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REPORT

Impacts of coral bleaching on pH and oxygen gradients across the coral concentration boundary layer: a microsensor study

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Abstract Reef-building corals are surrounded by complex microenvironments (i.e. concentration boundary layers) that partially isolate them from the ambient seawater. Although the presence of such concentration boundary layers (CBLs) could potentially play a role in mitigating the negative impacts of climate change stressors, their role is poorly understood. Furthermore, it is largely unknown how heat stress-induced bleaching affects O_2 and pH dynamics across the CBLs of coral, particularly in branching species. We experimentally exposed the common coral species *Acropora aspera* to heat stress for 13 d and conducted a range of physiological and daytime microsensor measurements to determine the effects of bleaching on O_2 and pH gradients across the CBL. Heat stress equivalent to 24 degree heating days (3.4 degree heating weeks) resulted in visible bleaching and significant declines in photochemical efficiency, photosynthesis rates and photosynthesis to respiration (*P*/*R*) ratios, whereas dark respiration and calcification rates were not impacted. As a consequence, bleached *A. aspera* had significantly lower (− 13%) surface O_2 concentrations during the day than healthy corals, with concentrations being lower than that of the ambient seawater, thus resulting in O_2 uptake from the seawater. Furthermore, we show here that *Acropora*, and potentially branching corals in general, have among the lowest surface pH elevation of all corals studied to date (0.041 units), which could contribute to their higher sensitivity to ocean acidification. Additionally, bleached *A. aspera* no longer elevated their surface pH above ambient seawater values and, therefore, had essentially no $[H^+]$ CBL. These findings demonstrate that heat stress-induced bleaching has negative effects on pH elevation and $[H^+]$ CBL thickness, which may increase the overall susceptibility of coral to the combined impacts of ocean acidification and warming.

Keywords *Acropora aspera* · Metabolism · Calcification · Diffusive oxygen flux · Concentration gradients · Heat stress

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Introduction

Coral reefs are in serious decline worldwide and increasingly threatened by rising atmospheric CO_2 concentrations and anthropogenic climate change (Hoegh-Guldberg et al. 2007). By the end of this century, ocean surface temperatures are expected to increase by 2.0 °C under the RCP 8.5 scenario, while ocean surface pH is predicted to decrease 0.3–0.4 units under the same scenario (IPCC 2013),

resulting in recurrent mass bleaching events and ocean acidification (Pandolfi et al. 2003; Hoegh-Guldberg et al. 2007; Hughes et al. 2017, 2018).

Over the past decades, research has identified a number of physiological mechanisms that enable reef-building corals to increase tolerance to warming and acidifying oceans, including heterotrophic plasticity (Grottoli et al. 2006; Towle et al. 2015), high levels of energy reserves (Rodrigues and Grottoli 2007; Schoepf et al. 2013, 2015b) and biological control over the chemistry of the calcifying fluid (McCulloch et al. 2012; Venn et al. 2013; Cai et al. 2016; Schoepf et al. 2017). Another aspect that has received much less attention in potentially enabling resistance to climate change stressors is the presence of a discrete concentration boundary layer (CBL) at the surface of many aquatic organisms (Hurd et al. 2011), including corals (Shashar et al. 1993; Kühl et al. 1995), which partially isolates them from the overlying ambient seawater. The differential velocity of seawater at the surface of the organism determines the potential thickness of the overlying diffusion boundary layer (DBL), while organism morphology and size determine whether this potential is achieved (Kühl et al. 1995; de Beer et al. 2000; Jimenez et al. 2011). Furthermore, due to the partial isolation of the DBL within the surrounding body of water, large concentration gradients of metabolites can form between the surface of an organism and the ambient seawater due to the organism's metabolism. In the context of ocean acidification (OA), for example, this means that marine calcifying organisms may be able to mitigate negative effects of OA on calcification by maintaining elevated daytime or overall higher pH in their CBL (Cornwall et al. 2014). Indeed, coralline algae were more resistant to OA when surface pH increased due to photosynthetic activity and thickening of the CBL via decreased flow speed (Cornwall et al. 2013, 2014). Furthermore, slow-flow CBL microenvironments may constitute a refuge from OA for calcifying organisms living on the surface of kelp blades (Noisette and Hurd 2018). Similarly, it was recently shown for corals that lower flow speeds and thicker CBLs increased surface daytime pH under ambient pH and OA conditions (Chan et al. 2016). However, our knowledge of how the coral CBL will be affected by various climate change stressors is currently very limited, and it is thus unclear whether CBL thickness could influence coral susceptibility to these stressors.

Healthy corals are surrounded by complex microenvironments whose chemical characteristics are strongly influenced by the organism's metabolism and display extreme diel fluctuations. Symbiont photosynthesis results in hyperoxia within the CBL during the day (Shashar et al. 1993; Kühl et al. 1995; de Beer et al. 2000; Al-Horani 2005; Wangpraseurt et al. 2012), accompanied by higher surface pH compared to ambient seawater due to the photosynthetic drawdown of CO_2 (Kühl et al. 1995; de Beer et al. 2000; Al-Horani et al. 2003a, b). This pattern is reversed at night, with hypoxia and low surface pH occurring within the CBL due to respiration (Shashar et al. 1993; Kühl et al. 1995; de Beer et al. 2000; Al-Horani et al. 2003a, b). Both oxygen concentrations and pH at the surface typically increase with increasing light due to enhanced metabolic activity (de Beer et al. 2000) and decrease as seawater velocity increases due to a thinning of the CBL (Kühl et al. 1995; de Beer et al. 2000; Jimenez et al. 2011; Chan et al. 2016).

Predictions of how corals will respond to future ocean change are complicated by the fact that they are based upon changes in the ambient seawater, rather than the corals' various microenvironments, such as the CBL or calcifying fluid, where important physiological processes occur. Given that these microenvironments differ substantially from the chemistry of the surrounding seawater under present-day and predicted future conditions (Kühl et al. 1995; de Beer et al. 2000; Agostini et al. 2013; Chan et al. 2016) and likely even influence one another, predictions for coral responses to future ocean change based on changes in the ambient seawater may not be applicable. This is particularly concerning in the light of emerging evidence that CBL dynamics can be linked to differential environmental tolerance in calcifying marine organisms (Cornwall et al. 2013, 2014). To date, only few studies have assessed how temperature, heat shock and/or OA impact the oxygen and pH microenvironment of corals (Al-Horani 2005; Agostini et al. 2013; Chan et al. 2016), demonstrating significant knowledge gaps in our understanding of the effects of climate change stressors on the CBL in corals. Critically, the impact of gradual heat stress (such as during natural bleaching events as compared to heat shock experiments) on CBL dynamics is currently unknown. This is because the only two studies examining temperature effects effectively heat-shocked their corals by exposing them to stressful temperatures only for the duration of the microsensor profiles or for 1 d, without gradual temperature acclimation (Al-Horani 2005; Agostini et al. 2013). As marine heatwaves and coral bleaching events become increasingly common (Hughes et al. 2017), this knowledge is urgently needed to identify mechanisms that may promote tolerance to climate change stressors. Additionally, it remains poorly understood how the CBL of branching corals such as the important framework-building genus *Acropora* will be affected by these stressors due to a preference for massive coral species in many microsensor studies (e.g. de Beer et al. 2000; Al-Horani et al. 2003b; Al-Horani 2005; Agostini et al. 2013). This preference has strongly biased our understanding of CBL dynamics towards the physiology of massive corals, which is

problematic in the context of climate change due to the typically higher heat resistance of massive corals (e.g. Baird and Marshall 2002).

To address these knowledge gaps, we exposed the common branching coral *Acropora aspera* to 13 d of heat stress (~ 1.5 °C above maximum monthly mean temperatures) in a laboratory experiment to induce coral bleaching. To prevent heat shock, the laboratory experiment was conducted in late summer when corals were seasonally acclimated to high temperatures and heat stress episodes are most likely to occur naturally. Oxygen and pH profiles were then conducted across the CBL to assess the impact of coral bleaching on the near-surface microenvironment of the coral. A range of other variables including whole-fragment photosynthesis, respiration, calcification rate and photochemical efficiency were measured to provide a physiological context for the interpretation of the oxygen and pH microsensor data.

Materials and methods

Six coral colonies of *A. aspera* were collected in April 2016 from the intertidal at Shell Island, Cygnet Bay, in the Kimberley region in north-western Australia (see Schoepf et al. 2015a for a detailed site description). Colonies were collected at least 10 m apart to increase the likelihood of selecting different genotypes. They were live-shipped to the University of Western Australia and maintained in indoor, flow-through aquaria at the Watermans Bay seawater facility. In July 2016, colonies were fragmented and glued onto plastic tiles. For the 10-month period prior to the start of the heat stress experiment in mid-May 2017, corals were maintained at seasonal Kimberley temperatures, with temperatures being adjusted every 2 weeks.

Mesocosm tank setup

Coral fragments (~ 10 cm) were maintained in 55-L transparent plastic tanks where seawater was being replaced at a rate of 0.5 L min^{-1}. Water motion was provided using a submersible pump (Macro Aqua, 3000 L h^{-1}) connected to a flow controller set at the highest speed. Temperature was maintained using titanium heaters (WeiPro, 500 or 1000 W) and controlled via ApexFusion software (Neptune Systems). The Apex temperature probes were calibrated 1–2 times a week using a high-precision thermometer (Fisher Scientific Traceable). Light was provided on a 12:12-h light:dark cycle (06:00–18:00 h) using 150 W LED lights (Ledzeal S150 Plus) with custom-designed LED arrangements and colours to ensure a light spectrum similar to shallow tropical reef environments. The lights were programmed to follow a natural diurnal light cycle, with gradual increases up to 560 µmol m^{-2} s^{-1} at noon (measured using an Apogee MQ-200 cosine-corrected planar PAR-meter). Relatively high maximum light levels were chosen because intertidal Kimberley corals regularly experience high light levels depending on tidal elevation, water clarity and cloud cover (Dandan et al. 2015). The incoming ambient seawater was pumped directly from 150 m offshore the Waterman's facility at ~ 12 m water depth and subsequently filtered through three sand filters (~ 20 µm nominal size). Corals were fed twice a week with live brine shrimp. HOBO v2 temperature loggers were deployed in each tank and continuously recorded seawater temperature every 5 min.

Heat stress experiment

In May 2017, tanks were assigned to either control or heat stress conditions ($n = 2$ replicate tanks per treatment). Starting on 2 May, temperature in the heat stress tanks was gradually ramped up over the course of 2 weeks at a rate of 1 °C per week to reach the bleaching threshold of ~ 32 °C (Schoepf et al. 2015a). These temperatures were sustained for 6 d and then elevated by 0.5 °C to increase thermal stress for a further 7 d (13 d of total heat stress), resulting in an average temperature of 32.32 °C (± 0.07 SE, $n = 13$, Fig. 1). Control tanks were maintained at 29.91 °C (± 0.06 SE, $n = 13$, Fig. 1), which is ~ 1 °C below the maximum monthly mean (MMM) temperatures at the collection site (Schoepf et al. 2015a). The heat stress experiment ended on 30 May 2017, and temperature in the heat stress tanks was returned to control temperature (i.e. ~ 29.9 °C). To quantify heat stress, daily average temperatures were used to calculate degree heating days (DHD) for the heat stress treatment (Maynard et al. 2008). Instead of long-term mean

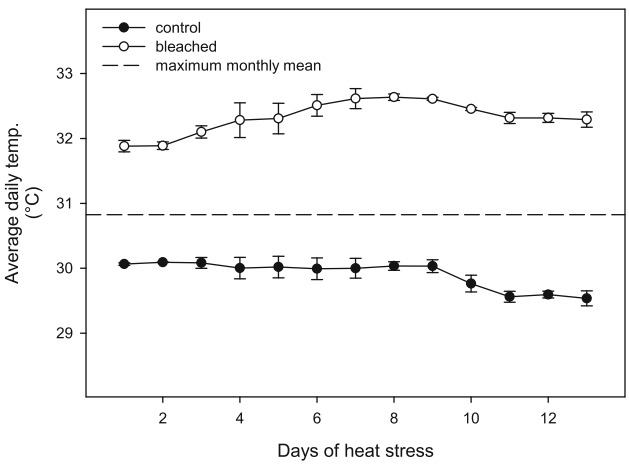

Fig. 1 Daily average temperatures in both control and heat stress (= bleached) tanks throughout the heat stress experiment. Mean ± 1 SE are shown. The dashed line indicates the maximum monthly mean (MMM) temperature at the collection site

summer temperatures, a MMM value of 30.827 °C (Schoepf et al. 2015a; NOAA 2016) was used to calculate DHD.

Physiological analyses

Photo-physiology

Maximum quantum yield (Fv/Fm) of chlorophyll a fluorescence in each coral fragment was measured on the last day of the heat stress experiment 1 h after simulated sunset to assess the photochemical efficiency in the dark-adapted state. Measurements were repeated 10 d after heat stress had ended to quantify whether significant recovery had occurred over the 10 d during which physiological measurements and microsensor profiles were conducted. All photochemical measurements were made using a Diving-PAM underwater fluorometer (Walz, Germany) with the following settings: measuring light intensity = 3, saturation pulse intensity = 12, saturation pulse width = 0.8 s, gain = 5 and damping = 2. Measurements were made at a constant distance of 2 mm from the coral tissue, approx. 2–3 cm below the tip.

Health score

Coral health was determined on the upper surface of the branches using the CoralWatch Coral Health Chart (Siebeck et al. 2006) at the end of the 13 d heat stress experiment.

Photosynthesis and respiration

Whole-fragment photosynthesis (P) and respiration (R) rates were determined over 10 d immediately following the heat stress experiment. At this time, both control and heat stress tanks were maintained at the same temperature (29.7 °C) to avoid confounding effects of temperature on P and R rates. Heat-stressed corals were incubated prior to control corals to minimise recovery effects. P and R rates were determined via oxygen production and consumption, respectively, by incubating corals in sealed, clear plastic chambers (1.75 L, Décor tellfresh). Chambers were placed in a water bath with temperature control to maintain constant temperatures. Turbulent water motion inside the chambers was achieved by placing the chambers on a submersible magnetic stirring plate (2Mag MIXdrive 6, John Morris Scientific, stir bar speed of 500 rpm). For control incubations, a clean coral skeleton on a tile (similar to the one used for the experimental corals) was placed inside the chamber. For light incubations, corals were exposed to constant light intensity of 560 µmol m^{-2} s^{-1} to match maximum light intensities in the culture tanks. Light incubations were conducted between ~ 10:00 and 14:00 h, whereas dark incubations were conducted between ~ 08:00 and 10:00 h and ~ 16:00–18:00 h. Corals were either dark-adapted overnight or for 1.5 h prior to the dark incubations (from ~ 14:30 to 16:00 h). Incubation duration varied from 50 min to 2 h depending on the size of the coral fragment to achieve a ~ 15% change in O_2 saturation. At the beginning and end of each incubation, oxygen (Orion Star A323 RDO/DO meter, Thermo Scientific), salinity (YSI 85), pH and temperature (Schott handylab pH12) were measured. Hourly oxygen data were converted from % O_2 saturation to µmol L^{-1} seawater using the equations of Garcia and Gordon (1992) and normalised to surface area (see below).

Calcification

Whole-fragment area-normalised calcification rates were determined using the buoyant weight technique (Jokiel et al. 1978) at the beginning and end of the 13 d heat stress experiment. Surface area was calculated using an established relationship between dry weight and surface area determined for the same coral species from the same location (Dandan et al. 2015).

Microsensor measurements

Microsensor measurements were made over 10 d immediately following the heat stress experiment on the same coral fragments that were used for physiological analyses. Microsensor profiles and P and R rates were measured on different days for a respective coral fragment to avoid excessive stress from manipulations. Profiles were conducted in a unidirectional, recirculating flume ($L \times W \times D$ = 180 × 20.5 × 21 cm, 18.5 cm water depth), with seawater velocity being controlled via a pump (Macro Aqua, 3000 L h^{-1}, connected to a flow controller) located at the end of the flume. Flow speed was ~ 3 cm s^{-1}. Flow straighteners (made of 5 × 1.2 cm long poly pipe pieces glued together with one layer of shade cloth) were placed 34 cm downstream of the flume entrance. Individual corals were placed in the centre of the working section of the flume during profiling and positioned in a way that ensured full exposure of the location on the coral where profiling was conducted to ambient water velocity. The addition of fresh seawater and aeration was temporarily discontinued during profiling to avoid interference with air bubbles and temperature fluctuations; however, the pump continued to operate to provide unidirectional water motion at a velocity of ~ 3 cm s^{-1}. Water temperature and light intensity were maintained constant at 29.63 °C (mean, ± 0.06 SE, n = 18) and ~ 600 µmol m^{-2} s^{-1}, respectively. Flume temperature was measured using a high-precision

thermometer at the beginning and end of a profile, or using a HOBO v2 temperature logger (logging interval every 30 s). Salinity of the incoming seawater was measured using a YSI 85 (average 36.8 ppt ± 0.03SE, n = 29). pH_T of the incoming seawater was determined using a pH meter (Schott handylab pH12) calibrated using TRIS buffer (Dickson et al. 2007) at three different temperatures. Average seawater pH_T was 8.03 (± 0.01 SE, n = 9), and this value was used to standardise pH microelectrode measurements (thus converting them from the NBS to the total scale). Oxygen concentrations of the ambient seawater in the flume were determined using the oxygen microsensors and converted to µmol L^{-1} using the equations of Garcia and Gordon (1992) (average 222 µmol L^{-1} ± 2.09 SE, n = 11).

A Unisense Microprofiling System (Unisense A/S, Denmark) was used to conduct separate pH and oxygen profiles. Oxygen concentrations were measured using an OX-25 Clark-type microsensor with a 20–30 µm tip diameter (< 2% stirring sensitivity, 90% response time < 4 s). Oxygen microsensors were calibrated with air-saturated seawater (100% O_2) and anoxic seawater prepared using sodium sulphite (0% O_2). pH was measured with a pH-50 microelectrode with a 40–60 µm tip diameter (90% response time < 10 s from pH 7 to pH 4) and an external reference electrode (Radiometer Analytical). pH microelectrodes were calibrated using NBS buffers 7 and 10. Only one profile per coral fragment was conducted. Sample size for pH profiles was 4, whereas it was 5 and 6 for oxygen profiles in bleached and healthy corals, respectively. Profiles were only conducted in the light due to logistical constraints imposed by the time required to conduct each profile and the necessity to complete all profiles within only a few days to minimise differences in recovery time after heat stress.

Microsensors were positioned between polyps (~ 1–2 mm apart) at the surface of the coral branches (referred to as 0 µm) growing at ~ 45° angle using the Unisense manual micro-manipulator and a hand-held magnifying glass. A 45-min acclimation period (after which pH and O_2 concentration at the surface of the coral had stabilised) occurred prior to starting vertical profiles. This was done to ensure that the CBL was in a stable state. Measurements of pH and O_2 were then taken every 100 µm above the coral surface up to 2000 µm, with a final measurement at 3000 µm. At each step, measurements were performed for 2 min so that the entire profile, excluding acclimation period, lasted for 44 min. Photographs were taken during each profile to ensure that pH and O_2 profiles were conducted on the same spot of each colony.

Concentration boundary layer (CBL) thickness was determined for each pH and O_2 profile, defined as the height above the surface of the coral at which the concentration of H^+ and O_2 was > 99% of the ambient seawater value (Hurd et al. 2011; Cornwall et al. 2013). In order to calculate this, O_2 concentrations and pH were standardised to ambient values to account for small variations among replicates (< 5% variation in concentrations). pH was standardised to H^+ concentration, as it is a logarithmic scale, using the conversion of $[H^+] = 10^{-pH}$. $[H^+]$ and $[O_2]$ were then standardised by dividing the concentration at any given profile location by the mean of the ambient seawater concentration (Hurd et al. 2011; Cornwall et al. 2013), then multiplying it by the mean pH or O_2 concentration of the ambient seawater for all profiles. This method was used instead of the linear method from Jorgensen and Revsbech (1985) because the linear fits overestimate CBL thickness for pH, particularly in organisms with morphologically complex surfaces (Cornwall et al. 2013).

The diffusive O_2 flux (J_{obs}) through the CBL was calculated according to Fick's first law of one-dimensional diffusion using the following equation from Nishihara and Ackerman (2007):

$$J_{obs} = -D\frac{C_b - C_s}{z} \quad (1)$$

with the O_2 diffusion coefficient D = 2.4732 and 2.5419 × 10^{-5} cm^2 s^{-1} (at 29.0 and 30.0 °C and a salinity of 37 ppt, respectively), O_2 concentrations in the ambient seawater (C_b, µmol cm^{-3}) and at the coral surface (C_s, µmol cm^{-3}), and the CBL thickness (z, cm). O_2 flux was then multiplied by 3600 to convert from µmol cm^2 s^{-1} to µmol cm^2 h^{-1} to facilitate comparison with literature values. When a CBL could not be detected, O_2 flux was set to 0 as well.

Statistical analyses

Since several response variables did not meet the assumptions of normality and homogeneity of variance, non-parametric one-way analysis of variance on ranks (Kruskal–Wallis test) was used to test for the effects of heat stress (2 levels: control, heat stress) on Fv/Fm, coral health score, photosynthesis, respiration, P/R ratios, calcification rate, pH and O_2 at the coral surface, thickness of the $[H^+]$ and O_2 CBL, diffusive O_2 flux and Fv/Fm at the end of the 10-d recovery period. Analyses were performed using SAS software, version 9.3 of the SAS System for Windows.

Model II linear regression (Legendre and Legendre 1998) was used to assess the relationship between net photosynthesis rates obtained using incubations and O_2 flux across the CBL measured using microsensors because both variables represented random variables and were measured with error. In such cases, model I regression using least squares underestimates the slope of the linear relationship

(Legendre and Legendre 1998). Ranged major axis (RMA) regression was used since variables were measured in different units. Regressions were computed using the "lmodel2" package with 99 permutations in R software (version 3.4.1). P values < 0.05 were considered significant.

Results

Heat stress and coral physiology

Control corals remained visibly healthy throughout the experiment (average health score of 4.5 ± 0.2 SE; Fig. 2b), whereas exposure to 23.6 DHD (3.4 degree heating weeks; including the temperature ramp-up phase) resulted in bleached, heat-stressed corals that were pale to white in colour at the end of the 13 d heat stress experiment (average health score of 1.7 ± 0.2 SE; Fig. 2b). However, no coral mortality occurred. Heat-stressed corals had significantly lower values than control corals in a range of response variables (Table 1): Fv/Fm was 26% lower (Fig. 2a), health scores were 62% lower (Fig. 2b), net photosynthesis rates were 62% lower (Fig. 2c) and *P/R* ratios were 66% lower (Fig. 2e). In contrast, dark respiration and calcification rates did not differ significantly between control and heat-stressed corals (Fig. 2d, f, Table 1).

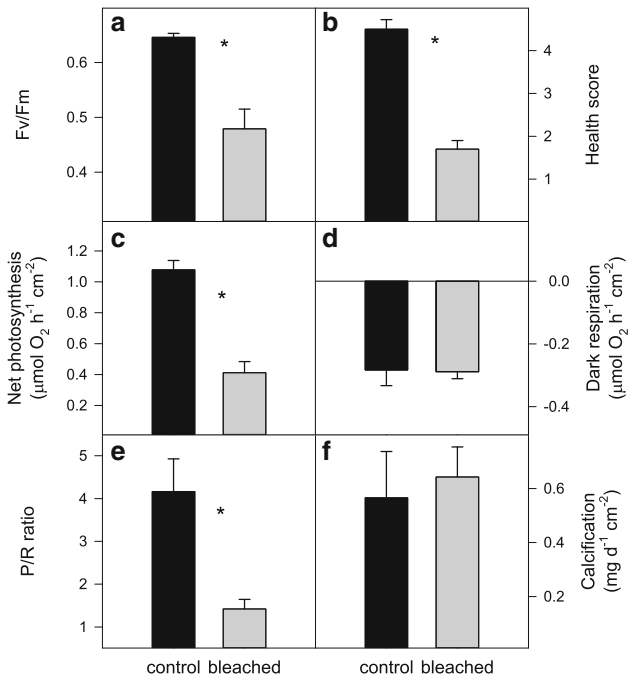

Fig. 2 **a** Photochemical efficiency (Fv/Fm), **b** coral health chart score, **c** net photosynthesis, **d** dark respiration, **e** photosynthesis/respiration ratio (*P/R* ratio) and **f** calcification rate in control and bleached *Acropora aspera* after 13 d of heat stress. Mean ± 1 SE are shown. Asterisks indicate significant differences between control and bleached corals (Table 1)

Table 1 Results from non-parametric Kruskal–Wallis one-way analysis of variance to assess the effect of heat stress on photochemical efficiency (Fv/Fm), coral health chart score, net photosynthesis (P), dark respiration (R), P/R ratio, calcification rate, O_2 concentration and pH_T at the coral surface, O_2 concentration boundary layer (CBL) thickness, $[H^+]$ CBL thickness, diffusive O_2 flux across the CBL and Fv/Fm by the end of the 10-day recovery period in *Acropora aspera*

Response variable	Degrees of freedom	X^2-statistic	p value
Fv/Fm	1	7.5000	**0.0062**
Health score	1	7.9327	**0.0049**
Net photosynthesis	1	5.3333	**0.0209**
Dark respiration	1	0.3333	0.5637
P/R ratio	1	5.3333	**0.0209**
Calcification	1	1.6333	0.2012
O_2 conc. surface	1	5.6333	**0.0176**
pH_T surface	1	3.0000	0.0833
O_2 CBL thickness	1	1.9276	0.1650
$[H^+]$ CBL thickness	1	2.2152	0.1367
Diffusive O_2 flux	1	5.6850	**0.0171**
Fv/Fm recovery	1	4.8109	**0.0283**

P values < 0.05 are highlighted in bold

Microsensor measurements

O_2 concentrations at the coral surface were highly variable but were nevertheless on average supersaturated with respect to ambient seawater concentrations in healthy corals (249 µmol L^{-1} ± 11 SE), and under-saturated in heat-stressed corals (216 µmol L^{-1} ± 3 SE) (Fig. 3a). Thus, heat-stressed corals had significantly lower (− 13%) O_2 concentrations at the coral surface than control corals (Fig. 4a, Table 1). The O_2 CBL thickness was 49% lower in heat-stressed (220 µm ± 132 SE) versus control corals (433 µm ± 102 SE) (Fig. 4b); however, this difference was not statistically significant because of high variability between replicates (Table 1).

Control corals maintained pH_T values at their surface that were highly variable but on average elevated (0.041 units ± 0.02 SE) above ambient seawater pH_T, whereas heat-stressed corals barely elevated surface pH_T above seawater values (i.e. less than the 0.02 unit error of the electrodes (Dickson et al. 2007); Fig. 3b). The difference in surface pH_T between heat-stressed and control corals was, however, not statistically significant (Fig. 4c, Table 1). Control corals had an average $[H^+]$ CBL thickness of 874 µm ± 411 SE, whereas CBL thickness was only 50 µm ± 29 SE in heat-stressed corals (Fig. 4d). Despite being 94% lower than in control corals, the difference in

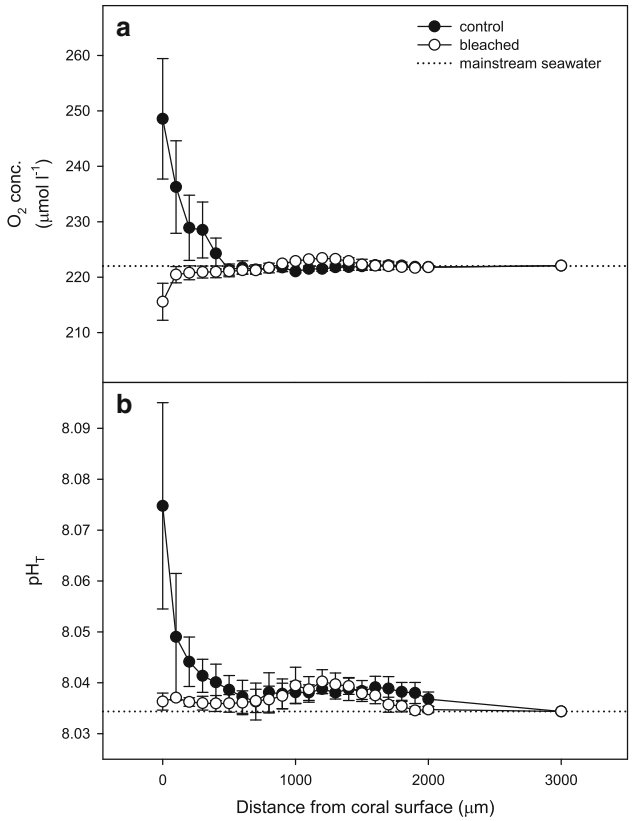

Fig. 3 Standardised **a** oxygen concentration and **b** pH_T at the coral surface (= 0 µm) across the concentration boundary layer and overlying seawater in control and bleached *Acropora aspera* after 13 d of heat stress. Mean ± 1 SE are shown

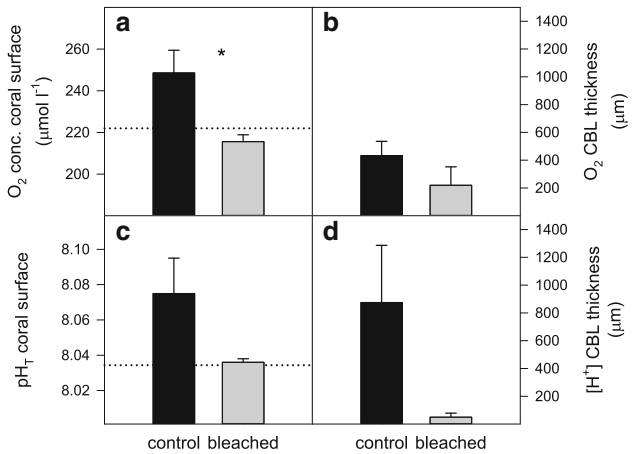

Fig. 4 **a** Standardised oxygen concentration at the coral surface, **b** thickness of the oxygen concentration boundary layer (CBL), **c** standardised pH_T at the coral surface and **d** thickness of the $[H^+]$ CBL in control and bleached *Acropora aspera* after 13 d of heat stress. Mean ± 1 SE are shown. Asterisks indicate significant differences between control and bleached corals (Table 1). Dotted lines indicate values for ambient seawater

$[H^+]$ CBL thickness between heat-stressed and control corals was not statistically significant due to high variability between different coral replicates (Table 1).

The diffusive O_2 flux across the CBL was positive (indicating O_2 production at the coral surface) in healthy corals (0.058 µmol cm^{-2} h^{-1} ± 0.02 SE), and negative (indicating O_2 uptake at the coral surface) in heat-stressed corals (− 0.038 µmol cm^{-2} h^{-1} ± 0.03 SE) (Fig. 5a). Thus, heat-stressed corals had significantly lower (− 166%) O_2 fluxes across the CBL than control corals (Table 1). Ranged major axis regression analysis showed that O_2 flux across the CBL was ∼ 84% lower than, but strongly correlated with, net photosynthesis rates ($y = 0.16x - 0.13$, $R^2 = 0.57$) (Fig. 5b).

Recovery

After 10 d of recovery (i.e. by the time all microsensor profiles were completed), heat-stressed corals had slightly increased Fv/Fm values compared to immediately after the heat stress experiment (Figs. 2a, 6); however, they still had significantly lower Fv/Fm (− 10%) than the control corals (Fig. 6, Table 1) and remained visibly bleached (V. Schoepf, personal observation).

Discussion

To our knowledge, this is the first study to assess how gradual exposure to heat stress impacts O_2 and pH dynamics across the coral concentration boundary layer, simulating conditions during natural bleaching events. Critically, this is also the first study to assess this in a branching coral species, despite the importance of the genus *Acropora* and other branching corals for reef-building.

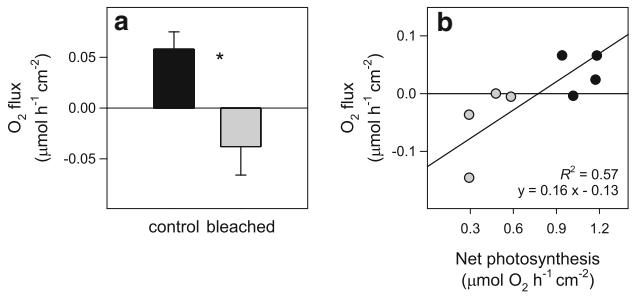

Fig. 5 **a** Diffusive oxygen flux across the concentration boundary layer and **b** relationship between net photosynthesis rates and diffusive oxygen flux in control and bleached *Acropora aspera* after 13 d of heat stress. In panel **a**, mean ± 1 SE are shown, and the asterisk indicates significant differences between control and bleached corals (Table 1). Positive fluxes indicate O_2 production at the coral surface, whereas negative fluxes indicate O_2 uptake. In panel **b**, the solid line represents the ranged major axis regression line

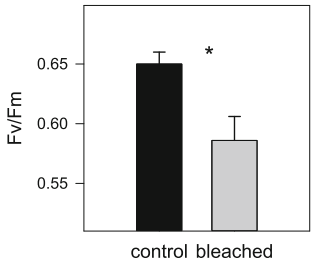

Fig. 6 Photochemical efficiency (Fv/Fm) in control and bleached *Acropora aspera* after 10 d of recovery. Mean ± 1 SE are shown. Asterisks indicate significant differences between control and bleached corals (Table 1)

Impacts of heat stress on the oxygen microenvironment

Our results show that bleached *A. aspera* corals had significantly lower surface O_2 concentrations than healthy corals during the daytime. Healthy corals maintained O_2 concentrations supersaturated with respect to ambient seawater, whereas bleached corals had O_2 concentrations that were lower than ambient seawater (Figs. 3a, 4a). These findings were a direct result of heat stress-induced declines in coral health and photosynthesis rates, yet unchanged respiration rates (Fig. 2a–d). Although *P*/*R* ratios remained just above 1 (Fig. 2e), negative O_2 fluxes at the coral surface (Fig. 5a) indicate that bleached corals were relying on O_2 uptake from the ambient seawater.

Our study further reports the first estimates of O_2 CBL thickness in bleached corals and demonstrates that heat stress may substantially reduce O_2 CBL thickness, although the difference of 49% was not statistically significant due to high variability among genotypes (Fig. 4b). Reduced O_2 CBL thickness could facilitate O_2 uptake from the ambient seawater into the bleached coral tissue, but it is currently poorly understood whether this would be beneficial for the coral. The reduced surface O_2 concentrations observed in bleached compared to healthy corals (Fig. 4a) suggest tissue O_2 concentrations are also reduced compared to ambient seawater during the day. This could lead to less oxidative stress, given that the production of reactive oxygen species (ROS) is directly related to the degree of hyperoxia (Jamieson et al. 1986) and is already amplified in bleached corals due to the light-enhancing properties of coral skeletons (Enríquez et al. 2005). By facilitating O_2 uptake from the ambient seawater into the coral tissue (Fig. 5a), a thinner O_2 CBL could then negate the reduction in the internal O_2 concentration. However, given that surface O_2 concentrations in bleached corals were not dramatically lower than ambient seawater, it remains unclear whether increased diffusive O_2 flux into the tissue due to a thinner O_2 CBL would be large enough to significantly influence tissue O_2 concentrations and ROS production.

Our findings are generally in good agreement with the only other study to date that has assessed the effects of stressful temperatures on O_2 dynamics across the CBL (Al-Horani 2005), even though their study used heat-shocked rather than bleached corals. Another difference between our study and Al-Horani (2005) is that at heat stress levels similar to those employed here (MMM + 1.5 °C), surface O_2 concentrations of the massive coral *Galaxea* still remained supersaturated with respect to ambient seawater, although they were nevertheless significantly lower than at average annual temperatures. This contrasting result could be attributed to differences in heat exposure, as corals in our study were subjected to heat stress for 13 d rather than just the duration of the microsensor profile. Our findings of significantly reduced O_2 fluxes in heat-stressed corals are also in broad agreement with two other studies (Al-Horani 2005; Agostini et al. 2013), although the temperature threshold at which O_2 fluxes become negative seems to vary between species and with bleaching severity. Given that both of these studies used massive corals, this could also reflect the typically higher heat tolerance of massive compared to branching corals (e.g. Baird and Marshall 2002), highlighting the need to conduct more microsensor work on branching corals.

It is difficult to compare our estimates of the O_2 CBL thickness in bleached corals to other studies since the only two studies assessing stressful temperature effects on coral O_2 microenvironments (Al-Horani 2005; Agostini et al. 2013) did not report the effects on O_2 CBL thickness. However, it is likely that the O_2 CBL thickness in these studies was also reduced, given that surface O_2 concentrations and diffusive O_2 fluxes significantly declined under stressful high temperatures (Al-Horani 2005; Agostini et al. 2013). For the healthy *A. aspera* corals in our study, an average O_2 CBL thickness of 433 µm (± 102 SE) is within the range reported for other coral species at incubation conditions similar to our study, e.g. ~ 430 µm in massive *Favites* and ~ 200 µm in branching *Pocillopora damicornis* at flow speeds of ~ 2 cm s^{-1} and high light levels of 800 µmol m^{-2} s^{-1} (Chan et al. 2016). Healthy massive *Galaxea* corals similarly had an average O_2 CBL thickness of 370 µm, but this was obtained under lower light levels (200 µmol m^{-2} s^{-1}) and higher flow speed (~ 5 cm s^{-1}) (Agostini et al. 2013).

We demonstrate here that diffusive O_2 fluxes obtained from microsensor spot measurements may potentially be used to estimate photosynthetic rates reflective of an entire coral fragment. Despite the vastly different measurement techniques and scale of integration, O_2 fluxes were strongly correlated with (R^2 = 0.57), although much lower (~ 84%) than photosynthetic rates obtained using

incubations (Fig. 5b). This finding is in general agreement with past observations by Kühl et al. (1995) for healthy corals, although they did not directly compare the two methods. We attribute the much lower values obtained using microsensor measurements to the fact that these measurements were taken on the upper surface of the coral branch. At high light intensities, such as the ones used in our study, these areas likely have lower photosynthetic rates than the underside of the branch due to photo-inhibition, particularly in bleached corals. Similarly, the degree of bleaching typically differs between more and less light-exposed areas, and bleached corals in our study were substantially paler on their upper surface than on their underside. Even in healthy corals, pigment concentrations are heterogeneous across coral fragments, thus resulting in different surface O_2 concentrations and physiological performance across small spatial scales (e.g. Kühl et al. 1995; Koren et al. 2016). Since photosynthetic rates obtained using incubations integrate all areas of the coral branch, not just the upper surface, whole-fragment photosynthetic rates are consequently much higher than microsensor-based O_2 fluxes. Finally, O_2 flux measurements were calculated using Fick's first law of one-dimensional diffusion. However, rugosity of the coral surface and the complex corallite morphology of *Acropora* corals may have resulted in three-dimensional diffusion, thus potentially underestimating O_2 fluxes. Although microsensors were positioned between corallites ($\sim$ 1–2 mm apart), this may have contributed to microsensor-based O_2 fluxes being much lower than whole-fragment photosynthetic rates obtained from incubations.

Impacts of heat stress on the pH microenvironment

Our study shows that branching *Acropora* corals have among the lowest surface pH values of all coral genera investigated to date. Even healthy *A. aspera* barely elevated pH above ambient seawater values (0.041 units on average), and bleached corals did not elevate surface pH at all (Figs. 3b, 4c). These findings were unexpected given the presence of large O_2 concentration gradients in healthy *A. aspera* corals, which are typically linked to corresponding pH gradients due to coral metabolism (Al-Horani et al. 2003a, b; Chan et al. 2016). It is possible that the high calcification rates of *Acropora* corals resulted in reduced surface pH elevation due to CO_2 production, and/or that *Acropora* corals internally regulate processes affecting tissue and surface pH more than other coral species. *Acropora* corals also elevate the pH of their calcifying fluid less than other corals (McCulloch et al. 2012). However, our findings are consistent with the only two other studies that have measured surface pH in *Acropora* to date and showed either very little ($\sim$ 0.05 units, Kühl et al. 1995) or no evidence (Cai et al. 2016) of pH elevation at the surface of healthy corals. While this could be the result of relatively high flow speed (5–6 cm s^{-1} and magnetic stirring, respectively) in past studies, similar observations from our study at much lower flow speed ($\sim$ 3 cm s^{-1}) confirm limited surface pH elevation in *Acropora*.

Given the limited surface pH elevation in healthy *A. aspera*, it was surprising that their average [H^+] CBL thickness was similar to that of other corals. For example, massive *Favia* corals had a CBL thickness of $\sim$ 750 µm at 5–6 cm s^{-1} flow speed, $\sim$ 22 °C and 350 µmol m^{-2} s^{-1} (Kühl et al. 1995), similar to our average of 874 µm ($\pm$ 411 SE) for healthy *A. aspera* at lower flow speed but higher temperature and light levels. However, only very few studies to date have reported [H^+] CBL thickness even in healthy corals, and comparisons across studies are difficult because CBL thickness as well as coral metabolism strongly depend on incubation conditions such as flow speed, light and temperature regimes.

Bleached *A. aspera* corals did not elevate surface pH above ambient seawater values, and therefore essentially had no [H^+] CBL (Figs. 3b, 4c, d). This was most likely a consequence of impaired photosynthesis rates, yet unchanged respiration rates and calcification rates (Fig. 2c–f), thus resulting in higher CO_2 concentrations and lower pH at the coral surface. No effects of heat stress on dark respiration and calcification rates have also been observed in other studies (e.g. Rodrigues and Grottoli 2007; Schoepf et al. 2014). The observed lack of surface pH elevation in bleached corals is in general agreement with the only other study that has assessed surface pH under a range of temperatures to date, although they did not study bleached corals (Al-Horani 2005). Al-Horani (2005) found that surface pH in massive *Galaxea* corals was stable and elevated above ambient seawater throughout the seasonal temperature range up to MMM temperatures + 1.5 °C. However, once temperatures increased further, surface pH declined steeply and corals no longer elevated surface pH under heat shock (Al-Horani 2005). Lack of surface pH elevation in bleached corals could therefore be a feature common to all symbiotic corals, independent of growth morphology and the degree of surface pH elevation in a healthy state.

It is possible that branching corals in general elevate surface pH less than massive corals. Chan et al. (2016) showed that *P. damicornis* elevated pH by only $\sim$ 0.07 units above seawater values at flow speeds similar to our study ($\sim$ 2 cm s^{-1}), whereas the massive coral *Favites* had consistently greater pH elevation than *P. damicornis* under all flow conditions ($\sim$ 0.4–0.55 units). The lower surface pH elevation in branching compared to massive corals is likely due to their different morphology (Chan et al. 2016), because morphology affects flow and thus the

microenvironment surrounding corals, although other factors such as different metabolic rates, surface roughness and tissue thickness could also play a role.

The lack of surface pH elevation in heat-stressed corals, and the low pH elevation in healthy *Acropora* or potentially all branching corals, has important implications for their resistance to ocean acidification. It is possible that the differences in surface pH elevation between branching and massive corals already contribute to known differences in OA susceptibility between taxa, with branching corals often being more susceptible than massive, plating or encrusting taxa (e.g. Edmunds et al. 2012; Schoepf et al. 2013). Furthermore, increased pH elevation at the coral surface could potentially mitigate negative effects of OA, as observed in coralline algae (Cornwall et al. 2013, 2014), but this mechanism will likely be restricted to massive corals. However, even in massive corals, marine heatwaves have the potential to impair such a potential protective mechanism, due to the strong negative effects of bleaching on surface pH elevation and [H^+] CBL thickness. As ocean surface temperatures continue to increase and mass bleaching events become increasingly common, this is of significant concern.

Areas for future research

Our study has provided novel insights into the O_2 and pH dynamics across the CBL in healthy and bleached branching corals, highlighting the strong negative effects of heat stress on surface O_2 and pH values, CBL thickness and diffusive O_2 fluxes across the CBL. Given the strong implications for coral health under combined ocean acidification and warming, our findings have also revealed critical knowledge gaps and research priorities for future microsensor work. This study has significantly advanced our understanding of the impacts of gradual heat stress and bleaching on the O_2 and pH microenvironment of corals, but we have not assessed this in darkness or under a range of flow conditions (e.g. high versus low-flow speed, or oscillatory versus unidirectional flow). More research is urgently required to identify the effects of combined ocean acidification and warming on O_2 and pH dynamics across the CBL, both in the light and dark and under a range of flow regimes. Furthermore, such research should ideally be coupled with detailed physiological and geochemical analyses to determine how potential differences in O_2 and pH dynamics across the CBL modulate environmental stress tolerance and physiological performance. Particular emphasis should be placed on potential links between O_2 and pH dynamics across the CBL and calcifying fluid chemistry, as these dynamics are likely linked to both pH and dissolved inorganic carbon (DIC) upregulation in the calcifying fluid, with DIC upregulation being most sensitive to bleaching-induced breakdown of the coral symbiosis (D'Olivo and McCulloch 2017). This research has the potential to significantly advance our understanding of calcification mechanisms under climate change and OA.

Finally, branching coral species should be a particular focus of future research, ideally in direct comparison with massive species, given their underrepresentation in the microsensor literature and strong differences in temperature and pH sensitivity as well as surface pH elevation. Future work should ideally investigate such mechanisms related to climate change resilience under a range of flow, light and oxygen conditions to assess implications for potential climate change refugia, such as, for example, low-flow habitats that may serve as refugia from ocean acidification (Cornwall et al. 2014; Hurd 2015).

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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