



Acclimation History of Elevated Temperature Reduces the Tolerance of Coralline Algae to Additional Acute Thermal Stress

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Increasing atmospheric CO₂ is driving major environmental changes in the ocean, such as an increase in average ocean temperature, a decrease in average ocean pH (ocean acidification or OA), and an increase in the number and severity of extreme climatic events (e.g., anomalous temperature events and heatwaves). Uncertainty exists in the capacity for species to withstand these stressors occurring concomitantly. Here, we tested whether an acclimation history of ocean warming (OW) and OA affects the physiological responses of an abundant, reef-building species of crustose coralline algae (CCA), *Porolithon cf. onkodes*, to chronic and acute thermal stress. To address this, we exposed algae to varying temperature and pH levels for 6 weeks and this chronic treatment experiment was followed by an acute exposure to an anomalous temperature event (+4–6°C from acclimation temperature). Net photosynthetic rate was negatively affected across all treatments by increasing temperature during the acute temperature event, however, algae acclimated to the control temperature were able to maintain photosynthetic rates for +4°C above their acclimation temperature, whereas algae acclimated to elevated temperature were not. Average relative change in O₂ produced resulted in a 100–175% decrease, with the largest decrease found in algae acclimated to the combined treatment of elevated temperature and reduced pH. We conclude that acclimation to chronic global change stressors (i.e., OW and OA) will reduce the tolerance of *P. cf. onkodes* to anomalous increases in temperature, and this may have implications for reef building processes.

Keywords: coralline algae, global change, acclimation history, thermal stress, net photosynthesis, ocean acidification, ocean warming

INTRODUCTION

Global change and increasing atmospheric CO₂ are driving multiple marine environmental changes such as ocean warming (OW) and ocean acidification (OA), which are both predicted to be major threats to marine organisms and ecosystems globally (Doney et al., 2012; Poloczanska et al., 2013). OW and OA can negatively impact phenological events, physiological processes of organisms, and their overall distributions (Doney et al., 2012; Poloczanska et al., 2013). Alongside OW and OA, the number and severity of extreme climatic events (e.g., heatwaves and anomalous temperature

events) are projected to increase, which have been found to have significant negative ecological impacts on species and ecosystems more broadly (Wernberg et al., 2016; Smale et al., 2019).

Tropical species inhabiting coral reef ecosystems are particularly susceptible to thermal stress as many reef organisms already exist at or near their upper thermal tolerance limit (Baker et al., 2008; Lough et al., 2018) and this could be exacerbated by OW in recent decades (Hoegh-Guldberg et al., 2007; Kroeker et al., 2020). Studies investigating the effects of warming on coral reef macroalgae are limited however (Pakker et al., 1995; Bennett et al., 2018; Cornwall et al., 2019), with even fewer experiments examining the effects of warming on reef-building crustose coralline algae (CCA) (Cornwall et al., 2019). CCA are a group of calcifying, red macroalgae that are the cement of reefs, aiding in building and maintaining the carbonate framework of coral reefs worldwide (Adey, 1998). To date, many OW studies on CCA have been done over short durations (Vásquez-Elizondo and Enríquez, 2016) and/or have not coupled warming with additional stressors such as OA (Anton et al., 2020) and subsequent anomalous temperature events, like heatwaves (Cornwall et al., 2019). From the current literature, we know that tropical coralline algae are likely less robust than fleshy macroalgae living in the same environment (Anton et al., 2020) and potentially equally or sometimes more robust than corals living in the same environment (Kornder et al., 2018; Cornwall et al., 2019; Anton et al., 2020). Furthermore, we know that increasing temperatures, even without the addition of global change drivers, can cause decreases in physiological processes, such as calcification and photosynthesis (Vásquez-Elizondo and Enríquez, 2016; Schubert et al., 2019). Despite the current knowledge on the responses of CCA to global change drivers both in isolation and in combination, it is still not known whether an acclimation history of OW and OA plays a role in the physiological responses of coralline algae to chronic and acute thermal stress.

The aim of this study was to investigate how acclimation history impacts the ability of a reef building species of CCA, *Porolithon* cf. *onkodes*, hereinafter, *P. onkodes*, to tolerate sudden, acute increases in temperature. Global change drivers, such as OW and OA, have been found to variably impact processes of coralline algae (Johnson and Carpenter, 2012; Kim et al., 2020; Page and Diaz-Pulido, 2020), however, how the acclimation history of these stressors will impact plasticity to additional acute stressors is less understood. In this study, *P. onkodes* was held under constant temperature and/or pH conditions that reflect IPCC global predictions, specifically the representative concentration pathway (RCP) 8.5 (IPCC, 2013), or present day control conditions for 6 weeks. After the 6-week acclimation period *P. onkodes* fragments were subjected to an acute temperature stress ($+1^{\circ}\text{C h}^{-1}$ until 33°C) whilst percent oxygen (O_2) was monitored, and calcification/dissolution was estimated as proxies for physiological stress. CCA as a group have been found to be variably, but largely negatively impacted under combined stressors of OW and OA (Martin and Gattuso, 2009; Brodie et al., 2014; Hofmann and Bischof, 2014; Scherner et al., 2016), therefore, it was hypothesized that a history of exposure to these two stressors will cause additive pressure during an acute

warming event, and therefore result in decreased O_2 production and increased skeletal dissolution.

MATERIALS AND METHODS

Crustose coralline algae fragments, $\sim 3\text{ cm} \times 3\text{ cm}$ in size, of the orange color morph of *P. onkodes*, were collected in October 2018 from the reef flat area of Yonge Reef ($14^{\circ}37'32.1''\text{S}$, $145^{\circ}38'06.2''\text{E}$), Great Barrier Reef (GBR), Australia between 1 and 3 m depth. After collection, CCA fragments were brought back to Lizard Island Research Station (LIRS) and kept in an outdoor, flow-through, common garden aquarium at constant temperature ($\sim 27.2^{\circ}\text{C}$), pH (8.0), and salinity (35 ppt), similar to seawater parameters measured at collection site. Seawater at LIRS is pumped from the reef lagoon into header tanks and then gravity fed into the aquarium system. Algae were thoroughly cleaned of epiphytes within 24 h of their collection. All fragments were examined under a dissecting microscope to ensure all were the same species based on morphological identification. Exposed calcium carbonate skeleton was sealed using Coral Glue[®] (Ecotech Marine). Light at collection site was between 520 and $650\ \mu\text{mol quanta m}^{-2}\text{ s}^{-1}$ at $\sim 3\text{ m}$ depth ($\sim 14:00$). Within the experimental tanks, unshaded natural light ranged from 100 to $200\ \mu\text{mol quanta m}^{-2}\text{ s}^{-1}$ dependent on time of day.

After CCA were acclimated in the common garden, ambient conditions, fragments were allocated to treatment tanks. Four experimental treatments were targeted and used during this experiment: ambient control (27.2°C and pH 8.0; $450\ \mu\text{atm } p\text{CO}_2$), reduced pH/elevated $p\text{CO}_2$ (27.2°C and pH 7.7; $1,000\ \mu\text{atm } p\text{CO}_2$), elevated temperature (29.5°C and pH 8.0; $450\ \mu\text{atm } p\text{CO}_2$), and the combined treatment of elevated temperature and reduced pH/elevated $p\text{CO}_2$ (29.5°C and pH 7.7; $1,000\ \mu\text{atm } p\text{CO}_2$). The experimental setup included an outdoor, flow-through, four-header sump (275 L each) system and twenty 32 L individual treatment tanks, allocated randomly to the four experimental treatments. Each header tank was supplied constantly with water from the reef lagoon and water from treatment sumps was continuously pumped into respective treatment tanks using Pondmaster 3600 Fountain Pumps (Pond One[®]). Light was provided naturally and measured daily, around 12:00, using an underwater quantum sensor LI-192 connected to a light meter LI-250 (LI-COR, United States), averaging about $140\ \mu\text{mol quanta m}^{-2}\text{ s}^{-1}$. In treatments where temperature and/or pH were manipulated, levels were increased over 7 days, ultimately reaching a 0.3 unit decrease in pH ($\sim 1,000\ \mu\text{atm } p\text{CO}_2$) and a 2.3°C increase in temperature, relating to levels predicted by the IPCC under RCP8.5 scenario for end of century levels (IPCC, 2019). *P. onkodes* fragments were held at constant experimental treatments for 6 weeks. pH was regulated by the use of pH-controllers (AquaController, Neptune Systems, United States) that injected either pure CO_2 or ambient air into the header sumps until desired value was reached. To obtain equivalent, desired pH value the offset for each of the Neptune Systems pH probes (one probe per treatment and offset accordingly after each measurement) was calculated from values obtained from a pH meter (Mettler Toledo, SevenGo Duo SG98)

paired with a pH electrode with integrated temperature probe (Mettler Toledo, InLab Routine Pro) calibrated on total scale (pH_T) using Tris–HCl buffers (Dickson et al., 2007). pH_T and temperature were measured twice daily (08:00 and 15:00) in each experimental tank and sump. Salinity was measured daily using a conductivity meter (Mettler Toledo, SevenGo Pro). Temperature was maintained and manipulated by use of titanium heaters (EcoPlus, Aqua Heat, 300 W) within each sump and small 50 W glass heaters (Aqua One®) within respective experimental tanks that were set to desired temperature. Temperature was adjusted daily to mimic ambient temperature based on season (publicly available data from Australian Institute of Marine Science)¹ and +2.3°C was added in addition to the adjusted seasonal temperature for elevated temperature treatments. Flow to each experimental tank was maintained at a rate of 20 L h⁻¹, allowing for complete turnover of the experimental tanks twice per hour. A submersible water pump (Resun® SP-1100 8 W) provided additional water movement within each experimental tank. Total alkalinity (A_T) was measured every 3 days, water was collected around 09:00, for the first week of the experiment, and then every 6 days following the initial week. A_T was determined through potentiometric titration on an automatic titrator (Mettler Toledo, T50) following standard operating procedures (SOP) 3b (Dickson et al., 2007). A_T, pH_T, temperature, and salinity were used to calculate the remaining carbonate chemistry parameters using the seacarb package version 3.2.12 (Gattuso et al., 2019) in R (Supplementary Table 1). High Mg-calcite saturation state was calculated for a 16.4% MgCO₃ following Diaz-Pulido et al. (2012). CCA mortality or changes in health (identified as a paling or bleaching of the pigmented layer) were monitored throughout the 6-week experiment.

After 6 weeks of acclimation, or preconditioning, to temperature and pH treatments, we tested the role of acclimation history on the response of *P. onkodes* to acute thermal stress by conducting short-term temperature experiments. Five fragments of *P. onkodes* from each of the four treatments were thoroughly cleaned under a microscope 48 h prior to incubations and then were gently brushed 2 h before incubations to remove epiphytes from surface of algae. Fragments were placed into sealed, acrylic, incubation chambers (~150 mL) filled with respective OW and OA treatment water (*n* = 5). Chambers were placed within a water bath on top of a magnetic stir plate. Light was maintained at 140 μmol quanta m⁻² s⁻¹ (MarsAqua 300 W LED, Full Spectrum), which was the average daily light intensity during the 6-week experiment. Temperature of the water bath was controlled via an immersion circulating heater (CORIO CD, Julabo). Each chamber was equipped with a magnetic stir bar to ensure water movement throughout incubation, a temperature sensor (Pt100), and a PreSens dipping O₂ optode (DP-PSt3). Optodes were connected to a 10-channel trace O₂ meter (OXY-10 SMA trace, G2, PreSens, Germany). O₂ sensors were calibrated prior to every incubation to 0% O₂, obtained through addition of sodium dithionite (Na₂S₂O₄), and 100% O₂ seawater, obtained through bubbling water with air. Before beginning the acute warming experiment, algae were held

at acclimation temperature for 30 min and dissolved O₂ was measured to establish O₂ production after 6-weeks in acclimation treatment. Once these values were obtained, temperature was lowered to closest whole number (i.e., 27°C or 29°C dependent on acclimation temperature treatment) and algae were allowed to acclimate again for 30 min before beginning the acute warming experiment. Temperature was then increased by 1°C h⁻¹ (a rate of about 0.017°C min⁻¹). Dissolved O₂ and temperature were continuously measured and recorded every minute throughout the incubation. Incubations lasted up to 6 h to allow for an ending temperature of 33°C within the incubation chambers from respective acclimation temperature. Temperature data on reef crest sites and surrounding reef environments have recorded temperatures of over 33°C or an increase of over +4°C within a daily cycle (Lough, 1998). Therefore, the maximum temperature of heating during this experiment allowed for an environmentally realistic temperature maximum for a day on a reef flat or crest in the Northern GBR. A seawater blank with no CCA was run with each incubation and used for normalization in data analysis. All incubations were started at the same time every day to ensure no diurnally caused differences in photosynthetic output and calcification. Net photosynthesis (NP) was calculated as follows:

$$NP = \frac{(sO_2 \times V)}{(SA)}, \quad (1)$$

where sO₂ is the slope of the linear regression line for change in O₂ versus time (μmol L⁻¹ h⁻¹) at each measurement temperature, *V* is the volume of the incubation chamber (L), and SA is the surface area (cm²) determined through aluminum foil technique (Marsh, 1970).

Crustose coralline algae net calcification (*g_n*, μmol CaCO₃ cm⁻² h⁻¹) was estimated using the TA anomaly technique (Smith and Key, 1975; Schneider and Erez, 2006; Anthony et al., 2013; Cohen et al., 2017). Prior to the start of the incubations, seawater was sampled from the experimental tanks (initial A_T) and directly from incubation chambers at the end of each acute temperature experiment (final A_T). Totalling two A_T samples (initial A_T and final A_T) for each individual and blank seawater chamber. Seawater samples were filtered through 20 μm filters into titration cups (47–55 mL of sample) and immediately processed through potentiometric titration. Net calcification was estimated based on the difference in A_T (ΔA_T), which was normalized to the density of seawater at sampling temperatures (i.e. 27.2, 29.5, or 33°C) after the incubations, assuming A_T was solely affected by calcification and dissolution processes, and for every 1 mol of CaCO₃ precipitated, A_T is reduced by 2 molar equivalents (Chisholm and Gattuso, 1991),

$$g_n = \frac{\Delta A_T V}{2 \Delta t S}, \quad (2)$$

where *V* is the volume of the incubation chamber (L), Δ*t* is the time between initial A_T and final A_T (h), and *S* is the surface area of the CCA (cm²). Changes in A_T ranged from 18.33 to 572.20 μeq^{-L}.

All data were analyzed in the statistical software R v3.6.1. The absolute value of O₂, or net photosynthesis, at acclimation

¹<https://weather.aims.gov.au/#/station/1166>

temperatures (27.2 and 29.5°C), and net calcification data were analyzed by two-way ANOVA (with pH and temperature treatments as main factors and fragments as replicates, $n = 4-5$). Although there was no mortality in any of the treatments, one sample is missing from the elevated temperature and reduced pH treatment due to a faulty O₂ probe. Net photosynthesis and calcification data were tested for normality using Shapiro Wilk's and Levene tests for homoscedasticity. Linear mixed effect model residuals were tested for normality through graphical analyses of residuals, using QQ normality plots. All data met tests of normality.

Relative change in O₂ was calculated by taking the starting, absolute O₂ value at respective acclimation temperature and subtracting that value from each following measurement during the acute warming experiment. To find the variation of the relative change in O₂ production at each ramp temperature due to acclimation treatment a linear mixed effects model was used with a random intercept. Acclimation temperature and pH were set as fixed factors/effects and sample as the random effect, which allowed for variation of sample effects across ramping temperatures. The linear mixed effect model was done using the *lme* function from the *nlme* package (v3.1.141) (Pinheiro et al., 2013) in R and if significant differences were detected, Tukey's pairwise tests were conducted using the *glht* function from the *Multcomp* package (v1.4.10) (Hothorn et al., 2016).

RESULTS

After 6 weeks in acclimation treatments, net photosynthesis, or O₂ production ($\mu\text{mol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$), was measured on adult fragments of *P. onkodes*. At the end of the 6-week acclimation period, no significant effect of acclimation temperature, pH, or the interaction of the two (ANOVA, $p = 0.427, 0.102,$ and 0.105 , respectively, **Supplementary Table 2** and **Figure 1**) was found. Absolute values of mean O₂ production ranged from 1.17 to 4.84 $\mu\text{mol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$ in samples acclimated to the control temperature of 27.2°C and ranged from 1.80 to 6.61 $\mu\text{mol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$ in samples acclimated to elevated temperature of 29.5°C (**Supplementary Table 2** and **Figure 1**).

O₂ production of *P. onkodes* across all treatments was adversely affected by the acute warming experiment (**Table 1** and **Figure 2**). Ramp temperature significantly affected the relative change in O₂, with O₂ primarily decreasing across all treatments during the acute temperature experiment (**Figure 2**). The decline in O₂, on average a 0.530 $\mu\text{mol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$ decrease with each degree of temperature increase, from starting acclimation temperature to the ending ramp temperature of 33°C can be attributed to ramp temperature (**Table 1**). There was a significant effect of acclimation temperature on O₂ production at particular ramp temperatures, seen in a steeper slope of O₂ production in the elevated acclimation temperature treatments than control temperature treatments, irrespective of pH treatment (**Figure 2** and **Supplementary Figure 1**). The slope for the elevated temperature treatment was 42% higher/steeper than that of the control treatment (**Supplementary Figure 1**). Acclimation pH independently and in combination with elevated acclimation

temperature and/or ramp temperature did not have a significant effect on the relative change of O₂ during the acute temperature experiment (**Table 1**).

Average CCA net calcification ranged from 0.90 to 1.35 $\mu\text{mol CaCO}_3 \text{ cm}^{-2} \text{ h}^{-1}$ (**Table 2**). There was no significant effect of the temperature or pH treatment on net calcification (ANOVA, $p = 0.079$ and 0.402 , respectively, **Supplementary Table 3** and **Supplementary Figure 2**).

DISCUSSION

An acclimation history of elevated temperature may exacerbate the adverse effects of acute increases in temperature on CCA productivity. Our study provides insight into the role of OW and OA acclimation history in the thermal tolerance of an abundant and important species of reef-building coralline algae. Extreme climatic events such as rapid temperature increases (e.g., heat waves) are predicted to occur more frequently in future oceans with increasing atmospheric CO₂ (Oliver et al., 2018) and are expected to have global impacts on marine ecosystems (Smale et al., 2019). Our findings show steady increases in temperature, only a few degrees above their summer average temperature, result primarily in decreases in amount of O₂ produced by *P. onkodes*. The degree of effect was largely dependent on the temperature *P. onkodes* was acclimated to, with those kept under elevated temperature ($\sim 2.3^\circ\text{C}$ above ambient control temperature) experiencing a more rapid loss of performance in terms of O₂ production. This study provides information on how this species of calcifying algae will fair in future oceans and sheds light on how increasing average ocean temperatures will play a role in the response of reef-forming species to acute, anomalous increases in temperature.

Exposure to chronic elevated temperature and/or reduced pH had a minimal effect on average O₂ production when measured at a static acclimation temperature. These results are in line with some studies on tropical coralline algae, where reduced pH (Comeau et al., 2016) or the combination of elevated temperature and reduced pH (Page and Diaz-Pulido, 2020) had little to no effect on O₂ production. On the other hand, there are also studies that have documented decreased photosynthetic rates in *P. onkodes* under high temperature and low pH in the southern GBR (Anthony et al., 2008), although this may have been due to the increased mortality in their experimental individuals (Diaz-Pulido et al., 2012). The final average O₂ production data from the chronic acclimation temperature experiment suggest this algae population is largely robust to static, chronic changes in their environment, even possibly benefitting from a combination of elevated temperature and reduced pH, where O₂ production was higher (5.07–44.86%) when compared to all other treatments, however not significantly. These results did not persist after *P. onkodes* was exposed to the acute warming experiment at the end of the 6-week acclimation.

A history of elevated temperature appeared to exacerbate the effects of acute thermal stress on *P. onkodes*. This response was predicted, as elevated, experimental acclimation temperature was expected to be an additive stressor when

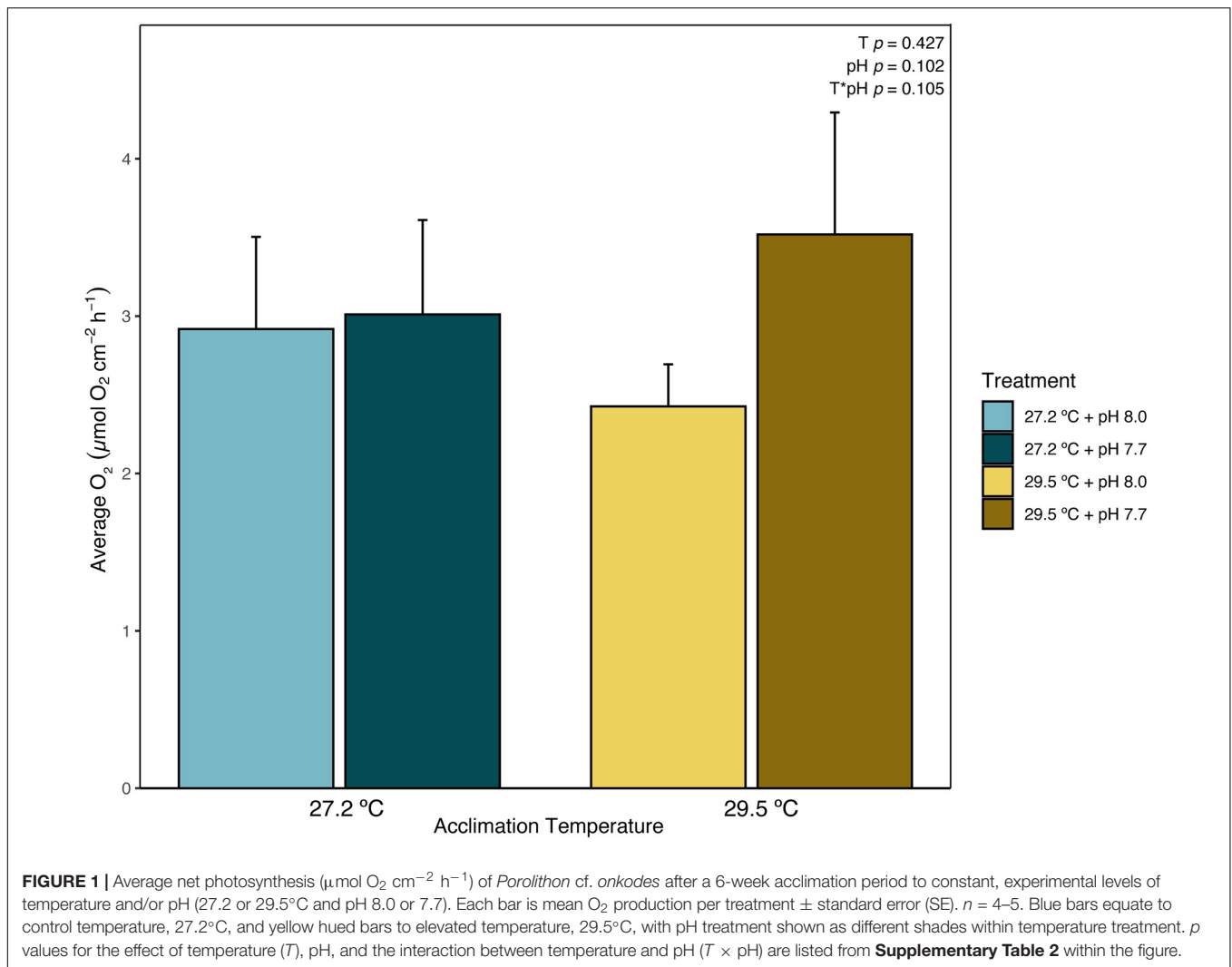


TABLE 1 | Summary of statistical information from a linear mixed effects model with a random intercept on the relative change of O_2 production during the acute warming experiment.

Factor	Value	df	t-value	P	Comparison
Relative change in O_2 production ($\mu\text{mol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$)					
Ramp temperature	-0.530	89	-7.263	<0.001	
Acclimation temperature	18.401	15	3.992	0.001	
pH	-2.472	15	-0.775	0.449	
Ramp temperature \times acclimation temperature	-0.590	89	-3.991	<0.001	C > H
Ramp temperature \times pH	0.091	89	0.873	0.385	
Acclimation temperature \times pH	-9.298	15	-1.453	0.165	
Ramp temperature \times acclimation temperature \times pH	0.325	89	1.579	0.118	

Significant results from linear mixed effect model are bolded. Comparison column obtained through slope comparison, with a flatter slope relating to less significant relative change in O_2 than a steeper slope (Figure 2 and Supplementary Figure 2). C (control/ambient 27.2°C) and H (high 29.5°C).

followed by an acute warming event. High temperature stress is understood to inhibit the photosynthetic apparatus and therefore photosynthetic processes (Mathur et al., 2014). There was a $\sim 125\%$ total relative decrease in O_2 production from starting acclimation temperature (27°C) to 33°C for *P. onkodes* acclimated to control temperature. In the individuals acclimated

to elevated temperature, this relative decrease was $\sim 100\%$ for the elevated temperature at control pH and $\sim 175\%$ for the elevated temperature at reduced pH. The treatment of reduced acclimation pH combined with elevated temperature seemingly intensified the alga's negative response to the acute warming experiment. Interestingly, *P. onkodes* acclimated to elevated

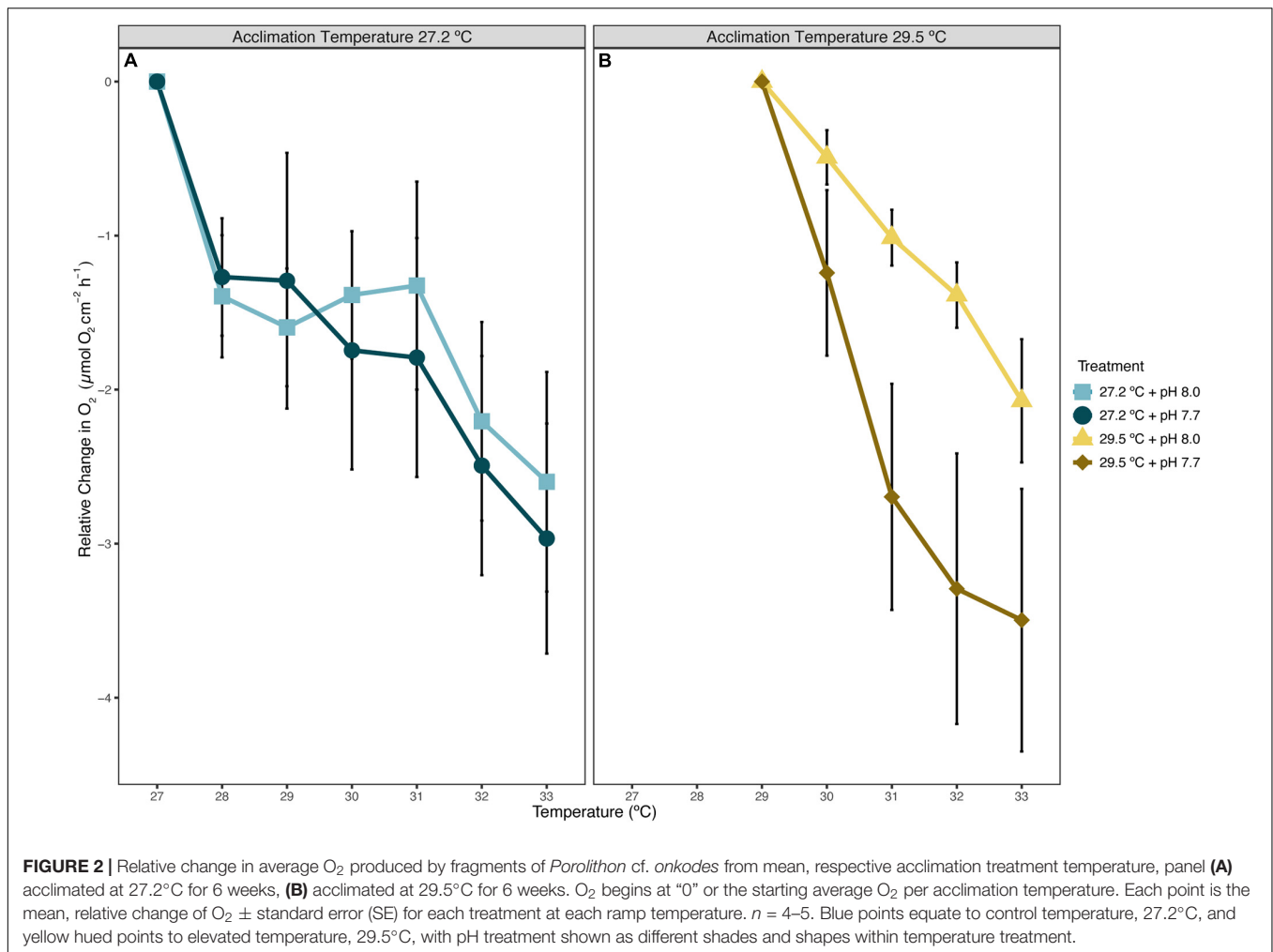


TABLE 2 | Summary of average net calcification ($\mu\text{mol CaCO}_3 \text{ cm}^{-2} \text{ h}^{-1}$) results from *Porolithon cf. onkodes* samples.

Treatment	Average net calcification ($\mu\text{mol CaCO}_3 \text{ cm}^{-2} \text{ h}^{-1}$)
27.2°C + 8.0 pH	1.18 \pm 0.06
29.5°C + 8.0 pH	0.90 \pm 0.22
27.2°C + 7.7 pH	1.35 \pm 0.19
29.5°C + 7.7 pH	0.94 \pm 0.16

Calcification was estimated by using the alkalinity anomaly technique. Results are shown as average for each treatment \pm standard error (SE).

temperature at control pH had the smallest relative change compared to other treatments, however, the slope of this change was 42% higher than the control temperature treatment, with an R^2 of 0.99 compared to an R^2 of 0.74, respectively. The steeper slope and higher R^2 value of the elevated temperature treatment is most likely linked to their acclimation history of elevated temperature, in which further increases in temperature resulted in a steady, unbroken reduction in O_2 . This result of a long-term increase in temperature followed by a short-term heatwave-like experiment inducing a reduction in physiological rates has been

seen previously in species of temperate articulated coralline algae (Rendina et al., 2019), but has not been documented for tropical reef-building corallines.

pH played a marginal role in magnifying the level of reduction in O_2 production resulting from increased acclimation temperature. This was seen most prominently when comparing the control treatment to the reduced pH treatment, where a reduction in pH resulted in a 12.4% larger decrease in O_2 production compared to the control pH treatments. This finding supports a meta-analysis that found CO_2 enrichment reduces photosynthesis in calcifying algae by 28% on average (Kroeker et al., 2013), however, this result only took into account three species of CCA out of the eight studies used to calculate this result. Some CCA specific studies have found elevated $p\text{CO}_2$ /reduced pH can increase photosynthesis (Semesi et al., 2009; Noisette et al., 2013; Ordoñez-Alvarez et al., 2018), therefore it was expected in the current study that an acclimation history of elevated $p\text{CO}_2$ /reduced pH might partially mitigate the physiological stress incurred by an acute warming event (Koch et al., 2013). Our experiment, however, demonstrated that reduced pH slightly exacerbated the negative effect of acute thermal stress on coralline algae.

P. onkodes never consumed O₂ faster than it was produced across any of the treatments. This result suggests some level of tolerance to acute, thermally stressful events both in current and future oceans. Additionally, unlike previous studies conducted with other calcifiers (Diaz-Pulido et al., 2012; Prada et al., 2017; Anderson et al., 2019), survivorship was not impacted amongst our algae. Our findings of O₂ consumption never overtaking O₂ production and no mortality are supported by previous work done on a tropical, Atlantic species of CCA, *Neogoniolithon*, that was exposed to elevated temperatures of 34°C, where positive net photosynthesis was maintained and no mortality occurred at elevated temperature, suggesting this species has a high thermal tolerance (Anderson, 2006). Our findings suggest that *P. onkodes* is able to largely withstand chronic, elevated levels of temperature up to +2.3°C and an increase in CO₂ of about 600 μatm (from current levels), although further, rapid and acute temperature increases may cause considerable physiological stress to the algae.

O₂ production results from the acute warming experiment suggest that acclimation history, particularly thermal, plays a role in the level of reduction seen in this specific physiological process, however, is this also seen in other crucial physiological processes of CCA? Data presented here did not show a significant effect of treatment condition on average net calcification. Temperature appeared to have more of an effect than pH on calcification in this study, with a marginal decrease in average net calcification under elevated temperature treatments. Although this result was not significant, it does support O₂ production findings from this study that temperature, not pH, has a larger impact on physiological processes of *P. onkodes*. These data suggest that, at least after a period of 6 weeks and following an acute increase in temperature, this population of *P. onkodes* is largely able to maintain net calcification rates. Calcification and dissolution values found in this study were comparable with previous studies that manipulated environmental parameters using CCA species from the GBR (Diaz-Pulido et al., 2012) and elsewhere (Martin and Gattuso, 2009). Results from studies measuring calcification after exposure to increased temperature and/or increased pCO₂/decreased pH vary (Martin and Gattuso, 2009; Diaz-Pulido et al., 2012; Vásquez-Elizondo and Enríquez, 2016; McNicholl et al., 2020). In coralline algae taxa, calcification can decrease significantly when temperature thresholds are crossed (Vásquez-Elizondo and Enríquez, 2016; Cornwall et al., 2019) and with elevated temperature and pCO₂ (Martin and Gattuso, 2009). In a Southern GBR population of *P. cf. onkodes*, calcification decreased under reduced pH levels, with this result being enhanced when combined with increased temperature (Diaz-Pulido et al., 2012). The population of *P. onkodes* examined in the current study responded differently from this Southern GBR population. It is possible that the difference in response of these two populations is due to the acute temperature stress the algae were exposed to at the end of the acclimation period and/or slightly different experimental conditions or varying techniques to measure net calcification (e.g., buoyant weight vs. TA anomaly).

In conclusion, our findings suggest thermally stressful events such as acute, anomalous increases in temperature or heatwaves can induce different ecophysiological responses

compared to those occurring from chronic (sustained), constant environmental changes, and understanding these responses could be more informative than those responses resulting from constant conditions (McCoy and Widdicombe, 2019). The wide thermal gradient that *P. onkodes* occupies may give them existing plasticity or tolerance to thermal stress, at least to constant levels of elevated temperature, however, when *P. onkodes* is further exposed to an acute, increase in temperature, this plasticity or tolerance does not persist. Future work into existing thermal tolerance, thermal limits, and standing genetic variation of populations of *P. onkodes* could generate illuminating data on phenotypes with different thermal tolerances and optima (Somero, 2010). This information could give us further insight into the fate of critical reef-building species in the face of rapid global change.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

TP and GD-P designed the experiment. TP conducted the experiment and analyzed the data, with guidance from GD-P and wrote the manuscript, with crucial guidance and input from GD-P. EB aided in collecting the algae, conducting and maintaining the experiment, and reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2021.660196/full#supplementary-material>

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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