to keep the cell pellet frozen and RNA was
251 extracted using RNAqueous-micro kits (ThermoFisher Scientific) following manufacturer's
252 instructions, except elution volume was 15 μL. DNA was removed *via* DNA-free DNA Removal
253 Kit (ThermoFisher Scientific). RNA was normalized using concentrations from a Quant-iT
254 PicoGreen dsDNA Assay (ThermoFisher Scientific) and total RNA was sent to the University of
255 Texas at Austin Genome Sequencing and Analysis Facility (GSAF), where it was prepared for
256 TagSeq following Meyer et al. [25] on a NovaSeq 6000 machine (single-end 100 bp).
257
258 *Gene expression analyses on* ex hospite Breviolum psygmophilum
259 Raw read processing of *ex hospite B. psygmophilum* TagSeq data followed methods
260 detailed in Part I except samples were only mapped to the *B. psygmophilum* reference
261 transcriptome. Principal component analysis (PCAs), GE plasticity, and GO-enrichment analyses
262 were conducted as detailed in Part I. A heat-map of genes with GO annotations related to

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263 photosynthesis was generated on the culture dataset with an unadjusted p-value <0.01 to restrict
264 the number of DEGs included. In addition, a heatmap of genes with GO annotations related to
265 oxidative stress was constructed, as these terms were consistently enriched in *ex hospite* GO
266 analyses.
267

268 ***III. Comparing* Breviolum psygmophilum *response* in *and* ex hospite**

269 To test the prediction that symbionts would respond more to temperature challenge *ex*
270 *hospite* compared to *in hospite*, *B. psygmophilum* GE datasets from Experiments I and II were
271 analyzed together. A batch effect correction was conducted on combined raw count data using
272 ComBat-seq [52], with a specified batch of experiment type (*in hospite* or *ex hospite*) and
273 temperature treatment (heat challenge, cold challenge, or control) as the treatment of interest.
274 Batch-corrected data were included in the same DESeq2 [39] model, which modeled a main effect
275 of the aggregate factor of treatment (cold challenge, heat challenge, or control) and sample type
276 (*in hospite* or *ex hospite*). Genes were only retained when present in at least 80% of samples (33/41
277 samples) at a mean count of 2 or higher, which left 1885 genes for downstream analyses.

278 Following PCAs detailed in Experiment I, combined symbiont count data were *rlog*-
279 transformed and used as input for a PCA to test the effect of the aggregate factor of temperature
280 treatment and sample type on GE. Significance was assessed with PERMANOVA, using the
281 *adonis2* function [package=vegan; 40]. GE plasticity was also calculated following methods
282 detailed above.
283
284
285

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286 **Results**

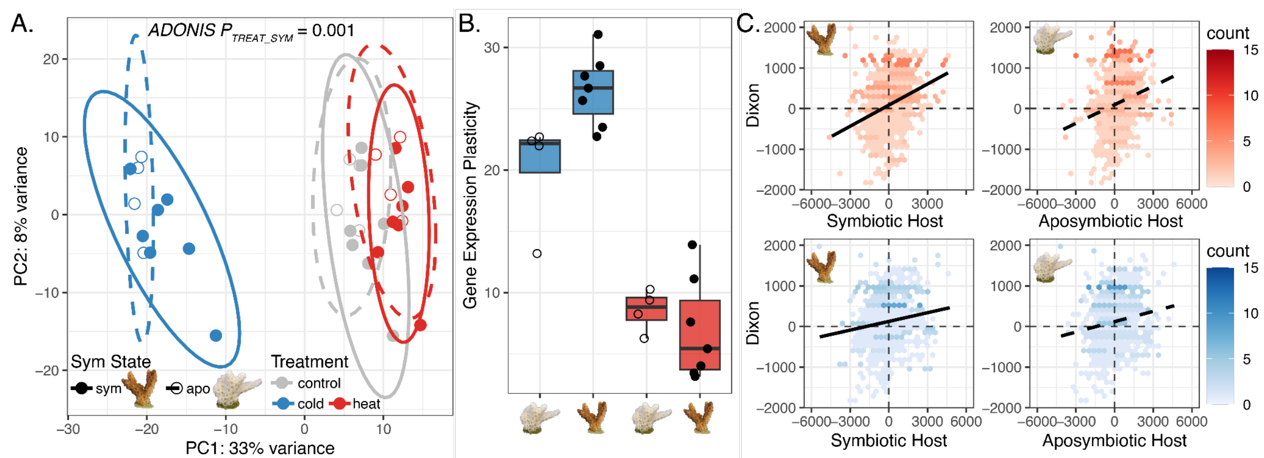
287 ***I. Independent responses of* Oculina arbuscula *and* Breviolum psygmophilum *to temperature***
288 ***challenges in symbiosis***

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

291 The aggregate factor of temperature treatment and symbiotic state had a significant effect
292 on *O. arbuscula* host gene expression patterns (Figure 2A; *ADONIS p*=0.001) with cold challenge
293 eliciting higher gene expression plasticity in both symbiotic (*Tukey HSD p*=0.013) and
294 aposymbiotic (*Tukey HSD p*<0.001) hosts compared to heat challenge (Figure 2B; *p*<0.001).
295 Symbiotic state did not influence gene expression plasticity within temperature treatments (cold
296 challenge, *Tukey HSD p*=0.218; heat challenge, *Tukey HSD p*=0.992; Figure 2B).

297 When comparing GO delta ranks of the 'type A' module from Dixon *et al.* [26] to symbiotic
298 and aposymbiotic *O. arbuscula* host delta-ranks from the thermal challenges, positive relationships
299 were observed for biological processes GO terms for all comparisons (Figure 2C).

300



301

302 **Figure 2. Symbiotic and aposymbiotic *Oculina arbuscula* host gene expression responses to**
303 **temperature challenges. A.** Principal component analysis (PCA) of gene expression of symbiotic
304 (solid point and line) and aposymbiotic (open points, dashed line) coral hosts under control (grey),

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305 cold (blue), and heat (red) challenge. The x- and y-axes represent the % variance explained by the
306 first and second PC, respectively. **B.** Gene expression plasticity of symbiotic and aposymbiotic
307 hosts under cold and heat challenges. **C.** Relationship between biological processes (BP) gene
308 ontology (GO) delta ranks from symbiotic (left) and aposymbiotic (right) hosts under heat (top)
309 and cold (bottom) challenge with BP delta-ranks of all "type A" studies from [26]. Positive slopes
310 represent "type A" environmental stress responses.
311

312 *Negative effects of cold challenge on* Breviolum psygmophilum *photosynthetic function*

313 ITS2 metabarcoding confirmed all *O. arbuscula* genotypes hosted a majority of *B.*
314 *psygmophilum* defining intragenomic variants (DIVs) (Figure S2). All but one individual hosted
315 100% *B. psygmophilum,* and all symbiotic *O. arbuscula* fragments hosted the same DIV of *B.*
316 *psygmophilum* (Figure S2). Photosynthetic efficiency (Fv/Fm) of *in hospite B. psygmophilum* was
317 reduced by temperature challenges through time (Figure 3A; $p<0.001$). By day 8 (cold
318 challenge=11°C, heat challenge=25°C), Fv/Fm had significantly declined in the cold challenge
319 relative to controls ($p=0.02$), but not in heat challenge ($p=0.09$). For the remainder of the
320 experiment, Fv/Fm was significantly reduced in both cold and heat challenge relative to the control
321 (Figure 3A; $p<0.001$ for all comparisons). Fv/Fm in cold challenge corals was more dramatically
322 reduced than under heat challenge, with fixed effect parameter estimates on day 14 of -0.065 in
323 heat challenge and -0.24 in cold challenge relative to control (Figure 3A).

324 Temperature challenges significantly affected gene expression profiles of *in hospite B.*
325 *psygmophilum* (Figure 3B; *ADONIS* $p=0.001$). However, no differences in gene expression
326 plasticity between symbionts in cold and heat challenge were observed (Figure 3C; *Tukey HSD*
327 $p=0.62$). Corroborating negative effects of cold challenge on *B. psygmophilum* Fv/Fm (Figure 3A),
328 six GO terms related to photosynthetic processes were underrepresented in cold challenge relative
329 to control conditions (photosystem [GO:0009521], photosynthesis, light harvesting
330 [GO:0009765], chlorophyll binding [GO:0016168], protein-chromophore linkage [GO:0018298],

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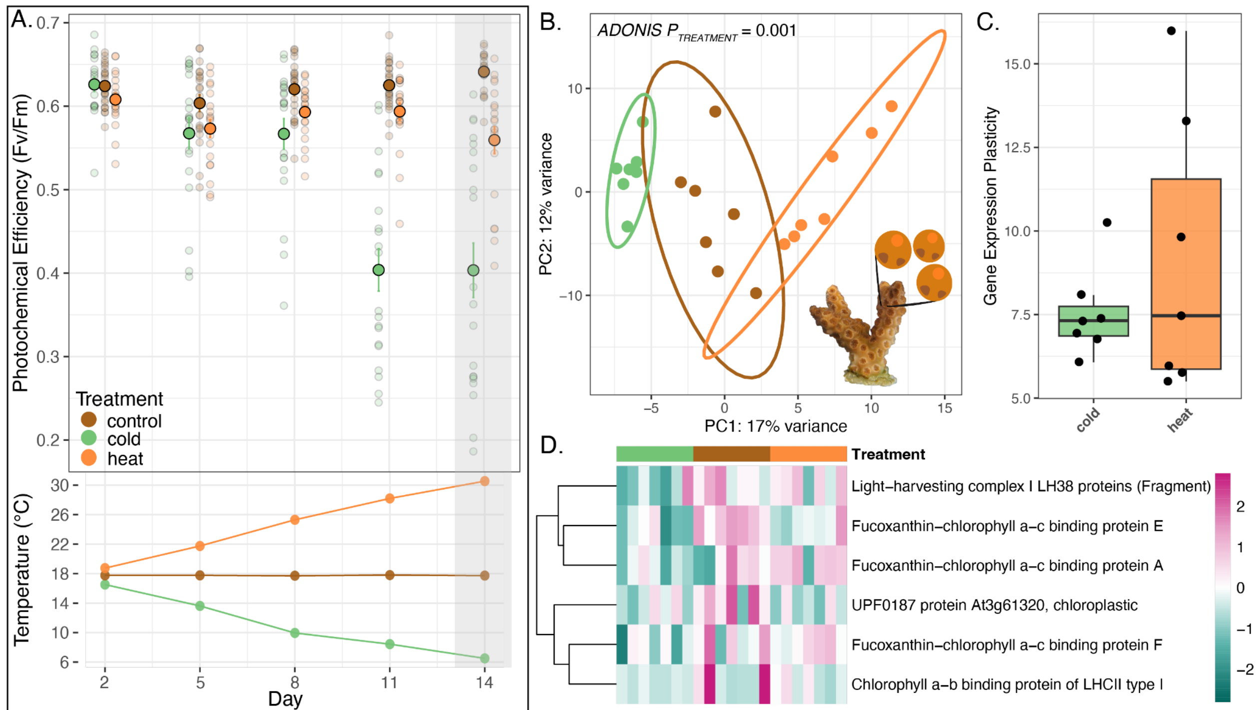
331 thylakoid membrane [GO:0042651], and tetrapyrrole binding [GO:0046906]). Six annotated genes
332 within these GO terms were downregulated under cold challenge (unadjusted p-value<0.10)
333 relative to control conditions and these genes included light-harvesting complex (LHC) and
334 chloroplast genes (Figure 3D).

335


336 **Figure 3. *In hospite* symbiont physiology and gene expression responses to temperature**
337 **challenges. A.** Photochemical efficiency (Fv/Fm, top panel) of *in hospite* symbionts through time
338 as temperatures diverged (bottom panel). Top: Large points represent mean Fv/Fm ± standard error
339 across treatments and transparent points represent a fragment's Fv/Fm at each time point. The gray
340 bar indicates the time point immediately prior to sampling for gene expression. **B.** Principal
341 component analysis (PCA) of gene expression of *in hospite* symbionts from A on day 15. **C.** Gene
342 expression plasticity of *in hospite* symbionts under cold (green) and heat (orange) challenge was
343 not significantly different (Tukey HSD p=0.62). **D.** Heatmap showing differentially expressed
344 genes (DEGs; unadjusted p-value<0.1) belonging to photosynthesis gene ontology (GO) terms,
345 where each row is a gene and each column is a sample. The color scale represents log2 fold change
346 relative to the gene's mean, where pink represents up-regulation and teal represents down-
347 regulation.
348
349
350

351

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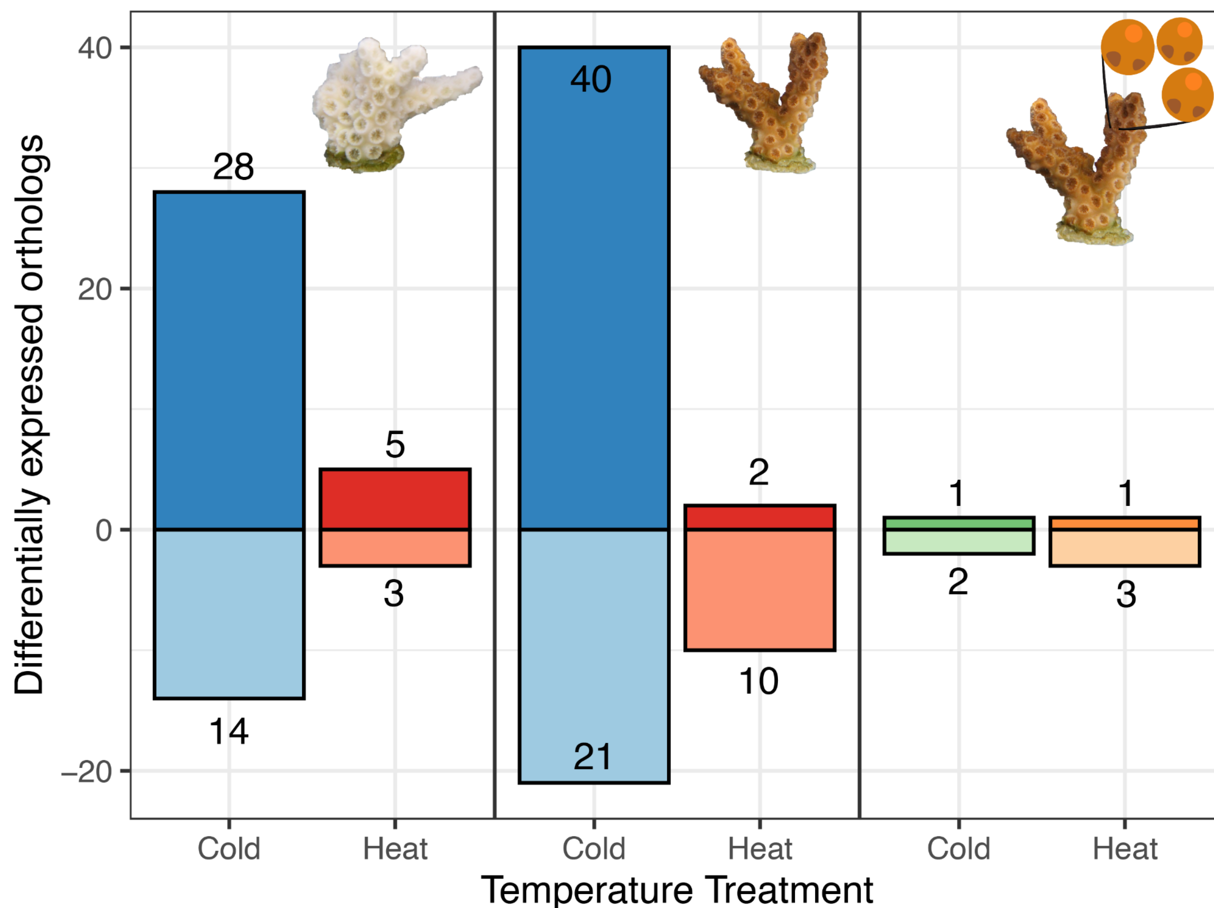
352 ***II. Comparing response of* Oculina arbuscula *and* Breviolum psygmophilum *in symbiosis***
353 ***using orthologous genes***

354 More differentially expressed orthologs (DEOs) were observed in symbiotic hosts
355 compared to aposymbiotic hosts under cold challenge (X^2 = 4.76; p=0.015), but not heat challenge
356 (Figure 4; X^2 = 0.482; p=0.24). Similarly, aposymbiotic hosts exhibited more DEOs compared to
357 *in hospite B. psygmophilum* under cold challenge (X^2 = 37.7; p<0.0001), but not heat challenge
358 (Figure 4; X^2 = 0.781; p=0.188). The number of DEOs was greater in symbiotic hosts compared
359 to *in hospite* symbionts under both cold challenge (X^2 = 64.3; p<0.0001) and heat challenge (Figure
360 4; X^2 = 3.23; p=0.036).



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362 **Figure 4. Coral hosts exhibit more differentially expressed orthologs (DEOs) than *in hospite*
363 symbionts under thermal challenges.** Bar plots representing the number of DEOs (positive
364 values=up-regulated, negative values=down-regulated) in response to temperature challenges in
365 aposymbiotic hosts (left), symbiotic hosts (center), and *in hospite* symbionts (right). Symbiotic *O.*
366 *arbuscula* had significantly more DEOs than *in hospite B. psygmophilum* under cold challenge
367 ($p<0.0001$) and heat challenge ($p=0.036$). Aposymbiotic *O. arbuscula* had significantly more
368 DEOs than *in hospite B. psygmophilum* under cold challenge ($p<0.0001$), but not heat challenge
369 ($p=0.188$). Symbiotic *O. arbuscula* had significantly more DEOs than aposymbiotic *O. arbuscula*
370 under cold challenge ($p=0.015$), but not heat challenge ($p=0.244$).
371
372
373

374 ***III.* Breviolum psygmophilum *response to temperature challenge out of symbiosis* - ex hospite**

375 Sanger sequencing confirmed that all parent cultures matched *B. psygmophilum* (GenBank
376 Accession ID LK934671.1) with 100% percent identity and 53-87% query coverage. *Breviolum*
377 *psygmophilum* cultures in all treatments were maintained in exponential growth phase throughout
378 the experiment (Figure S3B,C). Thermal challenges significantly affected gene expression patterns
379 of *ex hospite B. psygmophilum* (Figure 5A; *ADONIS* $p=0.001$). Gene expression plasticity was
380 greater in *ex hospite B. psygmophilum* under cold challenge relative to heat challenge (Figure 5B;
381 *Tukey HSD* $p=0.008$), whereas no significant effect was observed *in hospite* (Figure 3C).
382

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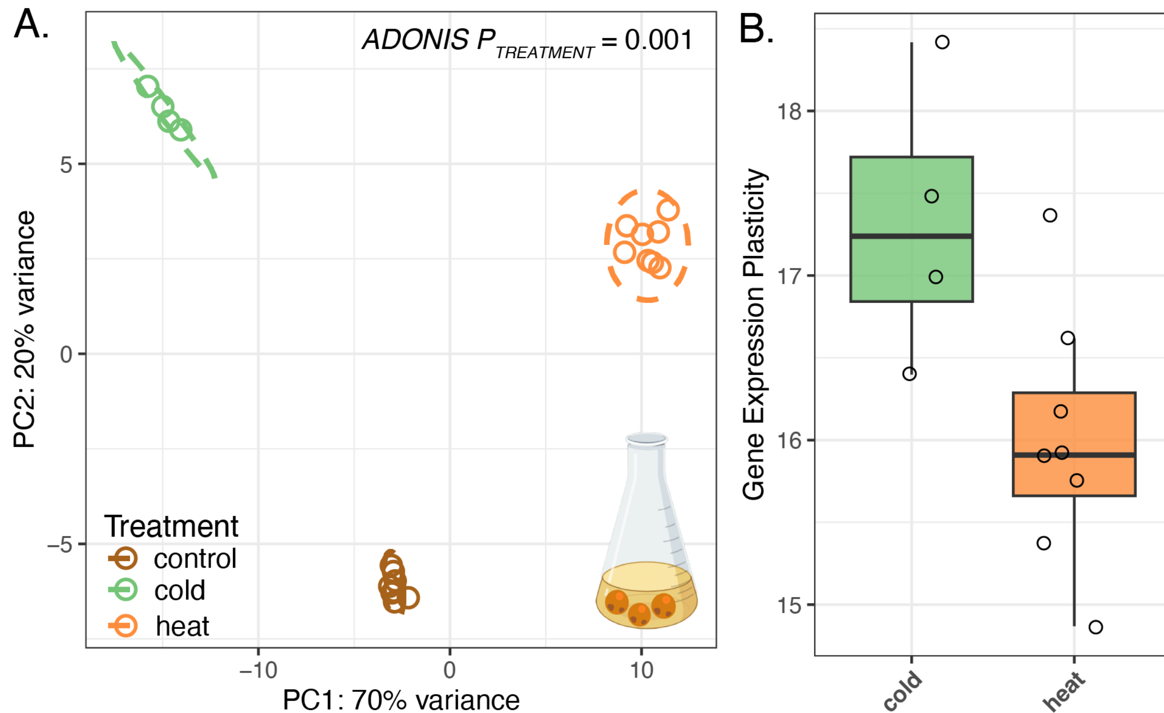

383

384 **Figure 5. *Ex hospite* symbiont gene expression responses to temperature challenges. A.**
385 Principal component analysis (PCA) of gene expression of *ex hospite* symbionts under temperature
386 challenges. **B.** Gene expression plasticity of symbionts *ex hospite* under cold (green) and heat
387 (orange) challenge. Gene expression plasticity was significantly greater under cold challenge
388 compared to heat challenge (*Tukey HSD* p=0.008). Culture icon created with BioRender.com.
389

390 Eight photosynthesis-related GO terms were underrepresented in *ex hospite B.*
391 *psygmophilum* under cold challenge (photosystem [GO:0009521], photosynthesis, light harvesting
392 [GO:0009765], chloroplast-nucleus signaling pathway [GO:0010019], photosynthesis
393 [GO:0015979], chlorophyll binding [GO:0016168], protein-chromophore linkage [GO:0018298],
394 thylakoid membrane [GO:0042651], and tetrapyrrole binding [GO:0046906]). A heat map of 59
395 DEGs (unadjusted p-value<0.01) under cold challenge belonging to these GO terms showcased a
396 small group of up-regulated genes and a larger group of down-regulated genes under cold
397 challenge (Figure 6A). Up-regulated photosynthesis-related genes included “Pentatricopeptide
398 repeat-containing proteins”, which are involved in RNA editing events in chloroplasts [53].

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399 Similar to *B. psygmophilum* in symbiosis (Figure 3C), genes involved in the LHC were down-
400 regulated under cold challenge (Figure 6A).

401 Five GO terms commonly associated with stress were differentially enriched in *ex hospite*
402 *B. psygmophilum* under cold challenge relative to control conditions (protein folding
403 [GO:0006457], cellular response to oxidative stress [GO:0034599], hydrogen peroxide metabolic
404 process [GO:0042743], unfolded protein binding [GO:0051082], cellular response to chemical
405 stress [GO:0062197]). A heat map of 69 DEGs under cold challenge (unadjusted p-value<0.01)
406 assigned to these GO terms and revealed two groups of genes, one down-regulated and one up-
407 regulated under cold challenge relative to control and heat challenge cultures (Figure 6B).

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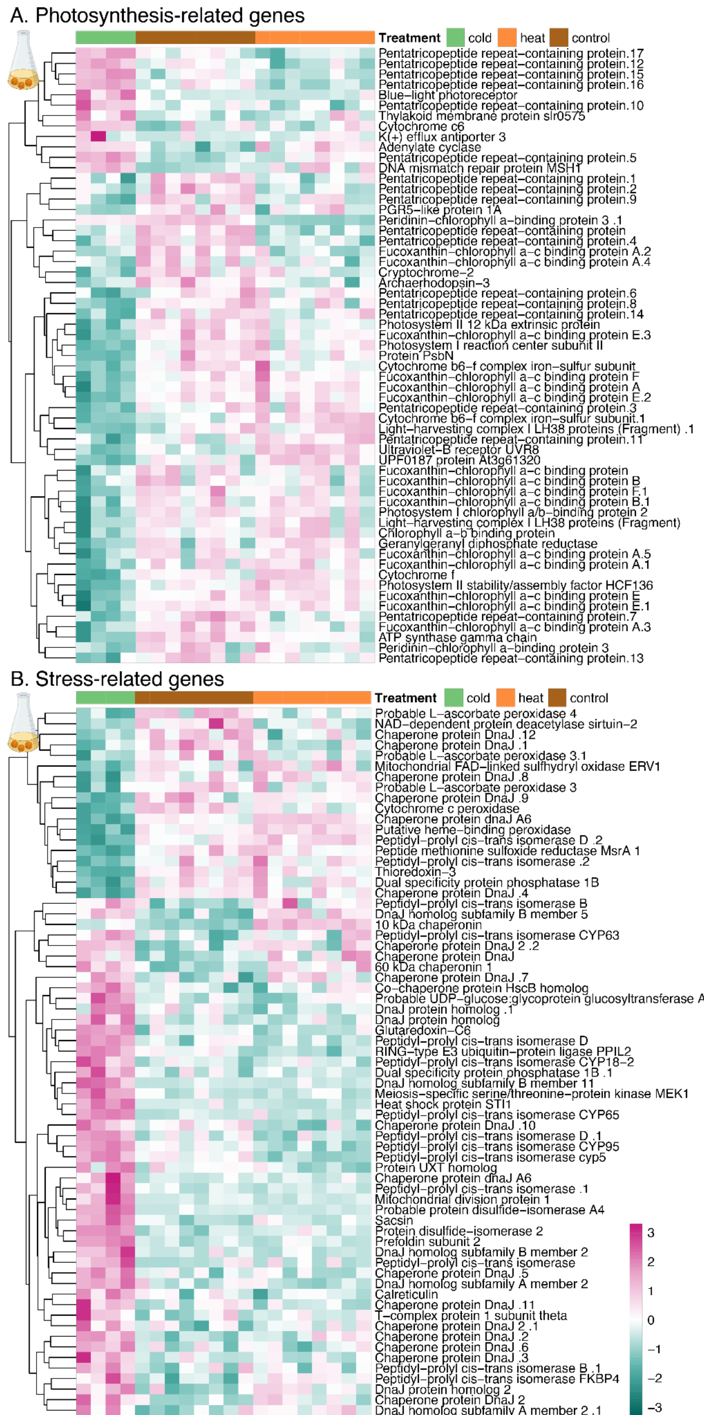

Figure 6. *Ex hospite* symbionts exhibit differential expression of photosynthesis and stress-related genes under cold challenge. Heatmap showing differentially expressed genes (DEGs; unadjusted p-value<0.01) belonging to photosynthesis (**A**) and stress (**B**) gene ontology (GO) terms, where each row is a gene and each column is a sample. The color scale represents log2 fold change relative to the gene's mean, where pink represents up-regulation and teal represents down-regulation. Culture icons created with BioRender.com.

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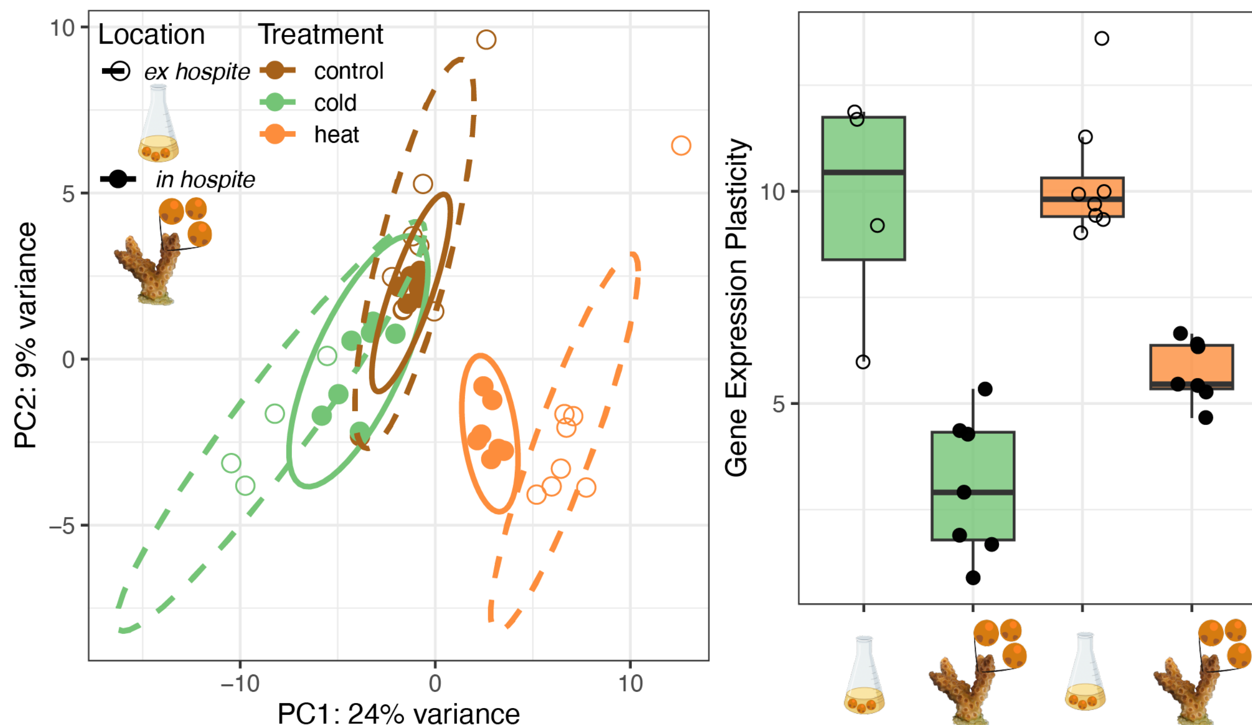
416 ***IV. Comparing responses of* in *and* ex hospite Breviolum psygmophilum**

417 When analyzing *in hospite* and *ex hospite B. psygmophilum* in the same DESeq2 model, a
418 significant effect of the aggregate factor of temperature treatment and symbiotic state was observed
419 (Figure 7A; *ADONIS* p=0.001). Temperature and symbiotic state also had significant effects on
420 gene expression plasticity (Figure 7B; p<0.0001), and *ex hospite B. psygmophilum* had
421 significantly higher gene expression plasticity compared to *in hospite B. psygmophilum*, both
422 under cold challenge (Tukey HSD p<0.0001) and heat challenge (Figure 7B; Tukey HSD
423 p=0.0001).



424

425 **Figure 7. *Ex hospite* symbionts respond more strongly to thermal challenges than *in hospite***
426 **symbionts. A.** Principal component analysis (PCA) of gene expression of *ex hospite* (open circles,
427 dashed lines) and *in hospite* (solid points and lines) symbionts under control, cold, and heat
428 temperatures. **B.** Gene expression plasticity of *ex hospite* and *in hospite* symbionts under cold
429 (green) and heat (orange) challenge. Gene expression plasticity was significantly greater in *ex*
430 *hospite* symbionts compared to *in hospite* symbionts under both cold challenge (Tukey HSD
431 p<0.0001) and heat challenge (Tukey HSD p=0.0001). Culture icons created with BioRender.com.
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436 **Discussion**

437 *Both aposymbiotic and symbiotic coral hosts exhibit classic environmental stress responses to*
438 *temperature challenges*

439 We leveraged genome-wide gene expression profiling of *in* and *ex hospite* facultative coral
440 hosts (*Oculina arbuscula*) and their algal symbionts (*Breviolum psygmophilum*) to disentangle the
441 independent responses of hosts and symbionts to divergent thermal challenges across symbiotic
442 states. In contrast to our prediction that symbiosis would alter the response of corals to thermal
443 challenge, we found that both heat and cold challenges elicited general ESRs ['type A', 26]
444 regardless of symbiotic state. Additionally, both symbiotic and aposymbiotic hosts exhibited
445 greater gene expression plasticity in response to cold challenge compared to heat, aligning with
446 previous work on the facultatively symbiotic coral *Astrangia poculata* when exposed to similar
447 temperature challenges [54]. Wuitchik et al. [54] found that aposymbiotic *A. poculata* in cold
448 challenge (6°C) exhibited five times more differentially expressed genes (DEGs) than in heat
449 (31°C), corroborating the higher gene expression plasticity and number of differentially expressed
450 orthologs (DEOs) observed in *O. arbuscula* under cold challenge. However, Wuitchik et al. [54]
451 found cold challenge elicited a more severe ESR response ['type A', 26]) than heat challenge
452 ['type B', 26], contrasting our findings that both thermal challenges elicited type A responses
453 across symbiotic states. This pattern suggests that, even though *O. arbuscula* exhibited higher gene
454 expression plasticity under cold challenge, corals in both temperature challenges were exhibiting
455 stress responses consistent with a tropical coral's ESR, highlighting the utility of *O. arbuscula* as
456 a calcifying model for symbiosis [36].

457 The type A response presented in Dixon et al. [26] is characterized by functional
458 enrichment of the coral ESR, including downregulation of cell division and upregulation of cell

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459 death, response to ROS, protein degradation, NF-κB signaling, immune response, and protein
460 folding. Specifically, type A datasets in tropical *Acropora* showcased upregulation of ROS and
461 protein folding [26]. This informed our hypothesis that temperature challenge would result in an
462 ESR-like response, akin to a type A response, in symbiotic *O. arbuscula* but not in aposymbiotic
463 individuals. Instead, we observed that both symbiotic and aposymbiotic *O. arbuscula* exhibited
464 type A responses under heat and cold challenge. This pattern could be the result of background
465 symbionts in aposymbiotic corals [as previously observed in aposymbiotic *Astrangia poculata*,
466 55] producing ROS and leading to a type A response. Alternatively, aposymbiotic corals may be
467 light-stressed as they lack shading from symbionts [*e.g.,* 56]. Additionally, the temperature
468 challenges applied here were relatively short, and it is possible that symbiotic and aposymbiotic
469 *O. arbuscula* would have exhibited differential responses if the challenges had been more extreme
470 in temperature or duration [57]. In general, facultatively symbiotic corals are understudied, and
471 future work should explore the responses of symbiotic and aposymbiotic corals under different
472 stressors (*i.e.,* light, nutrients) and for longer durations [as in 58].

473

474 *Evidence of host buffering in* O. arbuscula *holobionts*

475 We present three forms of evidence suggesting that *O. arbuscula* hosts are buffering their
476 algal symbionts under thermal extremes: 1. The coral host exhibited more differentially expressed
477 orthologs (DEOs) than its symbiont under cold challenge, 2. Stress-related genes were
478 differentially expressed in symbionts *ex hospite* but not *in hospite*, and 3. *Ex hospite* symbionts
479 exhibited higher gene expression plasticity in response to temperature challenges than *in hospite*
480 symbionts. Higher gene expression plasticity in coral hosts compared to symbionts in symbiosis
481 aligns with previous evidence suggesting that cnidarian hosts and their algal symbionts exhibit

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482 strong differences in the magnitude of gene expression responses under environmental challenges.
483 For example, Davies et al. [59] reported that the tropical coral *Siderastrea siderea* exposed to 95-
484 day temperature and ocean acidification challenges resulted in hosts consistently exhibiting greater
485 differential expression of highly conserved genes compared to their symbiont *Cladocopium*
486 *goreaui.* Barshis et al. [27] also found no changes in gene expression in either heat-susceptible
487 *Cladocopium* (type C3K) or heat-tolerant *Durusdinium* (type D2) in symbiosis with *Acropora*
488 *hyacinthus* following three days of high temperature exposure, which contrasted strong gene
489 expression responses in the host [60]. Corroborating these patterns, Leggat et al. [29] observed
490 that algae (*Cladocopium* C3) exhibited little change in expression of six stress and metabolic genes
491 compared to their hosts (*Acropora aspera*).

492 Symbiosis itself has also been observed to alter gene expression patterns and physiology
493 in Symbiodiniaceae algae. Here, we observed differential regulation of stress-related GO terms
494 under cold challenge *ex hospite*, and genes within those terms included up-regulation of a heat
495 shock protein (heat shock protein STI1) and a ubiquitin-related gene (RING-type E3 ubiquitin-
496 protein ligase PPIL2). These genes are both classic ESR genes in tropical corals [26] and their
497 differential regulation *ex hospite* highlights the potential benefits of a symbiotic lifestyle for
498 Symbiodiniaceae. Symbiosis mitigating Symbiodiniaceae stress responses have been previously
499 shown. For example, gene expression of *ex hospite Durusdinium trenchii* maintained at 28°C
500 exhibited enrichment of the GO term “response to temperature stimulus” relative to *in hospite D.*
501 *trenchii* in *Exaiptasia pallida,* which authors attributed to the protective microenvironment of the
502 symbiosome [61]. Additionally, Maor-Landaw et al. [62] compared gene expression of *Breviolum*
503 *minutum* in culture to *B. minutum* freshly isolated from *Exaiptasia diaphana* and observed down-
504 regulation of genes indicative of the protected and stress-reduced environment of the symbiosome.

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505 Specifically, pentatricopeptide repeats (PPR), which have been previously associated with
506 Symbiodiniaceae RNA processing in response to environmental stress and were included in the
507 repertoire of "stress responsive genes" in *Fugacium kawautii* [63], were down-regulated in freshly
508 isolated *B. minutum* [62]. These findings support our third piece of evidence for host buffering.
509 Together, these data suggest that *in hospite* symbionts respond less at the level of gene expression
510 to cope with temperature challenges compared to *ex hospite* symbionts, providing further evidence
511 that cnidarian hosts exert control over the symbiont's micro-environment under environmental
512 stress.
513
514 *Cold challenge elicited negative effects on photosynthesis of* ex hospite *and* in hospite *Breviolum*
515 *psygmophilum*
516 Although responses of *B. psygmophilum in hospite* were muted (*i.e.,* fewer DEGs and
517 DEOs) under temperature challenges compared to responses *ex hospite,* negative effects on
518 photosynthesis were observed at the level of phenotype (*in hospite* Fv/Fm) and gene expression
519 (both *in hospite* and *ex hospite*), particularly under cold challenge. *Ex hospite B. psygmophilum*
520 exhibited differential expression of photosynthesis and stress genes, including down-regulation of
521 light harvesting complex (LHC) genes under cold challenge. This aligns with previous work
522 investigating how symbiosis affects Symbiodiniaceae photosynthesis. For example, Bellantuono
523 et al. [61] found that photosynthetic processes were modified in *D. trenchii* living *in hospite*
524 compared to *ex hospite.* Specifically, GO terms related to photosynthesis (*i.e.*, photosynthesis,
525 photosystem II repair, and light harvesting) were enriched *in hospite* compared to *ex hospite*, which
526 may result from host carbon concentrating mechanisms increasing the availability of CO_2 *in*
527 *hospite*. In addition, we observed reduced Fv/Fm of *in hospite B. psygmophilum*, aligning with

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528 previous work demonstrating reduced Fv/Fm in cultured *B. psygmophilum* exposed to simulated
529 seasonal temperature declines (cooled from 26°C to 10°C and for two weeks before returning to
530 26°C) [64]. In that study, *B. psygmophilum* Fv/Fm recovered to pre-challenge values once
531 temperatures were returned to baseline, while other Symbiodiniaceae species that typically
532 associate with tropical corals failed to regain Fv/Fm following cold challenge [64]. This difference
533 was attributed to *B. psygmophilum's* symbiosis with corals in temperate/subtropical areas where
534 they experience larger annual temperature variation, aligning with a recent report of its wide
535 thermal breadth (16.15°C) compared to six other Symbiodiniaceae isolates [65]. Therefore, Fv/Fm
536 declines coupled with down-regulation of photosynthesis genes could represent *B.*
537 *psygmophilum*'s seasonal response to low temperatures, and if the cultures were returned to control
538 conditions, they may have recovered.

539 While we only observed strong phenotypic and gene expression responses of *B.*
540 *psygmophilum* under cold challenge and not heat challenge, these links between photosynthetic
541 disruption and transcriptional regulation of photosynthetic machinery align closely with previous
542 work on Symbiodiniaceae under heat stress. Previous work has highlighted that heat stress can
543 inhibit the synthesis and resulting mRNA pool of an antenna protein of the light harvesting
544 complex (acpPC) in Symbiodiniaceae [66]. Additionally, temperature anomalies can alter
545 thylakoid membrane fluidity, decoupling light harvesting and photochemistry, which suppresses
546 ATP synthesis and results in increased reactive oxygen species (ROS) in Symbiodiniaceae [67].
547 LHCs are well-known for transferring absorbed light energy to the photosynthetic reaction center,
548 but they also play an important role in photoprotection and have been linked to thermal sensitivity
549 in Symbiodiniaceae [66]. Decreasing the number of peripheral LHCs may serve as a
550 photoprotective mechanism under heat stress, as it ultimately decreases the light reaching

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551 photosynthetic reaction centers and reduces the risk of damage to D1 reaction center proteins [68].
552 It is possible that LHC down-regulation may represent a photoprotective mechanism in *B.*
553 *psygmophilum* under any thermal stress. While Symbiodiniaceae gene expression in response to
554 cold challenge remains largely unexplored, evidence suggests that cold challenge induces similar
555 photophysiology responses as heat challenge in Symbiodiniaceae [*e.g.,* 64, 69–72]. It is therefore
556 possible that the heat challenge explored here was not high enough or long enough to elicit a
557 similarly negative response as cold challenge. Indeed, Fv/Fm of *in hospite B. psygmophilum* under
558 heat challenge was declining, but remained significantly higher than in cold challenge. This pattern
559 aligns with Roth et al. [72], who found that cold challenge was more immediately harmful for
560 *Acropora yongei* symbiosis, but heat stress was more harmful in the long term. Although *ex hospite*
561 *B. psygmophilum* Fv/Fm was not quantified here, differential regulation of photosynthesis and
562 stress-related genes suggests an even stronger negative impact of cold stress on photosynthesis *ex*
563 *hospite.* Altogether, future work would benefit from longer and more extreme temperature
564 challenges to ensure that the entire thermal niche is investigated. Lastly, future studies quantifying
565 additional phenotypes in both *in* and *ex hospite B. psygmophilum* to determine if such convergent
566 responses to temperature challenge exist are warranted.
567

568 *Alternative hypotheses for "host buffering" and proposed future experiments*

569 Transcriptional regulation is only one molecular process involved in responding to thermal
570 stress. Mounting evidence suggests that a lack of transcriptional response under environmental
571 challenges could be the result of post-transcriptional and/or post-translational mechanisms in
572 Symbiodiniaceae. This includes evidence of microRNA (miRNA)-based gene regulatory
573 mechanisms in Symbiodiniaceae [28, 73], which aligns with the genomic evidence that

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574 dinoflagellates may be more capable of translational rather than transcriptional regulation [74].
575 Previous work in *Symbiodinium microadriaticum* highlighted that a lack of common transcription
576 factors and few DEGs could be attributed to small RNA (smRNA) post-transcriptional gene
577 regulatory mechanisms [28]. It has also been proposed that the lack of transcriptional regulation
578 in Symbiodiniaceae could be due to gene duplication as a mechanism to increase transcript and
579 protein levels of genes [75]. It is therefore possible that the lack of *in hospite* symbiont response
580 to thermal challenges found here is not evidence of 'host buffering', but instead that other post-
581 transcriptional or post-translational regulation occurs more commonly in Symbiodiniaceae *in*
582 *hospite*. Another unique aspect of Symbiodiniaceae genomes is *trans*-splicing of spliced leader
583 sequences, which converts polycistronic mRNAs (code for multiple proteins) into monocistronic
584 mRNAs (code for one protein) and potentially regulates gene expression [76, 77]. TagSeq cannot
585 account for splice variation [25], preventing us from considering these differences. Additionally,
586 lower depth of coverage of *in hospite B. psygmophilum* sequencing data captured here could have
587 limited our ability to detect transcriptional responses. Finally, comparing algae in culture to algae
588 in symbiosis inherently includes a confounding variable of nutritional status, as algae in culture
589 exist in nutrient replete conditions [78]. Below we highlight future studies and data needed to
590 further test the host buffering hypothesis.

591 Our work supports a scenario in which coral hosts modulate the environment of *in hospite*
592 Symbiodiniaceae algae to buffer their responses to temperature challenges; however, additional
593 experiments are needed to validate this hypothesis. First, replicating the temperature challenges
594 performed here but leveraging proteomic and gene expression profiling in parallel [*e.g.*, 79] will
595 be critical in establishing whether lack of gene expression of *in hospite* symbionts translates to a
596 lack of proteomic response under stress. Including nutrient controls, namely *ex hospite*

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597 Symbiodiniaceae in nutrient-depleted media that replicate the nutritional environment of the
598 symbiosome, are necessary to address the confounding variable of nutritional status. Additionally,
599 this proposed experiment should implement RNA extraction methods that prioritize obtaining and
600 sequencing equal amounts of genetic material from host and symbiont.

601 While understanding the response of subtropical corals to thermal extremes is valuable in
602 its own right, the facultative symbiosis, calcifying nature, and available genomic resources of *O.*
603 *arbuscula* make it a unique model for linking results to tropical coral responses as climate change
604 progresses [36]. If coral hosts are able to regulate the environments of their symbionts, and this
605 regulation in turn can serve to limit stress in the holobiont and ultimately reduce coral bleaching,
606 then this host buffering phenotype may allow for the identification of coral-algal pairings that will
607 be more resilient under future global change conditions.

608

609

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804

805 **Data Accessibility and Benefit-Sharing Section**

806 **Data Accessibility Statement**

807 Raw sequencing data will be deposited in the SRA upon publication (BioProject ID=XX). All
808 other data, code, and materials used in the analyses can be found on the Github repository
809 associated with this project (https://github.com/hannahaichelman/Oculina_Host_Sym_GE) and
810 additionally will be hosted on Zenodo upon publication (doi: X).

811

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816

817 **Author Contributions**

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