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RESEARCH ARTICLE

High light alongside elevated P_{CO_2} alleviates thermal depression of photosynthesis in a hard coral (*Pocillopora acuta*)

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ABSTRACT

The absorbtion of human-emitted CO_2 by the oceans (elevated P_{CO_2}) is projected to alter the physiological performance of coral reef organisms by perturbing seawater chemistry (i.e. ocean acidification). Simultaneously, greenhouse gas emissions are driving ocean warming and changes in irradiance (through turbidity and cloud cover), which have the potential to influence the effects of ocean acidification on coral reefs. Here, we explored whether physiological impacts of elevated P_{CO_2} on a coral–algal symbiosis (*Pocillopora acuta*–Symbiodiniaceae) are mediated by light and/or temperature levels. In a 39 day experiment, elevated P_{CO_2} (962 versus 431 µatm P_{CO_2}) had an interactive effect with midday light availability (400 versus 800 µmol photons m^{-2} s^{-1}) and temperature (25 versus 29°C) on areal gross and net photosynthesis, for which a decline at 29°C was ameliorated under simultaneous high-P_{CO_2} and high-light conditions. Light-enhanced dark respiration increased under elevated P_{CO_2} and/or elevated temperature. Symbiont to host cell ratio and chlorophyll *a* per symbiont increased at elevated temperature, whilst symbiont areal density decreased. The ability of moderately strong light in the presence of elevated P_{CO_2} to alleviate the temperature-induced decrease in photosynthesis suggests that higher substrate availability facilitates a greater ability for photochemical quenching, partially offsetting the impacts of high temperature on the photosynthetic apparatus. Future environmental changes that result in moderate increases in light levels could therefore assist the *P. acuta* holobiont to cope with the 'one–two punch' of rising temperatures in the presence of an acidifying ocean.

KEY WORDS: Scleractinia, Irradiance, Multiple stressors, Ocean acidification, Ocean warming, Symbiodiniaceae

INTRODUCTION

Atmospheric carbon dioxide concentrations are increasing as a result of human activities and the burning of fossil fuels. The oceans have absorbed two-thirds of anthropogenically emitted CO_2, and the resulting perturbation of seawater pH and carbonate chemistry (i.e. ocean acidification) is an unprecedented threat to global marine life (Kleypas et al., 1999). The elevation of the partial pressure of CO_2 in seawater (P_{CO_2}) results in an increase in proton and bicarbonate ion concentrations (Miller and Wheeler, 2012), while decreasing carbonate concentration and the aragonite saturation state (Ω_{arag}) (Miller and Wheeler, 2012). In coral–algal symbioses, the physiological impacts of elevated P_{CO_2} have included declines in adult and juvenile calcification (Albright and Langdon, 2011; Cohen and Holcomb, 2009; Zunino et al., 2017), symbiont density (Kaniewska et al., 2012) and lipid biomass (Wall et al., 2017), altered protein metabolism (Edmunds and Wall, 2014) and photophysiology (Iguchi et al., 2012; Noonan and Fabricius, 2016), and compromised sexual reproduction and recruitment (Albright et al., 2010).

Studies of the impacts of elevated P_{CO_2} on corals have most commonly focused on calcification; however, impacts on other aspects of coral–algal holobiont physiology are often more salient. For instance, a broad survey of physiological responses in the coral *Pocillopora acuta* demonstrated robustness of calcification under 961 µatm P_{CO_2} at low- and high-light conditions and found no changes in the density of the intracellular dinoflagellate symbionts (Symbiodiniaceae), total chlorophyll ($a+c_2$) concentrations or total biomass (Wall et al., 2017). However, declines (of up to 20%) in several forms of energy storage (lipids, protein and total energy content) were observed (Wall et al., 2017). Owing to the central role that the coral–algal symbiosis plays in energy acquisition and carbonate deposition in coral reefs, even moderate impacts on its physiology may result in significant changes to these ecosystems.

Physiological robustness or vulnerability to elevated P_{CO_2} by itself is noteworthy; however, it is important to recognise that elevated P_{CO_2} will co-occur alongside other environmental impacts. The impacts of climate change in increasing seawater temperature is well recognised (Hoegh-Guldberg et al., 2014), and the weather conditions that cause heat stress at coral reefs usually also cause very high levels of insolation (Skirving and Guinotte, 2000). However, climate change will have other direct impacts on light levels at reefs. Climate change is predicted to affect cloud cover (Schneider et al., 2019), atmospheric light attenuation (Haywood et al., 2011) and seawater turbidity [via sea level rise (Ogston and Field, 2010) and intensification of both dry spells and precipitation (Fischer and Knutti, 2015)]. Expected decreases in anthropogenic aerosol emissions will also affect irradiance as regulations for reducing air pollution are implemented globally (Rosenfeld et al., 2019; Sato and Suzuki, 2019). These multiple pathways of climate impact will cause irradiance to increase at some reefs and decrease at others, influencing the impact of warming on coral photoinhibition.

Indeed, interactive effects of light with elevated P_{CO_2}, temperature with elevated P_{CO_2}, or light with temperature on coral–algal holobiont physiology have been empirically demonstrated with outcomes that range from antagonistic to synergistic. For instance, Bahr et al. (2016) found that elevated P_{CO_2} reduced *P. damicornis* calcification; however, elevated P_{CO_2} also remedied the negative effect of high temperature on calcification seen at present-day P_{CO_2} levels (Bahr et al., 2016). Light and temperature also interact to shape symbiont photobiology *in hospite* (Ban et al., 2014), and light availability influences the susceptibility of coral calcification to P_{CO_2} effects in

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Received 10 February 2020; Accepted 12 August 2020

Journal of Experimental Biology (2020) 223, jeb223198. doi:10.1242/jeb.223198

List of symbols and abbreviations

chl *a*	chlorophyll *a*
CTAB	cetyl trimethylammonium bromide
DR	dark respiration
HCO_2	high P_{CO_2}
HL	high light
HT	high temperature
LCO_2	ambient/low P_{CO_2}
LEDR	light-enhanced dark respiration
LL	low light
LT	low temperature
P/R	photosynthesis to respiration ratio
P_{CO_2}	partial pressure of carbon dioxide
P_{gross}	gross photosynthesis
pH_T	pH on the total scale
P_{net}	net photosynthesis
PSII	photosystem II
qPCR	quantitative polymerase chain reaction
S/H	symbiont to host cell ratio

juvenile (Dufault et al., 2013) and adult corals (Suggett et al., 2013). Together, these lines of evidence suggest that combinations of light and temperature will mediate how the coral–algal symbiosis will respond physiologically to rising seawater P_{CO_2}.

Owing to the forecast upsurge in mass coral bleaching events under climate change, the combined effects of P_{CO_2}, light and temperature on symbiont densities in corals is of particular interest. Anlauf et al. (2011) documented a synergistic impact of temperature and P_{CO_2} on symbiont densities in new coral recruits, wherein elevated P_{CO_2} ameliorated the decrease in symbionts caused by high temperature stress. Elevated P_{CO_2} synergistically lowered coral luminance (a proxy of bleaching) in two coral species under naturally high irradiances (ca. 1000 µmol photons m^{-2} s^{-1}) (Anthony et al., 2008; but see Wall et al., 2014). Meta-analysis has shown that, in general, symbiont densities in corals decline under elevated P_{CO_2}, but there is not yet broad evidence for an interaction between high temperature and elevated P_{CO_2} in causing symbiont loss (Mason, 2018b).

Here, we explore the impact of simultaneous alteration of P_{CO_2}, light and temperature on a pocilloporid coral (*P. acuta*) in a factorial design to assess the influence of ecologically relevant scenarios for multiple stressors on performance of the coral–algal holobiont. We measured a range of physiological parameters on which P_{CO_2}, light and temperature are thought to have a major influence in the current understanding of coral–algal holobiont physiology (mechanisms summarised in Table 1): symbiont cell densities, chlorophyll *a* (chl *a*) concentrations, respiration rates, light-enhanced dark respiration, photosynthesis, photosynthesis to respiration (*P*/*R*) ratio and symbiont to host (S/H) cell ratio. Given the known or theoretical impacts of each stressor in isolation (Table 1), we hypothesised that the combined stressors of elevated P_{CO_2}, irradiance differences and elevated temperature will have an interactive effect on each aspect of physiology of the *P. acuta* holobiont. For symbiont densities, where single-stressor effects are the most clear-cut (Table 1), we hypothesised that this variable will see its most severe decline under the combination of high light, elevated P_{CO_2} and high temperature.

MATERIALS AND METHODS

Coral collection

Whole colonies of *Pocillopora acuta* (Lamarck 1816) were collected on 13 and 29 October 2014 (n=5 and 3 colonies, respectively) at 0.2–1.5 m depth, from the windward reef of Moku o Lo‘e in Kāne‘ohe Bay, O‘ahu, HI, USA. Colonies were identified as *P. acuta* through the prominent pigmented rings around polyps and acute branch tips that distinguish this species from the morphologically similar *P. damicornis* (Schmidt-Roach et al., 2014). Collections were performed in accordance with permitting guidelines of the Hawai‘i Department of Land and Natural Resources Division of Aquatic Resources under Special Activity Permit 2015–8. At the Hawai‘i Institute of Marine Biology, colonies were fragmented into nubbins (<5 cm), mounted onto bases (as per Wall et al., 2017) and maintained for 21–37 days in outdoor aquaria under ∼60% shade cloth (daily peak of 200–300 µmol photons m^{-2} s^{-1}: Putnam et al., 2016) in flow-through seawater pumped from Kāne‘ohe Bay and cooled to 26.05±0.01°C (mean±s.e.m., n=4869). Nubbins were then distributed among 24 indoor tanks (n=8 nubbins per tank with one from each colony). This sample size exceeds that used in most coral tank experiments (3 to 6 per tank), achieving a simulated power of 80% in the detection of a three-factor interaction on symbiont densities at an effect size of ca. -0.36×10^6 cells cm^{-2} (see online code). Nubbins were maintained at 25.78±0.04°C (n=168) under two light regimes (described below) for a tank acclimation period of 25 days prior to the commencement of the experiment. During this period (21 November 2014 to 15 December 2014), the differential between the ambient P_{CO_2} and the high P_{CO_2} treatment tanks was partly introduced (Fig. S1). Each tank also contained 14 additional *P. acuta* nubbins for other studies that were removed on day 32 of the experiment. Corals were not given supplemental food, but dietary items (dissolved organic matter, microbes and plankton <100 µm in size) were present in the flow-through seawater.

Experimental design

CO_2 treatments (431 and 962 µmol P_{CO_2}) reflect present-day P_{CO_2} and end-of-century ocean acidification under Representative Concentration Pathway 8.5 (Meinshausen et al., 2011). The high light treatment (15.7 mol photons m^{-2} day^{-1}) sits within the range of insolation (7-day moving average) seen during the 2014–2015 December–January–February (DJF) period at Moku o Lo‘e at 2 m depth. The low light treatment (7.5 mol photons m^{-2} day^{-1}) reflects lower light conditions seen in the more turbid northern Kāne‘ohe Bay during the 2014–2015 DJF period (Cunning et al., 2016). Temperature treatments (25°C and 29°C) reflect a non-stressful sea temperature (near the DJF climatological mean sea surface temperature) and a stressful temperature (2°C in excess of the maximum monthly mean) for the Hawaiian Islands (NOAA Coral Reef Watch, 2019).

Our exposure of *P. acuta* used a staged introduction of multiple stressors. Four treatments, consisting of two P_{CO_2} levels crossed with two light levels, were administered for 39 days (16 December 2014 to 23 January 2015). During the final 7 days of the experiment, each treatment was divided into two temperature levels (design illustrated in Fig. S2). Twelve 44.8 litre tanks received 400 µmol photons m^{-2} s^{-1} (LL) at the midday light maximum and 12 received 800 µmol photons m^{-2} s^{-1} (HL) at the midday light maximum (Aqua Illuminations Sol Light Emitting Diode lamps; C2 Development Inc., Ames, IA, USA). The photoperiod (12 h:12 h light:dark) consisted of sunrise at 05:00 h, 5 h of linear increase in light intensity, stable peak light from 10:00 to 12:00 h, 5 h of linear decrease in light intensity, and sunset at 17:00 h. During the first 4 days of the acclimation period, a longer duration of peak light during the daytime was used (07:00–15:00 h), with possible paling of tissue observed in the high light treatment. Each tank received flow-through seawater at a mean±s.e.m. of 431±8 µatm P_{CO_2} (ambient/low, LCO_2) or 962±27 µatm P_{CO_2} (high, HCO_2) (Table 2), at a flow rate of ca. 1.5 l min^{-1}. The procedures used for

Table 1. Mechanisms by which elevated light, elevated temperature and elevated P_{CO_2} could affect [increase (↑), decrease (↓) or no change (~)] the physiology of coral–algal symbioses

Aspect of physiology	Stressor	Direction of change in physiology	Mechanism	References
Respiration rate	High P_{CO_2}	↓	Reduced seawater pH may interfere with the disposal of protons from respiration	Jokiel (2011), Kaniewska et al. (2012), Mackey et al. (2015)
		↑	Elevated metabolism as a result of increased cost of cellular processes or growth under elevated P_{CO_2}	McCulloch et al. (2012)
	High light	↑	Enhances energy budget, alleviates metabolic depression	Davies (1980), Jacobson et al. (2016), Rogers (1979)
		~, ↓	Photobleaching, leading to reduced energy income and metabolic depression	Tremblay et al. (2012), Yakovleva and Hidaka (2004)
	High temperature	↑	Kinetic rate of respiratory reactions will increase	Gillooly et al. (2001)
		↓	Reduced energy acquisition and expenditure due to bleaching; thermally induced mitochondrial degradation	Dunn et al. (2012)
Light-enhanced dark respiration rate	High P_{CO_2}	↓	Decreased photorespiration; reduced seawater pH impacts proton disposal	Rowan et al. (1996), Mackey et al. (2015)
		↑	Greater photosynthate availability drives respiration following illumination	Crawley et al. (2010)
	High light	↑	Increased availability of photosynthate post illumination	Stokes et al. (1990)
	High temperature	↓,↑	As for respiration	
Symbiont density per unit area	High P_{CO_2}	↑,↓	Proliferation of symbiont cells initially occurs owing to enhanced photosynthesis; however, at extremes of light and/or temperature, CO_2 demand outpaces supply, leading to symbiont loss	Wooldridge (2009)
	High light	↓	Mild decrease during photoacclimation to high light	Muller et al. (2009)
		↓	Substantial decrease under photobleaching	Krämer et al. (2013)
	High temperature	↓	Apoptosis, shedding of zooxanthellate host cells, or reactive oxygen species production	Nii and Muscatine (1997), Gates et al. (1992), Tchernov et al. (2011), Dunn et al. (2002), Weis (2008)
		~	Heterotrophy or lipid stores may postpone or prevent bleaching	Anthony et al. (2009)
Chl *a* content per cell	High P_{CO_2}	↑	Elevated P_{CO_2} increases photoacclimation to low light	Crawley et al. (2010)
	High light	↓	Long-term decrease in chl *a* per cell as part of photoacclimation to high light	Cohen and Dubinsky (2015)
		↓	Photoinhibition and consequent pigment loss	Hoegh-Guldberg (1999)
	High temperature	↑	Symbiont loss results in decreased self-shading, increased investment in pigment	Caroselli et al. (2015)
		↓	Heat-impairment of PSII leads to reactive oxygen damage to pigments	Hoegh-Guldberg (1999)
Photosynthesis rate (area-standardised)	High P_{CO_2}	↑	Increased bicarbonate and CO_2 (*aq*) availability	Mackey et al. (2015)
		~	Inefficient carbon-concentrating mechanisms in some coral species	Noonan and Fabricius (2016)
		↓	Symbiont loss causes photophysiological dysfunction in those remaining in tissue	Anthony et al. (2008)
	High light	↑	Increased availability of light drives increased photosynthesis	Dubinsky and Falkowski (2011)
		↓	Photoinhibition at very high light levels	Hoegh-Guldberg (1999)
		~	Increase in quenching via photoprotective mechanisms	Gorbunov et al. (2001)
	High temperature	↓	Inhibits the protein synthesis-based repair of photo-damage to PSII and causes photoinhibition, damage to thylakoid membranes	Iglesias-Prieto et al. (1992), Iglesias-Prieto (1995), Takahashi et al. (2004); Warner et al. (1996); Warner et al. (1999)
Symbiont to host cell ratio	High P_{CO_2}	↑, ↓	As for symbiont density	
	High light	↓	As for symbiont density	
	High temperature	↑, ↓	Initial increase under moderate warming; decreases at high temperature	Cunning and Baker (2013)

That some stressors elicit both a positive and a negative effect on physiology is often because moderate doses of that stressor may be beneficial, and high doses (that exceed physiological tolerances) detrimental.

Journal of Experimental Biology (2020) 223, jeb223198. doi:10.1242/jeb.223198

modifying P_{CO_2} levels and for biweekly measurements of tank carbonate chemistry are reported in Wall et al. (2017). During the first stage (32 days), corals in each CO_2×light treatment (n=6 tanks treatment^{-1}) were maintained at seasonally ambient conditions of 24.59±0.06°C (mean±s.e.m., n=153). For the final 7 days, each CO_2×light treatment was split, with three tanks kept at 24.59±0.06°C (n=11; LT) and three tanks raised to 29.05±0.22°C (n=11; HT), using immersion and in-line aquarium heaters.

Alkalinity measurements performed on experimental treatment water at intervals over one 24 h period indicated diel variations in P_{CO_2} of ca. 150 and 400 µatm in the ambient P_{CO_2} and elevated P_{CO_2} treatments, respectively, with minima during midday (Fig. S3). As experimental tanks were downstream from the header sumps (where P_{CO_2} was controlled) and the residence time (duration to turnover 99% of water; Escobal, 1996) in each tank was moderate (137 min), diel P_{CO_2} fluctuations in each tank were likely a result of net respiration at night and net photosynthesis during the day. This compares with diel fluctuations of 200–300 µatm P_{CO_2} on the Kāne'ohe Bay barrier reef (Shamberger et al., 2011).

Respirometry

Respirometry measurements were performed on four nubbins per tank on days 36 and 37 (non-heated treatments) and days 38 and 39 (heated treatments), in two magnetically stirred 260 ml cylindrical Perspex chambers containing filtered seawater (0.25 µm) from the P_{CO_2} treatment relevant to each nubbin. To maintain the relevant temperature, chambers were controlled to 24.77±0.03°C (n=45) or 29.68±0.02°C (n=44) via immersion in a water bath. Following a period of low light acclimation (30 min at <10 µmol photons m^{-2} s^{-1}), each nubbin was incubated for ∼10 min in darkness (measuring dark respiration, DR), illuminated for ∼10 min at the midday light level of the relevant treatment (measuring daytime net photosynthesis, P_{net}), exposed to darkness again for ∼10 min (measuring light-enhanced dark respiration, LEDR) and then repatriated to its original treatment tank. Intra-chamber oxygen concentrations were determined at 1 s logging intervals with a Fospor fibre optic probe on a Neofox Oxygen Sensing System (Ocean Optics, Dunedin, FL, USA). Gross photosynthesis under midday light (P_{gross}) was calculated as P_{net}–LEDR (assuming no respiration of energy acquired via heterotrophy, and that LEDR approximates light respiration) (but see Stokes et al., 1990). Photosynthesis and respiration measurements were normalised to coral surface area. The ratio of photosynthesis to respiration (P/R ratio) was calculated by dividing P_{net} by DR.

Symbiont density, chl *a* and surface area

On the final day of the experiment, nubbins were snap-frozen in liquid nitrogen and stored at −80°C. Tissue was airbrushed off thawed nubbins with a pressurised stream of buffer at 4°C (0.4 mol l^{-1} NaCl, 0.05 mol l^{-1} EDTA) and the slurry was processed for ∼10 s with a T25 500 W variable speed homogeniser (IKA Works Inc., Wilmington, NC, USA). The presence of planula larvae (planulae), visible in the coral tissue as it was being removed, was noted when observed. Symbiont cells were pelleted (1 ml of slurry centrifuged at 4500 ***g*** for 5 min), extracted in 100% acetone (48 h at −20°C), and chl *a* was determined as per Jeffrey and Humphrey (1975). Chl *a* was normalised to coral surface area (chl *a* density) and to symbiont cell count (chl *a* per symbiont). Symbiont densities were determined by counts in a Neubauer haemocytometer chamber (n=2–4) (Reichert Inc., Buffalo, NY, USA) and normalised to coral surface area. Coral surface areas were estimated by double wax dipping (Vytopil and Willis, 2001).

S/H cell ratios

DNA was extracted from 300 µl of tissue slurry using a CTAB–chloroform protocol (dx.doi.org/10.17504/protocols.io.dyq7vv). Symbiont to host (S/H) cell ratios were determined by quantitative PCR (qPCR) on a StepOnePlus Real-Time PCR machine (Applied Biosystems, Foster City, CA, USA). We used taxon-specific primers that separately target the actin gene of dinoflagellate symbionts within Symbiodiniaceae clade C (genus *Cladocopium*) and clade D (genus *Durusdinium*) and *P. acuta* (primers and qPCR protocol as per Cunning and Baker, 2013). Clades C and D are the only clades typically detected in the genus *Pocillopora* (Cunning et al., 2013; Pochon et al., 2010; Stat et al., 2008, 2015; M. Stat, personal communication). qPCR reactions were excluded if they had a cycle threshold (C_T) within three cycles of the C_T (if any) observed in the respective negative control. We corrected the S/H cell ratios with the symbiont to host DNA extraction efficiency ratio for CTAB–chloroform extraction, 0.828 (Cunning and Baker, 2013). We assumed the same actin copy number (three) as measured in *P. damicornis* by these authors owing to its close taxonomic affinity to *P. acuta*. Actin gene copy numbers for clades C and D were

Table 2. Seawater physical and chemical parameters

Treatment	PAR (µmol photons m^{-2} s^{-1})	Temperature[a] (°C)	Temperature[b] (°C)	pH_T	TA (µmol kg^{-1})	P_{CO_2} (µatm)	HCO_3^- (µmol kg^{-1})	CO_3^{2-} (µmol kg^{-1})	Ω_{arag}
LCO_2–HL–HT	800	24.6±0.2	28.8±0.2	8.01±0.01	2174±4	420±17	1702±16	190±6	3.03±0.10
LCO_2–HL–LT	800	24.6±0.2	24.6±0.1	8.02±0.01	2177±4	410±16	1708±13	189±6	3.01±0.09
LCO_2–LL–HT	400	24.8±0.2	29.8±0.2	7.99±0.01	2173±4	444±16	1716±13	184±5	2.94±0.08
LCO_2–LL–LT	400	24.5±0.2	24.5±0.1	7.99±0.01	2176±4	449±13	1735±10	178±4	2.82±0.06
HCO_2–HL–HT	800	24.5±0.2	29.4±0.3	7.71±0.03	2179±6	974±72	1912±19	108±7	1.72±0.11
HCO_2–HL–LT	800	24.4±0.2	24.7±0.3	7.72±0.02	2181±6	919±44	1916±12	107±5	1.70±0.08
HCO_2–LL–HT	400	24.9±0.2	28.3±0.4	7.70±0.02	2179±5	984±47	1916±13	106±5	1.70±0.08
HCO_2–LL–LT	400	24.3±0.2	24.5±0.1	7.71±0.02	2181±5	960±53	1917±16	107±6	1.69±0.10

Values are expressed as means±s.e.m. pH_T (pH on the total scale), temperature (°C), total alkalinity (TA) and salinity were measured as per Wall et al. (2017). P_{CO_2}, HCO_3^- concentration, CO_3^{2-} concentration and Ω_{arag} (aragonite saturation state) were calculated from pH_T, TA, salinity and temperature using the R package seacarb. Treatments are low and high P_{CO_2} (LCO_2, HCO_2), low and high light (LL, HL), and low and high temperatures (LT, HT). Seawater chemistry parameters (pH_T, TA, P_{CO_2}, HCO_3^-, CO_3^{2-}, Ω_{arag}) had sample sizes of n=23–24 for all treatments, except for the treatments HCO_2–HL–LT and HCO_2–HL–HT (n=16). PAR, photosynthetically active radiation at midday.

[a]Temperature (°C) at time of water sampling during days 1–32 at sample size n=20–21, except for HCO_2–HL–HT and HCO_2–HL–LT (n=14).

[b]Temperature (°C) at time of water sampling during days 33–39 (n=2–3).

estimated using a singular value decomposition method (Cunning et al., 2016).

Statistical analysis

Differences between the two CO_2 treatments for carbonate chemistry variables (P_{CO_2}, pH_T and total alkalinity) were assessed via *t*-tests. To explore tank effects on these variables, one-way ANOVAs were performed to compare all tanks within each of the two CO_2 treatments. Analyses of physiological responses (symbiont density, chl *a* per symbiont, chl *a* density, P_{net}, P_{gross}, DR, LEDR, P/R and S/H ratios) were performed via mixed-effects models in the package lme4 (Bates et al., 2015) in R (https://www.r-project.org/). Temperature, light and P_{CO_2} were designated as fixed effects; tank and colony were designated as random effects, and random effects were retained or dropped according to the function {step} (lmerTest). Normality of distribution was checked using quantile–quantile plots and transforms were applied if needed, and homoscedasticity was checked using plots of residuals versus fitted values. Type III ANOVAs were performed in the package lmerTest (Kuznetsova et al., 2017). S/H cell ratio was log-transformed prior to analysis (Cunning and Baker, 2013). *Post hoc* tests of significant interactive effects were performed using least-square means with the Tukey method for *P*-value adjustment.

RESULTS

Treatment conditions

Seawater carbonate chemistry was effectively controlled to deliver consistent conditions at each P_{CO_2} level (Table 2). Mechanical issues in one HCO_2–HL–HT tank and one HCO_2–HL–LT tank towards the end of the experiment led to the *a priori* removal of these tanks and the nubbins within from further analyses, leading to final replication of two tanks per treatment for these treatments and three for all other treatments. The two carbonate chemistry treatments differed in P_{CO_2} ($P<0.001$) and pH_T conditions ($P<0.001$). Total alkalinity was not affected by CO_2 treatment ($P=0.147$). P_{CO_2} and pH_T did not differ among replicate tanks in the HCO_2 treatment ($P\geq0.668$). At ambient CO_2, P_{CO_2} and pH_T differed between two tanks (tank 14: 376 µatm P_{CO_2} and pH 8.05; tank 21: 498 µatm P_{CO_2} and pH 7.95) (one-way ANOVA $P\leq0.026$, *post hoc* $P=0.036$). However, these differences were within an acceptable range for the LCO_2 treatment and no pairwise difference in P_{CO_2} or pH_T was found between either tank and any other LCO_2 tanks (*post hoc* $P\geq0.064$), nor between any other pairs of LCO_2 tanks (*post hoc* $P\geq0.264$).

Physiological responses

Symbiont density was not affected by P_{CO_2} ($F_{1,21}=0.40$, $P=0.536$), but declined by 23 and 25% at high light ($F_{1,21}=18.20$, $P<0.001$) and high temperature ($F_{1,22}=30.69$, $P<0.001$), respectively (Tables 5 and 6). Symbiont density experienced no interactive effect among any factors ($F_{1,21}\leq3.78$, $P\geq0.065$) (Table 3). Chl *a* per symbiont did not change under high P_{CO_2} ($F_{1,14}=0.09$, $P=0.763$), decreased by 17% at high light ($F_{1,14}=21.82$, $P<0.001$) (Table 5) and increased by 24% at high temperature ($F_{1,14}=28.24$, $P<0.001$) (Table 6). Chl *a* per symbiont was not affected by an interaction among any factors ($F_{1,14}\leq0.51$, $P\geq0.485$) (Table 3). Chl *a* density declined by 36% at high light ($F_{1,20}=47.71$, $P<0.001$) (Table 5) but was not affected by P_{CO_2} ($F_{1,20}=0.68$, $P=0.418$), temperature ($F_{1,20}=3.02$, $P=0.097$) nor any interactive effects ($F_{1,20}\leq3.59$, $P\geq0.073$) (Table 3).

A significant interactive effect among all three factors was found for P_{net} ($F_{1,80}=5.26$, $P=0.024$). *Post hoc* tests indicated that high temperature caused declines in P_{net} at LCO_2–HL (of 40%) and HCO_2–LL (62%), but not at HCO_2–HL or LCO_2–LL (Fig. 1). Whilst main effects are difficult to interpret under a significant interaction, a significant main effect of temperature ($F_{1,80}=31.53$, $P<0.001$, decline of 41% at HT) (Table 6) but not light ($F_{1,80}=0.39$, $P=0.536$) or CO_2 ($F_{1,80}=1.04$, $P=0.311$) was found, and no two-factor interactions were detected ($F_{1,80}\leq1.85$, $P\geq0.178$) (Table 3).

A significant three-factor interaction among light, temperature and CO_2 affected P_{gross} ($F_{1,80}=4.08$, $P=0.047$). *Post hoc* tests found that high temperature depressed P_{gross} by 34% at HCO_2–LL, but not at LCO_2–LL, LCO_2–HL or HCO_2–HL (Fig. 2). For the main effects, P_{gross} increased by 12% at high P_{CO_2} ($F_{1,80}=4.78$, $P=0.032$) (Table 4) but did not change under high light ($F_{1,80}=1.44$, $P=0.233$) and decreased by 20% under high temperature ($F_{1,80}=12.72$, $P<0.001$) (Table 6). No two-factor interactions were detected ($F_{1,80}\leq1.79$, $P\geq0.184$) (Table 3).

Dark respiration was not affected by P_{CO_2} ($F_{1,76}=1.81$, $P=0.183$) or light ($F_{1,76}=1.21$, $P=0.276$), but increased by 24% at high temperature ($F_{1,76}=6.86$, $P=0.011$) (Table 6). No significant two- or three-factor interactions were found ($F_{1,76}\leq2.03$, $P\geq0.158$) (Table 3). LEDR increased by 15% at high P_{CO_2} ($F_{1,76}=5.05$, $P=0.028$) (Table 4), was not affected by light ($F_{1,76}=1.34$, $P=0.250$), and increased by 21% at high temperature ($F_{1,76}=6.63$, $P=0.012$) (Table 6). No significant interactive effects among any factors affected LEDR ($F_{1,76}\leq1.40$, $P\geq0.241$) (Table 3).

The P/R ratio was not affected by P_{CO_2} ($F_{1,76}=0.01$, $P=0.905$) or light ($F_{1,76}=0.24$, $P=0.629$), and decreased by 56% at high temperature ($F_{1,76}=26.47$, $P<0.001$) (Table 6). No two- or three-factor interactions affected P/R ratio ($F_{1,76}\leq3.25$, $P\geq0.075$) (Table 3).

The S/H cell ratio was not affected by P_{CO_2} ($F_{1,149}=1\times10^{-4}$, $P=0.991$) nor light ($F_{1,149}=4\times10^{-4}$, $P=0.984$), but increased by 65% at high temperature ($F_{1,149}=6.31$, $P=0.013$) (Table 6). No two- or three-factor interactions affected S/H cell ratio ($F_{1,149}\leq1.71$, $P\geq0.192$) (Table 3).

Though not statistically significant, S/H cell ratio values at HCO_2–LL–HT (0.16±0.04, mean±s.e.m.) and LCO_2–HL–HT (0.15±0.03) were higher compared with those at LCO_2–LL–HT

Table 3. *P*-values given by ANOVAs performed on linear models for all physiological response variables

Factor	Symbiont density (cells cm^{-2})	Chl *a* per symbiont (pg $cell^{-1}$)	Chl *a* density (µg cm^{-2})	S/H cell ratio	DR (µmol O_2 cm^{-2} h^{-1})	P_{net} (µmol O_2 cm^{-2} h^{-1})	P_{gross} (µmol O_2 cm^{-2} h^{-1})	LEDR (µmol O_2 cm^{-2} h^{-1})	P/R ratio
Temperature	2×10^{-5}	1×10^{-4}	0.097	0.013	0.011	3×10^{-7}	6×10^{-4}	0.012	2×10^{-6}
Light	3×10^{-4}	4×10^{-4}	9×10^{-7}	0.984	0.276	0.536	0.233	0.250	0.629
CO_2	0.536	0.763	0.418	0.991	0.183	0.311	0.032	0.028	0.905
T×L	0.065	0.657	0.073	0.969	0.419	0.178	0.184	0.876	0.126
T×CO_2	0.535	0.998	0.704	0.915	0.158	0.515	0.836	0.342	0.267
L×CO_2	0.910	0.522	0.838	0.359	0.162	0.249	0.288	0.241	0.894
T×L×CO_2	0.467	0.485	0.346	0.192	0.763	0.024	0.047	0.989	0.075

F statistics and degrees of freedom corresponding to each *P*-value are given in Table S1. T, temperature; L, light.

Journal of Experimental Biology (2020) 223, jeb223198. doi:10.1242/jeb.223198

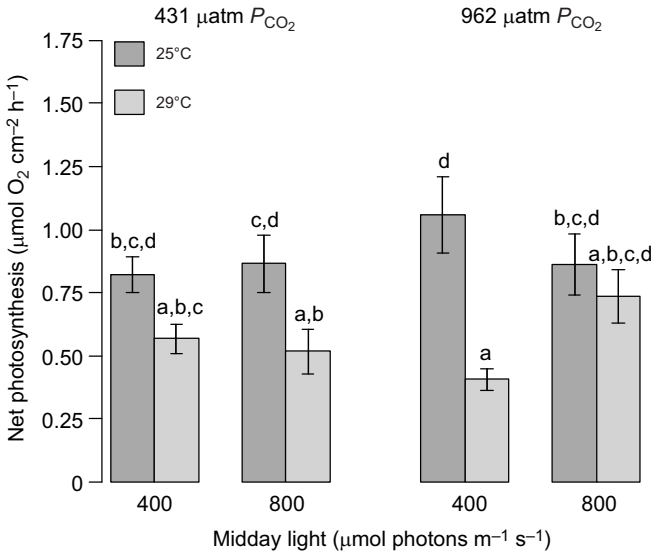

Fig. 1. Net photosynthesis in *Pocillopora acuta* is affected by a synergistic interaction of P_{CO_2}, light intensity and temperature. Bars are means±s.e.m. with sample sizes of n=6 (HCO_2–HL–LT), n=7 (HCO_2–HL–HT), n=11 (LCO_2–HL–LT) and n=12 (all other treatments). The light levels are the daily maxima in the light treatments (the light intensity between 10:00 and 12:00 h). Above the bars, treatments that share at least one letter are not significantly different from one another (P>0.05) in the *post hoc* test of the interactive effect of light, temperature and P_{CO_2}.

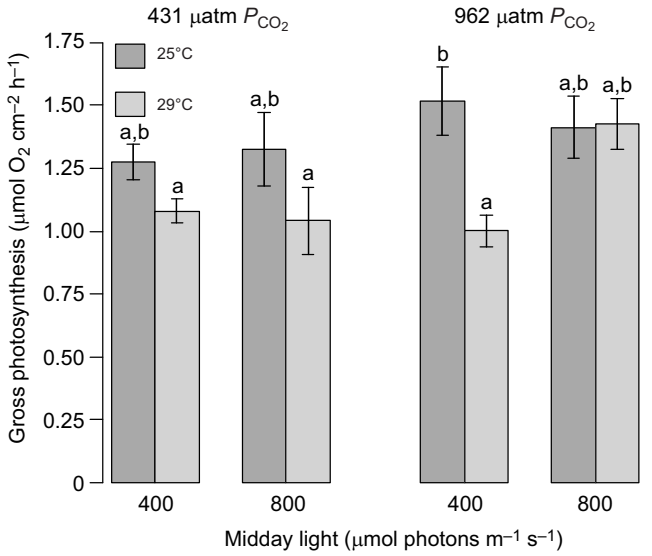

Fig. 2. Gross photosynthesis in *P. acuta* is affected by a synergistic interaction of P_{CO_2}, light intensity and temperature. These data suggest that the depression of photosynthesis, which may occur under high temperature (sixth bar from the left), is ameliorated by exposure to higher irradiance combined with ocean acidification (eighth bar from the left). Bars are means±s.e.m. with sample sizes of n=6 (HCO_2–HL–-LT), n=7 (HCO_2–HL–HT), n=11 (LCO_2–HL–LT) and n=12 (all other treatments). The light levels are the daily maxima in the light treatments (the light intensity between 10:00 and 12:00 h). Above the bars, treatments that share at least one letter are not significantly different from one another (P>0.05) in the *post hoc* test of the interactive effect of light, temperature and P_{CO_2}.

(0.10±0.03) and HCO_2–HL–HT (0.11±0.04). All treatments at 25°C had mean S/H cell ratios between 0.06 and 0.09.

DISCUSSION

In order to project how climate change will influence coral reef ecosystems, it is necessary to determine how multiple environmental parameters interact to attenuate and exacerbate climate change stressors. However, experimental explorations of such interactions are faced with the challenge that elevated P_{CO_2} owing to human activities is a chronic stressor, whereas high temperature is an acute phenomenon. As impacts of elevated P_{CO_2} may take some time to manifest (Anthony et al., 2008), the application of heat stress as a chronic stressor could obscure any effect of elevated P_{CO_2} (e.g. by continually exerting a strong downward pressure on symbiont population size). The present study utilised an experimental design for the analysis of the combined impacts of elevated P_{CO_2}, light and temperature that provided time for physiological changes to elevated P_{CO_2} and light to occur (over 32 days) before heat stress was applied (additional 7 days), thus providing the proper context of elevated P_{CO_2} and heat as chronic and acute impacts, respectively.

A significant three-way interaction between elevated P_{CO_2}, light and temperature was detected for net and gross photosynthesis. For P_{net}, declines were detected at 29°C versus 25°C within the P_{CO_2}–light combinations of LCO_2–HL and HCO_2–LL. Circumstantial evidence suggests that elevated S/H cell ratios may have been linked to depressed P_{net}. S/H cell ratio was elevated at 29°C versus 25°C (significant main effect); however, at LCO_2–HL and HCO_2–LL, this difference in S/H cell ratio was far more pronounced, 74–141% (trend only), compared with 18–24% in the other P_{CO_2}–light combinations. As the three-factor interaction for S/H cell ratio was not statistically significant, further work will be required to investigate this link.

For P_{gross}, a decline at 29°C versus 25°C was supported for HCO_2–LL but not LCO_2–HL or other P_{CO_2}–light combinations. Several explanations exist for why P_{net} and P_{gross} differed with respect to the LCO_2–HL treatment. Firstly, differences in light respiration among treatments, if any, may introduce real differences between P_{net} and P_{gross}. Secondly, LEDR, used here as a proxy for light respiration to calculate P_{gross}, may underestimate light respiration rates (Schrameyer et al., 2014). Finally, P_{gross}, as calculated here, contains measurement error from both P_{net} and LEDR and this added variation may have served to reduce the statistical power of the *post hoc* tests.

A consideration of how the experimental treatments may limit the light and dark reactions of photosynthesis could help to explain the interactive effects observed for P_{net} and P_{gross}. Increases in temperature are known to decrease the capacity for photosynthetic electron transport, such as by damaging the D1 protein (Warner et al., 1999). Under high light conditions, this can lead to overreduction of the remaining intact electron transport chains and to further damage through the production of oxygen radicals (Hoegh-Guldberg, 1999), decreasing the areal rate of photosynthesis (as was seen for P_{net} at

Table 4. Statistically significant main effects of P_{CO_2}

Parameter	Units	Value at 435 µatm P_{CO_2} (mean±s.e.m.)	Value at 961 µatm P_{CO_2} (mean±s.e.m.)	% change at high P_{CO_2}
P_{gross}	µmol O_2 cm^{-2} h^{-1}	1.18±0.05	1.32±0.07	12
LEDR	µmol O_2 cm^{-2} h^{-1}	−0.49±0.02	−0.56±0.03	15

Journal of Experimental Biology (2020) 223, jeb223198. doi:10.1242/jeb.223198

Table 5. Statistically significant main effects of light

Parameter	Units	Value at 400 µmol photons m^{-2} s^{-1} (mean±s.e.m.)	Value at 800 µmol photons m^{-2} s^{-1} (mean±s.e.m.)	% change at high light
Symbiont density	10^5 cells cm^{-2}	7.89±0.25	6.10±0.19	−23
Chl *a* density	µg cm^{-2}	3.64±0.12	2.32±0.07	−36
Chl *a* per symbiont	pg $cell^{-1}$	4.65±0.08	3.86±0.09	−17

elevated temperature and ambient P_{CO_2}). However, under high substrate availability (elevated P_{CO_2}), an enhanced capacity for Calvin–Benson activity may help to keep electron transport chains sufficiently open to cope with high light influx, offsetting the inhibiting effect of high temperature. Enhanced turnover rate of the Calvin–Benson cycle at moderately elevated temperature (Dusenge et al., 2018), and/or faster repair of damaged D1 proteins owing to an improved energetic status of the organism, might also contribute (Hoogenboom et al., 2012). This interpretation offers an explanation for why photosynthesis (both P_{net} and P_{gross}) increases with irradiance under elevated temperature combined with elevated P_{CO_2}. It is likely that these effects would only occur within a range of elevated light that is sufficient to deliver adequate reducing power, but still low enough to avoid substantive photodamage. As a case in point, under very high light conditions, ocean acidification and warming were found to synergistically decrease net photosynthetic productivity in *Porites lobata* by Anthony et al. (2008).

In previous studies involving fully crossed levels of P_{CO_2} and temperature, the effect of these variables on photosynthesis was independent in some coral species but interactive in others. In *Seriatopora hystrix*, P_{net} and P_{gross} decreased under high temperature and increased under high P_{CO_2}, with both stressors having an additive, but not interactive, effect (Noonan and Fabricius, 2016). In *Acropora millepora*, no interactive effect of temperature and acidification nor of light (at low levels) and acidification on P_{net} and P_{gross} has been found (Noonan and Fabricius, 2016; Vogel et al., 2015), but P_{gross} may decrease under acidification at high light (Kaniewska et al., 2012). In *Stylophora pistillata*, P_{net} increased under high temperature and decreased under high CO_2, but no interaction between the two factors was found (Reynaud et al., 2003). In *Acropora intermediata* and *P. lobata*, net productivity decreased over a gradient of low to high P_{CO_2} at ambient temperature, with warming interactively altering this pattern by effecting an increase in net productivity at intermediate P_{CO_2} in *A. intermediata* and exacerbating the size of the net productivity decline in *P. lobata* (Anthony et al., 2008). P_{gross} increased across a range of coral species at a natural CO_2 seep by 23–37% (Biscéré et al., 2019), moderately more than the increase in P_{gross} under acidification (12%) observed as a main effect in the present study. Physiological differences such as in carbon-concentrating mechanisms or symbiont photophysiology may account for inter-specific differences in P_{net} and P_{gross} responses (Brading et al., 2011).

Multiple stressors can have a compound effect on corals through interactive effects, where present, or through additive impacts where each stressor exhibits a main effect. The latter was seen with chl *a* per symbiont (discussed later) and LEDR. LEDR increased by 15% under elevated P_{CO_2}, and increased by 21% at elevated temperature. Increased LEDR can relate to several forms of photochemical quenching. Increased rates of respiration can occur as a result of increased Calvin–Benson cycle activity or production of ATP and NADPH by thylakoid electron transport under illumination (Parys and Jastrzębski, 2006). LEDR may also indicate oxygen consumption through the alternate photochemical quenching mechanisms of chlororespiration and the Mehler ascorbate–peroxidase cycle, or re-oxidation of spent electron carriers (Roberty et al., 2014; Stokes et al., 1990). Significantly, in a prior study of *Acropora muricata*, LEDR increased under acidification whilst DR remained constant, and evidence from xanthophyll cycling suggested that increased Mehler ascorbate–peroxidase cycle activity could have been the cause (Crawley et al., 2010). This may also occur in the *P. acuta* holobiont; however, the increase in P_{gross} under acidification leaves open the possibility of increased photosynthetic substrate as a cause of elevated LEDR. As LEDR increased under elevated temperature, likely owing to the increased kinetic rate of metabolic reactions (Gillooly et al., 2001), an additive impact of increased temperature and elevated P_{CO_2} on LEDR in some coral–algal holobionts is to be expected under future global change.

The additive impact on LEDR of increased temperature and P_{CO_2} may lead to energetic consequences for the *P. acuta* holobiont. In the case of the latter stressor, Wall et al. (2017) identified that lipids and energy content (measured per unit biomass and thus robust to the effects of skeletal extension) are depleted in *P. acuta* under acidification at two light levels. Depletion of energy reserves could be due to decreased availability of heterotrophically derived energy, or to an increased rate of energy usage, which, in most cases, will manifest as an increased rate of respiration. Our finding of increased LEDR under elevated P_{CO_2} suggests a greater rate of metabolism of photosynthates immediately after their production, and thus could provide a plausible hypothesis for the decreased energy reserves of *P. acuta* observed by Wall et al. (2017).

Maintenance of calcification rate may explain the lack of decline in DR and the increase in LEDR under elevated P_{CO_2}. The lack of decline in DR [also observed in *P. damicorn*is (Comeau et al., 2017)], and its increase under elevated P_{CO_2} at 29°C (Fig. 2) is notable as the increased

Table 6. Statistically significant main effects of temperature

Parameter	Units	Value at 25°C (mean±s.e.m.)	Value at 29°C (mean±s.e.m.)	% change at high temperature
Symbiont density	10^5 cells cm^{-2}	8.11±0.23	6.06±0.21	−25
Chl *a* per symbiont	pg $cell^{-1}$	3.85±0.07	4.77±0.09	24
P_{net}	µmol O_2 cm^{-2} h^{-1}	0.91±0.06	0.54±0.04	−41
P_{gross}	µmol O_2 cm^{-2} h^{-1}	1.38±0.06	1.10±0.05	−20
DR	µmol O_2 cm^{-2} h^{-1}	−0.48±0.03	−0.59±0.03	24
LEDR	µmol O_2 cm^{-2} h^{-1}	−0.47±0.02	−0.57±0.03	21
P/R ratio		2.31±0.26	1.01±0.09	−56
S/H cell ratio		0.08±0.01	0.14±0.02	65

seawater proton concentration under elevated P_{CO_2} could make respiration more energetically costly (Mackey et al., 2015). *Pocillopora damicornis* and *P. acuta* maintain their rate of calcification, normalised to both biomass and skeletal area, and experience declines in biomass or energy reserves, under elevated P_{CO_2} (Comeau et al., 2013; Comeau et al., 2014a,b; Wall et al., 2017). This could be a signature of the energetic cost of the maintenance of calcification under elevated P_{CO_2} (Comeau et al., 2013; Galli and Solidoro, 2018) and/or the increased energetic costs of respiration. In free-swimming larvae (i.e. a non-calcifying life stage) of *Pocillopora* spp., respiration rate displays a trend to decrease under elevated P_{CO_2} (Putnam et al., 2013), supporting the idea that the metabolic requirements of maintaining calcification under acidification contributes to stability in DR in adults.

Other vital processes may have also contributed to a changed energetic balance at high P_{CO_2}. Incipient planula larvae were observed within the adult tissue of many coral specimens at the end of this study. We were unable to determine from these observations whether P_{CO_2} level influenced the quantity of incipient larvae within coral tissues, the number of larvae released over the course of the experiment or their maturation time. However, their presence in this study suggests that gametogensis warrants investigation as a process that may potentially effect or be affected by changes in adult coral condition under elevated P_{CO_2}.

Our hypothesis that symbiont density loss would be greatest at the combination of elevated P_{CO_2}, high temperature and high light was not fully supported by the data. Symbiont density loss was the greatest under high light combined with high temperature, with statistical support, and the two stressors together had an additive but not interactive effect on this variable (also see Ban et al., 2014). Unlike light and temperature, elevated P_{CO_2} had no statistically significant impact on symbiont cells. However, a decline in symbiont densities under elevated P_{CO_2} has been shown in a meta-analysis across multiple studies and coral species (Mason, 2018b), indicating that an effect of P_{CO_2} on symbiont densities is often present. In our study, an effect of P_{CO_2} on symbiont densities may have required a greater period of incubation at high P_{CO_2} or may have been moderated by our particular combination of coral and symbiont species.

Of the three stressors tested (elevated P_{CO_2}, light and temperature), changes in temperature exerted the greatest number of main effects, significantly affecting almost every response variable. The ratio of P_{net} under midday irradiance to dark respiration declined to 1.01 at 29°C, which could suggest that phototrophy may not meet energy expenditure at 29°C if integrated over a full day–night cycle. The decrease in symbiont densities with an increase in temperature (from 25°C to 29°C) was accompanied by an increase in chl *a* content per symbiont cell. Increased chl *a* per symbiont under stressful temperature has been observed in some coral species [*Seriatopora caliendrum* (Baghdasarian et al., 2017), *S. hystrix* (Hoegh-Guldberg and Smith, 1989) and the temperate coral *Balanophyllia europaea* (Caroselli et al., 2015)], and likely indicates a photoacclimation response to decreased symbiont densities. In constrast to temperature, high light caused a decrease in both symbiont cell density and chl *a* per symbiont, possibly indicating some form of light-induced photosynthetic dysfunction, or rapid linear extension and concomitant thinning of symbiont densities and chl *a* per symbiont.

Whilst symbiont density decreased at high temperature, the S/H cell ratio increased. Decreasing symbiont density and increasing S/H cell ratio together suggest that host cell density per cm^2 decreased at elevated temperature, but at a rate greater than the loss of symbiont cells. This pattern is consistent with past observations of physiological responses of coral–algal holobionts to seasonal warming, which is thought to cause host cell loss, increasing the S/H ratio but also triggering a regulatory downwards adjustment in the symbiont population (Cunning and Baker, 2013). An alternative explanation, that S/H ratio increases owing to rapid linear extension and consequent thinning of symbiont cells, is unlikely as 29°C is above the calcification optima of Kāne‘ohe Bay corals (Jokiel and Coles, 1977). Increasing S/H cell ratios confirm the nuance revealed by chl *a* per symbiont that the symbiont density loss at high temperature is not yet indicative of a state of coral bleaching, though loss of host cells is a sign that bleaching may be imminent (Ainsworth et al., 2008).

It is clear that under future climates, where temperature and acidification have both already increased, the way that light is affected by climate change will prove to be important to *P. acuta*, impacting the degree to which warming will decrease photosynthetic rate. Light can decrease under climate change through sea level rise, which may cause increased tidal resuspension of sediments (Ogston and Field, 2010), through increased turbidity owing to stronger precipitation events (Fischer and Knutti, 2015), and through increased atmospheric water vapour content, which decreases light transmission (Haywood et al., 2011). In those cases, *P. acuta* will clearly not experience the alleviation of temperature-induced photosynthesis depression. In contrast, cloud cover could decrease in many areas owing to warming (McCoy et al., 2017; Schneider et al., 2019), and future reductions in aerosol emissions will tend to increase light penetration and reduce cloud cover (Rosenfeld et al., 2019; Sato and Suzuki, 2019). Changes in high cirrus clouds that trap heat but reduce irradiance could also be particularly important (Kärcher, 2017; Liou, 1986). We note that 800 µmol photons m^{-2} s^{-1} is relatively strong irradiance but still within the moderate range of light that reefs experience, and future increases in irradiance will only be beneficial to the extent that they move *P. acuta* into this range, but not beyond it (to photoinhibitive light levels).

Coral species/genotypes and their partner symbionts vary in their temperature tolerance and photophysiology, such as non-photochemical quenching and carbon concentrating mechanisms. As such, it is difficult to infer how general these responses would be among other coral species/symbiont combinations. However, increase in photosynthesis under acidification has been observed in some other species (Biscéré et al., 2019; but not all, see Sweet and Brown, 2016), and a decrease in photosynthesis at stressful temperatures is a common phenomenon (e.g. Hoegh-Guldberg, 1999). Therefore, whilst demonstrated in only one species here, a degree of commonality among corals in displaying the alleviation of thermally depressed photosynthesis by strong light combined with high P_{CO_2} is an intriguing possibility.

Conclusions

Owing to the roles of light and CO_2 as (respectively) power source and substrate for photosynthesis, and the role of temperature in optimising or disrupting this process, the influences of P_{CO_2}, light and temperature on marine photosynthesisers remains a research imperative under climate change. Of the three factors, temperature exerted the dominant influence on the physiology of the *P. acuta*–Symbiodiniaceae symbiosis. Importantly, these effects were observed at a heat stress level that had not caused bleaching, and hence may reflect physiological responses to moderate but year-round elevations in temperature under climate change. Interactions among all three factors were detected for some key physiological processes. Elevated P_{CO_2} caused an increase in photosynthesis in *P. acuta* (as found in

some other coral species), whilst stressful temperature caused a decrease. However, our results demonstrate that light can alter this impact: 800 µmol photons m^{-2} s^{-1} at high (but not ambient) P_{CO_2} ameliorated the decline in photosynthesis caused by high temperature, an effect that was clearly not seen at the lower light level. This is a significant finding, illustrating that the well-known negative impacts of high temperature on photosynthesis can be alleviated by moderately strong irradiance if accompanied by elevated seawater P_{CO_2}.

Changes in energetic status at the organism level could have flow over effects to the broader ecosystem. Coral–algal holobionts supply vast quantities of energy to other levels of the ecosystem, through the release of mucus (Crossland et al., 1980) and dissolved organic carbon (Crossland, 1987), and corallivory (Cole et al., 2011). The changes in the rate of photosynthesis but also of metabolism of energy (LEDR) at elevated P_{CO_2} observed in this study indicates the possibility of changes in the release of energy through these other pathways. Measurement of mucus release, dissolved organic carbon release and rates of corallivory under ocean acidification will be useful to ascertain the impacts of these holobiont-level changes on the coral reef ecosystem.

Acknowledgements
We thank D. Schar, J. Dulin, H. Putnam, R. Ritson-Williams, J. Davidson, B. Lenz, and W. Skirving for constructive discussions and logistical support, and G. Davies for performing DNA extractions and qPCR runs. Portions of this research formed a part of the PhD thesis of R.A.B.M. (Mason, 2018a). This is Hawai'i Institute of Marine Biology (HIMB) contribution number 1822 and School of Ocean and Earth Science and Technology (SOEST) contribution number 11136.

Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: R.A.B.M., C.B.W., R.C., S.D., R.D.G.; Methodology: R.A.B.M., C.B.W., R.C., R.D.G.; Software: R.C.; Formal analysis: R.A.B.M.; Investigation: R.A.B.M., C.B.W., R.C.; Resources: R.A.B.M., C.B.W., R.C., R.D.G.; Data curation: R.A.B.M., C.B.W., R.C.; Writing - original draft: R.A.B.M.; Writing - review & editing: R.A.B.M., C.B.W., R.C., S.D.; Visualization: R.A.B.M.; Supervision: S.D., R.D.G.; Project administration: R.A.B.M., R.D.G.; Funding acquisition: R.A.B.M., R.D.G.

Funding
This work was funded by grants to R.A.B.M. from the Linnean Society of New South Wales, the International Phycological Society, the Australian Wildlife Society and a Fulbright Scholarship. C.B.W. was supported by an University of Hawai'i Graduate Opportunity Grant, the UH Edmondson Research Fund, and Environmental Protection Agency (EPA) STAR Fellowship Assistance Agreement (FP-91779401-1). The views expressed in this publication have not been reviewed or endorsed by the EPA and are solely those of the authors. R.C. was supported by a National Science Foundation Postdoctoral Fellowship in Biology (NSF-DBI-1400787).

Data availability
Data and R scripts to replicate the analysis are openly accessible online (Mason et al., 2020) from Zenodo at doi:10.5281/zenodo.3526369.

Supplementary information
Supplementary information available online at
https://jeb.biologists.org/lookup/doi/10.1242/jeb.223198.supplemental

References
Ainsworth, T. D., Hoegh-Guldberg, O., Heron, S. F., Skirving, W. J. and Leggat, W. (2008). Early cellular changes are indicators of pre-bleaching thermal stress in the coral host. *J. Exp. Mar. Biol. Ecol.* **364**, 63-71. doi:10.1016/j.jembe.2008.06.032

Albright, R. and Langdon, C. (2011). Ocean acidification impacts multiple early life history processes of the Caribbean coral *Porites astreoides*. *Glob. Change Biol.* **17**, 2478-2487. doi:10.1111/j.1365-2486.2011.02404.x

Albright, R., Mason, B., Miller, M. and Langdon, C. (2010). Ocean acidification compromises recruitment success of the threatened Caribbean coral *Acropora palmata*. *Proc. Natl. Acad. Sci. USA* **107**, 20400-20404. doi:10.1073/pnas.1007273107

Anlauf, H., D'Croz, L. and O'Dea, A. (2011). A corrosive concoction: the combined effects of ocean warming and acidification on the early growth of a stony coral are multiplicative. *J. Exp. Mar. Biol. Ecol.* **397**, 13-20. doi:10.1016/j.jembe.2010.11.009

Anthony, K. R. N., Kline, D. I., Diaz-Pulido, G., Dove, S. and Hoegh-Guldberg, O. (2008). Ocean acidification causes bleaching and productivity loss in coral reef builders. *Proc. Natl. Acad. Sci. USA* **105**, 17442-17446. doi:10.1073/pnas.0804478105

Anthony, K. R. N., Hoogenboom, M. O., Maynard, J. A., Grottoli, A. G. and Middlebrook, R. (2009). Energetics approach to predicting mortality risk from environmental stress: a case study of coral bleaching. *Funct. Ecol.* **23**, 539-550. doi:10.1111/j.1365-2435.2008.01531.x

Baghdasarian, G., Osberg, A., Mihora, D., Putnam, H., Gates, R. D. and Edmunds, P. J. (2017). Effects of temperature and pCO_2 on population regulation of *Symbiodinium* spp. in a tropical reef coral. *Biol. Bull.* **232**, 123-139. doi:10.1086/692718

Bahr, K. D., Jokiel, P. L. and Rodgers, K. S. (2016). Relative sensitivity of five Hawaiian coral species to high temperature under high-pCO_2 conditions. *Coral Reefs* **35**, 729-738. doi:10.1007/s00338-016-1405-4

Ban, S. S., Graham, N. A. J. and Connolly, S. R. (2014). Evidence for multiple stressor interactions and effects on coral reefs. *Glob. Change Biol.* **20**, 681-697. doi:10.1111/gcb.12453

Bates, D., Mächler, M., Bolker, B. and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1-48. doi:10.18637/jss.v067.i01

Biscéré, T., Zampighi, M., Lorrain, A., Jurriaans, S., Foggo, A., Houlbrèque, F. and Rodolfo-Metalpa, R. (2019). High pCO_2 promotes coral primary production. *Biol. Lett.* **15**, 20180777. doi:10.1098/rsbl.2018.0777

Brading, P., Warner, M. E., Davey, P., Smith, D. J., Achterberg, E. P. and Suggett, D. J. (2011). Differential effects of ocean acidification on growth and photosynthesis among phylotypes of *Symbiodinium* (Dinophyceae). *Limnol. Oceanogr.* **56**, 927-938. doi:10.4319/lo.2011.56.3.0927

Caroselli, E., Falini, G., Goffredo, S., Dubinsky, Z. and Levy, O. (2015). Negative response of photosynthesis to natural and projected high seawater temperatures estimated by pulse amplitude modulation fluorometry in a temperate coral. *Front. Physiol.* **6**, 317. doi:10.3389/fphys.2015.00317

Cohen, I. and Dubinsky, Z. (2015). Long term photoacclimation responses of the coral *Stylophora pistillata* to reciprocal deep to shallow transplantation: photosynthesis and calcification. *Front. Mar. Sci.* **2**, 45. doi:10.3389/fmars.2015.00045

Cohen, A. L. and Holcomb, M. (2009). Why corals care about ocean acidification: uncovering the mechanism. *Oceanography* **22**, 118-127. doi:10.5670/oceanog.2009.102

Cole, A. J., Lawton, R. J., Pratchett, M. S. and Wilson, S. K. (2011). Chronic coral consumption by butterflyfishes. *Coral Reefs* **30**, 85-93. doi:10.1007/s00338-010-0674-6

Comeau, S., Edmunds, P. J., Spindel, N. B. and Carpenter, R. C. (2013). The responses of eight coral reef calcifiers to increasing partial pressure of CO_2 do not exhibit a tipping point. *Limnol. Oceanogr.* **58**, 388-398. doi:10.4319/lo.2013.58.1.0388

Comeau, S., Edmunds, P. J., Spindel, N. B. and Carpenter, R. C. (2014a). Fast coral reef calcifiers are more sensitive to ocean acidification in short-term laboratory incubations. *Limnol. Oceanogr.* **59**, 1081-1091. doi:10.4319/lo.2014.59.3.1081

Comeau, S., Carpenter, R. C., Nojiri, Y., Putnam, H. M., Sakai, K. and Edmunds, P. J. (2014b). Pacific-wide contrast highlights resistance of reef calcifiers to ocean acidification. *Proc. R. Soc. B Biol. Sci.* **281**, 20141339-20141339. doi:10.1098/rspb.2014.1339

Comeau, S., Carpenter, R. C. and Edmunds, P. J. (2017). Effects of pCO_2 on photosynthesis and respiration of tropical scleractinian corals and calcified algae. *ICES J. Mar. Sci.* **74**, 1092-1102. doi:10.1093/icesjms/fsv267

Crawley, A., Kline, D. I., Dunn, S., Anthony, K. and Dove, S. (2010). The effect of ocean acidification on symbiont photorespiration and productivity in *Acropora formosa*. *Glob. Change Biol.* **16**, 851-863. doi:10.1111/j.1365-2486.2009.01943.x

Crossland, C. J. (1987). In situ release of mucus and DOC-lipid from the corals *Acropora variabilis* and *Stylophora pistillata* in different light regimes. *Coral Reefs* **6**, 35-42. doi:10.1007/BF00302210

Crossland, C. J., Barnes, D. J. and Borowitzka, M. A. (1980). Diurnal lipid and mucus production in the staghorn coral *Acropora acuminata*. *Mar. Biol.* **60**, 81-90. doi:10.1007/BF00389151

Cunning, R. and Baker, A. C. (2013). Excess algal symbionts increase the susceptibility of reef corals to bleaching. *Nat. Clim. Change* **3**, 259-262. doi:10.1038/nclimate1711

Cunning, R., Glynn, P. W. and Baker, A. C. (2013). Flexible associations between *Pocillopora* corals and *Symbiodinium* limit utility of symbiosis ecology in defining species. *Coral Reefs* **32**, 795-801. doi:10.1007/s00338-013-1036-y

Cunning, R., Ritson-Williams, R. and Gates, R. D. (2016). Patterns of bleaching and recovery of *Montipora capitata* in Kāne'ohe Bay, Hawai'i, USA. *Mar. Ecol. Prog. Ser.* **551**, 131-139. doi:10.3354/meps11733

Davies, P. S. (1980). Respiration in some Atlantic reef corals in relation to vertical distribution and growth form. *Biol. Bull.* **158**, 187-194. doi:10.2307/1540930
Dubinsky, Z. and Falkowski, P. (2011). Light as a source of information and energy in zooxanthellate corals. In *Coral Reefs: An Ecosystem in Transition* (ed. Z. Dubinsky and N. Stambler), pp. 107-118. Dordrecht: Springer Netherlands.
Dufault, A. M., Ninokawa, A., Bramanti, L., Cumbo, V. R., Fan, T.-Y. and Edmunds, P. J. (2013). The role of light in mediating the effects of ocean acidification on coral calcification. *J. Exp. Biol.* **216**, 1570-1577. doi:10.1242/jeb.080549
Dunn, S. R., Thomason, J. C., Le Tissier, M. D. A. and Bythell, J. C. (2002). Detection of cell death activity during experimentally induced bleaching of the symbiotic sea anemone *Aiptasia* sp. In *Proceedings of the Ninth International Coral Reef Symposium, Bali. 23-27 Oct. 2000* (ed. M. K. Moosa, S. Soemodihardjo, A. Soegiarto, K. Romimohtarto, A. Nontji, Soekarno and Suharsono), pp. 145-153. Ministry of Environment of the Republic of Indonesia; Indonesian Institute of Sciences; International Society for Reef Studies.
Dunn, S. R., Pernice, M., Green, K., Hoegh-Guldberg, O. and Dove, S. G. (2012). Thermal stress promotes host mitochondrial degradation in symbiotic cnidarians: are the batteries of the reef going to run out? *PLoS ONE* **7**, e39024. doi:10.1371/journal.pone.0039024
Dusenge, M. E., Duarte, A. G. and Way, D. A. (2018). Plant carbon metabolism and climate change: elevated CO_2 and temperature impacts on photosynthesis, photorespiration and respiration. *New Phytol.*. **221**, 32-49. doi:10.1111/nph.15283
Edmunds, P. J. and Wall, C. B. (2014). Evidence that high pCO_2 affects protein metabolism in tropical reef corals. *Biol. Bull.* **227**, 68-77. doi:10.1086/BBLv227n1p68
Escobal, P. R. (1996). *Aquatic Systems Engineering: Devices and How they Function*, 2nd edn. Oxnard, CA: Dimension Engineering Press.
Fischer, E. M. and Knutti, R. (2015). Anthropogenic contribution to global occurrence of heavy-precipitation and high-temperature extremes. *Nat. Clim. Change* **5**, 560-564. doi:10.1038/nclimate2617
Galli, G. and Solidoro, C. (2018). ATP supply may contribute to light-enhanced calcification in corals more than abiotic mechanisms. *Front. Mar. Sci.* **5**, 68. doi:10.3389/fmars.2018.00068
Gates, R. D., Baghdasarian, G. and Muscatine, L. (1992). Temperature stress causes host cell detachment in symbiotic cnidarians: implications for coral bleaching. *Biol. Bull.* **182**, 324-332. doi:10.2307/1542252
Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M. and Charnov, E. L. (2001). Effects of size and temperature on metabolic rate. *Science* **293**, 2248-2251. doi:10.1126/science.1061967
Gorbunov, M. Y., Kolber, Z. S., Lesser, M. P. and Falkowski, P. G. (2001). Photosynthesis and photoprotection in symbiotic corals. *Limnol. Oceanogr.* **46**, 75-85. doi:10.4319/lo.2001.46.1.0075
Haywood, J. M., Bellouin, N., Jones, A., Boucher, O., Wild, M. and Shine, K. P. (2011). The roles of aerosol, water vapor and cloud in future global dimming/brightening. *J. Geophys. Res. Atmos.* **116**, D20203. doi:10.1029/2011JD016000
Hoegh-Guldberg, O. (1999). Climate change, coral bleaching and the future of the world's coral reefs. *Mar. Freshw. Res.* **50**, 839-866.
Hoegh-Guldberg, O. and Smith, G. J. (1989). The effect of sudden changes in temperature, light and salinity on the population density and export of zooxanthellae from the reef corals *Stylophora pistillata* Esper and *Seriatopora hysterix* Dana. *J. Exp. Mar. Biol. Ecol.* **129**, 279-303. doi:10.1016/0022-0981(89)90109-3
Hoegh-Guldberg, O., Cai, R., Poloczanska, E. S., Brewer, P. G., Sundby, S., Hilmi, K., Fabry, V. J. Jung, S. (2014). The Ocean. In *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel of Climate Change* (ed. V. R. Barros, C. B. Field, D. J. Dokken, M. D. Mastrandrea, K. J. Mach, T. E. Bilir, M. Chatterjee, K. L. Ebi, Y. O. Estrada and R. C. Genova et al.), pp. 1655-1731. Cambridge University Press.
Hoogenboom, M. O., Campbell, D. A., Beraud, E., DeZeeuw, K. and Ferrier-Pagès, C. (2012). Effects of light, food availability and temperature stress on the function of photosystem II and photosystem I of coral symbionts. *PLoS ONE* **7**, e30167. doi:10.1371/journal.pone.0030167
Iglesias-Prieto, R. (1995). The effects of elevated temperature on the photosynthetic responses of symbiotic dinoflagellates. In *Photosynthesis: from Light to Biosphere* (ed. P. Mathis), pp. 793-796. Netherlands: Kluwer Academic Publishers.
Iglesias-Prieto, R., Matta, J. L., Robins, W. A. and Trench, R. K. (1992). Photosynthetic response to elevated temperature in the symbiotic dinoflagellate *Symbiodinium microadriaticum* in culture. *Proc. Natl. Acad. Sci. USA* **89**, 10302-10305. doi:10.1073/pnas.89.21.10302
Iguchi, A., Ozaki, S., Nakamura, T., Inoue, M., Tanaka, Y., Suzuki, A., Kawahata, H. and Sakai, K. (2012). Effects of acidified seawater on coral calcification and symbiotic algae on the massive coral *Porites australiensis*. *Mar. Environ. Res.* **73**, 32-36. doi:10.1016/j.marenvres.2011.10.008
Jacobson, L. M., Edmunds, P. J., Muller, E. B. and Nisbet, R. M. (2016). The implications of reduced metabolic rate in resource-limited corals. *J. Exp. Biol.* **219**, 870-877. doi:10.1242/jeb.136044
Jeffrey, S. W. and Humphrey, G. F. (1975). New spectrophotometric equations for determining chlorophylls *a*, *b*, c_1, and c_2 in higher plants, algae, and natural phytoplankton. *Biochem. Physiol. Pflanz.* **167**, 191-194. doi:10.1016/S0015-3796(17)30778-3
Jokiel, P. L. (2011). Ocean acidification and control of reef coral calcification by boundary layer limitation of proton flux. *Bull. Mar. Sci.* **87**, 639-657. doi:10.5343/bms.2010.1107
Jokiel, P. L. and Coles, S. L. (1977). Effects of temperature on the mortality and growth of Hawaiian reef corals. *Mar. Biol.* **43**, 201-208. doi:10.1007/BF00402312
Kaniewska, P., Campbell, P. R., Kline, D. I., Rodriguez-Lanetty, M., Miller, D. J., Dove, S. and Hoegh-Guldberg, O. (2012). Major cellular and physiological impacts of ocean acidification on a reef building coral. *PLoS ONE* **7**, e34659. doi:10.1371/journal.pone.0034659
Kärcher, B. (2017). Cirrus clouds and their response to anthropogenic activities. *Curr. Clim. Change Rep.* **3**, 45-57. doi:10.1007/s40641-017-0060-3
Kleypas, J. A., Buddemeier, R. W., Archer, D., Gattuso, J.-P., Langdon, C. and Opdyke, B. N. (1999). Geochemical consequences of increased atmospheric carbon dioxide on coral reefs. *Science* **284**, 118-120. doi:10.1126/science.284.5411.118
Krämer, W. E., Schrameyer, V., Hill, R., Ralph, P. J. and Bischof, K. (2013). PSII activity and pigment dynamics of *Symbiodinium* in two Indo-Pacific corals exposed to short-term high-light stress. *Mar. Biol.* **160**, 563-577. doi:10.1007/s00227-012-2113-4
Kuznetsova, A., Brockhoff, P. B. and Christensen, R. H. B. (2017). lmerTest package: tests in linear mixed effects models. *J. Stat. Softw.* **82**, 1-26. doi:10.18637/jss.v082.i13
Liou, K.-N. (1986). Influence of cirrus clouds on weather and climate processes: a global perspective. *Mon. Weather Rev.* **114**, 1167-1199. doi:10.1175/1520-0493(1986)114<1167:IOCCOW>2.0.CO;2
Mackey, K., Morris, J. J., Morel, F. and Kranz, S. (2015). Response of photosynthesis to ocean acidification. *Oceanography* **25**, 74-91. doi:10.5670/oceanog.2015.33
Mason, R. A. B. (2018a). *Coral Responses to Temperature, Irradiance and Acidification Stress: Linking Physiology to Satellite Remote Sensing*. Brisbane: The University of Queensland. doi:10.14264/uql.2018.482
Mason, R. A. B. (2018b). Decline in symbiont densities of tropical and subtropical scleractinian corals under ocean acidification. *Coral Reefs* **37**, 945-953. doi:10.1007/s00338-018-1720-z
Mason, R. A. B., Wall, C. B., Cunning, R., Dove, S. G. and Gates, R. D. (2020). Data and code for: High light alongside elevated pCO_2 alleviates thermal depression of photosynthesis in a hard coral (*Pocillopora acuta*). *Zenodo*. doi:10.5281/zenodo.3526369
McCoy, D. T., Eastman, R., Hartmann, D. L. and Wood, R. (2017). The change in low cloud cover in a warmed climate inferred from AIRS, MODIS, and ERA-Interim. *J. Clim.* **30**, 3609-3620. doi:10.1175/JCLI-D-15-0734.1
McCulloch, M., Falter, J., Trotter, J. and Montagna, P. (2012). Coral resilience to ocean acidification and global warming through pH up-regulation. *Nat. Clim. Change* **2**, 623-627. doi:10.1038/nclimate1473
Meinshausen, M., Smith, S. J., Calvin, K., Daniel, J. S., Kainuma, M. L. T., Lamarque, J.-F., Matsumoto, K., Montzka, S. A., Raper, S. C. B., Riahi, K. et al. (2011). The RCP greenhouse gas concentrations and their extensions from 1765 to 2300. *Clim. Change* **109**, 213-241. doi:10.1007/s10584-011-0156-z
Miller, C. B. and Wheeler, P. A. (2012). *Biological Oceanography*, 2nd edn. Chichester: Wiley-Blackwell.
Muller, E. B., Kooijman, S. A. L. M., Edmunds, P. J., Doyle, F. J. and Nisbet, R. M. (2009). Dynamic energy budgets in syntrophic symbiotic relationships between heterotrophic hosts and photoautotrophic symbionts. *J. Theor. Biol.* **259**, 44-57. doi:10.1016/j.jtbi.2009.03.004
Nii, C. M. and Muscatine, L. (1997). Oxidative stress in the symbiotic sea anemone *Aiptasia pulchella* (Carlgren, 1943): Contribution of the animal to superoxide ion production at elevated temperature. *Biol. Bull.* **192**, 444-456. doi:10.2307/1542753
NOAA Coral Reef Watch (2019). *NOAA Coral Reef Watch 5km Virtual Station Time Series Data for the Hawaiian Islands*. College Park, MD: NOAA Coral Reef Watch. https://coralreefwatch.noaa.gov/product/vs/timeseries/polynesia.php (accessed 9 February 2019).
Noonan, S. H. C. and Fabricius, K. E. (2016). Ocean acidification affects productivity but not the severity of thermal bleaching in some tropical corals. *ICES J. Mar. Sci.* **73**, 715-726. doi:10.1093/icesjms/fsv127
Ogston, A. S. and Field, M. E. (2010). Predictions of turbidity due to enhanced sediment resuspension resulting from sea-level rise on a fringing coral reef: evidence from Molokai, Hawaii. *J. Coast. Res.* **26**, 1027-1037. doi:10.2112/JCOASTRES-D-09-00064.1
Parys, E. and Jastrzębski, H. (2006). Light-enhanced dark respiration in leaves, isolated cells and protoplasts of various types of C4 plants. *J. Plant Physiol.* **163**, 638-647. doi:10.1016/j.jplph.2005.05.009
Pochon, X., Stat, M., Takabayashi, M., Chasqui, L., Chauka, L. J., Logan, D. D. K. and Gates, R. D. (2010). Comparison of endosymbiotic and free-living *Symbiodinium* (Dinophyceae) diversity in a Hawaiian reef environment. *J. Phycol.* **46**, 53-65. doi:10.1111/j.1529-8817.2009.00797.x

Putnam, H. M., Mayfield, A. B., Fan, T. Y., Chen, C. S. and Gates, R. D. (2013). The physiological and molecular responses of larvae from the reef-building coral *Pocillopora damicornis* exposed to near-future increases in temperature and pCO_2. *Mar. Biol.* **160**, 2157-2173. doi:10.1007/s00227-012-2129-9

Putnam, H. M., Davidson, J. M. and Gates, R. D. (2016). Ocean acidification influences host DNA methylation and phenotypic plasticity in environmentally susceptible corals. *Evol. Appl.* **9**, 1165-1178. doi:10.1111/eva.12408

Reynaud, S., Leclercq, N., Romaine-Lioud, S., Ferrier-Pages, C., Jaubert, J. and Gattuso, J.-P. (2003). Interacting effects of CO_2 partial pressure and temperature on photosynthesis and calcification in a scleractinian coral. *Glob. Change Biol.* **9**, 1660-1668. doi:10.1046/j.1365-2486.2003.00678.x

Roberty, S., Bailleul, B., Berne, N., Franck, F. and Cardol, P. (2014). PSI Mehler reaction is the main alternative photosynthetic electron pathway in *Symbiodinium* sp., symbiotic dinoflagellates of cnidarians. *New Phytol.* **204**, 81-91. doi:10.1111/nph.12903

Rogers, C. S. (1979). The effect of shading on coral reef structure and function. *J. Exp. Mar. Biol. Ecol.* 41, 269-288. doi:10.1016/0022-0981(79)90136-9

Rosenfeld, D., Zhu, Y., Wang, M., Zheng, Y., Goren, T. and Yu, S. (2019). Aerosol-driven droplet concentrations dominate coverage and water of oceanic low-level clouds. *Science* **363**, eaav0566. doi:10.1126/science.aav0566

Rowan, R., Whitney, S. M., Fowler, A. and Yellowlees, D. (1996). Rubisco in marine symbiotic dinoflagellates: form ii enzymes in eukaryotic oxygenic phototrophs encoded by a nuclear multigene family. *Plant Cell* **8**, 539-553. doi:10.1105/tpc.8.3.539

Sato, Y. and Suzuki, K. (2019). How do aerosols affect cloudiness? *Science* **363**, 580-581. doi:10.1126/science.aaw3720

Schmidt-Roach, S., Miller, K. J., Lundgren, P. and Andreakis, N. (2014). With eyes wide open: a revision of species within and closely related to the *Pocillopora damicornis* species complex (Scleractinia; Pocilloporidae) using morphology and genetics. *Zool. J. Linn. Soc.* **170**, 1-33. doi:10.1111/zoj.12092

Schneider, T., Kaul, C. M. and Pressel, K. G. (2019). Possible climate transitions from breakup of stratocumulus decks under greenhouse warming. *Nat. Geosci.* **12**, 163-167. doi:10.1038/s41561-019-0310-1

Schrameyer, V., Wangpraseurt, D., Hill, R., Kühl, M., Larkum, A. W. D. and Ralph, P. J. (2014). Light respiratory processes and gross photosynthesis in two scleractinian corals. *PLOS ONE* **9**, e110814. doi:10.1371/journal.pone.0110814

Shamberger, K. E. F., Feely, R. A., Sabine, C. L., Atkinson, M. J., DeCarlo, E. H., Mackenzie, F. T., Drupp, P. S. and Butterfield, D. A. (2011). Calcification and organic production on a Hawaiian coral reef. *Mar. Chem.* **127**, 64-75. doi:10.1016/j.marchem.2011.08.003

Skirving, W. and Guinotte, J. (2000). The sea surface temperature story on the Great Barrier Reef during the coral bleaching event of 1998. In *Oceanographic Processes of Coral Reefs* (ed. E. Wolanski), pp. 301-313. CRC Press.

Stat, M., Morris, E. and Gates, R. D. (2008). Functional diversity in coral–dinoflagellate symbiosis. *Proc. Natl. Acad. Sci. USA* **105**, 9256-9261. doi:10.1073/pnas.0801328105

Stat, M., Yost, D. M. and Gates, R. D. (2015). Geographic structure and host specificity shape the community composition of symbiotic dinoflagellates in corals from the Northwestern Hawaiian Islands. *Coral Reefs* **34**, 1075-1086. doi:10.1007/s00338-015-1320-0

Stokes, D., Walker, D. A., Grof, C. P. L. and Seaton, G. G. R. (1990). Light enhanced dark respiration. In *Perspectives in Biochemical and Genetic Regulation of Photosynthesis* (ed. I. Zelitch), pp. 319-338. New York: Alan R Liss.

Suggett, D. J., Dong, L. F., Lawson, T., Lawrenz, E., Torres, L. and Smith, D. J. (2013). Light availability determines susceptibility of reef building corals to ocean acidification. *Coral Reefs* **32**, 327-337. doi:10.1007/s00338-012-0996-7

Sweet, M. J. and Brown, B. E. (2016). Coral responses to anthropogenic stress in the twenty-first century: an ecophysiological perspective. *Oceanogr. Mar. Biol. Annu. Rev.* *54*, 271-314.

Takahashi, S., Nakamura, T., Sakamizu, M., van Woesik, R. and Yamasaki, H. (2004). Repair machinery of symbiotic photosynthesis as the primary target of heat stress for reef-building corals. *Plant Cell Physiol.* **45**, 251-255. doi:10.1093/pcp/pch028

Tchernov, D., Kvitt, H., Haramaty, L., Bibby, T. S., Gorbunov, M. Y., Rosenfeld, H. and Falkowski, P. G. (2011). Apoptosis and the selective survival of host animals following thermal bleaching in zooxanthellate corals. *Proc. Natl. Acad. Sci. USA* **108**, 9905-9909. doi:10.1073/pnas.1106924108

Tremblay, P., Naumann, M. S., Sikorski, S., Grover, R. and Ferrier-Pagès, C. (2012). Experimental assessment of organic carbon fluxes in the scleractinian coral *Stylophora pistillata* during a thermal and photo stress event. *Mar. Ecol. Prog. Ser.* **453**, 63-77. doi:10.3354/meps09640

Vogel, N., Meyer, F. W., Wild, C. and Uthicke, S. (2015). Decreased light availability can amplify negative impacts of ocean acidification on calcifying coral reef organisms. *Mar. Ecol. Prog. Ser.* **521**, 49-61. doi:10.3354/meps11088

Vytopil, E. and Willis, B. (2001). Epifaunal community structure in *Acropora* spp. (Scleractinia) on the Great Barrier Reef: implications of coral morphology and habitat complexity. *Coral Reefs* **20**, 281-288. doi:10.1007/s003380100172

Wall, C. B., Fan, T.-Y. and Edmunds, P. J. (2014). Ocean acidification has no effect on thermal bleaching in the coral *Seriatopora caliendrum*. *Coral Reefs* **33**, 119-130. doi:10.1007/s00338-013-1085-2

Wall, C. B., Mason, R. A. B., Ellis, W. R., Cunning, R. and Gates, R. D. (2017). Elevated pCO_2 affects tissue biomass composition, but not calcification, in a reef coral under two light regimes. *R. Soc. Open Sci.* **4**, 170683. doi:10.1098/rsos.170683

Warner, M. E., Fitt, W. K. and Schmidt, G. W. (1996). The effects of elevated temperature on the photosynthetic efficiency of zooxanthellae *in hospite* from four different species of reef coral: a novel approach. *Plant Cell Environ.* **19**, 291-299. doi:10.1111/j.1365-3040.1996.tb00251.x

Warner, M. E., Fitt, W. K. and Schmidt, G. W. (1999). Damage to photosystem II in symbiotic dinoflagellates: a determinant of coral bleaching. *Proc. Natl. Acad. Sci. USA* **96**, 8007-8012. doi:10.1073/pnas.96.14.8007

Weis, V. M. (2008). Cellular mechanisms of cnidarian bleaching: stress causes the collapse of symbiosis. *J. Exp. Biol.* **211**, 3059-3066. doi:10.1242/jeb.009597

Wooldridge, S. A. (2009). A new conceptual model for the warm-water breakdown of the coral–algae endosymbiosis. *Mar. Freshw. Res.* **60**, 483-496. doi:10.1071/MF08251

Yakovleva, I. and Hidaka, M. (2004). Different effects of high temperature acclimation on bleaching-susceptible and tolerant corals. *Symbiosis* **37**, 87-105.

Zunino, S., Canu, D. M., Bandelj, V. and Solidoro, C. (2017). Effects of ocean acidification on benthic organisms in the Mediterranean Sea under realistic climatic scenarios: a meta-analysis. *Reg. Stud. Mar. Sci.* **10**, 86-96. doi:10.1016/j.rsma.2016.12.011