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The influence of diel carbonate chemistry fluctuations on the calcification rate of *Acropora cervicornis* under present day and future acidification conditions

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ABSTRACT

Ocean acidification (OA) will result in lower calcification rates for numerous marine taxa, including many species of corals which create important reef habitat. Seawater carbonate chemistry fluctuates over cycles ranging from days to seasons, often driven by biological processes such as respiration and photosynthesis. The magnitude of diel fluctuations varies spatially and may become more pronounced in the future due to OA. Due to technical constraints, OA experiments that incorporate diel variability into treatments are few in number. As a result, the degree to which coral reef organisms are influenced by ambient daily carbonate chemistry variability is poorly understood. Here we describe an experiment conducted in a novel seawater system which can independently manipulate carbonate chemistry in 16 separate aquaria, in real time, allowing precise control of the mean and magnitude of pH oscillations while minimizing pseudoreplication. Five genotypes of the threatened Caribbean coral *Acropora cervicornis* were subjected to a total of five pH treatments, 7.80 ± 0.20, 7.80 ± 0.10, and 7.80 ± 0.00, as well as 8.05 ± 0.10 and 8.05 ± 0.00. Those corals exposed to variable contemporary conditions (8.05 ± 0.10) calcified faster than those in current and future static treatment levels, which did not significantly differ from each other. Variable contemporary pH also resulted in faster growth rates than highly variable future conditions (7.80 ± 0.20), but were not significantly different than future conditions with the same ± 0.10 diel pH oscillation. These findings support the importance of incorporating diel variability into OA experiments and suggest that more variable natural ecosystems may yield higher calcification rates for corals.

1. Introduction

The global acidification of seawater (ocean acidification, OA) due to the anthropogenic increase in atmospheric CO_2 will have widespread ramifications for marine organisms and ecosystems (Fabry et al., 2008). Coral reef habitat, formed by the deposition of calcium carbonate by scleractinian corals, will be adversely affected due to OA-related depression in calcification (Chan and Connolly, 2013), and acceleration of dissolution (Enochs et al., 2016).

While the progressive decline in seawater pH is clear from open-ocean time series (Bates et al., 2014), shallow water systems are complicated by the influence of benthic organisms on carbonate chemistry, especially when water exchange is low (Hofmann et al., 2011). This biological control varies across spatial scales from centimeters (Gagliano et al., 2010) to kilometers (Manzello et al., 2012) and can be influenced by episodic events (Manzello et al., 2013) or by periodic oscillations with periods ranging from days (Price et al., 2012) to seasons (Shaw and Mcneil, 2014). Diel oscillations in pH are primarily due to light-mediated alteration in the balance of photosynthesis/respiration and calcification/dissolution. The magnitude of this fluctuation can vary greatly, and in some reef systems it can contribute to periodic exposure to conditions expected to occur by the end of the century due to OA (Shaw et al., 2012).

The implications of diel pH fluctuations on the organismal responses to OA are poorly understood and may be an important consideration for the persistence of coral reef ecosystems (Hogarth, 2006; Rivest et al., 2017b). If a coral's response to OA is driven by a depression in light enhanced calcification, then dynamic pH oscillations could facilitate

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more favorable daytime conditions, possibly acting as a temporal OA-buffering refuge. If, however, dark calcification is of paramount importance to a coral's response to OA, a higher amplitude nighttime reduction in pH could lead to a more dramatic OA-related depression in coral growth than previously predicted. The cumulative result of the decline in mean pH from OA, coupled with natural oscillations around that mean, implies that in certain highly variable environments periodic aragonite undersaturation, accompanied by abiotic dissolution, will be reached before the predictions of models that only consider a mean (Shaw et al., 2013b). Further, OA itself may increase the diel amplitude of natural carbonate chemistry oscillations by decreasing the buffering capacity of seawater, potentially leading to unforeseen ecosystem responses (Shaw et al., 2013a).

In order to incorporate natural variability into OA experiments, scientists have conducted in-situ studies on both small (Kline et al., 2012) and large spatial scales (Albright et al., 2018), relying on biological and physical processes to drive diurnal fluctuations. Additionally, naturally high-CO_2 systems due to physical (e.g., Crook et al., 2013; Fabricius et al., 2011) and biological forcing (e.g., Camp et al., 2017; Shamberger et al., 2014) have been employed to investigate dynamic OA conditions. These systems, however, may not always perfectly mimic diel oscillations found on normal reefs (e.g., Enochs et al., 2015).

Laboratory-based studies which experimentally manipulate diurnal pH oscillations are scarce relative to those considering static treatments, primarily due to technical difficulties with controlling carbonate chemistry in real-time. Previously, dynamic pH treatments have been achieved using three approaches. In the first, specimens have been physically transferred back and forth between artificially manipulated high and low pH at dawn and dusk (Comeau et al., 2014a; Dufault et al., 2012; Johnson et al., 2014) or have been automatically refreshed with different pH waters from statically controlled holding tanks (Cornwall et al., 2013). In this approach, the magnitude and phase of dynamic day/night oscillations are controllable but treatments can be artificially abrupt, as specimens are immediately exposed from one extreme to the next during the dawn/dusk transfer process.

In the second approach, diel variability is achieved via an upstream mesocosm containing a community of organisms which biologically force the carbonate chemistry. Both Camp et al. (2016) and Chan and Eggins (2017) have employed this methodology, subjecting corals to carbonate chemistry manipulated via seagrass and coral mesocosms, respectively. In the latter study, static treatments were also achieved by transferring water from the upstream mesocosm to separate aquaria at specific times of day which routinely experience a specific pH set point. While this approach accomplishes gradual diel changes, only one oscillating regime is attainable (that of the mesocosm) and treatment conditions may vary due to biological processes.

The third method of dynamic pH manipulation employees automated dosing of liquid reagents or CO_2 gas, linked with a feedback mechanism to identify when treatment conditions are met. Coupled with moving set points, this approach has been used to depress pH while retaining natural diurnal variability in 1300 L common garden tanks (Putnam et al., 2016), to alter pH fluctuations in 1600 L recirculating tanks (Jarrold and Munday, 2018), as well as to manipulate pH fluctuations (mean and amplitude) in 900 mL phytoplankton culture chambers (Golda et al., 2017). This method is the most flexible of the three, though there can be difficulties with gas delivery and treatment precision (Golda et al., 2017).

Studies which have directly manipulated seawater oscillations have investigated a suite of different taxa including algae (Cornwall et al., 2013; Johnson et al., 2014), coral (Chan and Eggins, 2017; Comeau et al., 2014a; Dufault et al., 2012; Putnam et al., 2016), fishes (Jarrold et al., 2017; Jarrold and Munday, 2018), and isopods (Alenius and Munguia, 2012), among others. Those that have focused on calcification have primarily employed an experimental design comparing responses to variable versus completely static pH. Dufault et al. (2012) found that diurnal oscillations (8.02 to 7.90) increased the calcification of *Seriatopora caliendrum* recruits, relative to static high (8.00) and low (7.88) pH treatments, which yielded non-significant differences. Chan and Eggins (2017) subjected adult *Acropora formosa* to static (7.8, 8.0, 8.2) and a naturally varying pH (7.8 to 8.2). Again, the variable system resulted in higher calcification rates vs. the contemporary (8.0) and future (7.8) treatments, though corals which were subjected to pH variability did not calcify significantly faster than in static 8.2 pH. Similarly, transferring the alga *Porolithon onkodes* across high (8.03) and low pH (7.87) conditions (day and night, respectively) resulted in enhancement of calcification relative to static low pH (7.86) but not static high pH (8.04) treatments (Johnson et al., 2014). Comeau et al. (2014a), however, measured calcification of *Acropora hyacinthus* in three static OA treatments (8.07, 7.88, 7.71), each paired with a variable treatment with a similar daily-averaged mean. The amplitude of the pH variation increased with pH depression and significant differences in calcification were only detected among the samples exposed to the most extreme static OA conditions (7.71) versus the most extreme variable OA conditions (8.07 to 7.47, day to night, respectively).

In contrast to the aforementioned studies, Camp et al. (2016) did not detect a significant influence of variability ($\sim \pm 0.05$ vs. $\sim \pm 0.2$) on present-day and acidified treatments on two species of Caribbean corals, *Acropora palmata* and *Porites astreoides*. Finally, an experiment conducted on a calcifying alga (*Arthrocardia corymbosa*) resulted in lower calcification rates in variable (± 0.4) vs. static pH conditions under present day (8.05) and future (7.65) mean levels (Cornwall et al., 2013). While the results are not always consistent across taxa, these studies indicate that differences in diel oscillation (or lack thereof) between experiments could potentially be responsible for some of the differences in OA-responses observed across prior studies (e.g., Okazaki et al., 2017).

Given the apparent differences between these studies and the limited incorporation of variability treatments into experimental designs, it is presently unclear how diurnal pH oscillation will influence the calcification of important reef-building corals, especially under future OA conditions. Here we describe the construction of a system for the precise manipulation of diurnally fluctuating seawater carbonate chemistry; a system which reproduces gradual diel oscillations (rather than abrupt changes) and does not rely on a biologically-forced header tank. This system was used to test the hypothesis that diel pH variability coupled with present day and future mean pH conditions influences the calcification of the threatened (Hogarth, 2006) staghorn coral *Acropora cervicornis* (Lamarck, 1816).

2. Methods

2.1. Experimental design

Between 40 and 49 fragments of *A. cervicornis* (225 total) were collected from five genotypes (Baums et al., 2009) present at two coral nurseries, south of Key Biscayne, Florida (25.3626 N, 80.1664 W, Genotypes A-C; 25.4888 N, 80.1091 W, Genotypes D,E) in March 2017. Prior to collection, colonies were grown from hanging trees and on cinder blocks in roughly six meters water depth (Lirman et al., 2014; Nedimyer et al., 2011). Fragments were each roughly five cm long and were selected to minimize the presence of multiple apical tips. Corals were transported back to the University of Miami CIMAS and NOAA AOML's Experimental Reef Laboratory, where they were affixed to four cm diameter grey PVC pucks, using cyanoacrylate adhesive. Corals were acclimated to indoor laboratory conditions mimicking those occurring in the field (24.3 °C, 8.05 pH) for two weeks, followed by a week of pH treatment ramping. Initial temperature was obtained from the field at the time of collection, initial pH data from the sites of collection was obtained from 1.5 month SAMI-pH logger (Sunburst Sensors) deployments in 2014 (Fig. S1). Replicates were randomly assigned to tanks and treatments, ensuring at least three corals per

genotype per tank.

Two mean pH treatments were selected to represent present day, as well as potential end of the century conditions (A2, IPCC, 2007). Three and two variability regimes were applied to future and present-day mean treatments, respectively, as follows: Low pH, high diel amplitude (7.80 ± 0.20, mean ± daily range); Low pH, mid diel amplitude (7.80 ± 0.10); Low pH, low diel amplitude (7.80 ± 0.00); High pH, mid diel amplitude (8.05 ± 0.10); High pH, low diel amplitude (8.05 ± 0.00).

Three tanks were randomly assigned to each treatment, except for the 7.80 ± 0.10 pH treatment, where only two tanks were assigned due to space availability. Oscillations in pH were calculated as sine waves with a 24 h wavelength. Minimum and maximum pH was achieved at 7:00 and 19:00 h, respectively, corresponding to the programmed sunrise and sunset. Treatment ramping was achieved by incrementally altering both mean pH and diel amplitude, to reach treatment targets after one week.

2.2. Aquarium system and control

Fourteen separate aquarium systems were used following the recommendations for statistical independence of treatment blocks in experimental ocean acidification laboratories detailed in Cornwall and Hurd (2016, design A-1). Fresh seawater was pumped from Biscayne Bay, filtered (one μm), brought to a constant temperature with a heat exchanger, and to a stable, low-CO_2 level using a Liqui-Cel membrane contactor, vacuum pump, and zero-CO_2 sweep gas. The flow of fresh seawater into each system was controlled by needle valves and monitored with optical gate flow meters (Micro-Flo, Serv-A-Pure), calibrated weekly. Flow was set to 150 mL per minute, resulting in a complete refresh of each tank system every 10 h.

Each of the 14 separate tank systems consisted of a 75 L glass aquarium in constant circulation with a 75 L sump tank where temperature and CO_2 treatments were applied. Corals were placed in the top tanks, which contained a circulation pump (Nanostream 6040, Tunze) to maintain constant water movement. Light was provided by 135 W LED arrays (Hydra 52 HD, Aqua Illumination), set with a three h dawn and dusk ramp and a six h, static mid-day light level. Peak photosynthetically active radiation (PAR) was initially set to 450 umol $m^{-2} s^{-1}$ (MQ-200, Apogee) and was lowered to 300 after two weeks due to concerns that high light levels were potentially contributing to a slight paling of some of the fragments. Temperature was measured in the top tank with a high-accuracy RTD sensor (TTD25C, ProSense) and was manipulated in the lower tank with a 300 W aquarium heater (TH-300, Finnex) and a titanium chiller coil (Hotspot Energy) plumbed to a sealed cold water source with an electronically actuated solenoid valve. Corals were fed once per week (Larval AP100, Ziegler).

Seawater pH (total scale) was measured several times per minute in each top tank with a high-accuracy, low-drift Durafet solid-state pH electrode (Honeywell) interfaced with Honeywell Universal Dual Analyzers (UDA 2182). Water samples were taken from each tank twice weekly for the analysis of carbonate chemistry (Dickson et al., 2007). Briefly, samples were sealed in borosilicate bottles, fixed with mercuric chloride and subsequently analyzed for salinity (DMA 5000 M, Anton Paar), as well as for dissolved inorganic carbon (DIC, AS-C3, Apollo SciTech), total alkalinity (A_T, AS-ALK2, Apollo SciTech), which were both corrected with certified reference materials. These parameters were used in conjunction with tank temperature to solve the carbonate system using CO2SYS (Lewis and Wallace, 1998) and to calculate pCO_2, pH, and the aragonite saturation state (Ω_{Arag}). Calculated pH was compared to that recorded by the Durafet at the time of sample collection and if the differences were > 0.02 pH units, sensors were calibrated to match the bottle samples.

A mixture of pure CO_2 and CO_2-free-air was introduced into each sump tank using a venturi injector and micro-bubbles were restricted from moving into the top tank using a system of baffles. Gas concentrations were controlled using two gas-specific (air and CO_2) mass flow controllers (GFC series, Aalborg) per tank system. Prior to injection, CO_2 was removed from treatment air using soda lime scrubbers, and air was passed through both particle filters (HF 20, Hankison) and a refrigerated compressed air dryer (Eaton). The concentration of CO_2 of treatment air was measured in real time using a nondispersive infrared CO_2 meter (LI-820, LI-COR) and the soda lime scrubber medium was replaced when the efficiency of CO_2 removal was compromised.

All system parameters (pH, temperature, water flow, air and CO_2 delivery rates) were logged every minute to a central computer via a USB connection through a modular hardware interface (CompactDAQ, National Instruments) and controlled in real-time using custom software written in LabVIEW (National Instruments). Time-dependent (hourly) pH set points were achieved using proportional-integral-derivative (PID) control and custom gas delivery algorithms.

2.3. Coral calcification

Calcification was measured using the buoyant weight technique (Jokiel et al., 1978), standardized to colony surface area as determined from 3D scanning (HDI Advance R2, 3D3 Solutions) at the beginning of the experiment following the methods of Enochs et al. (2014). Mass was measured using a calibrated analytical balance (0.0001 g precision, Ohaus). Corals were suspended from tungsten wire (0.05 mm) in a temperature-controlled seawater bath. Temperature was recorded during each mass measurement using a high-accuracy temperature probe (Digi-Sense) and salinity was measured once during each weighing session using a densitometer (DMA 5000 M, Anton Paar). Mass was recorded at the beginning the experiment (following ramping) and two weeks after treatments had been applied.

2.4. Statistics

Area-standardized coral calcification was analyzed using a fully-crossed ANOVA including interactions, with genotype and pH treatment as fixed effects and tank as a random effect. Assumptions of the test were analyzed both graphically and statistically (Levene's Test, $p = .95$; Shapiro-Wilk Test, $p = .76$). Tank effects were removed when their inclusion was found to increase the model AIC. Post hoc Tukey's Tests were used to test for differences between pCO_2 and genotype treatments. All statistics were run with R and R Studio (Team, 2015), using the lme4 package (Bates et al., 2015).

3. Results

3.1. Dynamic carbonate chemistry treatments

Acclimation, ramping (Fig. 1A), and the five carbonate chemistry treatments were successfully achieved using the experimental system (Fig. 1B). Temperature and salinity along with deviations in pH from programmed levels (as measured 59 min after each new hourly set point, Table 1) reveal close tracking of moving pH targets. Differences in pH measured by Durafet and that calculated from water bottle samples used for calibration are also shown in Table 1, reflecting the accuracy and stability of the solid state pH probes.

3.2. The influence of carbonate chemistry treatments on calcification

Carbonate chemistry treatments resulted in significant differences in calcification across the five genotypes (Table 2, Fig. 2). Post hoc analysis revealed that contemporary pH, diel oscillation (8.05 ± 0.10) conditions resulted in higher calcification ($p = .002$) than static maintenance at that pH level (8.05 ± 0.00). Calcification was also significantly greater in 8.05 ± 0.10 pH treatments vs. 7.80 ± 0.00 ($p = .004$) and 7.8 ± 0.20 ($p < .001$). A reduction in mean pH (8.05

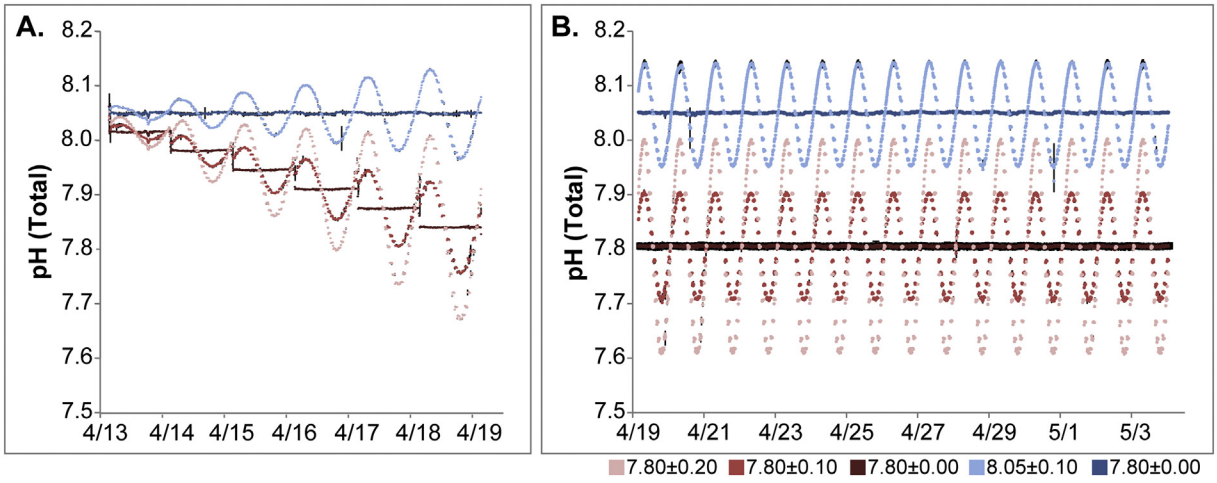

Fig. 1. Mean pH for each treatment, every ten minutes over the duration of treatment ramping (A.) and the experiment (B.). Error bars (small) are standard deviation. Treatments reflect pH means ± diel pH fluctuations. Values calculated from three tanks per treatment except 7.80 ± 0.10, with two tanks.

to 7.8) did not result in significantly different calcification rates for mid (± 0.10) or low (± 0.00) diel oscillation regimes.

3.3. The influence of genotype on calcification

There were strongly significant differences in calcification rates between genotypes but no significant interactions with pH treatments were detected (Table 2, Fig. 3). Across treatments, differences were driven by a single rapid (Genotype A, 0.976 ± 0.1820 mg cm^{-2} d^{-1}, mean ± SD) and a slowly calcifying genotype (Genotype C, 0.596 ± 0.1716), and the remaining three genotypes were not significantly different with respect to calcification (Genotypes B,D,E, 0.750 ± 0.1751).

Table 2
Results of ANOVA of calcification data from five genotypes of *Acropora cervicornis* subjected to five pH treatments. dF, degrees of freedom; SS, sum of squares; MS, mean square.

	df	SS	MS	F value	*P* value
Treatment	4	8.58E-07	2.15E-07	7.985	p < .001
Genotype	4	3.52E-06	8.80E-07	32.769	p < .001
Treatment: Genotype	16	4.35E-07	2.72E-08	1.012	0.445
Residuals	200	5.37E-06	2.69E-08		

4. Discussion

4.1. Experimental system

The aquaria and CO_2 dosing systems demonstrated precise control of seawater carbonate chemistry across multiple variability regimes. Treatment precision was greater than that achieved with other dynamic pH-stat systems which incorporated gas addition (Golda et al., 2017; Putnam et al., 2016). This level of control was achieved, in part, due to real-time pH feedback with stable solid state Durafet electrodes directly measuring total scale pH. We note that our system is the first to directly alter the concentration of CO_2 gas injection in real-time with mass flow controllers (versus solenoid valves) set according to PID algorithms.

Table 1
Mean temperature (°C), salinity (psu), and pH error by pH treatment and replicate tanks. Treatments reflect pH means ± diel pH fluctuations. pH set point deviation is calculated as the difference between tank pH (measured by Durafet) and the coded set point for the tank at that time. Values are every 6 h, one minute before the hour when the subsequent set point is applied. pH probe error calculated as the difference between the Durafet pH and that calculated from DIC and A_T. All pH values are total scale. Standard deviations in parentheses. Sample sizes in brackets.

pH Treatment/Tank	Temperature [*n* = 21,445]	Salinity [*n* = 5]	pH set point deviation [*n* = 15]		pH probe error [n = 5]		
			00:59	06:59	12:59	18:59	
8.05 ± 0.00	24.35 (0.060)	37.66 (0.223)	−0.001 (0.0010)	−0.001 (0.0012)	0.001 (0.0008)	0.000 (0.0008)	0.006 (0.0029)
Tank 1	24.34 (0.040)	37.68 (0.247)	0.001 (0.0008)	0.000 (0.0013)	0.001 (0.0029)	0.000 (0.0007)	0.014 (0.0079)
Tank 2	24.36 (0.081)	37.63 (0.206)	−0.002 (0.0020)	−0.002 (0.0016)	0.001 (0.0010)	−0.001 (0.0011)	−0.003 (0.0073)
Tank 3	24.35 (0.074)	37.67 (0.219)	−0.001 (0.0018)	−0.002 (0.0017)	0.001 (0.0012)	−0.001 (0.0015)	0.006 (0.0041)
8.05 ± 0.10	24.36 (0.071)	37.62 (0.212)	−0.003 (0.0008)	0.001 (0.0004)	0.002 (0.0012)	−0.008 (0.0023)	−0.001 (0.0065)
Tank 4	24.34 (0.048)	37.61 (0.200)	−0.004 (0.0007)	0.001 (0.0009)	0.001 (0.0011)	−0.014 (0.0046)	−0.009 (0.0082)
Tank 5	24.36 (0.078)	37.64 (0.218)	−0.003 (0.0014)	0.002 (0.0008)	0.001 (0.0031)	−0.006 (0.0027)	0.010 (0.0131)
Tank 6	24.39 (0.101)	37.61 (0.220)	−0.002 (0.0011)	0.002 (0.0008)	0.002 (0.0008)	−0.005 (0.0007)	−0.005 (0.0096)
7.80 ± 0.00	24.36 (0.068)	37.63 (0.212)	0.005 (0.0007)	0.005 (0.0004)	0.006 (0.0005)	0.005 (0.0005)	−0.003 (0.0100)
Tank 7	24.36 (0.079)	37.58 (0.217)	0.005 (0.0012)	0.005 (0.0012)	0.006 (0.0007)	0.004 (0.0007)	0.002 (0.0108)
Tank 8	24.34 (0.045)	37.66 (0.214)	0.006 (0.0009)	0.006 (0.0005)	0.007 (0.0009)	0.006 (0.0011)	−0.005 (0.0111)
Tank 9	24.38 (0.098)	37.60 (0.215)	0.004 (0.0009)	0.003 (0.0010)	0.005 (0.0008)	0.003 (0.0006)	−0.002 (0.0106)
7.80 ± 0.10	24.35 (0.049)	37.69 (0.261)	0.004 (0.0010)	0.006 (0.0011)	0.006 (0.0010)	0.003 (0.0007)	−0.007 (0.015)
Tank 10	24.33 (0.040)	37.78 (0.309)	0.003 (0.0014)	0.006 (0.0021)	0.005 (0.0018)	0.002 (0.0012)	−0.006 (0.0184)
Tank 11	24.36 (0.077)	37.61 (0.217)	0.005 (0.0009)	0.007 (0.0005)	0.006 (0.0014)	0.004 (0.0006)	−0.008 (0.0137)
7.80 ± 0.20	24.35 (0.055)	37.64 (0.212)	0.004 (0.0006)	0.009 (0.0005)	0.006 (0.0006)	0.001 (0.0007)	−0.010 (0.0144)
Tank 12	24.35 (0.062)	37.61 (0.217)	0.004 (0.0006)	0.009 (0.0012)	0.006 (0.0015)	0.001 (0.0014)	0.006 (0.0152)
Tank 13	24.33 (0.039)	37.69 (0.212)	0.004 (0.0007)	0.009 (0.0008)	0.006 (0.0008)	0.001 (0.0008)	−0.022 (0.0193)
Tank 14	24.38 (0.091)	37.61 (0.211)	0.003 (0.0009)	0.009 (0.0009)	0.006 (0.0006)	0.001 (0.0009)	−0.014 (0.0154)

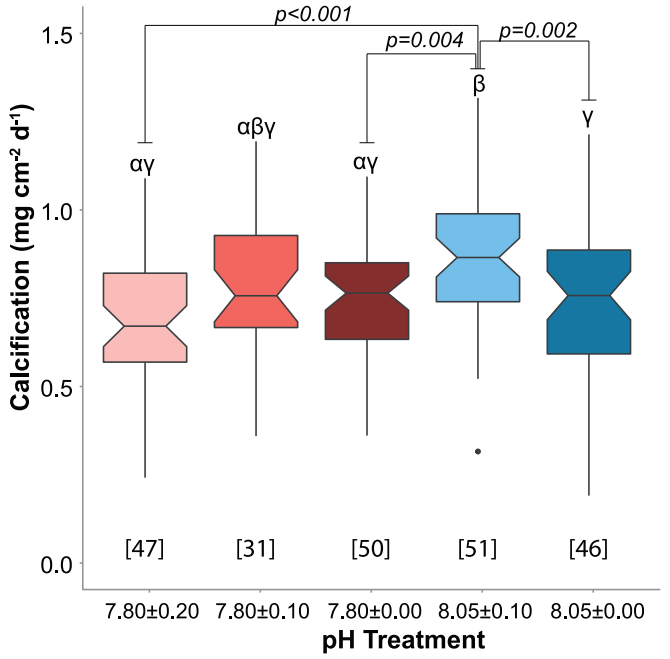

Fig. 2. Boxplot of calcification rates of *Acropora cervicornis* fragments subjected to five different treatments, incorporating alteration of mean and magnitude of diel fluctuations in seawater pH (mean ± amplitude). Values which share a Greek letter are not significantly different (p > .05). *P* values of significant relations are given above. Numbers in brackets are the sample sizes for each of the treatments.

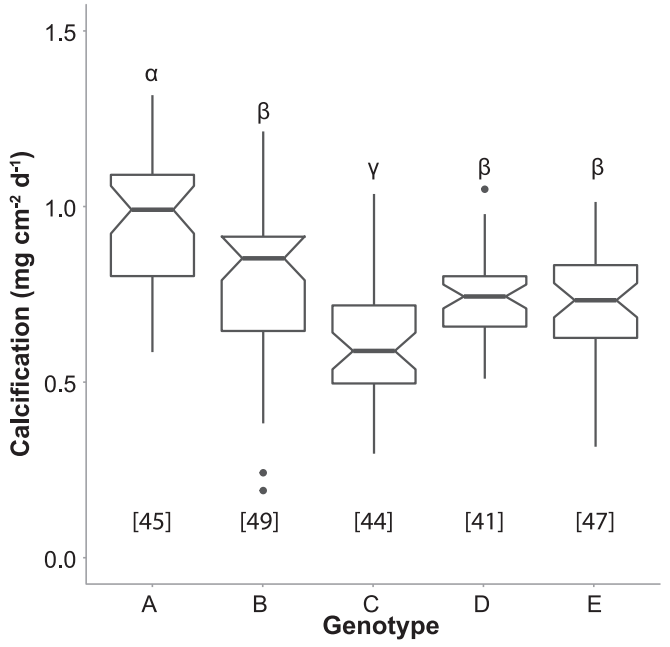

Fig. 3. Boxplot of calcification rates of five genotypes of *Acropora cervicornis*. Data are pooled across pH treatments. Values which share a Greek letter are not significantly different (*p* > .05). Numbers in brackets are the sample sizes for each of the genotypes.

This approach minimized overshooting during CO_2 injection, while ensuring rapid transitions to novel desired states.

It is worth noting that prior dynamic pH-stat systems have represented opposite ends of the size spectrum, from > 1000 L recirculating tanks (Jarrold et al., 2017; Jarrold and Munday, 2018; Putnam et al., 2016), to < 1 L culture jars (Golda et al., 2017). The system described herein is 150 L, with space for multiple coral replicates, while ensuring adequate space for independent tank/treatment replication. A further advantage to our approach is the semi-recirculating design, allowing a complete refresh of the treatment water twice a day and preventing the accumulation of potentially harmful metabolic byproducts.

The artificial control used in this and other dynamic pH-stat systems has several advantages over other approaches and can be easily integrated into many existing OA experimental systems. Firstly, our method is less labor-intensive to maintain relative to twice-daily (dawn and dusk) transferal of specimens between night and daytime treatments (Comeau et al., 2014a; Dufault et al., 2012). Physically transferring corals may unduly stress specimens due to handling or air exposure, and statistical independence may be difficult to achieve if specimens are moved across multiple aquaria and treatments. Further, abrupt transitions into extreme pH conditions may not sufficiently mimic real-world diel oscillations which can occur more gradually. While biological variation from an upstream mesocosm can mimic gradual real-world variability, problems may exist with the independence of replicates given that oscillations are applied by the same treatment source. Further, natural biological variability may lead to inter-day variance in the mean and magnitude of pH oscillations, potentially reducing the power to discern treatment effects. Finally, experimental systems controlled by the biological activity of larger mesocosms may be restricted in their ability to control differences in daily means or the amplitude of oscillations, thereby limiting investigation of future OA conditions.

4.2. Ecological implications of pH variability

Environmental variability can potentially lead to greater stress tolerance through acclimatization, adaptation, condition priming, or life history carry-over effects (Rivest et al., 2017b). While this has been demonstrated with temperature variability conferring a degree of thermotolerance (e.g., Oliver and Palumbi, 2011), it is presently not supported with respect to pH and OA tolerance of scleractinian corals (Camp et al., 2016; Comeau et al., 2014a; Rivest et al., 2017a). However, evidence from a species of coralline algae has shown that individuals collected from higher variability pH environments were more able to calcify in variable laboratory treatments faster than those collected from more constant pH sites.

Prior data from the nursery sites where corals were raised for this study suggest that our study specimens were not recently acclimatized to highly variable pH treatments, though there does appear to be a slight difference in the pH variability between collected sites (Fig. S1). We do not, however, have data from their original collection sites and it is impossible for us to completely eliminate the potential that adaptation or acclimatization influenced the responses described in this study. While we cannot draw conclusions related to pH variability conferring future resilience, this study does support the concept that variable pH environments may enhance *A. cervicornis* calcification in variable waters, especially in contemporary mean pH conditions. These findings are similar to that from the congeneric Pacific species *Acropora formosa* (Chan and Eggins, 2017) as well as recruits of *Seriatopora caliendrum* (Dufault et al., 2012). Enhancement relative to static conditions was not detectable among corals in the high or mid-variability when mean pH was set to 7.80. This finding is in contrast to Comeau et al. (2014a), who only detected an enhancement in the calcification rate of *Acropora hyacinthus* in oscillating vs. static pH in acidified conditions (7.71 vs. 8.07 to 7.47). The level of mean acidification and the magnitude of the pH oscillation in that study, however, were greater than in our most extreme 7.8 ± 0.20 pH treatment, indicating that differences may be detectable outside of the range we investigated (Johnson et al., 2014).

Physiological responses to OA, such as calcification, are not necessarily linearly related to aragonite saturation state. Tipping points can occur if an organism's capacity to buffer acidification stress is

overcome, whether it be at the cellular level, or outside the organism at the diffusion boundary layer (Cornwall et al., 2014; McCulloch et al., 2012; Ries et al., 2009; Ries et al., 2010). If oscillations are of sufficient magnitude that nighttime pH conditions exceed these biological thresholds for calcification, presumably the benefits conferred by elevated daytime pH could be obfuscated. Regression of bottle data taken as part of routine Durafet pH calibration indicates that undersaturation of aragonite ($\Omega_{arag} < 1$) would be achieved at a pH < 7.56 ($\Omega_{arag} = 5.1198$ *pH – 37.704, $R^2 = 0.99$). The most extreme treatment (7.80 ± 0.20) in this experiment only reached pH levels of 7.6, indicating that abiotic dissolution was likely not a strong driver in the patterns we observed. Nonetheless, if biological thresholds (e.g., cellular pathways not directly involved with calcification) are reached at saturation states higher than 1.0, the possibility exists that physiological stress during nighttime conditions may have interacted antagonistically with daytime calcification enhancement.

It is cautioned that the majority of previous studies addressing the impact of pH variability on coral calcification, including this one, have focused on species within the genus *Acropora* (Chan and Eggins, 2017; Comeau et al., 2014a). OA sensitivity across species may be correlated to functional traits such as calcification rate (Comeau et al., 2014b). Acroporid corals are generally much faster calcifiers than other species (e.g., data in Perry et al., 2012) and could potentially respond to diel oscillations in pH differently than other species. Further work is therefore needed to determine if these patterns hold true in other coral genera and other calcifying organisms (Rivest et al., 2017b).

4.3. Potential mechanisms by which variability influences a coral's response to OA

Chan and Eggins (2017) hypothesized that elevated daytime pH, coupled with higher calcification under high light conditions (e.g., Gattuso et al., 1999) was responsible for elevated calcification in their variable treatment. While the mechanism of light enhanced calcification is not fully agreed upon, it could be related to photosynthetic optimization of carbonate chemistry (abiotic) or direct photosynthetic enhancement of biological processes, including the production of ATP for energetically costly calcification (Chan and Eggins, 2017; Galli and Solidoro, 2018). According to this hypothesis, if daytime conditions are responsible, steady pH conditions equivalent to daytime peaks achieved in oscillating treatments would result in calcification rates similar to variable pH conditions. This was supported by data from Chan and Eggins (2017) where the variable treatment resulted in higher calcification than in static mean conditions but was not significantly different than in static maximum levels.

Dufault et al. (2012) also measured calcification rates at static levels equal to the minimum and maximum of their stepped oscillation and evaluated whether the daytime pH was responsible for the enhancement of calcification. When oscillating pH treatments resulted in higher calcification rates than static daytime levels, they concluded that daytime calcification was not solely responsible for this trend. Instead, the authors hypothesize that corals may accumulate DIC (primarily HCO_3^-) under higher pCO_2 nighttime conditions, which is then stored and used to enhance daytime calcification and photosynthesis (Dufault et al., 2012; Herfort et al., 2008). Interestingly, when the same experiment was repeated in reverse phase (i.e. lower pH during the day), growth (surface area of coral recruits) was 11% less than in natural phase oscillations, though no significant differences in weight were detected. By itself, this hypothesis leads to the counter-intuitive result of elevated calcification in more acidified water. While this is not strongly supported by the preponderance of OA calcification experiments (Chan and Connolly, 2013) several studies have recorded non-significant or positive relationships between calcification and OA (reviewed in Dufault et al., 2012).

Ultimately, these two hypotheses (light enhanced calcification and nighttime DIC sequestration) are not mutually exclusive and could contribute to different degrees, depending on the mean and magnitude of oscillations, light intensity (Enochs et al., 2014), as well as the physiology of the species/individuals involved, including their ability to regulate the chemistry at the site of calcification (Schoepf et al., 2017). The design of our study precludes the ability to directly address these mechanistic hypotheses, however, it is interesting to note the absence of significance between the 7.80 ± 0.20 and 8.05 ± 0.00 treatments. Peak daytime pH in the former (8.00) approaches the constant level of the latter (8.05), whereas nighttime pH in the variable treatment reaches 7.6, diverging from the static treatment by 0.45 pH units. Considering the absence of a significant difference in calcification between the two treatments, these data appear to support the importance of daytime pH conditions and light enhanced calcification proposed by Chan and Eggins (2017).

It is worth noting, however, that the 7.80 ± 0.20 conditions resulted in the lowest calcification rate of our study. If higher pH during daytime calcification was exclusively responsible for enhanced calcification, it would be expected that our 7.80 mean pH treatments would result in successively higher calcification, moving from ± 0.00, to ± 0.10, and finally to ± 0.20. This was not the case in our data, where none of the variability treatments were different than each other and the ± 0.10 pH treatment resulted in the highest calcification.

Two of the three low-pH treatments (± 0.20 and ± 0.00, but not ± 0.10) resulted in significantly lower calcification rates than in the 8.05 ± 0.10 treatment. Consistent with two of the previous OA/variability laboratory studies conducted on corals, no significant differences were detected between coral calcification rates in present day and future OA conditions, when diel oscillations were eliminated (Chan and Eggins, 2017; Dufault et al., 2012). This is in contrast to Comeau et al. (2014a) who found significant depression in calcification, only in the static extreme OA conditions (1000 μatm).

4.4. A conceptual model for investigating diel variance

If the relationship between saturation state or pH and calcification is non-linear (McCulloch et al., 2012; Ries et al., 2009; Ries et al., 2010) and holds across both light and dark calcification, the impact (enhancement vs. reduction, day vs. night respectively) can differ in magnitude, even with an equal oscillation around the mean (Fig. 4A). For example, a large increase in pH during the day may do little to increase calcification rate if the response region is near the asymptote, while an opposite but equal nighttime depression in saturation state could strongly reduce calcification if the response is near the horizontal inflection point. In this manner, it becomes apparent how mean pH conditions are relevant to the influence of diel variation, and why the two characteristics should be taken into account when predicting responses to OA stress.

It is important to also consider the ratio of day to night calcification when applying this model (Fig. 4B). If, for example, day and nighttime calcification are equal (1:1), and an oscillation is applied which leads to a linear doubling and halving of calcification (day and nighttime, respectfully), the net result of this diel carbonate variation would be no change (Fig. 4B). Conversely, if the ratio of day to night calcification is 3:1, the same percent change applied to both would yield a net increase of 62.5% (Fig. 4B). Gattuso et al. (1999) compiled data from 26 studies of photosynthetic scleractinian corals, yielding 108 ratios of day to night calcification. Values ranged from negative (due to dissolution) to 127, and the majority (71%) of these ranged from one to five. These data underscore the contribution of daytime calcification, while highlighting the great diversity in ratios across species and, potentially, environmental conditions such as light and temperature (Galli and Solidoro, 2018; Suggett et al., 2012). Interestingly, of the data considered, 12% had a ratio equal to or less than one, and these species may be less affected or even negatively influenced by diel oscillations in carbonate chemistry.

Ultimately, both physiology and carbonate chemistry dynamics are

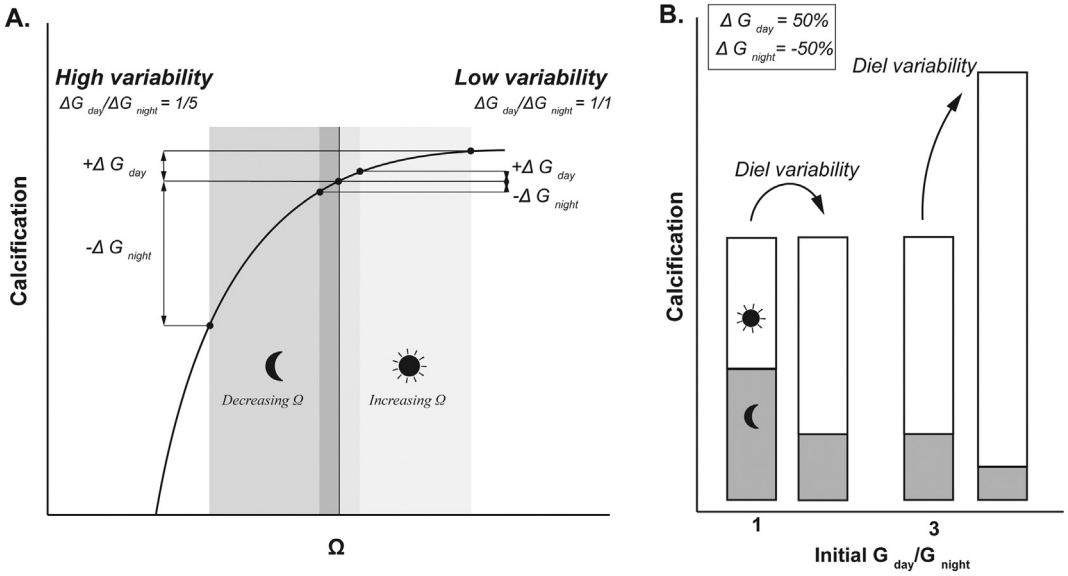

Fig. 4. Theoretical impacts of diurnal carbonate chemistry variability (Ω, saturation state) on calcification (G). A. The influence of a non-linear calcification/Ω relationship on a coral's response to diel variability. B. The influence of the day to night calcification ratio on the response to diel variability, assuming 50% enhancement of calcification during the day, and 50% reduction at night.

important in predicting calcification responses. With respect to the former, the balance of day vs. nighttime calcification should be considered, as well as the shape of the calcification response to Ω. With respect to carbonate chemistry, both amplitude and mean pH conditions are important in influencing the calcification response. As noted in Rivest et al. (2017b), future OA experiments should therefore attempt to characterize diel variability, in addition to the stability of mean carbonate chemistry conditions, to facilitate inter-experiment comparisons, meta-analyses, and broader conclusions.

4.5. Implications for OA and Acropora cervicornis

If the calcification response to OA conditions is primarily driven by daytime carbonate chemistry, dynamic systems may reach a critical CO_2 threshold at more advanced OA scenarios (Shaw et al., 2013b). Additionally, over longer time periods, routine exposure to extreme nighttime lows in pH could serve to drive selection for OA-tolerant genes and potentially help to buffer future deleterious impacts as proposed by Chan and Eggins (2017). Shallow water and restricted flow systems, which exhibit stronger biological forcing of carbonate chemistry, have more dynamic pH variability (Camp et al., 2017; Manzello, 2010; Page et al., 2017; Rivest et al., 2017b; Shaw et al., 2013b). Lagoons and inshore patch reefs may therefore demonstrate a degree of OA resistance, relative to fringing fore reefs and remote atolls with more stable ocean-driven carbonate chemistry conditions. It is cautioned, however, that mean pH may also be depressed in these environments relative to well-flushed, open systems (Camp et al., 2017; Shamberger et al., 2014). Despite these lower pH conditions, diverse coral communities have been documented in these habitats, reflecting a degree of resilience (Camp et al., 2017; Shamberger et al., 2014) that could have ramifications for future reef persistence (Schoepf et al., 2017).

Previous studies that have investigated the impacts of OA on *A. cervicornis* have yielded mixed results (Bedwell-Ivers et al., 2016; Enochs et al., 2014; Renegar and Riegl, 2005; Towle et al., 2015) and some experiments indicate a potential for the species to be resistant to high pCO_2 (Okazaki et al., 2017). Renegar and Riegl (2005) observed a significant reduction in calcification of *A. cervicornis* under elevated pCO_2 and 100% mortality when OA treatments were combined with both nitrate and phosphate enrichment. Enochs et al. (2014) also observed depressed calcification under high pCO_2 but no impact on linear extension. Towle et al. (2015) recorded significant effects of temperature and pCO_2 on the calcification of unfed colonies, but differences were not detectible when colonies were fed. In elevated temperatures (30.3 °C), Okazaki et al. (2017) observed *A. cervicornis* to switch from low calcification rates to dissolution, whereas in lower temperature treatments (27 °C), there were no strong influences of Ω and calcification remained slightly positive. Similarly, Bedwell-Ivers et al. (2016) did not detect an influence of OA on *A. cervicornis*, despite observing a significant effect on *Porites divericata*. Comparing and contrasting these studies using linear regression of calcification from a present-day baseline, Okazaki et al. (2017) detected a remarkable range, from 0 to 41% per one unit change in Ω.

The resistance to OA stress observed in some of these studies may be due, in part, to extraneous factors such as heterotrophy (Edmunds, 2011; Towle et al., 2015) or light (Enochs et al., 2014). Corals in this study were presented with food once a week and were kept under high light conditions, though incoming waters were filtered to one μm. It is difficult to assess whether corals in this study were energetically compromised without analysis of lipid content (Towle et al., 2015). However, the inability to distinguish significant differences in 8.05 vs. 7.80 mean pH treatments within the same variability regimes (± 0.10 and ± 0.00) suggests that mechanisms, such as feeding, could have contributed to OA resilience.

The potential also exists that differences in diel oscillation between *A. cervicornis* OA experiments could have contributed to variation in measured responses. While the majority of the aforementioned studies do not report diel variability in carbonate chemistry, Bedwell-Ivers et al. (2016) report strong diurnal oscillations (~0.15 pH units) in their high and low pH treatments, driven by the natural swings in their biologically controlled source water. Like this study and the 8.05 ± 0.10 vs. 7.8 ± 0.10 treatments, *A. cervicornis* fragments did not have lower calcification rates under more advanced OA conditions, suggesting that variability could be important for conferring resilience in the calcification response.

4.6. Genotypic differences

This study found strongly significant differences in the calcification rates of *Acropora cervicornis* genotypes, with the fastest calcifying at a

rate roughly 1.7 times that of the slowest. These data are supported by previous studies which have observed strong genotypic variation in the linear extension (Drury et al., 2017; Lirman et al., 2014; Lohr and Patterson, 2017; O'Donnell et al., 2017) and calcification (Kuffner et al., 2017; Lohr and Patterson, 2017) of the same species. Data from the Pacific congener *Acropora pulchra* suggests that intraspecific OA sensitivity is positively correlated with growth rate (Shaw et al., 2016). Therefore, the potential exists that this wide range in growth rates could yield an accompanying spread in OA sensitivity.

For the purposes of this study, we were not able to clearly attribute this difference to host genetics versus other characteristics of the holobiont, namely the composition of the associated *Symbiodinium* community. While variation in symbiont communities have been documented in hosts experiencing different thermal and nutrient regimes (Baums et al., 2010), others have observed a dominance of clade A (Drury et al., 2017), and a similarity in the growth rates of corals dominated by both clade A and C (Lirman et al., 2014).

4.7. Restoration implications

Given widespread efforts to grow and outplant *A. cervicornis* (Young et al., 2012), data pertaining to environmental factors influencing calcification rate can be used to maximize nursery production and reef restoration success. At contemporary mean pH conditions (8.05), a diurnal fluctuation of ± 0.10 pH units contributed to 17% higher calcification than static conditions. This suggests that natural carbonate chemistry variability can potentially contribute to coral growth today. In regions such as the Florida Keys, large-scale gradients in benthic cover such as seagrasses can influence carbonate chemistry and contribute to a more dynamic near-shore reef environment vs. those found offshore (Manzello et al. 2012). These natural gradients may contribute to restoration efforts and should be considered when evaluating nursery and outplanting sites.

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Declarations of interest

None.

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