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ORIGINAL PAPER

The effects of environmental history and thermal stress on coral physiology and immunity

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Abstract

Rising ocean temperatures can induce the breakdown of the symbiosis between reef building corals and *Symbiodinium* in the phenomenon known as coral bleaching. Environmental history may, however, influence the response of corals to stress and affect bleaching outcomes. A suite of physiological and immunological traits was evaluated to test the effect of environmental history (low vs. high variable pCO_2) on the response of the reef coral *Montipora capitata* to elevated temperature (24.5 °C vs. thermal ramping to 30.5 °C). Heating reduced maximum photochemical efficiency (F_v/F_m) and chlorophyll *a* but increased tissue melanin in corals relative to the ambient treatment, indicating a role of the melanin synthesis pathway in the early stages of thermal stress. However, interactions of environmental history and temperature treatment were not observed. Rather, parallel reaction norms were the primary response pattern documented across the two temperature treatments with respect to reef environmental history. Corals with a history of greater pCO_2 variability had higher constitutive antioxidative and immune activity (i.e., catalase, superoxide dismutase, prophenoloxidase) and F_v/F_m, but lower melanin and chlorophyll *a*, relative to corals with a history of lower pCO_2 variability. This suggests that reef environments with high magnitude pCO_2 variability promote greater antioxidant and immune activity in resident corals. These results demonstrate coral physiology and immunity reflect environmental attributes that vary over short distances, and that these differences may buffer the magnitude of thermal stress effects on coral phenotypes.

Introduction

The mutualistic symbiosis between scleractinian corals and dinoflagellates of the genus *Symbiodinium* underpins the function of hermatypic corals and their capacity to engineer tropical reef ecosystems (Putnam et al. 2017). Environmental disturbances destabilize this symbiosis and reduce the abundance of *Symbiodinium* cells and/or their photosynthetic pigments within coral tissues; a stress response called coral bleaching (Coles and Jokiel 1978). Elevated seawater temperatures have driven three global coral bleaching events to date (Hoegh-Guldberg et al. 2017), and ocean warming and the frequency of bleaching-level stress are predicted to increase as climate change intensifies (Heron et al. 2016; Hughes et al. 2017). While corals have persisted through considerable environmental change in the geologic record (Pandolfi and Kiessling 2014), the magnitude and rate of change in the thermal and chemical properties of seawater during the Anthropocene is unprecedented (Zeebe 2012; IPCC 2014; Hubbard 2015).

The response of corals to thermal stress is influenced by physical conditions that precede (Brown et al. 2002a; Middlebrook et al. 2008; Carilli et al. 2012; Guest et al. 2012; Ainsworth et al. 2016) and/or co-occur with elevated temperatures (Coles and Jokiel 1978; Dunne and Brown 2001; Nakamura and van Woesik 2001; Jokiel and Brown 2004; Anthony et al. 2008; Wiedenmann et al. 2012). It is recognized that organisms are equipped with diverse biochemical mechanisms to acclimate and adapt to physiological stress (Hochachka and Somero 2002), and in corals, evidence supports the role of the coral animal (Kenkel

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et al. 2013a) and *Symbiodinium* (Levin et al. 2016) in confronting environmental challenges (Edmunds and Gates 2008; Hume et al. 2016; Palumbi et al. 2014). For instance, corals experiencing thermal (Lesser 2004; Fitt et al. 2009; Kenkel et al. 2011, 2013b) and/or photo-stress (Brown et al. 2002b) can mitigate cellular damage by up-regulating stress proteins (i.e., fluorescent and heat-shock proteins) (Lesser 2004; Palmer et al. 2009; Louis et al. 2017). Coral immunity and pathogen defense mechanisms (e.g., melanin synthesis pathway) (Söderhäll and Cerenius 1998) are also dynamically regulated in response to bleaching stress (Mydlarz et al. 2009). Indeed, maintaining high baseline immunity and tissue-protective properties (e.g., antioxidative enzymes) may represent a conserved mechanism of coral physiological resilience to both disease and thermal stress (Weis 2008; Palmer et al. 2010; Louis et al. 2017).

Rising concentrations of carbon dioxide (pCO_2) and other greenhouse gases are driving global climate change by increasing air and ocean temperatures (IPCC 2014). In addition, the dissolution of atmospheric CO_2 in the upper ocean is disrupting seawater carbonate chemistry and causing ocean acidification (OA), which threatens the net calcification of coral reef ecosystems (Andersson and Gledhill 2013). Exposure to elevated pCO_2 also has the potential to influence coral immunity and the response of corals to warming temperatures (Anthony et al. 2008; Kaniewska et al. 2012). For instance, pCO_2 can exacerbate thermal stress effects and cause bleaching in some corals (Anthony et al. 2008; *but see* Wall et al. 2014; Noonan and Fabricius 2016). Additionally, corals in experimentally elevated pCO_2 conditions or from naturally high pCO_2-seeps show an upregulation of genes involved in oxidative stress and innate immune pathways (Kaniewska et al. 2012; Kenkel et al. 2017).

Natural field settings where elevated pCO_2 conditions are persistent (Fabricius et al. 2011; Albright et al. 2015; Padilla-Gamiño et al. 2016; Kenkel et al. 2017), or dynamic in nature (Drupp et al. 2013), can provide insight into the consequences of high pCO_2/low pH on marine taxa not apparent in short-term laboratory experiments (Calosi et al. 2013; Noonan and Fabricius 2016). Leveraging natural field settings with unique seawater properties can, therefore, clarify the influence of pCO_2 history on coral physiology and thermal stress responses (Noonan and Fabricius 2016). Within Kāne'ohe Bay (windward O'ahu, Hawai'i) a combination of factors (e.g., physical forcing, seawater residence time, watershed and oceanic biogeochemistry) (Lowe et al. 2009; Drupp et al. 2011, 2013; Shamberger et al. 2011) has created regions where corals are exposed to seawater pCO_2 projected to occur under end-of-the-century climate change scenarios (van Vuuren et al. 2011). As such, Kāne'ohe Bay provides an ideal natural setting to address the hypothesis that environmental history—specifically, regimes of contrasting pCO_2 variability (Drupp et al. 2011, 2013)—alters the biology of reef corals and their response to stress.

The dynamic interplay between multiple stressors, environmental history and physiological acclimatization shapes reef resilience in varying ways. The goal of this study was to test the interaction of environmental history in the context of pCO_2 variability and short-term thermal stress on the physiological, photochemical, and immunological responses of corals from two Kāne'ohe Bay reefs with contrasting pCO_2 conditions. Considering the potential for elevated pCO_2 to negatively influence coral performance and cause bleaching, we tested the hypothesis that corals from environments with a history of high variable pCO_2 would show greater sensitivity to thermal stress by exhibiting greater declines in photochemical efficiency, photopigment concentrations and *Symbiodinium* densities relative to corals from environments with a history of low variable pCO_2. We also expected corals from high variable pCO_2 environments to display increased antioxidative activity as a mechanism to mitigate cellular damage (Weis 2008), as well as greater immune activity measured by elevated melanin synthesis pathway activity (prophenoloxidase and melanin).

Materials and methods

Study site description

Reef selection was driven by previous characterization of the physical and chemical conditions occurring within the reef–lagoon system of Kāne'ohe Bay, O'ahu, Hawai'i (21°26′06.0″N, 157°47′27.9″W) (Lowe et al. 2009; Drupp et al. 2011, 2013; Shamberger et al. 2011). The hydrodynamics of Kāne'ohe Bay are highly heterogeneous due to different physical forcing (i.e., wave, wind, tidal) among bay regions (Lowe et al. 2009). In the southern lagoon (zone 6, sensu Lowe et al. 2009), geographic isolation and resistance to wave-driven forcing reduce seawater mixing and produce prolonged seawater residence times (ca. 30–60 days). Conversely, in the central lagoon (zone 5, sensu Lowe et al. 2009) seawater residence times are reduced (ca. 10 days) due to greater wave-driven forcing and oceanic influences (Lowe et al. 2009). The nexus of these physical factors and biological processes (i.e., photosynthesis/respiration, calcification/dissolution) produce distinct pCO_2 conditions within Kāne'ohe Bay (Drupp et al. 2011, 2013; Shamberger et al. 2011). The NOAA Pacific Marine Environmental Laboratory (PMEL) and the University of Hawai'i Coral Reef Instrumented Monitoring and CO_2-Platform (CRIMP) buoys provide high-resolution time-series data for water column carbonate chemistry and pCO_2, which have been applied to evaluate the relationship between physical forcing, nutrient input, and reef metabolism on air–sea CO_2 exchanges on

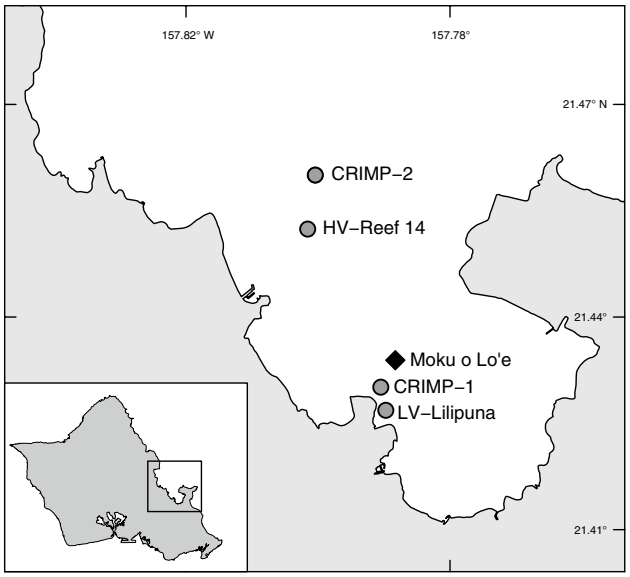

Fig. 1 Map of Kāne'ohe Bay on the windward side of the island of O'ahu, Hawai'i, USA, detailing locations of two reefs characterized by an environmental history of low variable pCO_2 (LV–Lilipuna) and high variable pCO_2 (HV–Reef 14), NOAA PMEL buoys (CRIMP-1 and CRIMP-2), and the Hawai'i Institute of Marine Biology (Moku o Lo'e)

reefs across O'ahu, including Kāne'ohe Bay (Fig. 1) (see De Carlo et al. 2007; Drupp et al. 2011, 2013; Shamberger et al. 2011). These data reveal water column pCO_2 in southern Kāne'ohe Bay (buoy: CRIMP-1, Sabine et al. 2012, or CRIMP–CO_2 buoy sensu Drupp et al. 2011) and adjacent to the barrier reef (buoy: CRIMP-2 buoy, sensu Drupp et al. 2013) are comparable (ca. 450 µatm pCO_2); however, the range in pCO_2 varies in these two regions of Kāne'ohe Bay, being 225–671 µatm pCO_2 at CRIMP-1 (i.e., low variable pCO_2) (Fig. 2a) and 196–976 µatm pCO_2 at CRIMP-2 (i.e., high variable pCO_2) (Fig. 2b) (Drupp et al. 2011, 2013).

Reefs adjacent to CRIMP-1 and CRIMP-2 buoy deployments were identified as the sites for this study. Corals were collected from an inshore fringing reef (21°25′36.8″N, 157°47′24.0″W) (Lilipuna) located in the southwestern basin of Kāne'ohe Bay, 350 m south of Moku o Lo'e [Coconut Island and the Hawaiian Institute of Marine Biology (HIMB)] and proximate to CRIMP-1 (Drupp et al. 2011), and an inshore patch reef (21°27′08.6″N, 157°48′04.7″W) (Reef 14) in the central lagoon of Kāne'ohe Bay and adjacent to CRIMP-2 (Drupp et al. 2013) (Fig. 1). Hereafter, the two reefs where corals were collected will be referred to as 'low variable pCO_2 Lilipuna' (LV–Lilipuna)—adjacent to CRIMP-1, experiencing prolonged seawater residence

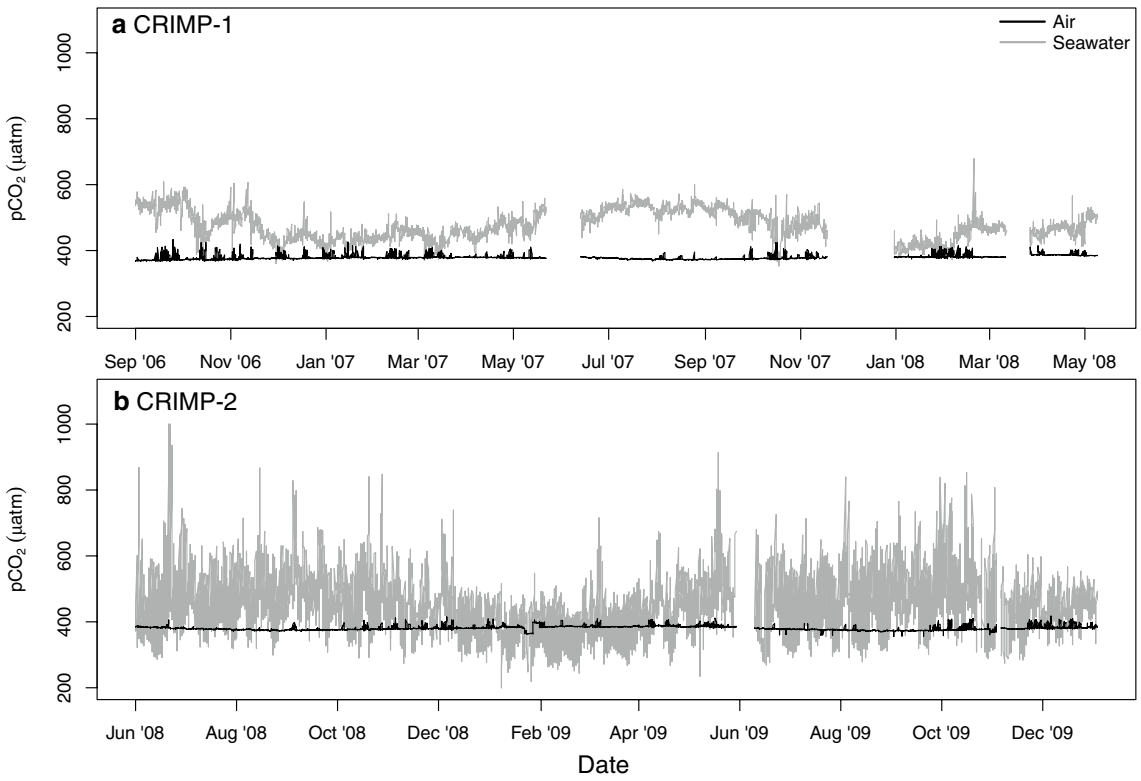

Fig. 2 Concentrations of carbon dioxide (µatm pCO_2) in seawater and air at CRIMP-1 (upper panel) and CRIMP-2 (lower panel) moored at two locations within Kāne'ohe Bay, O'ahu, Hawai'i, USA (see Fig. 1) (Data: NOAA PMEL; Drupp et al. 2011, 2013; Sabine et al. 2012; Sutton et al. 2016)

and low pCO_2 flux—and 'high variable pCO_2 Reef 14' (HV–Reef 14)—adjacent to CRIMP-2, experiencing short seawater residence and high pCO_2 flux.

Environmental monitoring

Seawater pCO_2 conditions proximate to each reef (LV–Lilipuna, HV–Reef 14) were sourced from quality-controlled, publically available NOAA PMEL-CRIMP CO_2-Platform moored buoys (https://www.pmel.noaa.gov/co2/story/Coral+Reef+Moorings) (Sabine et al. 2012; Sutton et al. 2016), using two deployment periods: CRIMP-1 (near LV–Lilipuna) 25 Nov 2005–16 Jun 2007, and CRIMP-2 (near HV–Reef 14) 11 Jun 2008–12 Jan 2010. During the present study's experimental period (Jan–Apr 2014), Hobo loggers (Onset Computer Corp., Bourne, Massachusetts) cross-calibrated to a certified digital thermometer (5-077-8, ± 0.05 °C, Control Company, Webster, Texas) recorded temperatures at each reef site at the depth of coral collection (< 1 m). Separately, a comparison of light availability at LV–Lilipuna and HV–Reef 14 was performed (Oct 2014–Dec 2014) at < 1 m for each reef using Odyssey photosynthetic irradiance loggers (Dataflow Systems Limited, Christchurch, New Zealand) cross-calibrated to a Licor (LI-1400, Lincoln, Nebraska) equipped with a cosine quantum sensor (LI-192) (Long et al. 2012). While collections of environmental data (i.e., pCO_2, temperature, PAR) are temporally distinct and reefs may not experience seawater with identical carbonate chemistry as measured at buoys, collectively, these data are useful in describing trends in environmental characteristics among the two reef locations and their relationship to coral performance.

Coral collection and laboratory treatments

Fifty *M. capitata* (Dana 1846) branch tips (ca. 4 cm in length) were collected from each reef on 5 February 2014 (State of Hawai'i Department of Land and Natural Resources, Special Activity Permit 2013-47); accordingly, holobiont biomass should be considered seasonally acclimated to Kāne'ohe Bay winter conditions (Fitt et al. 2000). Fragments were transported in seawater to HIMB and epoxied to plastic bases using Z-spar A788 splash zone compound in a flow-through water table. One day after collection, corals were transferred into two custom-built experimental flow-through aquaria (50 L; Aqualogic, Inc., North Haven, Connecticut) receiving sand-filtered seawater from Kāne'ohe Bay at a rate of ca. 0.2 L min^{-1} and maintained at ambient conditions of 36 salinity and ca. 24.5 °C. After 1 week of acclimation to laboratory conditions, corals ($N = 100$) were randomly allocated to four flow-through aquaria (50 L) (two replicate tanks $treatment^{-1}$) at a density of 25 fragments $tank^{-1}$.

Seawater temperatures in each tank were independently regulated using a combination of 100 W submersible heaters and a programmable solenoid controller that independently regulated the delivery of chilled water through an in-line mixing column (Multi Temp MT-1 Model #2TTB3024A1000AA, Aqualogic) receiving tank seawater. Temperature treatments represented ambient temperature conditions (24.5 °C: Ambient) for January–February 2014 (NOAA 2017) and a heated treatment gradually ramped to elevated temperatures (30.5 °C: Heated) (Fig. 3). Temperature ramping lasted 7 days and increased at a rate of ca. 0.75 °C day^{-1}. Corals were maintained at 30.5 °C for 2 days, which is near the upper thermal limit of Hawaiian reef corals (Coles et al. 1976; Coles and Jokiel 1978). The ramping regime was comparable to other studies (Middlebrook et al. 2010) and was implemented to avoid acute heat shock, ensuring observation of progressive heating effects on coral performance. Corals were exposed to treatments from 11 to 19 February 2014. ANOVA confirmed the establishment of two separate temperature treatments ($F_{1,239} = 231.300$, $P < 0.001$); temperatures did not differ among replicate heated tanks ($F_{1,130} = 0.018$, $P = 0.893$) but replicate ambient tanks differed by 0.24 °C ($F_{1,107} = 71.578$, $P < 0.001$).

During the experiment, temperature was monitored throughout the day using a certified digital thermometer (Fisher Scientific 15-077-8, ± 0.05 °C, Hampton, New Hampshire); photosynthetically active radiation (PAR) and salinity were measured daily at three time points (10:00, 12:00, 16:00 h) using a 4π-spherical quantum sensor (Li-Cor) and a conductivity meter (Model 63, YSI Inc., Yellow

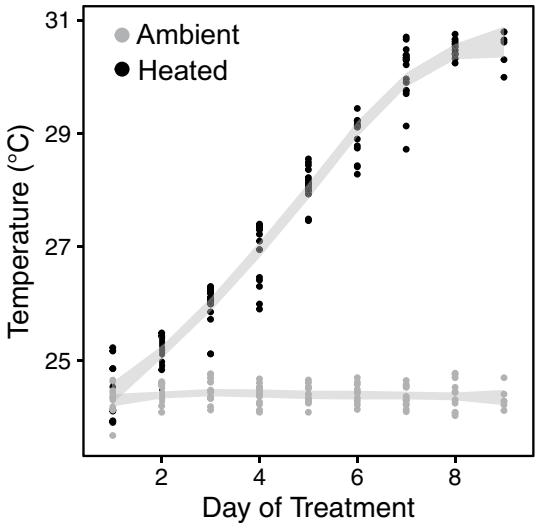

Fig. 3 Raw temperature measurements from experimental treatments representing an ambient (Ambient, gray symbols) and a progressively warming condition (Heated, black symbols). Shaded regions for each treatment indicate 95% confidence intervals of locally weighted least squares regression

Springs, Ohio), respectively. Light was supplied to each tank by LED-lamps (Sol Super Blue, Aqua Illumination, Ames, Iowa) on a 12-h light:12-h dark cycle and programmed to mimic diel changes in light intensities from sunrise (06:00 h) to sunset (18:00 h) (Gibbin et al. 2015). Photosynthetically active radiation (PAR) at the daily maximum (12:00 h) was ca. 750 µmol photons m^{-2} s^{-1}, and each treatment tank received a mean (± SE, $n = 19$) PAR of 452–467 ± 54–57 µmol photons m^{-2} s^{-1}. PAR did not differ among the four treatment tanks ($F_{3,72} = 0.014$, $P = 0.998$) or among days of the experiment ($F_{6,69} = 0.894$, $P = 0.505$).

Physiological metrics

Pulse amplitude modulation (PAM) fluorometry was used to measure temperature effects on the photochemical performance of *Symbiodinium* spp. *in hospite* for all corals on the 8th day of treatment exposure using a Diving-PAM (Waltz, GmbH, Effeltrich, Germany). The Diving-PAM was operated at a gain of 7, saturation intensity of 8, an electronic signal damping of 2; under these conditions, the signal to noise ratio was optimized and the minimum fluorescence was stabilized at ca. 400–700 (arbitrary units). The minimum (F_o) and maximum (F_m) fluorescence yield and the maximum photochemical efficiency (F_v/F_m) of dark-adapted PSII reaction centers were measured during the day at 14:00 h (which coincided with the period of peak PAR exposure (Jones and Hoegh-Guldberg 2001) following a 20-min dark acclimation period. Measurements were obtained using the 5-mm diameter fiber-optic probe positioned 5 mm above the surface of the coral tissue following F_o stabilization. Following 9 days of exposure, all corals were immediately snap-frozen in liquid nitrogen and stored at – 80 °C. A subset of corals were used for physiological assays (*Symbiodinium* density, chlorophyll *a* extraction, and total protein; $n = 10$–13 $treatment^{-1}$) and analyzed at HIMB. Another subset of corals were used in immunological assays (protein, melanin, prophenoloxidase, catalase, and superoxide dismutase; $n = 11$–12 $treatment^{-1}$) and analyzed at the University of Texas at Arlington. Corals remained at – 80 °C and were not thawed prior to analysis.

For physiological analyses, coral tissue was removed from the skeleton using an airbrush and filtered seawater (0.7 µm). The resulting coral tissue slurry was briefly homogenized and aliquots taken for each response variable, following Wall et al. (2017). The coral skeleton was placed in 10% bleach solution and allowed to dry at 60 °C before measuring the surface area of the skeleton by the paraffin wax-dipping technique (Stimson and Kinzie 1991). *Symbiodinium* densities were quantified by repeated cell counts ($n = 6$ $sample^{-1}$) using a haemocytometer, and cell densities were standardized to coral surface area (cells cm^{-2}). Chlorophyll *a* was extracted by centrifuging the tissue slurry (13,000 rpm × 3 min) and isolating the alga pellet, followed by adding 100% acetone and extracting at − 20 °C in darkness for 36 h. The pigment extract was measured spectrophotometrically ($\lambda = 630$ and 663 nm) and chlorophyll *a* concentrations were determined using equations for dinoflagellates (Jeffrey and Humphrey 1975). Chlorophyll *a* was standardized to surface area (µg cm^{-2}) and to the density of *Symbiodinium* cells (pg $cell^{-1}$). Total protein (soluble and insoluble) in the tissue slurry was measured using the Pierce BCA (bicinchoninic acid) Protein Assay Kit (Pierce Biotechnology, Waltham, Massachusetts). Solubilization of protein was achieved by adding 1 M NaOH to the tissue slurry, heating at 90 °C for 1 h, and neutralizing to pH ca. 7.5 using 1 N HCl. The total protein in three technical replicates $sample^{-1}$ was measured in a 96-well microtiter plate ($\lambda = 562$ nm) against a bovine serum albumin standard curve and standardized to coral surface area (mg protein cm^{-2}).

Immunological assays

Coral immunology was assessed following previously established protocols for protein extractions and enzymatic assays (Mydlarz et al. 2009, 2010; Palmer et al. 2010, 2011a; Mydlarz and Palmer 2011). Briefly, 3–4 mL of coral tissue slurry was obtained by airbrushing with coral extraction buffer (100 mM TRIS buffer + 0.05 mM dithiothreitol). The resulting slurry was homogenized for 1 min on ice using a hand-held tissue homogenizer (Powergen 125, Fisher Scientific, Waltham, Massachusetts). For melanin concentration estimates, 1 mL of the tissue slurry was freeze-dried for 24 h using a VirTis BTK freeze-dryer (SP Scientific, Warminster, Pennsylvania). The remaining slurry was centrifuged at 4 °C at 2500×*g* (Eppendorf 5810 R centrifuge, Hamburg, Germany) for 5 min to remove cellular debris, and enzymatic assays were performed on aliquots of the supernatant, representing a cell-free extract or soluble protein extract of the host coral. All assays were run in duplicates on separate 96-well microtiter plates using a Synergy HT multidetection microplate reader using Gen5 software (Biotek Instruments, Winooski, Vermont). Protein concentrations were estimated using the RED660 protein assay (G Biosciences, Saint Louis, Missouri) against a bovine serum albumin standard curve.

Antioxidant profile

Antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD) were measured. CAT is monitored as a change in absorbance after 25 mM hydrogen peroxide is added to crude protein extract and 50 µL of 10 mM PBS (pH 6.0). CAT activity was estimated as the mM H_2O_2 scavenged min^{-1} mg $protein^{-1}$. SOD activity was analyzed using a commercially available kit (SOD determination

kit #19160; Sigma-Aldrich, St. Louis, Missouri) following manufacturer's instructions and expressed as SOD activity mg protein^{-1}. SOD activity was estimated by comparing the absorbance of samples at 450 nm to a positive and negative standard after incubating 10 µL of crude protein extract with manufacturer-provided reagents.

Melanin synthesis pathway

Prophenoloxidase (PPO) activity and melanin (MEL) concentration per sample were used to study the melanin synthesis pathways. PPO activity was determined by incubating 20 µL of protein extract and 50 µL of 10 mM phosphate buffered saline (PBS) (pH 7.0) at room temperature with 20 µL of trypsin (0.2 mg mL^{-1} concentration) for 30 min. 20 µL of 25 mM L-DOPA (Sigma-Aldrich) was then added as a substrate. PPO activity was estimated as change in absorbance min^{-1} mg protein^{-1}. MEL concentration was estimated using a weighed freeze-dried portion of initial tissue slurry. Melanin was allowed to extract for 48 h in 400 µL of 10 M NaOH after a brief period of bead-beating with 1-mm glass beads. 65 µL of extracted melanin was used to determine endpoint absorbance at 495 nm and resulting values were standardized to a standard curve of commercial melanin (Sigma-Aldrich) and calibrated to µg melanin mg tissue^{-1}.

Statistical analysis

Dependent variables were analyzed using a two-way linear model using *lme* with temperature treatment (Ambient vs. Heated) and reef environmental history (LV–Lilipuna vs. HV–Reef 14) as fixed effects. Environmental data (light and temperature) were analyzed using a linear mixed effect model in the package *lme4* (Douglas et al. 2015) with site as a fixed factor and the repeated measure (sampling time) as a random factor. Analysis of variance tables (linear models) and analysis of deviance tables (linear mixed effect models) were calculated using Type-II sum of squares with Satterthwaite approximation of degrees of freedom using *lmerTest* (Kuznetsova et al. 2016). The assumptions of analysis of variance were confirmed by graphical inspection of residuals combined with Shapiro–Wilk's test and Levene's test and transformed where assumptions of ANOVA were not met. Transformations were selected using a Box–Cox power transformation using the package *MASS* (Box and Cox 1964; Venables and Ripley 2002). All analyses were performed in *R*, version 3.3.0 (R Development Core Team 2016). Experimental data and *R* code to reproduce figures and analyses are accessible on Zenodo (https://zenodo.org/record/1175034).

Results

Environmental data

During the experimental period (Jan–Apr 2014), temperature did not differ among the two study sites ($F_{1,12417} = 2.961$, $P = 0.085$). Similarly, the daily mean temperature (ca. 24.6 °C) ($F_{1,65} = 0.137$, $P = 0.713$) and maximum daily temperatures did not differ among the two reefs ($F_{1,65} = 0.013$, $P = 0.910$). However, on average LV–Lilipuna experienced a 0.12 °C lower daily minimum temperature during the experimental period ($F_{1,65} = 4.636$, $P = 0.035$) which led to an overall greater daily temperature range ($F_{1,65} = 5.250$, $P = 0.025$) at LV–Lilipuna (1.27 °C day^{-1}) compared to HV–Reef 14 (1.16 °C day^{-1}). Daily integrated light availability (Oct 2014–Dec 2014) was 10.6 mol photons day^{-1} and did not differ among the two study sites ($F_{1,68} = 0.004$, $P = 0.949$), but varied from 1.2 to 25.9 mol photons day^{-1} over this period.

Physiology responses

Corals appeared fully pigmented with polyps extended for most of the experiment. However, after 6 days corals in the heated treatment began to show visible signs of paling. After 8 days of treatment exposure F_o was significantly higher in corals from LV–Lilipuna relative to HV–Reef 14 ($F_{1,88} = 7.559$, $P = 0.007$) (Fig. 4a), and F_o tended to be higher in corals from heated treatments, although this trend was not significant ($F_{1,88} = 3.179$, $P = 0.078$). Conversely, F_m did not differ according to environmental history ($F_{1,88} = 1.955$, $P = 0.166$) but declined in corals from heated treatments ($F_{1,88} = 6.439$, $P = 0.013$) (Fig. 4b). F_v/F_m was higher in HV–Reef 14 corals ($F_{1,87} = 5.723$, $P = 0.019$) and declined in the heated treatments ($F_{1,87} = 44.562$, $P < 0.001$) (Fig. 4c) (Table 1). No environmental history × temperature treatment interactions were observed for F_o, F_m, or F_v/F_m ($F_{1,87} \geq 0.167$, $P \geq 0.178$) (Table 1).

Environmental history, temperature treatment, and their interaction did not affect total protein biomass ($F_{1,45} \geq 0.069$, $P \geq 0.519$) or *Symbiodinium* densities ($F_{1,44} \geq 0.025$, $P \geq 0.420$) (Table 1) (Fig. 5a, b). Conversely, chlorophyll *a* concentrations (µg cm^{-2}) were lowest in HV–Reef 14 corals relative to those from LV–Lilipuna (3.585 vs. 4.684 µg cm^{-2}) ($F_{1,45} = 6.175$, $P = 0.017$), and chlorophyll concentrations decreased in those corals exposed to heated treatments (3.667 vs. 4.687 µg cm^{-2}) ($F_{1,45} = 5.264$, $P = 0.026$). However, no environmental history × temperature treatment interactions were detected ($F_{1,45} = 0.024$,

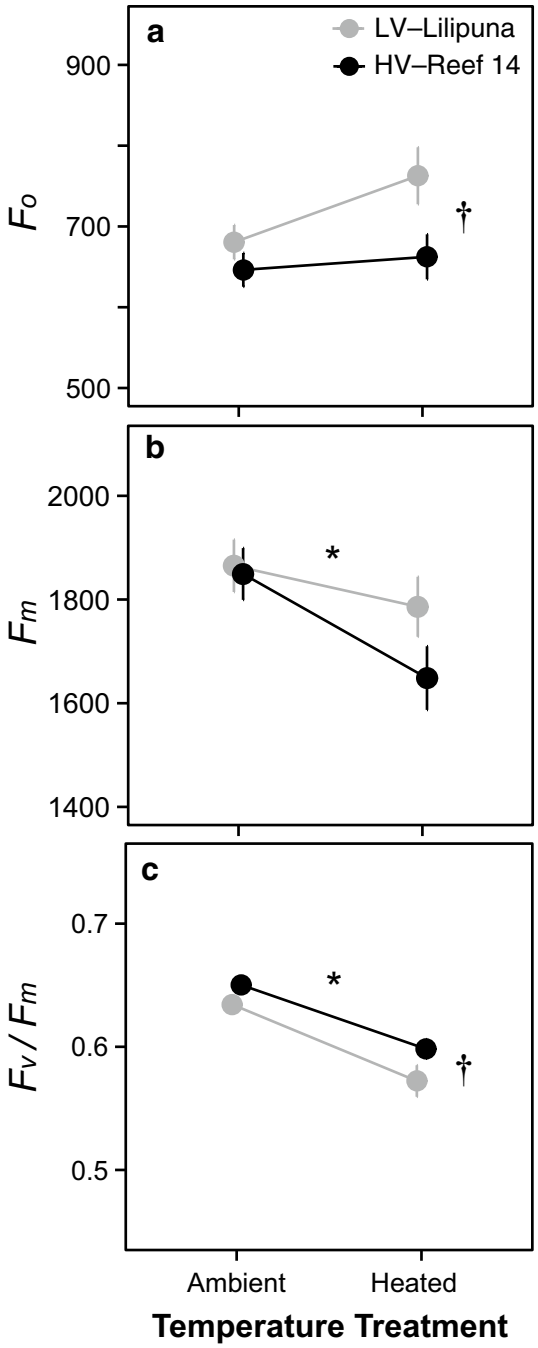

Fig. 4 **a** F_o (minimum fluorescence yield), **b** F_m (maximum fluorescence yield), and **c** F_v/F_m (maximum photochemical efficiency) of dark-adapted *Symbiodinium* photosystem II reaction centers in the coral *Montipora capitata* from two Kāne'ohe Bay reefs (LV–Lilipuna vs. HV–Reef 14) exposed to two temperature treatments (Ambient vs. Heated). Values represent mean ± SE (n = 20–25), and symbols indicate significant effects ($P < 0.05$) of temperature treatment (*) or reef environmental history (†)

$P = 0.877$) (Fig. 5c). The concentration of chlorophyll *a* normalized to *Symbiodinium* cell (pg chlorophyll *a* cell^{-1}) was not affected by environmental history ($F_{1,44} = 1.465$ $P = 0.233$), temperature treatment ($F_{1,44} = 1.006$, $P = 0.321$), or their interaction ($F_{1,44} = 0.547$, $P = 0.464$) (Table 1) (Fig. 5d).

Antioxidant and immunological responses

Superoxide dismutase activity (i.e., SOD) ($F_{1,43} = 6.912$, $P = 0.012$) and catalase activity (i.e., CAT) ($F_{1,43} = 5.648$, $P = 0.022$) differed according to environmental history. No temperature treatment ($F_{1,43} \geq 2.537$, $P \geq 0.068$) or environmental history × temperature treatment interactions were detected for SOD or CAT ($F_{1,43} \geq 0.007$, $P = 0.920$) (Table 2) (Fig. 6a, b). Pooled across temperature treatments, SOD and CAT activity, was 28 and 29% lower in LV–Lilipuna corals relative to HV–Reef 14 corals (Fig. 6a, b). Both prophenoloxidase activity (i.e., PPO) ($F_{1,43} = 5.447$, $P = 0.024$) and melanin synthesis activity (i.e., MEL) ($F_{1,43} = 9.054$, $P = 0.004$) differed according to environmental history ($F_{1,43} = 5.447$, $P = 0.024$) and temperature treatments ($F_{1,43} \geq 4.879$, $P \leq 0.033$), but not their interaction ($F_{1,43} \geq 0.771$, $P \geq 0.358$) (Table 2). PPO activity was 33% lower in corals from LV–Lilipuna relative to those at HV–Reef 14, and PPO declined by 48% in heated treatments (Fig. 6c). Environmental history and temperature treatment effects on PPO were directly opposite to MEL, where LV–Lilipuna corals exhibited 56% greater MEL activity relative to corals from HV–Reef 14, and MEL activity increased by 37% in response to heating (Fig. 6d).

Discussion

Thermal stress and bleaching can suppress coral immunity (Couch et al. 2008), leaving corals vulnerable to opportunistic infections and disease (Miller et al. 2009). The initial ability of corals to avoid thermal stress and to resist pathogenesis (i.e., constitutive antioxidative and immune activity) is an important driver of coral fate during environmental perturbation (Mydlarz et al. 2010; Palmer and Traylor-Knowles 2012). Therefore, the effect of environmental history on coral antioxidant profiles/immune activity has important implications for organismal performance, disease susceptibility, and responses to local and climate stressors (Couch et al. 2008). Environmental history is an important factor influencing the response of corals to physiological stress (Brown et al. 2000, 2002a; Ainsworth et al. 2016) and the capacity of corals to acclimatize and/or adapt to climate change (Palumbi et al. 2014; Dixon et al. 2015; Torda et al. 2017). As such, studying the ability of coral populations and *Symbiodinium* (Mayfield et al. 2012) to tolerate temperature variability (Maynard et al. 2008; Barshis et al. 2010), persistent high pCO_2 (Fabricius et al. 2011), and variable pCO_2 (Kenkel et al. 2017) is critical to understanding the

Table 1 Statistical analysis of environmental history and temperature treatment effects on *Symbiodinium* and *Montipora capitata* physiology

Dependent variable	Effect	SS	*df*	*F*	*P*
F_o	Env. history	0.247	1	7.559	**0.007**
	Treatment	0.104	1	3.179	0.078
	Env. history × treatment	0.060	1	1.842	0.178
	Residual	2.879	88		
F_m	Env. history	0.137×10^6	1	1.955	0.166
	Treatment	0.451×10^6	1	6.434	**0.013**
	Env. history × treatment	0.789×10^6	1	1.128	0.291
	Residual	6.158×10^6	88		
F_v/F_m	Env. history	0.014	1	5.723	**0.019**
	Treatment	0.106	1	44.562	**< 0.001**
	Env. history × treatment	0.394×10^{-3}	1	0.167	0.684
	Residual	0.206	87		
Protein cm^{-2}	Env. history	0.001	1	0.069	0.794
	Treatment	0.002	1	0.277	0.601
	Env. history × treatment	0.003	1	0.422	0.519
	Residual	0.352	45		
Symbiodinium cm^{-2}	Env. history	0.042×10^{11}	1	0.025	0.875
	Treatment	0.431×10^{11}	1	0.259	0.613
	Env. history × treatment	1.104×10^{11}	1	0.663	0.420
	Residual	73.270×10^{11}	44		
Chlorophyll *a* cm^{-2}	Env. history	14.219	1	6.175	**0.017**
	Treatment	12.121	1	5.264	**0.026**
	Env. history × treatment	0.056	1	0.024	0.877
	Residual	103.614	45		
Chlorophyll *a* $cell^{-1}$	Env. history	0.378	1	1.465	0.233
	Treatment	0.260	1	1.006	0.321
	Env. history × treatment	0.141	1	0.547	0.464
	Residual	11.361	44		

Env. history environmental history of low variable pCO_2 (LV–Lilipuna) or high variable pCO_2 (HV–Reef 14), *Treatment* ambient (24.5 °C) or heated (30.5 °C), F_o (minimum fluorescence yield), F_m (maximum fluorescence yield), and F_v/F_m (maximum photochemical efficiency) of dark-adapted *Symbiodinium* photosystem II reaction centers, *SS* sum of squares, *df* degrees of freedom

Bold *P* values represent significant effects ($P < 0.05$)

biology of reef corals in a world of rapid and unprecedented environmental change.

Montipora capitata from both reefs maintained their *Symbiodinium* densities, chlorophyll *a* per *Symbiodinium* cell, and total protein content when exposed to short-term thermal stress. However, chlorophyll *a* cm^{-2} and photochemical performance (i.e., F_m and F_v/F_m) declined in corals from both reefs when exposed to the heated treatment. The discrepancy in heating effects reducing chlorophyll *a* cm^{-2} but not affecting symbiont densities or chlorophyll *a* $cell^{-1}$ may in part be explained by methodology (i.e., sample variability, statistical power), in addition to biological processes (i.e., photoacclimation), differences in tissue and skeletal optical properties (Wangpraseurt et al. 2012), and the internal light environments where *Symbiodinium* reside. F_v/F_m differed according to reef environmental history, with higher F_v/F_m at HV–Reef 14 under ambient and heated conditions, suggesting environmental history affected properties of *Symbiodinium* photomachinery and rates of electron transport (Warner et al. 2010). The heated treatment reduced F_m and F_v/F_m, and indicates temperature-mediated damage to the photosynthetic machinery (Lesser 1997; Jones et al. 1998; Warner et al. 1999) and/or the activation of photoprotective mechanisms (Hoegh-Guldberg and Jones 1999; Osmond et al. 1999). The decreased photochemical performance, along with visible tissue paling and reduced chlorophyll *a* cm^{-2}, confirms *M. capitata* were experiencing stress commensurate with the onset of bleaching prior to the appreciable loss of symbiont cells. Declines in F_v/F_m often precede reductions in *Symbiodinium* or photopigment densities (Fitt et al. 2001; Rodrigues and Grottoli 2007). Indeed, short-term laboratory experiments have shown *M. capitata* maintains high symbiont densities despite loss of pigmentation from thermal (Rodrigues and Grottoli 2007) and ultraviolet (UV)

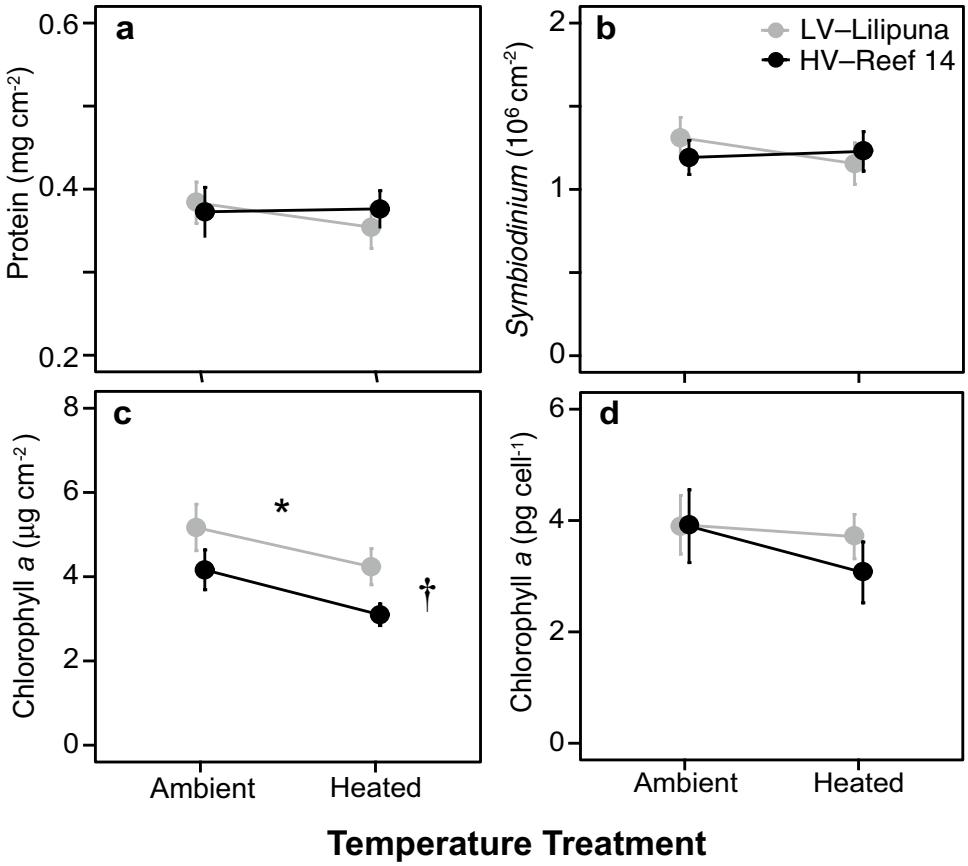

Fig. 5 Physiological responses of *Montipora capitata* from two Kāne'ohe Bay reefs (LV–Lilipuna vs. HV–Reef 14) exposed to two temperature treatments (Ambient vs. Heated). **a** Total protein content, **b** *Symbiodinium* densities, **c** chlorophyll *a* concentration per area of coral tissue, and **d** chlorophyll *a* per symbiont cell. Values represent mean ± SE (n = 10–13), and symbols indicate significant effects ($P < 0.05$) of temperature treatment (*) or reef environmental history (†)

Table 2 Statistical analysis environmental history and temperature treatment effects on antioxidant enzymes and immune activity of *Montipora capitata*

Dependent variable	Effect	SS	*df*	*F*	*P*
Superoxide dismutase (SOD)	Env. history	0.776×10^7	1	6.912	**0.012**
	Treatment	0.393×10^7	1	3.497	0.068
	Env. history × treatment	0.001×10^7	1	0.010	0.920
	Residual	4.827×10^7	43		
Catalase (CAT)	Env. history	39.576	1	5.648	**0.022**
	Treatment	17.780	1	2.537	0.119
	Env. history × treatment	0.045	1	0.007	0.936
	Residual	301.310	43		
Prophenoloxidase (PPO)	Env. history	1.542	1	5.447	**0.024**
	Treatment	3.908	1	13.800	**< 0.001**
	Env. history × treatment	0.243	1	0.859	0.359
	Residual	12.176	43		
Melanin (MEL)	Env. history	4.804×10^3	1	9.054	**0.004**
	Treatment	2.589×10^3	1	4.879	**0.033**
	Env. history × treatment	0.409×10^3	1	0.771	0.385
	Residual	22.815×10^3	43		

Env. history environmental history of low variable pCO_2 (LV–Lilipuna) or high variable pCO_2 (HV–Reef 14), *Treatment* ambient (24.5 °C) or heated (30.5 °C), *SS* sum of squares, *df* degrees of freedom

Bold *P* values represent significant effects ($P < 0.05$)

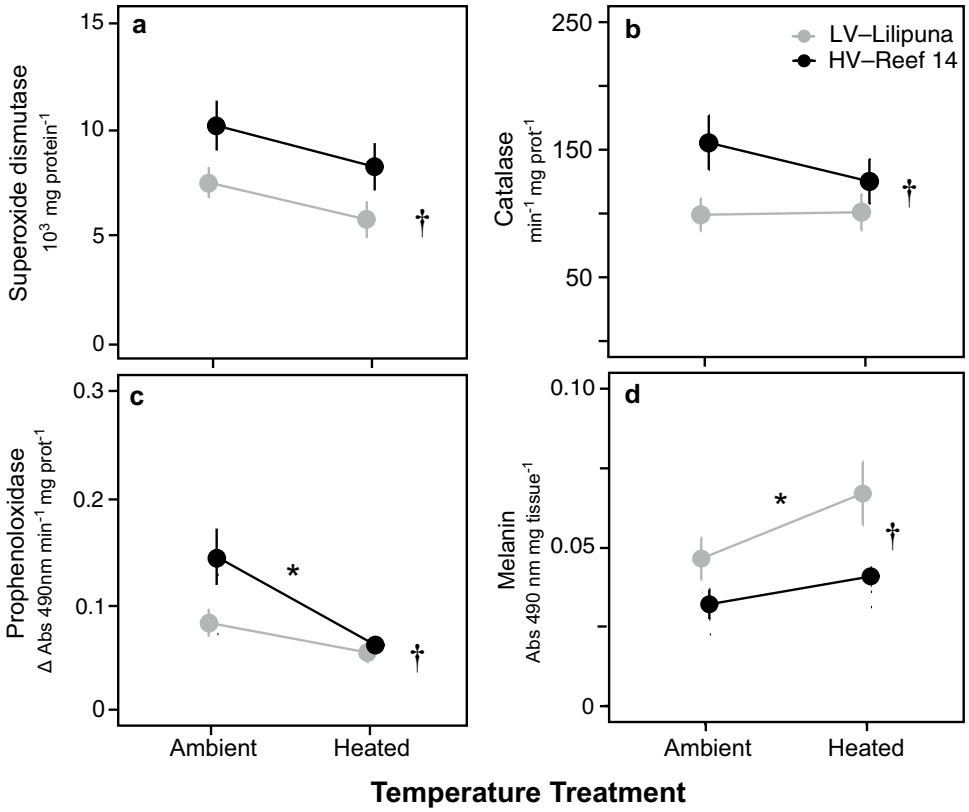

Fig. 6 Immunological responses of *Montipora capitata* from two Kāne'ohe Bay reefs (LV–Lilipuna vs. HV–Reef 14) exposed to two temperature treatments (Ambient vs. Heated). **a** Superoxide dismutase (SOD) concentration, **b** catalase (CAT) activity, **c** prophenoloxidase (PPO) activity, **d** melanin (MEL) concentration. Values represent mean ± SE ($n = 11$–12), and symbols indicate significant effects ($P < 0.05$) of temperature treatment (*) or reef environmental history (†)

radiation stress (Grottoli-Everett and Kuffner 1995), suggesting *M. capitata* and its *Symbiodinium* possess an especially robust capacity for photoacclimation.

In all corals sampled, levels of the antioxidative enzymes superoxide dismutase (i.e., SOD) and catalase (i.e., CAT) were not significantly affected by temperature treatments. Rather, significant differences were only observed as a function of reef environmental history. The lack of change in antioxidative activity was an unexpected observation, and we present three possible explanations for this result: (1) constitutive levels of antioxidants provided adequate protection during short-term thermal challenge; (2) antioxidative responses are a secondary form of defense not employed in the early stages of thermal stress, as such enzymes are energetically costly to produce (Palmer et al. 2011a); or (3) other compounds such as melanin (i.e., MEL) have dual function and exhibit some antioxidant activity (Nappi and Christensen 2005) providing sufficient protection against cellular damage during the onset of thermal stress. In other studies, the production of specialized antioxidative enzymes (e.g., SOD and CAT) is only induced after prolonged exposure (Downs et al. 2002). Melanisation may also function as a general acclimatization response to environmental perturbation. In this way, the melanin synthesis pathway may be an important immune parameter activated in corals exposed to periodic environmental stressors such as elevated irradiance and temperatures. Regardless, the observation of site-specific antioxidant profiles and melanin synthesis activity indicate phenotypic differences in corals at these two locations, potentially as a result of distinct pCO_2 histories at these locations. It is not known if the reef-specific differences in coral phenotypes observed here reflect mechanisms of acclimatization or local adaptation to a history of distinct environmental conditions; however, such lines of inquiry should be advanced to further our understanding of environmental history effects on corals.

The melanin synthesis pathway was responsive to heated treatments in corals from both reefs regardless of environmental history. This pathway begins with the proteolytic cleavage of inactive PPO to the active phenoloxidase (i.e., PO), and through a series of intermediate reactions ultimately leads to the production and deposition of melanin into coral tissues (Mydlarz and Palmer 2011; Nappi and Christensen 2005). PPO levels typically drop upon induction of melanin production as reserves of the latent enzyme are converted to their active form and consumed (Mydlarz et al. 2008; Palmer et al. 2011a, b). This agrees with results observed in the present study, where thermal stress caused reductions in coral tissue PPO and simultaneous increases in melanin production. However, other enzymes are also capable of completing the melanin synthesis pathway. For example, peroxidases can compete with PO for the hydroxylation of tyrosine and subsequent melanin deposition (Nappi

and Vass 1993; Nappi and Christensen 2005), although these enzymes were not measured here.

Melanin is a multifunctional compound that serves many roles. It is important for both wound healing (Palmer et al. 2011b) and pathogen encapsulation (Ellner et al. 2007; Mydlarz et al. 2008). It is also implicated in *Symbiodinium* photoprotection (Palmer et al. 2010, 2011a), as it is a known UV-absorbing molecule in mammals (Ortonne 2002; Sugumaran 2002). Similarly, invertebrate photoprotection is demonstrated in the water flea, *Daphnia* spp., where melanisation is positively correlated to UV exposure (Rautio and Korhola 2002). This photoprotective function was also recently confirmed in sponges, where melanin produced by symbiotic bacteria was protective against UV-induced intracellular reactive oxygen species (Vijayan et al. 2017). The sea fan, *Gorgonia ventalina*, displayed melanisation in response to elevated temperatures (Mydlarz et al. 2008), and higher constitutive levels of melanin and melanin-containing granular cells have also been documented in coral species considered resistant to thermal bleaching (Palmer et al. 2010). The exact role of coral melanisation in response to increased temperature has yet to be elucidated, however, and the causes and consequences of increased melanin synthesis activity under short-term and prolonged thermal stress have interesting implications for cellular adaptive mechanisms.

In the present study, we found that corals exhibit constitutive differences in photobiology, chlorophyll *a*, antioxidative enzymes, and immunity. However, environmental history effects did not interact with temperature treatments to alter thermal stress response trajectories. Therefore, while environmental history can shape the response of corals to bleaching stress (Brown et al. 2002a), the specific environmental conditions at the two reefs in the present study did not influence the biological response of corals to short-term thermal stress. Other physical or biological factors in addition to pCO_2 history may also be responsible for influencing the reef-specific responses observed here. Such factors may include pathogen infections or immune response elicitors (Palmer et al. 2011a) and their present and historical distribution within Kāne'ohe Bay (Aeby et al. 2010), as well as low coral/high bare substrate cover and dissolved inorganic nitrogen concentrations (Couch et al. 2008). However, such factors do not appear to have played a significant role in the present study. First, previous exposure to disease and physiological stress can elevate coral immune activity (Mydlarz et al. 2009; Palmer et al. 2011a), and historically, coral disease (i.e., *Montipora* white syndrome) prevalence is greater in southern Kāne'ohe Bay reefs proximate to LV–Lilipuna, relative to central and northern reefs (Aeby et al. 2010). However, we observed greater antioxidative (SOD and CAT) and immune (PPO) activity at HV–Reef 14 in central Kāne'ohe Bay. Therefore, historical disease prevalence does not explain greater antioxidant or immune activity at HV–Reef 14. Alternatively, it is possible immune activity in LV–Lilipuna corals surviving historically high disease pressure (southern Kāne'ohe Bay) is a consequence of resistance/immunity to immune activity elicitors. Second, coral cover at the two reefs are comparable (ca. 75%) and inorganic nutrients within Kāne'ohe Bay are not different from those measured on offshore reefs (Cox et al. 2006), suggesting the influence of coral cover and dissolved nutrients in explaining differences among corals in the present study may be minimal. Seawater temperature (Coles and Jokiel 1978) and flow speed influence coral performance (Dennison and Barnes 1988), and it is possible slightly cooler daily minimum temperature at LV–Lilipuna (0.12 °C) or other properties of seawater associated with residence time/flow (Lowe et al. 2009) exerted influence here. In addition, differences in holobiont traits due to seasonality (Fitt et al. 2000) or symbiont abundance (Cunning and Baker 2014) at the time when stress is applied can influence stress outcomes, and winter-acclimation may have attenuated heating effects in corals in the present study. Therefore, while pCO_2 history remains the most salient difference between LV–Lilipuna and HV–Reef 14 (Drupp et al. 2011, 2013) best explaining the distinct responses of corals to short-term heating, the influence of other physical factors should not be wholly dismissed.

Differences in symbiont communities among *M. capitata* colonies (Stat et al. 2011) can also influence physiological responses and stress outcomes (Sampayo et al. 2008; Cunning et al. 2016). *Montipora capitata* in the Main Hawaiian Islands are known to associate with both clade C and/or D *Symbiodinium* (Stat et al. 2013), namely C31 and D1-4-6 (*S. glynnii*) (Cunning et al. 2016; Wham et al. 2017). The latter are often found in corals from reefs with a history of thermal stress and/or variance and degraded water quality, such as Kāne'ohe Bay (Stat et al. 2013, 2015). Thus, the reef-specific effects reported here may result from a combination of several non-mutually exclusive factors including environmental history (Brown et al. 2002a), host genotypes (Barshis et al. 2010; Bongaerts et al. 2010), symbiont community (Sampayo et al. 2008), and microbial consortia (Morrow et al. 2015), as well as unidentified genetic mechanisms (i.e., gene expression plasticity, DNA methylation) (Kenkel and Matz 2016; Putnam et al. 2016).

The role of environmental history in shaping coral physiology remains an important and burgeoning field of inquiry (Brown et al. 2002a; Middlebrook et al. 2008; Kenkel et al. 2013a, b; Ainsworth et al. 2016; Kenkel and Matz 2016), especially in the context of thermal and pCO_2 stress (Fabricius et al. 2011; Noonan and Fabricius 2016; Gibbin et al. 2017; Kenkel et al. 2017). Environmental history and phenotypic plasticity are important considerations for predictions in the biology, ecology, and evolution of marine organisms (Gaylord et al. 2015; Torda et al. 2017). Here, distinct environmental histories of pCO_2 variability did not

interact with thermal stress to shape the suite of host and symbiont responses. Nevertheless, environmental history exerted strong influence over coral and *Symbiodinium* at both ambient and elevated temperatures, emphasizing differences among local reef environments even at small spatial scales are important in determining coral holobiont performance under favorable and challenging conditions. Finally, the melanin synthesis pathway was significantly upregulated during the early stages of thermal stress, and provides further evidence that melanisation is an important general stress response in corals exposed to warming seawater preceding the onset of symbiont losses.

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Compliance with ethical standards

Conflict of interest The authors declare they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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