Shifting Baselines: Physiological legacies contribute to the response of reef coral to frequent heat waves

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Key words: immunity, physiology, El Niño, bleaching, Symbiodiniaceae

Abstract

1. Global climate change is altering coral reef ecosystems. Notably, marine heat waves are producing widespread coral bleaching events that are increasing in frequency, with projections for annual bleaching events on reefs worldwide by mid-century.
2. The response of corals to elevated seawater temperatures can be modulated by abiotic factors at site of origin and dominant endosymbiont type, which can result in a shift in multiple coral traits and drive physiological legacy effects that influence the trajectory of reef corals under subsequent thermal stress events. It is critical, therefore, to evaluate the potential for shifting physiological and cellular baselines driven by these factors in *in situ* bleaching (and recovery) events. Here, we use the back-to-back regional bleaching events of 2014 and 2015 in the Hawaiian Islands and subsequent recovery periods to test the hypothesis that coral multivariate trait space (here termed physiotype, sensu (Van Straalen, 2003) shift in multiple bleaching events, modulated by both environmental histories and symbiotic partnerships (Symbiodiniaceae).
3. Despite fewer degree heating weeks in the first-bleaching event relative to the second (7 *vs*. 10), bleaching severity in a dominant reef building coral on Hawaiian reefs, *Montipora capitata,* was greater (~70% *vs*. 50% bleached cover) and differences due to environmental history (reef site) were more pronounced. Melanin, an immune cytotoxic response, provided an initial defense during the first event, potentially priming antioxidant activity, which peaked in the second-bleaching event (i.e., a legacy effect). While magnitude of bleaching differed, immune response patterns were shared among corals harboring heat-sensitive and heat-tolerant Symbiodiniaceae. This supports a pattern of increased constitutive immunity in corals resulting from repeat bleaching

events, with greater specialized enzymes (catalase, peroxidase, superoxide dismutase) and attenuated melanin synthesis.

4. This study demonstrates bleaching events have implications for reef corals beyond shaping their ecological assemblages. These events can change the magnitude and/or identity of response variables contributing to physiotype, thus generating physiological legacies carried over into the future. Quantifying baseline coral physiotypes and tracking their shifts will be critical to understanding and forecasting the effects of increased bleaching frequency on coral biology and ecology in the Anthropocene.

1. Introduction

Environmental change at local and global scales is degrading coral reef ecosystems. Notably, long-term trends in ocean warming and punctuated marine heat waves are destabilizing the symbiosis between corals and their endosymbiont algae (Symbiodiniaceae) and increasing coral bleaching events (Hughes et al., 2018a). The cumulative impacts of these episodic heat waves not only shift ecological and functional baselines (Hughes et al., 2019; McWilliam, Pratchett, Hoogenboom, & Hughes, 2020), but may also have legacy effects on coral biology, such that responses to bleaching events can be modulated by prior exposure (e.g., Brown, Dunne, Goodson, & Douglas, 2000). It is growing increasingly clear that susceptibility to bleaching in reef corals is based on the interaction of environmental histories (Brown et al., 2000; Safaie et al., 2018) and holobiont traits, including endosymbiont communities and genetics and physiology of the coral host (Barshis et al., 2013; Palmer, Bythell, & Willis, 2010; Palumbi, Barshis, Traylor-Knowles, & Bay, 2014; Sampayo, Ridgway, Bongaerts, & Hoegh-Guldberg, 2008).

Thermotolerant Symbiodiniaceae can confer bleaching resistance in some corals (Sampayo et al., 2008), and the Hawaiian coral *Montipora capitata* exhibits reduced bleaching when dominated by *Durusdinium* as opposed to *Cladocopium* Symbiodiniaceae (Cunning, Ritson-Williams, & Gates, 2016). However, corals are metaorganisms, and host properties such as constitutive immunity (Palmer, 2018a) and antioxidant capacity (Barshis et al., 2013) are also central to maintaining cellular homeostasis and preventing bleaching mortality (Palmer et al., 2010). As such, immunological processes are implicated as targets for natural selection and are integral to the future of reef-building corals in the face of climate change (Mydlarz, McGinty, & Harvell,

2010; Palmer, 2018a; Pinzón, Beach-Letendre, Weil, & Mydlarz, 2014). The nexus of these influential properties of coral stress responses – symbiont community and host immunity – and their role in repetitive natural bleaching and recovery events remains to be fully understood, especially with respect to the role of environmental history (Palumbi et al., 2014; Safaie et al., 2018). As global climate change increases the frequency and severity of coral bleaching events (Hughes et al., 2018), understanding the symbiotic, immune, and antioxidant responses of corals will be central to understanding the cumulative and latent effects of bleaching on the function of reef corals through time and enhancing our capacity to project community responses to thermal stress events.

The widely documented recent major bleaching of the Great Barrier Reef (2016-2017) reduced coral cover by >50% (Stuart-Smith, Brown, Ceccarelli, & Edgar, 2018), altered coral (Hughes et al., 2018b) and fish (Robinson, Wilson, Jennings, & Graham, 2019) assemblages, and disturbed the stock-recruitment capacity of reef corals (Hughes et al., 2019). As climate change continues to intensify, it is now critical to track shifts in the biology and function of corals through examination of multivariate trait-space, defined as 'physiotypes' by Van Straalen (2003; e.g., Figure 1a), the term which we use throughout this manuscript. Trait-space approaches have recently been applied to track changes in reef coral communities and their representative trait diversity following bleaching (Hughes et al., 2018b; McWilliam et al., 2020), and the generation of physiotype time series data will provide a critical match to further understand and interpret the growing collection of coral reef ecological time series datasets (De'ath, Fabricius, Sweatman, & Puotinen, 2012; Edmunds et al., 2014; Edmunds et al., 2014; McClanahan, Ateweberhan, & Omukoto, 2007). Together, these timeseries will allow us to integrate across cellular, ecological

105 and evolutionary scales and improve our mechanistic understanding of these scale linkages.
106 Importantly, tracking of multiple, interactive coral physiological traits provides the opportunity
107 to reveal the potential for beneficial acclimatization or negative legacy effects that may underlie
108 shifts in physiotype (e.g., Figure 1a) and testable mechanistic hypotheses underpinning resistance
109 or susceptibility to repeated stress (Figure 1b).
110
111 This integrative multivariate approach to tracking coral performance was utilized to test specific
112 hypotheses regarding targeted response variables and the linkage between environmentally-
113 driven physiological, symbiotic, and cellular (i.e., antioxidants, immunity) legacies as
114 mechanisms underpinning bleaching outcomes (Palmer et al., 2010; Palmer & Traylor-Knowles,
115 2012; Venn, Loram, & Douglas, 2008). Both the host and symbiont have enzymatic defenses to
116 mitigate oxidative stress (e.g., peroxidase, catalase, superoxide dismutase) (Venn et al., 2008)
117 caused by host (e.g., heat-damaged mitochondrial membranes, Dunn et al., 2012) and symbiont
118 (e.g., damage to the D1 protein of photosystem II (PSII)) (Jones, Hoegh-Guldberg, Larkum, &
119 Schreiber, 1998; Lesser, 1997) sources, ultimately leading to apoptosis and dysbiosis when
120 severe enough (Weis, 2008) (Figure 1b). Thermal stress also triggers host immune responses
121 (Mydlarz, Couch, Weil, Smith, & Harvell, 2009; Mydlarz et al., 2010; Pinzón et al., 2015), and
122 higher constitutive immunity (i.e., immune activity necessary for cellular homeostasis) reduces
123 coral thermal sensitivity and disease susceptibility (Palmer et al., 2010). In addition, the
124 melanin-synthesis pathway is an important component of host immunity that is active in wound
125 healing and pathogen invasion while also serving as a photoprotectant that may mitigate
126 bleaching stress (Mydlarz & Palmer, 2011; Palmer et al., 2010) (Figure 1b). However, corals
127 may mount different cellular mechanisms to combat bleaching due to energetic requirements

128 (Fuess, Mann, Jinks, Brinkhuis, & Mydlarz, 2018; Palmer, 2018b; Pinzón et al., 2015), stress
129 frequency (i.e, acute, chronic, repeated stress) (Ainsworth et al., 2016; Schoepf et al., 2015), or
130 as a function of histories of biotic and abiotic challenges that modulate constitutive immunity
131 (Mydlarz et al., 2009; Palmer, 2018b; Wall et al., 2018). For instance, there is evidence corals
132 may rely on different antioxidant and immunity mechanisms based on their environmental
133 histories, as lower melanin synthesis and higher antioxidant activity was observed in heat-
134 stressed corals from a reef with high pCO_2-variability compared to low pCO_2-variability (Wall et
135 al., 2018). Thus, pairing the measurement of bleaching metrics like cell density and chlorophyll
136 concentrations with immune and antioxidants provides a powerful and tractable approach within
137 a multivariate integrative framework.
138
139 The Hawaiian Islands, once thought to be a bleaching refuge for corals (Jokiel & Brown, 2004),
140 experienced severe bleaching in 2014 and 2015 across the populated Main and remote
141 Northwestern Hawaiian Islands (Bahr, Rodgers, & Jokiel, 2017; Couch et al., 2017). In
142 Kāneʻohe Bay, Oʻahu, Hawaiʻi, 7.1 degree heating weeks (DHW) were observed in 2014 and
143 10.2 DHW in 2015, beginning in August and July, respectively (*see* Methods), resulting in 45 ±
144 2% (mean ± SE) bleaching in the first event and 30 ± 4% bleaching in the second (Bahr et al.,
145 2017). An ecologically dominant reef coral, *Montipora capitata* (Dana 1846), showed
146 significant bleaching in both events (Figure 2a), with differences in bleaching responses
147 observed at the site and event level.
148
149 Using the opportunity presented by these natural bleaching events (Figure 2), we tracked coral
150 percent bleaching and physiotypes using a multivariate trait-space approach. We quantified the

151 response of *M. capitata* in Kāneʻohe Bay to the back-to-back bleaching events of 2014 and 2015
152 (i.e., bleaching in October 2014, recovery in February 2015, bleaching in October 2015, and
153 recovery in February 2016) at two Kāneʻohe Bay reefs (Lilipuna and Reef 14) with contrasting
154 environmental histories relating to seawater residence (10 – 20 d *vs*. >30 d), pCO_2 variability (ca.
155 300 *vs*. 600 μatm pCO_2 diel flux), and proximity to shore (Drupp, De Carlo, Mackenzie,
156 Bienfang, & Sabine, 2011; Drupp et al., 2013; Lowe, Falter, Monismith, & Atkinson, 2009).
157 We predicted that: (H1) immunity and antioxidant contributions to bleaching responses will
158 influenced by site environmental histories and symbiont communities such that greater bleaching
159 prevalence and attenuated immune responses will be observed in corals from the high variable-
160 pCO_2 site (Reef 14) and those association with thermally sensitive Symbiodiniaceae. We also
161 expected (H2) immunity and antioxidant activity would not occur with the same magnitude or
162 trajectory after repeated bleaching events due to legacy effects shaping acclimatory or stress
163 response pathways over time. Considering the wide-ranging functions of melanin in host
164 immunity (Palmer et al., 2008, 2010), we expected melanin synthesis to act as an acute and
165 broad-spectrum defense in physiologically stressed corals, with antioxidants having a more
166 specialized response after chronic or repeat bleaching stress. Finally, while the thermal tolerance
167 of symbiont communities is vital to coral bleaching sensitivity, evidence for differential
168 immunity or antioxidant capacities in coral holobionts associated with thermally tolerant
169 symbiont species is lacking. Given that the coral *M. capitata* is known to be dominated by two
170 genetically and physiologically contrasting Symbiodiniaceae, *Cladocopium* sp. and *Durusdinium*
171 *glynnii* (Cunning et al., 2016; Wall, Kaluhiokalani, Popp, Donahue, & Gates, 2020), we
172 predicted (H3) that holobionts harboring heat-tolerant *D. glynnii* would show higher immune and
173 antioxidant responses linked to greater bleaching resistance. To test these hypotheses, we

174 measured bleaching severity and recovery at each location using benthic surveys and measured
175 coral traits in coral fragments, including: physiological measurements of cellular bleaching
176 magnitude (symbiont density, areal- and cell-specific chlorophyll *a*, holobiont total protein and
177 total biomass), and completed enzymatic assays for mechanisms contributing to bleaching
178 resistance through coral host antioxidant capacity (peroxidase, catalase, superoxide dismutase),
179 and the host immune response of the melanin cascade (prophenoloxidase, melanin). This
180 approach allows for a more holistic description of coral physiotype that we tracked through two
181 bleaching and recovery periods.
182

183 **2. Materials and Methods**

184 *2.1 Site description*

185 Coral bleaching and recovery at two reef systems: a fringing reef (hereafter, 'Lilipuna'
186 [21°25′36.8″N, 157°47′24.0″W]) in southern Kāneʻohe Bay adjacent to the Hawaiʻi Institute of
187 Marine Biology (HIMB) on Moku o Loʻe, and an inshore patch reef (hereafter, 'Reef 14'
188 [21°27′08.6″N, 157°48′04.7″W]) in central Kāneʻohe Bay. These reefs sites were chosen due to
189 their unique environmental history of seawater pCO_2 and hydrodynamics (Drupp et al., 2013;
190 Wall et al., 2018). Seawater pCO_2 adjacent to the both locations (fringing reef at Lilipuna and
191 the patch reef Reef 14) is comparable (ca. 450 μatm), however, diel pCO_2 flux is significantly
192 higher (196 – 976 μatm pCO_2) in central Kāneʻohe Bay near Reef 14 relative to the Bay's
193 southern basin proximate to Lilipuna (225 – 671 μatm) (P. Drupp et al., 2011; Drupp et al.,
194 2013).
195

196 *2.2 Environmental monitoring*

Temperature (Hobo pendants, ± 0.53 °C accuracy, 0.14 °C resolution, Onset Computer Corp., Bourne, MA) and photosynthetic active radiation (PAR) loggers (Odyssey, Dataflow Systems Limited, Christchurch, New Zealand) were placed at Lilipuna and Reef 14 at a depth of 1 m. Loggers were periodically removed from the reef, cleaned, and replaced monthly. Temperature and PAR were recorded continuously at 15 min intervals from October 2014 – February 2016, however, gaps in environmental data do exist as a consequence of instrument failure and loss. Gaps in logger temperature data were supplemented with NOAA temperature data from the Moku o Lo'e station at HIMB (NOAA, 2019). PAR loggers were calibrated against a LI-1400 quantum meter (Li-Cor, Lincoln, Nebraska, USA) attached to a cosine LI-192 underwater quantum sensor. Temperature loggers were calibrated with a certified digital thermometer (5-077-8, ± 0.05 °C accuracy, Control Company, USA) and cross-calibrated against each other for standardization. Degree heating weeks (DHW) for the southern portion of Kāne'ohe Bay where our corals were collected were calculated in R using *in situ* Moku o Lo'e temperature data (NOAA, 2019), with the difference between mean half-week temperatures (i.e., mean hourly temp over 3.5 d) and the maximum monthly mean temperature of 27.7 °C, (Jokiel & Brown, 2004)] to determine 'hotspots'. DHW were determined as the number of hotspots >1 across a rolling 12 weeks window (i.e., 24 half-weeks) (NOAA, 2020). DHW for windward O'ahu and the Hawaiian Islands during the 2014 and 2015 bleaching events have been previously reported (Bahr et al., 2017; Sale, Marko, Oliver, & Hunter, 2019). The purpose of our calculations are to quantify the incurred heat stress using empirical calculation of DHW from temperature data collected proximate to our two reef locations.

2.3 Benthic surveys and coral collections

Four sampling periods were identified as corresponding to a 'bleaching period' following the point of maximum thermal stress (10 October 2014 and 12 October 2015) and a post-bleaching 'recovery period' approximately 4 months after peak seawater warming (11 February 2015 and 26 February 2016). In each time period, benthic surveys were conducted at each reef site using a 20 m transect and a line-point-intersect at 1 m intervals. Transects were positioned parallel to natural contours of the reef, being the north-south axis of the fringing reef (Lilipuna) and the east-west axis of patch reef (Reef 14). At each reef, transects (n = 2) were placed along the reef crest (1 – 2 m), and benthic community cover was recorded at the species level for reef corals (*Montipora capitata, Pocillopora* spp. [*P. acuta*, *P. damicornis*]*, Porites compressa*), and either crustose coralline algae (CCA), macroalgae, or sand/bare/turf. For corals, bleaching state was quantified categorically, being either non-bleached (i.e., appearing fully pigmented), or bleached (i.e., exhibiting degrees of tissue paling/pigment variegation or being wholly white). *M. capitata* cover was calculated as the proportion of total benthic cover, and *M. capitata* bleaching extent was calculated as proportion of total *M. capitata* colonies bleached. The relative position of transects at each reef was recorded to allow for repeat surveys within the same general location at each reef across survey periods.

In each sampling period, coral branch tips (<4 cm length) were collected from forty *M. capitata* coral colonies (n = 1 fragment colony^{-1}) along the reef crest at each reef site at a depth of ca. 1 m (State of Hawai'i Department of Land and Natural Resources, Special Activity Permit 2015-17 and 2016-69). Immediately post collection, corals were snap-frozen in liquid nitrogen and returned to HIMB and stored at -80 °C. While remaining frozen, each colony was split in half along its longitudinal axis. One-half of each coral fragment was stored at HIMB (-80 °C) until

243 processing for physiological assays and qPCR. The corresponding fragment-halves were used
244 for immunological assays and were shipped to the University of Texas at Arlington using a dry-
245 shipper charged with liquid nitrogen.
246
247 *2.4 DNA extraction and symbiont community analysis*
248 Symbiodiniaceae DNA was extracted by adding an isolate of coral tissue (500 μl) to 500 ul DNA
249 buffer (0.4 M NaCl, 0.05 M EDTA) with 2% (w/v) sodium dodecyl sulfate, following a modified
250 CTAB-chloroform protocol (Cunning et al., 2016) (dx.doi.org/10.17504/protocols.io.dyq7vv).
251 To determine the dominant genera of Symbiodiniaceae hosted by each *M. capitata* fragment, a
252 quantitative PCR (qPCR) (Cunning et al., 2016) was used to amplify Symbiodiniaceae actin gene
253 loci, with the corresponding number of actin gene copies in the Symbiodiniaceae genera
254 *Cladocopium* and *Durusdinium* (namely, ITS2 types C31, with C17 and C21 and D1-4-6
255 [*Durusdinium glynnii* (Wham, Ning, & LaJeunesse, 2017)]) known to be numerically dominant
256 in Kāne‘ohe Bay *M. capitata* (Cunning et al., 2016). Specificity of genus-level primers have
257 been previously validated using a combination of Symbiodiniaceae internal transcribed spacer
258 (ITS2) region of rDNA and actin gene sequencing (Cunning & Baker, 2013). Duplicate qPCR
259 reactions (10 μl) were run for each coral sample using a StepOnePlus platform (Applied
260 Biosystems) set to 40 cycles, a relative fluorescence (ΔR_n) threshold of 0.01, and internal cycle
261 baseline of 3 – 15. Symbiont genera detected in only one technical replicate were considered
262 absent. The relative abundance *of Cladocopium and Durusdinium* symbionts (i.e., C:D ratio) in
263 each sample was determined from the ratio of amplification threshold cycles (C_T) for
264 *Cladocopium* and *Durusdinium* (i.e., C_T^C, C_T^D) using the formula C:D = 2^(C_T^C - C_T^D), where
265 genus-specific C_T values are normalized according to gene locus copy number and fluorescence

266 intensity (Cunning et al., 2016). Coral colonies were determined to be *Cladocopium*- or
267 *Durusdinium*-dominated based on numerical abundance (>0.5 proportion) of each genus from
268 qPCR analysis (Innis, Cunning, Ritson-Williams, Wall, & Gates, 2018).
269
270 *2.5 Physiological metrics*
271 The extraction and processing of coral and symbiont tissues was performed following established
272 methods (summarized in Wall et al., 2018). Briefly, coral tissue was removed from the skeleton
273 using an airbrush filled with 0.2 μm filtered seawater, yielding ~10 – 30 ml of tissue slurry.
274 Extracted tissues were briefly homogenized and subsampled for the following physiological
275 metrics: symbiont cell densities, chlorophyll *a* concentrations, protein biomass, and the total
276 organic biomass determined from the ash-free dry weight (AFDW) of coral + algae tissues.
277 Coral tissue slurries were stored at -20 °C.
278
279 All physiological metrics were normalized to the surface area (cm^2) of coral skeleton using the
280 paraffin wax-dipping technique (Stimson & Kinzie, 1991). Symbiont cell counts were measured
281 by replicate cell counts (n = 6 – 10) on a haemocytometer, and expressed as symbiont cells cm^{-2}.
282 Chlorophyll *a* was quantified by concentrating algal cells through centrifugation (3,000 × *g* for 3
283 min) and extracting pigments in the algal pellet in 100% acetone for 36 h in darkness at -20 °C.
284 Spectrometric absorbance were measured (λ = 630 and 663 nm) using a 96-well quartz plate with
285 two technical replicates; chlorophyll concentrations quantified using the equations for
286 dinoflagellates (Jeffrey & Humphrey, 1975), normalized for path length and expressed as ug
287 chlorophyll *a* cm^{-2} and pg chlorophyll *a* symbiont $cell^{-1}$. Total protein concentration (soluble +
288 insoluble in the holobiont) was quantified using the Pierce BCA (bicinchoninic acid) Protein

289 Assay Kit (Pierce Biotechnology, Waltham, MA). Protein solubilization was achieved by adding
290 1 M NaOH and heating (90 °C) for 60 min, followed by the neutralizing to ca. pH 7.5 with 1 N
291 HCl. Protein was measured spectrophotometrically (λ = 562 nm) in a 96-well plate with three
292 technical replicates against a bovine serum albumin standard and expressed as mg protein cm^{-2}.
293 Total fraction of organic biomass was measured by drying a subsample of coral tissue at 60 °C
294 (48 h) in pre-burned aluminum pans followed by burning in a muffle furnace (450 °C) for 4 h.
295 The difference between the dried and burned masses is the AFDW and expressed as mg cm^{-2}.
296

297 *2.6 Immunity and oxidative stress metrics*

298 Immunology and oxidative stress metrics were determined using previously published protocols
299 for coral host tissues (Mydlarz et al., 2009; Mydlarz & Palmer, 2011; Palmer et al., 2011; Wall et
300 al., 2018). A 3 – 4 ml aliquot of coral tissue slurry was obtained by airbrushing with our coral
301 extraction buffer at pH 7.0 (100 mM TRIS buffer + 0.05 mM dithiothreitol). Tissue was
302 homogenized for 1 min on ice (hand held homogenizer, Powergen 125, Fisher Scientific,
303 Waltham, MA), and 1 ml of the resulting slurry was freeze-dried for 24 h (VirTis BTK freeze-
304 dryer, SP Scientific, Warminster, PA) and used for melanin concentration estimates. The
305 remaining slurry was centrifuged at 4 °C at 2,500 × *g* (Eppindorf 5810 R centrifuge, Hamburg,
306 Germany) for 5 min to remove cellular debris and most Symbiodiniaceae cells to achieve a host-
307 enriched cell-free extract. All colorimetric measurements were calculated using a Synergy 2
308 multi-Detection microplate reader with Gen5 software (BioTek, Winooski, VT, USA). All assays
309 were run in duplicate or triplicate on separate 96-well microtiter plates. Total protein
310 concentration of each coral cell-free extract was determined using the RED660 protein assay (G
311 Biosciences, Saint Louis, MO) with a bovine serum albumin standard curve.

312

313 To determine the concentration of melanin within each sample, the freeze-dried aliquots of
314 weighed and dried tissue were gently vortexed with 400 μl of 10 M sodium hydroxide (NaOH)
315 and left to extract for 48 h. Samples were bead-beaten with 1 mm glass beads and then vortexed
316 for 10 s and then centrifuged at 7,000 × *g* for 5min. For each sample, 65 μl of supernatant were
317 aliquoted in duplicate into a 96-well g-area microtiter plate (Greiner Bioone, Monroe, NC,
318 USA). The plates were read at an absorbance of 490 nm and the concentration of melanin was
319 determined using a standard curve (0 – 2 mg) of commercial melanin (Sigma-Aldrich, St. Louis,
320 MO) dissolved in 10 M NaOH for 48 h and treated the same as the samples on each microtiter
321 plate. Data presented are converted to mg melanin normalized to mg of tissue (Fuess et al.,
322 2018).

323

324 PPO activity was determined for each sample using duplicate 20 μl aliquots of coral cell-free
325 extract in clear 96-well microtiter plates, with 50 μl of 10 mM phosphate buffered saline at pH
326 7.0. To each well, 20 μl of trypsin (0.2 mg ml^{-1} concentration in deionized filtered water) was
327 added to activate PPO and the reaction was initiated by the addition of 20 μl of 25 mM L-1,3-
328 dihydroxyphenylalanine (L-DOPA; Sigma-Aldrich). The absorbance at 490 nm was measured
329 over 25 min and the change in absorbance during the linear portion of the reaction (typically 10 –
330 15 min) was normalized to mg protein and time for each sample (ΔAbs490 nm mg $protein^{-1}$ min^{-}
331 ^{1}) (Mydlarz & Palmer, 2011; Fuess et al., 2018).

332

333 Coral host oxidative stress was determined by measuring the scavenging activity of the coral
334 cell-free extracts to different substrates comparable to the antioxidants: peroxidase (POX),

335 catalase (CAT), and superoxide dismutase (SOD). Peroxidase activity (EC 1.11.1.7) was
336 determined for each sample using 10 μl aliquots of coral cell-free extract, in duplicate within 96-
337 well microtiter plates, diluted with 50 μl phosphate buffer (pH6.0). To each well, of 25 μl of 25
338 mM guaiacol in 10 mM PBS (pH 6.0) was added and the reaction was initiated with the addition
339 of 12.5 μl of 25 mM H_2O_2 high purity 30% hydrogen peroxide (H_2O_2; Sigma Aldrich). Guaiacol
340 is a phenolic compound with scavenging properties that is commonly used as a substrate for
341 peroxidase activity (Mydlarz & Harvell, 2007). The absorbance was read at 470 nm every min
342 for 15 min and the change over time was calculated for the linear part of the reaction (0 – 10
343 min) and normalized to mg protein in each sample the units for peroxidase activity are (ΔAbs470
344 nm mg protein^{-1} min^{-1}) (Mydlarz & Harvell, 2008).
345
346 Catalase activity (EC 1.11.1.6) was determined using the H_2O_2 depletion assay using 10 μl coral
347 cell free extract and 50 μl of 10 mM of pH 6.0 phosphate buffered saline (PBS) in duplicate
348 wells of a UV-transparent 96-well microtiter plate. The assay was activated by the addition of
349 10 μl 25 mM H_2O_2. The absorbance was measured at 240 nm over 15 min and the change in
350 absorbance over time (e.g., initial - final) was calculated for the linear part of the reaction
351 (typically 0 – 8 min) and converted to μmol H_2O_2 using a standard curve of known
352 concentrations, normalized per mg protein. CAT activity is presented as μmol H_2O_2 scavenged
353 mg protein^{-1} min^{-1} (Palmer et al., 2011).
354
355 Superoxide dismutase (EC 1.15.1.1) activity was calculated using the SOD Assay Determination
356 Kit-WST (Fluka, Switzerland) which includes Dojindo's highly water-soluble tetrazolium salt,
357 WST-1(2-(4- iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)- 2H-tetrazolium, monosodium

358 salt), that produces a water-soluble formazan dye with an absorbance peak at 450 nm upon
359 reduction with superoxide anion. 10 μL of extract was incubated with WST-1 and xanthine
360 oxidase, which produces superoxide anion. Inhibition of absorbance at 450 nm (scavenging of
361 the superoxide anion) was monitored in wells containing coral extracts and SOD standards and
362 compared to untreated samples. One SOD unit of activity (U) equates to 50% inhibition of
363 superoxide anion, and data are presented as SOD U mg protein^{-1} (Krueger et al., 2015).
364
365 *2.7 Statistical analysis*
366 Permutation analysis of variance (PERMANOVA) and non-metric multidimensional scaling
367 (NMDS) were performed using a balanced matrix of all physiology and immunity/antioxidant
368 responses (n = 10 responses) in the package *vegan* (Oksanen et al., 2019). PERMANOVA used
369 a scaled and centered matrix with Euclidean calculations of pairwise distances using *adonis2*.
370 The NMDS data matrix was double standardized using a Wisconsin and square root
371 transformation with a euclidean distance using *metaMDS*. Response variables showing
372 significant correlations (p<0.05) with NMDS axes were plotted as vectors using envfit command
373 in *vegan*. Coral 'physiotypes' were defined by convex hulls, with borders defined by the range
374 of points in the multivariate trait space (i.e., NMDS1 and NMDS2). Physiotypes were grouped
375 categorically by periods, or the interactions of Site, Symbiont, and Period. Physiotype centroids
376 used in trajectory plots were calculated as the mean of NMDS1 and NMD2 for each category.
377
378 Ecological benthic data (*M. capitata* total cover and bleached cover) were tested in a linear
379 model with Periods (two bleaching and two recovery events) and Sites (Lilipuna, Reef 14) as
380 fixed effects. Physiology, antioxidant, and immunity response variables were analyzed using a

linear model with Periods, Sites, and Symbiont community composition (*Cladocopium*- or *Durusdinium*-dominated) as fixed effects. Normal distribution and equal variance assumptions of ANOVA were examined by graphical representation of residuals and quantile:quantile plots. Where assumptions were not met, Box-Cox tests were performed (Box & Cox, 1964) and data transformations applied in the package *MASS* (Venable & Ripley, 2002). Analysis of variance tables for linear models were generated using type-II sum or squares with multiple comparisons using the package *emmeans* (Lenth, 2019). All analyses were performed in *R* version 3.6.1 (R Core Team, 2019). All data and code to generate figures and perform analyses are archived and openly available at Github (https://github.com/cbwall/Gates-Mydlarz-bleaching-recovery/releases/tag/v4).

3. Results

Coral cover at the two sites ranged from (mean ± SD) 63 ± 11% to 93 ± 11% from 2014-2016. Greater total bleached coral cover was observed in the first bleaching event (62 – 75 ± 9%) relative to the second (43 – 55 ± 16%) (i.e., during thermal stress) (Figure 2). Mean *M. capitata* cover was stable across time at Lilipuna (~40%) but was much more variable at Reef 14, falling from 91 ± 4% in October 2014 to 51 ± 7% in February 2016. In the two bleaching events (October 2014 and 2015) greater bleaching of *M. capitata* corals was observed at Reef 14, compared to Lilipuna (p=0.020).

PERMANOVA results testing effects of Period (bleaching event), Site (Lilipuna *vs*. Reef 14) and dominant Symbiodiniaceae species (*Cladocopium* sp. or *D. glynnii*) revealed significant differences of all main effects (p<0.001) and the interaction of Period-by-Site (p<0.001) and

404 Period-by-Symbiont community (p<0.001) (Figure 3 and 4, Table 1). In each of the four
405 periods, coral physiotypes occupied unique positions in multidimensional trait space (Figure 2),
406 with the location and trajectories of coral physiotypes in each period principally being a function
407 of symbiont community, and to a lesser extent, site of collection (i.e., environmental history and
408 physiological legacy) (Figure 3, 4).

409 Physiotypes of bleached corals relative to recovered corals showed stronger separation in 2014-
410 2015, which was apparent at both reef sites (Figure 3, top row) and paralleled patterns of greater
411 bleaching prevalence in the first-bleaching event (Figure 2c). Similarly, site effects on coral
412 physiotypes were most pronounced in the first bleaching event (Table 1, Figure 4). Despite
413 greater bleaching at Reef 14 in October 2014 (Figure 2b), shifts in bleaching and recovery
414 performance envelopes were most distinct at Lilipuna (Figure 4). At the physiological level,
415 separation of physiotypes during and after thermal stress was driven by bleaching sensitivity and
416 symbiont cells/chlorophyll concentrations (p<0.001, Figure 4 & S2, Table S1). "Bleached"
417 physiotypes observed in periods of temperature stress (October 2014 and 2015) generally aligned
418 with colonies hosting the more thermally sensitive *Cladocopium* sp., although in the first event
419 coral physiotypes at Reef 14 symbiont communities did not separate according to symbiont
420 communities (Figure 4). Nevertheless, across all sampling periods *M. capitata* colonies
421 dominated by *Durusdinium* symbionts had higher symbiont cell densities, less variable areal
422 chlorophyll concentrations, and lower chlorophyll per symbiont cell (i.e., pg chlorophyll cell^{-1})
423 compared to colonies dominated by *Cladocopium* symbionts (p<0.001, Figure S2). While
424 *Cladocopium* chlorophyll cell^{-1} oscillated across bleaching-and recovery periods, *Durusdinium*
425 pg chlorophyll cell^{-1} remained stable (p=0.010) and was also slightly higher at Reef 14 relative to
426 Lilipuna across all timepoints (p=0.001, Table S1). Coral protein and total biomass was variable

427 across the study, but each showed positive correlations with *Durusdinium*-dominated
428 physiotypes from Lilipuna in bleaching and recovery periods (Figure 4). Overall, protein was
429 9% higher in *Durusdinum*-dominated colonies (p=0.031) and 32% higher in Lilipuna corals
430 during first bleaching (October 2014) but equivalent at all sampling points thereafter (p=0.002).
431 Total biomass was lower during recovery periods (p<0.001) in addition to being 20 – 40% higher
432 at Lilipuna compared to Reef 14 in all periods (except in February 2015 recovery) (p<0.001) and
433 ~30% higher in *Durusdinium*-dominated colonies during the second bleaching, but equivalent
434 across all colonies in other periods (p=0.012) (Figure S2).

435 Immunity and antioxidant metrics (i.e., MEL, PPO, POX, CAT, SOD) differed through time in
436 response to repeat bleaching and recovery (Figure 3, Figure 4) (p<0.001), while the
437 environmental influence from the Sit and Symbiont communities were less pronounced (Figure
438 S2, S3, and Table S1, S2). In the first event, corals responded to thermal stress by increasing
439 melanin synthesis (p<0.001) (with corresponding declines in PPO precursors) and increasing
440 catalase (p<0.001). During recovery, prophenoloxidase (*post-hoc*: p<0.001) and superoxide
441 dismutase increased (*post-hoc*: p<0.001) as melanin and catalase declined (Figure 4, 5, S2, & S3,
442 Table S1, S2). Notably, the melanin pathway increased in all corals in the 2014-bleaching event
443 regardless of bleaching sensitivity (i.e., loss or retention of symbionts/chlorophyll), or symbiont
444 community, and was a significant cellular response that shaped coral physiotypes in the first
445 bleaching event (Figure 5, first row). Corals in the second bleaching experienced a 6-fold
446 increase in melanin (October 2015 *vs*. February 2015) along with corresponding reductions in
447 prophenoloxidase, however, melanin activity was significantly less compared to the high
448 melanin activity observed in the first bleaching period (0.015 *vs*. 0.003 mg melanin mg tissue^{-1} in
449 first- versus second-bleaching period). Conversely to lower melanin in the second bleaching was

a peak in catalase activity, which reached its highest level in 2015 bleaching (*post-hoc*, $p<0.001$) and was most pronounced in corals from Lilipuna compared to Reef 14 (*post-hoc*, $p<0.001$) (Table S2). The peak in catalase during 2015 bleaching corresponded with the lowest observed peroxidase activity, particularly for *Cladocopium*-dominated colonies (*post-hoc*, $p=0.007$). Subsequently in the 2016 recovery period, catalase declined by ~70% and peroxidase activity doubled, reaching peak activity similar to those observed in time periods. In contrast to other immunity and antioxidant responses, superoxide dismutase showed a unique trajectory, increasing progressively through time with each sampling period, being at its highest constitutive activity after repeat bleaching in the 2016 recovery, which was double that observed in the first bleaching period (October 2014) ($p<0.001$). Similar to catalase, superoxide dismutase was also slightly higher in corals from Lilipuna compared to those at Reef 14 ($p=0.028$).

4. Discussion

Ocean thermal anomalies are reshaping the structure and function of coral reefs the world over. Three mass bleaching events occurred since 2014 (Hughes et al., 2017), and climate models predict that by mid-century bleaching will be an annual phenomena (van Hooidonk, Maynard, & Planes, 2013). The stress responses of corals to ocean warming is based on a network of dynamic interactions at biological and environmental levels, that can influence how they respond to the physiological challenges posed by a warming planet (Suggett & Smith, 2019). Accordingly, there is a need for holistic measures of coral response that incorporate the influence of and environmental history (Palumbi et al., 2014) on physiological legacy effects. For example assessment of symbiont community (Suggett, Warner, & Leggat, 2017) and cellular memory (Brown, Dunne, Edwards, Sweet, & Phongsuwan, 2015) in the coral holobiont during and after thermal stress, to test for shifting physiological status that may ameliorate or exacerbate coral

473 bleaching. Thus, physiotype tracking – or multivariate physiological and molecular time series
474 data – provide the capacity for identification of the cellular mechanisms that contribute to
475 resilience, acclimatization, and/or resistance that can allow for better predictions of coral
476 ecological responses to intensifying climate change and other regional disturbances (Vercelloni
477 et al., 2020).

478 Symbiosis – via differential performance of genera and types (Sampayo et al., 2008; Stat et al.,
479 2008) and the potential for switching and shuffling (Buddemeier & Fautin 1993; Baker et al.,
480 2003) – provide one clear example of how environmental history can shift performance
481 baselines. In this study, the role of dominant Symbiodiniaceae on holobiont physiotype was
482 present, but the symbiont community effects were often Period- or Site-dependent. The high
483 symbiont and chlorophyll *a* densities in *M. capitata* colonies dominated by *D. glynnii* across
484 Periods aligns with the paradigm of high thermotolerance within the genus *Durusdinium*
485 (Cunning et al., 2016; Lesser, Stat, & Gates, 2013; Silverstein, Cunning, & Baker, 2017; Wham
486 et al., 2017). However, antioxidant and immunity metrics were equivalent in colonies differing
487 in dominant symbiont partner, demonstrating negligible effects on antioxidant and immune traits
488 counter to the expectation that these would be modulated by symbiont-derived bleaching
489 resistance. In fact, this similarity was despite a greater loss of symbiont cells and
490 photopigmentation in colonies dominated by *Cladocopium* relative to *Durusdinium*. Symbiont
491 PSII photodamge is thought to lead the cascade of cellular events that culminate in bleaching
492 (Weis, 2008). However, some Symbiodiniaceae (*Symbiodinium* sp., *Durusdinium trenchii*) have
493 been shown to resist expulsion from the host despite incurring photodamage (Kemp et al., 2011;
494 Silverstein et al., 2017). Therefore, host mechanisms regulating redox status may be equally
495 important in coral thermal stress responses and decoupled from symbiont photophysiological

function (Krueger et al., 2015). In our study, the similarities observed between the antioxidant and immune responses of *Cladocopium*- and *Durusdinium*-dominated *M. capitata* colonies during both bleaching and recovery periods implicate an integral role of host mechanisms in coral responses to bleaching and recovery despite the presence of functionally distinct symbiont communities.

Shifts in physiotypes across bleaching and recovery periods were influenced by symbiont communities and the physical environmental at each site, however, the significance of these predictors were not uniform across time and varied during first- and second-bleaching events. For instance, the effects of reef site were clear in the first event, possibly relating to greater bleaching in 2014, which reduced symbiont cells in both corals dominated by *Cladocopium* sp. and thermotolerant *D. glynii* at Reef 14. In the context of previous works, our data agrees with findings that DHW in Kāneʻohe Bay were lower in the first bleaching event relative to the second (5 – 7 DHW [2014] *vs*. 10 – 12 DHW [2015]) (Bahr et al., 2017), while the percentage of bleached coral cover was higher in 2014 at our study sites (70% [2014] *vs*. 50% [2015]) and in Kāneʻohe Bay as a whole (45 – 77% [2014] *vs*. 30 – 55% [2015]) (Bahr et al., 2017; Ritson-Williams & Gates, 2020) possibly due to greater cumulative temperature stress in 2014 (Bahr et al., 2017; Ritson-Williams & Gates, 2020). While this study and others agree that lower bleaching prevalence was observed in Kāneʻohe Bay during the second bleaching event, we show this corresponded with attenuated Site × Symbiont effects on coral physiotypes (Figure 4 *bottom row*), suggesting a positive feedback between this interaction and bleaching severity. Therefore, the symbiont community harbored by corals is integral to bleaching responses and coral physiotypes (Suggett et al., 2017), but the relative importance of symbiont effects are also tempered by present environmental conditions and site-specific environmental histories.

Environmental conditions between Lilipuna and Reef 14 did not differ in terms of light availability, seawater temperature, or DHW (Figures 2 & S1). Nevertheless, the hydrodynamics between these reefs are substantial, producing considerable differences in seawater residence and pCO_2 variability (Drupp et al. 2011, 2013). In addition, the fringing reef habitat of Lilipuna with a close proximity to shore and silt-dominated backreef benthos is in stark contrast to Reef 14, which is patch reef pinnacle in the middle of the Kāneʻohe Bay lagoon. Together, the persistence of long-term hydrodynamic and biogeochemistry conditions between these reefs have manifested as physiological legacies in resident *M. capitata*, which we demonstrate influence the response of corals to regional bleaching events. In the laboratory, we showed *M. capitata* from Reef 14 exhibited greater antioxidant activity and F_v/F_m but lower melanin compared to Lilipuna colones (Wall et al., 2018). We hypothesize these patterns observed in laboratory and field studies may be underpinned by energetic limitations due to pCO_2 histories. Immunity is energetically expensive (Palmer et al. 2018b) and studies show corals exposed to high-pCO_2 upregulate genes for energy reserve metabolism (Vidal-Dupiol et al., 2013) and can have lower lipid biomass compared to corals at ambient-pCO_2 (Wall, Mason, Ellis, Cunning, & Gates, 2017). In this case flow and pCO_2-history may be an important factor in the physiological legacies of Kāneʻohe Bay *M. capitata*, which had a greater impact on during the first bleaching event relative to the second.

Coral physiotypes are influenced by the physiological state and integrity of the coral-Symbiodiniaceae symbiosis, which includes (but is not limited to) pigmentation and symbiont densities. As expected, we observed symbiont loss/repopulation to drive changes in coral physiotypes between bleaching and recovery periods, and in support of our hypothesis, prior events had cumulative impacts on physiotype trajectories. Notably, coral physiotypes occupied

542 distinct spaces in each sampling period and showed changing trajectory through time (2014-
543 2016) with the terminus of the trajectory in 2016-recovery resulting in a coral trait space
544 intermediate between prior bleaching and recovery periods (Figure 1). These trajectories may be
545 driven by those responses active in either the first or second bleaching-recovery periods (such as
546 melanin), or by changes in constitutive immune activity that are cyclical (e.g., PPO and CAT), or
547 continuously increasing through time (e.g., SOD). For instance, changes in total biomass
548 generally declined in the aftermath of bleaching, being lower in recovery periods, but did not
549 follow patterns in symbiont or photopigment concentrations. Coral tissue biomass also increased
550 through time and was highest in the second year (on average pooled across all samples). The
551 progressive increases in constitutive antioxidant activity exemplified by superoxide dismutase,
552 may also have contributed to maintenance of biomass and therefore enhanced the potential for
553 overall coral colony survival (Thornhill et al., 2011). Therefore, the moderate increase in tissue
554 biomass through time, despite repeat stress events, may be a result of greater resilience in corals
555 surviving repeat bleaching events, in addition to seasonal and stress-dependent fluctuations in
556 coral tissue (Fitt, McFarland, Warner, & Chilcoat, 2000; Wall, Ritson-Williams, Popp, & Gates,
557 2019).

558 The observed differences in antioxidant and immune activity in corals during repeat bleaching
559 and recovery reveal that shifts in cellular priorities and mechanisms for dealing with bleaching
560 stress exist, which ultimately shapes coral physiotypes and their trajectories through time and
561 between stress states (Figure 5). In this case, increased catalase activity during bleaching may be
562 linked to reactive oxygen and nitrogen species and increased host apoptosis-like pathways
563 (Hawkins, Krueger, Becker, Fisher, & Davy, 2014; Krueger et al., 2015). Conversely, the
564 accumulation of superoxide dismutase is decoupled from the onset and subsidence of thermal

565 stress seen in catalase. This response may reflect the "cellular memory" of corals to thermal
566 stress, acting as a buffer to future oxidative stress (Barshis et al., 2013; Brown et al., 2015) to
567 reduce bleaching effects while also contributing to greater tissue retention and post-bleaching
568 survival. Both corals and Symbiodiniaceae have a broad array of superoxide dismutase isoforms
569 (Krueger et al., 2015; Richier, Sabourault, & Courtiade, 2006), which can be used to combat
570 reactive oxygen species originating from damage to photomachinery and host mitochondria that
571 together can trigger apoptosis (Dunn et al., 2012). The persistent increase of superoxide
572 dismutase through time thus suggests antioxidant "frontloading" (Barshis et al., 2013) is an
573 important strategy used by corals during repetitive thermal stress to reduce damage.

574 Engagement of the melanin pathway (collectively here as the prophenoloxidase reservoir and the
575 melanin product) is known to be an important generalized stress response for mitigating disease
576 and thermal stress effects in corals (Mydlarz, Holthouse, Peters, & Harvell, 2008; Palmer et al.,
577 2010; Wall et al., 2018). Likewise, exposure to thermal stress increases antioxidant activity in
578 corals and Symbiodiniaceae (Gardner et al., 2017). In the first-bleaching event, the melanin
579 pathway was observed as the primary cellular response to warming in all corals, regardless of
580 bleaching sensitivity or symbiont community. This initial peak in melanin synthesis further
581 supports the central role of this pathway as a generalized cellular response to periodic stress,
582 including wound repair, disease, photodamage, and bleaching, and/or a photoprotective role to
583 reduce excess excitation energy (Mydlarz et al., 2008; Palmer, Traylor-Knowles, Willis, &
584 Bythell, 2011; Palmer et al., 2010; Wall et al., 2018). Its subsequent decline suggests the
585 melanin cascade may i) prime the antioxidant response and no longer is required to act as a
586 primary stress response role, or ii) had its capacity overwhelmed in the second-bleaching,
587 resulting in corals primarily using antioxidant stress response mechanisms. Our findings are

588 more in line with the former. Under this thermal-priming hypothesis, sublethal thermal stress
589 and high variable temperature regimes can contribute to protective mechanisms in the coral
590 holobiont and bleaching resistance (Ainsworth et al., 2016; Barshis et al., 2013; Bellantuono,
591 Granados-Cifuentes, Miller, Hoegh-Guldberg, & Rodriguez-Lanetty, 2012; Safaie et al., 2018).
592 However, an increased frequency or magnitude of thermal stress may overcome acquired cellular
593 responses (or symbiont partnerships) that support coral resistance to bleaching and mortality
594 (Ainsworth et al., 2016).

595 In our study, the hierarchy of cellular stress responses shifts away from melanin-synthesis in
596 favor of antioxidant activity (Figure 5), which may reflect the specialized nature of antioxidants
597 in mitigating cellular damage and maintaining coral holobiont homeostasis (Murphy, Collier, &
598 Richmond, 2019). Antioxidant proteins are central to cellular stress response network
599 (Scandalios, 2002) and in corals there is clear evidence that antioxidant enzymes have a stress
600 inducible role, driven by both environmental stress and pathogen elicitors (Mydlarz & Harvell,
601 2007; Mydlarz et al., 2008; Palmer et al., 2011). Indeed, *M. capitata* exposed to thermal stress
602 showed greater antioxidant activity but lower melanin when primed by a history of high pCO_2-
603 variability (Wall et al., 2018). The increasing role of catalase, peroxidase, and superoxide
604 dismutase activity through time and in the second-bleaching event, compared to attenuated
605 melanin-synthesis pathway (Figure 5, S1, S2), further demonstrates that specialized antioxidative
606 enzymes are important for coping with chronic or persistent stress, as often reported in thermally
607 stressed corals (Gardner et al., 2017; Lesser, 1997). Such constitutive upregulation of cellular
608 defenses have also been observed in corals from areas of high thermal variance (Barshis et al.,
609 2013) and those acclimatized to warmer conditions before the onset of bleaching stress
610 (Bellantuono et al., 2012). In particular, the continued increases in superoxide dismutase and the

611 persistence of high levels of peroxidase in the final 2016-recovery period indicate that increasing
612 constitutive levels of select antioxidants is key to maintaining cellular homeostasis after repeated
613 or chronic thermal perturbations (Figure 5, S2). Such shifts in homeostatic mechanisms through
614 strategies like gene plasticity (Kenkel & Matz, 2016) or constitutive frontloading (Barshis et al.,
615 2013) reveal the important role of immunity, antioxidants, and their interplay in shaping
616 ecological and evolutionary trajectories of reef corals and the future of coral reefs.

617 In conclusion, we show coral physiotypes were distinct during each period of bleaching and
618 recovery from 2014-2016, and this indicates physiological legacy effects driven by chronic stress
619 or beneficial acclimation influence both bleaching and recovery trajectories. Bleaching was
620 strongly related to symbiont communities and their sensitivity to thermal stress, which was
621 influenced by site environmental history. However, physiotypes were shaped by the cascade of
622 immune and antioxidant activity in the bleaching and recovery periods irrespective of symbiont
623 communities, providing a mechanistic hypothesis for the shifting coral physiotype baseline.
624 Specifically, we observed changes in constitutive immunity from one year to the next in response
625 to extreme stress events, indicating a fundamental change in the homeostatic mechanisms
626 employed by corals. In an era of increasing frequency and magnitude of thermal stress, the
627 ongoing study of mechanisms such as frontloading of genes like heat shock proteins and
628 antioxidants to attenuate bleaching effects and support thermal resilience (Barshis et al., 2013) is
629 of great importance. Additionally, the examination of acclimatization of populations to
630 environmental stress that may be gained through gene expression plasticity (Kenkel & Matz,
631 2016), or epigenetic mechanisms (Durante, Baums, Williams, Vohsen, & Kemp, 2019; Eirin-
632 Lopez & Putnam, 2019; Liew et al., 2018) will be critical. Our study provides details of cellular
633 and physiological legacy effects in corals and evidence that environmental memory shapes the

homeostatic strategies of corals, which ultimately dictates a coral's ability to respond to future stress events.

Acknowledgements

The authors acknowledge funding support from an Environmental Protection Agency STAR Fellowship Assistance Agreement (FP-91779401-1) to C.B.W and NSF 1756623 (Biological Oceanography, Integrative and Ecological Physiology, and EPSCoR) to H.M.P. The views expressed in this publication have not been reviewed or endorsed by the EPA and are solely those of the authors. We also thank P.J. Edmunds for insightful comments on an earlier manuscript draft. This is SOEST contribution number xxx and HIMB contribution number xxx.

Author contributions

C.B.W., C.A.R., L.D.M., R.D.G., and H.M.P. designed the project. C.B.W., C.A.R., L.D.M., and H.M.P. wrote the manuscript, and C.B.W. statistically analyzed the data. Coral collections were performed by C.B.W. and A.D.W. Laboratory analyses were performed by C.B.W., C.A.R., A.D.W., B.E.L., and D.E.K.

Competing interests

The authors declare they have no competing interests.

Data availability

All data and code to generate figures and perform analyses are archived and openly available at Github (https://github.com/cbwall/Gates-Mydlarz-bleaching-recovery/releases/tag/v4).

657 **Tables**

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Table 1. Results of PERMANOVA testing the effects of repeated bleaching and recovery periods on *Montipora capitata* corals hosting two distinct symbiont communities at two reef locations.

Factor	*df*	*SS*	R^2	*F*	*p*
Period	3	855.740	0.297	48.491	**<0.001**
Site	1	60.996	0.021	10.369	**<0.001**
Symbiont	1	202.349	0.070	34.399	**<0.001**
Period × Site	3	69.003	0.024	3.910	**<0.001**
Period × Symbiont	3	60.101	0.021	3.406	**<0.001**
Site × Symbiont	1	6.861	0.002	1.166	0.293
Period × Site × Symbiont	3	19.048	0.007	1.079	0.360
Residual	273	1605.902	0.558		
Total	288	2880.000	1.000		

Period = sequential bleaching and recovery events from October 2014 – February 2016, *Site* = Lilipuna or Reef 14, *Symbiont* = *Cladocopium* sp. or *Durusdinium glynnii* dominated symbiont community. *SS* = sum of squares; *df* = degrees of freedom; bold *p* values represent significant effects ($p < 0.05$).

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663 **Figures**
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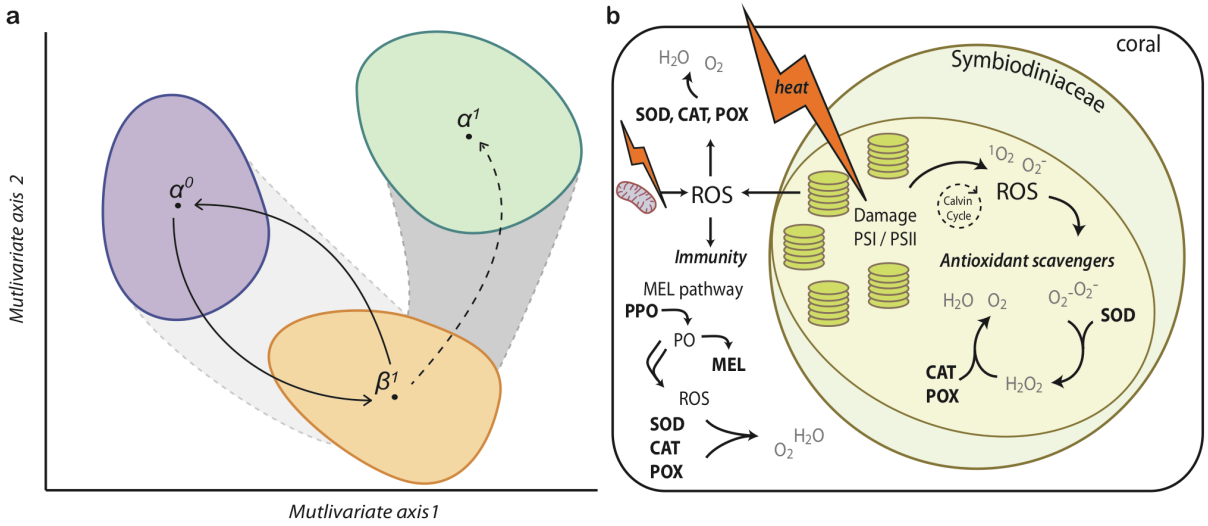

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669 **Figure 1.** (a) Multivariate analyses identify changes in organism physiotype through space and
670 time by accounting for the variation in multiple traits (Van Straalen, 2003). Transitions of
671 organisms from initial (α^0) to stressed states (β^1), such as during bleaching in response to a heat
672 wave, and their recovery to initial (α^0) (i.e., physiological resilience) or altered states (α^1).
673 Alternatively the absence of physiotype shifts may be observed in stress resistant individuals
674 (i.e., maintained at α^0). (b) Conceptual model of melanin and antioxidant activity in coral and
675 Symbiodiniaceae under heat stress (indicated by lightning bolts). Reactive oxygen species
676 (ROS), oxygen singlets ($^1O_2$) and superoxide (O_2^-), generated by symbiont photochemical
677 dysfunction and host mitochondria membrane damage, are neutralized by a combination of
678 antioxidant scavenger enzymes (e.g., superoxide dismutase [SOD], catalase [CAT], and
679 peroxidase [POX]). Host immunity via the melanin-synthesis pathway can also scavenge ROS
680 during intermediate steps that lead to the synthesis of melanin (MEL). Oxidative bursts during
681 the melanin-synthesis pathway can also create ROS that act as antimicrobials, which may also
682 lead to priming, or antioxidant enzymes up-regulation (shown in bold letters and dark lines).
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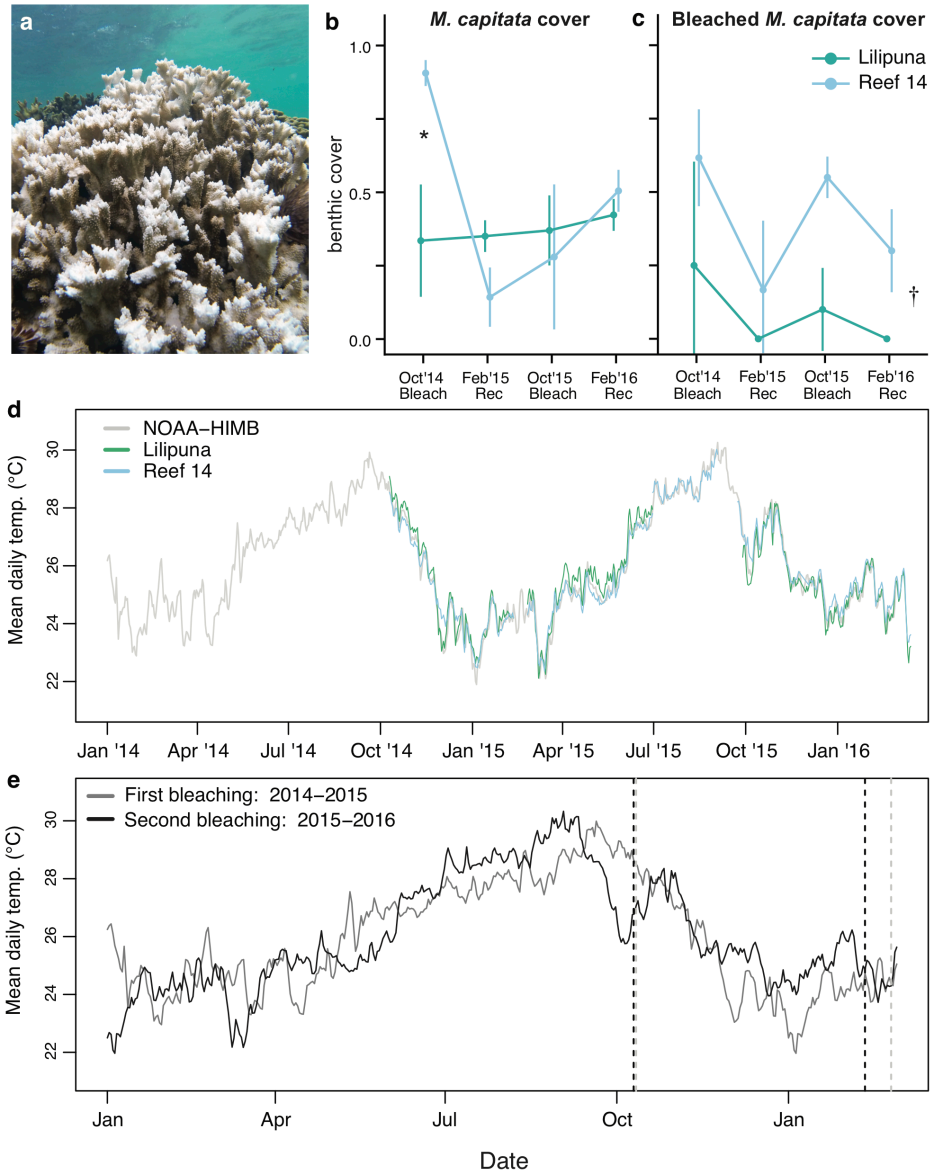

Figure 2. *Montipora capitata* cover and bleaching patterns at two reefs in relation to thermal regimes. (a) A bleached *M. capitata* colony and (b) benthic surveys of absolute *M. capitata* at two sites (Lilipuna and Reef 14) during and after thermal stress in 2014-2015 and 2015-2016. (c) The proportion of bleached *M. capitata* colonies scored during (October) and after (February) thermal stress (values are mean ± SD, *n* = duplicate transects). Symbols indicate differences among sites within a period (*) and among sites (†). (d) Overlay of mean daily temperatures at Lilipuna and Reef 14 and the NOAA-HIMB Moku o Loʻe buoy from January 2014 - January 2016 and (e) a comparison of temperature ramping and cooling between the first and second bleaching events relative to our collection dates. Vertical dashed lines indicate coral collections during bleaching and recovery periods in the first (*black,* 2014-2015) and second (*gray*, 2015-2016) events.

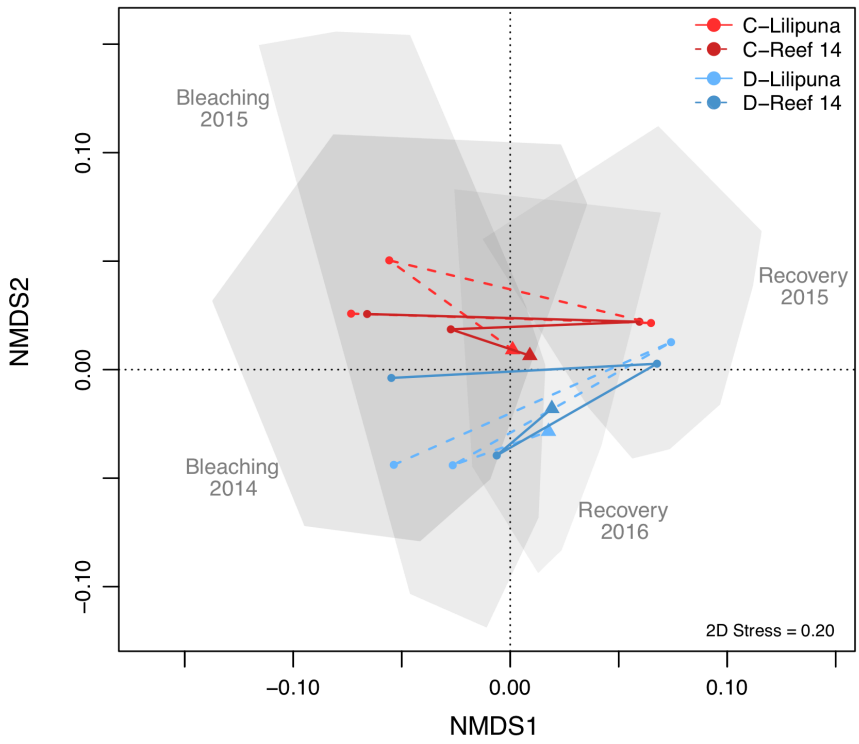

Figure 3. Non-metric multidimensional scaling (NMDS) analyses of coral performance envelopes during bleaching stress and post-bleaching recovery. *Montipora capitata* corals dominated by *Cladocopium* sp. (red) or *Durusdinium glynnii* (blue) symbionts from two sites (*solid lines* Lilipuna, *dashed lines* Reef 14). Convex hulls represent the coral physiotype (i.e., NMDS point clusters) of all corals in each time period, with points indicating mean centroids of physiotype polygon for each group. Lines show trajectories of mean centroids for each group across the four periods (Bleaching 2014, Recovery 2015, Bleaching 2015, Recovery 2016) and triangle arrowheads indicate trajectory termini.

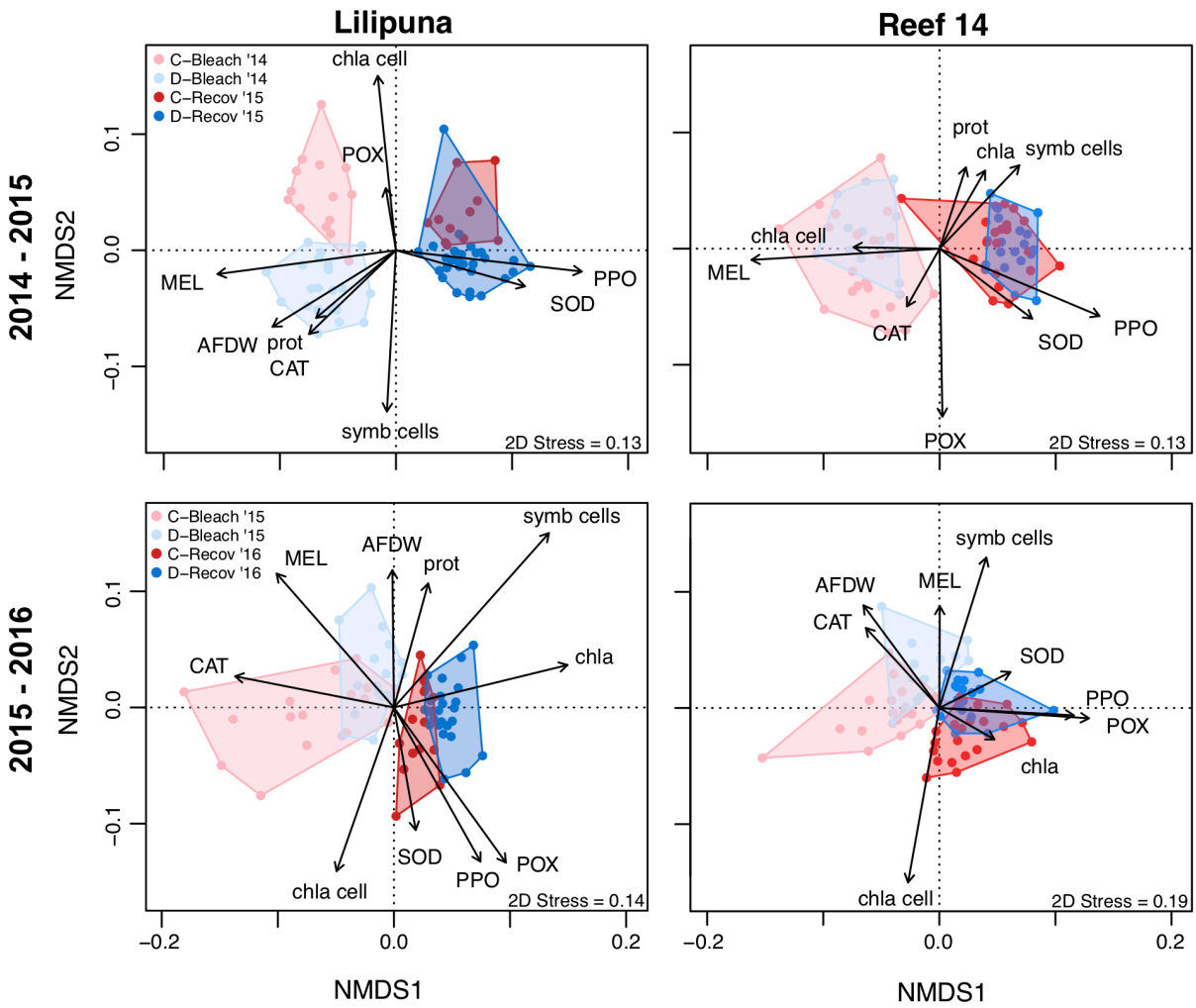

Figure 4. Non-metric multidimensional scaling (NMDS) analyses identify coral physiotypes separated by dominant symbiont type (colors), bleaching-recovery periods (lighter and darker shades), sites (columns), and events (rows). *Montipora capitata* corals dominated by *Cladocopium* sp. or *Durusdinium glynnii* symbionts from Lilipuna (left panel) and Reef 14 (right panel) during bleaching (Bleach) and recovery (Recov) periods. Biplot vectors (black arrows) represent significant physiology and immunity responses ($p < 0.05$) according to squared correlation coefficients (r^2). AFDW = ash-free dry weight biomass (mg gdw^{-1}), CAT = catalase, MEL = melanin, POX = peroxidase, PPO = prophenoloxidase, SOD = superoxide dismutase, chla = chlorophyll *a* μg cm^{-2}, chla cell = chlorophyll *a* per symbiont cell, prot = protein mg cm^{-2}, symb cells = symbionts cm^{-2}.

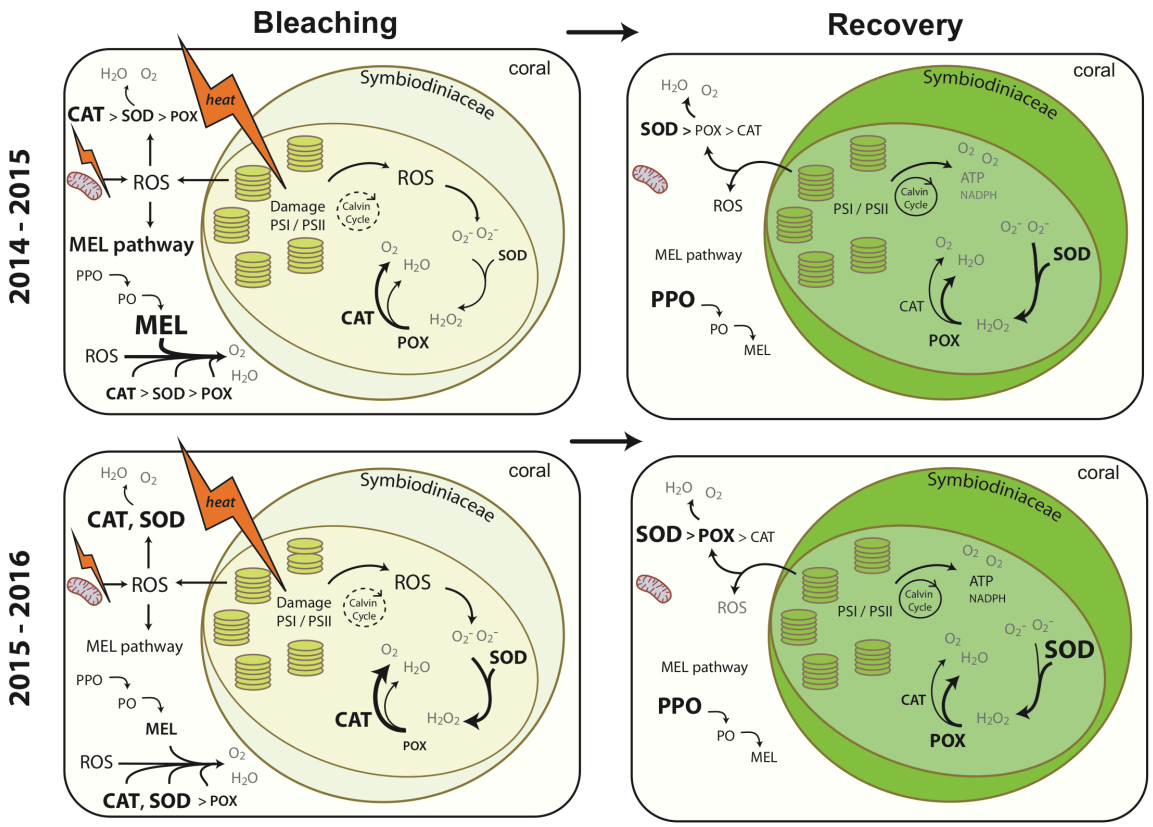

Figure 5. Schematic of mechanistic coral responses to repeat bleaching and recovery leading to shifted physiotypes. In the first event (*top row*) thermal stress leads to a substantial increase in MEL along with modest spikes in CAT and SOD. Corals in the first recovery showed increases in PPO (as the precursor to the melanin-pathway) as well as SOD and POX, while CAT activity declined. In the second event (*bottom row*), CAT and SOD spike with modest contributions of MEL and a general decline in POX. Corals in the second recovery had the highest levels of SOD across all time points, an increase in POX and a sharp decline in CAT (*see* Figure 4 for abbreviations).

References

Ainsworth, T. D., Heron, S. F., Ortiz, J. C., Mumby, P. J., Grech, A., Ogawa, D., … Leggat, W. (2016). Climate change disables coral bleaching protection on the Great Barrier Reef. *Science*, *352*(6283), 338–342. doi: 10.1126/science.aac7125

Bahr, K. D., Rodgers, K. S., & Jokiel, P. L. (2017). Impact of Three Bleaching Events on the Reef Resiliency of Kāneʻohe Bay, Hawaiʻi. *Frontiers in Marine Science*, *4*, 435. doi: 10.3389/fmars.2017.00398

Baker, A. C. (2003). Flexibility and Specificity in Coral-Algal Symbiosis: Diversity, Ecology, and Biogeography of *Symbiodinium*. *Annual Review of Ecology, Evolution, and Systematics*, *34*, 661–689.

Barshis, D. J., Ladner, J. T., Oliver, T. A., Seneca, F. O., Traylor-Knowles, N., & Palumbi, S. R. (2013). Genomic basis for coral resilience to climate change. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(4), 1387–1392. doi: 10.1073/pnas.1210224110

Bellantuono, A. J., Granados-Cifuentes, C., Miller, D. J., Hoegh-Guldberg, O., & Rodriguez-Lanetty, M. (2012). Coral Thermal Tolerance: Tuning Gene Expression to Resist Thermal Stress. *PloS One*, *7*(11), e50685–14. doi: 10.1371/journal.pone.0050685

Box, G. E. P., & Cox, D. R. (1964). An Analysis of Transformations. *Journal of the Royal Statistical Society. Series B, Statistical Methodology*, *26*(2), 211–243. doi: 10.1111/j.2517-6161.1964.tb00553.x

Brown, B. E., Dunne, R. P., Edwards, A. J., Sweet, M. J., & Phongsuwan, N. (2015). Decadal environmental "memory" in a reef coral? *Marine Biology*, *162*(2), 479–483. http://link.springer.com/10.1007/s00227-014-2596-2

Brown, B. E., Dunne, R. P., Goodson, M. S., & Douglas, A. E. (2000). Bleaching patterns in reef corals. *Nature*, *404*(6774), 142–143. doi: 10.1038/35004657

Buddemeier, R. W., & Fautin, D. G. (1993). Coral bleaching as an adaptive mechanism. *Bioscience*, *43*(5), 320–326.

Couch, C. S., Burns, J. H. R., Liu, G., Steward, K., Gutlay, T. N., Kenyon, J., … Kosaki, R. K. (2017). Mass coral bleaching due to unprecedented marine heatwave in Papahānaumokuākea Marine National Monument (Northwestern Hawaiian Islands). *PloS One*, *12*(9), e0185121. doi: 10.1371/journal.pone.0185121

Cunning, R., & Baker, A. C. (2013). Excess algal symbionts increase the susceptibility of reef corals to bleaching. *Nature Climate Change*, *3*, 259–262. doi: 10.1038/nclimate1711

Cunning, R., Ritson-Williams, R., & Gates, R. D. (2016). Patterns of bleaching and recovery of *Montipora capitata* in Kāne ‘ohe Bay, Hawai ‘i, USA. *Marine Ecology Progress Series*, *551*, 131–139. http://www.int-res.com/abstracts/meps/v551/p131-139/

De’ath, G., Fabricius, K. E., Sweatman, H., & Puotinen, M. (2012). The 27-year decline of coral cover on the Great Barrier Reef and its causes. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(44), 17995–17999. doi: 10.1073/pnas.1208909109

Drupp, P. S., De Carlo, E. H., Mackenzie, F. T., Sabine, C. L., Feely, R. A., & Shamberger, K. E. (2013). Comparison of CO_2 Dynamics and Air–Sea Gas Exchange in Differing Tropical Reef Environments. *Aquatic Geochemistry*, *19*(5-6), 371–397. doi: 10.1007/s10498-013-9214-7

Drupp, P., De Carlo, E. H., Mackenzie, F. T., Bienfang, P., & Sabine, C. L. (2011). Nutrient Inputs, Phytoplankton Response, and CO_2 Variations in a Semi-Enclosed Subtropical

Embayment, Kaneohe Bay, Hawaii. *Aquatic Geochemistry*, *17*(4), 473–498. doi: 10.1007/s10498-010-9115-y

Dunn, S. R., Pernice, M., Green, K., Hoegh-Guldberg, O., & Dove, S. G. (2012). Thermal stress promotes host mitochondrial degradation in symbiotic cnidarians: are the batteries of the reef going to run out? *PloS One*, *7*(7), e39024. doi: 10.1371/journal.pone.0039024

Durante, M. K., Baums, I. B., Williams, D. E., Vohsen, S., & Kemp, D. W. (2019). What drives phenotypic divergence among coral clonemates of *Acropora palmata*? *Molecular Ecology*, *28*(13), 3208–3224. doi: 10.1111/mec.15140

Edmunds, P. J., Adjeroud, M., Baskett, M. L., Baums, I. B., Budd, A. F., Carpenter, R. C., … Gates, R. D. (2014). Persistence and Change in Community Composition of Reef Corals through Present, Past, and Future Climates. *PloS One*, *9*(10), e107525. http://dx.plos.org/10.1371/journal.pone.0107525.s001

Edmunds, P. J., Pochon, X., Levitan, D. R., Yost, D. M., Belcaid, M., Putnam, H. M., & Gates, R. D. (2014). Long-term changes in *Symbiodinium* communities in *Orbicella annularis* in St. John, US Virgin Islands. *Marine Ecology Progress Series*, *506*, 129–144. doi: 10.3354/meps10808

Eirin-Lopez, J. M., & Putnam, H. M. (2019). Marine Environmental Epigenetics. *Annual Review of Marine Science*, *11*, 335–368. doi: 10.1146/annurev-marine-010318-095114

Fitt, W. K., McFarland, F. K., Warner, M. E., & Chilcoat, G. C. (2000). Seasonal patterns of tissue biomass and densities of symbiotic dinoflagellates in reef corals and relation to coral bleaching. *Limnology and Oceanography*, *45*(3), 677–685. doi: 10.4319/lo.2000.45.3.0677

Fuess, L. E., Mann, W. T., Jinks, L. R., Brinkhuis, V., & Mydlarz, L. D. (2018). Transcriptional analyses provide new insight into the late-stage immune response of a diseased Caribbean

coral. *Royal Society Open Science*, *5*(5), 172062. doi: 10.1098/rsos.172062

Gardner, S. G., Raina, J.-B., Nitschke, M. R., Nielsen, D. A., Stat, M., Motti, C. A., … Petrou, K. (2017). A multi-trait systems approach reveals a response cascade to bleaching in corals. *BMC Biology*, *15*(1), 117. doi: 10.1186/s12915-017-0459-2

Hawkins, T. D., Krueger, T., Becker, S., Fisher, P. L., & Davy, S. K. (2014). Differential nitric oxide synthesis and host apoptotic events correlate with bleaching susceptibility in reef corals. *Coral Reefs* , *33*(1), 141–153. doi: 10.1007/s00338-013-1103-4

Hughes, T. P., Anderson, K. D., Connolly, S. R., Heron, S. F., Kerry, J. T., Lough, J. M., … Wilson, S. K. (2018a). Spatial and temporal patterns of mass bleaching of corals in the Anthropocene. *Science*, *359*(6371), 80–83. doi: 10.1126/science.aan8048

Hughes, T. P., Kerry, J. T., Álvarez-Noriega, M., Álvarez-Romero, J. G., Anderson, K. D., Baird, A. H., … Wilson, S. K. (2017). Global warming and recurrent mass bleaching of corals. *Nature*, *543*(7645), 373–377. doi: 10.1038/nature21707

Hughes, T. P., Kerry, J. T., Baird, A. H., Connolly, S. R., Chase, T. J., Dietzel, A., … Woods, R. M. (2019). Global warming impairs stock–recruitment dynamics of corals. *Nature*, *568*(7752), 387–390. doi: 10.1038/s41586-019-1081-y

Hughes, T. P., Kerry, J. T., Baird, A. H., Connolly, S. R., Dietzel, A., Eakin, C. M., … Torda, G. (2018b). Global warming transforms coral reef assemblages. *Nature*, *556*(7702), 492–496. doi: 10.1038/s41586-018-0041-2

Innis, T., Cunning, R., Ritson-Williams, R., Wall, C. B., & Gates, R. D. (2018). Coral color and depth drive symbiosis ecology of *Montipora capitata* in Kāneʻohe Bay, Oʻahu, Hawaiʻi. *Coral Reefs* , *37*(2), 423–430. doi: 10.1007/s00338-018-1667-0

Jeffrey, S. W., & Humphrey, G. F. (1975). New spectrophotometric equations for determining

chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. *Biochemie Und Physiologie Der Pflanzen: BPP*, *167*(2), 191–194. doi: 10.1016/S0015-3796(17)30778-3

Jokiel, P. L., & Brown, E. K. (2004). Global warming, regional trends and inshore environmental conditions influence coral bleaching in Hawaii. *Global Change Biology*, *10*(10), 1627–1641. doi: 10.1111/j.1365-2486.2004.00836.x

Jones, R. J., Hoegh-Guldberg, O., Larkum, A. W. D., & Schreiber, U. (1998). Temperature-induced bleaching of corals begins with impairment of the CO_2 fixation mechanism in zooxanthellae. *Plant, Cell & Environment*, *21*(12), 1219–1230.

Kenkel, C. D., & Matz, M. V. (2016). Gene expression plasticity as a mechanism of coral adaptation to a variable environment. *Nature Ecology & Evolution*, *1*(1), 14. doi: 10.1038/s41559-016-0014

Krueger, T., Fisher, P. L., Becker, S., Pontasch, S., Dove, S., Hoegh-Guldberg, O., … Davy, S. K. (2015). Transcriptomic characterization of the enzymatic antioxidants FeSOD, MnSOD, APX and KatG in the dinoflagellate genus *Symbiodinium*. *BMC Evolutionary Biology*, *15*, 48. doi: 10.1186/s12862-015-0326-0

Krueger, T., Hawkins, T. D., Becker, S., Pontasch, S., Dove, S., Hoegh-Guldberg, O., … Davy, S. K. (2015). Differential coral bleaching—Contrasting the activity and response of enzymatic antioxidants in symbiotic partners under thermal stress. *Comparative Biochemistry and Physiology, Part A*, *190*(C), 15–25. doi: 10.1016/j.cbpa.2015.08.012

Lenth, R. V. (2019). Russell Lenth (2019). emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.3.5. https://CRAN.R-project.org/package=emmeans. *Journal of Statistical Software*, *69*(1), 1–33. https://CRAN.R-

project.org/package=emmeans

Lesser, M. P. (1997). Oxidative stress causes coral bleaching during exposure to elevated temperatures. *Coral Reefs* , *16*(3), 187–192. doi: 10.1007/s003380050073

Lesser, M. P., Stat, M., & Gates, R. D. (2013). The endosymbiotic dinoflagellates (*Symbiodinium* sp.) of corals are parasites and mutualists. *Coral Reefs* , *32*(3), 603–611. doi: 10.1007/s00338-013-1051-z

Liew, Y. J., Zoccola, D., Li, Y., Tambutté, E., Venn, A. A., Michell, C. T., … Aranda, M. (2018). Epigenome-associated phenotypic acclimatization to ocean acidification in a reef-building coral. *Science Advances*, *4*(6), eaar8028. doi: 10.1126/sciadv.aar8028

Lowe, R. J., Falter, J. L., Monismith, S. G., & Atkinson, M. J. (2009). A numerical study of circulation in a coastal reef-lagoon system. *Journal of Geophysical Research*, *114*(C6), 997. doi: 10.1029/2008JC005081

McClanahan, T. R., Ateweberhan, M., & Omukoto, J. (2007). Long-term changes in coral colony size distributions on Kenyan reefs under different management regimes and across the 1998 bleaching event. *Marine Biology*, *153*(5), 755–768. doi: 10.1007/s00227-007-0844-4

McWilliam, M., Pratchett, M. S., Hoogenboom, M. O., & Hughes, T. P. (2020). Deficits in functional trait diversity following recovery on coral reefs. *Proceedings. Biological Sciences / The Royal Society*, *287*(1918), 20192628. doi: 10.1098/rspb.2019.2628

Murphy, J. W. A., Collier, A. C., & Richmond, R. H. (2019). Antioxidant enzyme cycling over reproductive lunar cycles in *Pocillopora damicornis*. *PeerJ*, *7*, e7020. doi: 10.7717/peerj.7020

Mydlarz, L. D., & Harvell, C. D. (2007). Peroxidase activity and inducibility in the sea fan coral exposed to a fungal pathogen. *Comparative Biochemistry and Physiology. Part A,*

Molecular & Integrative Physiology, *146*(1), 54–62. doi: 10.1016/j.cbpa.2006.09.005

Mydlarz, L. D., & Palmer, C. V. (2011). The presence of multiple phenoloxidases in Caribbean reef-building corals. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology*, *159*(4), 372–378. doi: 10.1016/j.cbpa.2011.03.029

Mydlarz, L. D., Couch, C. S., Weil, E., Smith, G., & Harvell, C. D. (2009). Immune defenses of healthy, bleached and diseased *Montastraea faveolata* during a natural bleaching event. *Diseases of Aquatic Organisms*, *87*(1-2), 67–78. doi: 10.3354/dao02088

Mydlarz, L. D., Holthouse, S. F., Peters, E. C., & Harvell, C. D. (2008). Cellular Responses in Sea Fan Corals: Granular Amoebocytes React to Pathogen and Climate Stressors. *PloS One*, *3*(3), e1811–e1819. doi: 10.1371/journal.pone.0001811

Mydlarz, L. D., McGinty, E. S., & Harvell, C. D. (2010). What are the physiological and immunological responses of coral to climate warming and disease? *The Journal of Experimental Biology*, *213*(6), 934–945. doi: 10.1242/jeb.037580

NOAA. (2019, August 14). Retrieved August 14, 2019, from National Data Buoy Center, Station MOKH1 - 1612480 - Mokuoloe, HI website: https://www.ndbc.noaa.gov/station_page.php?station=mokh1

NOAA. (2020, April 20). Coral Reef Watch, NOAA Satellite and Information Services, https://coralreefwatch.noaa.gov/satellite/methodology/methodology.php

Oksanen, J., Guillaume, F. B., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., … Wagner, H. (2019). *vegan: Community Ecology Package*. 631–637. https://cran.r-project.org/web/packages/vegan/index.html

Palmer, C. V. (2018a). Immunity and the coral crisis. *Communications Biology*, *1*, 91. doi: 10.1038/s42003-018-0097-4

Palmer, C. V. (2018b). Warmer Water Affects Immunity of a Tolerant Reef Coral. *Frontiers in Marine Science*, *5*, 253. doi: 10.3389/fmars.2018.00253

Palmer, C. V., & Traylor-Knowles, N. (2012). Towards an integrated network of coral immune mechanisms. *Proceedings. Biological Sciences / The Royal Society*, *279*(1745), 4106–4114. doi: 10.1098/rspb.2012.1477

Palmer, C. V., Bythell, J. C., & Willis, B. L. (2010). Levels of immunity parameters underpin bleaching and disease susceptibility of reef corals. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*, *24*(6), 1935–1946. doi: 10.1096/fj.09-152447

Palmer, C. V., McGinty, E. S., Cummings, D. J., Smith, S. M., Bartels, E., & Mydlarz, L. D. (2011). Patterns of coral ecological immunology: variation in the responses of Caribbean corals to elevated temperature and a pathogen elicitor. *The Journal of Experimental Biology*, *214*(Pt 24), 4240–4249. doi: 10.1242/jeb.061267

Palmer, C. V., Traylor-Knowles, N. G., Willis, B. L., & Bythell, J. C. (2011). Corals Use Similar Immune Cells and Wound-Healing Processes as Those of Higher Organisms. *PloS One*, *6*(8), e23992–11. doi: 10.1371/journal.pone.0023992

Palumbi, S. R., Barshis, D. J., Traylor-Knowles, N., & Bay, R. A. (2014). Mechanisms of reef coral resistance to future climate change. *Science*, *344*(6186), 895–898. doi: 10.1126/science.1251336

Pinzón C, J. H., Beach-Letendre, J., Weil, E., & Mydlarz, L. D. (2014). Relationship between Phylogeny and Immunity Suggests Older Caribbean Coral Lineages Are More Resistant to Disease. *PloS One*, *9*(8), e104787. doi: 10.1371/journal.pone.0104787

Pinzón, J. H., Kamel, B., Burge, C. A., Harvell, C. D., Medina, M., Weil, E., & Mydlarz, L. D.

(2015). Whole transcriptome analysis reveals changes in expression of immune-related genes during and after bleaching in a reef-building coral. *Royal Society Open Science*, *2*(4), 140214. doi: 10.1098/rsos.140214

R Core Team. (2020). *R: A language and environment for statistical computing*. https://www.R-project.org

Richier, S., Sabourault, C., Courtiade, J., Zucchini, N., Allemand, D., & Furla, P. (2006). Oxidative stress and apoptotic events during thermal stress in the symbiotic sea anemone, *Anemonia viridis*. *The FEBS Journal*, *273*(18), 4186–4198. doi: 10.1111/j.1742-4658.2006.05414.x

Ritson-Williams, R., & Gates, R. D. (2020). Coral community resilience to successive years of bleaching in Kāneʻohe Bay, Hawaiʻi. *Coral Reefs* . doi: 10.1007/s00338-020-01944-4

Robinson, J. P. W., Wilson, S. K., Jennings, S., & Graham, N. A. J. (2019). Thermal stress induces persistently altered coral reef fish assemblages. *Global Change Biology*, *25*(8), 2739–2750. doi: 10.1111/gcb.14704

Safaie, A., Silbiger, N. J., McClanahan, T. R., Pawlak, G., Barshis, D. J., Hench, J. L., … Davis, K. A. (2018). High frequency temperature variability reduces the risk of coral bleaching. *Nature Communications*, *9*(1), 1671. doi: 10.1038/s41467-018-04074-2

Sale, T. L., Marko, P. B., Oliver, T. A., & Hunter, C. L. (2019). Assessment of acclimatization and subsequent survival of corals during repeated natural thermal stress events in Hawaiʻi. *Marine Ecology Progress Series*, *624*, 65–76. doi: 10.3354/meps13031

Sampayo, E. M., Ridgway, T., Bongaerts, P., & Hoegh-Guldberg, O. (2008). Bleaching susceptibility and mortality of corals are determined by fine-scale differences in symbiont type. *Proceedings of the National Academy of Sciences of the United States of America*,

105(30), 10444–10449. doi: 10.1073/pnas.0708049105

Scandalios, J. G. (2002). Oxidative stress responses--what have genome-scale studies taught us? *Genome Biology*, *3*(7), REVIEWS1019. doi: 10.1186/gb-2002-3-7-reviews1019

Schoepf, V., Grottoli, A. G., Levas, S. J., Aschaffenburg, M. D., Baumann, J. H., Matsui, Y., & Warner, M. E. (2015). Annual coral bleaching and the long-term recovery capacity of coral. *Proceedings. Biological Sciences / The Royal Society*, *282*(1819), 20151887. doi: 10.1098/rspb.2015.1887

Silverstein, R. N., Cunning, R., & Baker, A. C. (2017). Tenacious D: *Symbiodinium* in clade D remain in reef corals at both high and low temperature extremes despite impairment. *The Journal of Experimental Biology*, *220*(Pt 7), 1192–1196. doi: 10.1242/jeb.148239

Stat, M., Morris, E., & Gates, R. D. (2008). Functional diversity in coral–dinoflagellate symbiosis. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(27), 9256–9261.

Stimson, J., & Kinzie, R. A. (1991). The temporal pattern and rate of release of zooxanthellae from the reef coral *Pocillopora damicornis* (Linnaeus) under nitrogen-enrichment and control conditions. *Journal of Experimental Marine Biology and Ecology*, *153*(1), 63–74. doi: 10.1016/S0022-0981(05)80006-1

Stuart-Smith, R. D., Brown, C. J., Ceccarelli, D. M., & Edgar, G. J. (2018). Ecosystem restructuring along the Great Barrier Reef following mass coral bleaching. *Nature*, *560*(7716), 92–96. doi: 10.1038/s41586-018-0359-9

Suggett, D. J., & Smith, D. J. (2019). Coral bleaching patterns are the outcome of complex biological and environmental networking. *Global Change Biology*. doi: 10.1111/gcb.14871

Suggett, D. J., Warner, M. E., & Leggat, W. (2017). Symbiotic Dinoflagellate Functional

Diversity Mediates Coral Survival under Ecological Crisis. *Trends in Ecology & Evolution*, 1–11. doi: 10.1016/j.tree.2017.07.013

Thornhill, D. J., Rotjan, R. D., Todd, B. D., Chilcoat, G. C., Iglesias-Prieto, R., Kemp, D. W., … Fitt, W. K. (2011). A Connection between Colony Biomass and Death in Caribbean Reef-Building Corals. *PloS One*, *6*(12), e29535. doi: 10.1371/journal.pone.0029535

van Hooidonk, R., Maynard, J. A., & Planes, S. (2013). Temporary refugia for coral reefs in a warming world. *Nature Climate Change*, *3*(5), 508–511. doi: 10.1038/nclimate1829

Van Straalen, N. M. (2003). Ecotoxicology becomes stress ecology. *Environmental Science & Technology*, *37*(17), 324A – 330A. doi: 10.1021/es0325720

Venable, W. N., & Ripley, B. D. (2002). Modern Applied Statistics with S. Springer. *New York*.

Venn, A. A., Loram, J. E., & Douglas, A. E. (2008). Photosynthetic symbioses in animals. *Journal of Experimental Botany*, *59*(5), 1069–1080.

Vercelloni, J., Liquet, B., Kennedy, E. V., González-Rivero, M., Caley, M. J., Peterson, E. E., … Mengersen, K. (2020). Forecasting intensifying disturbance effects on coral reefs. *Global Change Biology*. doi: 10.1111/gcb.15059

Vidal-Dupiol, J., Zoccola, D., Tambutté, E., Grunau, C., Cosseau, C., Smith, K. M., … Tambutté, S. (2013). Genes related to ion-transport and energy production are upregulated in response to CO_2-driven pH decrease in corals: new insights from transcriptome analysis. *PloS One*, *8*(3), e58652. doi: 10.1371/journal.pone.0058652

Wall, C. B., Kaluhiokalani, M., Popp, B. N., Donahue, M. J., & Gates, R. D. (2020). Divergent symbiont communities determine the physiology and nutrition of a reef coral across a light-availability gradient. *The ISME Journal*. doi: 10.1038/s41396-019-0570-1

Wall, C. B., Mason, R. A. B., Ellis, W. R., Cunning, R., & Gates, R. D. (2017). Elevated pCO_2

affects tissue biomass composition, but not calcification, in a reef coral under two light regimes. *Royal Society Open Science*, *4*(11), 170683. doi: 10.1098/rsos.170683

Wall, C. B., Ricci, C. A., Foulds, G. E., Mydlarz, L. D., Gates, R. D., & Putnam, H. M. (2018). The effects of environmental history and thermal stress on coral physiology and immunity. *Marine Biology*, Vol. 165. doi: 10.1007/s00227-018-3317-z

Wall, C. B., Ritson-Williams, R., Popp, B. N., & Gates, R. D. (2019). Spatial variation in the biochemical and isotopic composition of corals during bleaching and recovery. *Limnology and Oceanography*, *64*(5), 2011–2028. doi: 10.1002/lno.11166

Weis, V. M. (2008). Cellular mechanisms of Cnidarian bleaching: stress causes the collapse of symbiosis. *The Journal of Experimental Biology*, *211*(Pt 19), 3059–3066. doi: 10.1242/jeb.009597

Wham, D. C., Ning, G., & LaJeunesse, T. C. (2017). *Symbiodinium glynnii* sp. nov., a species of stress-tolerant symbiotic dinoflagellates from pocilloporid and montiporid corals in the Pacific Ocean. *Phycologia*, *56*, 396–409. doi: 10.2216/16-86.1