

1 Positive genetic associations among fitness traits support evolvability of
2 a reef-building coral under multiple stressors

3 Running title: Trait covariance in reef corals under stress

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23

24 **Abstract**

25

26 Climate change threatens organisms in a variety of interactive ways that requires simultaneous
27 adaptation of multiple traits. Predicting evolutionary responses requires an understanding of the
28 potential for synergistic interactions among stressors and the genetic variance and covariance
29 among fitness-related traits that may reinforce or constrain an adaptive response. Here we
30 investigate the capacity of *Acropora millepora*, a reef-building coral, to adapt to multiple
31 environmental stressors: rising sea surface temperature, ocean acidification, and increased
32 prevalence of infectious diseases. We measured growth rates (weight gain), coral color (a proxy
33 for Symbiodiniaceae density), and survival, in addition to nine physiological indicators of coral
34 and algal health in 40 coral genets exposed to each of these three stressors singly and combined.
35 Individual stressors resulted in predicted responses (*e.g.*, corals developed lesions after bacterial
36 challenge and bleached under thermal stress). However, corals did not suffer substantially more
37 when all three stressors were combined. Nor were tradeoffs observed between tolerances to
38 different stressors; instead, individuals performing well under one stressor also tended to perform
39 well under every other stressor. An analysis of genetic correlations between traits revealed
40 positive co-variances, suggesting that selection to multiple stressors will reinforce rather than
41 constrain the simultaneous evolution of traits related to holobiont health (*e.g.*, weight gain and
42 algal density). These findings support the potential for rapid coral adaptation under climate
43 change and emphasize the importance of accounting for corals' adaptive capacity when
44 predicting the future of coral reefs.

45

46

47 **Introduction**

48 Reef-building corals are experiencing unprecedented declines due to changing environmental
49 conditions, such as rising sea surface temperatures that lead to coral bleaching, and ocean
50 acidification that impairs calcification (Andersson & Gledhill, 2012; Hoegh-Guldberg et al.,
51 2007). Climate change has also indirectly led to increasingly prevalent coral diseases, which are
52 often attributed to the increased abundance and virulence of bacterial pathogens (Ben-Haim,
53 2003; Maynard et al., 2015; Pruzzo et al., 2010). In the face of these stressors, corals are left with
54 few options but to move, adapt, or die. A number of studies have documented corals' capacities
55 to expand their ranges to more suitable habitats (Makino et al., 2014; Yamano, Sugihara, &
56 Nomura, 2011). Models that simulate future coral cover under different climactic scenarios
57 increasingly include estimates of adaptive capacity, such as simulated directional genetic
58 selection (Logan, Dunne, Eakin, & Donner, 2014) or predictions of the spread and persistence of
59 alleles conferring thermal-tolerance (Bay, Rose, Logan, & Palumbi, 2017; Matz, Treml,
60 Aglyamova, & Bay, 2018). These studies primarily focus on a single environmental challenge
61 (thermal stress) and do not predict interactive effects among simultaneous stressors or account
62 for genetic associations between multiple tolerance traits.

63 Adaptation to rapidly changing conditions requires standing phenotypic variation upon
64 which selection can act, provided that this heterogeneity has a genetic basis (*i.e.*, that it is
65 heritable). Although a large and ever-growing number of studies examine mean responses of
66 coral species to individual effects of climate change (Marubini, Ferrier-Pages, & Cuif, 2003;
67 Okazaki et al., 2017), few have measured standing genetic variation and heritability of these
68 responses (Dixon et al., 2015; Kenkel, Setta, & Matz, 2015; van Oppen, Császár, Berkelmans,
69 Ralph, & Frankham, 2010; Vollmer & Kline, 2008; S Wang et al., 2009) and even fewer have
70 assessed variation in multiple stress tolerance phenotypes (Shaw, Carpenter, Lantz, & Edmunds,
71 2016). Corals exhibit remarkable variation in stress tolerance traits upon which selection could
72 theoretically act (Baums et al., 2013; Dixon et al., 2015; Wright et al., 2017). However,
73 univariate analyses assessing a single stress-response phenotype, such as mortality under
74 bacterial challenge or bleaching under thermal stress, fail to fully describe the genetic basis of
75 the phenotypes under selection. Selection is an inherently multivariate process that acts
76 simultaneously on sets of functionally related traits. Indeed, centuries of animal and plant
77 breeding have demonstrated that selection on one trait will often result in changes in another
78 correlated trait (Rauw, Kanis, Noordhuizen-Stassen, & Grommers, 1998; Zhao, Atlin, Bastiaans,

79 & Spiertz, 2006). Commercial demand for multiple-stress-tolerant crops has driven extensive
80 research on stressor combinations in plants (Pandey, Irulappan, Bagavathiannan, & Senthil-
81 Kumar, 2017). In these plants, combinations of abiotic stressors and pathogens result in either
82 resistance or susceptibility to disease, depending on the intensity or duration of stress (Pandey,
83 Ramegowda, & Senthil-Kumar, 2015). Positive associations between different stressors can be
84 attributed to shared pathways. For example, some biotic and abiotic stressors stimulate the same
85 defense-related endogenous signals (Mithöfer, Schulze, & Boland, 2004). Alternatively, stress
86 responses may compete for demands on energetic reserves, resulting in a negative association in
87 tolerances, or a trade-off (Sokolova, 2013).

88 The prospects for future reef-building corals are exceedingly pessimistic without rapid
89 adaptation to a number of simultaneous stressors. This capacity for adaptation is determined by
90 the answer to an outstanding question: does success under one type of stress come at a cost of
91 susceptibility to a co-occurring environmental challenge? To address this critical knowledge gap,
92 we quantified the capacity for *Acropora millepora*, a model representative of a keystone group of
93 marine organisms that are among the most vulnerable to climate change (Reusch, 2014), to adapt
94 to simultaneous stressors. Multiple coral colonies (n = 40, hereafter referred to as “genets”) were
95 split into replicate clonal fragments (n = 5 per treatment) that were exposed to elevated
96 temperature (30°C), increased $p\text{CO}_2$ (pH = 7.8, 700 ppm CO_2), bacterial challenge (10^6 CFU mL⁻¹
97 *Vibrio owensii*), a combination of these three stressors at the same levels, or a control condition
98 (27°C, pH = 8.0, 400 ppm CO_2 , no added *V. owensii*). We measured a comprehensive suite of
99 coral host and algal traits to assess each genet’s performance in each condition and constructed a
100 genetic variance–covariance matrix to identify potential genetic trade-offs or reinforcements
101 between phenotypes.

102

103 **Methods and Materials**

104 *Study organism and aquarium conditions*

105 Forty-one colonies of *Acropora millepora* were sampled between October – December 2014
106 from Davies Reef lagoon (78 km offshore; 18°50’11’’S, 147°38’41’’E), Rib Reef (56 km
107 offshore; 18°28’55’’S, 146°52’15’’E), Pandora Reef (16 km offshore; 18°48’44’’S,
108 146°25’59’’E), and Esk Island (24 km offshore; 18°46’04’’S, 146°30’57’’E). These colonies
109 were transferred to holding tanks at the National Sea Simulator system at the Australian Institute
110 of Marine Science (Townsville, Queensland, Australia). After approximately two weeks

111 acclimatization, each colony was fragmented into 25 replicate fragments (“nubbins”), which
112 were mounted on aragonite plugs and placed on replicate trays. Trays were maintained in six
113 indoor holding tanks which were supplied with 0.2 μM filtered seawater (FSW) at 27°C. Three
114 lights (AI Aqua Illumination, USA) were suspended above each tank providing an average
115 underwater light intensity of 180 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ on a 10-/14-hour light–dark cycle. Corals
116 were fed freshly hatched *Artemia nauplii* twice daily and cleaned three times a week to prevent
117 algal growth. Coral nubbins were acclimated to these conditions for 3–5 months, depending on
118 the date of collection. Unique genets were later confirmed via 2b-RAD genotyping (Shi Wang,
119 Meyer, McKay, & Matz, 2012). The final total genets for each sampling location are as follows:
120 Davies (n = 10), Rib (n = 10), Pandora (n = 14), and Esk (n = 6). Algal symbiont types were
121 investigated by ITS-2 sequencing for eight of the coral colonies (Howe et al., submitted).

122

123 *Experimental treatments and sample preparation*

124 On 2 March 2015, coral nubbins (25 per genet) were placed into 25 50 L treatment tanks fitted
125 with 3.5-W Turbelle nanostream 6015 pumps (Tunze, Germany) with flow through seawater at
126 $\sim 25 \text{ L hour}^{-1}$ at the same temperature and light conditions as in the previous holding tanks. Initial
127 weights for each nubbin were obtained following the method described by (Spencer Davies,
128 1989). Tanks (n = 5 per treatment) were allocated to the following treatments: elevated
129 temperature (30°C), increased $p\text{CO}_2$ (700 ppm, pH = 7.8), bacterial challenge (10^6 CFU mL^{-1}
130 *Vibrio owensii* DY05), a combined treatment (30°C, 700 ppm, 10^6 CFU mL^{-1} *V. owensii*), and
131 control (27°C, 400 ppm, pH = 8.0, no bacteria). This isolate of non-quarantined *V. owensii* had
132 been recently sampled during an infectious disease outbreak in cultured lobsters at the research
133 facility. Temperature and $p\text{CO}_2$ were gradually increased in their respective tanks over the course
134 of a week to 30°C and 400 ppm (pH = 8.0). The bacterial challenge was conducted in separate
135 isolated tanks (no flow through) and consisted of a daily six-hour incubation. *Vibrio owensii* was
136 added at a final concentration of 10^6 CFU mL^{-1} to every bacterial challenge tank, including the
137 combined treatment (which was also maintained at 30°C and 700 ppm $p\text{CO}_2$ for these six hours).
138 Corals were then returned to their respective treatment tanks until the next day’s bacterial
139 challenge and the bacterial challenge tanks were treated with 20% bleach for at least 30 minutes.
140 Coral fragments were photographed daily to quantify bleaching via the Coral Health Chart
141 (Siebeck, Marshall, Klüter, & Hoegh-Guldberg, 2006) and lesion progression. Net oxygen
142 production and the change total alkalinity under light were measured for randomly selected

143 genets following methods described in (Strahl et al., 2015). Fragments exhibiting any tissue loss
144 or that were fully bleached and exhibiting algal growth were removed from treatment tanks,
145 buoyant weighed, and preserved in liquid nitrogen. The time of death (day post initial exposure)
146 was recorded at each instance. The experiment continued for 10 days, until approximately 21%
147 mortality was recorded over all treatments. Eleven days after the initial challenge, all surviving
148 corals were photographed, buoyant weighed, preserved in liquid nitrogen, and stored at -80°C
149 until sample processing.

150 Tissue was removed from coral skeletons using an air gun and 0.2 µM filtered seawater
151 and homogenized for 60 seconds using a Pro250 homogenizer (Perth Scientific Equipment,
152 Australia). A 1 mL aliquot of the tissue homogenate was centrifuged for 3 minutes at 1500 ×g at
153 4°C and the pellet was stored at -80°C for chlorophyll analyses. The remaining homogenate was
154 centrifuged for 3 minutes at 1500 ×g at 4°C to separate host and symbiont fractions. The
155 fractions were frozen in 96-well tissue culture plates and stored at -80°C. Coral skeletons were
156 rinsed with 10% bleach then dried at room temperature (~24°C). Skeletal surface area was
157 quantified using the single paraffin wax dipping method (Stimson & Kinzie, 1991) and skeletal
158 volume was determined by calculating water displacement in a graduated cylinder.

159

160 *Physiological trait assays*

161 Assays were conducted to detect cellular and metabolic activity changes within the
162 Symbiodiniaceae or coral host tissue in response to the treatment. All standards and samples
163 were loaded as duplicates, and absorbance was recorded with a Cytation 3 multi-mode
164 microplate reader (BioTek, Winooski, USA) and analyzed using Gen5 software (BioTek,
165 Winooski, USA).

166 To quantify chlorophyll concentrations, tissue homogenate algal pellets were resuspended
167 in 1 mL chilled 90% acetone. The homogenate was sonicated on ice for 10 seconds at 40%
168 amplitude, left in the dark for 20 minutes, and centrifuged for 5 minutes at 10,000 ×g at 4°C. A
169 200 µL aliquot of sample extract was loaded to a 96-well plate, and absorbance was recorded at
170 630 and 663 nm. Chlorophyll a and c2 concentrations were calculated with the equations in
171 Jeffrey & Haxo (1968) and were normalized to surface area:

$$172 \text{ Chlorophyll a } (\mu\text{g ml}^{-1}) = 13.31 \times A_{663} - 0.27 \times A_{630}$$

$$173 \text{ Chlorophyll c2 } (\mu\text{g ml}^{-1}) = 51.72 \times A_{630} - 8.37 \times A_{663}$$

174

175 A commercial colorimetric protein assay kit (DCTM Protein Assay Kit, Bio-Rad,
176 Hercules, USA) was used to quantify total protein content of the coral host tissue. A 100 μL
177 aliquot of Symbiodiniaceae-free coral tissue sample was digested using 100 μL sodium
178 hydroxide in a 96-well plate for 1 hour at 90°C. The plate was centrifuged for 3 minutes at 1500
179 $\times g$. Following manufacturer's instructions, 5 μL digested tissue was mixed with 25 μL alkaline
180 copper tartrate solution and 200 μL dilute Folin reagent in a fresh 96-well plate. Absorbance at
181 750 nm was recorded after a 15-minute incubation. Sample protein concentrations were
182 calculated using a standard curve of bovine serum albumin ranging from 0 and 1000 $\mu\text{g mL}^{-1}$.

183 Carbohydrate content of the Symbiodiniaceae-free coral tissue was estimated following
184 the method by Masuko et al. (2005) that measures monosaccharides, including glucose, which is
185 the major photosynthate translocated between symbionts and host corals (Burriesci, Raab, &
186 Pringle, 2012). A 50 μL aliquot of coral tissue was mixed with 150 μL concentrated sulfuric
187 acid and 30 μL 5% phenol in a 96-well plate for 5 minutes at 90°C. After another 5-minute
188 incubation at room temperature, absorbance at 485 nm was recorded. The total carbohydrate
189 concentrations of samples were calculated using a standard curve of D-glucose solutions ranging
190 from 0 to 2000 $\mu\text{g mL}^{-1}$.

191 To analyze non-fluorescent chromoprotein content, a 30 μL aliquot of coral tissue was
192 loaded to a black/clear 384-well plate and absorbance was recorded at 588 nm. Mean absorbance
193 was standardized to sample protein content.

194 The activity of catalase (CA), a reactive oxygen species scavenging enzyme (Lesser,
195 2006), was measured by estimating the change in hydrogen peroxide (H_2O_2) substrate
196 concentration. A 20 μL aliquot of coral tissue was mixed with 30 μL 50 mM phosphate-buffered
197 saline solution (PBS; pH 7.0) and 50 μL 50 mM H_2O_2 in a 96-well plate. CA was calculated as
198 the change in absorbance at 240 nm every 30 seconds over the linear portion of the reaction
199 curve for 15 minutes and was standardized to sample protein content:

$$200 \quad \text{CA (mg protein}^{-1}\text{)} = \text{Initial } A_{240} - \text{Final } A_{240}$$

201
202 The change in coral color was estimated using photographs taken during the experiment
203 with a Nikon D300 digital camera. Brightness values were measured over the entire front and
204 back sections of each nubbin using image analysis software (ImageJ, NIH). Corals become
205 brighter (whiter) when they lose darkly colored algal symbionts, so changes in brightness reflect
206 changes in Symbiodiniaceae densities (Beer, Loya, Winters, Holzman, & Blekman, 2009). A

207 standard curve of brightness values was constructed using standard coral color cards that were
208 present in each image. Brightness values were standardized to color cards to normalize for
209 differences across photo sessions.

210

211 *Statistical analyses*

212 The time of death was noted for each individual nubbin. Survival was modeled as time of death ~
213 treat + reef + (1|tank) using *coxme* package in R 3.3.1 (R Core Team, 2016; Therneau, 2012),
214 where treatment was specified as the presence or absence of elevated heat, bacteria, or increased
215 $p\text{CO}_2$ (*i.e.*, bac = 1 for “bacteria” and “all” treatments; heat = 1 for “heat” and “all” treatments).
216 All subsequent analyses were performed on lesion-free (alive) corals. Data were log-transformed
217 using powers determined by Box-Cox transformations performed using the *powerTransform*
218 function in the R package *car* (Fox & Weisberg, 2001). Linear mixed-effects models
219 implemented using the R package *nlme* (Pinheiro, Bates, DebRoy, & Sarkar, 2017) tested the
220 effects of treatments, treatment interactions, and reef-of-origin on trait values. The *stepAIC*
221 function in the R package *MASS* (Venables & Ripley, 2002) determined which terms to include
222 in the best-fit model. Principal components analysis on mean-centered and variance-scaled
223 values was performed using the *prcomp* function in base R. Pearson correlations between trait
224 values were calculated using the *cor* function in base R, and correlation heatmaps were
225 constructed using the *corrplot* function. The genetic variance–covariance matrix was constructed
226 using the R package *MCMCglmm* (Hadfield, 2010). Trait data were mean-centered and variance
227 scaled. The multivariate model was fit for four traits (growth, color, chlorophyll c2, and
228 carbohydrate) with treatment as a fixed effect and genet as a random effect, using the *idh*
229 variance structure. The model was run for 20000 iterations after a 5000 iteration burn-in, storing
230 the Markov chain after 20 iteration intervals. Partial regression coefficients for each trait on
231 binomial survival outcome were modeled using a categorical *MCMCglmm* model with genet as
232 a random effect. The selection gradient was composed of these partial regression coefficients,
233 scaled to unit variance. Predicted changes in trait values ($\Delta\bar{z}$) were calculated using the
234 multivariate breeder’s equation (Lande & Arnold, 1983):

$$235 \quad \Delta\bar{z} = \mathbf{G}\beta$$

236 \mathbf{G} is the genetic variance–covariance matrix and β is the selection gradient.

237

238

239 **Results**

240 Mean responses to treatments

241 *Survival*

242 Corals in the control condition experienced the lowest mortality at 13.5%, followed by increased
243 temperature (21.5%), combined treatment (23.7%), bacteria challenge (26.4%), and elevated
244 $p\text{CO}_2$ (27.4%). Mortality rates were compared using the *coxme* package in R (R Core Team,
245 2016; Therneau, 2012). Treatment was specified as the presence or absence of each stressor. The
246 Cox proportional hazards model included each treatment, interactions among treatments, and
247 reef as fixed effects, with tank as a random effect. Bacteria challenge significantly increased
248 mortality rates (hazard ratio [HR] = 3.32, $p = 0.018$), while a weak interaction between bacteria
249 challenge and increased temperature slightly improved survival odds (HR = 0.17; $p = 0.09$).
250 Elevated $p\text{CO}_2$ ultimately caused the most mortality, but mortality rates in corals under this
251 condition were indistinguishable from control corals throughout the first week of the experiment
252 (Figure 1A). Colonies from Rib reef had the lowest mortality (16.5%). Colonies from Davies and
253 Esk reefs had higher mortality rates (HR = 0.54, $p = 0.007$ and HR = 0.58, $p = 0.01$, respectively)
254 than those from Rib (Supplementary Figure 1).

255

256 *Physiological Responses*

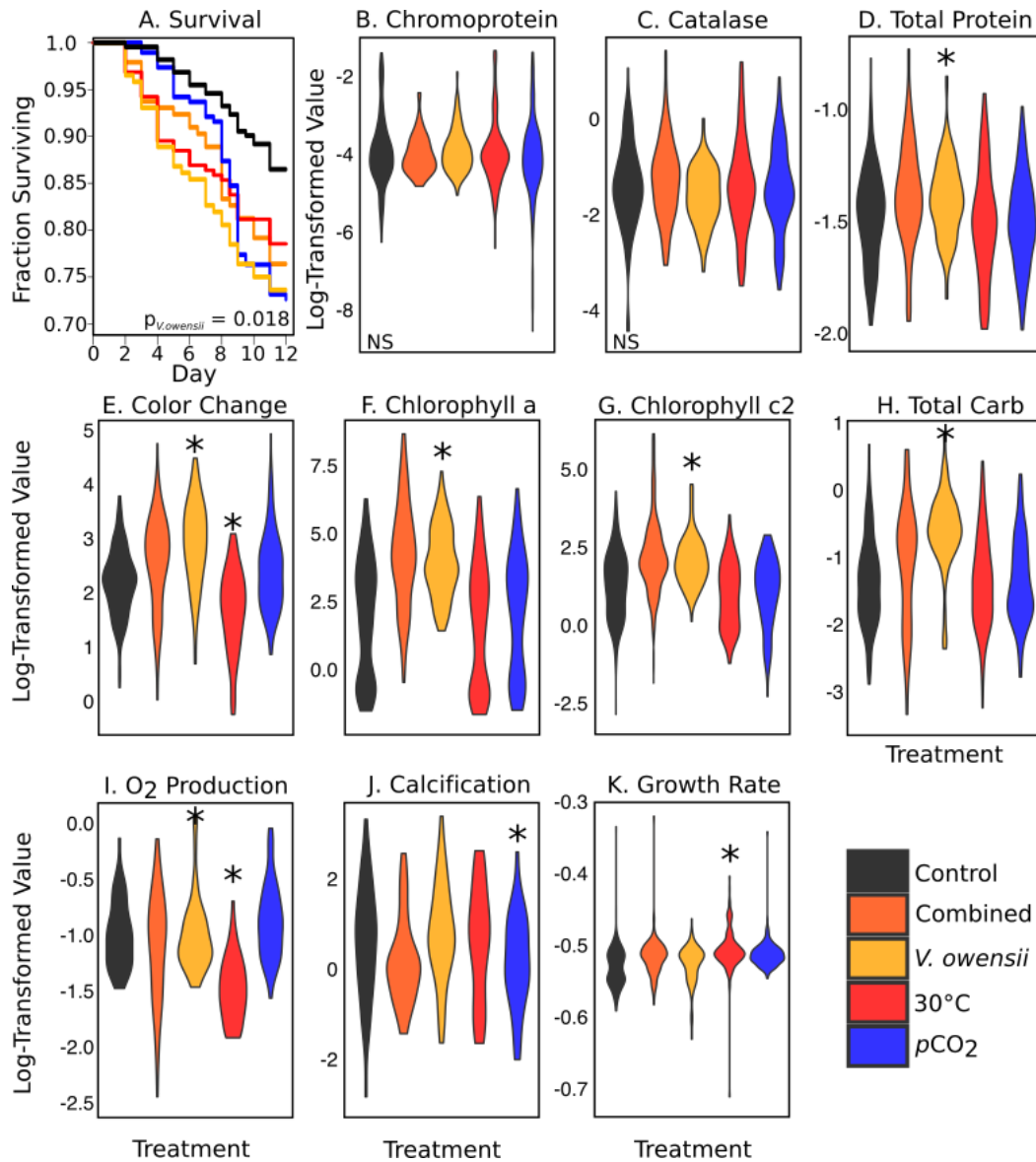
257 We measured the following algal and host-associated traits from surviving coral fragments: coral
258 color (an indicator of Symbiodiniaceae densities), algal chlorophyll a and chlorophyll c2 content,
259 total host carbohydrate, total host protein, host catalase activity, host chromoprotein content,
260 oxygen production (indicator of photosynthetic rate), instant calcification rate, and change in
261 buoyant weight (skeletal growth).

262 Chromoprotein content and catalase (Figure 1B–C) serve as proxies for coral innate
263 immune response. We found no significant effect of treatment on either of these measurements.
264 However, there was a weak interaction between the effects of elevated temperature and bacterial
265 treatment, which tended to increase catalase activity ($\beta = 0.33$, $p = 0.06$).

266 Bleaching was calculated as a log-transformed change in color in photographs
267 standardized to the Coral Health Chart (Siebeck et al., 2006). Bacteria treatment made corals
268 significantly darker ($\beta = 0.70$, $p < 0.001$), whereas corals became lighter (*i.e.*, bleached) in the
269 elevated temperature treatment ($\beta = -0.54$, $p < 0.001$; Figure 1E). These findings are
270 corroborated by chlorophyll measurements: bacterial treatment increased chlorophyll a ($\beta = 2.1$;

271 $p < 0.001$; Figure 1F) and c2 ($\beta = 1.0$, $p = 0.001$; Figure 1G) content in the algal fraction of the
272 coral tissue. Bacteria treatment increased the carbohydrate content in the coral host tissues ($\beta =$
273 0.73 , $p < 0.001$; Figure 1H). We also observed a weak negative interaction between elevated
274 temperature and bacterial challenge on carbohydrate content ($\beta = -0.54$, $p = 0.059$).

275 Photosynthetic and instant calcification rates were measured for a smaller subset of the
276 coral genets. As expected, the elevated temperatures reduced photosynthetic rates ($\beta = -0.51$, $p <$
277 0.001 , Figure 1I). Coincident with improved algal traits under bacterial challenge (Figure 1E–G),
278 the bacterial treatment rescued photosynthetic rates under elevated temperatures and there was a
279 positive interaction between these two stressors ($\beta = 0.40$, $p = 0.04$; Figure 1I). Only the elevated
280 $p\text{CO}_2$ treatment affected instant calcification rates, decreasing them on average ($\beta = -0.48$, $p =$
281 0.01 ; Figure 1J). Buoyant weights of each fragment were measured at the beginning of the
282 experiment and when each fragment was removed from the experiment. Corals in the elevated
283 temperature treatment experienced moderately increased growth rates ($\beta = 0.017$, $p = 0.04$,
284 Figure 1K). Buoyant weights were not significantly affected by any other treatment.



285

286 **Figure 1: Mean responses to treatment.** (A) Survival fraction over the duration of the

287 experiment. Colors correspond to treatment (see inset key). (B–K) Box-Cox power transformed

288 trait values separated by treatment (indicated by color in legend). NS = no significant effect of

289 treatment. Asterisks indicate a significant ($p < 0.05$) effect of the indicated treatment. (B)

290 Chromoprotein content ($A_{588} \cdot \mu\text{g protein}^{-1}$). (C) Catalase activity ($\Delta\text{H}_2\text{O}_2 \cdot \text{mg protein}^{-1} \cdot \text{min}^{-1}$).

291 (D) Total protein content ($\text{mg} \cdot \text{cm}^{-2}$). (E) Coral fragment color change (final–initial score).

292 Chlorophyll a (F) and c2 (G) content ($\mu\text{g} \cdot \text{cm}^{-2}$). (H) Total carbohydrate ($\text{mg} \cdot \text{cm}^{-2}$). (I) Oxygen

293 production ($\mu\text{g O}_2 \cdot \text{cm}^{-2} \cdot \text{min}^{-1}$). (J) Instant calcification rate ($\mu\text{mol CaCO}_3 \cdot \text{cm}^{-2} \cdot \text{min}^{-1}$). (K)

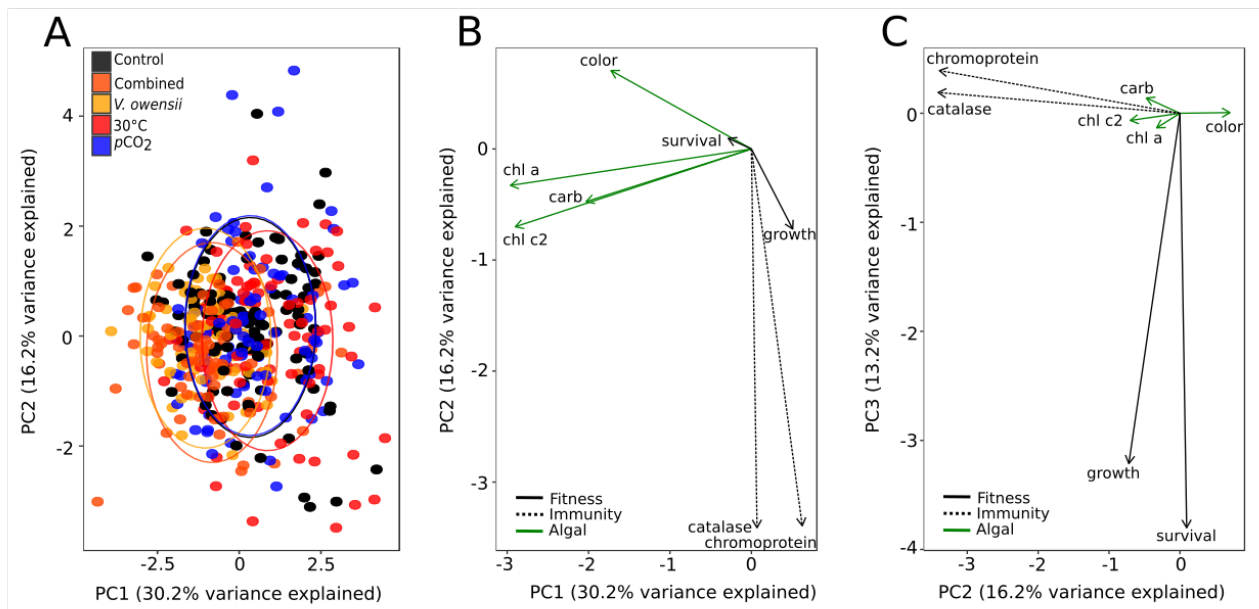
294 Buoyant weight growth rate ($\% \Delta \text{weight g} \cdot \text{day}^{-1}$).

295

296 Phenotypic space, correlations, and evolvability calculations

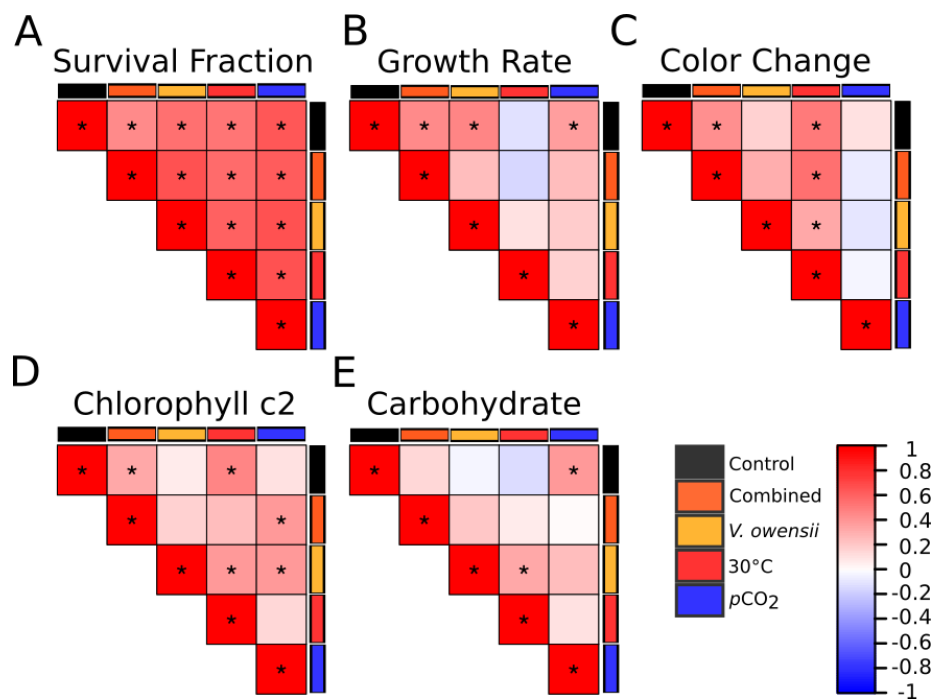
297 The lack of synergistic treatment effects on coral fitness proxies provides encouraging evidence
298 for an individual coral's capacity to resist multiple stressors. To investigate whether a population
299 of corals can adapt to multiple threats, we looked for potential tradeoffs by measuring
300 correlations between stressor effects across genets.

301 Principal components analysis explored patterns in phenotypic space of 429 individual
302 fragments (Figure 2A) with complete datasets for growth, color change, chlorophyll a,
303 chlorophyll c2, chromoprotein, catalase, and survival fraction (the proportion of fragments
304 surviving for the genet in each respective treatment). The first principal component explained
305 30.2% of the variation and separates samples by differential algal responses to treatment (Figure
306 2A–B). The second PC explained 16.2 % of the variation and separates samples by host immune
307 enzyme responses (chromoprotein and catalase activity). Holobiont fitness metrics (growth and
308 survival fraction) are projected in the third PC, which represents about 13% of the total variance
309 (Figure 2C).



310
311 **Figure 2: Principal components analysis.** (A) Principal components analysis based on
312 physiological trait data for 429 fragments. (B) Loadings for traits along the first two (B) and
313 second and third (C) principal components axes. Data point colors represent the treatment in
314 which the coral fragment was placed. Colors and line shapes of loadings identify traits related to
315 algal parameters (green), immune enzyme activity (dashed), or general coral fitness (black).
316

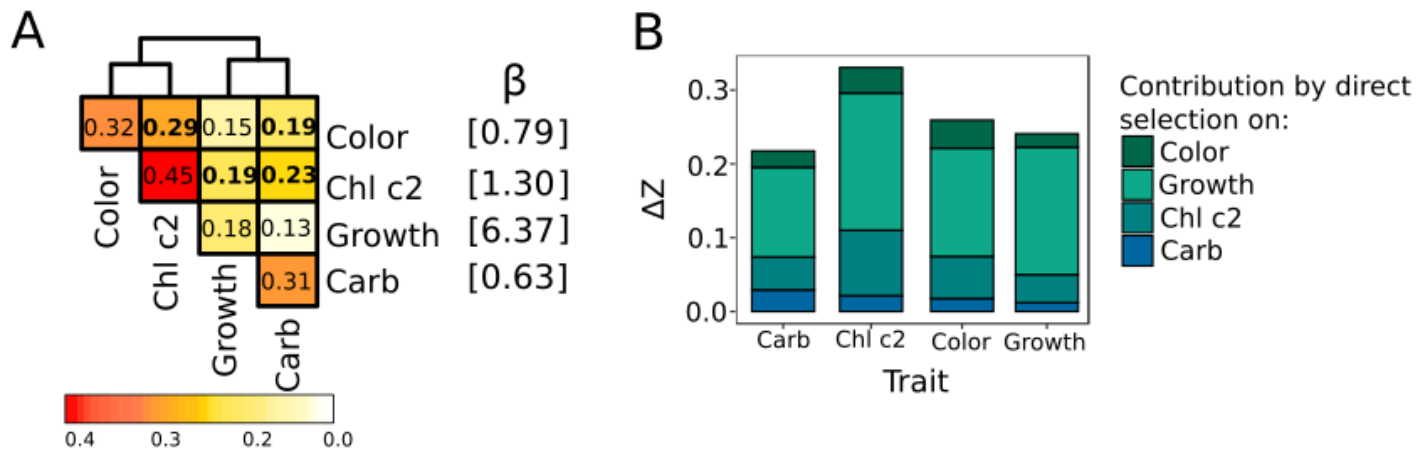
317 We calculated Pearson correlations by genet across treatments for five traits with the
318 most complete data and clearest link to fitness: survival fraction, growth rate, color change,
319 chlorophyll c2 content, and total host carbohydrate (Figure 3). The correlation heatmaps show
320 many positive and statistically significant correlations between trait pairs and very few negative
321 correlations, none of which were statistically significant (Figure 3). Survival fractions for each
322 genet are significantly positively correlated among treatments (Figure 3A). In other words,
323 individuals that show high fitness characteristics under one stressor also perform well under
324 other stressors.
325



326
327 **Figure 3:** Pearson correlation heatmaps based on scaled average (A) survival fraction, (B)
328 growth rate, (C) color change, (D) chlorophyll c2 content, and (E) carbohydrate content for 39
329 genets. Colored bars indicate the treatment measurement. Colors within the heatmap squares
330 represent the magnitude and direction of the Pearson correlation according to the key. Significant
331 ($p < 0.05$) correlations are indicated with asterisks.

332

333 Pairwise comparisons do not adequately capture the overall genetic covariance of traits in
 334 the population. To further explore the evolvability of these traits, we constructed a genetic
 335 variance–covariance matrix (**G** matrix) by selecting four traits that describe various aspects of
 336 host and symbiont fitness: color change, weight gain, chlorophyll c2 content, and carbohydrate
 337 content. All genetic correlations between these traits were positive (Figure 4A).



338
 339 **Figure 4:** (A) Left: Genetic variance–covariance matrix for four fitness traits across five
 340 treatments in 39 genets. The color in the inset key and number within each box quantifies trait
 341 variances (diagonal elements) and covariances between paired traits (off-diagonal elements).
 342 Bold font denotes significant associations. Right: Selection gradient (β), a vector of partial
 343 regression coefficients for each trait on survival. (B) Changes in trait means (ΔZ) per unit of
 344 selection for higher survival under multiple stressors. Each stacked bar is composed of the direct
 345 effect of selection on the trait and effects of selection on each of the genetically correlated traits.
 346

347 In multivariate trait space, the response of a trait to selection may deviate from the direction
 348 of selection due to influences of genetically associated traits. Depending on the shape of genetic
 349 variance–covariance matrix (**G** matrix) and selection strength on individual traits, fitness traits
 350 may or may not be able to evolve in concert. To investigate this issue in our coral, we calculated
 351 a selection gradient (a vector of partial regression coefficients standardized to unit length) by
 352 regressing the four traits against binomial survival (1 = fragment survived; 0 = fragment died).
 353 All selection coefficients were positive (Figure 4A), although only growth was significantly
 354 associated with survival ($p < 0.001$). We then applied the multivariate breeder's equation to
 355 estimate how trait values would change given our **G** matrix and selection for higher survival
 356 under moderate stressors (Figure 4B). Since all selection coefficients and covariances were

357 positive, the change in every trait over one generation was also positive (Figure 4B). This result
358 implies that fitness traits should be able to co-evolve together and moreover, reinforce each
359 other's evolution, *i.e.*, corals with high survival rates would also tend to produce offspring with
360 increased growth rates, carbohydrate content, Symbiodiniaceae densities, and chlorophyll
361 content under different stressors.

362 To demonstrate this reinforcement effect, we decomposed the total predicted change in each
363 trait (the total height of each bar in Figure 4B) into the contribution of selection directly on that
364 trait or on the three covarying traits. For example, a predicted increase in chlorophyll c2 content
365 is mostly driven by selection on growth rate, which is more strongly correlated with survival and
366 is genetically correlated with chlorophyll c2 content (Figure 4A). The model predicts less
367 improvement in color (a proxy of algal symbiont density) and host carbohydrate content because
368 these traits covary less with growth rate and are not themselves strongly associated with survival
369 (Figure 4A).

370

371 **Discussion**

372 Mean effects of single and combined stressors

373 Each experimental treatment largely resulted in the expected mean response. Elevated
374 temperatures reduced algal parameters (Symbiodiniaceae densities, chlorophyll a and c2
375 content), analogous to a natural coral bleaching event. Bacterial challenge resulted in the
376 development of lesions, but also caused an unexpected increase in algal traits. Although a meta-
377 analysis of coral stress responses predicted that most stressors act synergistically (Ban, Graham,
378 & Connolly, 2014) and other empirical studies have documented compromised coral immunity
379 and increased prevalence of coral disease concurrent with thermal stress (Palmer, 2018; Selig et
380 al., 2007), we did not observe detrimental interactive effects of the combined challenge. In fact,
381 the combination of treatments tended to improve holobiont health relative to a single stress. For
382 example, bacterial challenge alone caused more mortality than the combined treatment (Figure
383 1A). A tempting hypothesis to explain this phenomenon is that elevated temperature exposure
384 prior to pathogen exposure primed the coral's stress response system to mitigate oxidative stress
385 associated with launching an innate immune response (Lu, Wang, & Liu, 2015).

386 We also saw that coral bleaching was minimized in the combined treatment relative to
387 thermal stress alone (Figure 2A), possibly as a result of heterotrophic feeding (Bourne, Morrow,
388 & Webster, 2016): both coral host and algal symbiont had access to extra nutrients (bacterial
389 inoculations triply washed in sterile seawater) in the combined treatment. Further supporting this

390 hypothesis is the unexpected observation of increased algal traits in the bacteria-only treatment.
391 Reef-building corals feed on bacteria (Houlbrèque & Ferrier-Pagès, 2009) which could act as a
392 beneficial nutrient source to encourage algal productivity (Rädecker, Pogoreutz, Voolstra,
393 Wiedenmann, & Wild, 2015; Sawall, Al-Sofyani, Banguera-Hinestroza, & Voolstra, 2014).
394 Heterotrophic compensation has been investigated as a method by which corals withstand
395 extended bleaching events (Baumann, Grottoli, Hughes, & Matsui, 2014; Grottoli, Rodrigues, &
396 Palardy, 2006; Hughes & Grottoli, 2013), but the capacity for heterotrophic feeding to prevent
397 bleaching deserves more attention.

398

399 Best performing genets tolerate multiple stressors

400 Pairwise correlations of trait values in each treatment revealed mostly positive associations
401 across all genets. Importantly, survival rates were significantly positively correlated under most
402 treatments (Figure 2 and Figure S2), indicating the absence of tradeoffs with respect to an
403 individual's ability to withstand different disturbances. Individuals that could survive one
404 stressful condition tended to be able to manage other stressors as well. There were a few
405 exceptions involving Symbiodiniaceae: color under bacterial challenge was negatively correlated
406 with survival under bacterial challenge or under thermal stress, and also negatively correlated
407 with growth under control conditions and under bacterial challenge. Again, Symbiodiniaceae
408 density measurements in corals exposed to *Vibrio* in this experiment may reflect the ability of a
409 coral to improve algal traits due to bacteria presence as a result of increased heterotrophic
410 feeding.

411 Given the drastically different phenotypic responses to each stressor applied in this study
412 (e.g., symbiont expulsion vs. tissue loss), it is reasonable to expect to observe tradeoffs in a
413 coral's ability to manage each challenge. Presumably these unique stressors require the coral to
414 employ distinct molecular response mechanisms, which come at a metabolic cost. This study
415 cannot offer a mechanism to explain the positive associations in stress tolerances observed in
416 these corals, but the literature offers promising areas for further investigation. In corals, the
417 oxidative theory for bleaching under thermal stress posits that ROS accumulation damages cells
418 and triggers symbiont expulsion (Lesser, 1997). ROS are also produced in response to immune
419 challenges to exert antimicrobial activity and stimulate immune signaling pathways (Bogdan,
420 Röllinghoff, & Diefenbach, 2000). Innate immune activation limits pathogen growth but also
421 poses immunopathological risk to the host; thus, a maximal immune response is not always

422 optimal (Viney, Riley, & Buchanan, 2005) and antioxidant mitigation of self-harm is necessary
423 for survival (Knight, 2000). Elevated $p\text{CO}_2$ has also been shown to trigger oxidative stress in
424 corals (Davies, Marchetti, Ries, & Castillo, 2016) and oysters (Tomanek, Zuzow, Ivanina,
425 Beniash, & Sokolova, 2011). Given the critical role of managing oxidative stress in responses to
426 thermal stress, elevated $p\text{CO}_2$, and bacterial challenge, the robustness of a coral's antioxidant
427 defense system to prevent self-harm may underlie tolerance to all three of these stressors. A
428 recent study in rice identified an allele of the transcription factor Ideal Plant Architecture 1
429 (IPA1) that simultaneously confers improved growth and immune function by toggling between
430 phosphorylation states that drive expression in distinct subsets of gene targets (Wang et al.,
431 2018). Future studies should critically evaluate the nature of shared pathways in coral stress
432 responses.

433 Another consideration is the contribution of the algal symbiont towards holobiont health.
434 In this study, colonies primarily hosted *Cladocopium* symbionts (almost exclusively), but also
435 contained lower abundances of *Brevolium*, *Durusdinium*, and/or *Gerakladium* symbionts
436 (<0.1% of the total community) (Howe et al., submitted). Notably, one colony contained a larger
437 proportion of *Durusdinium* and was among the worst performing colonies in this experiment.
438 Though we do not observe a clear contribution of any one symbiont type on overall host health
439 in this study, as other studies have demonstrated (Rouzé, Lecellier, Saulnier, & Berteaux-
440 Lecellier, 2016; Silverstein, Cuning, & Baker, 2017), future studies should continue to
441 investigate how algal symbionts modify coral responses to environmental stressors.

442

443 Positive genetic associations of fitness traits suggest holobiont adaptability

444 Multivariate analyses describe how fitness traits may change under directional selection. The
445 variance–covariance structure estimated in this study indicates that our focal species possesses
446 the genetic heterogeneity and flexibility to respond to multiple selective pressures. The positive
447 genetic covariances between four traits associated with holobiont fitness (growth,
448 Symbiodiniaceae density, chlorophyll c2 content, and total carbohydrate content) argue for
449 reinforced evolution of all traits (Figure 4). Growth rate is most strongly associated with survival
450 under climate change stressors and therefore would evolve rapidly due to direct selection
451 pressure. Although directional selection is weaker for other traits (algal density, carbohydrate
452 content, and chlorophyll c2 content), genetic correlation with growth rate would result in their
453 increases as well. However, it is important to note that our experiment cannot disentangle genetic

454 associations that are due to host, symbiont, or their specific combination. Our conclusions
455 regarding the effect of selection on fitness traits would hold only if genetic interactions between
456 host and symbiont do not contribute much to fitness trait variation (*i.e.*, if most of the trait
457 variance is attributable to a simple sum of variances due to host and symbiont). More research on
458 study systems where holobiont genetic composition can be manipulated (*e.g.*, coral recruits) is
459 necessary to resolve this issue.

460

461 Considerations for future reefs

462 The management implications of these findings are two-fold. Firstly, adaptive processes should
463 not be ignored in ecological climate modeling. Dire estimates for future coral cover are often
464 derived from experiments in which a coral from today is placed under conditions predicted
465 decades into the future (Okazaki et al., 2017). *A. millepora* can reach reproductive maturity as
466 early as three-years after fertilization (Baria, dela Cruz, Villanueva, & Guest, 2012) and thus,
467 modeling strategies based on end-of-the-century climate scenarios ignore dozens of generations
468 of potential adaptive evolution. Our results suggest that this adaptation can proceed because coral
469 fitness traits tend to reinforce, rather than constrain, adaptation toward improved fitness under
470 multiple environmental challenges. Secondly, our results should be taken into account during
471 efforts to spread adaptive genetic variation by propagating, translocating, and breeding genets
472 that have survived a natural stress event, although latent effects of the stress event may impact
473 thermal tolerance into the future. Regardless of the method used to select broodstock for coral
474 reseeded, our results strongly suggest that colonies should be selected for restoration in a
475 manner that does not jeopardize corals' natural ability to adapt, for example through a narrowing
476 of the gene pool. In the absence of severe bottlenecks in genetic diversity, natural selection will
477 continue selecting for corals that thrive despite multiple harassments brought about by climate
478 change. However, this adaptation will only proceed if reproduction is maintained under
479 increasingly hostile conditions and until adaptive genetic variation starts running out (Matz et al.,
480 2018), and therefore our findings should not undermine the critical urgency to limit
481 anthropogenic climate change.

482

483

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490

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