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Acclimatization to high-variance habitats does not enhance physiological tolerance of two key Caribbean corals to future temperature and pH

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Corals are acclimatized to populate dynamic habitats that neighbour coral reefs. Habitats, such as seagrass beds, exhibit broad diel changes in temperature and pH that routinely expose corals to conditions predicted for reefs over the next 50–100 years. However, whether such acclimatization effectively enhances physiological tolerance to, and hence provides refuge against, future climate scenarios remains unknown. Also, whether corals living in low-variance habitats can tolerate present-day high-variance conditions remains untested. We experimentally examined how pH and temperature predicted for the year 2100 affects the growth and physiology of two dominant Caribbean corals (*Acropora palmata* and *Porites astreoides*) native to habitats with intrinsically low (outer-reef terrace, LV) and/or high (neighbouring seagrass, HV) environmental variance. Under present-day temperature and pH, growth and metabolic rates (calcification, respiration and photosynthesis) were unchanged for HV versus LV populations. Superimposing future climate scenarios onto the HV and LV conditions did not result in any enhanced tolerance to colonies native to HV. Calcification rates were always lower for elevated temperature and/or reduced pH. Together, these results suggest that seagrass habitats may not serve as refugia against climate change if the magnitude of future temperature and pH changes is equivalent to neighbouring reef habitats.

1. Introduction

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Tropical coral reefs are increasingly threatened from both ocean warming and acidification as atmospheric pCO_2 concentrations continue to rise [1]. However, how these two factors will interact to drive future productivity and distribution of reef building corals remains unclear [2,3]. Evidence suggests that a rise in temperature can increase coral metabolic and/or growth rates [4], but eventually push corals past their physiological limits, resulting in mortality [1]. Lower pH appears to enhance the metabolism of select coral endosymbionts [5], but may increase the energetic cost of calcification and growth for the coral host [6,7]. Consequently, research efforts have focused on attempting to de-convolve the impacts of pH and temperature, while also trying to understand the potential synergistic interactions of combined stressors.

Coral reefs can have inherently high or low environmental variance. Deeper outer- and fore-reef habitats typically have relatively stable physico-chemical conditions. In contrast, shallow reefs are highly dynamic where

bathymetry, benthic composition, extreme weather events and tidal cycles can create natural oscillations in pH and temperature [8–10]. The scale of these oscillations are habitat specific (e.g. reef-flat versus seagrass or mangroves [8–12]) with daily temperature fluxes up to 2.5°C and pH variance exceeding 0.5 units [8,9]. Over longer temporal scales, these habitats are subject to greater oscillations [12]. Consequently, corals within shallow habitats routinely experience periods of extreme pH (7.8 [9,13]) and temperature (greater than 33°C [9,12]) considered representative of future reef conditions under IPCC climate change scenarios. It is, therefore, plausible that corals persisting in high-variance habitats are better conditioned to tolerate periods of less favourable environmental conditions (e.g. enhanced physiological control) through expansion of physiological performance ranges [7].

Increased physiological tolerance to anomalous temperature and/or pH exposure has been shown for corals populating habitats with high environmental variance in some [11,14,15], but not in all studies [16,17]. Such contrasting observations could reflect that few studies have included both temperature and pH as experimental variables, despite the simultaneous threat of ocean warming and acidification, with even fewer experiments replicating the natural daily oscillations (frequency and range) of temperature and pH inherent to coral habitats. Experiments have been performed to account for ambient pH variability for coral recruits (*Seriatopora caliendrum* [18]) and mature corals (*Acropora hyacinthus* [11]) when grown under dynamic rather than steady-state pCO_2 conditions, but do not consider the simultaneous role of temperature variance. Conversely, the interactive role of temperature and acidification has been examined for massive- [2,7,19] and branching-corals [2], but do not replicate the ambient variance in temperature and pH. Only Dove *et al.* [20] have incorporated daily and seasonal variance for both temperature and pH within their study on patch reef communities, predicting serious implications for coral reef systems under future climate change [20]. However, how their observations hold across systems with inherently different scales of natural variance remains untested.

To date, no study has compared the physiological response of corals from relatively low (LV, seagrass) versus high (HV, outer-reef) variance habitats, as characterized by natural daily oscillations of both temperature and pH. It is unknown whether corals populating HV pH–temperature habitats have inherently greater tolerance to pH–temperature stress that is predicted for reefs of the future. Whether corals populating LV pH–temperature habitats can acclimatize to HV pH–temperature conditions is also unresolved. Addressing these unknowns is fundamental to understanding how present-day dynamic reef systems will respond to future changes in environmental conditions [18]. To address these unknowns, we conducted a multifactorial manipulative experiment on two dominant Caribbean coral species, one cosmopolitan to HV and LV habitats (*P. astreoides*) versus a species only found in the LV habitat (*A. palmata*). Both species were exposed to current mean temperature and/or pH as well as future predicted mean temperature and/or pH for HV and LV habitats, but superimposing the natural variance of either habitat onto the predicted mean temperature and pH (following [20]). We also assessed the physiological tolerance based on key metabolic traits.

2. Material and methods

(a) Study location and test organisms

The study was conducted on the north coast of Little Cayman (Cayman Islands) at two sites comprising two habitats: outer-reef (LV) on the reef-terrace (19°41.53, 80°03.50) and coral inhabited seagrass (HV) within the adjacent shallow lagoon *ca* 0.5 km inshore (19°41.48, 80°03.26). Detailed environmental characterization between March and April 2013, informed the target control conditions within the study (details in §2c).

The manipulation study was conducted between May and July 2014. Two study organisms were selected: *A. palmata*, which was only found at the LV outer-reef habitat ($n = 40$) and *Porites astreoides*, which populated both the HV outer-reef and LV seagrass habitats ($n = 40$ colonies per habitat). On the reef, these two coral species accounted for $45.2 \pm 0.3\%$ of relative coral cover. Removed fragments were less than 5 cm, carefully collected from a depth of *ca* 1.5 m.

(b) Experimental design

Eight experimental treatment conditions were created within controlled laboratory conditions (electronic supplementary material, figure S1). For both HV and LV, treatments consisted of: (i) a control, (ii) elevated temperature only, (iii) reduced pH only, and (iv) elevated temperature-reduced pH. Each treatment was superimposed onto the ambient diel variance of temperature and pH for the HV versus LV habitats. The eight experimental treatments were each replicated across five independent aquaria (2 L), with one fragment of: (i) *A. palmata* (LV), (ii) *P. astreoides* (LV), and (iii) *P. astreoides* (HV) per aquarium. All aquaria were maintained under an ambient 12 L : 12 D photoperiod [2], with average daylight PAR of 400–500 μmol photons $m^{-2} s^{-1}$ (representative of *in situ* conditions; electronic supplementary material, table S1). Light was measured using three HOBO Pendant temperature/light loggers (Microdaq, USA), with values averaged and converted to PAR using the daylight coefficient [21]. Daily NO_3^- measurements were taken using a NO_3^- probe (Orion, USA) with all treatments exposed to relatively low levels throughout the experiment (mean $\pm$ s.e. of $n = 59$ per habitat), HV $= 0.95 \pm 0.03$ μM, LV $= 1.06 \pm 0.04$ μM (electronic supplementary material, table S1).

Ambient *in situ* diel variance of pH and temperature for the LV and HV habitats were recreated for experimentation. *In situ* diel records of temperature and pH were used to determine seven time points representing the range of conditions and hence scale of variance. Mean temperature and pH at each time point served as the basis of recreating ambient habitat variance *ex situ* (see electronic supplementary material, figure S2) in one of two ways: (i) for the LV habitat, four 250 l reservoirs of non-filtered outer-reef habitat (LV) natural seawater supplied all treatment aquaria; (ii) for the HV seagrass habitat, water from the reservoir was pumped into a 45 l sump containing seagrass and carbonate sediment (collected from the *in situ* seagrass habitat; electronic supplementary material, figure S1). The sump contained a pump and aerator, which subsequently supplied water to all HV treatment aquaria. Temperature was controlled for both the LV and HV treatments using a water bath with heaters (Aquael, Poland) to achieve the target conditions for each time period.

Additional elevated temperature and/or reduced pH 'future scenario' treatments were created by superimposing the pH and temperature conditions predicted for 2100 onto the natural diurnal trends from the LV and HV reservoirs (as per [20], figure 1). Elevated temperature was achieved using additional heaters to re-create the diurnal oscillations. Temperature was continuously measured over the duration of the experiment using a HOBO temperature/light logger verified daily with a temperature probe (Ocean Optics, England).
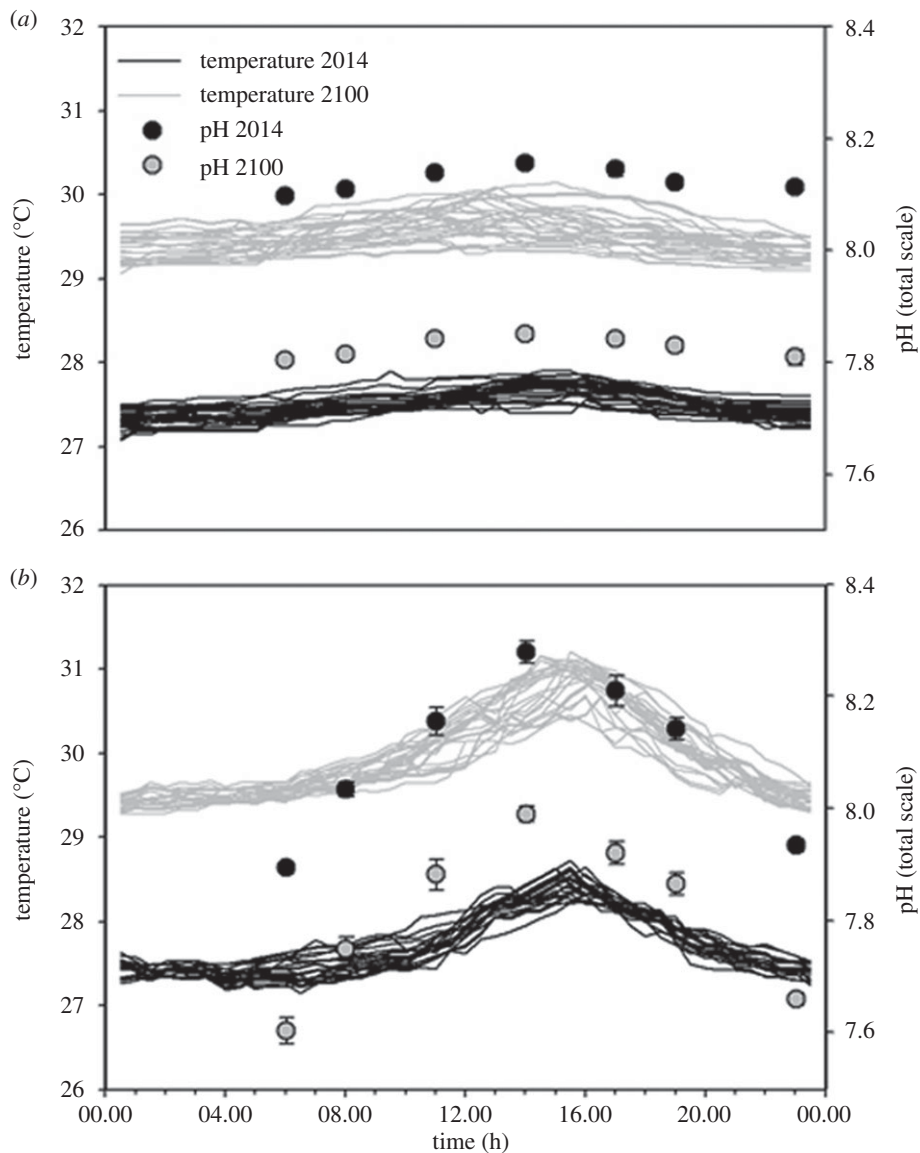
Figure 1. The average pH and temperature aquarium conditions for: (a) the outer-reef low-variance (LV) and (b) seagrass high-variance (HV) habitats of Little Cayman, Cayman Islands, BWI, for both present day and 2100 (under A1B scenario estimates). The 2100 conditions (grey) represent an approximate temperature **Q6** increase ± s.e. of $2.2 \pm 0.03°C$ and pH decrease of 0.3 ± 0.02 units. pH was measured from daily discrete water samples over the seven time periods, while temperature was measured continuously with a HOBO pendant temperature/light 64 K data logger set at a 30 min interval.

For the reduced pH treatments, feeds from either the LV or HV source water were tapped into aquaria, where pH was altered by subsequently manipulating the seawater by equimolar additions of strong acid ($1 \, mol \, l^{-1}$ HCl), NaOH and Na_2CO_3 (Thermo-Fisher Scientific, USA) [22]. Volumes of HCl and HCO_3^-, required to adjust pCO_2 and pH to the chosen target values, were first calculated from the measured ambient state of the carbonate system in seawater using CO2SYS [22]. For each of the seven daily time periods (electronic supplementary material, figure S2), water within each aquarium was flushed with new manipulated seawater. To ensure that the target pH was achieved and that total alkalinity (TA) was maintained, pH and TA were tested before every water exchange from discrete water samples. pH (total) was measured using the Orion Ross Ultra Glass Triode Combination Electrode (Fisher Scientific, UK) calibrated with Tris buffers (accuracy ca ±0.002) using the potentiometric technique [23]. An open-cell potentiometric titration procedure was used to measure TA using a Titrino titrator (Metrohm, UK) with accuracy and precision of ca less than or equal to $2 \, \mu mol \, kg^{-1}$ (verified with Dickson standards).

Based on these manipulations, the future scenario treatments achieved a temperature increase of $2.2 \pm 0.03°C$ and pH decrease of 0.3 ± 0.02 units (mean ± s.e. conditions at the seven diurnal

time points over the 59-day experimental period; $n = 413$; electronic supplementary material, figure S2). Our method of manipulating the pH and temperature was able to recreate the predicted changes of 2100 under IPCC A1B scenarios incorporating the natural diel trends of each habitat (figure 1), including aragonite saturation state (Ω_{arg}) (electronic supplementary material, figure S3 and table S1). Together, these treatments yielded a daily mean (± s.e.) ($n = 59$) Ω_{arg} for the HV and LV habitats of 3.79 ± 0.04 and 2.94 ± 0.02, respectively, for present-day conditions versus 2.33 ± 0.08 and 2.24 ± 0.03 for 2100 scenarios. Under both the HV and LV treatments, greater daily variance in both temperature and pH was experienced for the tanks exposed to the 2100 level conditions (temperature: $t_6 = 6.36$, $p < 0.001$, pH: $t_6 = 4.44$, $p < 0.005$, see the electronic supplementary material).

The eight experimental treatments were conducted over three phases: (i) recovery (3 days), where corals were initially removed from their *in situ* environment and left to recover in the laboratory under their present-day ambient, i.e. native LV versus HV treatment conditions; (ii) acclimatization (21 days, as per [24]) where the full set of present-day or future scenario pH and temperature treatments were applied, and finally, (iii) experimentation (35 days) where corals continued to be exposed to all treatments (total experimental duration, $n = 59$

days; electronic supplementary material, figure S4). All corals were sampled for zooxanthellae and chlorophyll a concentrations, and incubated to measure rates of photosynthesis, respiration and calcification (see §2d). (i) At the end of the experimental period (t_e) to evaluate for the treatment effect, but also (ii) at the end of the recovery period (t_0) to determine that physiological properties had not drifted in the controls as a result of the experimental set-up. Buoyant mass measurements were taken at the end of the acclimatization period (t_i) and t_e to establish an average daily growth rate (electronic supplementary material, figure S4).

(c) Carbonate chemistry baseline

At each location, discrete water sampling was conducted over 18 days between March and April 2012 to establish the natural diurnal trends in pH and temperature. Samples were collected over 24 h, at 3 h intervals starting at sunrise ($n = 144$ per site). Seawater carbonate chemistry was measured following the Carbon Dioxide Information Analysis Centre protocols [23], with carbonate parameters (pCO_2, TCO_2 and Ω_{arg}) calculated from TA and pH, as described in [21]. Discrete water samples were also collected weekly at the seagrass (HV) and outer-reef (LV) sites during the experimental period ($n = 8$; electronic supplementary material, table S1).

(d) Physiological measurements

Coral replicates were weighed in seawater (buoyant weight) at t_i and t_e with a Ohaus Scout-Pro balance (accuracy 0.01 g). Skeletal dry weight was demined as per [25] and normalized to surface area calculated using the advanced geometric technique (AGT) [26]. Density was determined at the end of the experimental period using the calculated mass and volume determined from three-dimensional scans [27]. Growth rates established from buoyant mass corresponded closely to rates established from the TA method (electronic supplementary material, figure S5) and we, therefore, present data obtained only from the TA method. Daily-integrated rates were simultaneously obtained for all colonies from 8×3 h incubations (and three control chambers that contained only seawater) sequentially conducted throughout a 24 h period at t_0 and t_e (as previously detailed [21]). Each colony was incubated in a closed 500 ml chamber, which was manually stirred every hour using a stir-bar and magnet. Net photosynthesis (P_N) and respiration rates (R) were determined (each 4×3 h incubations at t_0 and t_e), where O_2 was measured at the start and end of each incubation using a Foxy-R O_2 probe (Ocean Optics, England). Dark incubations were conducted immediately after exposure to light by covering the incubation chambers with custom-made blackout bags (see [21]). Changes in TA and O_2 for each chamber and 3 h incubation were corrected for any changes in TA or O_2 from the seawater controls ($n = 3$), to yield hourly rates for calcification (G, mmol $CaCO_3$ m^{-2} h^{-1}) or P_N and R (mmol O_2 m^{-2} h^{-1}) [21]. Gross productivity (P_G) was calculated by the addition of net photosynthesis and respiration.

Three colonies of each species from each experimental treatment were randomly selected for surface area determination by three-dimensional scans [27]. This method showed that AGT underestimated surface area and thus a species-specific correction factor was applied to all surface area measurements to account for this difference: *A. palmata*: $r^2 = 0.974$, $n = 24$, $p = 0.001$, AGT $= -0.93 + (0.97 \cdot$ three-dimensional scan); *P. astreoides*: $r^2 = 0.965$, $n = 48$, $p = 0.010$, AGT $= -1.08 + (0.97 \cdot$ three-dimensional scan).

(e) Chlorophyll a and zooxanthellae counts

Tissue was removed from each nubbin with a water pik using GF/F-filtered seawater; the area of tissue removed was quantified through corrected AGT [26]. Tissue slurry was homogenized using a Pasteur pipette and a small aliquot taken for cell quantification using microscopy [28]. A second aliquot was filtered and extracted in methanol for 24 h at 4°C, and chlorophyll a quantified on the pigment extracts using a USB 2000$^+$ Spectrophotometer (Mikropack, HL-1000) and the equations of [29] for dinoflagellates.

(f) Statistics

Linear regression was used to compare the rates of calcification between t_0 and t_e, to relate calcification calculated with the TA anomaly method to the buoyant mass technique, and to relate surface area measured by AGT and the three-dimensonal scanning method. A t-test was conducted to assess whether rates of calcification at t_0 were different from rates of calcification at t_e.

(i) Multi-model comparison

Within the study, *P. astreoides* was found at both habitats, whereas *A. palmata* was only found at one, and thus the experiment was not fully factorial. In addition, exploratory analysis of the data identified significant third-order interaction terms (such as pH, temperature and species). The large number of terms in a third-order ANOVA and the unbalanced design raised concerns that an ANOVA may be affected by over-fitting or ill-conditioning [30]. For these reasons, our results were analysed in two ways: (i) by ANOVA with restrictions on the variables (electronic supplementary material, table S2 and S3), and (ii) with a set of nested nonlinear models using the multi-model selection framework (electronic supplementary material). The Akaike information criterion of linear models (ANOVA) and nonlinear models was compared, (electronic supplementary material, table S4–S6), with a difference of 0–2 considered negligible [30]. Model simplification, by removing non-significant variables (e.g. tank variance of 2100 versus present-day, see the electronic supplementary material), was undertaken to compare models with progressively simplified fixed effects to select the most appropriate model (see the electronic supplementary material). Confidence intervals (CIs) were calculated by the log-profile method [30], and from these we obtained bounds on the corresponding p-values. pH and temperature variation for the present-day and 2100 treatments were tested by Kolmogorov–Smirnov on the residuals of both the ANOVA and nonlinear model (electronic supplementary material, table S7–S8 and figure S6).

3. Results

(a) Carbonate chemistry manipulation

To ensure that experimental HV (seagrass) and LV (outer-reef) present-day control treatments were comparable to the ambient habitat conditions, we compared calcification rates at t_0 with those at t_e of the experimental period. Calcification rates across all species and treatments were unchanged between t_e and t_0 (slope not significantly different to one, i.e. 1 : 1), thus demonstrating that ambient conditions were well conserved throughout the experimental period (figure 2). Calcification rates for all species based on the TA anomaly method versus buoyant mass were significantly correlated ($r^2 = 0.791$, $n = 120$, $p = 0.001$), with their relationship described by the equation: buoyant mass (mmol m^{-2} d^{-1}) = 4.33 + (0.864 · alkalinity depletion (mmol m^{-2} d^{-1})) (electronic supplementary material, figure S5 and table S9).

(b) Native habitat and species response

Daily mean calcification rates for colonies maintained under their native conditions were 9.3% higher (CI: 7.8–10.7%) for
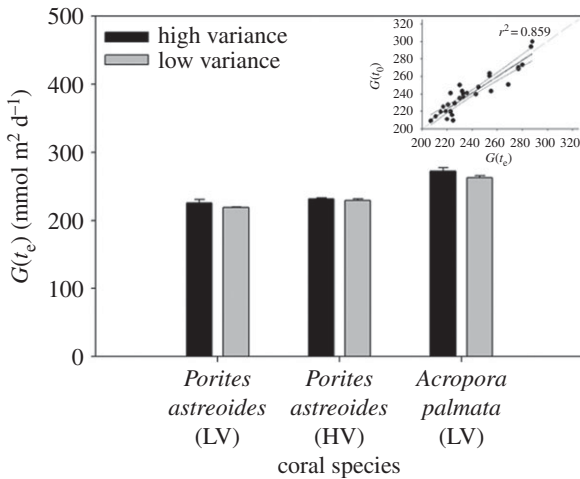
Figure 2. Average ($\pm$ s.e.) daily calcification rates (G) at the end of the experiment (t_e) for: *A. palmata* (outer-reef, low-variance (LV) habitat), *P. astreoides* (LV), and *P. astreoides* (seagrass, high-variance (HV) habitat) within the HV and LV control tanks. A regression between rates of G at the start (t_0) and t_e of the experiment show strong colinearity between rates ($r^2 = 0.859$, $n = 30$, $p = 0.001$, $G(t_0)$ (mmol m^{-2} d^{-1}) $= 18.55 + 0.93G(t_e)$ (mmol m^{-2} d^{-1}).

A. palmata (table 1 and electronic supplementary material, S9) (268.5 ± 1.2 mmol m^{-2} d^{-1}) compared with both native HV and LV *P. astreoides* (231.6 ± 5.6 mmol m^{-2} d^{-1}, 218.6 ± 7.4 mmol m^{-2} d^{-1}, respectively, table 1 and figure 2). Respiration and photosynthesis rates were *ca* 9.0% (CI: 7.2–10.3%) and 7.0% (CI: 5.2–7.9%) higher, respectively, for *P. astreoides* compared with *A. palmata* (table 1). Transplantation of *P. astreoides* colonies from HV and LV habitats into LV and HV control tanks, respectively, did not induce a change in calcification (figure 2). Thus, colonies native to the LV outer-reef sustained calcification in the HV seagrass control tanks. Colonies originating from HV, however, showed no enhanced calcification rates under the more stable LV control (electronic supplementary material, table S9).

Consistently, across all observations (photosynthesis, respiration and calcification) native habitat gave a small positive effect (less than +4% for corals native to HV seagrass) which in only a single case (photosynthesis) rose above the threshold for statistical significance (table 1). Thus, the response to 'future scenarios' was independent of LV (outer-reef) or HV (seagrass) growth history. However, colonies from the LV habitat all experienced an increase in skeletal density under the reduced pH treatments ($t = -3.79$, $p = 0.005$; electronic supplementary material, figure S7). No significant difference was detected in the skeletal densities of the controls between the HV and LV treatments, independent of species.

Overall, physiological responses to experimental treatments were similar for both *A. palmata* and *P. astreoides* (figure 3 and electronic supplementary material, table S9). pH had a larger influence on coral calcification than temperature (table 1). Reduced pH treatments decreased calcification by 38.7% (CI: 36.3–41.0%), whereas elevated temperature treatments decreased calcification by 20.4% (CI: 17.8–22.9%). The greatest decrease in calcification was observed under the combined elevated temperature-reduced pH treatment. However, the nonlinear model indicated that any interactive effect of pH and temperature was negligible (3.2%, CI: −0.8 to 7.1%, table 1), because an additive response of temperature and pH independently explained the majority of trends within the

dataset (see the electronic supplementary material). Consequently, the additive response of temperature and pH stress resulted in a decrease in calcification of 59.1%.

Respiration did not change in response to any treatment (within 5.5% of the control; figure 3 and table 1) and, therefore, changes in net photosynthesis were mirrored as changes in gross photosynthesis. Net photosynthesis fell under all reduced pH treatments (pH: 17.3%, CI: 14.8–19.7; pH + temp: 26.5% CI: 22.5–30.6%). When temperature was elevated to 2100 scenarios but pH was maintained at present-day levels, net photosynthesis was elevated (8.3%, CI: 5.6–11.1%, table 1).

(c) Treatment variance

Corals native to both HV (seagrass) and LV (outer-reef) environments experienced significant, but very small (less than 5%) reductions in all metabolic parameters under the HV treatment conditions (table 1). The treatment response was the same for LV-grown *A. palmata* exposed to HV treatment conditions, as well as the reciprocal transplantation of HV or LV grown *P. astreoides* (figure 3 and table 1).
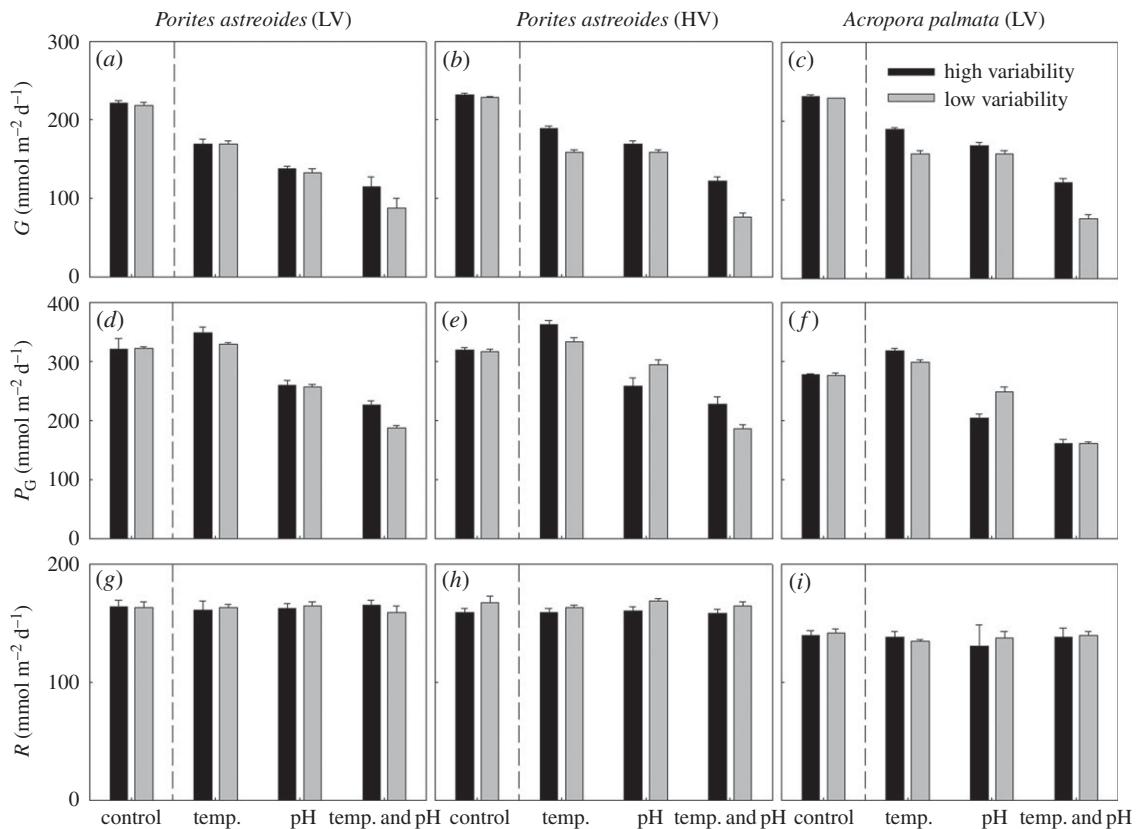
(d) Metabolic coupling

Across treatments, calcification rates were closely coupled to gross photosynthesis rates, independent of species and native habitat, with 83% of calcification explained by the covariance with gross photosynthesis ($r^2 = 0.831$, $n = 90$, $p = 0.01$, figure 4). For *P. astreoides*, changes in gross photosynthesis were generally independent of the zooxanthellae density (zooxanthellae never declined by less than 10% of the control, figure 4). Similarly, *A. palmata* colonies exposed to the elevated temperature treatment exhibited a decrease in gross photosynthesis that was independent of zooxanthellae density. However, *A. palmata* exposed to reduced pH, or combined elevated temperature-reduced pH treatments exhibited a decrease in gross photosynthesis that corresponded with a loss in zooxanthellae density (figure 4; as well as decrease in chlorophyll *a*, 10–23%, electronic supplementary material, table S9). Visual observations support these trends, with paling observed among the *A. palmata* fragments beginning on day 14 of the experiment (E. Camp, personal observation). Q3 Thus, while the treatment-induced declines in calcification were independent of host taxa (figure 4), they correspond with very different modes of symbiont response: a loss of productivity per zooxanthellae cell for *P. asteoides* (and *A. palmata* under elevated temperature alone) versus a loss of zooxanthellae cells (but general maintenance of productivity per zooxanthellae cell) under reduced pH for *A. palmata* (figure 4).

4. Discussion

Recent studies have suggested that populations grown under more variable pH [11] or temperature [15] environments enhance resistance of corals to anomalous pH and temperature stress. Shallow reef habitats are often characterized by variable temperature and pH as a result of coupling between environmental factors and benthic metabolism [20]. However, we observed the same impact of elevated mean temperature and/or reduced pH on metabolic rates, regardless of acclimatization to HV (seagrass) or LV (outer-reef) habitats. This was

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Table 1. Statistical results for the interval estimate and significance of temperature, pH and temperature–pH combined on the physiology (photosynthesis, respiration and calcification rates) for the corals *A. palmata* (native to outer-reef), *P. astreoides* (native to outer-reef) and *P. astreoides* (native to seagrass), under both high and low experimental treatment variance. For each of the metabolic parameters (photosynthesis, respiration and calcification) model estimates are shown for different variable interactions (species, habitat, treatment variance, temperature and pH). Models were simplified to remove any non-significant parameters (see the electronic supplementary material). Confidence intervals (CIs) are indicated at 95% for the model estimates as are significance (p) values. Experimental parameters represented in the model are indicated by dummy-coding.

model parameters		photosynthesis (%)				respiration (%)				calcification (%)			
		est.	CI low	CI high	Sig.	est.	CI low	CI high	sig.	est.	CI low	CI high	sig.
variable	interactions	299	293	305	***	151	147	155	***	249	244	254	***
baseline conditions	species	6.5	5.2	7.9	***	8.7	7.2	10.3	***	−9.3	−10.7	−7.8	***
	habitat	−0.4	−2.5	0.9		−0.6	−3.3	1.6		2	0	3.9	
temperature	temperature	8.3	5.6	11.1	***	−1.5	−4.7	3.5		−20.4	−22.9	−17.8	***
	temperature: treatment var.	3.4	1.6	5.2	**	−0.4	−2.7	1		1.7	−0.1	3.5	
	temperature: habitat	1.2	−2.8	4		0.3	−0.7	3.6		1.1	−2.6	3.6	
pH	pH	−17.3	−19.7	−14.8	***	−2.2	−5.5	1.1		−38.7	−41	−36.3	***
	pH: treatment var.	−6.3	−8.1	−4.5	***	−3.2	−5.4	−0.9	*	−1.9	−3.7	−0.1	*
	pH: habitat	3.8	1.3	6.2	**	3.2	−0.1	6.5		3.7	1.4	6	**
temperature and pH	temperature and pH	−26.5	−30.6	−22.5	***	1.5	−3.6	6.1		3.2	−0.8	7.1	
	temperature and pH: treatment var.	3.7	0.6	6.9	*	4.4	0.5	8.3	*	3.8	0.7	7	*
	temperature and pH: habitat	0.5	−1.1	4.5		−4.6	−9.3	0		0.1	−0.2	4	

significance

level	*p*-value
<0.05	*
<0.01	**
<0.001	***

model representation

variables	dummy-coding	experimental parameter
species	1	*P. astreoides*
	−1	*A. palmata*
habitat	1	seagrass
	−1	outer-reef
treatment variance	1	high
	−1	low
temperature	0	present-day ambient
	+1	+2.2°C
pH	0	present-day ambient
	+1	−0.3 pH units

effect estimate

shading	effect description	change
	small increase	+5 to +10%
	small decrease	−5 to −10%
	medium decrease	−10 to −20%
	large decrease	> −20%

**Figure 3.** The metabolic response of corals across experimental treatments. Daily rates of respiration (R), gross productivity (P_G) and calcification (G) for *Porites astreoides* (outer-reef, low-variance (LV) habitat) (4. *a,d,g*), *P. astreoides* (seagrass, high-variance (HV) habitat) (4. *b,e,h*) and *A. palmata* (LV) (4. *c,f,i*) relative to the controls, for both the HV and LV treatments. Daily rates ($\pm$ s.e., $n = 5$) were determined at the end of the experiemnt (t_e). Corals were from Little Cayman, Cayman Islands, BWI, with control conditions resprentative of present-day *in situ* conditions for the seagrass and outer-reef, while experimental conditions best represent the temperature increases and pH decreases estimated under the IPCC A1B scenario.

true for *P. astreoides* found in both habitats, but also for *A. palmata* found only in the LV outer-reef, which under our experimental conditions acclimatized to present-day HV conditions. Therefore, the absence of *A. palmata* from the HV seagrass habitat would suggest that some factor other than variance itself restricts its ability to survive in this environment (e.g. low sexual reproductive success of *A. palmata* [31]).

Within our study, reduced pH had a larger negative impact on photosynthesis and calcification rates relative to the elevated temperature treatment (figure 3). This outcome is consistent with recent observations that calcification rates decline under lower seawater pH scenarios [1], with little-to-no upregulation in calcification from elevated temperature [2]. Both *A. palmata* and *P. astreoides* in our experiment exhibited a large decrease in calcification across all treatments (independently of LV and HV). These observations are consistent with previous reports of a significant decrease in calcification (*ca* 40%) of *P. astreoides* along an increasingly acidic gradient [16] and under lowered pH conditions within laboratory studies [32]. Similarly, *Acropora* spp. have exhibited reduced calcification in experimental acidification scenarios [33]. Such responses are not always observed, with feeding on particulate organics [19] and/or elevated inorganic nutrient availability [34] in particular found to ameliorate the impacts of low pH on *Porites* spp. calcification. However, our experimental set-up was designed to replicate the *in situ* conditions with low inorganic nutrient availability (NO_3^- concentrations less than 1.1 μM) and supplied with organic particulate by the regular exchange of native ambient seawater.

The reduced pH treatments resulted in denser coral calcification for the HV populations, although it is not currently clear why HV corals were more densely calcified when they experienced similar reductions in calcification as the LV populations. HV conditions did result in periods of extended low pH (at night) and consequently, low Ω_{arg} (close-to/or below the saturation threshold). This response appears to mirror abiotic aragonite precipitation, where low Ω_{arg} (pH) conditions have been shown to induce shorter and wider crystal formation in tightly packed bundles, compared with longer thinner crystals under 'normal' Ω_{arg} [6].

For the pH and combined pH/temperature treatments, calcification and photosynthesis were coupled across all treatments. Interestingly, however, the elevated temperature treatment stimulated photosynthesis (as per [35]) for both species examined but was not accompanied by enhanced calcification rates, resulting in an uncoupling of the photosynthesis/calcification relationship. While the cause of this latter response is not entirely clear, Anthony *et al.* [2] observed increased productivity for *Acropora* sp. without an overall decrease in calcification rates for an intermediate warming scenario, roughly equivalent to future treatment levels employed within our study [2]. This is potentially indicative of exceeding thermal windows that govern metabolic processes (e.g. inorganic carbon acquisition [2]). Thermal tolerance windows are species-specific [36] and, perhaps, explain why some studies [35], but not others [37] have observed an increase in calcification with increased temperature. Coral species can potentially sustain calcification through upregulation of pH of the
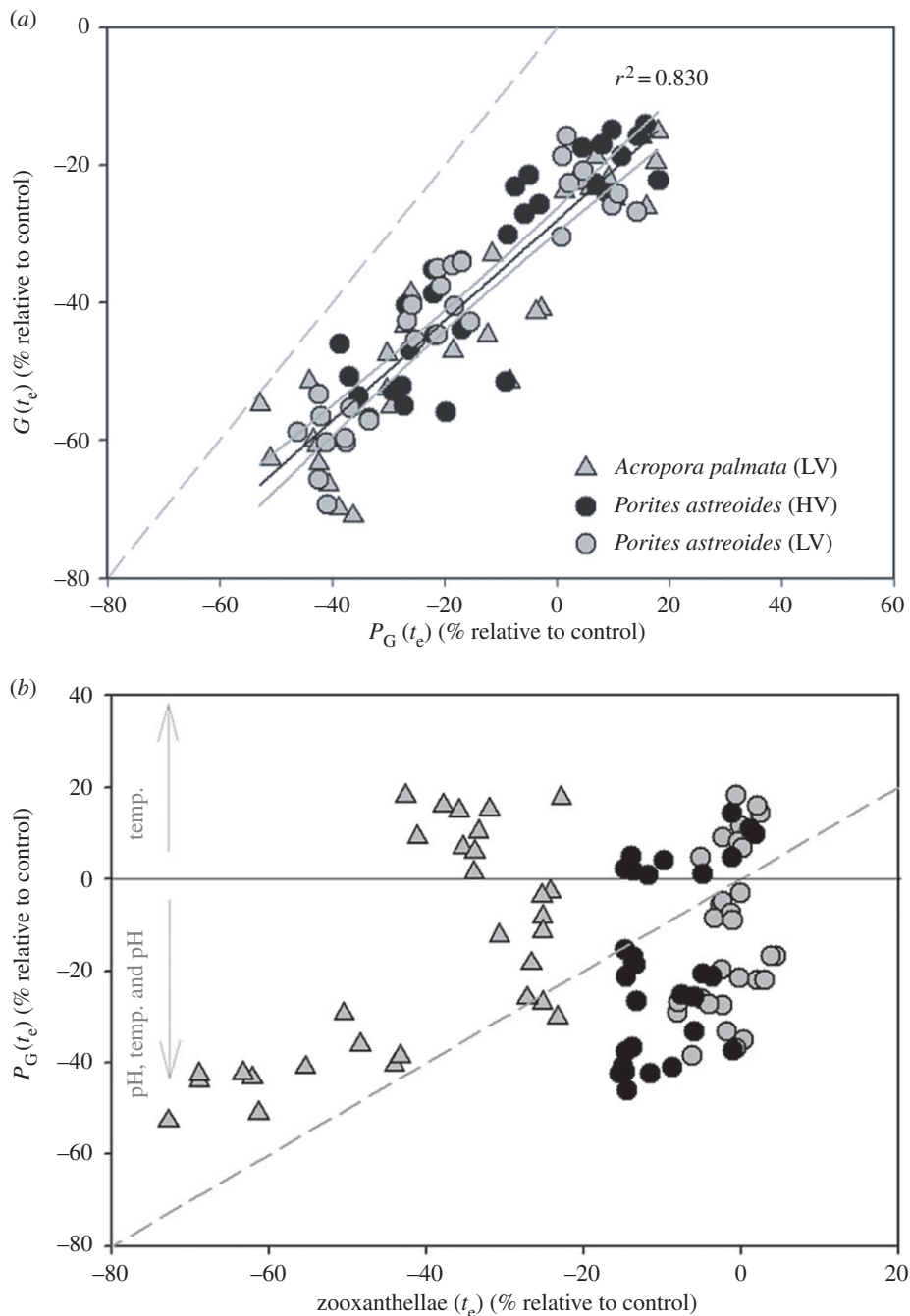
Figure 4. Gross productivity (P_G) versus calcification (G) and zooxanthellae per cent change for *A. palmata* (outer-reef, low-variance (LV) habitat), *P. astreoides* (seagrass, high-variance (HV) habitat) and *P. astreoides* (LV) from Little Cayman, Cayman Islands, BWI. (*a*) Per cent change in P_G versus G and (*b*) Per cent change in P_G plotted against the per cent change in zooxanthellae. Relative per cent change ($\pm$ s.e.) was determined by averaging the experimental replicates per treatment ($n = 5$) and standardizing by the control at the end of the experiment (t_e).

calcifying fluid [7], although this likely comes with an energetic cost [6]. Consequently, corals that are unable to maintain photosynthesis owing to reduced thermal tolerance are likely to experience a decrease in calcification rates, when respiration also remains unchanged as our results demonstrate.

For *P. astreoides*, changes of gross photosynthesis rates over the experimental treatments were independent of zooxanthellae density as cells become less productive with the various stressors. In contrast, *A. palmata* under reduced pH exhibited a loss of productivity that was associated with a loss of symbiont cell density (and coloration). Such acidification-induced bleaching has been observed by Anthony *et al.* [2] for both massive and branching corals, but the cause has not been

fully resolved [2]. For example, depigmentation could stem from direct impacts of acidosis [38], disruption to the carbon-concentration mechanisms [39], or disruption to the photoprotective mechanism of corals through reduction of PGPase [40]. Even so, a key difference between the two species, we tested, appears to be an inherent association with different symbiont genotypes. A search of the 'Coral Trait' database (https://coraltraits.org; search 11 November 2015) demonstrates that *A. palmata* has a highly conserved association with *Symbiodinium* ITS2 type A3 throughout the Caribbean [41], whereas *P. astreoides* has a wider symbiont pool, including types A3, A4, A4a, B1, C3 and Cla-j [41,42]. *Symbiodinium* genotypes, in particular those in Clade A have very different

responses to pH [5] and or temperature [43] and may partly explain why *A. palmata* and *P. astreoides* are affected by temperature and pH stress in very different ways (despite the same net outcome of a reduction in photosynthesis and calcification); this notion warrants more targeted investigation.

Central to how corals will survive pH/temperature stress predicted for reefs under future climate scenarios is whether the rate of adaptation exceeds the rate of environmental change [44]. Within our experiment, we exposed corals to altered environmental conditions of $2.2 \pm 0.03°C$ and a pH decrease of 0.3 ± 0.02 units over 59 days; consequently, any adaptation process exceeding this period will not have been identified. The overall lack in effect of environmental growth history (LV versus HV) on the metabolic rate measurements is consistent with Comeau *et al.* [11], who observed that coral calcification was independent of native Ω_{arg} variance [11]. Similarly, coral populations from the Florida Reef tract exposed to large highly dynamic diel and seasonal fluctuations in pH experienced no reduced effect to elevated $p\mathrm{CO}_2$ conditions expected under acidification [17]. It is conceivable that the differences in environmental growth history may be too small to affect coral physiology, i.e. we observed the same physiological response for corals native to HV and LV in terms of metabolic rate measurements.

Variance in pH and/or temperature did not influence calcification rates, despite the very different Ω_{arg} profiles of each habitat. Under present-day conditions, the seagrass habitat could thus be described as providing a 'buffering' service [9,12], because the daily net Ω_{arg} was elevated by 1.52 units compared with the reef system, thereby maintaining favourable conditions that are being lost elsewhere (a refuge, *sensu* [45]). However, when 2100 pH and temperature conditions were superimposed, the HV seagrass and LV outer-reef achieved similar daily net Ω_{arg}, because the daytime elevation in pH and Ω_{arg} of the seagrass habitat did not compensate for the low night-time conditions. Similarity in coral metabolic response when exposed to 2100 conditions, irrespective of their environmental history, questions whether seagrass habitats can sustain a buffering

role if they ultimately experience the same changes in pH as predicted for the open-ocean.

Whether dissolution of carbonate sediment [10] or enhanced daytime productivity of photoautotrophs [12] can offset the influence of future acidification on inshore habitats remains to be seen. Seagrass beds, as for our HV habitat, typically appear to respond favourably to high-CO_2 with increased reproduction, rhizome biomass and growth of new shoots [46]. As a result, a positive feedback scenario might be expected as ocean acidification progresses, whereby, the amplification of pH by seagrass may increase to moderate the decline in pH. Future research and long-term monitoring is necessary to determine if the status of seagrass as 'winners' with ocean acidification can counteract the decline in pH and provide refuge to corals within or near seagrass beds. A true 'ecological scale' experiment will clearly be required to resolve whether acidification and warmer waters combined will drive greater metabolic variance in shallow habitats; however, we have shown for the first time that variance does not enhance tolerance of *A. palmata* or *P. astreoides* when maintained under present-day variance regimes of pH and temperature. Species appear to have very different photosynthesis responses coupled to a common calcification response across pH and temperature treatments. Shallow water systems with inherently variable pH and/or temperature have been proposed as possible future climate change refugia [12], but the results from our species and system examined would not support this view.

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