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Resilience of Kimberley coral reefs to climate and environmental extremes: past, present and future

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WAMSI Kimberley Marine Research Program

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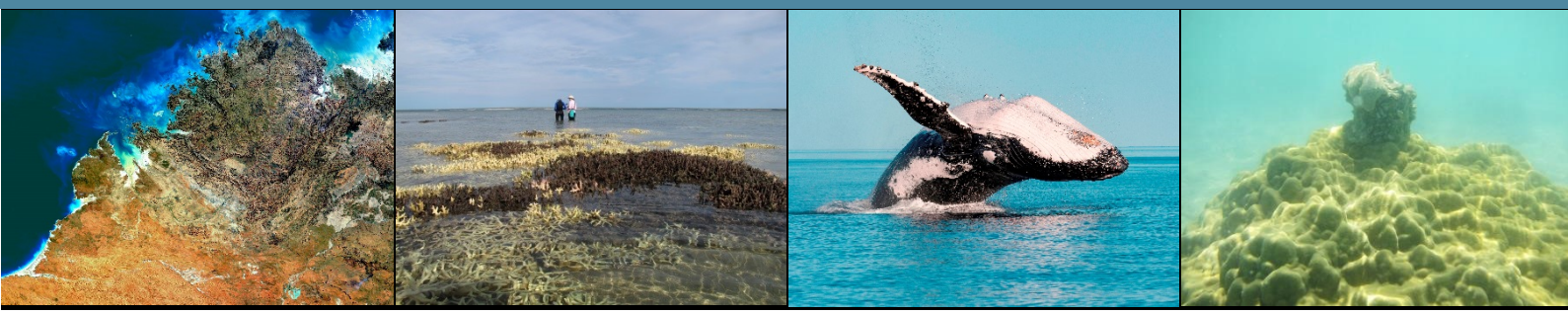

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THE UNIVERSITY OF WESTERN AUSTRALIA

ARC CENTRE OF EXCELLENCE Coral Reef Studies

WAMSI Kimberley Marine Research Program

Initiated with the support of the State Government, the Kimberley Marine Research Program is co-invested by the WAMSI partners to provide regional understanding and baseline knowledge about the Kimberley marine environment. The program has been created in response to the extraordinary, unspoilt wilderness value of the Kimberley and increasing pressure for development in this region. The purpose is to provide science based information to support decision making in relation to the Kimberley marine park network, other conservation activities and future development proposals.

Front cover images (L-R)

Image 1: Satellite image of the Kimberley coastline (Image: Landgate)

Image 2: Bleached coral communities at Shell Island, Cygnet Bay (Image: Chris Cornwall, UWA)

Image 3: Humpback whale breaching (Image: Pam Osborn)

Image 4: Massive *Porites* coral in Cygnet Bay with plug covering the core hole (Image: Verena Schoepf, UWA)

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Contents

Executive Summary

The Kimberley region in northwest Australia is a naturally extreme environment that features abundant and highly diverse coral reefs. However, it is currently unknown how Kimberley corals can survive within these extreme conditions and whether this affects their calcification rates and resilience in the face of climate and environmental change. The overall objectives of this project were to understand how corals, the key ecosystem engineers on tropical reefs, have adapted and will respond in the future to the extreme variations in physical (e.g., light, temperature, water motion) and chemical (e.g., pCO_2, oxygen, and nutrients) conditions characteristic of the Kimberley coastal region.

Through a series of field and laboratory experiments, we aimed to:

(1) Study seasonal calcification rates of common Kimberley corals over two years;

(2) Assess their thermal tolerance and establish the first bleaching thresholds for this region; and

(3) Reconstruct their resilience to historical climate and environmental extremes using geochemical proxies in coral cores.

Despite experiencing more extreme environmental conditions, common Kimberley corals overall calcified at rates that were comparable or faster than those from similar corals at a more typical tropical reef, namely Ningaloo Reef located ~1200 km southwest of Cygnet Bay (Chpt 2). The effects of tidal exposure and season, however, were highly species-specific: branching *A. aspera* grew more slowly in the environmentally more extreme intertidal than in the subtidal, whereas massive *D. favus* and *T. geoffroyi* grew faster in the intertidal environment.

Further, growth rates of branching *A. aspera* were reduced in summer compared to winter, suggesting that the combination of high summer temperatures and environmental extremes due to the large tidal amplitude resulted in an atypical seasonal behaviour. In contrast, the massive corals showed either no seasonal response or a more complex behaviour. Overall, these findings demonstrate that Kimberley corals generally exhibit high resilience of calcification to extreme temperature variations but the exact mechanisms of adaptation and/or acclimatization are strongly taxon dependent.

Detailed physiological measurements showed that Kimberley corals are highly susceptible to heat stress and coral bleaching despite being adapted to a naturally extreme temperature environment (Chpt 3 and 4). The earliest onset of bleaching (i.e., chronic photoinhibition resulting in significant decreases in Fv/Fm) already occurred when corals were exposed to heat stress for only a few days and the first visible paling was observed after only 3-5 days.

Further, in the branching *Acropora* corals, exposure to heat stress corresponding to ~20 degree heating days resulted in up to 75% mortality or severe losses of symbiont cells and chlorophyll a per surface area in the surviving corals. In contrast, massive *Dipsastraea* corals also experienced significant loss of symbiont cells and chlorophyll a, but all corals survived and losses were not as pronounced as in *Acropora*.

Based on these results, our best estimate of a coral bleaching threshold for Kimberley corals is ~32°C average daily temperature for only a few days, which is only ~1°C higher than maximum monthly mean (MMM) temperatures. Overall, this confirms that corals already tolerant of naturally higher and more variable temperature environments are nonetheless living precariously close to their physiological limits for enduring thermal stress and that the upper thresholds for coral bleaching and survival are remarkably consistent at 1-3°C above regional MMM, regardless of location (e.g. Oliver and Palumbi 2011; Riegl et al. 2011).

Importantly, intertidal corals of both species were generally more resistant to heat stress than the subtidal corals. Since all corals harboured the same genetic type of *Symbiodinium* (clade C) independent of origin or treatment, this indicates that the native thermal environment plays a critical role in shaping coral thermal tolerance. Specifically, the highly fluctuating intertidal environment (up to 7°C daily temperature variation) promotes greater resistance to heat stress than the more moderate subtidal environment (2-3°C daily

temperature variation (Dandan et al. 2015).

These findings were confirmed during the first documented, regional-scale bleaching event in the Kimberley region in March/April 2016. We conducted aerial bleaching surveys of ~30 reefs between Montgomery Reef and Sunday Island which were ground-truthed using in situ surveys. Most surveyed reefs had 30-60% bleaching and these data contributed to an Australia-wide analysis of the 2016 bleaching event, which was recently published in *Nature* (Hughes et al. 2017). At Shell Island, bleaching was more severe and widespread in the subtidal than the intertidal. Consequently, six months later this had resulted in dramatic mortality of branching *Acropora* corals, whereas most corals in the intertidal had recovered. Although this natural bleaching event represents a major disturbance, chances for reef recovery are good given the absence of many other stressors in the Kimberley. These findings are currently being prepared for publication in a peer-reviewed scientific journal.

The calcification rates of massive *Porites* spp. coral were relatively stable (~1.2 to ~1.6 g/cm^2/yr) for the past ~100 years (Chpt 5). No significant trend was observed, despite a slight warming trend in the reconstructed annually-resolved seawater temperatures since 1919 and more variable temperatures since the 1970s.

Similar to tropical corals living in less extreme environments, the carbonate chemistry of the calcifying fluid of the Kimberley coral was substantially elevated above ambient seawater values. The DIC_{cf} was ~2-fold enriched relative to ambient seawater, whereas the calcifying fluid pH_{cf} was ~8.5, an elevation of ~0.5 pH units above seawater pH. The elevated DIC_{cf} is attributed to the additional supply of metabolic CO_2 which combined with pH_{cf} up-regulation leads to elevated aragonite saturation states (Ω_{cf}) of ~16 to ~20 that promote coral calcification.

However, a recent warming trend from 2011 to 2016 may have negatively affected coral metabolism, since the DIC_{cf} exhibited significant decreases as well as subdued seasonal variability during this period. The pH_{cf} nevertheless remained at an elevated level and increased as DIC_{cf} was depleted. Therefore, the aragonite saturation state (Ω_{cf}) of the calcifying site remained relatively undisturbed since the increase in pH_{cf} largely offset the decrease in DIC_{cf}.

Overall, these findings demonstrate that key calcification mechanisms in Kimberley corals are not compromised by the extreme environmental conditions, resulting in high and stable calcification rates as observed in corals from less extreme reef environments (see also Dandan et al. 2015). Nevertheless, recent ocean warming between 2011 and 2016 has negatively affected the critical relationship between coral algal symbionts and the animal host, which not only threatens calcification rates but also coral reef survival, as the natural bleaching event in summer 2016 demonstrated.

Implications for management

Kimberley corals are arguably Australia's most stress-resistant corals, and have adapted their calcification rates and overall physiology to the naturally extreme environment of the Kimberley. They should therefore be considered regional and national priorities for long-term coral health monitoring and further research into the mechanisms enabling such remarkable stress resistance in reef-building coral. Intertidal coral communities, in particular, should be the focus of awareness and protection efforts as their naturally higher heat resistance resulted in significantly higher survival and recovery than subtidal, less heat-tolerant coral communities during the first documented mass bleaching event in the region. This event, however, also highlighted that the threat of ocean warming and marine heatwaves to Kimberley corals is real; thus, it is critical to minimize local stressors to boost coral resilience, particularly during heat stress events. Coral cores have also provided clear evidence of sediment pulses into the coral reefs at Cygnet Bay. The sediment input is strongly correlated with river discharge of flood waters during major cyclonic events. Given the negative impacts of increased sediment and nutrient concentrations on water quality and coral health, monitoring of sediment/nutrient input into the Fitzroy River catchment should be a key priority.

The present-day seasonal and historical coral calcification rates measured here provide an important baseline

and thus the basis for evaluating how coral growth responds to changes in environmental conditions.

Further, the first estimate of an experimentally based bleaching threshold for this region is of critical importance during times where heat stress events are increasing in frequency. Furthermore, coral health surveys conducted before, during and after the first natural bleaching event in the Kimberley region in 2016 provide important baseline data on coral bleaching abundance, bleaching susceptibility, and coral community dynamics associated with disturbance-related recovery and mortality.

Key residual knowledge gaps

1. Given the first documented, regional-scale bleaching event in the Kimberley in 2016, how long will it take for Kimberley coral reefs to recover from this event and how does recovery differ between species and different environments (e.g. intertidal versus subtidal coral communities)?
2. What are the physiological, genetic, genomic and biogeochemical mechanisms that enable the high resilience of Kimberley corals to heat and other environmental stressors such as pH fluctuations, and are they indicative of physiological plasticity and thus high acclimation capacity, or do they represent fitness trade-offs?
3. How is cryptic species diversity in corals linked to functional diversity, particularly with respect to climate change stressors?
4. How do environmental conditions such as seawater temperature and carbonate chemistry change over daily/tidal/seasonal/annual/decadal time scales, both past, present and future in habitats exposed to different tidal influence?
5. What is the capacity of Kimberley corals to acclimate and/or adapt to ongoing climate change and ocean acidification, and do they have the potential to be used for proactive management approaches, such as assisted evolution via selective breeding experiments or assisted translocation to restore degraded reefs?

1 Introduction

Globally, coral reefs are in decline due to increasing pressures from the rapidly changing physical and biogeochemical conditions leading to degradation of the marine environment (Hoegh-Guldberg et al. 2007). Climate change and CO_2 driven ocean warming are now, for example, causing more severe and frequent coral bleaching events; the latter a direct consequence of the unusually high sea surface temperatures that are being increasingly manifested during particularly strong positive and negative phases of the El Niño Southern Oscillation or ENSO (e.g., the heat wave that occurred along the WA coastline during the 2011 La Niña event (Moore et al. 2012) or the mass worldwide bleaching that occurred during the 1998-99 El Niño event (Hughes et al. 2003)).

Rapidly rising levels of atmospheric CO_2 are also causing ongoing reductions of seawater pH and a concomitant decline in the carbonate/bicarbonate ratio of the surface oceans. This latter process, commonly termed 'ocean acidification', has the potential to cause major reductions in rates of calcification.

On a local-scale there are arguably even more dramatic changes, with human activities such as overfishing, land-clearing of river catchments and development of coastal regions resulting in increased terrestrial runoff, higher suspended sediment loads together with increased levels of nutrients. Furthermore the general degradation of water quality combined with direct disturbances to marine ecosystem is leading to an increased prevalence of diseases, outbreaks of crown of thorns plagues, and in extreme cases threshold crossing ecosystem phase-shifts from coral to algal dominated systems.

How the combined effects of these global as well as local environmental/climate changes are being expressed within the natural range of diurnal and seasonal changes in temperature and water chemistry is still difficult to decipher. Furthermore there is the key and still unanswered question of whether coral reefs will be able to adapt, acclimatise, or otherwise defend themselves against this increasing array of environmental stresses.

Coral reefs in the Kimberley are an ideal natural laboratory to investigate these questions; not only because of their intrinsic value to regional Australian Indigenous culture, but also because this coastal environment is one of the naturally most extreme coral reef environments in the world and can thus provide insights into the mechanisms and limits of coral stress tolerance. The Kimberley is characterised by extremely high and rapidly changing temperatures (22°C - 32°C) and high light as well as episodically high sediment loading and terrigenous runoff from cyclone induced flooding. Furthermore, the Kimberley has extreme tides (>10 m) that create expansive intertidal zones with highly energetic (>1 m/s) currents as well as even more extreme physical regimes associated with frequent cyclones. An additional factor is the repetitive isolation of the shallow intertidal habitats that are especially abundant throughout the coastal zones of the Kimberley and are also subject to extreme changes in water temperature (25°C to 37°C), water chemistry, and light on time scales of just hours (Dandan et al. 2015).

Since the impact of local human activities and regional climate change on Kimberley ecosystems is likely to increase with time, there is an imperative to gain a proper understanding of the past, present and future environmental and ecosystem drivers. This requires an assessment of the critical factors controlling the health and abundance of Kimberley coral reefs; knowledge that is prerequisite for proper resource assessment, planning and management of the region. The overall objectives of this project are to understand how corals, the key ecosystem engineers on tropical reefs, have adapted and will respond in the future to the extreme variations in physical (e.g., light, temperature, water motion) and chemical (e.g., pCO_2, oxygen, and nutrients) conditions characteristic of the Kimberley coastal region.

Through a series of field and laboratory experiments, we aimed to:

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coral cores.

This report is organized into four research chapters. Chapter 2 addresses Aim 1 and shows that Kimberley corals have highly resilient calcification rates despite the extreme temperature environment. Chapters 3 and 4 address Aim 2 and provide insights into the heat tolerance of Kimberley corals, as well as the impacts of the 2015/16 marine heatwave on Kimberley coral reefs. Chapter 5 addresses Aim 3 and presents historic temperature records for the inshore Kimberley region that were reconstructed from the geochemical composition of a coral core and have important implications for climate change in the region. The research chapters are followed by the acknowledgments and a summary describing the communication activities and outputs produced during this project. The report concludes with an appendix providing detailed responses to questions outlined in the Kimberley Marine Research Program Science Plan.

2 Resilience of coral calcification to extreme temperature variations in the Kimberley region, northwest Australia

Dandan SS, Falter JL, Lowe RJ, and McCulloch MT (2015) Resilience of coral calcification to extreme temperature variations in the Kimberley region, northwest Australia. Coral Reefs, doi: 10.1007/s00338-015-1335-6 (open access)

2.1 Introduction

Corals living in the nearshore Kimberley region of Western Australia are exposed to average monthly temperatures of around 22°C in July to over 31°C in December, and exceed 30°C on average for around five months each year (Richards et al. 2015). They are also subject to diurnal tidal amplitudes of up to 11 m (Kowalik 2004) that can expose corals to potentially stressful and damaging levels of temperature and light (Anthony et al. 2007). Furthermore, water motion can become stagnant during such low tide excursions (Lowe et al. 2015), decreasing rates of oxygen export and increasing oxidative stress (Lesser and Farrell 2004; Anthony and Kerswell 2007; Mass et al. 2010), as well as increasing the temperature of coral tissue above already elevated ambient levels (Fabricius 2006; Jimenez et al. 2008). Thus, intertidal and near-shore environments along the Kimberley coast provide a challenging thermal environment to which corals have adapted, yet to date we have found no record of extensive coral bleaching on a regional scale along the Kimberley coast. Despite its potential value for understanding coral growth under extreme conditions, this area has received little attention beyond conducting habitat surveys (Rosser and Veron 2011; Wilson and Blake 2011; Richards et al. 2015). Instead, greater focus has been placed on the off-shore reefs to the north of the Kimberley coast, such as Scott Reef, due to their potential as natural gas reserves (e.g., Cooper et al. 2010; Gilmour et al. 2013).

Numerous studies have already examined the community structure (Craig et al. 2001; Riegl et al. 2011), physiology (Smith et al. 2007; Putnam and Edwards 2011), and/or genomics (Oliver and Palumbi 2009; Barshis et al. 2013) of corals living in various high temperature environments. In the present study we measured seasonal changes in the calcification rates of three different coral species: the branching *Acropora aspera*, the flabello-meandroid *Trachyphyllia geoffoyi*, and the massive *Dipsastraea favus*, in both subtidal and intertidal environments over a 17-month period (April 2011 through September 2012; Figure 2) where diurnal ranges in mean hourly temperature reached up to ~7°C. We show that the corals within these high and variable temperature environments grow and calcify at rates comparable to similar species of hermatypic corals living in the more moderate temperature environments of Coral Bay in Ningaloo Reef, Western Australia.

2.2 Materials and Methods

Our goal was to compare the growth rates of three different taxa of scleractinian coral (*Acropora aspera, Favia favus*, and *Trachyphyllia geoffroyi*) from two intertidal sites and one subtidal site seaward of Shenton Bluff on the north side of Cygnet Bay in the Kimberley Region (Figure 1). The two intertidal sites differed by by depth and isolation from exchange during low phases of the tide.

2.2.1 Environmental data

We deployed HOBO Pro v2 Water Temperature Data Loggers (U24-002, ±0.2C) at all three sites and Hobo Water Level Loggers – U20-001-02-Ti (Onset, MA USA ±1.5cm) at Isolated and Subtidal pools sampling at 10-min intervals from April 2011 through September 2012. Downwelling light (scalar PAR irradiance) incident to the benthos was measured at each site during each survey using an Odyssey Photosynthetic Irradiance Recording System (Dataflow Pty., NZ), which logs light in the 400-700nm range. Each of these sensors had previously been calibrated under water against a factory-calibrated LiCor 192SA PAR sensor back at UWA. Downwelling light was also measured RBR XR-420CTPAR (RBR Ltd., Ontario, Canada) using the same LiCor sensor at the Isolated site to check the calibration of the Odyssey sensors. Salinity (±0.002) and temperature (±0.002) were also measured at the most tidally isolated site using the XR-420CTPAR.

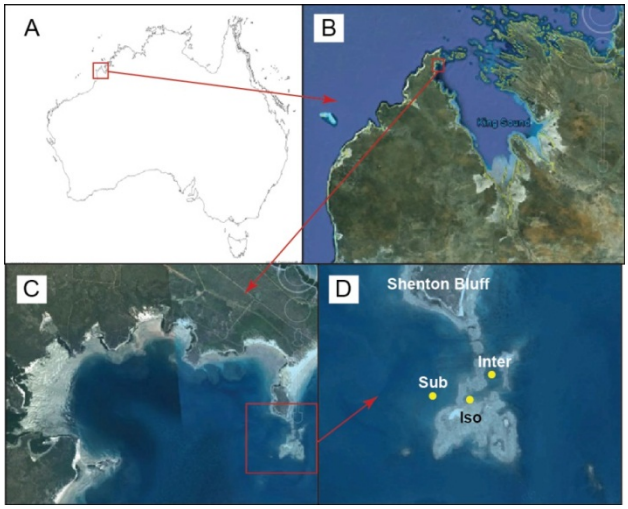

Figure 1. Map of A) Australia, B) Dampier peninsula in the Kimberley region, C) Cygnet Bay, and D) Shenton Bluff with the three study sites.

Water samples were collected during all field surveys and sampled as close as possible to the deployed corals at each of the three sites using a 10-L bucket., Water samples intended for the analysis of total alkalinity and dissolved inorganic nutrients were filtered in the field using 0.7 µm glass fiber filters (Whatman GF/F) and stored immediately in the dark and on ice. Total alkalinity (±5 ueq./L) was measured using an approach based on the spectrophotometric method of Yao and Byrne (1998). pH on the Total scale (±0.02) was measured using a Schott Handylab 12 pH meter equipped with a Blueline 24 pH electrode (SI analytics GmbH, Germany). All other carbonate chemistry parameters (e.g. DIC, pCO2, and Ωar) were calculated from measured pH, TA, and temperature using the CO2SYS program (Lewis et al. 1998).

2.2.2 Calcification rates

Branching coral fragments of *Acropora aspera , Trachyphyllia geoffroyi* and *Favia favus* were attached to acrylic tiles (8×8 cm2) using the marine epoxy Z-SPAR (Splash Zone, St. Louis, MO, USA). After several months had passed, the mounted coral fragments were then removed from the field site, the individual coral tiles were cleaned of any epiphytic growth, and the new buoyant weight of the coral fragment was measured. Changes in fragment buoyant weight were converted to changes in dry skeletal mass assuming a density of 2.93 g cm-3 for aragonite (Jokiel et al. 1978; Chalker et al. 1985) and a seawater density calculated from temperatures and salinities recorded at the time of buoyant weighing. For the branching *A. aspera* we estimated total surface area of the fragment as the sum of the surface areas of many branches that were geometrically represented as cylinders of individual heights and diameters; an approach used and validated in prior studies (Bak and Meesters 1998; Naumann et al. 2009). For the massive *T. geoffroyi* and *F. favus* we measured surface area using the foil technique (Marsh 1970, Veal et al. 2010). We used the resulting measured changes in dry weight (ΔM, derived from buoyant weight), initial and final surface areas estimated from the surface area-mass relationships to calculate the areal rate of net calcification (mg CaCO3 cm-2 d-1) between each growth interval.

2.2.3 Statistical Analysis

Two separate mixed-effects models examining the influence two independent factors on calcification rate were run for each of the three genera studied (either *Acropora*, *Dipsastraea*, or *Trachyphyllia*) with one common factor being each individual season (winter, summer, then winter). The second factor we considered was location based on one of either two separate spatial scales: local (inter-tidal versus subtidal at Cygnet Bay only) or regional (Cygnet Bay versus Coral Bay, subtidal habitats only). Given that all independent factors considered were categorical and therefore dimensionless, all coefficients generated by the mixed effect model were thus in units of g cm^{-2} yr^{-1}. We did not perform a cross-regional comparison of calcification rates between *T.*

geoffroyi at Cygnet Bay and *L. hemprichii* at Coral Bay, given that they were too taxonomically different from one another. Nonetheless, we still report calcification rates for *L. hemprichii* in this paper for reference in future work. All statistical analyses were performed using the software SPSS v22 (IBM, Armonk, USA).

2.3 Results

2.3.1 Environmental conditions

We collected our measurements during spring tides when the duration of intertidal isolation or 'slack water period' was ~2 h for the intermediate pool and ~4 h for the isolated pool. Daily average water temperatures at the subtidal site at Cygnet Bay tidal pools ranged from 21.9°C in the peak of winter (June–July) to 31.7 °C in the peak of summer (December–January) and averaged ~31 °C for 38 d between December 2011 and January 2012 (Figure 2). Differences between the mean daily temperatures of each of the intertidal sites and the subtidal site were not that substantial when averaged over the course of the winter and summer seasons (~0.1 °C). However, diurnal variations in water temperature were far more pronounced at the intermediate and isolated sites than at the subtidal site where maximum solar heating and cooling occurred and particularly during spring low tides. For example, the diurnal range in mean hourly temperatures (maximum minus minimum) exceeded 3 °C for 41 % of the year at the isolated site, 24 % of the year at the intermediate site, and only 1.6 % of the year at the subtidal site and further reached up to ~7 °C at the isolated site during summer. The highest hourly temperature recorded throughout the study was 37.3 °C at the isolated site in December 2011. Thus, during the summer of 2011–2012, daytime temperatures would frequently exceed 33 °C at the intermediate site (on 19 d) and 34 °C at the isolated site (on 14 d). Night-time excursions were generally much smaller than the corresponding daytime elevations, exhibiting only a 1–2 °C drop in temperature. Salinity ranged between 33.6 and 34.4 for the post-winter field surveys and averaged 34.1 for the post-summer survey. Maximum daily light levels at the subtidal and intermediate sites ranged from ~1000 to 1800 µmol m^{-2} s^{-1} depending on tidal elevation, water clarity, and cloud cover; however, maximum daily light levels in the isolated site reached ~2100–2400 µmol m^{-2} s^{-1} in March 2012 due to its particularly shallow depth at low tide (0.2–0.3 m).

There was substantial within-season variation in water column carbonate chemistry due to our measurements being limited to discrete sampling during daylight hours and at low tide when rates of net production and net calcification are highest and the water column shallowest (~0.2 m in the isolated habitat), thus making it difficult to discriminate between the effects of diurnal and seasonal variation in our data. Nonetheless, given that these samples were collected at the same sites, at the same time of day (~8:00–11:00) and during the same phase of tide, we doubt that there was much seasonal bias in TA beyond what would be expected from seasonal changes in salinity (~0.9 % or ~19 µeq kg^{-1} from summer to winter versus ~10 µeq kg^{-1} observed). In summer, average daytime pH ranged from 8.06 in the subtidal to 8.12 in the isolated pool, while in winter it ranged from 8.11 at the subtidal site to 8.17 at the isolated site.

Although daytime pH was significantly higher in winter than in summer by ~0.05, differences of this magnitude are unlikely to have a marked effect on rates of coral calcification given the ability of most coral to biologically elevate pH at the site of calcification by ~0.5 units. Consequently, during the day the partial pressure of dissolved carbon dioxide in the water column (pCO_2) reached values that were below atmospheric values, and aragonite saturation state (Ω_{ar}) reached values that averaged between 4.0 and 4.25 for the intertidal sites and between 3.5 and 3.9 at the subtidal site.

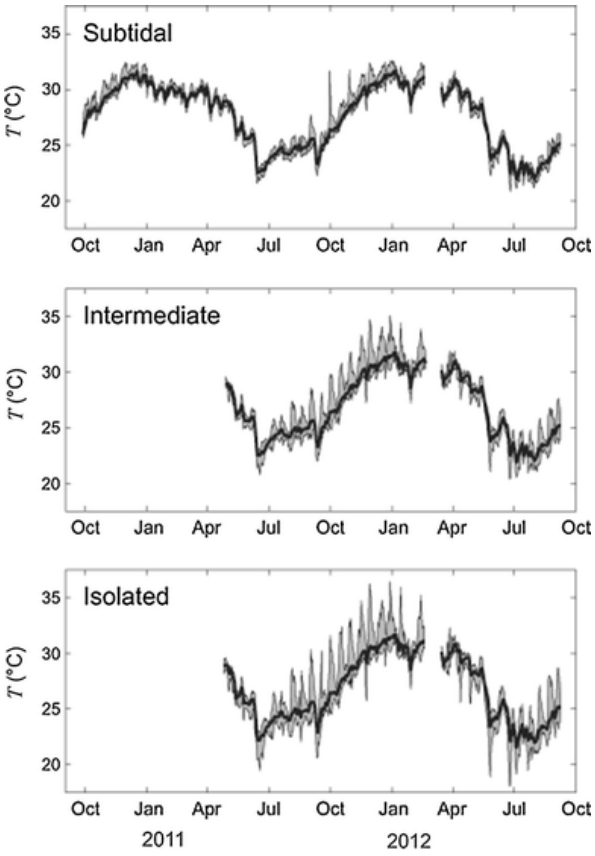

Figure 2 Water column temperatures recorded at the three Cygnet Bay sites: subtidal, intermediate, and isolated sites from September 2010 through August of 2012. The heavy black line represents daily average temperatures, while the grey regions represent the range between hourly minimum and maximum temperatures for each day. All growth experiments began in April 2011. Note the large tidally driven cycles in temperature of ~4 °C that are superimposed upon the seasonal cycle resulting in summer maximum temperatures in the isolated pools of up to 35 °C

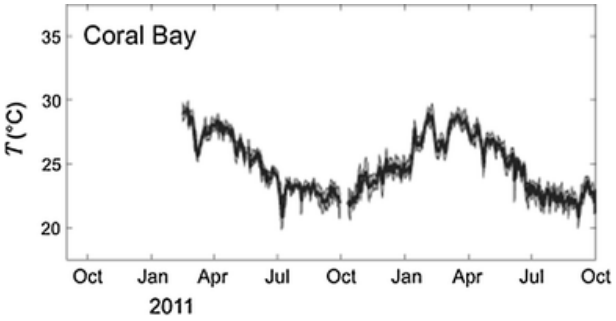

Figure 3 Water column temperatures recorded at the Coral Bay site from February 2011 through October 2012. The heavy black line represents daily average temperatures, while the grey regions represent the range between hourly minimum and maximum temperatures for each day. Note that the scale of the y-axis is the same as in Figure 2

At Coral Bay, water column temperatures at our study site averaged ~23.3 °C in winter (May through October) and ~25.7 °C in summer (November through April; Figure 3). Unlike the three sites at Cygnet Bay, the diurnal range in mean hourly temperatures (maximum minus minimum) at the Coral Bay site never exceeded 3 °C during the year-long measurement period. Benthic light levels reached maximum hourly irradiances of around 1100–1200 µmol m^{-2} s^{-1} in summer and 800–850 µmol m^{-2} s^{-1} in winter. Salinity at Coral Bay exhibited a negligible difference between season (34.8 in winter and 34.9 summer). Total alkalinity at the backreef site in Coral Bay averaged 2286 µeq kg^{-1} in summer and 2222 µeq kg^{-1} in winter, whereas daytime pH averaged 8.09 in summer and 8.15 in winter. Thus, daytime pCO_2 values were lower than atmospheric in both seasons: 289 µatm

in summer and 351 µatm in winter. Given the opposing effects of temperature and pH, Ω_{ar} values were not significantly different on average between summer and winter (3.6–3.7).

2.3.2 Calcification rates

The total bioactive surface area for all morphologies (branching, flabello-meandroid, and massive) was significantly and positively correlated with fragment mass ($r^2 = 0.58–0.96$, $p \leq 0.01$). Calcification in *A. aspera* at Cygnet Bay was significantly affected by both the degree of tidal exposure and individual season even though seasonal patterns in coral growth rates were mixed across sites (Figure 4). Nonetheless, on an annual basis, *A. aspera* at the isolated site calcified at rates that were ~30 % lower than at either the subtidal or intermediate sites (0.40 ± 0.03 vs. 0.58 ± 0.03 and 0.59 ± 0.02 gcm^{-2} yr^{-1}), mainly as a result of slower calcification in summer (0.73 ± 0.08 mg cm^{-2} d^{-1} vs. 1.34 ± 0.04, and1.59 ± 0.08 mg cm^{-2} d^{-1} in winter).

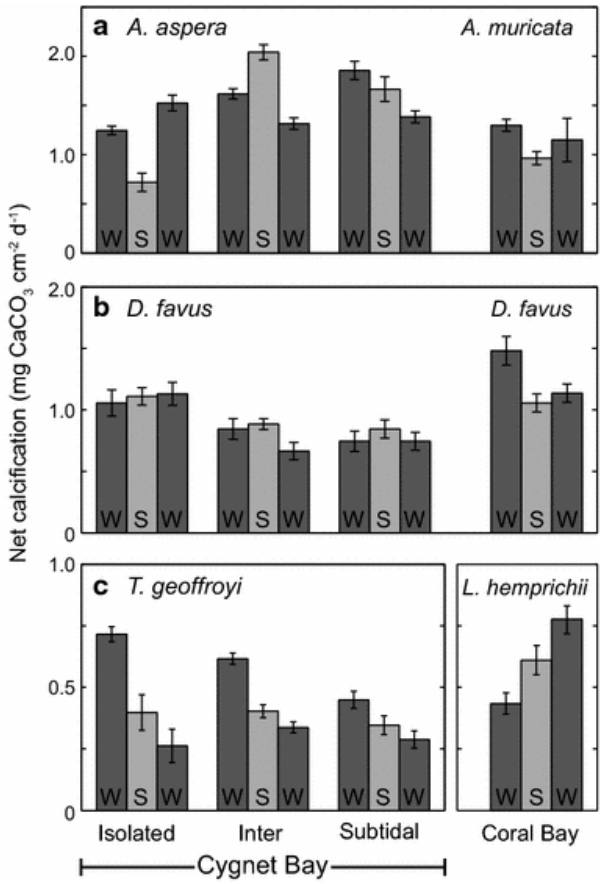

Figure 4 Average net calcification rates (±SE) for specimens of *Acropora aspera* and *Acropora muricata* (a), *Dipsastraea favus* (b), *Trachyphyllia geoffroyi* (c), and *Lobophyllia hemprichii* (bottom) over summer (September to March) and winter (March or April to September) at sites at Cygnet Bay in the Kimberley Region and at Coral Bay along Ningaloo Reef from April 2011 to September 2012

Unlike *A. aspera*, calcification in *D. favus* was not significantly influenced by season at either Cygnet Bay or Coral Bay (Figure 4); however, it was influenced by the intertidal location at Cygnet Bay. In further contrast with *A. aspera*, calcification in *D. favus* was fastest at the most tidally exposed site: 0.40 ± 0.03 g cm^{-2} yr^{-1} (1.10 ±0.07 mg cm^{-2} d^{-1}) or ~30 % faster than rates at the intermediate and subtidal sites (0.30 ± 0.02 and 0.29 ±0.02 g cm^{-2} yr^{-1}, respectively). For T. *geoffroyi*, both season and tidal exposure influenced calcification rates; however, the 'seasonal' effect was manifest mainly as a significant overall downward trend in calcification rates at all sites in Cygnet Bay ((Figure 4). For instance, there was no apparent seasonal dependency when comparing summer rates to average winter rates (0.33 ± 0.04 and 0.35 ± 0.04 mg cm^{-2} d^{-1}). Similar to *D. favus*, calcification in *T. geoffroyi* was ~30 % faster at the tidally exposed sites (0.16 ± 0.02 and0.15 ± 0.01 g cm^{-2} yr^{-1} at the isolated and intermediate sites, respectively) than at the subtidal site (0.12 ±0.01 g cm^{-2} yr^{-1}).

The influence of both region and individual season was significant when comparing rates of calcification in *A. aspera* at Cygnet Bay to *A. muricata* at Coral Bay as well as between *D. favus* at both sites. Calcification in *A. muricata* at Coral Bay was 14 % faster in the winter than summer (Δcalc. = 0.12 ± 0.07mg cm^{-2} d^{-1}), but still 42 % slower than in *A. aspera* at the subtidal site of Cygnet Bay (0.34 ± 0.02 vs.0.59 ± 0.02 g cm^{-2} yr^{-1}). Calcification in *D. favus* at Coral Bay was also 24 % faster in winter than summer (Δcalc. = 0.26 ± 0.07 mg cm^{-2} d^{-1}) and 52 % faster than in *D. favus* at the subtidal site of Cygnet Bay (0.44 ± 0.03 vs. 0.29 ± 0.02 g cm^{-2} yr^{-1}). Calcification rates in *L. hemprichii* at Coral Bay increased steadily 0.06 mg cm^{-2} d^{-1} or at an average annual calcification rate of 0.23 ± 0.02 g cm^{-2} yr^{-1} (Figure 4). Looking across all sites and both regions, we found that *Acropora* spp. generally calcified the fastest (0.34 ± 0.02 to 0.59 ±0.02 g cm^{-2} yr^{-1}), followed by *D. favus* (0.29 ± 0.02 to0.44 ± 0.03 g cm^{-2} yr^{-1}), *L. hemprichii* (0.23 ± 0.02g cm^{-2} yr^{-1}), and *T. geoffroyi* (0.12 ± 0.01 to 0.16 ±0.02 g cm^{-2} yr^{-1}).

2.4 Discussion and Conclusions

Despite experiencing more extreme environmental conditions, common Kimberley corals overall calcified at rates that were comparable or faster than those from similar corals at a more typical tropical reef, namely Ningaloo Reef located ~1200 km southwest of Cygnet Bay. The effects of tidal exposure and season, however, were highly species-specific: branching *A. aspera* grew more slowly in the environmentally more extreme intertidal than in the subtidal, whereas massive *D. favus* and *T. geoffroyi* grew faster in the intertidal environment.

Further, growth rates of branching *A. aspera* were reduced in summer compared to winter, suggesting that the combination of high summer temperatures and environmental extremes due to the large tidal amplitude resulted in an atypical seasonal behaviour (Figure 4). In contrast, the massive corals showed either no seasonal response or a more complex behaviour (Figure 4). Overall, these findings demonstrate that Kimberley corals generally exhibit high resilience of calcification to extreme temperature variations but the exact mechanisms of adaptation and/or acclimatization are strongly taxon dependent.

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3 Limits to the thermal tolerance of corals adapted to a naturally extreme, highly fluctuating temperature environment

Schoepf V, Stat M, Falter JL and McCulloch MT (2010) Limits to the thermal tolerance of corals adapted to a naturally extreme, highly fluctuating temperature environment. Scientific Reports 5:17639, doi: 10.1038/srep17639 (open access)

3.1 Introduction

Coral reefs are in serious decline worldwide (Hoegh-Guldberg et al. 2007) and increasingly suffer from episodes of thermally induced stress or coral bleaching, which leads to the breakdown of the vital endosymbiosis with dinoflagellates in the genus *Symbiodinium* spp. (Hoegh-Guldberg and Smith 1989; Glynn 1996). Corals typically obtain the majority of their metabolic requirements from photosynthetic carbon translocated from their endosymbionts (Muscatine et al. 1981); thus, the loss of these symbionts via bleaching significantly reduces their ability to meet key metabolic needs and can ultimately lead to death if continued for a prolonged period of time. As surface ocean temperatures have already increased on average by 0.6°C since preindustrial times and are projected to increase by at least another 2°C under a business as usual scenario by the year 2100 (IPCC 2013), coral bleaching events are expected to increase in frequency and intensity over the coming decades (Teneva et al. 2012; Frieler et al. 2013). This raises the question of whether corals are capable of acclimatising and/or adapting to not only rising ocean temperatures but also more frequent extreme thermal events, and if so, whether these processes will be fast enough to keep pace with the rapid rates of ocean warming that are currently occurring.

The majority of coral reefs occur in tropical latitudes between 22°S and 22°N and thus only experience relatively limited seasonal changes in water temperatures (4-5°C) and average maximum temperatures of ~30°C (Kleypas et al. 1999). However, coral reefs also exist in much more extreme temperature environments such as the Persian Gulf where the seasonal temperature range can be >20°C (14-36°C) and daily mean summer temperatures can reach 34-35°C for several months (Riegl et al. 2012). The existence of such communities demonstrates that corals can adapt to a large range of temperatures and that thermal tolerance is therefore a plastic trait which is influenced by their natural thermal environment. Consequently, upper limits of thermal tolerance also vary significantly over both large and small spatial scales (Coles et al. 1976; Hume et al. 2013).

Furthermore, corals living in thermally more variable environments such as those found in back reefs or on reef flats are often found to be more resistant to temperature stress and bleaching compared to corals from thermally more stable environments such as the fore reef (Goreau and Macfarlane 1990; Oliver and Palumbi 2011; Castillo et al. 2012; Palumbi et al. 2014), although this is not always the case (Berkelmans and Willis 1999). Therefore, thermally variable environments seem to enhance coral thermal tolerance beyond their adaptation to specific mean summer temperatures and can therefore promote increased resistance to climate change. This influence of environment on resilience also has important implications for predicting the occurrence of future bleaching events as models based on historical temperature variability rather than climatological maxima were found to have the highest predictive power (Donner 2011).

Beyond thermal environment and history, the degree to which coral can resist bleaching is also influenced by many biological aspects such as its morphology, the genetic type of *Symbiodinium*, tissue characteristics and heterotrophic plasticity. For example, branching corals are typically more susceptible to bleaching than massive corals (Marshall and Baird 2000; Loya et al. 2001), and corals hosting *Symbiodinium* clade D are often, though not always, more tolerant of thermal stress than corals hosting other symbiont types (Berkelmans and van Oppen 2006; Stat and Gates 2011). Thick tissues and high levels of stored energy reserves also promote further resistance to bleaching (Thornhill et al. 2011; Grottoli et al. 2014), while the capacity to increase heterotrophic feeding during bleaching can help some corals avoid resource limitation and starvation (Grottoli et al. 2006; Bessell-Browne et al. 2014).

Corals living in naturally extreme environments can provide important insight into the mechanisms underlying

coral resistance to thermal stress. However, our present knowledge of these mechanisms has come mainly from a few sites (e.g., the southern Persian Gulf and the back reef pools of American Samoa). Given the importance of understanding how corals will ultimately respond to current rates of ocean warming, it is therefore critical to study the growth of reef-building corals in as wide a range of naturally extreme environments as possible. The little-known Kimberley region in northwest Australia is a naturally extreme environment that supports unusual and highly diverse coral reefs (Rosser and Veron 2011; Richards et al. 2015), yet remains poorly studied due to its remote location and difficulty of access. This region is characterized by the largest tropical tides in the world (up to 10 m during spring tides), strong currents and turbid waters (Purcell 2002; Rosser and Veron 2011; Richards et al. 2015). Due to the extreme tides, intertidal corals often experience significant short-term temperature fluctuations of up to 7°C daily as well as aerial exposure for several hours (Purcell 2002; Rosser and Veron 2011; Richards et al. 2015) without exhibiting any obvious signs of stress. In addition, Kimberley corals are adapted to naturally high mean water temperatures that exceed 30°C for five months of the year (Rosser and Veron 2011). Thus although the Kimberley region is comparable to other naturally extreme environments such as the back reef pools of American Samoa (Craig et al. 2001), though not quite as warm as the Persian Gulf (Riegl et al. 2012), it differs in being a much more dynamic and variable environment. In particular, the large tidal range and frequent aerial exposure of intertidal corals provides a unique set of environmental conditions to study the scope and limits for coral thermal tolerance and adaption in the face of climate change.

The existence of coral reefs in such naturally extreme and variable environments is encouraging in view of global warming, but it remains unclear if corals adapted to such environments exhibit unusually high thermal tolerance or if this puts them even more at risk by living closer to the upper temperature limit of corals. Therefore, the goal of this study was to experimentally assess the thermal tolerance of two common Kimberley corals (branching *Acropora aspera* and massive *Dipsastraea* sp. (formerly *Favia* (Budd et al. 2012)) from both intertidal and subtidal environments (Figure 1) to variable and elevated water temperatures. We hypothesised that (1) Kimberley corals have higher bleaching thresholds than expected based on local mean summer temperatures due to the naturally extreme thermal environment, (2) corals from the intertidal environment would be more resistant to thermal stress than subtidal corals due to more pronounced daily temperature fluctuations (Figure 1, see Methods), and (3) *Dipsastraea* would be more resistant than *Acropora* independent of their original environment. To test these hypotheses, corals were subjected to either ambient control temperatures, ambient +2°C or ambient +3°C for 11 days in outdoor flow-through seawater tanks during which we followed changes in key metrics of both symbiont and coral physiology.

3.2 Materials and Methods

3.2.1 Collection Sites and Thermal Environment

Coral fragments of branching *Acropora aspera* and massive *Dipsastraea* sp. (formerly *Favia* (Budd et al. 2012)) were collected in April 2014 from Shenton Bluff, Cygnet Bay, Kimberley region, Western Australia. They were collected from shallow depth (<2 m) in two different thermal environments, the intertidal and subtidal. The intertidal environment (16°28’45.8”S, 123°2’41.3”E) is a small shallow pool (ca. 200 x 100 m) that becomes isolated from the surrounding waters of King Sound during low tides. The associated slack water period lasts for up to 4 hours and the shallower corals become exposed to air during this time while the submerged corals are subject to stagnant flow condition. Temperature logger data from 2011-2013 showed that the daily variation in seawater temperatures in this pool is up to 7°C, while the seasonal range is 22°C to 31.5°C based on a 7-day moving average of daily mean temperatures (Figure 1). Maximum monthly mean (MMM) temperatures of 30.9°C and 31.2°C were recorded in December 2011 and February 2013, respectively. In contrast, the subtidal environment (16°28’46.8”S, 123°2’36.6”E) represents a more moderate thermal environment that experiences only up to 3°C daily temperature variation although the seasonal temperatures range in the subtidal is the same as in the intertidal (22°C to 31.5°C; Figure 1). Similarly, MMM temperatures were 31.1, 30.8 and 31.3°C °C in December 2010, December 2011 and February 2013, respectively. Corals in this environment are never exposed to air during low tides.

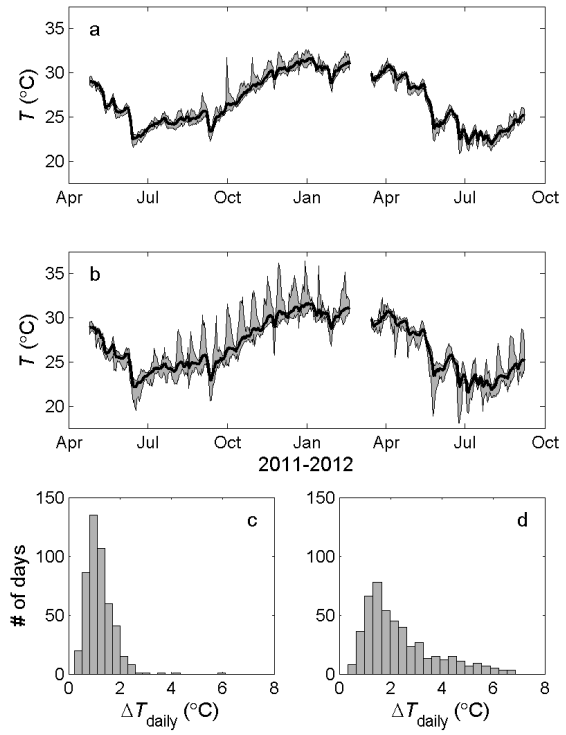

Figure 1. Temperature data for the (a) subtidal and (b) intertidal environment from 2011-12. Histograms show the number of days with a certain daily temperature range (ΔTdaily) in the (c) subtidal and (d) intertidal environment for the same time period. In panels a and b, the bold black line shows the mean daily temperature, while the hourly max. and hourly min. temperature for each day is shown as a grey envelope around the daily mean.

Colonies (n=12 for *Acropora*, n=10 for *Dipsastraea*) were selected at least 10 m apart to increase the probability that different genotypes of the same species were selected. Four fragments were collected from each parent colony per environment and species, one for each of the three temperature treatments and a fourth fragment stored in 100% ethanol to determine the *Symbiodinium* type of each parent colony back at the UWA (see below). Coral fragments were then glued onto plastic tiles and maintained in shaded outdoor, flow-through seawater tanks (see below). Corals were allowed to acclimate at ambient seawater temperature (day: ~32°C, night: ~30°C) for 1 week prior to the start of the experiment. During that time, they were stained with alizarin red at a concentration of ~5 mg/L for 9 hours during daylight.

3.2.2 Coral Bleaching Experiment

The bleaching experiment was conducted from 25 April to 5 May 2014 (11 days). Since seasonal variation in bleaching thresholds can occur (Berkelmans and Willis 1999), we wanted to test the thermal resilience of the Kimberley coral at the end of the summer when temperature stress is most likely to occur. Bleaching thresholds are as yet unknown for coastal Kimberley regions and were therefore estimated to be >32°C based on MMM data from temperature loggers deployed in previous years.

Coral fragments were randomly assigned to each of three temperature treatments: (1) ambient control (day: 31.9°C, night: 29.9°C), (2) ambient +2°C and (3) ambient +3°C. Each temperature treatment consisted of two separate 43 L flow-through tanks (one for each environment) fed from one 140 L sump where temperature was controlled using titanium heaters (Wei Pro, 1000 W) connected to a temperature controller (Auber Instr. TD100A). Temperature was gradually increased by 0.6°C per day until the target temperature was achieved to prevent heat shock. Importantly, a maximum daily temperature variation of 4-5°C was maintained in all treatments (Figure 2) to better mimic the naturally variable thermal conditions. HOBO temperature loggers recorded seawater temperature every 15 min in all six tanks.

Since water flow can significantly affect thermal tolerance (Nakamura et al. 2005), tanks were designed as miniflumes (length 117 cm, width 25 cm, height 29 cm; water depth 15 cm) to allow for more realistic flow conditions. Two submersible pumps (Macro Aqua) per tank generated flow rates of 12-15 cm s-1 (determined from the timed passage of dye). Seawater renewal rate was 3 L min-1 for each treatment, resulting in a turnover time of ~15 minutes per treatment. Incoming seawater was filtered to a nominal size of 10 µm so although corals were not fed during the experiment, they nonetheless had access to some natural particulate (<10 µm) and dissolved organic matter as well as dissolved inorganic nutrients provided by the incoming seawater. Shade cloth reduced incoming maximum photosynthetically active radiation (PAR) levels to 500 and 400 µmol m-2 s-1 just below the water surface and at the bottom of the tanks, respectively (measured using an Apogee MQ-200 cosine-corrected planar PAR-meter).

Although we had planned on conducting the experiment for several weeks, the experiment was ended after 11 days due to significant mortality in some treatments (see Results). After the experiment was terminated, all corals were frozen, transported back to the University of Western Australia (UWA) under liquid nitrogen and stored at -80°C until further analysis.

3.2.3 Monitoring and Characterisation of Treatment Conditions

Seawater temperature, salinity and conductivity were measured daily in all six tanks (two per treatment) using a YSI 85 multi-sensor. Seawater samples for total alkalinity (TA) and nutrient samples were taken from each of the six tanks every three days. pH was measured in each tank within 15 min of collecting the water samples using a Schott handylab pH 12 pH meter. Water samples were filtered using glass fibre filters with 0.7 µm nominal pore size (Whatman GF/F), collected in screw-top Nalgene HDPE bottles and stored frozen until analysis. TA was determined by titration from a spectrophotometrically determined end-point pH (Yao and Byrne1998). Treatment xCO2 (dry air), aragonite saturation state (Ωarag), and pHT were calculated using the program CO2SYS (Lewis and Wallace 1998) based on measured pH and alkalinity. An aliquot of the water samples collected for TA analysis was used to measure concentrations of ammonium (NH_4^+, ±0.2 µM), nitrate (NO_3^-, ±0.05 µM) and phosphate (HPO_4^{2-}, ±0.02 µM) using a QuikChem 8500 Series 2 Flow Injection Analysis (FIA) System (Lachat Instrument, USA) according to standard colorimetric methods as provided by the manufacturer.

In addition to calculating average day and night water temperatures for each temperature treatment, degree heating days (DHD) and heating rate (HR) were calculated (Maynard et al. 2008). Although these indicators of thermal stress are not typically used in an experimental context, we decided to use them here as they provide a measure of cumulative thermal stress and are thus more useful in characterising the experimental heating treatments and facilitating comparison with in situ bleaching events. Since long-term mean summer temperatures (LMST) are not available for the Kimberley region and experimental control temperatures are more relevant in an experimental context, DHD were calculated as follows:

$$DHD = \Sigma (THeating - TControl) \quad (1)$$

where THeating is the average daily temperature in the respective heating treatment and TControl is the average daily temperature in the control treatment over the course of the bleaching experiment. To account for the rate of temperature increase, HR was also calculated as follows:

$$HR = DHD / \Sigma days (THeating > TControl) = DHD / \#experimental\ days \quad (2)$$

3.2.4 Physiological and Genetic Analyses

Mortality. Coral mortality was visually assessed for each fragment daily in the morning and during fluorescence measurements at noon.

Endosymbiont type. Initial algal endosymbiont types were determined from small (1-2 cm) biopsies, which were removed from all parent colonies sampled during coral collection and stored in 100% ethanol. To detect any changes in symbiont type occurring during the experiment, biopsies were removed from each surviving coral fragment at the end of the experiment and stored in 100% ethanol. *Symbiodinium* in five samples per treatment and species collected at the start and end of the experiment (unless less than five fragments per treatment survived) were genotyped. The same coral colonies were analysed for all intertidal and subtidal temperature treatments, respectively.

Total DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer's instructions with an initial overnight incubation at 56°C. Symbiodinium chloroplast 23S rDNA domain V was amplified in PCR using forward 23S1 (5′ GGC TGT AAC TAT AAC GGT CC 3′) and reverse 23S2 (5′ CCA TCG TAT TGA ACC CAG C 3′) primers (Zhang et al. 2000). PCR reactions contained 0.5U JumpStartTM Taq DNA polymerase (Sigma-Aldrich), 1 X PCR buffer, 1 mM MgCl2, 20 µg BSA, 0.2 mM each dNTP, 0.2 □M each primer, and 1 µl DNA template made up to a 30 µl volume with sterile deionized water. PCR was performed in an Eppendorf Mastercycler® with 5 min at 94°C followed by 35 cycles of 30 s at 95°C, 30 s at 55°C, and 1 min at 72°C, and ended with a final 10 min extension at 72°C. 23S rDNA amplicons were purified and sequenced in both directions at the Australian Genome Research Facility (Perth node). Chromatograms were inspected and edited in Geneious 6.1.6. Chloroplast 23S rDNA haplotypes were identified by performing a nucleotide BLAST search in NCBI.

Photophysiology. Effective quantum yield (ΔF/Fm') of chlorophyll a fluorescence in each coral fragment was measured daily at noon (except for day 2) to assess the photochemical efficiency of coral in the light-adapted state. Maximum quantum yield (Fv/Fm) of chlorophyll a fluorescence in each coral fragment was also measured daily 1 hour after sunset (except for day 2) to assess the photochemical efficiency in the dark-adapted state. 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 = 6 and 5 for Acropora and Dipsastraea, respectively, and damping = 2. Measurements were made at a constant distance of 3 mm from the coral tissue. The maximum excitation pressure over photosystem II (Qm) (Iglesias-Prieto et al. 2004), which is an indicator of symbiont performance at peak sunlight, was calculated as Qm = 1 – (ΔF/Fm') / (Fv/Fm), with values close to 1 indicating photoinhibition and values close to 0 indicating light-limitation of photosynthesis under maximum irradiance.

Tissue biomass, chlorophyll and symbiont density. Coral tissue was removed from the skeleton using either an airbrush (*Acropora*) or a waterpik (*Dipsastraea*). A 3-6 ml aliquot of the resulting tissue slurry was then dried at 60°C in pre-combusted aluminium pans to constant weight and ashed in a muffle furnace at 500°C for 4 hours (Fitt et al. 2000). Ash-free dry weight (=tissue biomass) was determined as the difference between dry and ash weight and standardized to surface area, which was estimated using the simple geometry technique for *Acropora* and the aluminium foil technique (Marsh 1970) for *Dipsastraea*. The remaining tissue slurry was separated into animal host and symbiont fraction via centrifugation. Chlorophyll a and c2 were extracted in 100% acetone in the dark at 4°C for 24 hours, determined spectrophotometrically using the equations of Jeffrey and Humphrey (1975) and standardized to both surface area and cell density. Symbiont cell density was calculated using 8 replicate counts on an improved Neubauer hemocytometer and standardized to surface area.

3.2.5 Statistical Analyses

Non-parametric one-way analysis of variance (ANOVA) was used to test for significant differences in tank conditions (i.e., average day and night temperature, THeating - TControl, pHT, pCO2, total alkalinity, saturation state and nutrient concentrations) between intertidal and subtidal tanks within each temperature treatment.

For Fv/Fm and Qm, generalized linear mixed model (GLMM) analysis was used to test for the effect of time (=days of heating), temperature, and environment for each species individually. Time was fixed with ten levels (days 1, 3-11 – no measurements were performed on day 2), temperature was fixed with three levels (ambient, ambient +2°C, ambient +3°C) and environment was fixed with two levels (intertidal, subtidal). Parent colony was a random factor nested within environment. For chlorophyll a and c2 (per area and per cell), endosymbiont density and tissue biomass, GLMM analysis was used to test for the effects of temperature, environment and parent colony for each species individually. Tukey adjusted p-values were used for post hoc tests when main effects were significant. When a significant interaction was observed, multiple pair-wise comparisons were conducted using Tukey adjusted p-values.

Since all fragments were exposed to identical conditions except temperature during the bleaching treatments, any differences in the observed responses were due to temperature and environment effects alone and independent of seasonal variation. P-values ≤0.05 were considered significant. Statistical analyses were performed using SAS software version 9.3.

3.3 Results

For 1 week prior to the start of the experiment, all corals were allowed to acclimate to ambient treatment conditions, including the daily temperature variation of 4-5°C (see Methods). All corals appeared visibly healthy at the beginning of the experiment, and all ambient control corals appeared to remain healthy throughout the experiment (see also Figs. 3, 4A, B). Average day and night temperature, degree heating days, heating rate, pHT, pCO2, total alkalinity, saturation state and nutrient concentrations for each of the six tanks are summarized in Table 1. None of these parameters differed significantly between intertidal and subtidal tanks subject to the same temperature treatment (Table S1). Thus, any observed differences in the response of intertidal versus subtidal corals within each temperature treatment can be attributed to differences in their in situ habitat and are independent of tank effects.

Temperature profiles for each temperature treatment are shown in Figure 2. From day 6 to 9, unusual weather conditions associated with storms, high cloud cover and strong winds resulted in cooler water temperatures, particularly in the ambient +3°C treatment where the heater struggled to maintain the high temperature under these conditions (Figure 2). Therefore, this treatment was not consistently higher than the ambient +2°C treatment over the entire course of the experiment, resulting in similar average day and night temperatures, degree heating days and heating rate (Table 1). However, temperatures in the +3°C treatment were higher than in the +2°C treatment on days 1, 4, 5, 10 and 11 and during night 4 (Figure 2), thus resulting in an overall more variable and stressful treatment, the effect of which became evident in the physiological data (e.g., chlorophyll a fluorescence, Figs. 3, 4; see below).

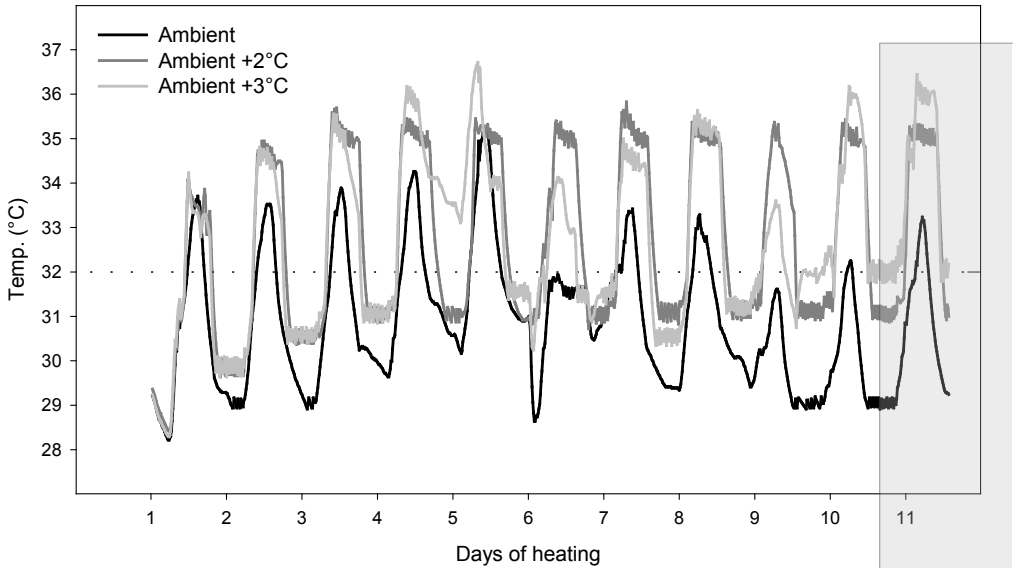

Figure 2. Temperature profiles for each heating treatment over the course of the bleaching experiment. The dashed line indicates the presumed bleaching threshold of 32°C, whereas the shaded area indicates days with unusual weather conditions due to storms, high cloud cover and strong winds.

3.3.1 Photophysiology and Mortality

Acropora. Active chlorophyll a fluorescence is generally the preferred method for detecting the initial onset of heat-stress induced coral bleaching (Warner et al. 1999). Over the duration of the experiment, maximum photosynthetic quantum yield (Fv/Fm) of *Acropora* corals decreased significantly with time in both heat stress treatments (+2°C and +3°C, Table S2), while remaining high and relatively constant in the ambient controls regardless of whether the corals were from the intertidal or subtidal environment (Figure 3A,B). Furthermore, Fv/Fm of heat-stressed corals from the +3°C treatment declined sooner and reached significantly lower values compared to corals in the +2°C treatment for much of the experiment. This trend was also reflected in the excitation pressure over photosystem II (Qm) (Figure 3C, D, Table S2), which is the ratio of the effective quantum yield at midday relative to the maximum quantum yield (see Methods). However, the gap between the greater decline in Fv/Fm as well as the greater increase in Qm of corals from the +3°C versus the +2°C treatment narrowed during the last days of the experiment (Figure 3A, B).

Despite these overall similar trends, subtidal *Acropora* showed much greater declines in Fv/Fm than intertidal *Acropora* corals, with Fv/Fm being 51-57% lower in heat-stressed subtidal *Acropora* relative to controls after 11 days of heat stress and only 32-33% lower in heat-stressed intertidal *Acropora* (Figure 3A, B, Table S2). This increased susceptibility to heat stress in subtidal versus intertidal heat-stressed *Acropora* was also evident in much higher values of Qm throughout much of the experiment (Figure 3C, D, Table S2).

Within 5-6 days of heat stress, many *Acropora* corals became highly susceptible to rapid tissue necrosis (RTN), which resulted in tissue sloughing and death within 24-48 hours (Figure 3E, F). Similar to trends in photophysiology, mortality occurred both earlier and at a higher rate in subtidal versus intertidal corals (Figure 3E, F). By the end of the experiment, 75% of all heat-stressed subtidal *Acropora* had died (+2°C and +3°C treatments, Figure 2F), whereas only 50-58% of all heat-stressed intertidal *Acropora* had died (Figure 2E). Importantly, neither subtidal nor intertidal *Acropora* in the ambient control treatment developed RTN or died (Figure 3E, F).

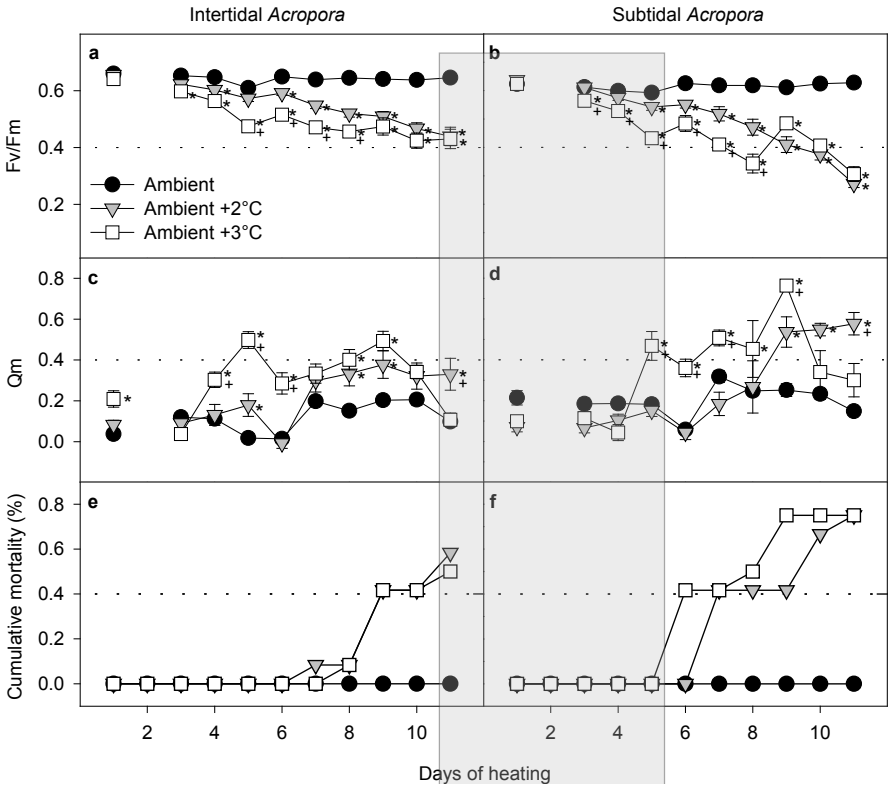

Figure 3. Photochemical efficiency (Fv/Fm) (a, b), excitation pressure over photosystem II (Qm) (c, d) and cumulative mortality (e, f) of intertidal and subtidal *Acropora aspera*. Mean ± SE are shown for a-d. Asterisks indicate a significant difference from the ambient control treatment, whereas + indicates a significant difference between ambient +2 and +3°C treatments. The dashed reference lines were added to highlight differences between intertidal and subtidal corals. The shaded area indicates days with unusual weather conditions due to storms, high cloud cover and strong winds.

Dipsastraea. Similarly to *Acropora*, all heat-stressed *Dipsastraea* corals from both environments showed significant declines in Fv/Fm over the course of the experiment, while control corals maintained high and relatively stable values (Figure 4A, B, Table S3). However, intertidal *Dipsastraea* showed similar declines in Fv/Fm in both the +3°C and the +2°C treatment (Figure 4A), whereas subtidal *Dipsastraea* from the +3°C treatment had significantly lower Fv/Fm values than corals in the +2°C treatment from day 4 onward (Figure 4B). Furthermore, subtidal *Dipsastraea* overall experienced greater declines in Fv/Fm than intertidal corals, with 37-48% lower values relative to controls observed in subtidal corals at the end of the experiment compared to only 30-32% lower values in intertidal corals (Figure 4A, B).

Levels of Qm in heat-stressed *Dipsastraea* corals were generally lower than in *Acropora* regardless of their original environment (Figure 4C, D). Further, intertidal heat-stressed *Dipsastraea* generally experienced similar Qm values as the controls for the majority of the experiment (Figure 4C, Table S3). In contrast, subtidal *Dipsastraea* in the ambient +2°C treatment had significantly lower Qm values than the controls on 5 out of the 11 days (days 4, 6, 8, 9, and 10; Figure 4D). Subtidal *Dipsastraea* in the ambient +3°C treatment had significantly higher Qm than the controls on day 3, but otherwise did not differ significantly from the controls (Figure 4D).

In stark contrast to *Acropora*, none of the *Dipsastraea* corals from either the elevated or ambient temperature treatments developed RTN or died despite being maintained in the same tanks as the *Acropora* corals (Figure 4E, F).

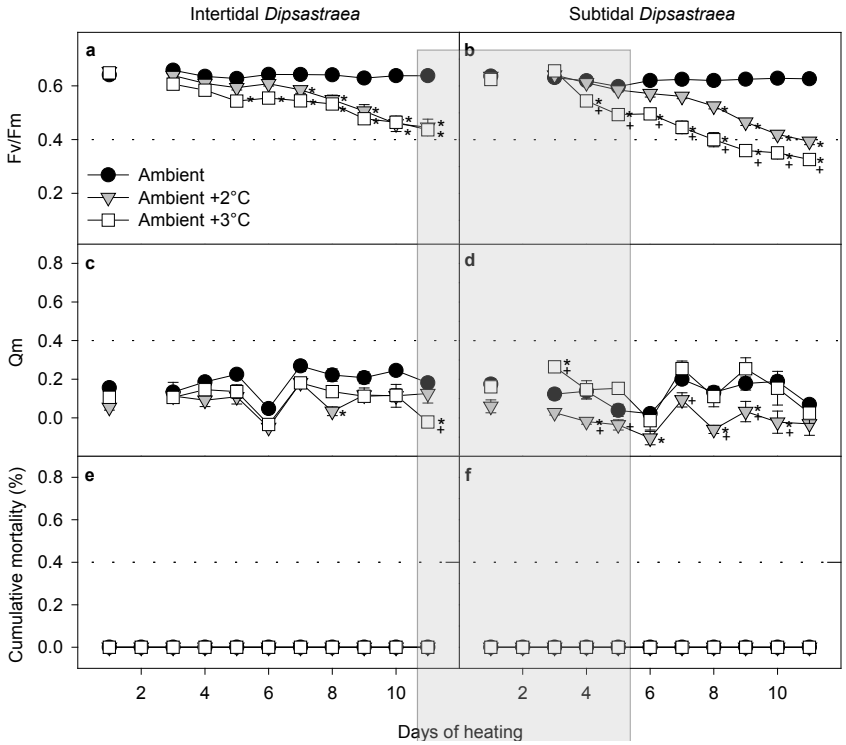

Figure 4. Photochemical efficiency (Fv/Fm) (a, b), excitation pressure over photosystem II (Qm) (c, d) and cumulative mortality (e, f) of intertidal and subtidal *Dipsastraea* sp. Mean ± SE are shown for a-d. Asterisks indicate a significant difference from the ambient control treatment, whereas + indicates a significant difference between ambient +2 and +3°C treatments. The dashed reference lines were added to highlight differences between intertidal and subtidal corals. The shaded area indicates days with unusual weather conditions due to storms, high cloud cover and strong winds.

3.3.2 Endosymbiont Type

A total of 79 *Symbiodinium* chloroplast 23S rDNA sequences were recovered from the coral fragments used in the experiment. All sequences belonged to clade C *Symbiodinium* and were thus independent of species, treatment or environment. There were two unique clade C haplotypes: 76 sequences were identical to Cp1 (accession number FJ461478 (Stat et al. 2009)), and three sequences represented a novel haplotype Cp20 (KT223627) that is a single base pair different to Cp1. The three coral fragments with *Symbiodinium* Cp20 all originated from the same parent colony (subtidal *Acropora* #8).

3.3.3 Chlorophyll a, Symbiont Density and Tissue Biomass

Acropora. Area-normalized chlorophyll a concentrations were significantly lower in heat-stressed *Acropora* corals relative to ambient controls (Figure 5A, Table S4), with this effect being more pronounced in colonies from the subtidal versus intertidal environment (-73% and -91% versus -51% and -60% for the +2°C and +3°C treatments, respectively; Table S4). The effect of heat stress on chlorophyll a concentrations was much less pronounced when normalizing per symbiont cell rather than per surface area for both the subtidal and intertidal colonies: heat-stressed intertidal *Acropora* in the ambient +2 and +3°C treatments had only 14% and 21% lower concentrations than the controls, respectively, but in heat-stressed subtidal *Acropora* they were 30% higher and 38% lower, respectively (Figure 5B, Table S4). This more damped response in chlorophyll a per cell versus area was due to significant decline in symbiont densities within heat-stressed corals (Figure 5C, Table S4), the effect of which was again more pronounced in colonies from the subtidal versus intertidal environment (-79% and -86% versus -58% and -65% for the +2°C and +3°C treatments, respectively). Finally, tissue biomass was not significantly influenced by either temperature or environment (Figure 5D, Table S4); however, heat-stressed intertidal *Acropora* corals tended to have a 23-26% lower biomass than the controls (Figure 5D).

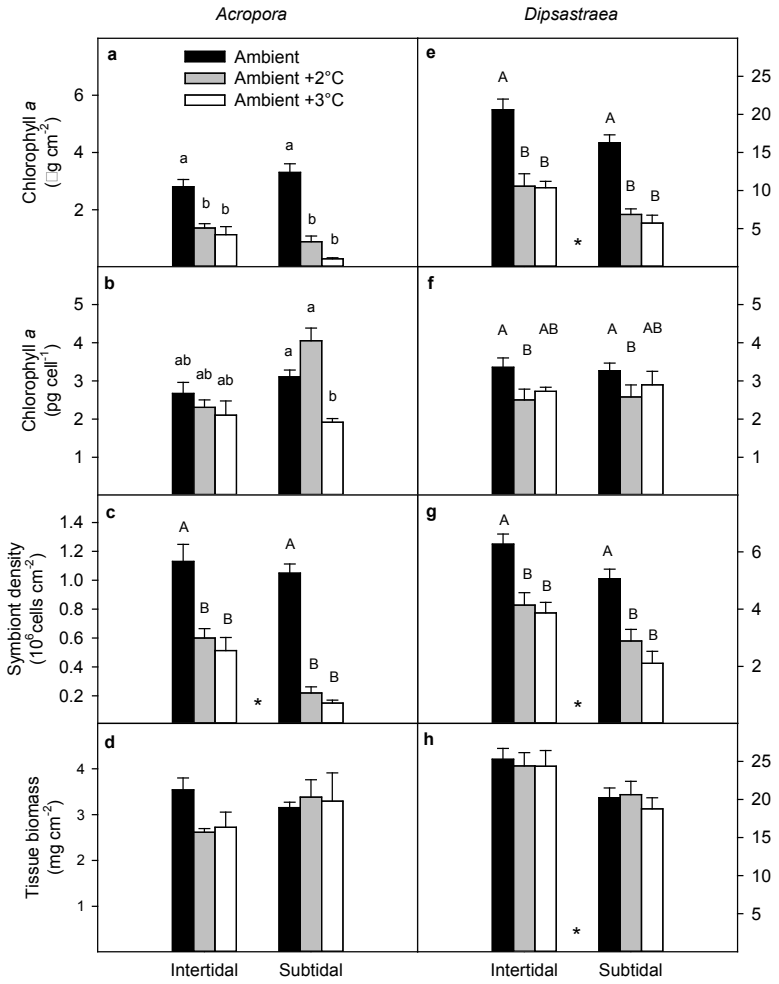

Figure 5. Chlorophyll a normalized to (a, e) surface area and (b, f) symbiont cells, symbiont density (c, g) and tissue biomass (d, h) of intertidal and subtidal *Acropora aspera* and *Dipsastraea* sp. Mean ± SE are shown. Asterisks indicate significant effects of environment, whereas upper case letters indicate significant temperature effects. Lower case letters indicate results from Tukey-adjusted multiple pairwise comparisons when there was a significant interaction between environment and temperature. Statistical results in Table S4. Note the different scales for the two corals except in panels b and f.

<u>*Dipsastraea.*</u> Heat-stressed corals had significantly lower area-normalized chlorophyll a concentrations than the controls, and subtidal corals generally had lower concentrations than intertidal corals (Table S4, Figure 5E). Specifically, heat-stressed intertidal *Dipsastraea* in the ambient +2 and +3°C treatments had 49% and 50% lower concentrations than the controls, respectively, whereas concentrations were 58% and 65% lower in subtidal *Dipsastraea*, respectively (Figure 5E). Similar to *Acropora*, the effect of heat stress on *Dipsastraea* chlorophyll a concentrations was much less pronounced when normalizing per symbiont cell rather than per surface area for coral from both environments, and corals in the ambient +2°C treatment had the lowest concentrations (Figure 5F, Table S4). This was generally due to significant declines in symbiont density in heat-stressed *Dipsastraea* corals, with more pronounced declines in heat-stressed subtidal corals (-43% and -58% versus -34% and -38% in the +2°C and +3°C treatments, respectively; Figure 5G, Table S4). Finally, tissue biomass was significantly higher (+19%) in intertidal than subtidal *Dipsastraea* corals, however no temperature effect was observed (Table S4, Figure 5H).

3.4 Discussion and Conclusions

3.4.1 How resistant are Kimberley corals to heat stress and bleaching?

The present study is the first to examine the thermal tolerance of corals growing in the remote Kimberley region of north-western Australia, which represents a naturally extreme thermal environment that is in many aspects comparable to other extreme environments such as the Persian Gulf or the tide pools of American Samoa. Despite the fact that corals growing in this region experience large daily temperature variability (up to 7°C) and temperature extremes of up to 37°C (Figure 1), branching *Acropora* and massive *Dipsastraea* corals were highly susceptible to coral bleaching when exposed to temperatures of 2°C or more above their maximum monthly mean of ~31°C for just several days. For *Acropora*, the amount of heat stress accumulated by the end of the experiment (>20 degree heating days) further resulted in up to 75% mortality due to rapid tissue necrosis and tissue sloughing, potentially due to increased sensitivity to the pathogen *Vibrio harveyi* (Luna et al. 2007).

Our results show that common reef-building corals of the Kimberley can tolerate temperature extremes at which corals from more commonly occurring reef environments severely bleach and die, yet nevertheless remain highly susceptible to temperature increases of ~2°C above their maximum monthly mean. They are further consistent with findings from other naturally extreme environment such as the back-reef environment of Ofu Island in American Samoa: heat stress experiments performed on *Acropora* corals showed that temperatures of only 2°C above the regional maximum monthly mean caused substantial mortality (up to ~50%) after six days of exposure equivalent to only 11 degree heating days (Oliver and Palumbi 2011). Similar levels of mortality occurred in subtidal Kimberley *Acropora* corals after exposure to comparable heat stress (Figure 3F). Further, coral reefs in the Persian Gulf experienced a series of natural bleaching events between 1996 and 2011 (~2°C above MMM for several weeks), which resulted in wide-spread mass mortality of *Acropora* corals, severe reductions in coral cover and shifts in coral community composition (Burt et al. 2011; Riegl et al. 2011; Coles and Riegl 2013). Collectively, these results suggest that corals already tolerant of naturally higher and more variable temperature environments are nonetheless living precariously close to their physiological limits for enduring thermal stress and that upper thresholds for coral bleaching and survival are remarkably consistent at 1-3°C above regional MMM regardless of location (Coles and Riegl 2013).

It is difficult to establish a single, well-defined temperature as the bleaching threshold for the Kimberley given the highly fluctuating thermal environment and the significant daily temperature variation in our experiment as well as the gradual changes in various physiological metrics that occurred at different times over the course of the study. Clearly, average day and night temperatures of ~32 and ~30°C, respectively, are well tolerated by these corals without causing chronic photoinhibition as indicated by high and stable photochemical efficiency (Fv/Fm) in the controls over the course of the experiment (Figs. 3, 4A, B). This was the case for both corals and both environments and despite ambient peak water temperatures reaching up to 35°C for short time periods (Figure 2). In contrast, exposure to elevated temperatures of +2°C above the MMM resulted in chronic photoinhibition within just 4 days (Figs. 3, 4A, B) and substantial mortality for *Acropora* corals after 11 days (50-75%, Figs. 3E, F). This suggests that the bleaching threshold lies somewhere between 32-33°C (mean daily temperature, exposure of several days), a threshold that is relatively consistent with NOAA's approach of defining bleaching thresholds as MMM temperatures +1°C.

The highest bleaching thresholds reported for reef environments to date come from the Persian Gulf and are 2-3°C higher than what we have found for the Kimberley (34-36°C vs. 32-33°C) (Riegl et al. 2011; Riegl et al. 2012; Coles and Riegl 2013). However, MMM temperatures in the Persian Gulf are several degrees higher than in the Kimberley with corals spending 4-5 months every year at daily mean temperatures of >30°C and about 2 months at >33°C (Riegl et al. 2012). Combined with the extreme seasonal variation of up to 20°C (Riegl et al. 2012), this seems to underlie the extremely high thermal tolerance of Persian Gulf corals. Unfortunately, as yet there is not enough data on the physiological changes these corals undergo under normal and bleaching conditions with which to compare our own results or those from American Samoa.

3.4.2 The role of the thermal environment in determining bleaching resistance

Intertidal corals of both species generally showed higher bleaching resistance and symbiont health than subtidal corals as demonstrated by more modest declines in photochemical efficiency (Fv/Fm), pigment concentrations and symbiont densities as well as lower excitation pressure over photosystem II (Qm), at least for *Acropora* (Figs. 3-5). More importantly, the survival rate of intertidal *Acropora* was higher than that of subtidal *Acropora* under the same levels of heat stress.

Even before the onset of tissue necrosis and death, the bleaching mechanism in *Acropora* differed significantly according to which environment the parent colonies originated from. Heat-stressed intertidal *Acropora* bleached predominantly through the loss of *Symbiodinium* cells whereas subtidal *Acropora* were able to partially compensate for the greater loss of *Symbiodinium* through increased concentrations of chlorophyll a in the remaining symbionts (Figure 5A-C). Overall, the decline in both symbiont cells and chlorophyll a per cell in subtidal *Acropora* in the ambient +3°C treatment indicates that they experienced greater photodamage than those in the ambient +2°C treatment (Figure 5B, C). These results are further consistent with higher values of Qm in the +3°C versus +2°C treatment (Figure 3D). In contrast, both intertidal and subtidal heat-stressed *Dipsastraea* predominantly bleached by losing *Symbiodinium* cells rather than chlorophyll a per cell (Figure 5E-G). Such species- and habitat-specific differences in the bleaching mechanism are consistent with other studies (Hoegh-Guldberg and Smith 1989; Warner et al. 1996).

Surprisingly, heat-stressed intertidal *Acropora* showed a trend of up to 26% lower tissue biomass than the controls whereas heat-stressed subtidal *Acropora* were able to maintain their tissue biomass (Figure 5D). This may indicate that the superior ability of intertidal *Acropora* to cope with heat stress comes from an ability to access stored energy reserves such as lipid and protein. These energy reserve pools make up a significant portion of coral tissue biomass (Schoepf et al. 2013) and can play an important role in promoting bleaching resistance and recovery (Grottoli et al. 2014). In *Dipsastraea*, higher overall levels of tissue biomass in intertidal compared to subtidal corals could therefore have contributed to their increased bleaching resistance (Figure 5H). We expect, however, that depletion of energy reserves would be even greater in both *Dipsastraea* and *Acropora* under the more prolonged periods of heat stress that normally precede major natural bleaching events (weeks to months).

The increased thermal tolerance of intertidal versus subtidal corals to heat stress in the present study is consistent with reports showing that corals from back-reef environments are more resistant to thermal stress than corals from the fore reef (Goreau and Macfarlane 1990; Oliver and Palumbi 2011; Castillo et al. 2012; Palumbi et al. 2014), although not in all cases (Berkelmans and Willis 1999). Since back-reef environments typically experience much larger fluctuations and extremes in temperature and other parameters, this is consistent with our findings of increased thermal tolerance for intertidal compared to subtidal corals because the intertidal environment represents much more extreme temperature conditions than the subtidal (Figure 1, see Methods).

Importantly, the genetic type of *Symbiodinium* did not differ between environments and temperature treatments. Thus, this study confirms that more extreme fluctuations in temperature enhance bleaching resistance even without undergoing substantial changes to the symbiont genotype. However, it is less clear whether this enhancement in thermal stress resistance is the result of acclimation, natural selection and/or adaption of the coral holobiont given that the two environments are within <500 m and the intertidal pool is flushed during high tides. Genetically distinct coral host populations can exist between lagoon and reef slope environments (Barshis et al. 2010), and even in the absence of genetic population substructures, genetic differences can still provide a mechanism for increased heat tolerance (Barshis et al. 2013; Bay and Palumbi 2014). Further genetic studies are needed to determine whether the same is true at our study location.

3.4.3 Other factors determining thermal tolerance

The genetic type of *Symbiodinium* can play a significant role in determining thermal tolerance because some types (e.g. within clade D) have been found to perform better at high temperatures than others (Jones and

Berkelmans 2010; Stat and Gates 2011). Further, there is evidence that more extreme temperature environments often support higher abundance of corals hosting clade D (Stat and Gates 2011). It may therefore be surprising that the thermally tolerant Kimberley corals in this study all hosted clade C (chloroplast 23S type Cp1 with the exception of one subtidal *Acropora* colony that hosted Cp20); however, prior studies have also found that symbionts in *Acropora* corals from the Kimberley are dominated by clade C (Thomas et al. 2014) and that *Acropora* in Western Australia generally has a high symbiont specificity for clade C across a large latitudinal range (Silverstein et al. 2011; Thomas et al. 2014). We know of no other studies that have analysed symbiont type in Western Australian *Dipsastraea* corals, but Pacific congeners are also typically dominated by clade C (LaJeunesse et al. 2003).

This and other studies are making it increasingly clear that resistance to heat stress can be achieved without the presence of clade D. For example, *Symbiodinium* C3 dominates corals in the southern Persian Gulf, one of the hottest environments in the world supporting coral growth (Hume et al. 2013; Hume et al. 2015), although it was recently shown that this particular C3 variant from the Gulf represents a new thermotolerant species (S. thermophilum) (Hume et al. 2015). Similarly, Porites lobata in American Samoa hosted C15 independent of whether they grew on the fore reef or the warmer and more variable back reef (Barshis et al. 2010). Significant functional diversity also exists within clade C(Fisher et al. 2012) but this was not assessed in our study. Further, it has been shown that *Symbiodinium* C1 can be adapted locally to high temperatures (Howells et al. 2011) and that increased resistance to thermal stress can be achieved without changes in symbiont type due to acclimation of the coral holobiont (Bellantuono et al. 2012; Palumbi et al. 2014). It is therefore likely that both *Symbiodinium* and the coral host are locally adapted to the high temperature environment of the Kimberley, and that this is enhanced by the extreme temperature fluctuations of the intertidal environment (Figure 1).

Another important factor determining thermal tolerance is coral morphology. Branching *Acropora* in this study was much more susceptible to coral bleaching and mortality than massive *Dipsastraea*, results consistent with well-established patterns of morphologically dependent bleaching susceptibility (Marshall and Baird 2000; Loya et al. 2001), which are hypothesised to result from differences in tissue thickness. Typically, massive corals have thicker tissues than branching corals, which is consistent with more than 5 times higher tissue biomass per area in *Dipsastraea* compared to *Acropora* (Figure 5D, H). Thicker tissues can provide increased protection from light, more efficient self-shading of the symbiont cells and higher levels of energy reserves, thus improving overall resistance to light and heat stress (Loya et al. 2001; Thornhill et al. 2011).

3.4.4 Implications for the future of Kimberley coral reefs

In contrast to the wide-spread use of constant temperature regimes in bleaching experiments, all treatments in the present study experienced significant daily temperature variation (up to 5°C), thus mimicking in situ conditions experienced by these corals. This is also important because lower night temperatures can significantly reduce bleaching and mortality during periods of thermal stress (Mayfield et al. 2013). High flow rates, which are characteristic for Kimberley coral reefs, can further help reduce mortality and photoinhibition during thermal stress (Nakamura et al. 2005) and are therefore critical to properly assess bleaching susceptibility in a given reef habitat. The use of mini-flumes in this study provided experimental corals with moderate flow (12-15 cm s-1), which likely helped to moderate the amount of thermal stress received. However, we did not simulate aerial exposure and stagnant flow which would likely have further augmented heat and photooxidative stress during low tide slack water periods. It is therefore possible that during natural bleaching events in this region, bleaching susceptibility and mortality may be even higher than observed in this experiment, particularly in the intertidal and shallow subtidal when coinciding with mid-day spring tides. On the other hand, the high turbidity of Kimberley waters could also potentially mitigate light and heat stress to some extent and it remains to be determined how these factors play out during natural bleaching events.

Overall, our findings and those from previous work (Riegl 2003; Oliver and Palumbi 2011; Guest et al. 2012; Bay and Palumbi 2014; Grottoli et al. 2014; Palumbi et al. 2014) clearly show that corals exhibit significant potential for acclimatization and/or adaption and that the thermal (micro)environment plays a key role in this process. Specifically, highly variable temperatures rather than just high mean temperatures alone appear to enhance

the tolerance of coral to thermal stress. Such adaptive processes have important implications for predicting the spatial and temporal patterns of future coral bleaching events and may significantly delay the onset of frequent severe bleaching events worldwide (Teneva et al. 2012).

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3.6 Appendices

Supplemental Table S1. Results from one-way ANOVAs to test for significant differences in tank conditions between intertidal and subtidal tanks within each temperature (temp.) treatment. df=degrees of freedom, SS=sum of squares, TA = total alkalinity, Ω_{arag} = saturation state for aragonite.

Variable	Temp.	df	SS	*F*-statistic	*P*-value
Day Temp.	Ambient	1	0.0036	0.0046	0.9467
	Ambient +2°C	1	0.2728	0.8392	0.3705
	Ambient +3°C	1	0.1473	0.1592	0.6941
Night Temp.	Ambient	1	0.0006	0.0012	0.9726
	Ambient +2°C	1	0.2426	0.4478	0.5110
	Ambient +3°C	1	0.0277	0.0241	0.8782
$T_{Heating}$ - $T_{Control}$	Ambient	n/a	n/a	n/a	n/a
	Ambient +2°C	1	0.0006	0.0012	0.9728
	Ambient +3°C	1	0.0024	0.0034	0.9541
pH_T	Ambient	1	0.0000	0.0059	0.9424
	Ambient +2°C	1	0.0001	0.0678	0.8074

	Ambient +3°C	1	0.0002	0.5294	0.5072
pCO_2	Ambient	1	1.3067	0.0004	0.9855
	Ambient +2°C	1	118.1041	0.0834	0.7871
	Ambient +3°C	1	113.7962	0.2112	0.6697
TA	Ambient	1	10.1140	0.0613	0.8166
	Ambient +2°C	1	0.2166	0.0020	0.9664
	Ambient +3°C	1	8.8088	0.0708	0.8033
Ω_{arag}	Ambient	1	0.0000	0.0000	1.0000
	Ambient +2°C	1	0.0017	0.0250	0.8820
	Ambient +3°C	1	0.0014	0.0375	0.8559
Ammonium	Ambient	1	0.0012	0.1370	0.7301
	Ambient +2°C	1	0.0005	0.0232	0.8862
	Ambient +3°C	1	0.0039	3.3611	0.1407
Nitrate	Ambient	1	0.0013	1.2535	0.3256
	Ambient +2°C	1	0.0000	0.0628	0.8145
	Ambient +3°C	1	0.0036	1.0627	0.3609
Phosphate	Ambient	1	0.0000	0.0672	0.8082
	Ambient +2°C	1	0.0000	0.0240	0.8843
	Ambient +3°C	1	0.0001	0.0723	0.8014

Supplemental Table S2. Results from two generalized linear mixed model analyses to test for the effects of time, temperature (=Temp.), and environment (=Env.) on Fv/Fm and Qm of *Acropora aspera*. *P*-values ≤0.05 are highlighted in bold. Num df = numerator degrees of freedom, den df = denominator degrees of freedom.

Effect	Num df	Den df	*F*-statistic	*P*-value
Fv/Fm				
Temp.	2	44	370.39	**<0.0001**
Time	9	480	189.50	**<0.0001**
Temp. x Time	18	480	63.41	**<0.0001**
Env.	1	22	34.45	**<0.0001**
Temp. x Env.	2	44	5.41	**0.0079**
Env. x Time	9	480	7.80	**<0.0001**
Temp. x Env. x Time	18	480	6.51	**<0.0001**
Qm				
Temp.	2	44	22.05	**<0.0001**
Time	9	477	57.17	**<0.0001**
Temp. x Time	18	477	18.96	**<0.0001**
Env.	1	22	3.18	0.0882
Temp. x Env.	2	44	0.33	0.7224
Env. x Time	9	477	4.61	**<0.0001**
Temp. x Env. x Time	18	477	5.45	**<0.0001**

Supplemental Table S3. Results from two generalized linear mixed model analyses to test for the effects of time, temperature (=Temp.), and environment (=Env.) on Fv/Fm and Qm of *Dipsastraea* sp. *P*-values ≤0.05 are highlighted in bold. Num df = numerator degrees of freedom, den df = denominator degrees of freedom.

Effect	Num df	Den df	*F*-statistic	*P*-value
Fv/Fm				
Temp.	2	36	45.16	**<0.0001**
Time	9	485	176.94	**<0.0001**
Temp. x Time	18	485	44.36	**<0.0001**
Env.	1	18	5.88	**0.0260**
Temp. x Env.	2	36	3.10	0.0573
Env. x Time	9	485	6.97	**<0.0001**
Temp. x Env. x Time	18	485	4.82	**<0.0001**
Qm				
Temp.	2	36	21.23	**<0.0001**
Time	9	485	23.06	**<0.0001**
Temp. x Time	18	485	2.55	**0.0005**
Env.	1	18	1.64	0.2172
Temp. x Env.	2	36	9.00	**0.0007**
Env. x Time	9	485	3.26	**0.0007**
Temp. x Env. x Time	18	485	0.96	0.5091

Supplemental Table S4. Results from generalized linear mixed models to test for the effects of temperature (=Temp.) and environment (=Env.) on chlorophyll *a* per area, chlorophyll *a* per cell, symbiont density (=Dens.) and tissue biomass (=Biom.) of *Acropora aspera* and *Dipsastraea* sp. Post hoc Tukey tests were used when main effects (but no interaction terms) were significant. *P*-values ≤0.05 are highlighted in bold. Num df = numerator degrees of freedom, den df = denominator degrees of freedom. NB=ambient control, BL=ambient +2°C, BH=ambient+3°C. IT=Intertidal, ST=subtidal.

Factor	Effect	Num df	Den df	*F*-statistic	*P*-value	Tukey
Acropora aspera						
Chl *a* area^{-1}	Temp.	2	13	55.22	**<0.0001**	
	Env.	1	22	2.45	0.1317	
	Temp. x Env.	2	13	6.07	**0.0137**	
Chl *a* cell^{-1}	Temp.	2	13	9.26	**0.0032**	
	Env.	1	22	1.10	0.3055	
	Temp. x Env.	2	13	4.60	**0.0308**	
Dens.	Temp.	2	13	44.95	**<0.0001**	NB > BL=BH
	Env.	1	22	4.36	**0.0486**	IT > ST
	Temp. x Env.	2	13	1.23	0.3233	
Biom.	Temp.	2	13	2.40	0.1294	
	Env.	1	22	0.52	0.4778	
	Temp. x Env.	2	13	2.44	0.1264	
***Dipsastraea* sp.**						
Chl *a* area^{-1}	Temp.	2	36	110.72	**<0.0001**	NB > BL=BH
	Env.	1	18	9.64	**0.0061**	IT > ST
	Temp. x Env.	2	36	0.19	0.8297	
Chl *a* cell^{-1}	Temp.	2	36	5.72	**0.0069**	NB=BH > BH=BL
	Env.	1	18	0.04	0.8392	
	Temp. x Env.	2	36	0.17	0.8481	
Dens.	Temp.	2	36	65.42	**<0.0001**	NB > BL=BH
	Env.	1	18	9.17	**0.0072**	IT > ST
	Temp. x Env.	2	36	0.78	0.4667	
Biom.	Temp.	2	36	0.47	0.6275	
	Env.	1	18	7.42	**0.0139**	IT > ST
	Temp. x Env.	2	36	0.26	0.7736	

4 Impacts of the 2015/16 marine heatwave on Kimberley coral reefs

4.1 Introduction

Coral reefs are in serious decline worldwide due to a combination of increasing local and global anthropogenic pressures (Wilkinson 2008, Pandolfi et al. 2011). Rising atmospheric CO_2-concentrations are causing ocean warming, which leads to more intense and frequent mass coral bleaching events. To date, three global mass bleaching events (1998, 2010, and 2015/16) have been documented since the 1980s and were associated with El Niño-Southern Oscillation (ENSO) driven warming events (Hoegh-Guldberg 1999, Eakin et al. 2016, Hughes et al. 2017), highlighting the sensitivity of corals to marine heatwaves (Hobday et al. 2016). Bleaching most commonly occurs during periods of thermal stress when corals lose their algal dinoflagellate symbionts (*Symbiodinium* spp.), resulting in a pale or white appearance of the coral colony (Hoegh-Guldberg & Smith 1989, Porter et al. 1989, Jokiel & Coles 1990). Given that the majority of scleractinian corals meets most of their metabolic demand from carbon derived from symbiont photosynthesis (Muscatine et al. 1981), bleaching results in severe resource limitation and thus significantly weakens them. While bleached corals can sometimes recover, the physiological damage caused during bleaching often results in extensive coral mortality (McClanahan et al. 2004, Depczynski et al. 2013, DeCarlo et al. 2017). Therefore, warming-related mass bleaching events are among the greatest threats to coral reefs today (Hoegh-Guldberg et al. 2007, Hughes et al. 2017). Since these events can lead to mass mortality on regional to global scales, they impact both the diversity and functioning of coral reef ecosystems (Baker et al. 2008), and also threaten their socio-economic services on which millions of people worldwide depend (Moberg & Folke 1999).

In 2015/16, unusually high ocean temperatures associated with one of the strongest El Niño events on record triggered an unprecedented global coral reef crisis, resulting in what would become the third documented global mass bleaching event (NOAA 2015). This event was predicted to impact 38% of the world's coral reefs and has become the longest and most severe mass bleaching event on record (NOAA 2015, Hughes et al. 2017). It has impacted coral reefs in all three major ocean basins (Eakin et al. 2016, Perry & Morgan 2017, Hughes et al. 2017, DeCarlo et al. 2017, Xie et al. 2017, McClanahan 2017), and caused back-to-back bleaching in several locations for the first time (Eakin et al. 2016, CoE 2017). Coral reefs in the South China Sea, for example, experienced unprecedented mass bleaching with 40% coral mortality in 2015 (DeCarlo et al. 2017), while in the Maldives live coral cover declined by 75% due to severe bleaching in 2016 (Perry & Morgan 2017). Similarly, the Great Barrier Reef experienced the worst bleaching event in its history in 2016 (Hughes et al. 2017), followed by another severe bleaching event just one year later (CoE 2017).

In late 2015, the U.S. National Oceanic and Atmospheric Administration (NOAA)'s Coral Reef Watch predicted significant coral bleaching and/or mortality (alert levels 1 and 2) for most coral reefs in Western Australia (WA) during the austral summer 2016. NOAA's bleaching forecasts showed that the greatest heat stress would occur along the northern WA coast, particularly in the remote Kimberley region. This macrotidal region is one of the most extreme natural coral reef environments in the world, with tides up to 12m, strong tidal currents, turbid waters and sea surface temperatures (SST) exceeding 30°C for five months per year (Purcell 2002, Rosser & Veron 2011, Richards et al. 2015, Dandan et al. 2015). Highly diverse coral reefs exist throughout the Kimberley despite these extreme conditions (Richards et al. 2015). Interestingly, the highly fluctuating temperatures of intertidal reef habitats (up to 7°C daily) have been shown to enhance coral thermal tolerance (Schoepf et al. 2015), consistent with other work on thermally variable reef environments (Oliver & Palumbi 2011, Castillo et al. 2012). However, Kimberley corals were nevertheless not immune to severe heat stress simulated in a tank experiment (Schoepf et al. 2015), raising the question how they would respond to a natural bleaching event.

To date, regional-scale mass bleaching has been documented in WA only once during a strong La Niña event in 2010/11 (Abdo et al. 2012, Moore et al. 2012, Pearce & Feng 2013, Caputi et al. 2014, Zhang et al. 2017), but not during strong El Niño years (e.g. 1997/98, 2010) that caused mass bleaching in many other locations around the world. Although some offshore oceanic coral atolls in northern WA (e.g. Scott Reef) did bleach severely in 1998 (Goreau et al. 2000), WA has largely been considered to be at low risk from bleaching during

strong El Niño events. During the La Niña-driven heatwave in 2010/11, seawater temperatures exceeded normal summer temperatures by an average of 3°C for along the WA coast between 22°S (Ningaloo Reef) and 34°S (Cape Leeuwin) (Pearce & Feng 2013). This resulted, for example, in 12–100% bleaching at the Houtman Abrolhos Islands (Smale & Wernberg 2012, Abdo et al. 2012) and 79–92% at Ningaloo Reef (Depczynski et al. 2013). However, coral reefs in northern WA escaped the marine heatwave and bleaching. This included the Kimberley region. The predictions by NOAA's Coral Reef Watch for the austral summer 2016 raised significant concern for northern WA, given that regional-scale mass bleaching has never occurred in WA during a strong El Niño. We conducted extensive coral health surveys at three sites in the Kimberley region between January 2015 and May 2016 in response to NOAA predictions. Since El Niño events will likely increase in frequency and intensity due to climate change (Cai et al. 2014), understanding how these extreme climatic events impacts coral reefs in northwestern WA is critical to predict their persistence under continued ocean warming and climate change.

4.2 Material and Methods

4.2.1 Survey sites

Surveys were conducted in the subtidal and intertidal environments at Shell Island, Cygnet Bay (16°28'46.8"S, 123°2'36.6"E) in January, April and October 2016. These environments are described in detail elsewhere (Dandan et al. 2015, Schoepf et al. 2015). The tidal range in Cygnet Bay is ~8m. At low tide, the intertidal represents a shallow tide pool with a slack water period of several hours, resulting in extreme temperature fluctuations (up to 7°C daily, maxima of up to 37°C) and regular aerial exposure of shallow corals. In contrast, the subtidal is a more moderate temperature environment where aerial exposure of coral occurs only a few days per year during extreme low tides (Dandan et al. 2015). Branching *Acropora* colonies dominate coral cover in both environments. *In situ* seasonal temperatures in both environments are similar and range from ~22.0°C to 31.5°C (daily averages) (Dandan et al. 2015). Bleaching thresholds for both intertidal and subtidal corals were experimentally established to be ~32°C, ~1°C above the local MMM (Schoepf et al. 2015). We therefore used the long-term MMM of 30.827°C from NOAA's 5-km virtual station North Western Australia (NOAA Coral Reef Watch, version 2) for our analyses. The surveys in April 2016 were conducted concurrently with extensive aerial surveys of the region, covering the region between Montgomery Reef and the Dampier Peninsula (Hughes et al. 2017).

4.2.2 Bleaching surveys and sea surface temperature monitoring

Aerial surveys of ~30 reefs in the southwestern Kimberley were conducted between the Dampier Peninsula and Montgomery Reef on three days in late April 2016 when bleaching was particularly visible. We used light aircraft, flying at an elevation of approximately 150 m or less. Each reef was assigned by visual assessment to one of five categories of bleaching severity, using the same protocols as aerial surveys conducted on the Great Barrier Reef in 1998 and 2002: 0, < 1% of corals bleached; 1, 1–10%; 2, 10–30%; 3, 30–60%; and 4, > 60% of corals bleached. The accuracy of the scores was assessed by underwater ground-truthing (see next section).

For the in situ surveys, four to six 15m transects were conducted at randomized locations via intertidal walking. Care was taken to ensure that all transects were conducted at a similar depth. High-resolution photos of a 50×50 cm quadrat were taken every 0.5-1m along the transect line.

HOBO v2 temperature loggers (± 0.2°C; Onset Computer Corp) were deployed at each site except the remote Montgomery Reef, and continuously recorded in situ water temperature every 15 minutes during the study period (i.e., from 22 October 2015 to 06 April 2016 at Cygnet Bay). This time period was chosen to assess heat stress during the 12 weeks prior to the first survey time point and between the first and second survey time point. For Montgomery Reef, satellite-derived SSTs from NOAA's 5-km virtual station North Western Australia were used for a similar time period as at nearby Cygnet Bay (9 November 2015 to 23 April 2016). To assess cumulative heat stress, degree heating days (DHD) (Maynard et al. 2008) were calculated as the sum of all positive temperature anomalies (i.e., daily average SST exceeding the local MMM) over the previous 12 weeks;

this was found to provide more realistic estimates of heat stress than NOAA's methodology of accumulating only positive temperature anomalies ≥1°C (see Discussion). Large DHD values were converted to Degree Heating Weeks (DHW) by dividing by 7. The bleaching threshold was set at 1°C above the local MMM, which is generally thought to be the threshold for bleaching in most coral species (Glynn & D'Croz 1990, Atwood et al. 1992).

4.2.3 Photoquadrat analysis

Photoquadrats were analyzed using the software photoQuad(Trygonis & Sini 2012) by one person to keep observer bias equal across all photos. The outline of the quadrat within the photo was manually defined. Substrate type was defined using stratified random point counts (100 points), such that the spawn canvas was divided into sub-cells and points spawned within each cell in a random manner. This method ensures that at least one point is present in each sub-cell. The following types of substrate were distinguished: hard coral, soft coral, seaweed/seagrass/encrusting coralline algae/turf, sand/rubble, rock, and unknown. The 'unknown' category applied to quadrat areas that could not be unequivocally assigned to a substrate category since high water turbidity and rapidly changing water levels created challenging conditions. Hard corals were identified to genus level when possible and further assigned a morphology (i.e., branching, plate-like/plating, encrusting and mounding/sub-massive/massive). Each coral colony was scored using the following four health categories as a categorical bleaching score (McClanahan et al. 2004): unbleached (UB), moderately bleached (M: <50% of the colony bleached or colony pale), severely bleached (S: >50% bleached), and dead (D).

4.2.4 Statistical analysis

Prior to multivariate statistical analysis, the count data from the analyses of the photoquadrats were converted to percent abundance data and then square root transformed. The four health categories (UB, M, S, D) across all coral genera were statistically tested for differences between sites and time periods using Permutational Multivariate Analysis of Variance (PERMANOVAs), the Bray-Curtis similarity index and 9999 iterations. Transects served as replicates for each site. A two-way PERMANOVA was conducted to compare coral health across all genera between sites (two levels: intertidal and subtidal) and time points (three levels: January, April and October 2016). Additional one-way PERMANOVAs were conducted to test the effect of time on coral health across all genera in the intertidal and subtidal, respectively. Post-hoc pairwise comparisons were calculated, with p-values adjusted using the sequential Bonferroni correction. A third one-way PERMANOVA tested the effect of site on coral health across all genera in October 2016 only. Principal Component Analyses (PCA) were used to visualize the data. For statistical analyses, the software PAST, version 3.15, was used (Hammer *et al*., 2001). *P*-values ≤0.05 were considered significant.

4.3 Results

4.3.1 Patterns of heat stress

The general patterns of heat stress were similar at each site in Shell Island, Cygnet Bay. The corals started to experience positive DHW values, and thus heat stress, as early as in November 2015 as temperatures started to exceed the local MMM. By the first survey time point in January 2016, subtidal and intertidal corals had been exposed to 2.8 and 3.4 DHW, respectively. Heat stress increased to 4.3 DHW in the subtidal and to 4.5 DHW in the intertidal by the second survey time point in April, and peaked in the beginning of May 2016 (6.3 and 5.8 DHW, respectively). After that, DHW values started to decline at both sites and were back to zero by the end of July, indicating the end of the heat stress period. Thus, the corals no longer experienced any heat stress by the third survey time point in October 2016 (Figure 1).

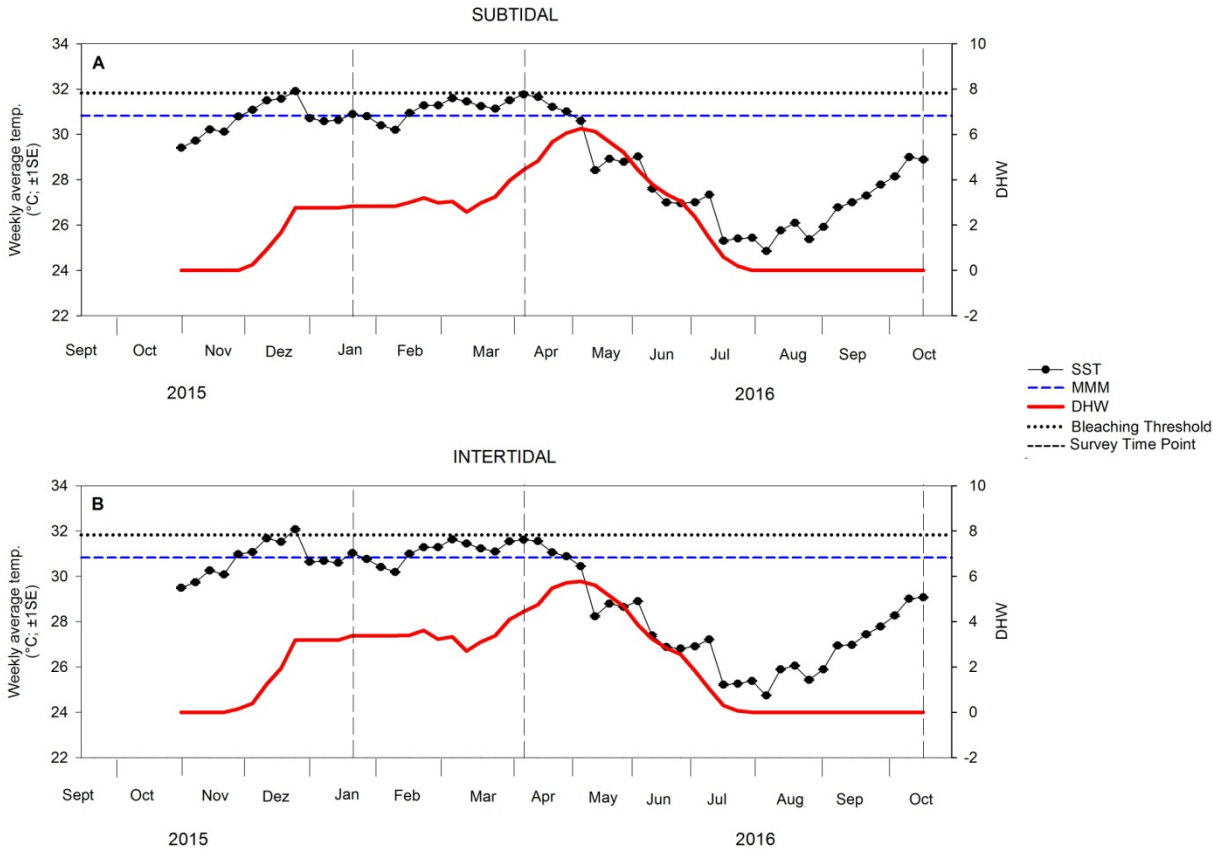

Figure 1: *In situ* sea surface temperature (SST) and Degree Heating Weeks (DHW) in the subtidal (A) and intertidal (B) at Shell Island, Cygnet Bay from October 2015 until October 2016. The Maximum Monthly Mean temperature (MMM) was set to 30.827 °C by NOAA Coral Reef Watch (2017, Version 2). Error bars are smaller than the symbols for the *in situ* sea surface temperature. The coral bleaching threshold was set to 32°C by Schoepf *et al*. (2015). Vertical dashed lines indicate the survey time points in January, April and October 2016.

4.3.2 Aerial bleaching surveys

The majority of coral reefs surveyed in the southwestern Kimberley had ~50% bleaching in April 2016 (Figure 2).

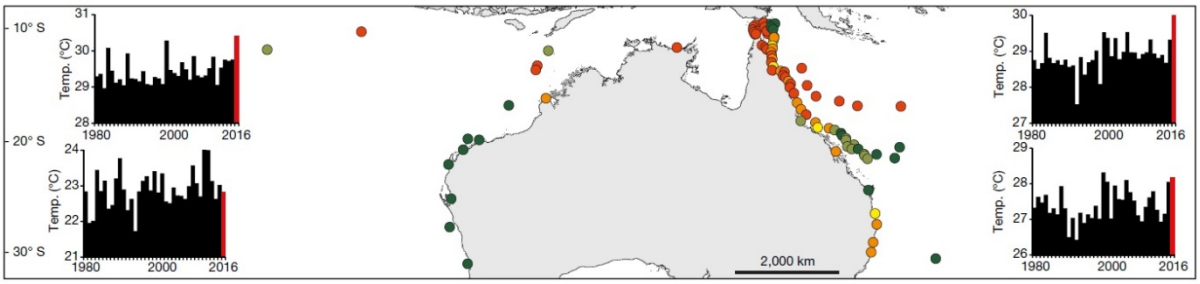

Figure 2. Bleaching severity during March to early April 2016 on both sides of Australia, including the Coral Sea and the eastern Indian Ocean. Colour bleaching scores: dark green (< 1% of corals bleached), light green (1–10%), yellow (10–30%), orange (30–60%), red (> 60%). Bar graphs show mean sea surface temperatures during March for each year from 1980 to 2016 for northern and southern latitudes on either side of Australia. The red bar highlights the north–south disparity in 2016. From Hughes et al. 2017.

4.3.3 Coral health over time

The heat stress period that caused severe bleaching in the Kimberley in April 2016 led to different health conditions of corals in the subtidal and intertidal of Shell Island ~ six months after the bleaching (Figs. 3, 4).

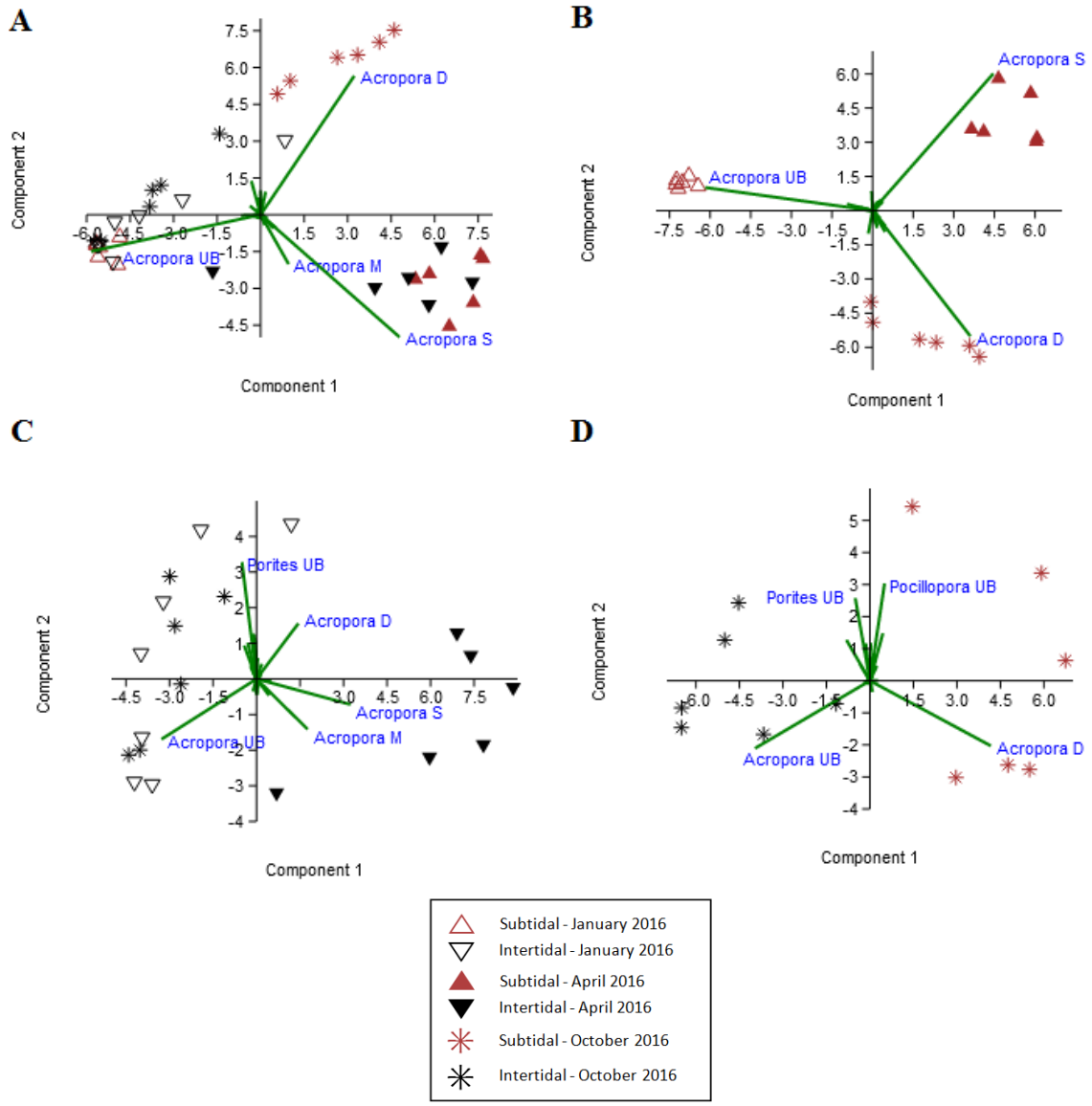

Figure 3: Principal Component Analyses (PCA) of coral health across all genera for A) the two sites (intertidal and subtidal) and the three time points (January, April and October 2016), B) the three time points in the subtidal, C) the three time points in the intertidal, and D) the subtidal and intertidal site in October 2016 only. UB = unbleached, M = moderately bleached, S = severely bleached, D = dead. The symbols represent individual transects. The greatest influence on overall coral health is symbolized by vectors, naming coral genera and their associated health status.

When comparing coral health over time in the subtidal site only, a significant effect of time was observed, with all three time points being significantly different and clearly separated from each other (Table 1, Figure 3B). January transects clustered along the vector for healthy *Acropora*, consistent with 94.3 ± 2.7 % of the live coral cover being comprised of healthy corals (Figure 4). April transects clustered along the vector for severely bleached *Acropora*. By this time point, 5 ± 2.5 % and 75.6 ± 4.8 % of the live coral cover were moderately and severely bleached, respectively (Figure 4). October transects clustered along the vector for dead *Acropora*. By this time point, 71.3 ± 10.6 % of the live coral cover were dead. Only 28.4 ± 10.5 % survived this extreme climatic event (Figs. 3A, 4).

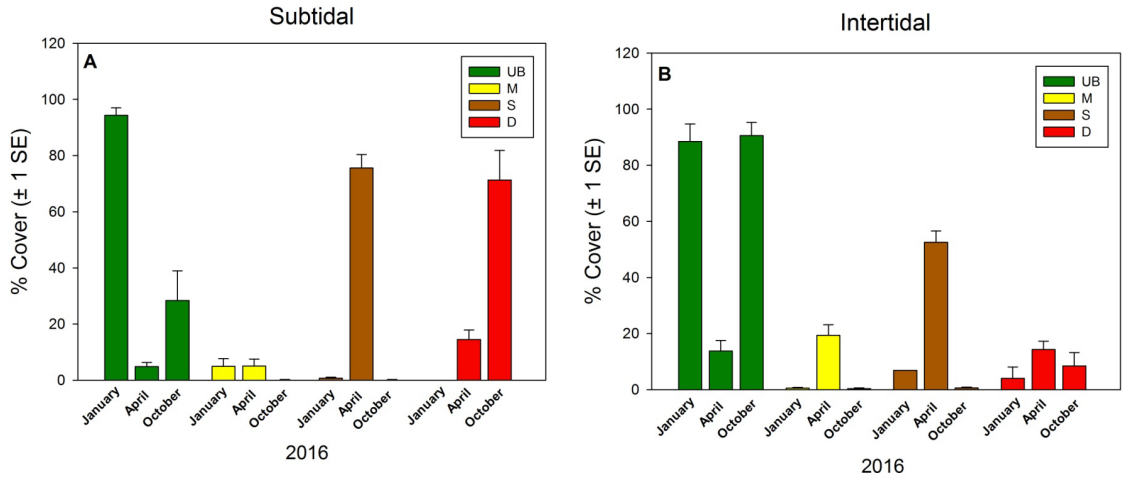

Figure 4: Changes in coral health (% of coral cover) pooled for all coral genera between January, April and October 2016 in the A) subtidal and B) intertidal, respectively. U = unbleached, M = moderately bleached, S = severely bleached, D = dead.

When comparing coral health over time in the intertidal site only, a significant effect of time was also observed (Table 1, Figure 3C). However, at this site, only the April time point differed significantly from the other two survey time points. This was because the April transects clustered along the vector for bleached and dead *Acropora*. By this time point, 19.36 ± 3.8 %; of the live coral cover were moderately bleached, 52.6 ± 4 % were severely bleached, and 14. 28 ± 3 % were found dead (Figure 4). In contrast, the January and October transects both clustered along the vector for unbleached *Acropora* and *Porites*. In January, 88.5 ± 6.2 % of the live coral cover were healthy and by October, 90.6 ± 4.7 % were again unbleached (Figs. 3, 4). Only 8.5 ± 4.8 % of the corals had died in consequence of the heat stress by October (Figs. 3, 4). Because of this high percentage of unbleached corals, the intertidal was significantly different from the subtidal in October (Table 1, Figure 3D).

Table 1: Results of the various Multivariate Permutational Analyses of Variance (PERMANOVA). Bold p-values indicate significant differences (p≤0.05). N.A. = not applicable

Multivariate Analysis	**Factor**	***df***	**F-Values**	**p-Values**	**Pairwise results (p-values)**
Two-Way PERMANOVA to assess the effect of site and time on coral health	Site	1	4.3467	**0.0038**	N.A.
	Time	2	17.445	**0.0001**	
	Interaction	2	3.3913	**0.0009**	
One-Way PERMANOVA to assess the effect of time on coral health in the subtidal	Time	2	16.13	**0.0001**	January vs April: **0.0014** April vs October: **0.0020** January vs

					October: **0.0037**
One-Way PERMANOVA to assess the effect of time on coral health in the intertidal	Time	2	7.265	**0.0001**	January vs April: **0.0007** April vs October: **0.0020** January vs October: 0.2206
One-Way PERMANOVA to assess the effect of site on coral health in October 2016	Site	2	8.543	**0.0024**	Intertidal ≠ Subtidal

4.4 Discussion

We show here that marine heatwaves associated with extreme climatic events such as the record-strength 2015/16 El Niño have the potential to cause unprecedented regional-scale mass bleaching, even in coral reef regions that harbour naturally heat-tolerant corals and have escaped mass bleaching in previous El Niño years. This occurred in the macrotidal Kimberley region in northwestern Australia during the austral summer of 2016. This region features highly diverse and naturally stress-resistant coral reefs that thrive under conditions that corals from more typical reef environments would usually not survive (e.g. long aerial exposure, daily temperature fluctuations of up to 7°C and temperature maxima of up to 38°C during low tide (Dandan et al. 2015, Schoepf et al. 2015). A recent study showed that these highly fluctuating temperatures enhance the thermal tolerance of Kimberley corals (Schoepf et al. 2015). Nevertheless, Kimberley coral reefs experienced unprecedented mass bleaching in April 2016 in response to severe heat stress (~4-9 DHW, Figure 1), with more than 71-80% of live coral cover being bleached at our two Kimberley study sites (Figure 3). Aerial surveys of 25 reefs, conducted over the same time period, further confirmed the regional scale of this mass bleaching event and showed that most reefs in the southern Kimberley had 30-60% bleaching (Hughes et al. 2017). This demonstrates that even naturally heat-resistant corals from extreme temperature environments such as the Kimberley region are not immune to marine heatwaves and extreme climatic events.

These findings are consistent with experimental work on Kimberley corals showing that heat stress equivalent to ~3 DHW resulted in severe bleaching and mortality, although heat stress in that study was applied over a much shorter time period (<2 weeks) (Schoepf et al. 2015). Coral communities in other naturally extreme temperature environments, such as the Persian/Arabian Gulf where corals have the world's highest bleaching thresholds (~35-36°C), are also not immune to severe heat stress and have suffered from multiple episodes of bleaching associated with significant mortality over the last three decades (Coles & Riegl 2013). This suggests that even naturally heat-resistant corals are significantly threatened by continued ocean warming, as it is currently unclear whether they can increase their heat tolerance over the time scales required to cope with future climate change.

The marine heatwave causing the 2016 mass bleaching in the Kimberley was characterized by long-lasting exposure to small positive temperature anomalies that rarely exceeded the local MMM by more than 1°C (Figure 1a-c). SSTs already rose above the local MMM in November 2015, resulting in increasing heat stress and DHW from that point onwards. As a consequence, some coral genera (i.e. *Seriatopora* and *Stylophora*) were already severely bleached in January 2016 but did not substantially influence overall coral community health due to their overall low abundance. Thus, Kimberley reefs experienced cumulative heat stress for ~5 months in a row, demonstrating that even small positive temperature anomalies can cause severe bleaching and mortality when persisting for a long period of time. This was confirmed by the significant bleaching of massive corals, although they are typically more resistant to heat stress (Loya et al. 2001).

The choice of the MMM value can have a huge influence on calculated heat stress and DHW values even if *in situ* temperature data are available. We are confident that NOAA's MMM of 30.827°C for the virtual station North Western Australia is appropriate since it is in close agreement with experimentally established bleaching thresholds (MMM +1°C) of ~32°C for Kimberley corals (Schoepf et al. 2015). However, NOAA's climatology changes from time to time and has only recently been updated, emphasizing the challenge of defining baseline temperatures that corals are adapted to. The latest version now lists a much lower MMM (29.903°C) for this station than version 2 which we used for this study. This lower MMM value is most likely much too conservative because Kimberley corals do not show any signs of visible bleaching or declines in their photochemical efficiency (Fv/Fm) when exposed to daily average temperatures of ~31°C for almost two weeks (Schoepf et al. 2015). While we cannot exclude the possibility that Kimberley corals may have acclimatized to rising SSTs, these discrepancies highlight the importance of *in situ* temperature records and physiological data, especially in complex macrotidal reef environments that create particular challenges for satellite-derived SST monitoring.

The large spatial scale of the 2016 mass bleaching event in northern WA is, to the best of our knowledge, unprecedented. Kimberley offshore oceanic atolls (e.g. Scott Reef) have bleached previously (Gilmour et al. 2013, AIMS 2016); however, the vast inshore Kimberley region has escaped any bleaching prior to 2016. While it is possible that such events may have gone unnoticed or undocumented due to the remoteness of this region, this is unlikely given that local Aboriginal people have no record of such an event in their oral history (V. Schoepf and C. Cornwall, pers. comm.). The 2015/16 El Niño coincided with an extremely unusual and dry wet season in the Kimberley, and also with the most extreme tides of the year. This likely resulted in increased temperature, light and UV stress as well as longer aerial exposure of shallow corals. Furthermore, the absence of major storms and cyclones would have prevented mitigation of both heat and light stress (Carrigan & Puotinen 2014). The combination of these factors most likely contributed or exacerbated heat stress (Takahashi & Murata 2008), thus resulting in unprecedented bleaching.

On small spatial scales, the bleaching susceptibility of Kimberley coral communities differed significantly depending on small-scale differences in their thermal environment. During peak heat stress, coral health of subtidal coral communities at Cygnet Bay had declined significantly more than in intertidal communities, as indicated by a much higher percentage of severely bleached corals (Figure 3). This was the case despite similar exposure to heat stress (4.3 and 4.5 DHW in the subtidal and intertidal, respectively) and community composition (dominated by *Acropora* corals). These observations confirm experimental work showing that subtidal *Acropora* and *Dipsastraea* corals at Cygnet Bay have a lower thermal tolerance than their intertidal counterparts (Schoepf et al. 2015). Although both intertidal and subtidal environments have similar average temperatures, they differ substantially with regards to daily temperature fluctuations and the frequency of aerial exposure during low tide (Schoepf et al. 2015). Given that symbiont types did not differ between intertidal and subtidal corals (Schoepf et al. 2015), our findings provide further evidence that extreme temperature fluctuations (up to 7°C daily in the intertidal) represent a mechanism that enhances coral thermal tolerance (Oliver & Palumbi 2011, Castillo et al. 2012, Schoepf et al. 2015).

We also show here that naturally heat-resistant Kimberley corals have a remarkable capacity to fully recover from severe bleaching (up to 80 %) within less than six months (Figs 2, 3). However, this rapid and complete

recovery was not observed in all reef habitats and strongly depended on known differences in coral heat tolerance driven by spatial variations in the thermal environment. Corals in the intertidal showed a very strong recovery capacity as ~ 91 % were found unbleached less than six months after the bleaching event. In contrast, ~71 % of the corals in the subtidal did not survive this extreme climatic event. This was the case, although both sites were exposed to similar heat stress (4.5 and 4.3 DHW in the IT and ST, respectively) during the bleaching and had a relatively similar bleaching response. Furthermore, both sites had a similar community composition (> 71 % branching *Acropora* spp.) prior to the bleaching event, indicating that the heat stress susceptibility of the entire coral community should have been roughly similar. This suggests that other environmental and/or biological factors influenced the different recovery capacities.

The higher recovery capacity of intertidal compared to subtidal corals is consistent with known differences in heat tolerance between intertidal and subtidal coral populations at the study site (Schoepf et al 2015a). Specifically, the higher heat tolerance of intertidal corals was attributed to the more variable and extreme thermal environment of the intertidal as it is known that strong daily temperature fluctuations can increase the resistance of corals to heat stress (Oliver & Palumbi, 2011; Schoepf et al., 2015a). Kimberley intertidal corals experience highly fluctuating temperatures (up to 7 °C daily) and long periods of aerial exposure (Rosser & Veron, 2011; Richards et al., 2015; Schoepf et al., 2015a) whereas subtidal corals experience a more stable temperature regime and are only exposed to air for a few hours during extreme spring tides (Rosser & Veron, 2011; Schoepf et al., 2015a). Although bleaching was also extensive in the intertidal, the higher heat tolerance of intertidal corals likely resulted in significantly fewer severely bleached coral and the subsequent rapid and complete recovery observed here. These findings are consistent with a study from American Samoa which showed that corals from moderately variable back-reef pools suffered much higher mortality during heat stress than corals from a highly variable pool (Oliver & Palumbi, 2011). Thus, coral communities growing in a thermally variable environment, like the intertidal on Shell Island, may be the winners under future ocean warming. Further research is required to determine the physiological and genetic mechanisms underlying the higher heat tolerance and recovery capacity of intertidal corals, and to follow the long-term recovery of the subtidal coral community.

The 2016 mass bleaching event in the Kimberley region is the first such event to occur in WA during a strong El Niño year (Zhang et al. 2017). Although offshore oceanic atolls in northwestern Australia (e.g. Scott Reef) bleached during El Niño events before (Gilmour et al. 2013, AIMS 2016), regional-scale mass bleaching in WA has to date only occurred once during a strong La Niña year in 2010/11 (Abdo et al. 2012, Moore et al. 2012, Pearce & Feng 2013, Caputi et al. 2014, Zhang et al. 2017). The 2010/11 heatwave primarily affected mid to southern WA, devastating coral communities from Ningaloo Reef to Rottnest Island. This study shows that the geographic footprint of the 2010/11 and 2016 mass bleaching events in WA differed substantially. Bleaching patterns across these two events suggest that northern WA is particularly at risk of bleaching during strong El Niño years, whereas mid to southern WA is vulnerable during strong La Niña years (Zhang et al. 2017). Moreover, our findings highlight that regional-scale mass bleaching may now occur in WA during both El Niño and La Niña events (Zhang et al. 2017). As El Niño Southern Oscillation events will likely become more frequent and intense with continued climate change (Cai et al. 2014), these findings have significant implications for the future resilience of coral reefs in WA.

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5 Climate change as registered by Sr/Ca, Li/Mg, δ^{11}B and B/Ca systematics in an ~100-year old *Porites* coral from the thermally extreme Kimberley region of northwestern Australia

Chen X, McCulloch MT, Wei G. Climate change as registered by Sr/Ca, Li/Mg, δ^{11}B and B/Ca systematics in an ~100-year old *Porites* coral from the thermally extreme Kimberley region of northwestern Australia. Manuscript in review for *Paleoceanography*.

5.1 Introduction

Rising levels of CO_2 are driving both warming and acidification of the world's oceans (IPCC, 2014) and posing significant and growing risks to the sustainability of coral reef ecosystems (Hughes et al., 2017). Thus, global warming acting together with severe and more frequent El Niño events is now repeatedly subjecting coral reef ecosystem to regimes of thermally induced stress and resultant coral bleaching (Heron et al., 2016; Hughes et al., 2017; Spalding and Brown, 2015). However, whether corals living in naturally thermally extreme environments may potentially be better adapted to withstand the increasing effects of global warming is poorly understood, especially on decadal and longer timescales over which warming is occurring.

There are few constraints available on the combined effects of ocean warming and acidification on the key process of coral calcification (De'ath et al., 2009; Hoegh-Guldberg et al., 2007). For the tropical reef systems, it has been show that over longer timescales the response of coral calcification to climate change can vary significantly among different environments; for instance, a long-term declining calcification in the inner Great Barrier Reef while outer-shelf reefs show a trend of increasing calcification (D'Olivo et al., 2013). For the naturally extreme reef environment, less is known about either the long-term responses of coral calcification or their capability to better adapt and/or acclimate to the climate change.

The skeletal geochemistry of scleractinian corals is sensitive to both external environment changes and the processes controlling internal biomineralization (Cohen and McConnaughey, 2003; Gagan et al., 2000; Gagnon et al., 2012; Trotter et al., 2011). Therefore, the geochemical proxy information preserved within the skeleton of long-lived corals can be used to properly quantify the coral responses to the ongoing climate change. To assess the impacts of ocean warming on the reef environment, coral Sr/Ca and Li/Mg proxies serve as important tools to generate reliable temperature reconstruction (Smith et al., 1979; McCulloch et al., 1999; Fowell et al., 2016; Montagna et al., 2014). In addition, the newly developed δ^{11}B and B/Ca proxies offer a mechanistic approach to resolve the carbonate chemistry of coral calcifying fluid (CF) where coral calcification takes place, helping to unveil the key processes that chemically control coral calcification (McCulloch et al., 2017). Briefly, coral δ^{11}B is thought to constrain the pH of extracellular calcifying fluid (pHcf) (Trotter et al., 2011; McCulloch et al., 2012), and B/Ca ratios is an indicator for CO_3^{2-} ion and thus dissolved inorganic carbon in the calcifying fluid (i.e. DICcf) when combined with pHcf estimated from δ^{11}B (Holcomb et al., 2015; McCulloch et al., 2017). By examining the δ^{11}B and B/Ca systematics in tropical *Porites* spp. corals, it is found that corals can interactively up-regulate the DICcf and pHcf in the CF to achieve higher aragonite saturation state and thus rapid but stable calcification, with DICcf being more than twice of seawater DIC (DICsw) with inversely varying levels pHcf of from ~8.3 to ~8.5 compared to seawater pH of ~ 8.0 (McCulloch et al., 2017). Here we provide new evidence of the ability of corals to up-regulate the carbonate chemistry at their site of calcification under extreme levels mainly naturally occurring thermal stress. We show that this ability to up-regulate the essential carbonate chemistry parameters (DICcf and pHcf) controlling the calcifying fluid composition is critical in determining their resilience to the climate change.

The Kimberley region is an ideal environment to conduct such studies, being a naturally extreme environment with abundant and highly diverse range of corals species as well as coral reef structures (Dandan et al., 2015; Richards et al., 2015). Corals from this region are regularly subject to extreme levels of thermal stress, with large seasonal temperature variability which ranges from ~22 °C to over 31 °C and prolonged periods during

the summer >30 °C (Dandan et al., 2015; Richards et al., 2015). Moreover, the highly dynamic tidal regimes of the Kimberley which can exceed 12 m over tidal cycles also leads to a large daily temperature variations of from ~ 30 °C to >36°C (Dandan et al., 2015). In this study, we report both high-resolution (seasonal) and annually-resolved geochemical records (i.e. Sr/Ca, Li/Mg, $\delta^{11}B$, and B/Ca) from a long-lived (~100-year old) *Porites* coral living in the nearshore Kimberley region of northwest Australia in order to investigate the long-term temperature variability and the coral resilience in this thermally extreme environment. We show that by utilizing these proxies, the impacts of long-term climate change in thermally extreme the reef ecosystems can be evaluated and hence the resilience and responses of coral reefs in such environments can be better understood.

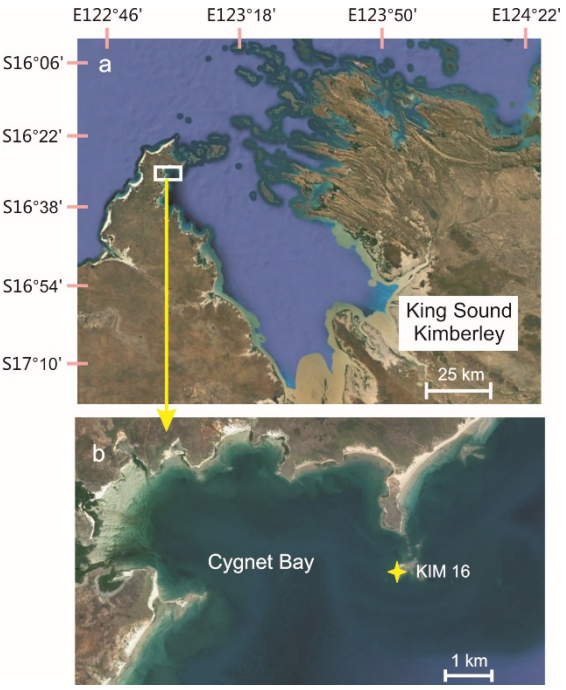

Figure 1: Location of *Porites* coral core offshore Shell Island, Cygnet Bay, analysed for historical temperature (SST) and Fitzroy River discharge into King Sound.

5.2 Materials and Methods

5.2.1 Coral sampling

Coral core KIM16 was drilled in April 2016, from a living massive *Porites* spp. coral colony at a water depth of 2 m in low tide, on the subtidal zone of Shenton Bluff (also known as Shell Island) which is located on the north end of Cygnet Bay, Kimberley region of northwest Australia (Figure 1). The core was cut into slices (~5 mm thick) along the plane of the vertical growth axis, and X-rayed to reveal the density and annual growth bandings that were used as a guide for sampling. As the growth banding was not always clear and regular, linear extension rates were measured on clear bandings to calculate the average for each section which then was used as a reference for collecting annual subsamples along the main growth axis. Additionally, high-resolution subsamples were collected continuously at an ~ 1.4 mm interval from the top for a period of ~20 years (i.e. 1995-2015).

5.2.2 Coral calcification rate

Coral calcification rate (g cm^{-2} yr^{-1}) was obtained by the product of the linear extension rate and the density. The linear extension rate was measured directly using the X-ray negative prints, and the skeletal density was measured by using Coral-X-radiograph Densitometry System.

5.2.3 Geochemical analyses

Elemental ratios (B/Ca, Sr/Ca, Li/Mg) were measured for both high-resolution and annually-resolved samples. The measurements were undertaken at the University of Western Australia by using Q-ICPMS (X-series II, Thermo Fisher Scientific) followed the method presented in Holcomb et al. (2015). About 10 mg powders were dissolved in 0.51 N HNO_3 (prepared from sub-boiling distilled (Savillex DST-1000) HNO_3), with sub-aliquots diluted in 2% HNO_3 spiked with a calibration solution to a final concentrations of ~100 ppm Ca and 10 ppm Ca respectively, according to the element suite to be measured (e.g. Holcomb et al., 2015). The remaining sample solution was then used in boron purification described below. The coral standard JCp-1 with certified B/Ca value (0.4596 mmol/mol, Hathorne et al. (2013a)) was used as an internal standard.

The top 7-year high-resolution samples and the annually-resolved samples representing the period from 1919 to 2016 were determined for their boron isotopic composition, which was measured via MC-ICPMS (NU Plasma II, Cameca instruments) at the University of Western Australia, following the methods described in McCulloch et al., (2014). The dissolved sample was loaded onto the preconditioned cation and anion columns which contain 0.6 mL of AG50W-X8 resin and 1.0 mL of AG1-X8 resin, respectively, to remove both cation and anion matrix existing in the carbonate solution (e.g. Ca^{2+}, Sr^{2+}, and SO_4^{2-} ions). The columns were then eluted with 4× 0.5 mL of 0.075 N HNO_3, yielding an ~200 ppb boron solution ready for $\delta^{11}B$ measurement using MC-ICPMS. Typical operating conditions and measurement strategies are summarized in McCulloch et al., (2014). Sample measurements were bracketed by ERM AE121 ($\delta^{11}B$ = 19.9 ± 0.6%, BAM, Germany), and coral standard JCp-1 (*Porites* spp.: Geological Survey of Japan) was chemically treated repeatedly and measured along with the coral samples to monitor the analytical quality of the samples. The measurements yielded $\delta^{11}B$ values of 24.60 ± 0.24 ‰ (2 SD) for JCp-1, consistent with previously reported values within analytical errors (e.g. Foster et al., 2006; Gonfiantini et al., 2003; McCulloch et al., 2014; Wang et al., 2010).

5.2.4 Boron geochemical proxies for carbonate chemistry

Boron isotopic systematics. Boron exists as two forms in aqueous solutions, boric acid $B(OH)_3$ and borate ion $B(OH)_4^-$, with the proportions being pH dependent. Boron isotopes consist of ^{11}B (~80%) and ^{10}B (~20%), and are commonly defined as $\delta^{11}B = (({}^{11}B/{}^{10}B)_{sample}/({}^{11}B/{}^{10}B)_{SRM951}-1)\times 1000$, with isotopic fractionation between the boron species also being pH dependent. As is confirmed by recent co-precipitation experiments (Mavromatis et al., 2015) and first-principles theoretical calculations (Balan et al., 2016), tetragonal B (i.e. $B(OH)_4^-$) is the predominant structural species that substitutes for CO_3^{2-} in aragonite. Therefore, boron isotopic compositions of coral skeleton ($\delta^{11}B_{carb}$) inherit the composition of borate ion ($\delta^{11}B_{B(OH)4^-}$) in the extracellular calcifying fluid (CF), and then serve as an archive of pH_{cf}, according to the following the equation (Zeebe and Wolf-Gladow, 2001):

$$pH_{cf} = pK_B - \log\left[\frac{\delta^{11}B_{sw}-\delta^{11}B_{carb}}{\alpha_{B3-B4}\times\delta^{11}B_{carb}-\delta^{11}B_{sw}+1000\ (\alpha_{B3-B4}-1)}\right] \qquad \text{Eq. (1)}$$

where $\delta^{11}B_{sw}$ is the B isotope composition of seawater ($\delta^{11}B_{sw}$ = 39.61‰; Foster et al., 2010) and the B isotope fractionation factor ($\alpha_{B3\text{-}B4}$) is 1.0272 estimated by Klochko et al., (2006). The B dissociation constant (pK_B) is well defined with a value of 8.597 at 25 °C and a salinity of 35 (Dickson, 1990a). The temperature dependence of the calculated pH_{cf}, was estimated using Sr/Ca and Li/Mg multiproxy-SST (see Section 3), and the mean salinity of 34 as determined by Dandan et al. (2015).

B/Ca ratios. The incorporation of $B(OH)_4^-$ where it substitutes for CO_3^{2-} in the aragonite lattice during $CaCO_3$ precipitation is a pH dependent process, with the partition coefficient given by $K_D = (B/Ca)_{CaCO3}/ (CO_3^{2-}/ B(OH)_4^-)_{cf}$. From the experimental constraints $K_D = 0.00297\exp(-0.0202(H^+)_T)$ (Holcomb et al., 2016; McCulloch et al., 2017). Given the limited range in pH_{cf} of from ~8.1 to 8.5 gives a corresponding limited range in K_D of from 2.65 to 2.75 (x10^{-3}). This is similar to the systematic error (~ ±7%) in the experimentally determined K_D (Holcomb et al., 2016; McCulloch et al., 2017). Thus combined with pH_{cf} derived from $\delta^{11}B_{carb}$ and assuming that $(B)_{cf}$ is equal to total boron concentration in seawater, the concentration of carbonate ion within the calcifying

fluid ($(CO_3^{2-})_{cf}$) can be calculated using B/Ca ratios with the following equation:

$$(CO_3^{2-})_{cf} = K_D \times (B(OH)_4^-)_{cf}/(B/Ca)_{CaCO_3} \qquad \text{Eq. (2)}$$

Thus with both $\delta^{11}B$ and B/Ca ratios the pH_{cf} and $(CO_3^{2-})_{cf}$, and hence the complete carbonate system parameters can be determined. Calculations use carbonate species dissociation constants of Mehrbach et al., (1973) as re-fit by Dickson and Millero (1987), and the $KHSO_4$ from Dickson (1990b) and the aragonite solubility constants of Mucci (1983). Aragonite saturation state of the CF (Ω_{cf}) is calculated using $(CO_3^{2-})_{cf}$ with the assumption that $(Ca^{2+})_{cf}$ is similar to seawater.

5.2.5 Environmental data

For recent observational temperatures, satellite 0.25 × 0.25 degrees monthly Optimum Interpolation Sea Surface Temperature (OISST) records are adopted (Reynolds et al., 2007), and are compared with the in-situ sea surface temperature (SST) recorded from September 2010 onward (Dandan et al., 2015). Although exhibiting a smaller seasonal amplitude the high-resolution, OISST is found to strongly correlate with the in-situ SST. To correct this reduced seasonal amplitude, the longer adjusted OISST record is used for geochemical proxy calibration with the correction obtained by the linear calibration between OISST and in-situ logger temperature.

A SeaFET Ocean pH sensor (±0.05 pH) was deployed at the coral site from August to October 2016. The in-situ measured pH_T (total scale) shows a strong correlation with water temperature, yielding a seasonal relationship of $pH_{sw} = -0.011 \times T + 8.31$ ($r^2 = 0.79$, n=20992). As indicated by Dandan et al., (2015), seawater pH in Cygnet Bay is higher in winters and lower in summers, with seasonal pH range of ~0.05. Such seasonal variations in seawater pH are consistent with that in the open ocean, reflecting the seasonal changes in temperature as the main driver of the carbonate reaction coefficients and pCO_2 solubility.

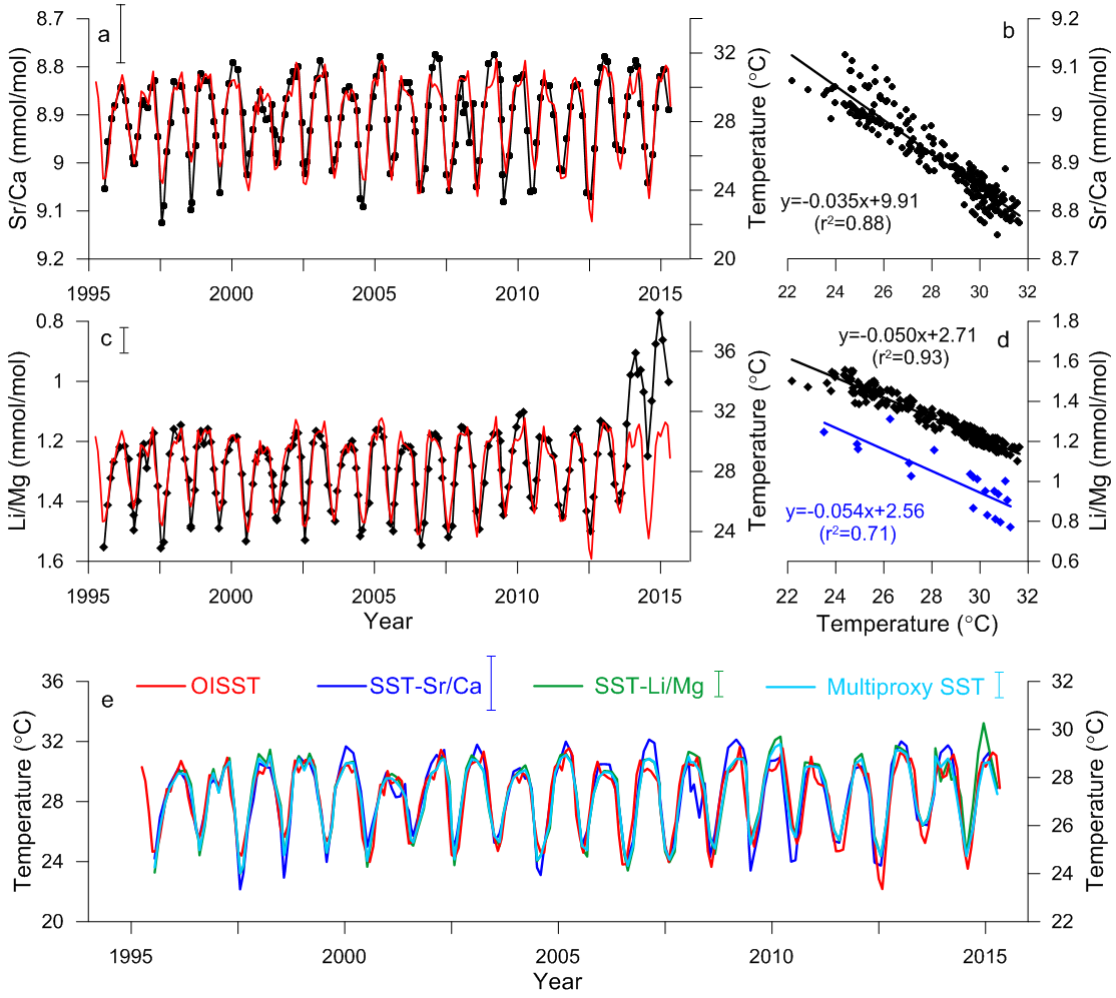

Figure 2. Sr/Ca and Li/Mg ratios calibrations: a, Sr/Ca ratios vs. adjusted OISST; b, regression analysis of Sr/Ca ratios and temperature; c, Li/Mg ratios vs. adjusted OISST; d, regression analysis of Li/Mg ratios and temperature (black: bulk data; blue: 2014/15 section); e reconstructed SSTs by each calibration.

5.3 Results

5.3.1 Sr/Ca and Li/Mg ratios and temperature calibration

High-resolution Sr/Ca and Li/Mg ratios for KIM16 coral are presented in Figure 2. Both ratios exhibit strong seasonal cycles in phase with temperature, but the amplitude of Li/Mg cycles follows more closely the monthly variability of the adjusted OISST than that of Sr/Ca cycles (Figure 2a&c). During the period between 2014 and 2015, however, the Li/Mg ratios show significant offsets with the adjusted OISST, with ~20-30% lower values as well as larger variation amplitude (Figure 2c). These anomalously lower Li/Mg values are also apparent in the annual records (not shown), and will be discussed in detail in Section 4.1. If data for the anomalous are excluded, Sr/Ca and Li/Mg ratios are well correlated on both seasonal and annual scales, with correlation coefficients of 0.93 and 0.66, respectively (Table 1).

To establish the Sr/Ca-temperature relationship, the maximum Sr/Ca values are matched to the minimum temperature and the minimum Sr/Ca values are matched to the maximum temperature. Assuming linear growth between the maximum and the minimum, the intervening SST can then be correlated with the interpolated Sr/Ca ratios. Using this approach an approximately linear relationship is found between Sr/Ca and SST, yielding a regression equation of SST (°C) = $-0.035 \times$Sr/Ca (mmol/mol) + 9.91 (r^2 = 0.88; n = 238; Figure 2b). Accordingly, the calculated monthly-resolved Sr/Ca-SST is shown in Figure 2e.

Following the same approach, linear regressions are also found between Li/Mg ratios versus SST (Figure 2d), with a stronger linear relationship r^2 of 0.93 (n = 218) before 2014 and reduced r^2 of 0.71(n = 20) after 2014. Accordingly, the Li/Mg-SST is calculated and shown in Figure 2e. The Li/Mg-SST calibration is further compared with other multispecies Li/Mg-temperature calibration (Fowell et al., 2016; Montagna et al., 2014), and found to broadly fit the exponential trend (Figure 3a), yielding a new exponential regression: Li/Mg = 5.53exp(–0.051×T) (r^2 = 0.96, n= 299). This equation is then used to derive a new Li/Mg-SST (referred to henceforth as the "multispecies Li/Mg-SST") shown in Figure 3b. The derived multispecies Li/Mg-SST show generally good consistency with the linear Li/Mg-SST and the adjusted OISST before 2014, while as already noted after 2014 the anomalous Li/Mg data significantly bias the SST reconstruction (Figure 3b).

To better constrain temperature variations, multiple linear regressions are carried out by using an online Regression Tools (http://www.xuru.org/rt/TOC.asp), which combines Sr/Ca and Li/Mg ratios to generate multiproxy SST calibrations. The derived regression equations are SST (°C) = –4.69×Sr/Ca (mmol/mol) – 15.75×Li/Mg (mmol/mol) + 90.57 (r^2 = 0.91; n = 218; before 2014), and SST (°C) = –25.22×Sr/Ca (mmol/mol) – 3.27×Li/Mg (mmol/mol) + 255.97 (r^2 = 0.93; n = 20; after 2014). The resultant multiproxy SST shows excellent agreement with the adjusted OISST as well as the individual Sr/Ca- and Li/Mg-SST (Figure 2e).

5.3.2 Annual temperature reconstruction

Annual SST records are calculated by utilizing the seasonal calibrations respectively, and are shown in Figure 3a. Generally, the single-proxy and multiproxy SSTs are broadly consistent within the analytical precision. The annual SST reconstruction is dominated by interannual to interdecadal variability with relatively smaller fluctuations before 1970s and larger variability thereafter, and exhibits a gradually increasing trend from 1919 to the present (~0.009±0.003°C/yr). The annual temperature is normalized to the period 1961 to 1990 and demonstrates positive anomalies in the recent decades with the average anomaly of ~0.65°C (Figure 3b).

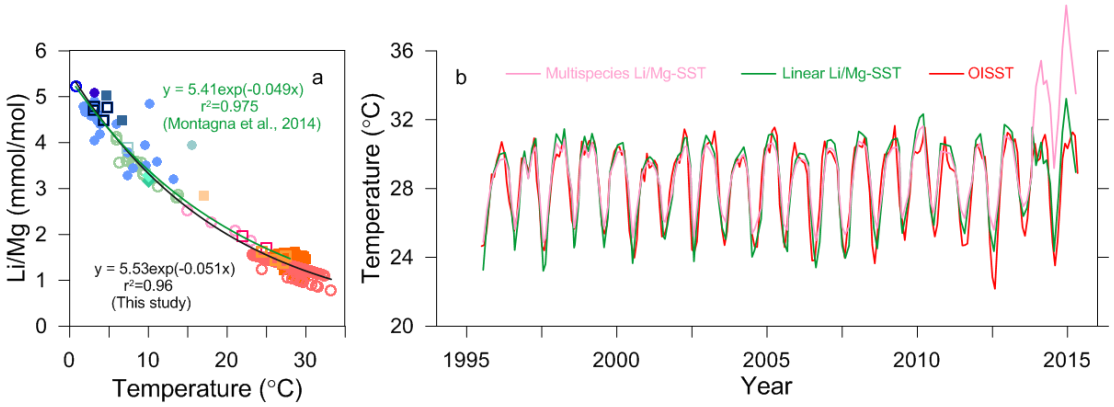

Figure 3. Coral multispecies Li/Mg-T calibration: a, Exponential regression analysis of Li/Mg ratios for a wide range of coral species at seawater temperature between 0 ºC and 31ºC; data from Case et al. 2010, Fowell et al. 2016 and Montagna et al. 2014; b, reconstructed Li/Mg-SST and the adjusted OISST.

5.3.3 Boron systematics and the CF carbonate chemistry

The DIC_{cf} and pH_{cf} in the coral calcifying fluid are calculated by using $\delta^{11}B_{carb}$ and B/Ca ratios according to the Eq. (1) and Eq. (2), respectively, and are shown in Figure 5&6. The concentration of DIC_{cf} is about ~2- to 2.6-fold higher than that of seawater (~1850 to ~1882 µmol/kg, Dandan et al., 2015), and the pH_{cf} is up-regulated by ~0.5 pH units relative to ambient seawater pH (~8.00) (Figure 5b, c& 6c, d). Enhancement in both DIC_{cf} and pH_{cf} leads to elevated aragonite saturation state in the calcifying site with Ω_{cf} varying from ~16 to ~20 (Figure 5d&6e). On seasonal timescales, the carbonate chemistry in CF all exhibits strong seasonal cycles, with the DIC_{cf} changing in phase with temperature and pH_{cf} varying in an opposite trend with temperature. Furthermore, the seasonal variability of pH_{cf} is much larger than that expected from artificial experiments (pH_{cf}^{*}(calc) = 0.32pH_T +

5.2; Trotter et al., 2011; McCulloch et al., 2012) which shows a subdued variation in pH_{cf} (Figure 5c, dashed line). These findings are similar to that of McCulloch et al., (2017) where DIC_{cf} and pH_{cf} exhibit out-of-phase variability on seasonal scale, with higher DIC_{cf} and lower pH_{cf} values occurring in summers and lower DIC_{cf} and higher pH_{cf} values occurring in winters, and thereby produce an enhanced and relatively stable level of Ω_{cf} (Figure 5e). On annual timescales, the carbonate chemistry of CF shows interannual to interdecadal variations with the amplitude comparable to that on seasonal timescale, except the DIC_{cf} which shows reduced variability though a significant shift occurred in 1990s (Figure 6). The DIC_{cf} and pH_{cf} still exhibit antithetical variations on longer timescale, but the Ω_{cf} no longer co-varies with DIC_{cf} as it does on seasonal timescale.

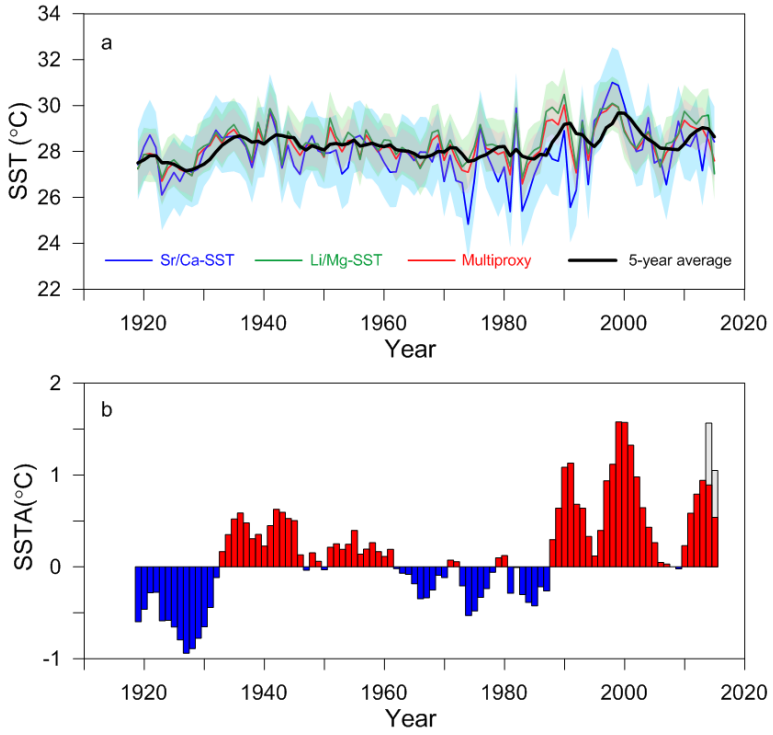

Figure 4. Annual temperature records in Kimberley region: a, SST reconstructions based on coral Sr/Ca, Li/Mg, and the combined multiproxy, respectively. Bold line is the five-year running average of the multiproxy-SST. The shaded areas indicate the errors for each SST reconstruction. b, multiproxy-SSTA normalized to the 1961-1990 period. The grey bars stand for the temperature anomaly calculated from Sr/Ca-SST to supplement the aberrant Li/Mg values in 2014-2015.

Based on the IpHRAC model which combines internal pH regulation of the calcifying fluid with abiotic calcification (McCulloch et al., 2012), theoretical calcification rate (G) can then be quantified as $G = k(\Omega_{cf} -1)^n$ (Burton et al., 1977), where the constants k and n being temperature dependent constant and order of the reaction, respectively. The calculated calcification rate changes in phase with temperature on seasonal scale (Figure 5e), and shows reduced variability on annual timescale (Figure 6b). Compared with the measured calcification rate (Figure 6b, blue curve), the calculated (Figure 6b, brown curve) is consistently lower, but both the measured and calculated rates are sub-parallel and demonstrate relatively stable variability for the past century. The calculated calcification rate varies within the range of 0.6 to 1.0 g $cm^{-2}yr^{-1}$(Figure 6d, brown curve), whereas the measured rate fluctuates from ~1.2 to 1.6 g $cm^{-2}yr^{-1}$, with the consistent offset being attributed to limitations in the accuracy of the inorganic calcification rate parameters. The latter rate is slightly lower compared to the coral living in more typical tropical reef environments (De'ath et al., 2009; D'Olivo et al., 2013).

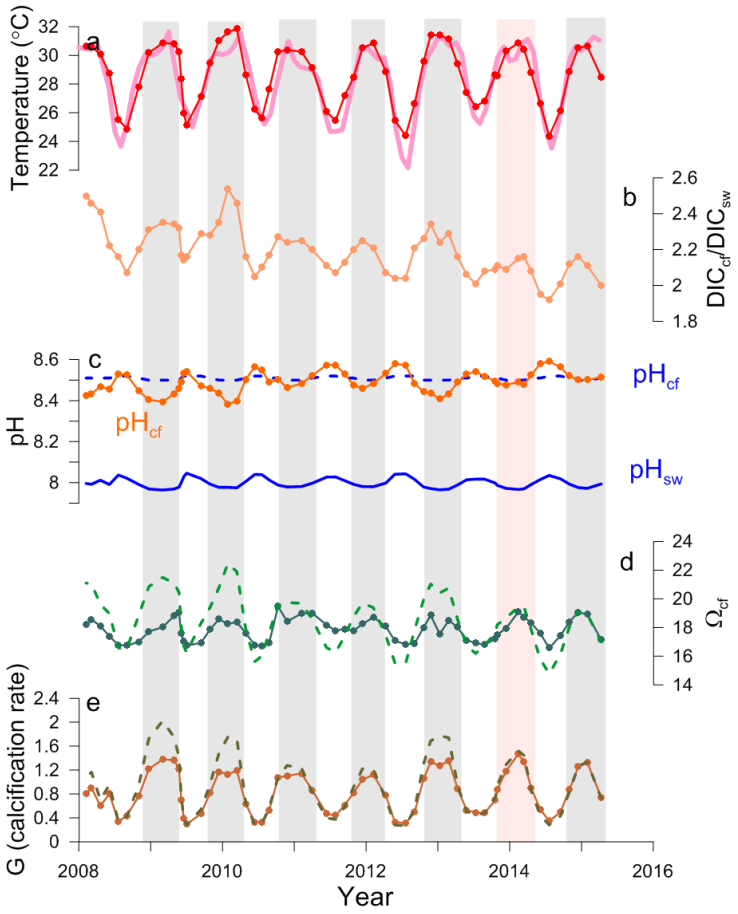

Figure 5. Seasonal variations of temperature and carbonate chemistry in coral calcifying fluid estimated from geochemical records for *Porites* coral from Kimberley region of northwest Australia: a, temperature; b, pH_{cf}; c, DIC_{cf}/DIC_{sw}; d, aragonite saturation state (Ω_{cf}); e, theoretical calcification rate (G). Solid lines indicate the variation of pH_{sw} (blue) as calculated from pH_{sw} = -0.011T +8.31 (see Section 2.4), and the adjusted OISST (pink), respectively. Dashed lines indicate parameters calculated according to less variable pH_{cf}^{*}derived from the artificial experiments that pH_{cf}^{*} (calc) = 0.32pH_T +5.2 (McCulloch et al., 2012; Trotter et al., 2011). The gray bars indicate the summer time of each year and the pink one indicates the depressed DIC_{cf}/DIC_{sw} and pH_{cf} during intensified heat stress in 2013/2014.

5.4 Discussion

5.4.1 SST reconstruction in the Kimberley region

Anomalous Li/Mg ratios. The anomalously low Li/Mg ratios in the 2014-2015 section are present in both the high-resolution and annually-resolved records. The X-ray of this section shows normal growth bands with the Sr/Ca, U/Ca and Ba/Ca ratios measured in this section all being within normal ranges. Such abnormal decreases in Li/Mg ratios have also been reported by Hathorne et al. (2013b), and are ascribed to "a biological effect on the incorporation into the biogenic aragonite" (Hathorne et al., 2013b). A closer inspection of the elemental compositions in this section shows that the decreased Li/Mg ratios arise mainly from the greatly elevated Mg but little-affected Li contents. It is noted that during the 2015-2016 summer the Kimberley was subject to an unprecedented bleaching event suggesting the possibility of a thermal stress related influence on the Mg/Ca ratio. The offset is also outside the range of the multispecies Li/Mg calibration and thus cannot be reconciled with an SST-based bias (Figure 2e & 3b).

The abrupt changes in coral skeletal Mg concentration have been reported on both very fine (micrometer) (Case et al., 2010; Holcomb et al., 2009; Meibom et al., 2004, 2008) and coarser (micrometer) scales (Clarke et

al., 2017; Frankowiak et al., 2013; Lazreth et al., 2016). On micrometer scale, increased proportion of the center of calcification (COC) in the section of the skeleton would lead to significant increase in Mg contents, as the COC tends to be Mg enriched (Case et al., 2010; Holcomb et al., 2009; Meibom et al., 2004, 2008). On millimeter scales, both skeletal diagenesis and heat stress can cause great variations in skeletal Mg abundance, but such changes are normally accompanied with changes in other trace elements (Clarke et al., 2017; Frankowiak et al., 2013; Lazreth et al., 2016). For instance, the transformation of aragonite to calcite skeleton can induce decreases in Sr, B, and Li, with concomitant increases in Mg contents (Frankowiak et al., 2013; Lazreth et al., 2016). The heat stress can inhibit coral growth and therefore lead to anomalous increases in Sr/Ca ratios and decreases in Mg/Ca ratios (Clarke et al., 2017), or the breakdown in the seasonality of geochemical proxies (D'Olivo and McCulloch, 2017).

In our study, however, the anomalous increase in Mg abundance without accompanying offsets in other elements (e.g. Sr, U, Li, and B) suggests that such changes may be caused by a Mg specific factor. Although the increased temperature in 2014-2015 may create an intensified heat stress to Kimberley corals, the increased Mg/Ca ratios and less affected growth rate are in contrary to what has been found in more typical tropical corals under thermal influences (Clarke et al., 2017; D'Olivo and McCulloch, 2017). While the underlying mechanism remains unclear, the potential biological effects on Mg incorporation suggest that anomalous data with Mg/Ca > 5 mmol/mol should be excluded or treated separately when carrying out Li/Mg temperature calibration. Consequently, these data are not considered in the following discussion.

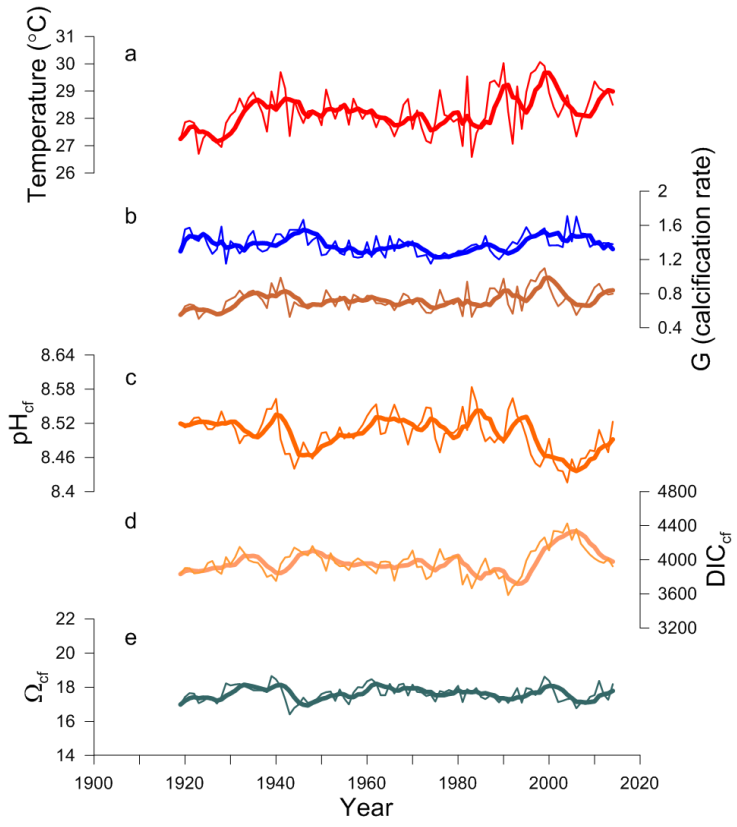

Figure 6. Annual variations of carbonate chemistry in coral calcifying fluid estimated from geochemical records for *Porites* coral from Kimberley region of northwest Australia: a, pH_{cf}; b, DIC_{cf}/DIC_{sw}; c, Ω_{cf}; d, theoretical calcification rate (G); e, temperature. Bold lines represent 5 year running averages.

Assessment of Li/Mg-SST and Sr/Ca-SST systematics in a thermally extreme environment. The well-fitted Kimberley Li/Mg calibrations with the universal exponential curve (Figure 3a) corroborates the Li/Mg-temperature relationship on both intercolonial and interspecies basis regardless of their inhabiting environment. However, the generated multispecies Li/Mg-SST time-series seems to slightly overestimate the winter minimum temperature while the summer maximum temperature is basically in good agreement with the adjusted OISST (Figure 3b). Therefore, site-specific Li/Mg-temperature calibration is still required to improve the reliability of Li/Mg-based temperature reconstruction.

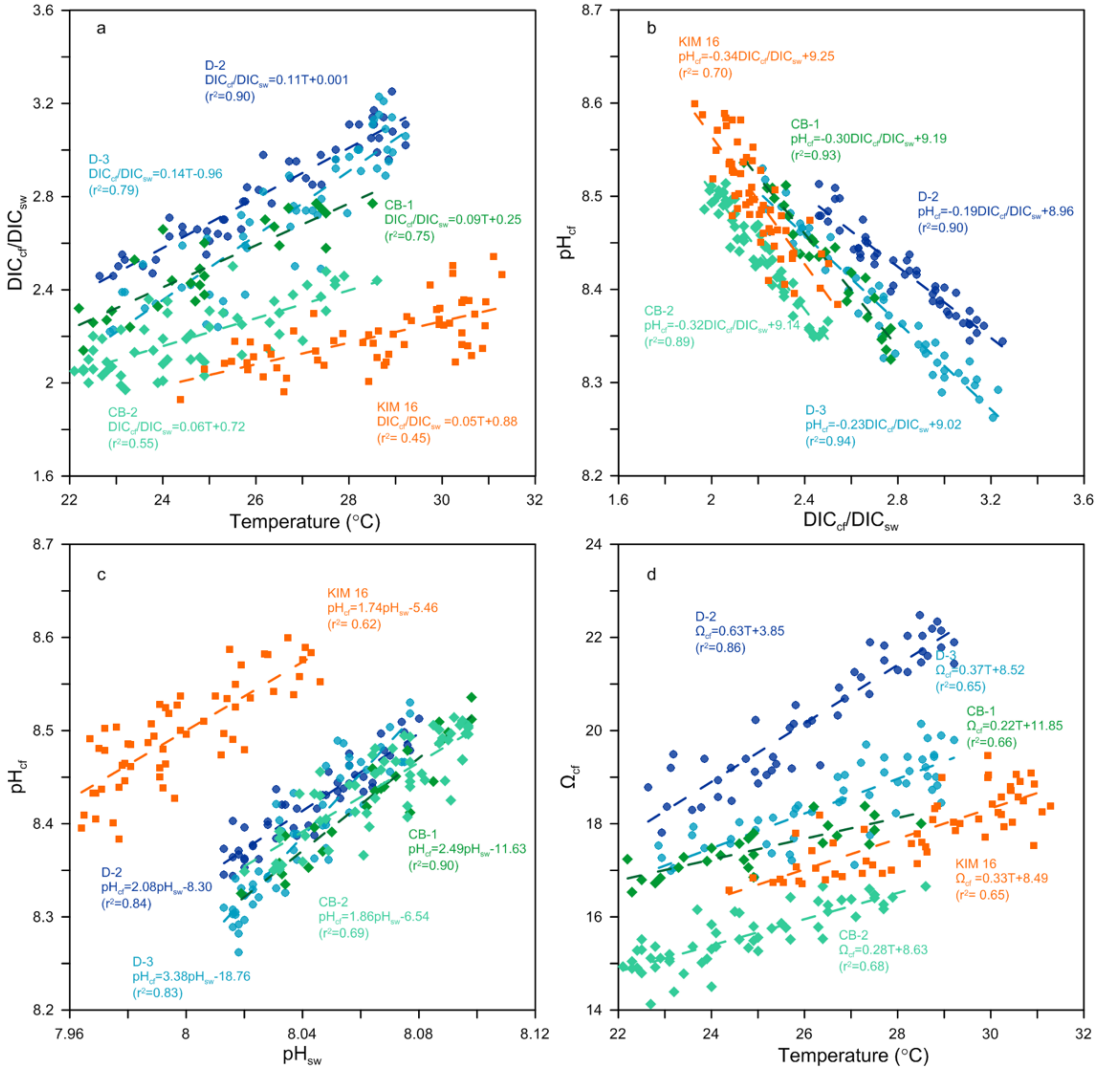

Figure 7. Relationships between coral internal carbonate system parameters, temperature among five *Porites* coral colonies from different reef sites: a, DIC_{cf}/DIC_{sw} vs. temperature; b, pH_{cf} vs. DIC_{cf}/DIC_{sw}; c, pH_{cf} vs. pH_{sw}; d, Ω_{cf} vs. temperature. Coral D-2 and D-3 are from Great Barrier Reef (Davies Reef), and coral CB-1 and CB-2 are from Ningaloo Reef, Western Australia (McCulloch et al., 2017).

The reconstructed SST records using site-specific Sr/Ca and to a lesser entent Li/Mg calibrations are all consistent with the adjusted OISST (Figure 2e), confirming the fidelity of coral Sr/Ca and Li/Mg thermometers in a naturally extreme environment. The Sr/Ca-temperature sensitivity in Kimberley is ~0.035 mmol/mol/ºC (Figure 2b), considerably lower than that found in most tropical corals which average at ~0.060 mmol/mol/ºC (Gagan et al., 2012, and the references therein). The Li/Mg-temperature sensitivity (~0.050 mmol/mol/ºC) is comparable to that found in other *Porites* corals (~0.048 and ~0.060 mmol/mol/ºC; Hathorne et al., 2013), but

is significantly different to *Siderastrea siderea* corals (~0.097 and ~0.033 mmol/mol/ºC for forereef and backreef, respectively; Fowell et al., 2016). These all highlight the importance of site- and species-specific calibrations for coral-based temperature reconstruction (Fowell et al., 2016; Hathorne et al., 2013). Importantly though by applying the monthly-resolved proxy calibrations to annual SST reconstruction, it is found that the reconstructed annual SST using combined multiproxy approach (i.e. Li/Mg and Sr/Ca) produces an improved and arguably more robust temperature estimate than that derived from single-proxy determination (Figure 4).

Table 1 Intercorrelations between coral geochemical records on both seasonal and annual resolution. *

		B/Ca	Sr/Ca	Li/Mg (bulk data)	Li/Mg (without anomalous data)
Seasonal	$\delta^{11}B$	0.8	0.48	-	0.64
	B/Ca		0.8	0.35	0.87
	Sr/Ca			0.81	0.93
Annual	$\delta^{11}B$	0.8	0.34	-	0.22
	B/Ca		0.57	0.33	0.39
	Sr/Ca			0.54	0.66

*Correlation coefficients with *p* value below 0.05 are shown.

Annual temperature variations in the thermally extreme environment. Estimates of the Sr/Ca and Li/Mg multiproxy-SST appear to show a long-term trend towards warmer temperature of recent decades, (Figure 4), which agrees with the other coral-based temperature reconstructions in the Indian Ocean (Zinke et al., 2008, 2014). Superimposed on this trend are significant interannual to decadal fluctuations, of which the amplitude is also enhanced after the 1970s. In addition, the positive temperature anomalies of the past ~20 years which are as high as ~1.5 ºC compared to the 1961-1990 mean suggest an intensified thermal stress in Kimberley region under the global warming background.

Table 2 Intercorrelations between carbonate chemistry in coral calcifying fluid. *

	DIC_{cf}	Ω_{cf}	G	T
pH_{cf}	-0.94	-0.42	-0.73	-0.71
DIC_{cf}		0.51	0.7	0.6
Ω_{cf}			0.82	0.71
G				0.89
pH_{cf}	-0.86	0.39	0.26	-0.47
DIC_{cf}			0.22	0.28
Ω_{cf}			0.57	0.28
G				0.94

*Correlation coefficients with *p* value below 0.05 are shown.

5.4.2 Calcifying Fluid (CF) carbonate chemistry and calcification in Kimberley coral

Seasonal dynamics in coral CF carbonate chemistry. Although corals are subject to extreme thermal influences in Kimberley region, typical compositions and seasonal cycles in the carbonate chemistry of coral CF are observed (Figure 5). The 2-fold enrichment in DIC_{cf} corroborates the significant contribution from metabolic CO_2 (Erez, 1978; Furla et al., 2000; Holcomb et al., 2009), and it combined with up-regulated pH_{cf} act together to maintain a significantly enhanced aragonite saturation state in coral CF, with Ω_{cf} being ~5 to 6-fold higher than that of ambient seawater. Such features are similar to those found in corals growing in more typical tropical oceans (McCulloch et al., 2017), which emphasizes the inherent commonality of the interactions between coral metabolism and internal carbonate chemistry. Additionally, this also suggests that such interactions in Kimberley corals are maintained at 'normal levels' even under the most thermally extreme environmental conditions.

Table 3 Intercolonial comparison of coral internal carbonate chemistry

	DIC_{cf}/DIC_{sw}	DIC_{cf} μmol/kg	pH_{cf}	ΔpH [b]	Ω_{cf}
D-2 [a]	3.04	5549	8.42	0.38	20
D-3 [a]	2.99	5318	8.40	0.36	18
CB-1 [a]	2.69	4883	8.43	0.37	17
CB-2 [a]	2.33	4322	8.44	0.37	16
KIM16	2.33	4073	8.49	0.49	17

a Data from McCulloch et al., 2017.

b $\Delta pH = pH_{cf} - pH_{sw}$.

However, as can be seen from Figure 5b, the DIC_{cf} has decreased gradually by ~10% from ~2010 to the present. This magnitude of decrease in DIC_{cf} is unlikely induced by the changes in seawater DIC_{sw}, possibly suggestive of a decline in the coral's ability to concentrate DIC in the calcifying fluid. This is consistent with the observation that, the DIC_{cf} decreases are accompanied by significant increases in monthly summer water temperature in Cygnet Bay (Figure 2&S2), which has exceeded the maximum monthly mean (31 ºC) in Kimberley region (Schoepf et al., 2015). In particular, during 2013/14 the increased summer and winter temperatures have resulted in not only reduced DIC_{cf}/DIC_{sw}, but also weakened seasonal variability. Although such temperature increases did not trigger any bleaching in this coral, the declines in coral internal DIC_{cf} provides evidence that the recent warming has induced an intensified level of stress on the corals and zooxanthellae metabolism leading to the progressive reduction in DIC_{cf}/DIC_{sw} observed in the Cygnet Bay *Porites*. These findings also suggest that metabolic CO_2 production is not simply a linear response to increasing temperature. Once the temperature increases above a critical threshold level, this deleteriously affects metabolic functions of corals and their symbionts, for example by declines in photochemical efficiency or tissue biomass, with the DIC_{cf}/DIC_{sw} being impacted.

Nevertheless, it seems that pH up-regulation is barely if at all affected by the increased temperature, since pH_{cf} still remains at an elevated level and shows an increasing trend in response to the decreased metabolic DIC_{cf} input (Figure 5b, c). Therefore, the aragonite saturation state shows only relatively small disturbances by the increasing temperature (Figure 5d). This implies that coral can manipulate its internal carbonate chemistry to maintain a favorable calcifying environment even when coping with increasing temperature. Thus of breakdown the ability of corals to up-regulate the pH of their calcifying fluid due to the loss of metabolic support for Ca-ATPase pump is likely to be catastrophic and represents the major vulnerability of corals as future levels of heat stress increase at a rate that exceeds the capacity of corals to acclimatise.

Long-term variations in coral calcification and the CF carbonate chemistry. Although the nearshore Kimberley region exhibits a clear warming trend, no significant trend is found the coral calcification rate over the past century (Figure 6 a&b). With respect to the coral internal CF carbonate chemistry, the DIC_{cf} shows subdued variability compared to that on seasonal scale, except a significant enhancement occurring around the 1990s (Figure 6d). The long-term trend of DIC_{cf} seems to follow the temperature, showing a marginal increase from the 1920s to the present, while in certain periods DIC_{cf} shows opposite variations with the temperature, especially during the recent decade. Therefore, the overall relationship between DIC_{cf} and temperature is weak, with a correlation coefficient of 0.31 ($p < 0.001$; n= 96) (Table 2), significantly lower compared to that on seasonal timescale (r = 0.60; $p < 0.000001$; n = 58) (Table 2). This suggests that the more limited annual temperature range and possibly thermal stress may attenuate the relationship between DIC_{cf} and temperature on longer timescales. In contrast to DIC_{cf}, pH_{cf} shows a long-term decreasing trend on the annual timescale, and it remains well correlated to DIC_{cf} with a significant correlation coefficient of –0.87 ($p< 0.0000001$, n= 96) which consistent with the strong seasonal inter-relationship between them. Therefore, similar to what we observed on seasonal timescale the antithetical variations in DIC_{cf} and pH_{cf} help to maintain a relatively stable Ω_{cf} on the longer timescale. Nevertheless, it is noted that the influences of DIC_{cf} on Ω_{cf} seems to be weakened, and pH_{cf} becomes the dominant controller of the variation of Ω_{cf} on annual scale (Table 2). The positive relationship between pH_{cf} and Ω_{cf} is opposite that found on seasonal scale, which is possibly due to the relatively smaller variability of DIC_{cf} on annual timescale that does not fully account the changes in carbonate ions (CO_3^{2-}) which is induced by pH_{cf} variations (Table 2).

According to the IpHRAC model, reduced variability in Ω_{cf} leads to a generally more stable rate of calcification. This is consistent with the measured rates of calcification (Figure 6b blue curve) on annual timescales (Figure 6b, brown curve), with). However, the systematic differences between the theoretical IpHRAC model and measured rates of calcification indicate that this is not simply an inorganically controlled process but also subject to strong biological or physicochemical controls. For example the skeletal organic matrix also play important roles in coral calcification by controlling energetic factors during crystal synthesis, for example, by providing sites for nucleation and directing the growth morphology (Cuif et al., 1996; Teng et al., 1998; Von Euw et al., 2017). Additionally calcification rates are strongly dependent on the coral architecture, in particular the effective surface area over which symbionts are distributed. Therefore, it is not surprising that there can be large offsets between the calculated and the measured calcification rate. Nevertheless, these two records show divergent variations in certain periods, especially during the warming events. For instance, during the warming trend in early 1990s coral calcification is suppressed despite that the predicted calcification rate being increased. Similarly, during the recent warming (from 2005 onward), the calcification rate tends to also decrease contrary to the predicted increase. These all signify that the responses of coral calcification to environment changes are biologically limited instead of directly controlled by the thermodynamics of inorganic precipitation. This is consistent with the observation that increased levels of thermal stress is impairing coral growth despite maintenance of elevated levels of Ω_{cf}. This is consistent with elevated 'threshold' levels of Ω_{cf} being an essential pre-requisite for calcification, is not necessarily rate limiting (D'Olivo and McCulloch, 2017). Clearly other physiologically dependent factors such rates of production of metabolites, DIC etc are rate limiting especially under conditions of increased thermal stress.

Intercolonial comparisons. The CF carbonate chemistry of Kimberley coral is compared to that of Davies and Ningaloo reef from previous study (Figure 7) (McCulloch et al., 2017). The DIC_{cf}/DIC_{sw} is found to vary significantly on both intercolonial and regional basis (Figure 7a; Table 3). Generally, Davies reef tends to have higher DIC_{cf}/DIC_{sw} levels than that in Ningaloo reef and Kimberley. Large differences also exist between colonies from the same reef site (e.g. Ningaloo CB-1 and CB-2; Table 3). Different DIC_{cf}/DIC_{sw} levels may reflect different coral's ability to concentrate DIC (i.e. production of metabolic CO_2) where coral tissue biomass and symbionts density may play important roles. The lower DIC_{cf}/DIC_{sw} in Kimberley *Porites* (KIM16) likely suggests the reduced coral production of metabolic CO_2 under high temperature stress. Unlike DIC_{cf}/DIC_{sw}, pH_{cf} exhibits minor differences among colonies, though the pH_{cf} in KIM16 tends to be slightly higher than others (Figure 7b; Table 3). The reduced variability of pH_{cf} among colonies compared to DIC_{cf} gives rise to the suggestion that the

reverse relationship between pH_{cf} and DIC_{cf} observed on temporal scale does not hold on an intercolonial basis. This supports the contention that the Ca-ATPase pumping may play an important role in maintaining critical levels of pH_{cf} up-regulation among colonies (Georgiou et al., 2015; McCulloch et al., 2012; Trotter et al., 2011). Interestingly, the ΔpH (pH_{cf}-pH_{sw}) of Kimberley coral is about 0.1 pH unit higher than that of other colonies (Figure 7c; Table 3), indicating a higher coral pH_{cf} up-regulation capability in Kimberley region. While analyses of more coral colonies are needed to fully understand the underlying mechanism, it is clear that elevated levels of pH_{cf} in Kimberley coral have largely offset the adverse impacts of reduced DIC_{cf} on Ω_{cf}, resulting in a 'normal' level of elevated Ω_{cf} among (Figure 7d). This highlights the coral's ability to regulate its internal carbonate chemistry to maintain a favorable calcifying environment.

5.5 Conclusions

In this study, we reconstruct the century-long temperature record based on Sr/Ca and Li/Mg multiproxy, and examine the seasonal and annual variability of carbonate chemistry in the coral calcifying fluid by using B/Ca and $\delta^{11}B$ of a *Porites* spp. coral in the thermally extreme Kimberley region. Our main conclusions are as follows.

(1) The site-specific Sr/Ca and Li/Mg temperature calibrations can generate reliable SST reconstructions in high temperature reef settings, but the anomalous values (2014 to 2015) found in Li/Mg ratios suggest that aberrant Mg/Ca ratios need to be screened before utilizing Li/Mg-SST reconstruction. Importantly, multiproxy SST calibration by combining both Sr/Ca and Li/Mg ratios can provide both improved precision as well as reliability for both seasonal and annual temperature reconstruction.

(2) For the past century, Kimberley region has experienced a gradual increase and intensified variability of temperature, with an average positive anomaly of ~0.65°C in the recent decades, reflecting elevated temperatures and possibly enhanced levels of thermal stress. Nonetheless, the longer-term coral calcification rate is relatively stable and not apparently affected by the warming.

(3) Under such highly thermal stress, typical seasonal variations in CF carbonate chemistry, i.e. DICcf, pHcf, Ωcf, are observed in Kimberley region, similar to that found in more typical tropical coral reef environment, confirming the inherent commonality in the interaction between coral metabolism and internal carbonate chemistry. The generally lower DICcf/DICsw values in Kimberley coral compared to that in central Great Barrier and Ningaloo reef, however suggests that in thermally extreme environments the production of metabolic CO_2 is inhibited. Furthermore, under recent warming, coral DICcf in Kimberley is in decline and exhibit subdued seasonal variability, a likely indicator of enhanced thermal stress. However, the pHcf still remains at an elevated level and acts to keep elevated but near constant levels of Ωcf, highlighting coral's ability to manipulate the internal carbonate chemistry for calcification.

5.6 References

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7 Communication

7.1 Students supported

Sana Dandan, PhD Student at UWA, 2010-2014

Morane Le Nohaïc, visiting MSc Student from France, Thesis title “Impacts of the 2015/16 El Niño on coral reefs in Australia’s naturally extreme Kimberley region”. Completed 2016

Maria Jung, visiting MSc Student from the University of Bremen, Bremen, Germany

Xuefei Chen, visiting student from the State Key Laboratory of Isotope Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou, China

7.2 Journal publications

Dandan SS, Falter JL, Lowe RJ, and McCulloch MT (2015) Resilience of coral calcification to extreme temperature variations in the Kimberley region, northwest Australia. Coral Reefs, doi: 10.1007/s00338-015-1335-6

Schoepf V, Stat M, Falter JL and McCulloch MT (2015). Limits to the thermal tolerance of corals adapted to a naturally extreme, highly fluctuating temperature environment. Scientific Reports 5:17639, doi: 10.1038/srep17639

7.3 Submitted manuscripts

Le Nohaïc M, Ross CL, Cornwall CE, Comeau S, McCulloch M, Schoepf V. Marine heatwave causes unprecedented regional mass bleaching of thermally resistant corals in northwestern Australia. In revision for *Scientific Reports*

Chen X, McCulloch MT, Wei G. Climate change as registered by Sr/Ca, Li/Mg, δ^{11}B and B/Ca systematics in an ~100-year old *Porites* coral from the thermally extreme Kimberley region of northwestern Australia. In review for *Paleoceanography*

7.4 Presentations

Schoepf V, Stat M, Le Nohaïc M, Jung M, McCulloch MT (2017) Will corals from the naturally extreme Kimberley region be able to cope with climate change? Australian Marine Science Association Conference, Darwin, Australia (July 2-6)

Schoepf V (2017) Life on Australia’s wildest coral reefs. Public Forum, Coral Reef Futures Symposium, ARC Centre of Excellence for Coral Reef Studies, Canberra, Australia (June 15)

Schoepf V, Stat M, Falter J, McCulloch M (2016) Thermal tolerance of corals from the naturally extreme Kimberley region in northwest Australia. 13th International Coral Reef Symposium, Honolulu, Hawaii (June 19-24)

Schoepf V (2015) How resistant are Kimberley corals to marine heatwaves? Presentation for the Kimberley Science Program - Teacher Workshop, The University of Western Australia (December 7)

Schoepf V (2015) Thermal tolerance of corals from the Kimberley region, a naturally extreme environment. Future of Marine Ecosystems Symposium, ARC Centre of Excellence for Coral Reef Studies, Hobart, Tasmania (October 7).

Schoepf V (2015) Resilience of Kimberley coral reefs to climate and environmental extremes: past, present and future. WAMSI Conference, 1 May, Perth, Western Australia

McCulloch MT (2014) Is there a future for coral reefs in a high-CO2 world? Royal Society of Western Australia Centenary Event, 16 June, SciTech, West Perth, Western Australia

Schoepf V (2014) Resistance of Kimberley corals to ocean warming. Cygnet Bay Pearl Farm, 15 May, Dampier Peninsula, Western Australia

7.5 Other communications achievements

2017 – Dr Schoepf was featured in the video "WA Corals in Crisis", an educational video for Western Australia's science communication website "Particles"; the interview features unique coral reefs in Western Australia and highlights threats

2016 – General media articles and radio interviews about Kimberley "super" corals

https://www.researchgate.net/blog/post/could-super-corals-help-scientists-find-a-way-to-protect-the-great-barrier-reef

http://www.3cr.org.au/womenontheline/episode-201606060830/focus-coral-bleaching

http://www.aqob.com.au/details.php?p_id=1094&seo=Kimberley_corals_part_2&menuid=&submenuid=&categoryid=&listid=771&slistid=

http://www.aqob.com.au/details.php?p_id=1093&listid=771&slistid=&seo=Bleaching_resistant_Kimberley_corals?&menuid=&submenuid=

http://www.theage.com.au/technology/sci-tech/supercoral-may-take-heat-off-great-barrier-reef-bleaching-20160615-gpjf42.html

2016 – ARC Centre of Excellence for Coral Reef Studies media releases, articles and radio interviews related to mass bleaching on Great Barrier Reef and the Kimberley region (selected links)

http://www.sciencewa.net.au/topics/perspectives/item/4149-perspective-local-coral-reefs-battle-bleaching-conditions

http://www.3cr.org.au/womenontheline/episode-201606060830/focus-coral-bleaching

http://www.sueddeutsche.de/politik/meeresbiologin-stress-unter-wasser-1.3019650

https://www.coralcoe.org.au/only-7-of-the-great-barrier-reef-has-avoided-coral-bleaching/

https://www.coralcoe.org.au/coral-death-toll-climbs-on-great-barrier-reef/

http://www.nytimes.com/2016/05/30/world/australia/bleaching-coral-death-great-barrier-reef.html?_r=0

https://insideclimatenews.org/news/07062016/coral-bleaching-alarms-scientists-climate-change-global-warming-great-barrier-reef

http://www.theage.com.au/environment/great-barrier-reef-bleaching-survey-shows-93-per-cent-of-reef-bleached-20160419-goaeli.html

2016 – German/French documentary about Dr Schoepf's Kimberley research

Part of the documentary series "Frauen und Ozeane" ("Women and Oceans") produced by German/French TV broadcaster ZDF/ARTE; Dr Schoepf was one of five female marine scientists featured in five 45 min portraits (alongside Dr Sylvia Earle and others); released in March 2016; international version in preparation

http://www.arte.tv/guide/de/055217-002-A/frauen-und-ozeane

2015 – Media release about Schoepf et al. 2015 paper in *Scientific Reports*

https://theconversation.com/even-the-super-corals-of-australias-kimberley-are-not-immune-to-climate-change-51484

http://www.abc.net.au/news/2015-12-03/wa-super-coral-may-recover-faster-after-bleaching-event/6995568

http://www.natureworldnews.com/articles/18528/20151203/coral-bleaching-reefs-adapted-warm-waters-threatened-climate-change.htm

https://underthecblog.org/2015/12/03/even-in-so-called-super-corals-temperature-is-still-kryptonite/

7.6 Knock on opportunities created as a result of this project

Dr Schoepf was invited to participate as a Topic Editor for the Frontiers Research Topic "The Future of Coral Reefs Subject to Rapid Climate Change: Lessons from Natural Extreme Environments", *Frontiers in Marine Science*, 2016/17 – a perspectives/review paper is currently in preparation

Dr Schoepf received research funding from the PADI Foundation ($6,200) and a UWA Research Collaboration Grant ($26,785) to investigate physiological and genomic mechanisms of heat tolerance in Kimberley corals (collaboration with Dr Luke Thomas from Stanford University and Dr Michael Stat from Curtin University)

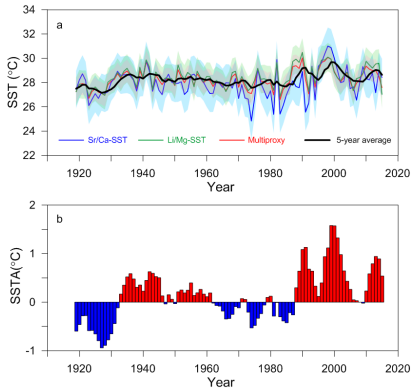
7.7 Key methods for uptake (ie advisory committee, working group, website compendium of best practice.)

WAMSI Lunch and learn seminar and follow on discussion with KMRP Advisory Committee – July 17, 2017

8 Appendices

Appendix 1. This project directly addresses the following questions outlined in the Kimberley Marine Research Program Science Plan.

Key Question
Informed Response
1. How sensitive are calcification rates to seasonal and diurnal changes in temperature, light and pH? How do these relationships for the Kimberley compare with corals living in more moderate tropical reef environments (e.g., Ningaloo and the Great Barrier Reef)? Despite experiencing more extreme environmental conditions, common Kimberley corals overall calcified at rates that were comparable or faster than those from similar corals at a more typical tropical reef, namely Ningaloo Reef located ~1200 km southwest of Cygnet Bay. The effects of tidal exposure and season, however, were highly species-specific: branching *Acropoa aspera* grew more slowly in the environmentally more extreme intertidal than in the subtidal, whereas massive *Dipsastraea favus* and *Trachyphyllia geoffroyi* grew faster in the intertidal environment. Further, growth rates of branching *A. aspera* were reduced in summer compared to winter, whereas the massive corals showed either no seasonal response or a more complex behavior. Overall, these findings demonstrate that Kimberley corals generally exhibit high resilience of calcification to extreme temperature variations but the exact mechanisms of adaptation and/or acclimatization are strongly taxon dependent.
2. What are the physiological mechanisms that enable the inshore Kimberley corals to have such high temperature tolerance and hence avoid the destructive effects of bleaching? Can these physiological characteristics be transferred to other, more sensitive coral reefs (e.g., Ningaloo) and on what timescales? Strong daily fluctuations in temperature and other environmental variables, such as those present in intertidal habitats, significantly enhance the heat tolerance of Kimberley corals. While this higher heat tolerance does not impart immunity to coral bleaching, it dramatically improves survival and recovery from mass bleaching events. Since intertidal corals host the same symbiont types as the more heat-sensitive subtidal corals, our findings suggest that the genetic and physiological mechanisms underlying the high heat tolerance of intertidal corals are a complex interplay between all partners of the coral symbiosis. Further research is required to identify the relative contribution of each partner to heat tolerance in Kimberley corals, as well as the time scales required to achieve such resilience.
3. How have environmental conditions in the inshore Kimberley region changed over the past 50 to 100 years? The most profound changes that were registered in the long-lived (1919 to 2016) *Porites* coral collected and analysed as part of this study are: 1. From the 1930's to late 1970's at the coral site (near Shell Island) temperatures (via Sr/ca and Li/Mg proxies) were relatively constant on an annual basis. From the 1980's to present x3 ~ 0.65^{o}C pulses of warming occurred, the most recent from 2012 to 2016 was associated with bleaching. The other two pulses were in the late 1980's – early 1990's and late 1990's (see figures below).



2. We found that the measured annual calcification rate closely follows temperature with generally small ~±10% variations recorded in the *Porites* since the 1930's. However, this coral would need to be resampled to properly see the full impact of the 2016 bleaching event.
3. The other major change has been in the sediment runoff into King Sound as registered at the Shell Island coral site (see discussion below)

4. How often do major flood events occur and how did coral calcification and coral reef growth respond? Has the input of sediments and consequently nutrients into Kimberley inshore habitats increased due to changes in land use?

Major flood events are generally caused by cyclonic events and, depending on their pathway, recorded by river flow levels measured upstream in the Fitzroy at gauging stations. The *Porites* coral core records both sediment pulses from these flood events (via Ba/Ca) and salinity changes (via combined Sr/Ca and $\delta^{18}O$ proxies). We found that sediment input was closely linked to both salinity decreases as well as river discharge, indicating the profound importance of cyclonic events on the discharge of the Fitzroy (largest river in Australia when in flood). We further note that this occurs despite the very large tides in King Sound and hence expected rapid dissipation/diminution of flood events. The two largest sediment input events recorded in the coral occurred during the floods of 1986 and 2002. We also note that the baseline levels of sediment input were abnormally elevated from the 1960's through to 1980 likely reflecting a 'temporary ?' degradation of the catchment due to increased grazing during this period. This aspect of the study clearly points to the importance of the 'state' and hence management of the Fitzroy River catchment in determining sediment/nutrient input into the corals reefs at Cygnet Bay (Shell Island).

5. How will the coral reefs of the Kimberley region respond to increasing water temperatures, ocean acidification and frequency of cyclones with ongoing climate change?

The first documented mass bleaching in the inshore Kimberley region in 2016 highlighted that Kimberley corals are threatened by marine heatwaves and climate change, despite their remarkable ability to withstand temperature extremes over short time scales. Although intertidal corals were able to recover from this mass bleaching event remarkably fast, subtidal corals suffered from extensive mortality. As such, mass bleaching events will become increasingly frequent, Kimberley coral reefs are as threatened as other tropical coral reefs by ongoing climate change. Historic calcification data show that Kimberley coral have not been affected by declines in seawater pH to date. However, further research is required to identify how resistant they are to future ocean acidification and whether Kimberley corals have the ability to further increase their heat tolerance via acclimatisation and/or adaptation.

6. What are the ultimate physical, biogeochemical thresholds that will limit the long-term existence of the Kimberley inshore coral reef systems?

The unprecedented mass bleaching event in the Kimberley in 2016 highlights that the increasing frequency of

marine heatwaves and extreme climatic events is one of the key threats that will limit the long-term existence of Kimberley inshore coral reefs. Continued monitoring and further studies of both the sub- and intertidal reefs from this unique region (especially following recovery the 2016 and likely further bleaching events?) will be required to properly address this longer-term question.

NEW QUESTIONS POSED BY MANAGERS

What information and findings can we use to get across the message that we need to understand the importance of coral fragility as well as resilience to manage its conservation properly?

Kimberley corals are arguably Australia's most stress-resistant corals, and have adapted their calcification rates to the naturally extreme environment of the Kimberley. They should therefore be placed under the highest level of protection, and should be considered regional and national priorities for further research into the mechanisms enabling such remarkable stress resilience in reef-building coral. Intertidal coral communities, in particular, should be the focus of awareness and protection efforts as their naturally higher heat resistance resulted in dramatically enhanced survival and recovery during the first documented mass bleaching event in the region. This event, however, also highlighted that Kimberley corals are threatened by ocean warming and marine heatwaves; thus, it is critical to minimize local stressors to boost coral resilience, particularly during heat stress events.

Clear evidence was also found of sediment pulses into the corals reefs at Cygnet Bay. The sediment input is strongly correlated with river discharge of flood waters during major cyclonic event, pointing to the importance of the 'state' and hence management of the Fitzroy River catchment in determining sediment/nutrient input and hence water quality for the Kimberley reefs.