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Environmental legacy effects and acclimatization of a crustose coralline alga to ocean acidification

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ABSTRACT

Prior exposure to variable environmental conditions is predicted to influence the resilience of marine organisms to global change. We conducted complementary 4-month field and laboratory experiments to understand how a dynamic, and sometimes extreme, environment influences growth rates of a tropical reef-building crustose coralline alga and its responses to ocean acidification (OA). Using a reciprocal transplant design, we quantified calcification rates of the Caribbean coralline *Lithophyllum* sp. at sites with a history of either extreme or moderate oxygen, temperature, and pH regimes. Calcification rates of *in situ* corallines at the extreme site were 90% lower than those at the moderate site, regardless of origin. Negative effects of corallines originating from the extreme site persisted even after transplanting to more optimal conditions for 20 weeks. In the laboratory, we tested the separate and combined effects of stress and variability by exposing corallines from the same sites to either ambient (Amb: pH 8.04) or acidified (OA: pH 7.70) stable conditions or variable (Var: pH 7.80-8.10) or acidified variable (OA-Var: pH 7.45–7.75) conditions. There was a negative effect of all pH treatments on *Lithophyllum* sp. calcification rates relative to the control, with lower calcification rates in corallines from the extreme site than from the moderate site in each treatment, indicative of a legacy effect of site origin on subsequent response to laboratory treatment. Our study provides ecologically relevant context to understanding the nuanced effects of OA on crustose coralline algae, and illustrates how local environmental regimes may influence the effects of global change.

1. Introduction

Ocean acidification (OA) is one of the foremost global threats to the persistence of coastal marine habitats, particularly calcifier-dominated ecosystems such as coral reefs [1,2]. A tremendous research effort over the last decade has identified predominantly negative effects of decreasing pH on calcifying organisms and the ecosystems they support [3,4]. Although the magnitude and direction of responses vary by taxa [5,6], the majority of reef-builders calcify less under OA, and can even shift to net dissolution [7,8]. Negative effects of OA on biogenic calcification will likely have cascading repercussions for ecosystem function, persistence of habitat frameworks, and survival of associated biodiversity [9–12]. Understanding the factors that influence the response of foundational taxa to OA is essential to improving our predictions for how coral reefs, and the goods and services they provide to millions of people, will be impacted as OA intensifies in the coming decades [13–15]. Past and present exposure to variability in environmental conditions may be a key contributing factor to how the effects of global change will manifest at local scales [16], but remains poorly understood in the context of OA [17].

Environmental variability is inherent in many nearshore ecosystems, where the physicochemical properties of seawater can vary cyclically over multiple spatial and temporal scales [18,19]. Physical processes, including upwelling and tides [20], and biological processes, such as photosynthesis and respiration [21], can cause fluctuations in seawater pH, temperature, and dissolved oxygen (DO) concentrations over predictable time scales [22–24], and can regularly cause local conditions to surpass those predicted to occur in the future ocean under global change. For example, nearshore coral reefs with limited water circulation, such as those found in lagoons, tidal flats, or semi-restricted embayments may frequently experience changes in pH that exceed the 0.2 pH change predicted for end of century with OA [1,17]. Reefs without restricted water flow, but with a high abundance of photosynthetic taxa,

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have similar fluctuations driven by diel cycles in photosynthetic and respiratory modulation of CO_2 concentrations, and thus pH [25]. These dynamic habitats provide an opportunity to explore the role of past and present exposure to pH variability on the performance of key reef taxa and their subsequent responses to OA, and will shed light on how natural variability regimes may influence resilience to global changes stressors and community trajectories in the future ocean.

Historical exposure to environmental variability has the potential to mediate organismal responses to anomalous conditions, potentially increasing tolerance or heightening sensitivity to subsequent stress [26]. Much of the support for this theory is grounded in studies exploring the role of temperature variability in shaping the organismal effects of warming and, on coral reefs, has focused predominantly on scleractinian coral responses. Temperature variability is generally expected to enhance coral resilience to thermal stress [27] and decrease bleaching susceptibility [28], though there are some exceptions (reviewed in [21]). This concept has been generally accepted and is often now incorporated into coral restoration practices where thermally tolerant genotypes are being selectively propagated to build or restore reef habitat [29]. The same theory is also posited to apply to pH variability facilitating OA tolerance, though the few studies to address this question have yielded mixed results [17]. Contrary to predicted theory, prior exposure to pH variability does not necessarily increase coral tolerance to OA [30–33]. Empirical studies that explore the role of pH variability in determining the responses of other key reef taxa to OA are needed to fill these knowledge gaps [17].

The advent and broader implementation of autonomous sensors has increased our awareness and understanding of the scope of pH variability on coral reefs [34]. In addition to including the environmental history of focal taxa in experimental frameworks, incorporating variability regimes into the design of lab-based global change experiments is becoming increasingly important. The majority of OA studies, to date, have simulated reductions in mean pH based on predictions for the open ocean where environmental conditions are relatively stable over time, and have failed to incorporate the diel pH cycling typical of coastal ecosystems. Currently, it is unclear if stable pH reductions are an accurate representation of the potential effects of OA on organisms that reside in naturally dynamic conditions of nearshore habitats. Incorporating variability that is inherent to the respective study system is an important step in improving the ecological relevance and accuracy of global change experiments [16].

Relatively little is known about the effects of variability and global change on key reef builders other than corals, such as calcifying algae. Crustose coralline algae are a critical component of benthic ecosystems globally, and on coral reefs they serve a suite of ecologically important roles where they contribute to reef accretion and stability [35] and facilitate coral settlement [36,37]. Coralline algae secrete the most soluble polymorph of $CaCO_3$, high-Mg calcite [38], and as a result are thought to be among the most sensitive marine taxa to OA [3,39]. Although most crustose coralline algae respond negatively to pH reductions, there is some variability in responses across species [40]. History of exposure to natural pH variation in the environment, in addition to current exposure to variability, may contribute to the documented differential effects of OA within these taxa [30]. Inconsistencies and the lack of adequate information on environmental histories across studies may thus be confounding our understanding of coralline responses to OA. Coralline algae responses to OA will likely have cascading ecological implications, and understanding the biological and physical mechanisms underpinning variation in responses is important for predicting the ecosystem-scale effects of OA.

Here, we use an *in situ* reciprocal transplant study and a controlled laboratory experiment to quantify the effects of environmental history and pH variability on the calcification response of a Caribbean reef-building crustose coralline alga to OA. Our first objective was to use reciprocal transplants across a known site gradient in Bocas del Toro, Panama to test the effects of environmental regimes on *in situ* calcification of *Lithophyllum* sp. at sites with either extreme/variable environmental conditions or moderate conditions. Our second objective was to conduct a concurrent lab experiment with corallines from the same sites to test the hypothesis that prior exposure to extreme conditions would increase coralline tolerance to OA. Our third objective was to incorporate diel pH cycling into OA treatments to test the effects of pH fluctuations relative to current and predicted pH conditions on coralline calcification. By monitoring calcification rates of *Lithophyllum* sp. at multiple time points, we explore the potential for acclimatization to local site conditions *in situ* and for acclimatization to pH treatments in the lab. Our results demonstrate how environmental legacies and pH variability shape the responses of crustose coralline algae to OA, and have important implications for understanding the effects of global stressors in the context of naturally variable environments.

Table 1
Summary statistics from long-term monitoring at the Outer Bay (moderate) (n = 169) and Inner Bay (extreme) sites (n = 185). Sampling was conducted by the Smithsonian Marine Global Earth Observartory (MarineGEO) monitoring program every 1–2 weeks from Jan 2016-2020. Data were collected at 3–4 m depth adjacent to transplant sites.

Site		pH_{NBS}	Temperature (°C)	Dissolved Oxygen (mg L^{-1})
Outer Bay	Mean	8.16	28.7	6.35
	SD	0.16	0.9	0.27
	Range	0.86	4.6	1.67
	Min	7.70	26.3	5.63
	Max	8.55	30.9	7.30
Inner Bay	Mean	8.16	29.4	6.38
	SD	0.17	1.0	0.42
	Range	0.97	5.5	3.84
	Min	7.56	26.7	3.76
	Max	8.53	32.2	7.60

2. Methods

2.1. Site description

This study was conducted from June-November 2017 at the Smithsonian Tropical Research Institute's (STRI) Bocas del Toro Research Station on the Caribbean coast of Panama (Fig. 1b). Almirante Bay is a semi-enclosed bay where open ocean water is exchanged through two inlets [41] (Fig. 1b). A series of islands and mangrove cays further limit water circulation to the innermost reaches of the bay [42]. As a result, the environmental conditions at sites in the inner bay are more extreme and variable with respect to temperature, salinity, oxygen, and pH than outer bay locations closer to the inlets [43–46].

Crustose coralline algae were collected from two sites in Almirante Bay, each representative of either moderate or more extreme, variable conditions. The site we categorize as moderate, Hospital Point (9.3326, -82.2164), was adjacent to one of the two major inlets of water and is hereafter referred to as the Outer Bay or moderate site (Fig. 1b). The site we categorize as having more extreme, variable conditions, Cayo Roldan (9.22025, -82.3231), is hereafter referred to as the Inner Bay or extreme site, and is located at the inner reaches of Almirante Bay (Fig. 1b). Both sites have been thoroughly characterized in previous studies, with the Outer Bay representing mean conditions and moderate environmental variability characteristic of shallow coral reefs (<3-4 m) in Almirante Bay, and the Inner Bay site representing warmer, more acidic, and less oxygenated conditions that have higher ranges of variability over seasonal and diel cycles [44,45] (see Tables 1, 2). From weekly long-term monitoring data from 2011 to 2020 the Inner Bay site on average experienced lower pH (pH 7.92) than the Outer Bay site (pH 8.11) [44] and warmer, more variable temperatures (26.8–31.4 °C versus 26.2–30.7 °C) [46]. Furthermore, over the same time period the Inner Bay site was exposed to more stressful oxygen conditions 53% of the time, while the Outer Bay site experienced similarly stressful conditions only 3% of the

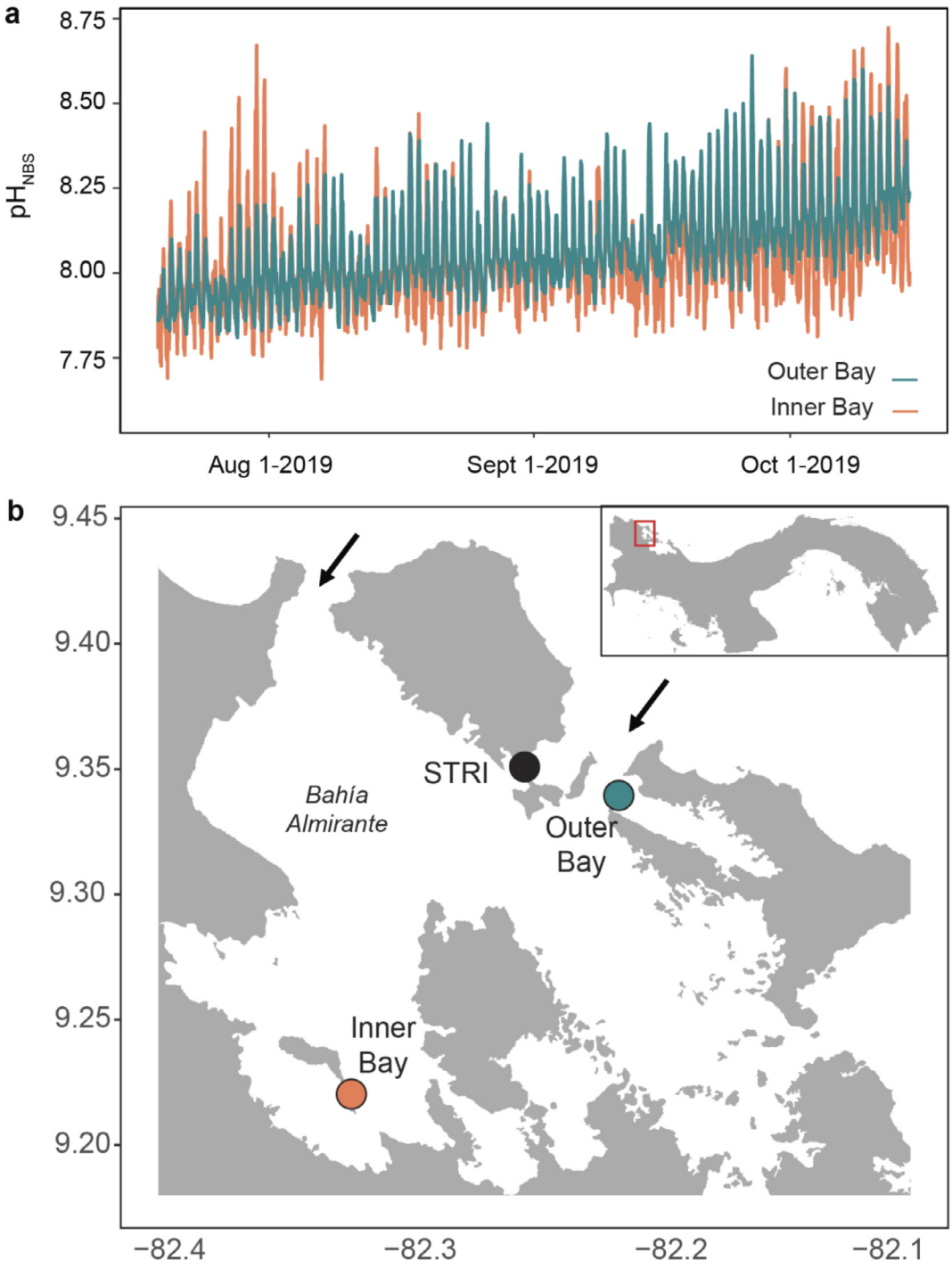

Fig. 1. (a) Diel variability in pH was monitored at the Inner Bay and Outer Bay sites where the crustose coralline alga *Lithophyllum* sp. was collected for *in situ* reciprocal transplants in the field and for the OA laboratory experiment. Each of the two primary inlets for ocean water to the semi-enclosed bay are noted by black arrows. (b) The Inner Bay site is located near the mainland in the Bay of Almirante, Bocas del Toro Panama, and is characterized by extreme, variable environmental conditions, compared to the more moderate Outer Bay site. Lab experiments were conducted at the Smithsonian Tropical Research Insitute's (STRI) Bocas del Toro Research Station.

time [45]. Notably, the Inner Bay sites have been subject to occasional episodes of acute hypoxia, corresponding with late boreal summer and early boreal fall. The first event was documented in 2010 [47], and the second in 2017 during the second half of the reciprocal transplant study [48,49]. Though the spatial and temporal extent of these events is unknown, the field transplants from the Inner Bay site were likely affected by warmer, more acidic, and hypoxic conditions that accompany major hypoxic episodes, and the reoccurrence of these conditions over time further contribute to our classification of this site as extreme.

2.2. Sample collection

We used a common crustose coralline alga, *Lithophyllum* c.f. *intermedium*, in the *in situ* reciprocal transplant study and OA laboratory ex-

Table 2
Mean (±SD) summary statistics from diel monitoring at the Outer Bay (moderate) and Inner Bay (extreme) sites. Statistics first were calculated over each day of sampling, and averages of those summary statistics are presented below. Outer Bay: n = 109 (pH), n = 202 (Temp) n = 145 (DO); Inner Bay: n = 109 (pH), n = 250 (Temp), n = 192 (DO). Significance at $p < 0.05$ is noted in bold.

Site		pH_{NBS}	Temperature (°C)	Dissolved Oxygen (mg L^{-1})
Outer Bay	Mean	8.05 ± 0.11	29.4 ± 0.86	5.50 ± 0.79
	Range	0.33 ± 0.12	0.9 ± 0.43	4.52 ± 2.03
	Min	7.92 ± 0.09	29.0 ± 0.82	3.72 ± 0.93
	Max	8.25 ± 0.17	29.9 ± 1.02	8.24 ± 1.71
Inner Bay	Mean	8.01 ± 0.09	29.6 ± 0.9	5.24 ± 0.65
	Range	0.43 ± 0.16	1.0 ± 0.5	5.79 ± 2.41
	Min	7.83 ± 0.09	29.1 ± 0.8	2.83 ± 0.96
	Max	8.26 ± 0.18	30.1 ± 1.0	8.61 ± 1.89

periment. This coralline species identification was performed through morphological assessments and confirmed by an expert in coralline taxonomy [50] (personal communication, R. Steneck). Given the difficulty of species identifications of crustose coralline algae without genetic sequencing, we take a conservative approach and hereafter refer to corallines used in this study as *Lithophyllum* sp.

Lithophyllum sp. is a heavily calcified, reef-building coralline common on the shallow coral reefs of Almirante Bay, where it grows as a continuous crust on available hard substrata, and often coats small pebbles on shallow fringing reefs (personal observation, M. Johnson). Individuals for laboratory and field experiments were collected as pebbles covered contiguously by *Lithophyllum* sp. from 2–3 m depth in unshaded habitats at the Inner and Outer Bay sites. Corallines were returned to the wet lab facilities at STRI and maintained in constant flow-through seawater and ambient light. Crusts were cleaned of epiphytes with a soft-bristle brush and allowed to recover from collection for 3–4 days before the start of experiments. Corallines used for the *in situ* reciprocal transplant study were outplanted to their origin site or to the transplant site within 3 days of collection.

2.3. *In* situ *reciprocal transplants*

To explore the impact of environmental regimes on coralline calcification *in situ, Lithophyllum* sp. was reciprocally transplanted between the Inner and Outer Bay sites following initial buoyant weight measurements in the lab. Ten individuals from each site were transplanted to the opposite site (hereafter referred to as transplant site) and ten were returned to their site of origin (hereafter referred to as origin site) (Fig. 1b). Individuals were labeled and secured with zip ties to plastic egg crate racks that were mounted on cinder blocks. Racks were deployed at the same depth (2–3 m) and location of collection at each site.

Corallines were buoyant weighed at the start of the experiment (prior to outplanting), after 10 weeks, and again after 20 weeks. Buoyant weight was converted to actual weight based on the density of calcite (2.71 g) following [51]. Because we were interested in potential for acclimatization over time, we present the overall calcification rate (0–20 weeks) and the calcification rates for the first and second halves of the experiment separately (0–10 weeks, 10–20 weeks). Calcification rates were calculated as the change in weight normalized to initial weight and duration for each time period, and are expressed as mg $CaCO_3$ mg^{-1} day^{-1}.

2.4. *Environmental monitoring*

Environmental conditions at each site are presented from two monitoring efforts. To characterize prevailing patterns at the focal sites at a diel temporal resolution, we deployed autonomous loggers at each site to measure temperature and pH_{NBS} (HoboOnset, MX2501), conductivity (HoboOnset, U24-002), and DO (HoboOnset, U26-001) every hour. Instruments were deployed at 3 m depth at the transplant sites from July through November 2019, directly matching the seasonal timeframe of the reciprocal transplant study. Instruments were maintained and calibrated monthly following manufacturer protocols. Dissolved oxygen data were corrected by conductivity and temperature using HoboPro Software. Because the autonomous instruments were not available to us in 2017, the diel monitoring data do not directly represent the conditions experienced by corallines during the reciprocal transplant study. However, the 2019 diel monitoring data does shed light on the scope of diel variability typical for each site, and the long-term monitoring data (described below) reveal that conditions for all parameters were similar in 2017 and 2019. Raw hourly pH data illustrate the differences in diel pH cycling at the two focal sites (Fig. 1a). Diel summary statistics (i.e., mean, range, minimum, maximum) were used in all analyses and subsequent figures. pH data logged after Oct 15, 2019 were excluded from analyses due to possible sensor drift.

Long-term monitoring of temperature, pH, and dissolved oxygen (DO) occurred from January 2016 through January 2020 (STRI Physical Monitoring Program). The temporal frequency of sampling varied from weekly to biweekly depending on weather conditions. Surveys occurred at sites adjacent to the Outer and Inner Bay sites through depth profiles with a handheld multiparameter sonde (YSI EXO and EXO2, optical DO sensors: accuracy 0.1 mg L^{-1}; temperature sensors: accuracy 0.01 °C; pH sensors: accuracy 0.02 units, Yellow Springs Instruments). The instruments were calibrated before sampling days following manufacturer protocols. pH was calibrated with NBS buffers and is presented on the NBS scale (pH_{NBS}) for all environmental measurements (diel and long-term). Sample measurements were taken between 0800 and 1100, and sites were measured in the same order every sampling day. Only data from the depths at which outplants were positioned (3–4 m) are presented. Although the temporal resolution of these data is coarse, they provide long-term annual and seasonal trends for 4 consecutive years at the focal sites.

2.4. *OA laboratory experiment*

The OA experiment was conducted in the temperature controlled wet lab at the Bocas del Toro Research Station (BRS). *Lithophyllum* sp. individuals were exposed to one of four treatments for 16 weeks: ambient control (Amb), variable (Var), stable OA (OA), and OA variable (OA-Var). Three corallines from each site were co-mingled within 2.8 L plastic aquaria that received a constant flow of treatment seawater, with 6 replicate aquaria per treatment. The control treatment consisted of untreated, filtered seawater from the BRS seawater system (~8.04 total scale pH). The OA treatment simulated a stable acidified daily mean pH (7.70), which represents ocean conditions predicted for the end-of-the century in business as usual representative carbon pathways [1], and is the approximate minimum pH at the Inner Bay site from the long-term monitoring data (Table 1). The variable treatments overlaid the ambi-

ent and OA treatments with diel changes in pH by 0.3 units, a range in pH that has been observed on some coral reefs, including the reefs in Almirante Bay (Table 2) [17,21,44,51]. The variable treatment targeted a daily pH range of ~7.8–8.1, while the variable OA treatment targeted a daily range of ~7.45–7.75 to incorporate diel cycling concurrent with acidification. The variability regime and acidification in the OA-Var treatment approximate the environmental conditions of the extreme site, based on long-term and diel monitoring data (Tables 1, 2), which illustrates how present-day conditions at the Inner Bay present an analogue for future ocean conditions.

The experimental setup is detailed in [53], and described briefly here. Treatment aquaria were positioned haphazardly within a flow-through seawater table with each aquarium containing a mini pump (Homasy 300 L hr^{-1}) to maintain circulation within tanks, and tanks shuffled every 2–3 days to reduce potential position effects. Temperature control of the air in the aquarium facility was set to 28 °C, which regulated tank temperatures. Lighting was supplied by 8, 7-color programmable LEDs (Aquaillimunation, Hydra 52) evenly spaced across all tanks. Lights were programmed to simulate the ambient 12:12 h light-dark cycle, with increasing intensity over four hours starting at 0600 to simulate sunrise and decreasing intensity over four hours from 1400 to simulate sunset. Light intensity was adjusted to simulate midday photosynthetically active radiation (PAR) intensities at collection sites at ~2–3 m depth (~270 μmol photon $m^{-2}s^{-1}$). A LiCor meter (Li-1400) and cosine quantum sensor (Li-193) were used to measure PAR in the field and in the lab when tank positions were shuffled.

Seawater flowed continuously into each aquarium at a rate of 15.7 mL min^{-1} from either the ambient seawater line or treatment header tanks. pH was manipulated in header tank reservoirs by bubbling ambient seawater with pure CO_2, using a pH-feedback to control gas injection into one header tank per treatment. A pH probe in each header tank measured and logged pH every minute during the experiment and was connected to a solenoid valve and CO_2 gas through an aquarium controller (Neptune Systems, Apex Aquacontroller). The controller was set to the targeted pH values, and when pH increased above the target, the pH probe triggered a solenoid valve to open and release pure CO_2 into the header tank. In the variable treatments, the controller was programmed to adjust target pH values to include stepwise changes in pH at 1–2 h intervals to simulate natural cycling in pH, with peak highs during midday and peak lows at night. Neptune controller pH probes were calibrated every week with NBS buffers following factory protocol (pH 7.00, 10.00), and corrected to total scale pH (pH_T) by cross-calibration with the Ross triode (described below). To minimize initial shock responses to pH treatments, all corallines were initially exposed to ambient conditions and then the pH of treatments was decreased by 0.05 units a day until reaching target values.

Carbonate chemistry of treatment and header tanks was determined from direct measurements of pH_T, temperature, salinity, and total alkalinity (A_T). Daily discrete measurements for pH, temperature, and salinity were taken between 0930-1030. pH was measured with a Ross Ultra Triode connected to an Orion Star pH meter. The Ross triode was calibrated weekly with NBS buffers and pH measurements were corrected to total scale pH using weekly calibrations against certified reference material with a known pH (Tris Buffer in synthetic seawater, Batch T30, A. Dickson Scripps Institution of Oceanography). Temperature was measured with a traceable digital thermometer accurate to ±1 °C (Thomas Traceable Kangaroo thermometer) and salinity was measured with a handheld YSI (YSI-63-10). To characterize the actual (versus programmed) diel cycle in each treatment, and ground truth pH recorded by the aquacontroller, we took discrete measurements of pH and temperature with the handheld probes every hour for one full 24 h cycle. Due to logistical constraints we were unable to measure pH of the ambient treatment continuously. Instead, we used the daily discrete measurements to represent ambient treatment conditions and the 24 h sampling to verify the absence of notable pH cycling.

Water samples were collected from header tanks, a subset of treatment aquaria, and the ambient seawater line every week for determinations of A_T. Seawater was siphoned from header tanks or aquaria into 250 mL borosilicate bottles. Samples were equilibrated to laboratory room temperature and analyzed within 12 h of collection. Any samples that could not be analyzed within this time period were poisoned with 200 μL of saturated mercuric chloride solution upon collection to prevent biologically-driven changes in seawater chemistry. Open-cell potentiometric titrations for A_T were conducted with an automated titrator (Mettler Toledo DL15) fit with a DG115-SC pH probe (Mettler Toledo) calibrated daily with NBS buffers (pH 4.00, 7.00, 10.00). Titrations were performed with certified HCl titrant (A. Dickson) and followed standard operating protocol (SOP 3b) [54]. Measurements were quality checked for accuracy against certified reference material (Reference Material for Oceanic CO_2 measurements, Batch 158, A. Dickson) at the beginning, middle, and end of each set of titrations. The mean accuracy of A_T measurements was within 0.86% of reference material ($n = 25$). The remaining carbonate parameters were calculated with the package *seacarb* in R v 3.4.2 [55] from measurements of temperature, salinity, pH_T, and A_T.

Net calcification rate of coralline fragments was determined by the change in buoyant weight as described above. For the lab experiment, fragments were buoyant weighed initially, after 8 weeks, and again after 16 weeks. Calcification rates are expressed as mg $CaCO_3$ mg^{-1} day^{-1}, and are presented as calcification rate over the full 16-week experiment, and calcification rates for the first and second halves of the experiment, 0–8 weeks and 8–16 weeks, respectively.

2.5. Statistical analyses

All analyses were performed in R v 4.0.2 [56]. For the diel environmental monitoring data from 2019, we calculated daily mean, range, and minimum pH and DO for each site. The overall average pH of each treatment for the duration of the experiment was calculated from the daily discrete measurements (between 0930-1030) that were averaged across all tanks within a treatment for each day (Table 3). We similarly calculated daily minima and maxima from hourly averages of pH_T (Neptune) to determine the average diel cycle in each treatment (Table 4). For the ambient treatment, for which there were no continuous pH measurements, the average pH range was calculated over one 24 h sampling period (Table 3). T-tests were performed to test for site effects with each variable. Long-term environmental data are presented as raw data, with Loess smoothing to visualize trends over time. Differences between Outer Bay and Inner Bay long-term site averages were evaluated with separate t-tests for temperature, DO, and pH_{NBS}.

Treatment effects on calcification rates were analyzed with linear mixed effects models constructed with the package *lme4* [57]. To evaluate model assumptions, normality of calcification rates was assessed by visual inspection of residuals and Q-Q plots, and homoscedasticity was evaluated with Levene's test. *In situ* calcification rates (overall and at each time point) were square root transformed to meet model assumptions, and calcification rates from the laboratory OA experiment were transformed with a Box-Cox transformation. Untransformed data are presented in figures to aid interpretation. For the field transplant experiment, overall *in situ* calcification rate (0–20 weeks) was modeled as a function of Origin site and Transplant site as fixed effects, and the interaction of the two factors. A linear mixed effects model was used to evaluate *in situ* calcification rates at the two time points (0–10 weeks and 10-20 weeks) as a function of Origin Site, Transplant Site, and Time Point as fixed effects, the factorial interactions of all factors, and Individual included as a random effect to account for repeated measures over time. For the lab OA experiment, overall calcification rate was modeled as a function of Site and OA Treatment as fixed interactive effects and Tank as a random effect nested within OA Treatment. Calcification rate over time was modeled as a function of Site, OA Treatment, and Time as fixed interactive effects, Individual as a random effect, and Tank as a random effect nested within treatment. The significance of fixed effects

Table 3
Mean physical parameters (± SE) for each treatment from discrete daily measurements. Temperature, salinity, and pH_T were measured daily ($n = 82$). Light and water flow were measured every 4-5 days after tank positions were haphazardly shuffled ($n = 18$). Replicate tank values ($n = 6$ per treatment) were averaged for each day, and these means were averaged to yield overall daily treatment means. Total alkalinity (A_T) was measured every 5–7 days from header tanks and ambient seawater ($n = 14$). pCO_2 and Ω_c were derived from measured values of A_T, salinity, temperature, and pH_T using the package *seacarb* in R. Treatments were ambient (Amb), variable (Var), ocean acidification (OA), and ocean acidification with variability (OA-Var). Light† = photosynthetically active radiation (PAR, μmol photon m^{-2} s^{-1}), pH_T‡ = total scale, DIC = dissolved inorganic carbon, Ω_c = saturation state of calcite.

Treatment	Temp (°C)	Salinity (PSU)	Light†	Flow (L min^{-1})	pH_T‡	A_T (μmol kg^{-1})	pCO_2 (μatm)	DIC (μmol kg^{-1})	Ω_c
Amb	29.2 ± 0.1	32.2 ± 0.16	288 ± 5	15 ± 3	8.04 ± 0.004	2134 ± 11	386 ± 4	1841 ± 3	5.21 ± 0.06
Var	28.9 ± 0.1	32.2 ± 0.16	298 ± 4	17 ± 2	7.76 ± 0.005	2126 ± 13	815 ± 10	1967 ± 5	3.02 ± 0.03
OA	29.0 ± 0.1	32.2 ± 0.16	302 ± 6	13 ± 3	7.70 ± 0.004	2132 ± 15	918 ± 6	1990 ± 6	2.68 ± 0.03
OA-Var	28.8 ± 0.1	32.2 ± 0.16	280 ± 5	18 ± 1	7.58 ± 0.006	2128 ± 14	1241 ± 12	2022 ± 5	2.09 ± 0.03

Table 4
Mean (± SE) minimum and maximum pH_T over a diel cycle for laboratory ocean acidification treatments. Treatments were ambient (Amb), variable (Var), ocean acidification (OA), and ocean acidification with variability (OA-Var). pH is presented on the total scale (pH_T).

Treatment	Minimum pH_T	Maximum pH_T	Range
Amb	7.92 ± 0.007	8.05 ± 0.006	0.13
Var	7.67 ± 0.005	7.94 ± 0.004	0.27
OA	7.68 ± 0.004	7.72 ± 0.005	0.04
OA-Var	7.45 ± 0.009	7.75 ± 0.010	0.30

Table 5
Results of linear models evaluating *in situ* calcification rates of the reciprocal transplant study. Overall calcification rate (0–20 weeks) was modeled as a function of Origin Site and Transplant Site as fixed interactive effects. Calcification rates over time (first half: 0–10 weeks, second half: 10–20 weeks) were modeled as a function of Origin Site, Transplant Site, and Time Point as fixed interactive effects. Significance at $p < 0.05$ is noted in bold.

Fixed Effect	F	*p*
Overall Calcification Rate (0-20 weeks)		
Origin Site	0.03	0.870
Transplant Site	17.61	**< 0.001**
Origin x Transplant	1.95	0.173
Calcification Rate (0-10 weeks & 10-20 weeks)		
Origin Site	0.02	0.888
Transplant Site	25.78	**< 0.001**
Time Point	29.35	**< 0.001**
Origin x Transplant	2.47	0.126
Transplant x Time	10.92	**0.002**
Origin x Time	0.30	0.587
Origin x Transplant x Time	0.08	0.777

was determined from type II analysis of variance tables with Satterthwaite's method. Significant differences between treatments were determined by Tukey's post-hoc pairwise contrasts using least-square means in the R package *emmeans* [58].

3. Results

3.1. Environmental monitoring

Diel hourly sampling for pH, temperature, and DO illustrate higher variability and extreme values of pH and DO (Fig. 2) at the Inner Bay site compared to the Outer Bay site at a diel resolution, but no differences in temperature between sites. The diel range of pH and DO at the Inner Bay site was significantly higher than at the Outer Bay site (range pH: $p < 0.001$; range DO: $p < 0.001$), and was matched by significantly lower diel mean and minimum values for pH (mean pH: $p = 0.014$; min pH: $p < 0.001$) and DO (mean DO: $p < 0.001$; min DO: $p < 0.001$) (Table S1). The range of daily averages in pH_{NBS} was 0.11 units higher at the Inner Bay site than at the Outer Bay site (0.43 vs 0.33), which corresponded with an average minimum pH_{NBS} at the Inner Bay site of 7.83 vs 7.92 at the Outer Bay site. Similar patterns were detected for DO concentrations, with a greater range and lower minimum at the Inner Bay site (see Table 2 for all summary statistics). Lower pH and DO conditions at the diel scale (Fig. 2) illustrate how conditions at the Inner Bay site can be considered more extreme and variable than the more moderate conditions at the Outer Bay site.

The Inner Bay (extreme) site was significantly warmer than the Outer Bay (moderate) site in the long-term monitoring data from 2016-2020 ($p < 0.001$), with no difference in average pH ($p = 0.887$) or DO concentrations ($p = 0.403$) (Fig. 3; Table S2). Average (±SD) temperature at the Inner Bay site was 29.4 ± 1.0 °C and ranged from 26.7–32.2 °C. Conversely, temperature at the Outer Bay site was only slightly cooler at 28.7 ± 0.9 °C on average, but was less variable and ranged from 26.3–30.9 °C (see Table 1 for all summary statistics). The long-term average DO concentrations were not significantly different between sites (Table S2). However, the lack of difference in DO site means potentially underestimates the impact of anomalous hypoxic conditions that occurred in the acute event in 2017 during the reciprocal transplant study or low-oxygen conditions that could have occurred at night [44,45]. A substantial decline in DO concentrations, paired with lower pH conditions, are clear in the long-term monitoring data during this time period (Fig. 3). Although weekly to biweekly sampling failed to resolve diel differences across sites, these long-term trends show how corallines collected from the Inner Bay site experienced warmer and more variable temperatures at a seasonal to annual scale than those collected from the Outer Bay site (Fig. 3).

3.2. In situ *reciprocal transplants*

In the reciprocal field transplant experiment, overall *in situ* calcification rates of *Lithophyllum* sp. were significantly lower at the Inner Bay site after 20 weeks of outplanting (Transplant Site, $p < 0.001$), regardless of whether corallines originated from the Inner or Outer Bay sites (Origin Site, $p = 0.906$) (Table 5; Fig. 4a). Individuals outplanted to their original site (transplant controls) trended towards having higher calcification rates than the transplants at the same site, though this interaction was not significant (Origin x Transplant, $p = 0.164$) (Table 5). On average, calcification rates were ~33 to 56% lower in corallines at the Inner Bay for the site controls and the transplants, respectively, relative to their counterparts at the Outer Bay site (Fig. 4a).

To evaluate potential for *Lithophyllum* sp. to acclimatize to *in situ* environmental conditions, we monitored calcification rates over the first and second halves of the experiment (0–10 weeks and 10–20 weeks post-transplant) (Fig. 4b). There was a significant interactive effect of time point and transplant site on calcification rates (Transplant x Time, $p = 0.004$) (Table 5), and no interactions among origin site and time point, origin site and transplant site, or in the three-way interaction among all factors (Table 5). The significant time and transplant site in-

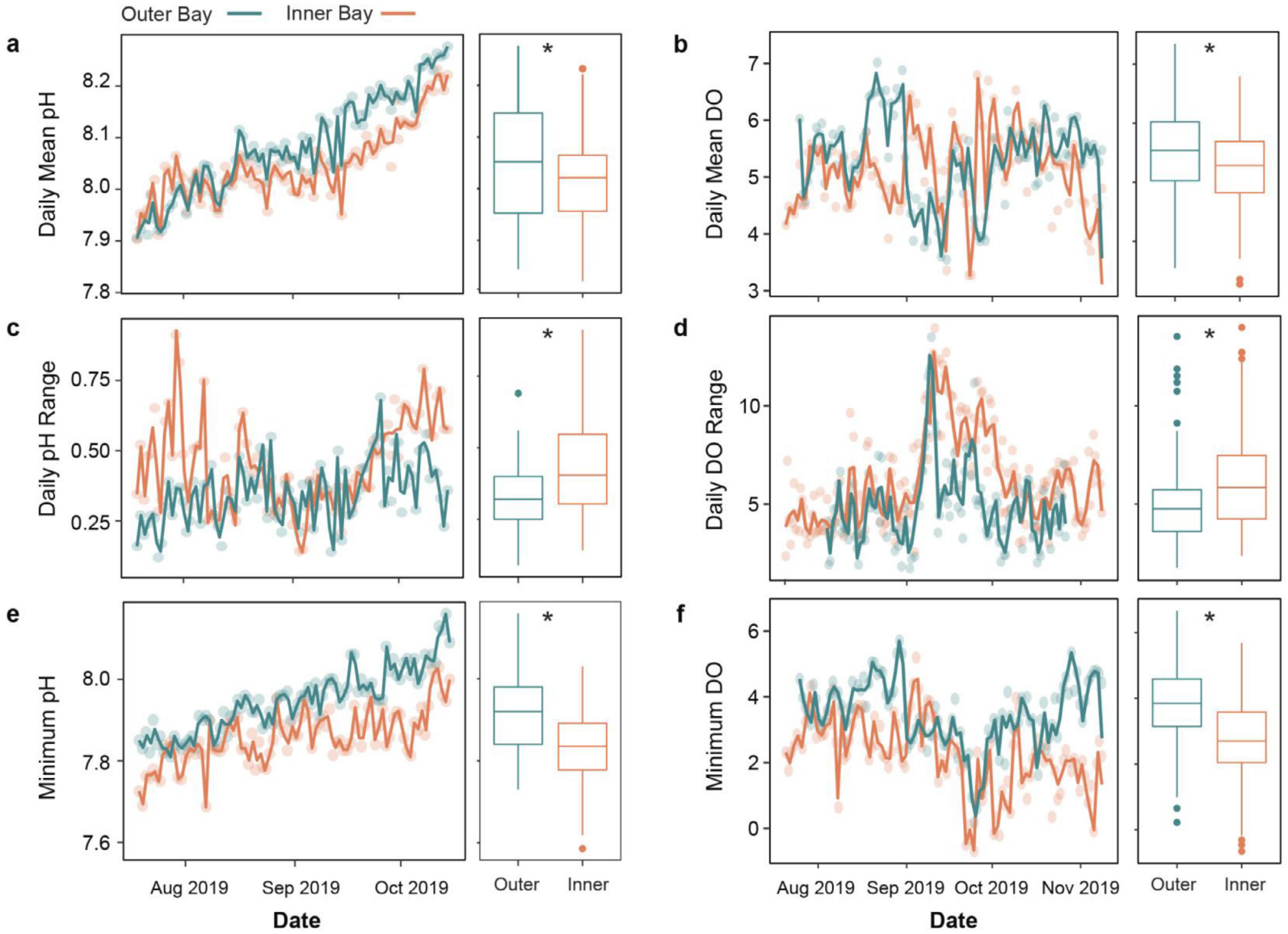

Fig. 2. Diel monitoring data from the Inner (orange) and Outer Bay (blue) sites. Data are presented as the average for each day sampled from Nov – July 2019. (a) pH is on the NBS scale, (b) dissolved oxygen (DO) is in in mg L^{-1}. (c) Daily average pH range, (d) daily average DO range, (e) daily average minimum pH, and (f) the daily average minimum DO. Solid lines indicate Loess smoothing of the daily averages for each parameter, which are represented by lighter colored dots. In box plots, the center line indicates the median, the box limits the interquartile range, the whiskers the minimum and maximum values, and the dots the outliers. An asterisk on the box plot indicates that the parameter was significantly different between the Inner and Outer Bay sites at $p < 0.05$.

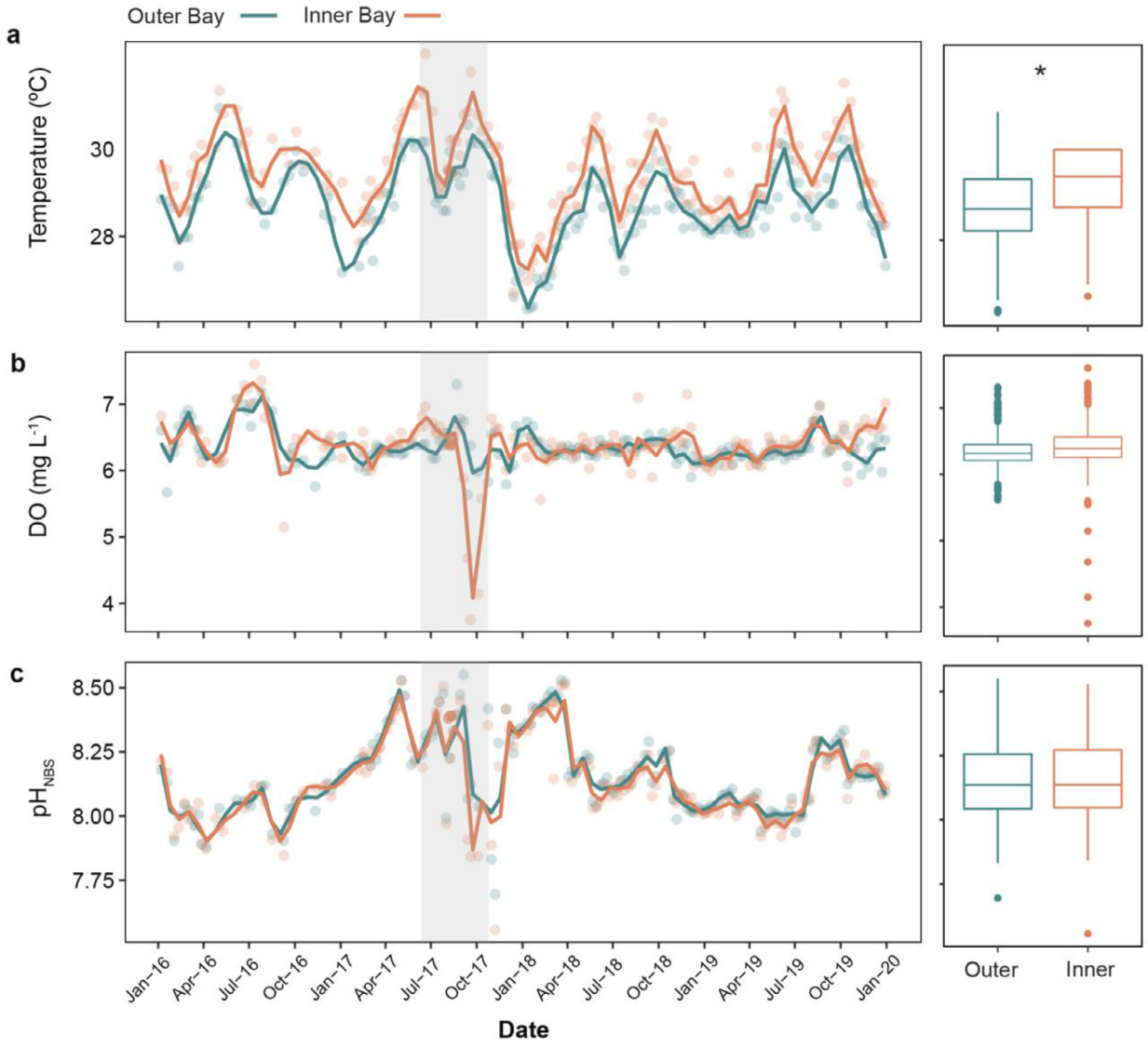

Fig. 3. Long-term monitoring data from the Smithsonian Institution's MarineGEO monitoring program for (a) temperature (°C), (b) dissolved oxygen (DO) concentrations in mg L^{-1}, and (c) pH on the NBS scale. Monitoring occurred at sites adjacent to the Inner (orange) and Outer Bay (blue) sites every 1-2 weeks from January 2016 through January 2020. Solid lines indicate Loess smoothing of the raw data, which are represented by lighter colored dots. In box plots, the center line indicates the median, the box limits the interquartile range, the whiskers the minimum and maximum values, and the dots the outliers. The shaded area highlights the time period of the recriprocal transplant study (July – Nov 2017). An asterisk on the box plot indicates that the parameter was significantly different between the Inner and Outer Bay sites at $p < 0.05$.

teraction was driven by different magnitudes of calcification response at the Inner Bay site and Outer Bay site during the first versus second half of the experiment. Although calcification rates trended towards being lower at the Inner relative to Outer Bay site during the first half of the experiment, this result was not significant (Tukey's test, $p > 0.05$). During the second half of the experiment, however, *Lithophyllum* sp. calcified significantly less at the Inner Bay site regardless of origin (Tukey's test, $p < 0.05$), with both transplants and site controls calcifying ~70 or 90% less in the Inner Bay relative to their counterparts in the Outer Bay (Fig. 4b).

3.2. OA laboratory experiment

Mean hourly pH is presented to visualize the diel patterns in treatment conditions for one representative month during the laboratory OA experiment (Fig. 5a), and over one 24 h sampling period (Table 4; Fig. 5b).

Lithophyllum sp. overall calcification rates (0-16 weeks) in the lab varied significantly depending on their site of origin (Origin, $p < 0.001$) and OA treatment (Treatment, $p < 0.001$), with no interaction between origin site and treatment (Origin x Treatment, $p = 0.648$) (Table 6). Corallines from the Inner Bay site calcified ~35–45% less than the Outer Bay corallines in all but the Ambient treatment (Fig. 6a). Corallines originating from both sites showed the same pattern of response to OA treatments, with highest overall calcification rates in the Ambient treatment and lowest calcification rates in the OA-Var treatment (Fig. 6a). The Var and stable OA treatment depressed calcification rates to similar magnitudes. For the Outer Bay corallines, Var, OA, and OA-Var decreased calcification rates by ~19%, 12%, and 43%, respectively, relative to Ambient controls (Fig. 6a). The magnitude of treatment effect was greater in corallines from the Inner Bay site, with corresponding rates that were ~37%, 37%, and 63% lower than the respective Ambient control (Fig.6a).

For calcification rates at the two time points (0-8 weeks, 8-16 weeks), there was a significant interactive effect of origin site and time point,

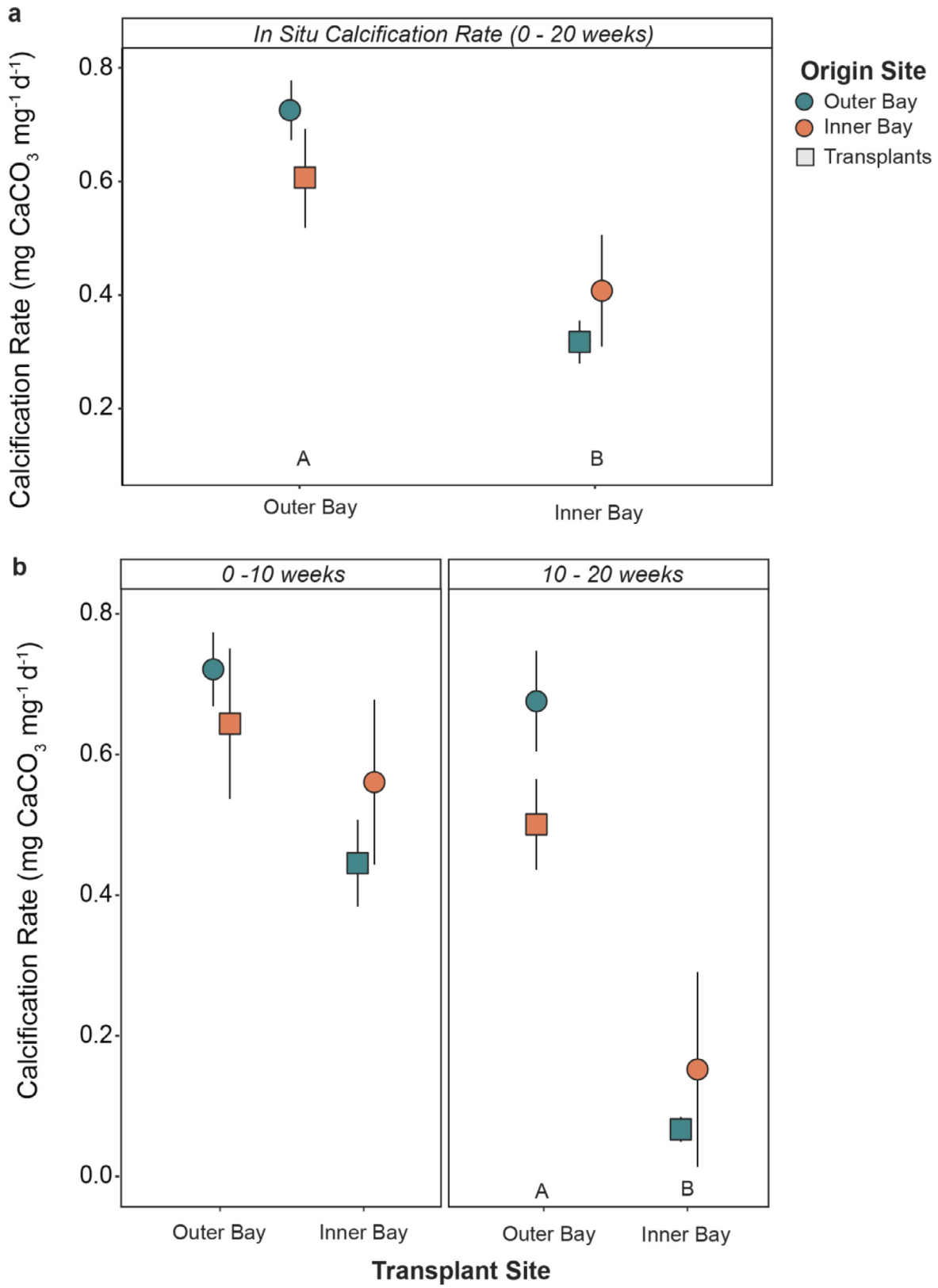

Fig. 4. (a) Mean (±SE) *in situ* calcification rates of *Lithophyllum* sp. for the full 20-week transplant study, (b) for weeks 0-10 (left) and for weeks 10-20 (right). Circles indicate site controls (returned to origin site) and squares indicate transplants. Blue represents corallines originating from the Outer Bay site and orange represents corallines originating from the Inner Bay site. Different letters indicate significant differences between transplant sites. Upper case letters indicate effects are the same for both origin sites. Significance is reported from tukey's tests at $p < 0.05$.

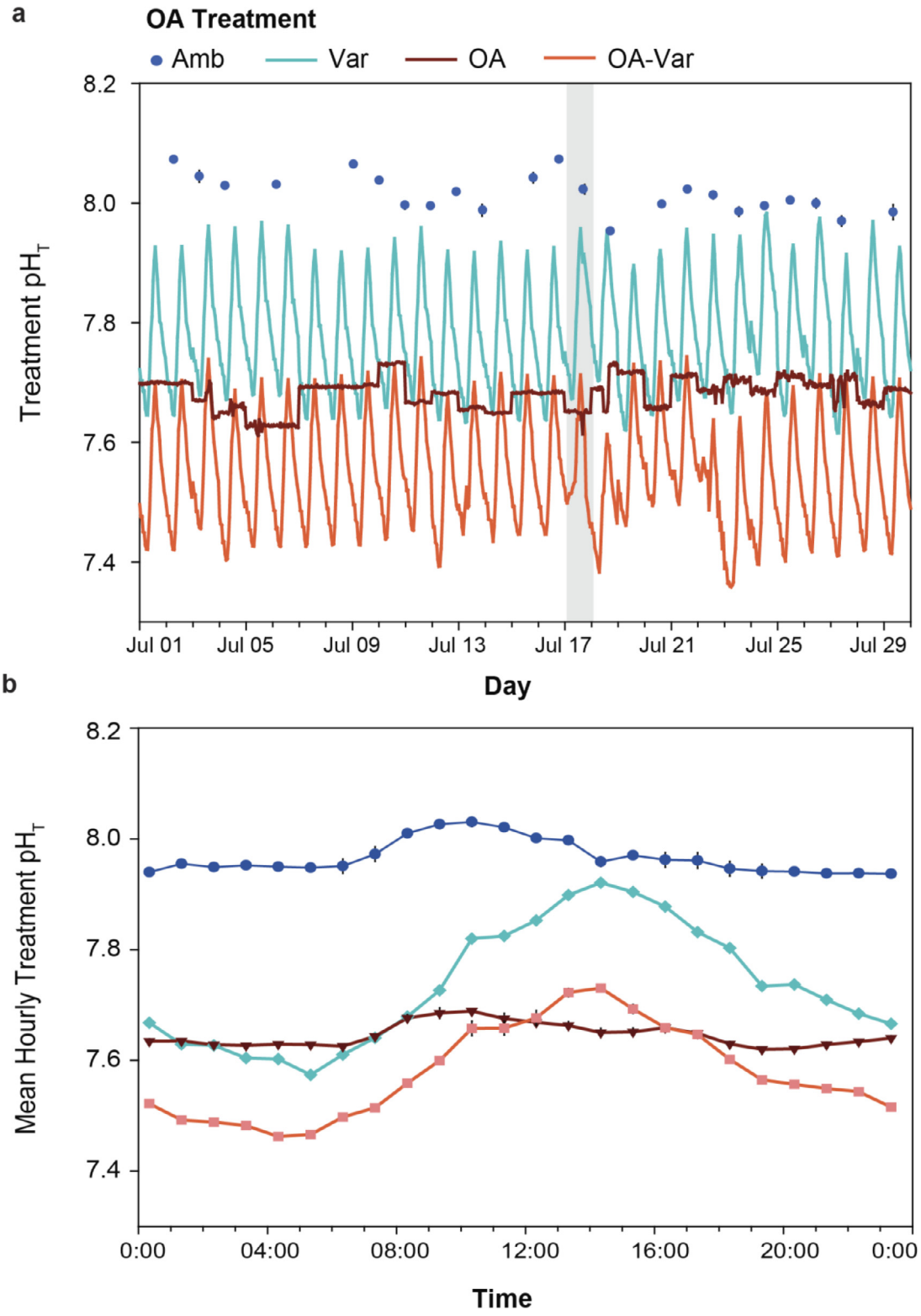

Fig. 5. (a) Mean treatment pH_T (total scale) for one month of the laboratory experiment. The lines represent the mean hourly pH of each header tank for the respective pH treatments. Due to logistical constraints no continuous pH data are available for the ambient treatment. The blue dots represent the mean (±SE) pH for the ambient treatment from daily discrete measurements (averaged across all ambient tanks each day). The shaded date indicates measurements of (b) mean (±SE) hourly pH_T in treatment tanks taken from discrete measurements every hour over one 24 h period. Treatments are referred to as Amb (ambient), Var (variable), OA (stable OA), and OA-Var (OA variable). Small error bars may be occluded by symbols.

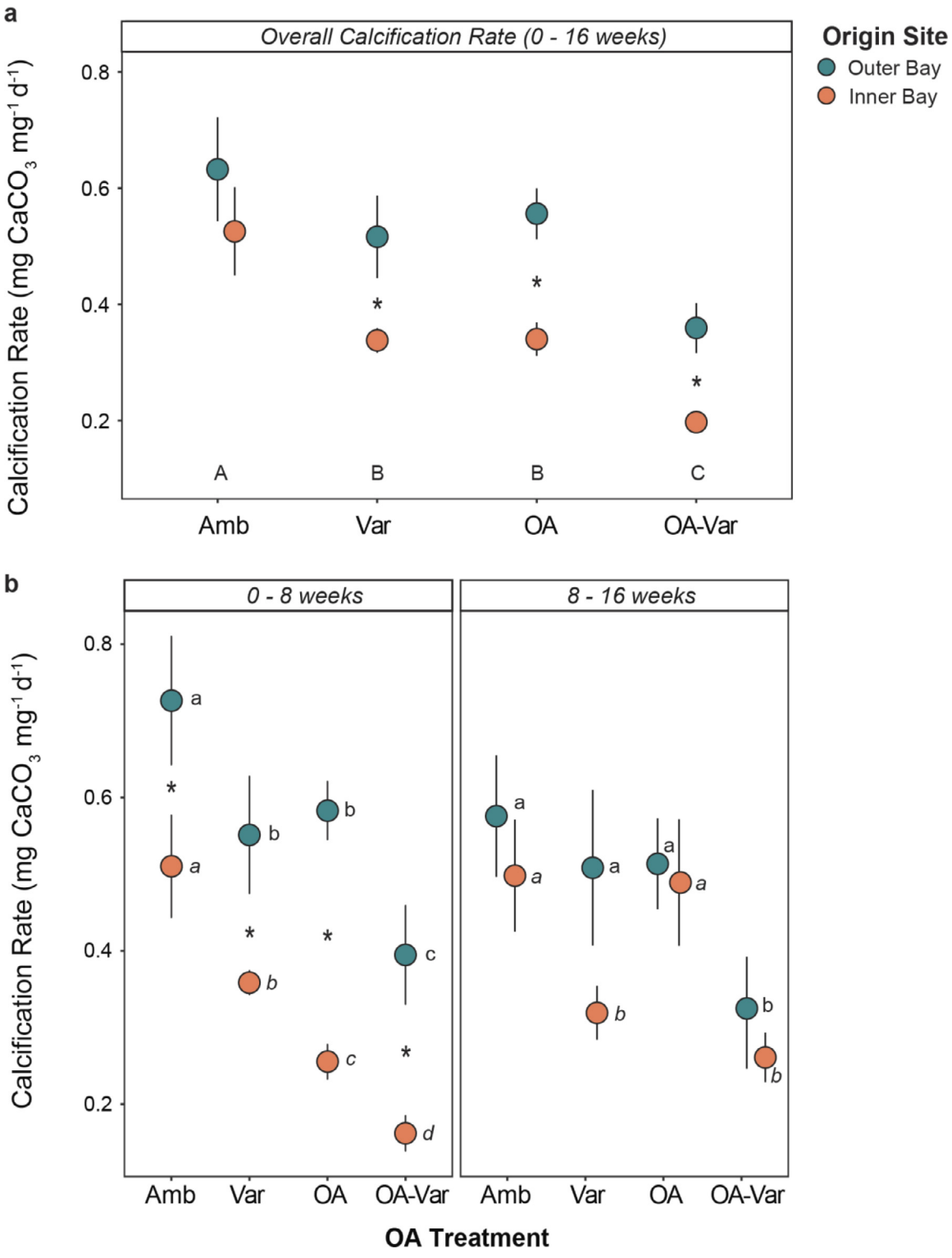

Fig. 6. (a) Mean (±SE) calcification rates of *Lithophyllum* sp. for the full 16-week lab experiment, and for (b) weeks 0–8 (left) and (c) weeks 8–16 (right) in response to OA treatments. Blue represents corallines originating from the Outer Bay site and orange represents corallines originating from the Inner Bay site. Different letters indicate significant differences between treatments. Upper case letters indicate effects are the same for both origin sites. Lower case letters indicate significant differences between origin sites for each treatment. The asterisk indicates a significant difference between the Outer Bay and Inner Bay corallines within a treatment. Significance is reported from tukey's tests at $p < 0.05$.

Table 6
Results of linear models evaluating calcification rates of corallines in the laboratory ocean acidification experiment. Overall calcification rate (0-16 weeks) was modeled as a function of Origin Site and Treatment as fixed interactive effects. Calcification rates over time (0–8 weeks, 8–16 weeks) were modeled as a function of Origin Site, Treatment, and Time Point as fixed interactive effects. Significance at $p < 0.05$ is noted in bold.

Effect	F	*p*
Overall Calcification Rate (0-16 weeks)		
Treatment	13.65	**< 0.001**
Origin Site	14.63	**< 0.001**
Origin Site x Treatment	0.55	0.648
Calcification Rate (0-8 weeks & 8-16 weeks)		
Treatment	12.76	**< 0.001**
Origin Site	16.66	**< 0.001**
Time Point	1.49	0.225
Origin Site x Treatment	0.06	0.978
Time Point x Treatment	2.37	0.073
Origin Site x Time Point	13.64	**< 0.001**
Origin Site x Treatment x Time Point	1.98	0.120

indicating that corallines from the two sites differed in their response to the treatments over the course of the experiment (Origin x Time Point, $p < 0.001$). There were also significant main effects of OA treatment (Treatment, $p < 0.001$) and origin site (Origin, $p < 0.001$). Other interactive effects were not significant (Table 6). The significant interaction between origin and time was driven by different responses of the corallines from the Inner Bay site to OA treatments during the first and second halves of the experiment, but largely similar responses of the Outer Bay corallines to treatments at each time point (Fig. 6b).

Interpretation of the main treatment and site effects is confounded by the significant interaction between time and origin, and here we describe the consistent patterns in the data. The site origin effect was likely due to significantly lower calcification rates in Inner Bay corallines relative to their Outer Bay counterparts in every OA treatment in the first half of the experiment (0–8 weeks) (Table 6). Calcification rates were ~35–45% (Fig. 6b) lower in Inner Bay corallines relative to the Outer Bay counterparts in their respective OA treatments. Lower calcification rates in Inner Bay corallines relative to Outer Bay corallines in the Ambient control treatment over the first half of the lab experiment indicates that there was initially a negative effect on calcification simply due to originating from the Inner Bay site. The significant OA treatment effect was due to lower calcification rates for corallines in the OA treatments, though the magnitude of effect varied depending on treatment and origin (Fig. 6b). For example, Inner Bay coralline calcification rates incrementally decreased with exposure to increasingly acidified treatments (i.e., Var> OA> OA-Var) (Fig. 6b). Outer Bay corallines were negatively affected by OA treatments to a lesser extent, with similarly low rates in the Var and OA treatments, and lowest calcification rates in the OA-Var treatment (Fig. 6b). For corallines from both sites, calcification rates were lowest under OA and variability combined (OA-Var), with 46% and 66% less calcification in Outer and Inner Bay corallines, respectively. During the second half of the experiment (8–16 weeks), *Lithophyllum* sp. calcification rates did not vary significantly by origin site (Fig. 6b), except for a 39% decrease in calcification in corallines from the Inner Bay versus the Outer Bay site in the Var treatment. Corallines from both sites calcified ~46–49% less in the OA-Var treatment relative to their respective Ambient controls.

A noteworthy difference in response of corallines to OA treatments over time was evident in the Inner Bay coralline calcification rates during the first versus second halves of the experiment. Inner Bay *Lithophyllum* sp. calcification rates were the same in the first and second time periods within both the Var and OA-Var treatments, and overall each was lower than the control. In contrast, the calcification rates during the second time period (8–16 weeks) increased by 85% in the stable OA treatment relative to their initial time period (0–8 weeks). With increasing time of exposure, calcification rates of corallines from the Inner Bay site in the stable OA treatment came to match the calcification rates of the respective Ambient controls, as well as calcification rates of the Outer Bay corallines in the same stable OA treatment (Fig. 6b), which provides evidence of acclimatization to the laboratory treatments.

4. Discussion

Here we show how natural variation in environmental conditions can influence the calcification rates of an important Caribbean coralline alga, and modulate its subsequent response to OA in the lab. We found that corallines calcified more slowly at an Inner Bay site characterized by extreme, variable environmental conditions, relative to an Outer Bay site where conditions were more moderate over both diel and annual time scales. This negative site effect carried over to lab experiments, where corallines from the Inner Bay site continued to calcify more slowly than Outer Bay corallines in ambient conditions and were more negatively affected by OA treatments for the first 8 weeks of the experiment. The legacy effect of origin site dissipated in the latter half of the 16-week lab experiment, and ultimately these corallines were the only ones to show signs of acclimatization to OA. The ability of the Inner Bay corallines to increase calcification rates in the stable OA treatment over time suggests that longer exposure to stable conditions, even if acidified, may facilitate a positive acclimatization response. Together our results reveal that history of exposure to extreme, variable conditions modulates the response and acclimatization potential of reef-building coralline algae to future OA conditions.

4.1. Repercussions of living in an extreme, variable environment

Our first objective was to explore how *in situ* exposure to different environmental regimes affected calcification rates of an ecologically important coralline alga. Two reciprocal transplant sites spanned a known gradient in environmental conditions, and the presence of resident populations of the focal species, *Lithophyllum* c.f. *intermedium*, at each site indicates that this species naturally persists under the range of environmental conditions incorporated in our study. Diel and long-term monitoring data over 4 years provide evidence that the Inner Bay site in Almirante Bay is generally characterized by warmer water temperatures, and lower and more variable pH and DO concentrations (Fig. 2, 3). Both *Lithophyllum* sp. transplants and transplant controls at the Inner Bay site had significantly lower calcification rates than Outer Bay corallines, which demonstrates that Inner Bay conditions impaired growth rates and further supports our classification of the Inner Bay site as “extreme”.

The concept that extreme, variable environments could facilitate acclimatization to environmental stress is pervasive in the literature [17]. However, there is also evidence that such conditions can negatively affect resident organisms when tolerance thresholds are surpassed [58]. For example, high thermal variability on a coral reef can decrease the size and abundance of the free-living crustose coralline alga *Lithophyllum kotschyanum* [60]. Our results support the idea that extreme stress can lead to long-term declines in organismal function, as evidenced by the significant decrease in *Lithophyllum* sp. calcification rates at the Inner Bay site. Although variable environments might facilitate survival under more stressful conditions in some other organisms, the sublethal negative impact associated with persistence under variable conditions should be considered when evaluating the potential of these habitats to provide refuge from global change stressors [59,61].

The negative Inner Bay site effect intensified over time, with *Lithophyllum* sp. demonstrating extremely low calcification rates at the Inner Bay site during the second half of the field experiment. This timing corresponded to a period of warmer water temperatures, lower oxygen concentrations, and lower pH, as well as an acute hypoxic episode

that caused mortality of corals on the adjacent, deeper reef (Fig. 3) [45,48,49]. The hypoxic event was documented at depths below 4 m, which was evident by a clear demarcation on the benthos where the shoaling of hypoxic waters stopped [48,49]. The transplants were located at 2–3 m depth and sat above the oxycline that marked the most extreme conditions, but were likely affected by stressful conditions associated with the event that are apparent in the long-term monitoring data (Fig. 3). The co-variance of numerous related biotic and abiotic conditions in the field is common, but makes it difficult to interpret their relative importance in causing the lower calcification rates observed at the Inner Bay site. By pairing the reciprocal transplant study with our controlled laboratory experiment, we gain a more comprehensive understanding of the potential contribution of pH to coralline calcification responses in the field.

4.2. Legacy effects of an extreme environment

Our second objective was to quantify how history of exposure to extreme, variable conditions affected coralline responses to laboratory OA conditions and to simultaneously incorporate exposure to pH diel cycling into our experimental framework. The *Lithophyllum* sp. response to OA treatments over time strongly depended on the site of origin, and history of exposure to a more variable habitat did not initially confer tolerance to OA. Instead, we found that prior exposure to extreme conditions translated to an initial heightened sensitivity to stable and variable OA conditions in the lab. These negative legacy effects were apparent in the lower calcification rates of *Lithophyllum* sp. from the Inner Bay in all lab treatments relative to Outer Bay corallines for the first 8 weeks of the experiment. The term “legacy effects” has been widely used in the terrestrial literature [62,63,64], and refers to a biological response determined by environmental history that persists after conditions have improved. In our study, this biological legacy effect manifested as slower calcification by Inner Bay corallines, even under optimal conditions in the lab (i.e., ambient control treatment).

We propose two hypotheses for why negative legacy effects could persist after corallines from the Inner Bay site were released from stressful environmental conditions. First, lower calcification rates at the Inner Bay site may have resulted from physiological impairment or damage to basic biological functions due to environmental stress [65], which would require time and energetic resources to repair. Second, persistence of *Lithophyllum* sp. in extreme conditions at the Inner Bay site may have been due to adaptive molecular mechanisms [66]. For example, mechanisms of acclimatization to pH reductions could include up- or downregulation of physiological machinery involved in governing pH in the intracellular calcifying space (i.e., carbonic anhydrase, proton pumps, and calcium pumps) [67]. If regulation is driven by gene expression, those changes take time to manifest and can be energetically costly, which may explain the dissipation of negative legacy effects during the second half of the experiment [30].

Studies reporting on the effects of environmental history on crustose coralline algal responses to environmental stress are particularly limited, and a few others have similarly found nuanced or contrasting coralline responses to simulated OA and pH variability. For example, *Hydrolithon reinboldii* from a highly variable reef environment was not more resilient to OA than counterparts with a history of exposure to more stable pH conditions [30], and although history of exposure to a variable pH environment increased calcification under variable pH conditions, it did not confer resilience to stable OA in *Porolithon onkodes* [68]. Combined with the results from our study, history of exposure to extreme or variable environments appears to influence the effects of OA on coralline algae, but responses are mixed and can range from negligible to heightened sensitivity. Environmental history is not always accounted for in OA experiments, and our results incorporating long-term site effects emphasize how different historical exposures can cause disparate lab results.

4.3. pH variability and acclimatization

Our third objective was to test the effect of exposure to variable pH regimes on coralline algae, while incorporating lower mean pH to simulate OA and accounting for potential to acclimatize to lab conditions. In addition to historical exposure to pH variability (i.e., environmental history), present exposure to variability can have an equally important role in shaping organismal responses to simulated OA [24]. The prevailing theory that exposure to pH variability increases the resilience of coral reef organisms to OA [16,69,70] has been explicitly tested in only a few studies, with equivocal results (reviewed in [17]). Even fewer studies have addressed this question using tropical crustose coralline algae (except see [30,68]).

We found that, for individuals originating from both sites, the overall effect (0-16 weeks) of stable OA and ambient pH variability was the same, whereas OA combined with variability had the most severe negative effects. This is particularly interesting because in the variable treatment the pH, on average, was higher than the pH of the stable OA treatment for at least 16 hours of the day (Fig. 5). Two other studies have likewise found that ambient pH variability and stable OA conditions reduced growth of coralline algae by the same amount, while OA combined with variability had the strongest negative effect [24,53]. A possible explanation for similar responses to different pH regimes is that the negative effects of low pH on calcification at night outweighed potential benefits of higher pH on calcification during the day. Another possible explanation is that constantly changing pH either elicited a stress response or inhibited the initiation of an acclimatization response [67,71]. These findings suggest that the minimum pH, rather than the average, may determine calcification rates, and concurs with field data from the tropical central Pacific where minimum pH is a key determinate of net calcification rates of *in situ* early successional communities on corals reefs that are dominated by crustose coralline algae [52,72]. Accounting for the variability of acidification regimes in experimental designs, monitoring programs, and future projections is therefore critical to accurately evaluating the potential impacts of OA on ecologically important taxa.

Duration of exposure to treatments also influenced the effects of pH variability and OA on *Lithophyllum* sp. calcification. This is evident in different calcification responses when data were normalized over the full duration of the experiment (0–16 weeks) versus over the first (0–8 weeks) and second halves (8–16 weeks) separately. By considering calcification rates over two time periods, we detected the potential for *Lithophyllum* sp. from the Inner Bay site to increase calcification and acclimatize to stable OA conditions, a response that would have been missed by considering only the overall calcification rate. In this instance, prior exposure to extreme, variable conditions seems to have primed their response to stable reduced pH, but only after 8-16 weeks of exposure. This supports the idea that past exposure to environmental variability can eventually be overcome with acclimatization. Future experiments should continue to explore how responses to OA treatments change over time and consider sampling frequency and duration of experiments when interpreting results.

4.4. Conclusion

In summary, our results provide insight into the potential long-term ramifications of exposure to extreme, variable environmental conditions, and reveals that history of exposure to such conditions can have legacy effects reflecting a biological cost, even when the environment improves. Further, our study provides important evidence countering the theory that prior exposure to variability explicitly confers resilience to OA. Instead, we found that corallines from the Inner Bay, more extreme, site were initially more sensitive to stable and variable pH reductions. However, those negative legacy effects dissipated over time as Inner Bay corallines acclimatized to stable OA conditions. Our study contributes to a growing body of literature demonstrating that the ef-

fects of OA are highly complex. We show that some of the complexity and variability of within-species responses to OA may be due to environmental history of an organism, the incorporation of pH variability in OA treatments, and the duration of exposure to experimental conditions. These factors should be incorporated in future experiments and monitoring programs to facilitate more accurate predictions of organismal responses to OA in the coming decades, particularly for organisms inhabiting nearshore habitats where environmental conditions can be highly dynamic.

Author contributions

MDJ conceived and designed the study, MDJ and LMRB conducted the field and laboratory experiment, NL deployed and maintained sensors, MDJ conducted analyses and wrote the manuscript, AHA provided funding support, all authors contributed to manuscript revisions.

Data availability

Raw data associated with this study are available on Smithsonian figshare at: https://doi.org/10.25573/data.14597142.v1.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ecochg.2021.100016.

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