High-frequency temperature variability mirrors fixed differences in thermal limits of the massive coral *Porites lobata* (Dana, 1846) (120 Character Limit)

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Summary Statement (15-30 Words):

Corals native to highly variable habitats demonstrate greater thermal tolerance than corals from less variable habitats after 36-days of acclimation to thermally stable or variable common garden treatments.

Abstract (250 words)

Spatial heterogeneity in environmental characteristics can drive adaptive differentiation when contrasting environments exert divergent selection pressures. This environmental and genetic heterogeneity can substantially influence population and community resilience to disturbance events. Here, we investigated corals from the highly variable back reef habitats of Ofu Island in American Samoa that thrive in thermal conditions known to elicit widespread bleaching and mortality elsewhere. To investigate the hypothesis that thermal variability is the driving force shaping previously observed differences in coral tolerance limits in Ofu, specimens of the common Indo-Pacific coral *Porites lobata* (Dana, 1846) from locations with differing levels of thermal variability were acclimated to low and high thermal variation in controlled common garden experimental aquaria. Overall, there was minimal effect of the acclimation exposure. Corals native to the site with the highest level of daily variability grew fastest, regardless of acclimation treatment. When exposed to lethal thermal stress, corals native to both variable sites contained elevated levels of heat shock proteins and maintained photosynthetic performance for 1-2 days longer than corals from the stable environment. Despite being separated by < 5 km, there was significant genetic differentiation among coral colonies (F_{ST} = 0.206, p < 0.0001; nuclear ribosomal DNA), while *Symbiodinium* phylotypes were all ITS2-type C15. Our study demonstrates consistent signatures of adaptation in growth and stress resistance in corals from naturally thermally variable habitats, emphasizing that existing genetic diversity of corals is an important asset in strategies to protect and manage coral reef ecosystems in the face of global change.

Introduction

Heterogeneous environments can drive adaptive diversification when contrasting environmental conditions exert strong divergent selection pressures and individual niches are not abundant enough to favor the evolution of overall plasticity (e.g., Dempster, 1955; Kawecki and Ebert, 2004; Levene, 1953; Ravigné et al., 2004). Local adaptation is expected to evolve in populations with limited connectivity, but if environmentally driven selection is strong enough, adaptive differentiation can still accumulate despite ongoing gene flow (Feder et al., 2012; Hoey and Pinsky, 2018). In the marine environment, reproduction via broadcast spawning and gamete mixing at the sea surface means dispersal potential (i.e., gene flow) among neighboring habitats can often be high, with larval neighborhoods sizes of many marine organisms estimated at > 10 km (e.g., Palumbi, 2004; Pinsky et al., 2017). Thus, for small-scale population differentiation to be driven by selection in the sea, certain genotypes must preferentially settle in optimal habitat-types, or sub-optimal settlers must have reduced fitness via strong post-settlement selection (Dempster, 1955; Levene, 1953; Ravigné et al., 2004).

Despite an established theoretical framework, the functional dynamics of adaptation and natural selection in most species remain unknown and these processes are particularly complex in reef building corals due to the symbiotic nature of the organism (Baird et al., 2007; Pandolfi et al., 2011). For example, adaptation of coral endosymbiotic algae, *Symbiodinium* spp., is known to confer varying degrees of thermal tolerance (Howells et al., 2012), and *Symbiodinium* diversity within individual host coral species can vary across thermal environments (D'angelo et al., 2015; Oliver and Palumbi, 2011). The specifics of how the genetic diversity of the coral host contributes to adaptation,

however, is relatively unknown (Baird et al., 2009; Baird et al., 2007; Barshis et al., 2010; Dixon et al., 2015; Hoegh-Guldberg et al., 2007; but see Lundgren et al., 2013; Matz et al., 2018). Adaptation to environmental change, including climate shifts, has been demonstrated in other organisms (Hancock et al., 2011; Hoffmann and Sgro, 2011; Sanford and Kelly, 2011), and recent evidence for corals suggests that adaptive differences in coral thermal tolerance are heritable (Dixon et al., 2015; Kenkel et al., 2015; Meyer et al., 2009); lending credence to the idea of evolutionary rescue (sensu Bell and Gonzalez, 2009) of corals from climate impacts.

The back reef pools on Ofu Island, American Samoa, represent a natural laboratory for investigations of adaptation and acclimatization of corals to contrasting environments due to their high diurnal variation and small-scale heterogeneity in environmental characteristics (e.g., temperature, pH, flow, dissolved O2; Barshis et al., 2010; Craig et al., 2001; Smith et al., 2007). For example, the highly variable (HV) back reef pool of Ofu undergoes daily temperature fluctuations of up to 5.6 °C and reaches daily extremes of > 35 °C (mean daily range 1.59 °C ± 0.42 SD). In contrast, the adjacent less variable forereef has seasonal maximum daily temperature fluctuations of 1.8 °C (mean daily range is 0.6 ± 0.2 SD; Craig et al., 2001; Smith et al., 2008; unpublished data). Corals from among these thermal habitats have phenotypic differences consistent with local adaptation of thermal performance, including increased prevalence of heat-tolerant clade D *Symbiodinium* (e.g., Acropora spp., Pocillopora spp., Pavona spp.; Cunning et al., 2015; Oliver and Palumbi, 2009), constitutive turning-on of genes involved in cellular stress defense (Barshis et al., 2013), fixed and plastic responses following field transplantation (Palumbi et al., 2014; Smith et al., 2007; Smith et al.,

106 2008), and small-scale (< 5 km) genetic differentiation of coral hosts (Barshis et al.,
107 2010; Bay and Palumbi, 2014).

108 In the massive coral *Porites lobata* (Dana, 1846), host genotypes were subdivided
109 across small spatial scales (< 5 km), while all *Symbiodinium* sequences matched ITS2
110 phylotype C15 (Barshis et al., 2010). The genetic differentiation of the host mirrored
111 fixed differences in the cellular stress response (Barshis et al., 2010) and growth
112 characteristics (Smith et al., 2007) suggestive of genetic adaptation to differences in the
113 amount of diurnal environmental variability between back-reef pools; however, upper
114 thermal limits were not tested in previous *P. lobata* studies. Here, we explore whether
115 high-frequency thermal variability (defined here as diurnal or shorter variation *sensu*
116 Safaie et al., 2018) is the environmental factor that differentiates growth and thermal
117 tolerance of *P. lobata* colonies from contrasting habitats on Ofu. We used a common-
118 garden laboratory acclimation experiment to test the hypothesis that corals from different
119 thermal habitats have unique responses to daily thermal variation.
120

121 **Materials and Methods**

122 *Study site, sample collection and transport*

123 Corals were collected during May 2007 from three sites on Ofu and Olosega
124 islands in the territory of American Samoa (14˚11’ S, 169˚40’ W). These islands host
125 diverse communities of ~85 shallow reef-building coral species, many of which are
126 consistently exposed to atypically high seawater temperatures (Craig et al., 2001) and
127 irradiances (Smith and Birkeland, 2007). Two back reef sites, a High Variability (HV)
128 and Medium Variability (MV) pool, and one low-variability forereef site (forereef) were

129 selected based on general differences in environmental characteristics (Craig et al., 2001;
130 Piniak and Brown, 2009; Smith et al., 2007; Smith and Birkeland, 2007; Smith et al.,
131 2008). Briefly, the HV pool is smaller, shallower, more thermally variable, and
132 experiences higher water flow than the MV pool, while the forereef is relatively more
133 stable than the HV and MV pools.

134 A pneumatic hole saw drill was used to remove n=30, 19 mm diameter cores from
135 the upward facing surfaces of each of n=5 source colonies in each site (total n=150
136 cores). Source colonies were of similar size (1-1.5 m diameter) and at least 5 m apart to
137 minimize potential for sampling the same clone (i.e., genet). Cores were affixed to nylon
138 bolts with Z-Spar, Splash Zone marine epoxy (Carboline Company, St Louis, MO) and
139 placed in the MV pool for a seven-day recovery period prior to shipping. Cores were
140 wrapped in plastic bags and wet paper towels with a minimal amount of seawater,
141 shipped in insulated coolers to the environmental simulation aquarium facility at San
142 Francisco State University's Estuary & Ocean Science Center in Tiburon, CA, and
143 immediately placed in experimental aquaria. All corals were collected and exported under
144 applicable permits from the National Park of American Samoa (NPSA-2006-SCI-0001)
145 and the Department of Marine and Wildlife Resources and imported under the authority
146 of the US Fish and Wildlife Service.
147
148 *Coral acclimation conditions*
149 The cores from each source colony were divided into two groups of 15 and held
150 in two separate experimental aquaria at a constant temperature of 28 ± 0.5 ºC and average
151 irradiance of 260 µmol quanta m^{-2} sec^{-1} (12hr light/dark cycle) for a 28 day recovery

period. Algal growth was removed from the nylon bolts daily during the recovery period using a toothbrush. Following the recovery period, corals were exposed to one of two different thermal conditions for 35 days: “variable” or “stable”. In the variable aquarium, temperatures fluctuated between 27 and 32 ºC during the afternoon of each day (mean temperature = 28.2 °C) while the other aquarium was set to remain stable at 28.5 ºC (Fig 1). The specific temperatures and amplitude of the treatments were chosen to reflect average water temperature and daily range extremes of natural summer temperature profiles of the forereef and back reef sites (Barshis et al., 2010; Craig et al., 2001; Smith et al., 2008). Due to equipment malfunction, there were two days of the acclimation when the stable aquarium and variable aquarium reached the same high temperature and three days where the stable aquarium reached temperatures below that of the variable aquarium (Fig S1). After the 35-day acclimation period, coral growth, photophysiology, and protein stress biomarkers were assessed.

We acknowledge that it would have been preferable to directly replicate the acclimation tanks and treatments, however it was both logistically and financially prohibitive to do so. Each system cost many thousands of dollars to achieve such manipulable temperature control and the acclimation period (28 + 36 days) was of such an extended duration that successive field collections and trials were unable to be performed. Additionally, both aquaria had a constant flow of water from the same 5,000 L recirculating water source, likely preventing any substantial differences in water chemistry between tanks. Each tank was continuously fed from this water source throughout the experiment at a flow rate of ~4 tank volumes per day. This same system has been used successfully in other published studies (e.g., Paganini et al., 2014).

Furthermore, we believe the concordance between this study's lab-based results and those of prior field experiments demonstrating strong fixed effects of origin in this species and minimal effects of acclimation treatment corroborate the assertion of little to no confounding influence of the single tank replicates on the results of the study.

Growth

New tissue growth was measured as the distance the growth margin had extended down the sides of each coral core since original sampling; measured linearly down the four cardinal sides of each core using calipers. The four measurements were averaged and analyzed using a single average value for each individual core.

Photophysiology

Chlorophyll *a* fluorescence of *Symbiodinium* sp. was measured using a pulse-amplitude modulated (PAM) fluorometer (DIVING-PAM, Walz GmbH, Germany). PAM fluorometry is a rapid, non-invasive technique which assesses the photosynthetic efficiency of photosystem II (PSII) reaction centers which can be used as a proxy for assessing the health of the symbiotic association (Fitt et al., 2001). DIVING-PAM parameters and measurements were made following a previous study (Piniak and Brown, 2009); initial fluorescence measurements (*F*) were between ~150–400 units and maximum fluorescence (F'*m*) was measured using a saturating light pulse (0.8 s, ~8000 umol quanta m^{-2} s^{-1}). Maximum quantum yield [(*Fm* - *Fo*)/*Fm*, or *Fv* / *Fm*] was measured for dark-adapted samples at the end of each experimental day 45 min after all lights had been turned off.

198

199 *Thermal challenge*

200 After the 35-day acclimation period, a "temperature ramp" was performed. This
201 consisted of placing five replicate cores from all source colonies and treatments in the
202 variable temperature aquarium (baseline 27, peak 32 ºC) for one day and subsequently
203 raising the baseline and peak temperatures by 2 °C every 24 hrs for four additional days
204 with a final temperature fluctuation of 35 - 40 ºC and total experimental duration of 120
205 hrs (Figs. S1, 3). PAM measurements were taken each day at 21:15 after 45 min of dark-
206 adaptation. A single core from each source colony and acclimation treatment was
207 sacrificed for protein analyses following PAM measurements each night, flash frozen in
208 liquid nitrogen, and stored at -80 °C until analyzed as described below.

209

210 *Protein biomarkers: Hsp70 and ubiquitin-conjugates*

211 Each coral core was flash frozen in liquid nitrogen and the tissue layer (up to 1 cm
212 below surface) was removed with bone cutting pliers and placed in a pre-frozen, 50 ml
213 stainless steel mixing jar (Glennmills, Clifton, NJ). The tissue and skeleton of each tissue
214 layer was crushed using a TissueLyser® (Qiagen, Valencia, CA) at 25 rpm for 5 s, and the
215 powdered samples were transferred to individual 2.5 ml cryovials and stored at -80 ºC
216 until further analyses.

217 Between 280-380 mg of crushed tissue was added to a prechilled 2 ml
218 microcentrifuge tube before adding 750 µL of chilled 50 mM phosphate buffer (K_2HPO_4
219 + KH_2PO_4; pH 7.8). Samples were vortexed and centrifuged at 2,000 x *g* for 5 min to
220 separate out host and algal endosymbiont (*Symbiodinium*) tissue fractions. The

supernatant (host fraction) was removed and placed on ice while the remaining pellet (skeletal debris and *Symbiodinium* fraction) was washed three times with fresh phosphate buffer before re-suspension in a final volume of 500 µL of phosphate buffer, sonicated for 5 min, and briefly centrifuged to remove skeletal debris. Aliquots were removed from both host and *Symbiodinium* fractions and stored at -80 ºC until further analyses.

Levels of heat shock protein 70 (hsp70) and ubiquitin-conjugated proteins were assessed via western blot for both host and *Symbiodinium* protein fractions as described previously (Barshis et al., 2010). All samples were assayed in triplicate and a single average concentration per sample was analyzed.

Host genetic analyses

To assess the potential influence of host genotype on physiological responses, the internal transcribed spacer region (ITS) of nuclear ribosomal DNA was amplified and sequenced from each individual source colony. Primer sequences, polymerase chain reaction conditions, and sequencing methods were performed as described previously (Barshis et al., 2010). Resulting sequences were inspected using Sequencher version 4.5 (Gene Codes Corp., Ann Arbor, MI) and aligned using Bio-edit (Hall, 2001) and by eye. Population genetic structure was estimated using an analysis of molecular variance (AMOVA) in Arlequin 2.0 (Schneider, 2000). A molecular phylogenetic network was constructed using the median-joining algorithm and maximum parsimony post-processing calculation in NETWORK ver 4.5.0.0 (Fluxus Technology Ltd., Polzin and Daneschmand, 2003).

Statistical analyses

Within a common garden framework, comparisons between transplant groups are designed to assess acclimation potential versus genetic/epigenetic control over the response variables. Comparisons between acclimation treatments examine environmental effects (i.e., phenotypic plasticity), while comparisons between source colony origins and individuals examine potential genetic or epigenetic influence on the response variables (DeWitt and Scheiner, 2004; Schluter, 2000; Smith et al., 2007).

Growth, photosynthetic efficiency, and western blot biomarker levels were assessed from field collections prior to shipping (field baseline), following the acclimation to the differing temperature profiles of the two experimental aquaria (acclimation baseline), and during the temperature ramp. For the field baseline and acclimation baseline tests, all variables were tested against the fixed factors of source colony origin and acclimation treatment in a two-way ANOVA (aov) with source colony individual (i.e., genotype) included as a random factor. Post-hoc analyses of significant main effects were computed using the lsmeans function in R v3.2.2 (R_Core_Team, 2015). Individual clonal replicates within time points were averaged prior to the ANOVA and plotting to avoid pseudoreplication. Assumptions of normality and homoscedasticity were tested via the shapiro.test and fligner.test functions in R v3.2.2 (R_Core_Team, 2015), respectively. For comparisons across time points, a repeated measures framework was used incorporating source colony identity (i.e., individual genotype) as a unit of repeated measure, allowing for a between-subjects test of origin and within-subjects tests of acclimation and day. Post-hoc analyses of multiple comparisons were computed using the lsmeans function in R v3.2.2 (R_Core_Team, 2015).

Results

Initial acclimation: temperature

The stable treatment had a slightly higher mean temperature and lower standard deviation than the variable treatment (28.54 ± 0.63 and 28.15 ± 1.20 ºC for the stable and variable tanks respectively; Figs. 1, S1). On average, the daily range of the variable tank was 11.84 times greater than the daily range of the stable tank. Of the 33 acclimation days for which temperature records were available, the variable tank had a daily range greater than 3 ºC on 24 days (73 %), while the daily range of the stable tank exceeded 3 ºC on only one day due to a heater malfunction (Fig. S1). Irradiance levels did not appear to differ between the two tanks with an average irradiance of 263 and 259 µmol quanta m^{-2} sec^{-1} for the stable and variable tank respectively.

Initial acclimation: growth

New tissue extension during the acclimation period was affected by source colony origin ($p = 0.0106$; Fig. 2, Table S1). HV source colonies grew fastest overall, with an average tissue extension of 10.85 mm ± 2.72 SD and 11.66 mm ± 2.23 SD in the stable and variable tanks respectively compared to MV source colonies (8.07 mm ± 2.62 SD and 7.60 mm ± 2.01 SD) and forereef source colonies (6.06 mm ± 2.00 SD and 8.01 mm ± 1.80 SD) for the stable and variable treatments respectively. There was no significant difference in growth between acclimation treatments for corals from any origin ($p = 0.0977$; Fig. 2, Table S1).

290 *Temperature ramp: photophysiology*

291 Measurements of maximum quantum yield (*Fv/Fm*) were significantly affected by
292 source colony origin, day, and an origin x day interaction ($p = 0.0036$, <0.0001, <0.0001
293 respectively; Fig. 3, Table S2). On days three and four of the temperature ramp, corals
294 from the thermally stable forereef had markedly lower effective quantum yield (Fv/Fm)
295 compared to back-reef corals regardless of acclimation treatment (Fig. 3, Table S2).
296 There was no effect of acclimation treatment throughout the temperature ramp. By the
297 end of the ramp (day 5), corals from all populations had little to no fluorescence signature
298 (Figs. 3, Table S2).
299
300 *Hsp70 and ubiquitin-conjugates:* Symbiodinium *fraction*
301 Hsp70 levels in the *Symbiodinium* fraction of field-collected samples were
302 different among origins ($p = 0.0259$, Table S3A), with forereef levels 3.5 times lower
303 than MV corals ($p = 0.0249$; Fig. 4, Table S3A). Ubiquitin-conjugate levels were also
304 different among origins ($p = 0.0352$; Fig. 5, Table S4A), with forereef levels 10.3 times
305 lower than HV corals ($p = 0.0439$; Fig. 5, Table S4A). Following acclimation, both
306 origin, acclimation, and origin * acclimation effects were observed ($p = 0.0398$, $p <$
307 0.0001, and $p = 0.0093$ respectively; Table S3B) in *Symbiodinium* hsp70 levels, with 3.3
308 times lower levels in the stable vs variable acclimation treatment ($p < 0.0001$; Table S3B)
309 and 10.2 times higher in the HV vs. MV or forereef variable acclimated corals ($p =$
310 0.0029, $p = 0.0032$ for MV and forereef contrasts respectively; Table S3B).
311 *Symbiodinium* ubiquitin-conjugates were not different amongst origins or acclimation
312 treatments following acclimation (Fig. 5, Table S4B). During the temperature ramp, a

mix of origin and acclimation effects were observed, with variable and contrasting responses across groups throughout the experiment (Figs. 4, 5, Tables S3C, S4C).

Hsp70 and ubiquitin-conjugates: Host fraction

Neither host hsp70 nor ubiquitin-conjugate protein levels were different in the field-collected samples (Figs. 6, 7, Tables S5A, S6A). Following acclimation, host hsp70 levels were similar amongst origins but 1.4 times higher on average in stable-acclimated corals ($p = 0.0029$; Fig 5, Table S5B), while ubiquitin-conjugates were 2.2 times lower on average in stable-acclimated corals ($p = 0.0012$; Fig 7, Table S6B). Host hsp70 levels were 2.9 and 2.5 times higher in HV vs. forereef corals on days 2 and 4 of the heat ramp respectively ($p = 0.0129$, $p = 0.0404$; Fig 6, Table S5C), and there was a significant origin x day interaction in host ubiquitin-conjugate levels ($p = 0.0080$), though no significant individual contrasts (Fig 7, Table S6C).

Host genetic analyses

A 368 base pair (bp) fragment of the internal transcribed spacer region (ITS) was amplified from 15 individuals (n=5 per origin) and subsequently cloned for a total of 77 cloned sequences (NCBI accession numbers xxxxxx – xxxxxx). These 77 sequences were comprised of 28 unique haplotypes: one shared between the HV and MV pools, one shared between the HV pool and forereef, and eight, nine, and nine unique to the HV, MV, and forereef sites respectively (Fig. 8). An analysis of molecular variance (AMOVA) revealed significant population subdivision among all three populations ($F_{ST} = 0.2061$, $p < 0.0001$). Pairwise F_{ST} comparisons were highest between the MV pool and

the other two populations (F_{ST} = 0.2483 and 0.2319, p < 0.0001 for HV and forereef respectively), while the HV pool and forereef showed lower but still significant subdivision (F_{ST} = 0.0509, p < 0.03). This was qualitatively evident in the phylogenetic network construction, which showed a more explicit separation between MV haplotypes versus HV and forereef haplotypes (Fig. 8).

Discussion

The influence of high-frequency variability on coral physiological tolerance limits

Here, we found that increasing the amount of high-frequency thermal variability (i.e., diurnal or shorter time-scales) for 36 days of acclimation had little to no effect on coral growth, photophysiology, thermal tolerance, or protein biomarker response (Figs. 2-7). The predominant signal in our data was that of source population origin, in that corals from back reef habitats (HV and MV) with consistent high-frequency variability in thermal and other environmental characteristics grew faster and had elevated thermal tolerance limits compared to corals from the more thermally stable forereef, regardless of acclimation treatment. Taken together, these results suggest real differences in thermal tolerance limits between back reef corals that have routinely been exposed to high-frequency environmental variability and forereef corals native to a less-variable environment. The disparity between the lack of acclimation effects and strong origin effects speaks to the potential for chronic exposure to high-frequency variability to exert differential selection pressure over very small spatial scales (< 5 km).

The most widely-used models of coral bleaching impacts and thermal tolerance differences rely on island-scale or regional level data (e.g., the 5 km pixel width of

359 NOAA Coral Reef Watch; Heron et al., 2016). However, our findings demonstrate
360 substantial differences in coral thermal tolerances across hundreds of meters to a few
361 kilometers. This follows previous results from Ofu corals in the highest variability back
362 reef habitats showing meter-scale differences in increased prevalence of heat-tolerant
363 clade D *Symbiodinium* (e.g., Acropora spp., Pocillopora spp., Pavona sp.; Cunning et al.,
364 2015; Oliver and Palumbi, 2009), constitutive turning-on of genes involved in cellular
365 stress defense (Barshis et al., 2013), acclimation gains in thermal tolerance following 12+
366 months of exposure to the HV pool (Palumbi et al., 2014), and small-scale (< 5 km)
367 genetic differentiation of coral hosts consistent with local adaptation (Barshis et al., 2010;
368 Bay and Palumbi, 2014).
369 A number of other studies across the globe have found similar small-scale
370 differences in physiological tolerance limits between corals from habitats with
371 contrasting amounts of short-term environmental variability. For example, *Porites*
372 *astreoides* corals from inshore environments with high-frequency thermal variability in
373 the Florida Keys bleached less during thermal stress (Kenkel et al., 2013), demonstrated
374 increased flexibility in gene expression modulation (Kenkel and Matz, 2016), and
375 increased growth rates that were heritable between generations (Kenkel et al., 2015)
376 compared to corals from lower variability offshore sites (~ 7 km away). Similarly, Pineda
377 et al. (2013) found decreased mortality in *Stylophora pistillata* on protected (shoreward)
378 vs. exposed (seaward) sides of reefs in the central Red Sea following a natural bleaching
379 event in 2010. Despite being separated by < 300 m, the protected sides of the reefs had
380 greater high-frequency thermal variability than exposed sites presumably due to
381 decreased wind-driven mixing (Pineda et al., 2013). Similar increased stress tolerance

382 was observed in inshore vs. offshore populations of *Montastrea annularis* in Belize
383 (Castillo and Helmuth, 2005), which was subsequently linked to long-term declines in
384 growth rates in offshore populations of this species over the past few decades (Castillo et
385 al., 2012). A recent large-scale meta-analysis of in-situ temperature records and bleaching
386 surveys from 5 reef regions around the globe found that greater amounts of high-
387 frequency temperature variability were correlated with reduced bleaching severity and
388 bleaching prevalence (Safaie et al., 2018), suggesting the trends observed in the various
389 single-site, single-species studies may be valid at the global and whole-reef community
390 scales.

391 There are a few notable exceptions to this pattern, however, with high variability
392 and low variability populations of *Acropora palmata* and *Porites astreoides* in the
393 Cayman Islands exhibiting a nearly identical response to increased heat and pCO_2
394 exposure (Camp et al., 2016), and exposure to greater high-frequency thermal variability
395 eliciting bleaching rather than resilience in *Pocillopora meandrina* and *Porites rus* in
396 Moorea, French Polynesia (Putnam and Edmunds, 2011). While the specific threshold
397 above which high-frequency variability increases resilience and/or the tipping points
398 between beneficial exposures versus chronic stress remain to be determined, our data
399 corroborate a growing body of evidence from multiple ocean basins, coral species,
400 genera, and habitat types suggesting a mostly beneficial role of high-frequency variability
401 in increasing coral resilience to thermal stress. Thus, it is conceivable that that differing
402 degrees of environmental variability may exert divergent selection pressures across these
403 small-scales and drive adaptive differentiation.
404

Is temperature variability really the most important driver?

Despite the overall effects of source colony origin, however, we found little evidence that acclimation to high-frequency temperature variability altered thermal tolerance limits in this species. In contrast to the lack of acclimation observed herein, multiple studies of *Acropora* spp. have demonstrated increased thermal tolerance following short-term (days to weeks) exposure to elevated temperatures. *Acropora nana* from a single back-reef population on Ofu exposed to variable temperatures (29-33 °C) bleached less and had a muted gene expression response compared to corals acclimated to 29 °C after just 7-11 days of exposure to the variable thermal regime (Bay and Palumbi, 2015). Similarly, *Acropora millepora* preconditioned to a 10-day mild stress (3 °C below the experimentally determined bleaching threshold) bleached less during subsequent heat stress than non-preconditioned corals (Bellantuono et al., 2012b) and exhibited a muted gene expression response as well (Bellantuono et al., 2012a), similar to that seen in variable acclimated *Acropora nana* (Bay and Palumbi, 2015) and HV *A. hyacinthus* (Barshis et al., 2013) in Ofu. Lastly, *Acropora aspera* preconditioned to a 48 hr pre-stress (31 °C) bleached less and maintained elevated photosynthetic efficiency during a subsequent 6-day heat stress (34 °C) compared to non-preconditioned corals (Middlebrook et al., 2008).

Most prior thermal-acclimation work in corals has focused on branching species in the genus *Acropora*, due to their ubiquity on the reef and known variation in thermal sensitivity (e.g., Loya et al., 2001; van Woesik et al., 2011). In contrast, massive coral species such as *Porites lobata*, are thought to be more thermally tolerant due to greater tissue thicknesses (Loya et al., 2001), increased mass transfer rates (Loya et al., 2001;

Nakamura and Van Woesik, 2001), and elevated metabolism (Gates and Edmunds, 1999) compared to most branching coral species (primarily *Acropora* and *Pocillopora*). Thus, as a species with a massive morphology, *Porites lobata* may have a greater innate temperature tolerance range to begin with, simply tolerating the environment when faced with new conditions versus the physiological acclimation seen in Acroporids. However, the consistent origin effects on growth, thermal tolerance, and cellular response suggests that the differing amounts of high-frequency variability in environmental characteristics between the back reef and forereef habitats do influence thermal tolerance limits in *P. lobata*, though perhaps over longer timescales than those under investigation.

Significant origin effects in common garden experiments are generally attributed to potential genotypic (i.e., adaptive) influence on the response variable (DeWitt and Scheiner, 2004; Sanford and Kelly, 2011; Schluter, 2000). However, long-term acclimatization, developmental plasticity, and/or epigenetics can similarly cause apparent origin effects. Corals are long-lived organisms, and based on the size (>1 m diameter) of the colonies used in this study, we roughly estimate the minimum age of the source colonies to be > 60 years old (based on > 500 mm radius and ~8 mm/year growth rate sensu Houck et al., 1977; Potts et al., 1985). Decadal-scale 'environmental memory' was recently observed in the massive coral *Coelastrea aspera*, with former west sides of colonies (experimentally turned to face east) that had been previously exposed to high-irradiance levels retaining four times the *Symbiodinium* during a natural bleaching event compared to un-manipulated east-facing/low-irradiance sides of colonies; despite 10 years of conditioning to the low-irradiance eastern orientation and identical *Symbiodinium* phylotypes (Brown et al., 2015). This certainly raises the possibility that

long-term conditioning to the high-frequency environmental variability of the Ofu back-reef could have long-lasting acclimation effects on *P. lobata* thermal tolerance limits that may not have been altered by our 36-day exposure.

However, we did observe acclimation effects on host and *Symbiodinium* protein biomarkers, particularly hsp70 (Figs. 4-7; Tables S3-S6). While differences across acclimation treatments were variable in magnitude and direction depending on the marker and day, the host hsp70 response demonstrated an interesting pattern relative to the fluorescence response. On the final day of the acclimation treatment, host hsp70 levels were lower in the variable vs. stable acclimated corals (Fig. 6, Table S5B), suggesting reduced need for chaperone activity following variable thermal exposure. However, the initial acclimation effect was supplanted by a strong origin effect with the greatest host hsp70 increase in HV corals on days 2 and 4 of the temperature ramp (Fig. 5, Table S5C), corresponding to the greater maintenance of photosynthetic efficiency in HV corals on days 3 and 4 (Fig. 3, Table S2). It is notable that a similarly rapid and higher induction of hsp70 was observed in back reef vs. forereef corals in our previous field study following transplantation (Fig. 4A from Barshis et al., 2010). Thus, it is tempting to speculate that the larger and more rapid hsp70 increases in HV corals during the temperature ramp might signify a higher capacity for maintenance of homeostasis under thermal stress. While not conclusive evidence for or against a mechanism of long-term acclimatization vs. local adaptation, the acclimation and origin effects in protein response observed herein and previously (Barshis et al., 2010) demonstrate the ability of these corals to respond to high-frequency thermal variability over short time-scales as well as potential evolutionary constraints on that ability related to population of origin.

474 Alternatively, the increased thermal tolerance limits of back-reef corals may have
475 been influenced very early on either via developmental canalization post-settlement in the
476 back-reef, parental effects, and/or epigenetic acclimatization. Both maternal effects and
477 signatures of differential epigenetic modification have been recently observed in
478 *Pocillopora damicornis*, with larvae from parents exposed to high temperature and pCO_2
479 exhibiting metabolic acclimation during subsequent stress compared to larvae from un-
480 exposed parents (Putnam and Gates, 2015) and increased levels of DNA methylation in
481 adults following high pCO_2 exposure (Putnam et al., 2016), suggesting that the observed
482 larval acclimation could have been caused by epigenetic modification. In Ofu, however,
483 larvae from back reef parents would have to settle/disperse back to the pool of origin for
484 epigenetic modification from parents to positively affect the response of the offspring. If
485 there was epigenetic modification of larvae from back reef parents but the larvae all
486 dispersed outside the HV and MV pools, then there would be no positive contribution to
487 the phenotype of the next generation.
488 While long-term acclimatization, parental effects, and/or epigenetic modification
489 could explain the thermal tolerance differences between our populations, none of these
490 processes would likely cause the genetic differentiation among populations seen here.
491 The significant genetic subdivision among all three populations suggests the presence of
492 a physical or environmental barrier to gene flow between the HV, MV, and forereef
493 populations, strong divergent selection pressures, or potential cryptic species/genepools
494 across the habitats in Ofu. Reduced connectivity across such a small spatial scale (~500
495 m -1 km between HV and MV, and ~5 km between HV/MV and the forereef) is unlikely
496 to be due to a physical barrier alone, as the water masses in the back reef appear to be

497 well-mixed during the daily high tide cycle and well-within the spatial range of
498 dispersing larvae. Bay and Palumbi (2014) observed a similar pattern of genetic
499 differentiation between HV and MV *Acropora hyacinthus*, though only at a subset of
500 outlier single nucleotide polymorphisms (SNPs) putatively responding to selection. They
501 posited a mechanism involving strong spatial balancing selection, wherein the contrasting
502 environmental pressures of each habitat exert high selection pressure on settling coral
503 larvae from a common gene pool (sensu a protected polymorphism via an environment x
504 genotype association; Bay and Palumbi, 2014; Levene, 1953; Ravigné et al., 2004;
505 Sanford and Kelly, 2011). van Oppen et al. (2018) found a similar pattern of
506 differentiation and outlier loci separating reef flat and reef slope *Pocillopora damicornis*
507 on Heron Island in Australia and posited a similar mechanism of environmentally driven
508 selection. The ITS locus sequenced herein is unlikely to be a direct target of selection,
509 though differentiation at this locus could be correlated with the specific gene targets
510 responding to selection.

511 *Conclusions*

512 The limited acclimation response, enhanced thermal tolerance capacity of back
513 reef corals, differential biomarker response, and significant genetic differentiation
514 observed in the present study are all consistent with a model of post-settlement selection
515 and adaptation of coral genotoypes to the greater amount of high-frequency
516 environmental variability in the MV and HV pools. However, the lack of acclimation in
517 thermal tolerance limits following 35 days of exposure to *temperature* variability alone,
518 calls into question whether differences in high-frequency temperature exposures among
519 habitats are the driving force behind these differences. Differences in the amount of high-

frequency temperature variability remains the common factor across the multiple experiments on Ofu (Barshis et al., 2013; Barshis et al., 2010; Bay and Palumbi, 2014; Craig et al., 2001; Cunning et al., 2015; Oliver and Palumbi, 2011; Palumbi et al., 2014; Smith et al., 2007), the Red Sea (Pineda et al., 2013), Florida Keys (Kenkel et al., 2013; Kenkel et al., 2015; Kenkel and Matz, 2016), meso-american barrier reef (Castillo and Helmuth, 2005; Castillo et al., 2012), Australia (van Oppen et al., 2018), and the variety of sites examined in Safaie et al. (2018). Future research should focus on assessing the potential influences of other environmental drivers on the observed differences in thermal limits, as well as the relative contributions of long-term acclimatization and/or developmental canalization. Additionally, the magnitude of F_{ST} differentiation observed herein makes it difficult to contextualize the scale of genetic differentiation across populations. Future in-depth genetic analysis of massive *Porites* populations from a variety of habitat types may provide a clearer picture of the potential for cryptic genotype x environment associations in this taxonomic group.

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Competing interests

The authors declare no competing or financial interests.

Data Accessibility:

All raw data, analyses, and scripts are available as an electronic notebook:

(https://github.com/BarshisLab/Poriteslobata-thermal-adaptation). DNA sequences have also been deposited in NCBI Genbank (accession #'s XXXXXXX).

Author Contributions

DJB, JHS, CB, RJT, and RDG conceived and designed the research. DJB and JHS conducted the research. CB, RJT, RDG, and JHS funded the research. DJB and JHS performed the analyses. DJB, JHS, CB, RJT, and RDG wrote the paper.

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Figure Legends

Figure 1. Daily mean (squares), minimum and maximum (dark circles) ± 95% confidence intervals of each acclimation aquarium during the 35-day acclimation period (left-hand axis) and boxplot of daily temperature range (right-hand axis) of each acclimation aquarium.

Figure 2. Linear tissue extension measurements taken after the 35-day acclimation period. Values are category means ± 1 SD. Statistical significance at $p < 0.05$ (*)is presented for comparison of source colony origins (O).

Figure 3. Pulse Amplitude Modulated fluorometry (PAM) measured maximum quantum yield (Fv/Fm) of corals during 5 days of the ramping temperature exposure. PAM measurements were taken at the end of the experimental day following ≥ 45 minutes of dark adaptation. The experimental temperature profile is shown as the solid black line and on the right-hand axis. Squares, triangles, and circles represent source colonies from the High Variability (HV) pool, Medium Variability (MV) pool, and forereef (FR) respectively and open and shaded symbols are for stable and variable acclimation treatments, respectively. Values are category means ± 1 SD. Statistical significance at $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***) is presented for overall comparisons of

source colony origin (O), acclimation treatment (A), day (D), and the various interactions (e.g., OxA) along the left hand y-axis, while within-day contrasts are presented along the x-axis.

Figure 4. *Symbiodinium* heat shock protein 70 levels across the entire sampling period: field baseline, post-acclimation, and days 1-5 of the temperature ramp. All values are relative to a single control extract. Values are category means ± 1 SD. Symbols and significance values are denoted as in Figure 3.

Figure 5. *Symbiodinium* ubiquitin-conjugate protein levels across the entire sampling period: field baseline, post-acclimation, and days 1-5 of the temperature ramp. All values are relative to a single control extract. Values are category means ± 1 SD. Symbols and significance values are denoted as in Figure 3.

Figure 6. Coral host heat shock protein 70 levels across the entire sampling period: field baseline, post-acclimation, and days 1-5 of the temperature ramp. All values are relative to a single control extract. Values are category means ± 1 SD. Symbols and significance values are denoted as in Figure 3.

Figure 7. Coral ubiquitin-conjugate protein levels across the entire sampling period: field baseline, post-acclimation, and days 1-5 of the temperature ramp. All values are relative to a single control extract. Values are category means ± 1 SD. Symbols and significance values are denoted as in Figure 3.

Figure 8. Maximum parsimony phylogenetic network reconstruction of ITS rDNA haplotypes drawn in NETWORK ver 4.5.0.0 (Fluxus Technology Ltd.; Polzin & Daneschmand 2003). Haplotypes shown in red, yellow, and blue are from HV, MV, and forereef populations respectively. Diameter of circles at each node is proportional to the number of individuals with identical sequences. Haplotypes that co-occur in the same individual are connected by colored curves. Mutations are shown in red on each branch with the number corresponding to the base pair position, hypothetical intermediate haplotypes are designated by black circles. NCBI accession numbers for all sequences used in this study are XXXXX-XXXXX.

Supplementary Figure Legends

Figure S1. Experimental tank temperatures measured every 15 min during the 36-day acclimation period. The variable tank is shown in red and the stable tank in blue.

Figure 1

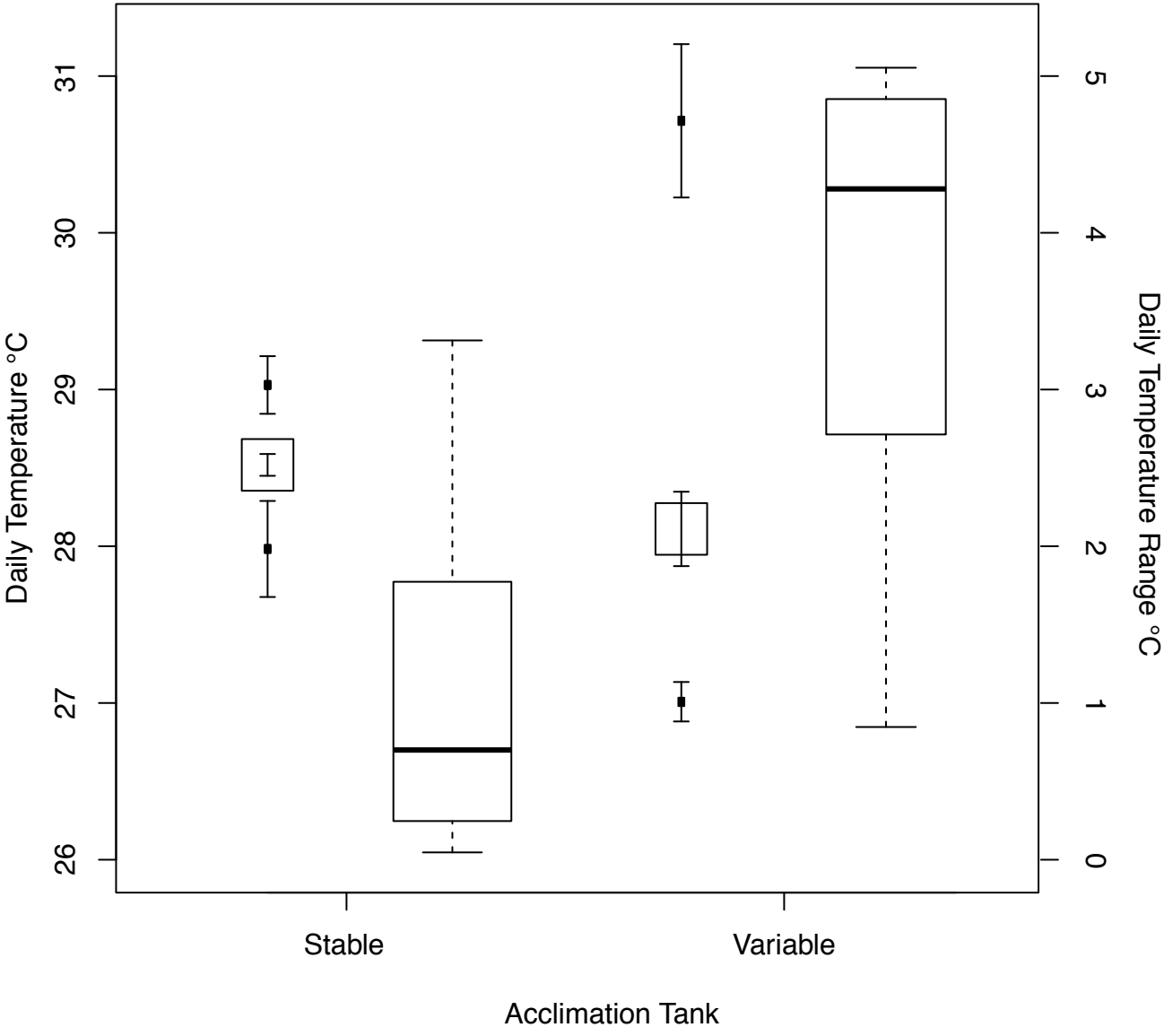
Daily Temperature °C
26
27
28
29
30
31
Daily Temperature Range °C
0
1
2
3
4
5
Stable
Variable
Acclimation Tank

Figure 2

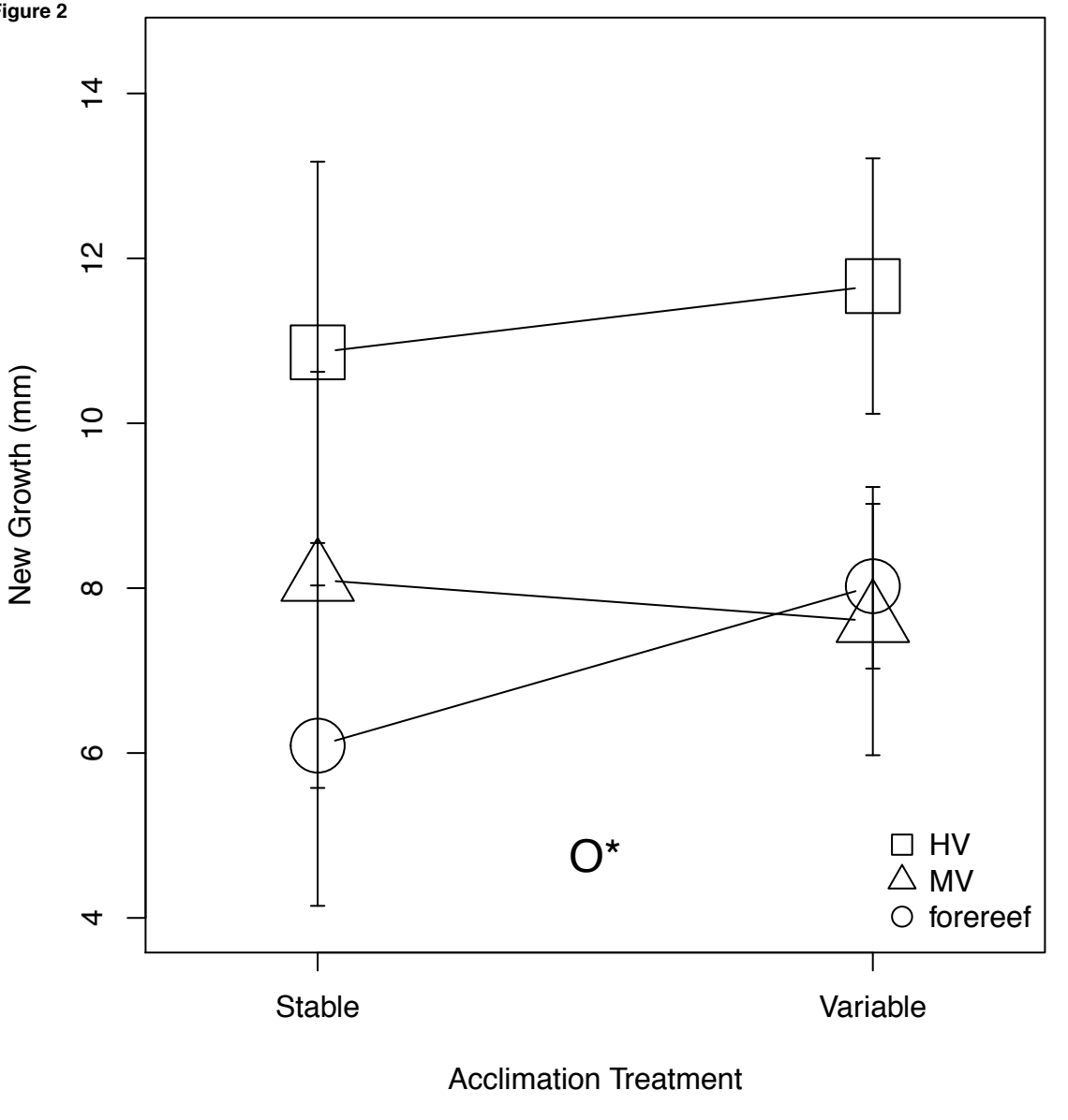
New Growth (mm)
4
6
8
10
12
14
Stable
Variable
Acclimation Treatment
O*
HV
MV
forereef

Figure 3

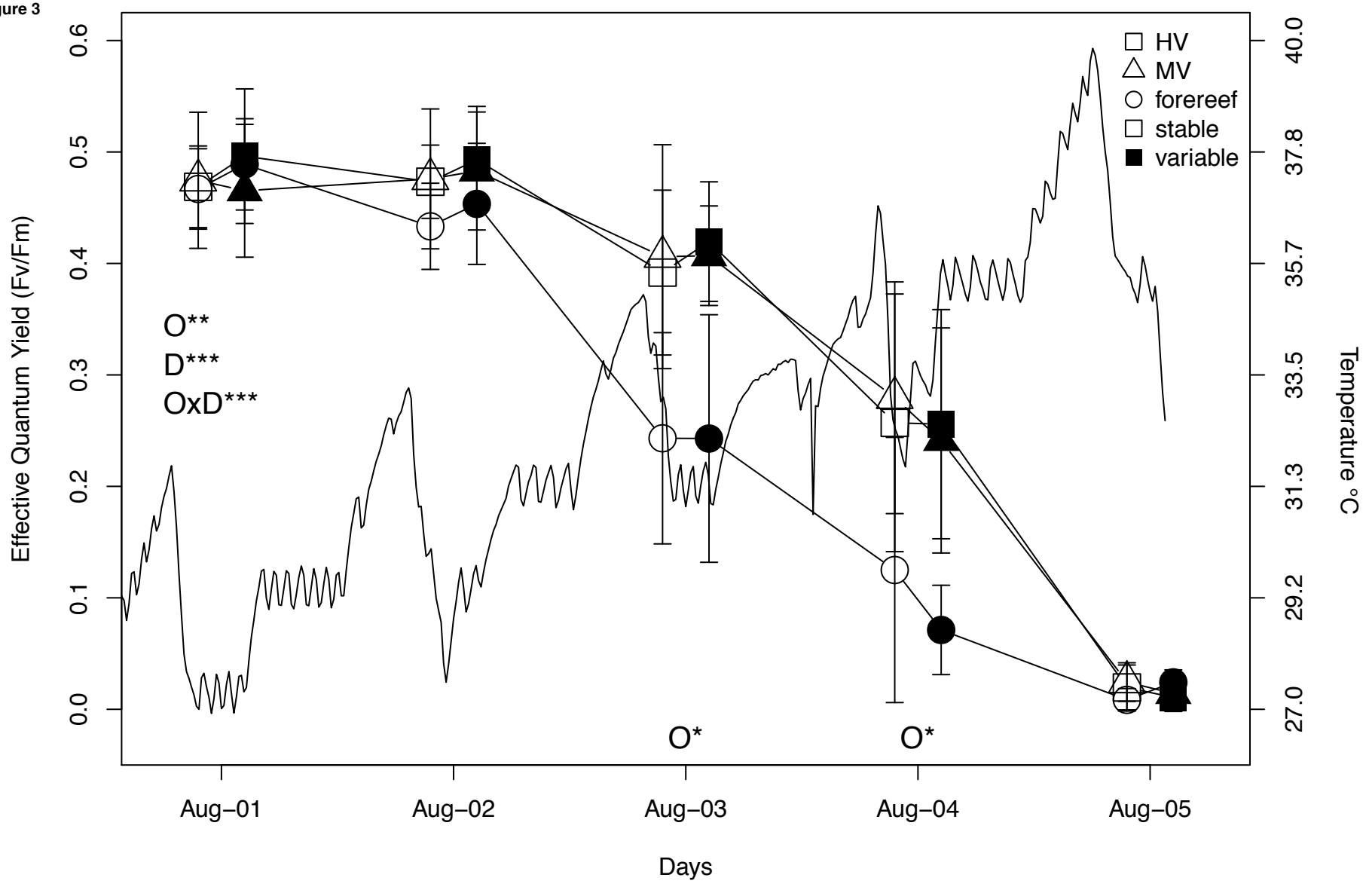
HV
MV
forereef
stable
variable
O**
D***
OxD***
O*
O*
Effective Quantum Yield (Fv/Fm)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
Temperature °C
27.0
29.2
31.3
33.5
35.7
37.8
40.0
Aug–01
Aug–02
Aug–03
Aug–04
Aug–05
Days

Figure 4

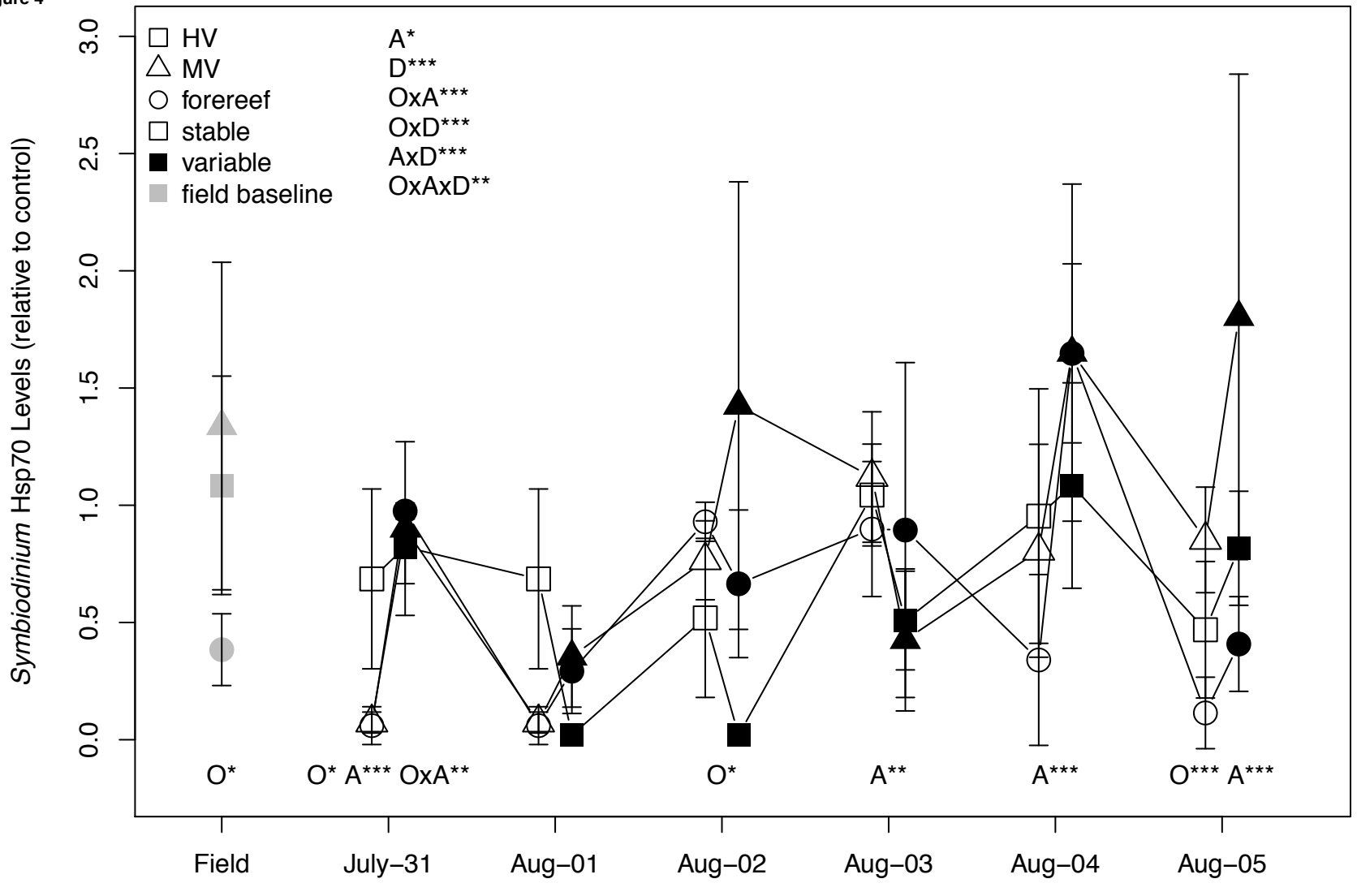
HV
MV
forereef
stable
variable
field baseline
A*
D***
OxA***
OxD***
AxD***
OxAxD**
Symbiodinium Hsp70 Levels (relative to control)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
O*
O* A*** OxA**
O*
A**
A***
O*** A***
Field
July–31
Aug–01
Aug–02
Aug–03
Aug–04
Aug–05

Figure 5

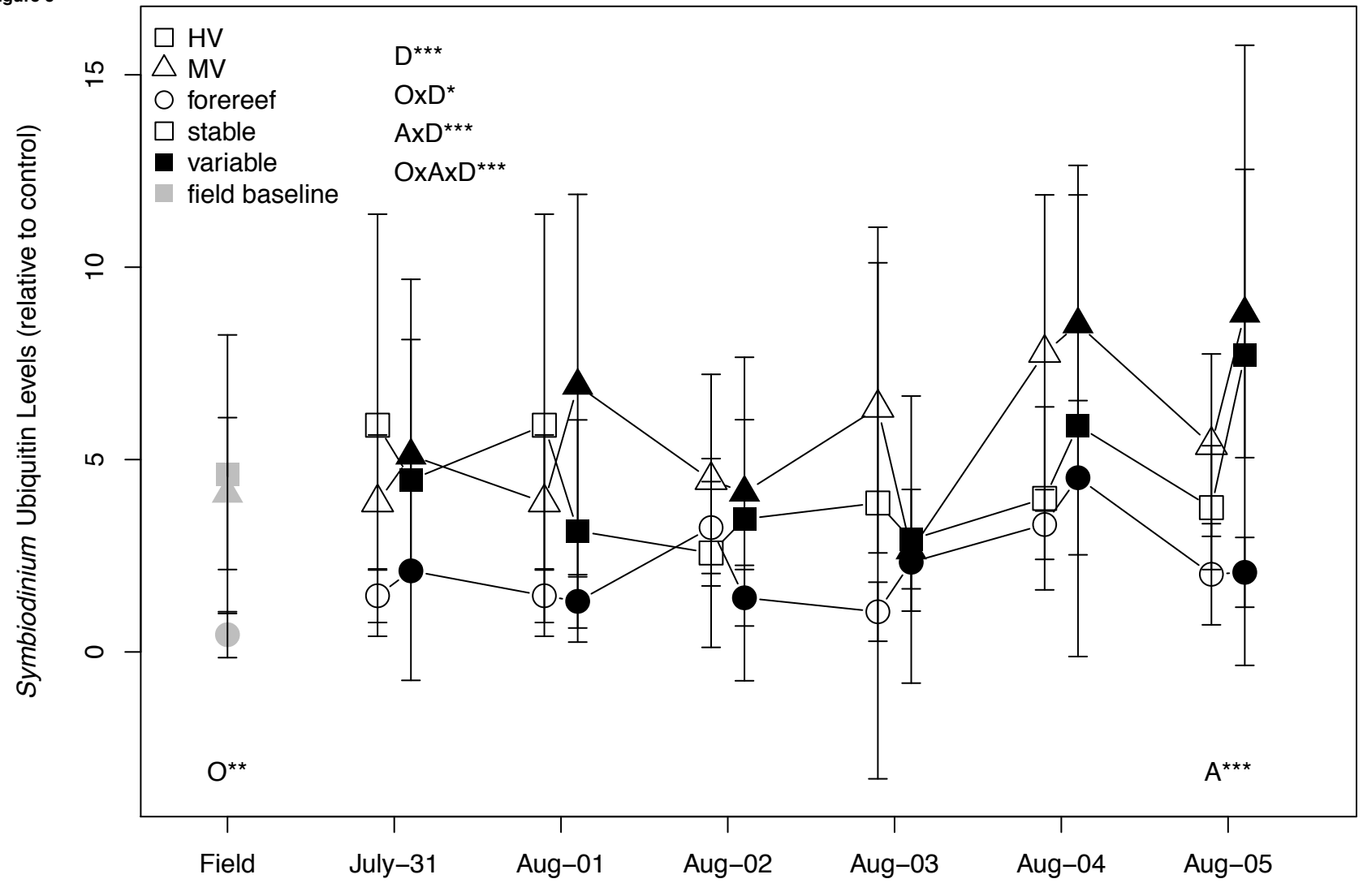
HV
MV
forereef
stable
variable
field baseline
D***
OxD*
AxD***
OxAxD***
O**
A***
Symbiodinium Ubiquitin Levels (relative to control)
0
5
10
15
Field
July–31
Aug–01
Aug–02
Aug–03
Aug–04
Aug–05

Figure 6

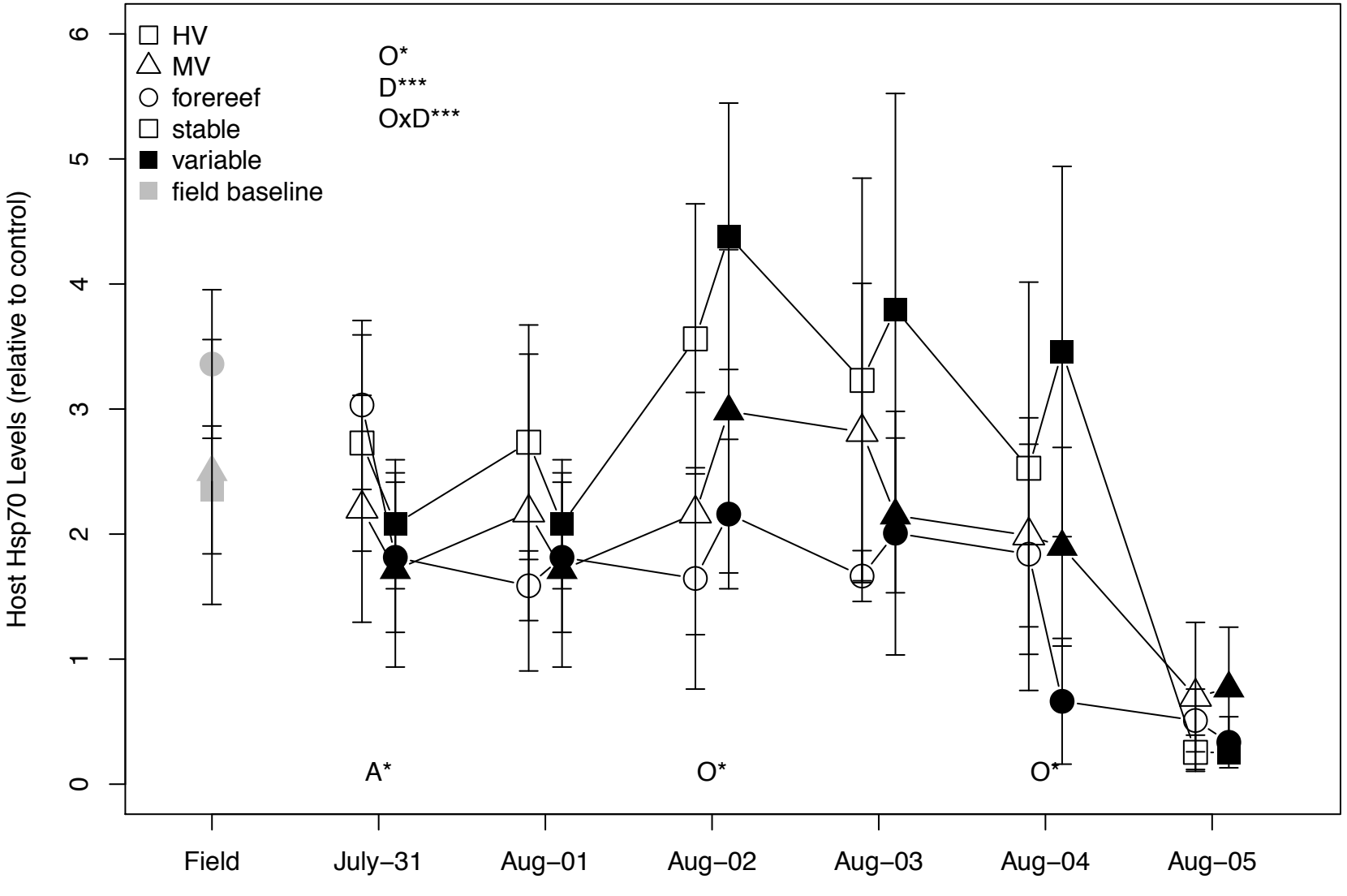
HV
MV
forereef
stable
variable
field baseline
O*
D***
OxD***
A*
O*
O*
Host Hsp70 Levels (relative to control)
0
1
2
3
4
5
6
Field
July–31
Aug–01
Aug–02
Aug–03
Aug–04
Aug–05

Figure 7

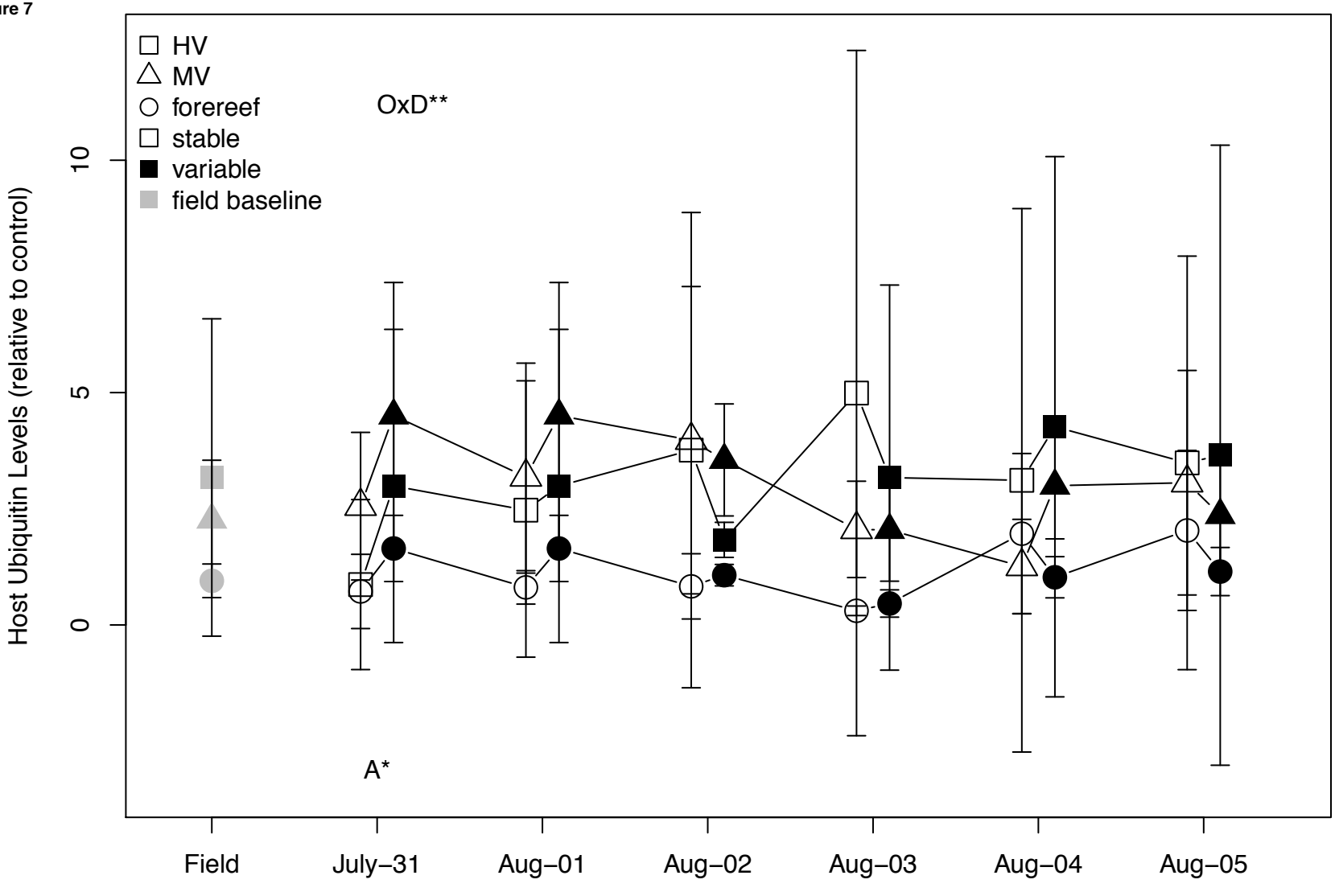
HV
MV
forereef
stable
variable
field baseline
OxD**
A*
Host Ubiquitin Levels (relative to control)
0
5
10
Field
July–31
Aug–01
Aug–02
Aug–03
Aug–04
Aug–05

Figure 8