Coral Reefs (2020) 39:259–269
https://doi.org/10.1007/s00338-019-01881-x

REPORT

Physiological effects of heat and cold exposure in the common reef coral *Acropora millepora*

J. J. V. Nielsen[1,2,3] · C. D. Kenkel[1,4] · D. G. Bourne[1,2,3] · L. Despringhere[5] · V. J. L. Mocellin[1] · L. K. Bay[1,2]

Received: 3 September 2018 / Accepted: 29 November 2019 / Published online: 13 January 2020

Abstract Reef-forming corals are under threat globally from climate change, leading to changes in sea temperatures with both hot and cold events recorded and projected to increase in frequency and severity in the future. Tolerance to heat and cold exposure has been found to be mutually exclusive in other marine invertebrates, but it is currently unclear whether a trade-off exists between hot and cold thermal tolerance in tropical corals. This study quantified the changes in physiology in *Acropora millepora* from the central Great Barrier Reef subjected to three temperature treatments; sub-lethal cold, ambient and sub-lethal heat (23.0 °C, 27.0 °C and 29.5 °C, respectively). After 10 weeks, pigment content and Symbiodiniaceae density increased in cold-treated corals but decreased in heat-treated corals relative to corals at ambient conditions. Heat-treated corals gained less mass relative to both ambient and cold-treated corals. These results indicate that the physiological condition of *A. millepora* corals examined here improved in response to mild cold exposure compared to ambient exposure and decreased under mild heat exposure despite both these temperatures occurring in situ around 15% of the year. The energetic condition of corals in the hotter treatment was reduced compared to both ambient and cooler groups, indicating that corals may be more resilient to mild cold exposure relative to mild heat exposure. The results indicate that the corals shifted their resource allocation in response to temperature treatment, investing more energy into skeletal extension rather than maintenance. No evidence of thermal tolerance trade-offs was found, and cold thermal tolerance was not lost in more heat-tolerant individuals. An enhanced understanding of physiological responses of corals at both ends of the thermal spectrum is important for predicting the resilience of corals under projected climate change conditions.

Keywords Thermal tolerance · Coral · Energetic condition · Cold exposure · Trade-offs

Topic Editor Mark Vermeij

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00338-019-01881-x) contains supplementary material, which is available to authorized users.

✉ J. J. V. Nielsen
josephine.nielsen@my.jcu.edu.au

[1] Australian Institute of Marine Science, PMB #3, Townsville MC, QLD 4810, Australia

[2] AIMS@JCU, Australian Institute of Marine Science, James Cook University, Townsville, QLD 4811, Australia

[3] College of Science and Engineering, James Cook University, Townsville, QLD 4811, Australia

[4] Department of Biological Sciences, University of Southern California, 3616 Trousdale Parkway, Los Angeles, CA 90089, USA

[5] Université de Perpignan Via Domitia, 66100 Perpignan, France

Introduction

Coral reefs are under increasing threats globally from climate change and anthropogenic perturbations (De'ath et al. 2012; Hughes et al. 2017). When temperatures exceed the upper and lower thresholds of coral tolerance, the symbiotic relationship between the coral host and the endosymbiotic Symbiodiniaceae is disrupted (Visram and Douglas 2007; Paz-García et al. 2012). The loss of Symbiodiniaceae and/or their photosynthetic pigments leads to a breakdown in the symbiotic relationship, characterised by decreased

photosynthesis of the symbiont and subsequent reduced energy uptake by the coral host, a phenomenon referred to as bleaching (Weis 2008). Level of bleaching severity and mortality depends upon the interaction between light intensity, the duration of the thermal perturbation as well as the accumulation of degree heating weeks (DHW, Howells et al. 2013; Ainsworth et al. 2016).

The direct relationship between increased sea surface temperatures (SST) and coral bleaching is well established (Berkelmans and van Oppen 2006) with high levels of mortality documented across the northern and central regions of the Great Barrier Reef (GBR) following marine heatwaves in 2016 and 2017 (Hughes et al. 2017). Most studies of coral thermal tolerance have focussed on the physiological effects of heat stress (Gates et al. 1992; Baird and Marshall 2002; Baker et al. 2008). However, temperature projections indicate the continued prevalence of severe cold events (Vavrus et al. 2006) as highlighted by a cold snap occurring in winter 2017–2018 in Florida. The continued occurrence of cold events in addition to increasingly rapid transitions between El Niño and La Niña events will effectively widen the thermal envelopes that corals will need to successfully cope with (Vavrus et al. 2006; Eakin et al. 2009; Cai et al. 2015). If restoration initiatives such as assisted gene flow (moving warm-adapted individuals to cooler, yet warming reefs) proceed, translocated corals are more likely to be exposed to stressful cold rather than heat conditions (Howells et al. 2013). Thus, there are multiple reasons why it is important to understand the effects of temperature exposure at both ends of the thermal spectrum, and this study provides an examination of physiological responses to moderate, yet chronic cold thermal stress events.

Coral thermal tolerance varies substantially within and among species. Oliver and Palumbi (2011) showed that corals of the same species (*Acropora hyacinthus*) from different thermal environments had significantly different thermal tolerances, suggesting some level of local adaptation. Similar patterns have now been described across habitats and latitudes (e.g. Berkelmans and Willis 1999; Howells et al. 2011; Oliver and Palumbi 2011). Additionally, the Symbiodiniaceae type harboured by the coral impacts thermal tolerance with clade D known to confer a 1.0 °C increase in upper bleaching thresholds (Berkelmans and van Oppen 2006). This increased tolerance incurs trade-offs with other traits as corals hosting clade D demonstrate reduced growth (Mieog et al. 2009). This highlights the influence of the Symbiodiniaceae community on the overall thermal tolerance and physiological condition of the coral holobiont.

Despite evidence of significant cold bleaching events globally (Lirman et al. 2011; Paz-García et al. 2012), very few studies have examined the physiological effects of cold exposure on coral physiology (but see Jokiel and Coles 1977, Roth et al. 2012). The effects of cold stress can manifest themselves rapidly (Lirman et al. 2011; Roth et al. 2012), and in some localised instances, the reported mortality from cold stress has been greater than that resulting from heat-induced stress (Jokiel and Coles 1977). Some authors have suggested that corals exposed to cold will eventually acclimatise (Roth et al. 2012; Roth and Deheyn 2013), while others indicate that long exposure to cold conditions leads to greater mortality than high-temperature exposure (Jokiel and Coles 1977; Higuchi et al. 2015). Studies have demonstrated commonalities in the physiological responses of corals to cold and heat stress, but these are not always consistent (Table 1, Jokiel and Coles 1977; Roth et al. 2012). Exposure to hot and cold thermal stresses both result in photo-acclimatory responses and damage to photosystem II (Saxby et al. 2003; Pontasch et al. 2016). *In hospite*, Symbiodiniaceae densities have been found to both decrease and increase (Saxby et al. 2003; Roth et al. 2012; Rodríguez-Troncoso et al. 2014) in response to cold exposure (Table 1). Within the coral host, both heat- and cold-induced stresses lead to decreased respiration and calcification (Higuchi et al. 2015), reduced growth (Howells et al. 2011; Roth et al. 2012) and reduced heterotrophic feeding (Rodolfo-Metalpa et al. 2008). Consequently, the physiological effects of heat and cold responses are not yet resolved and require further study.

To assess the physiological effects of chronic thermal stress, this study simultaneously compared hot and cold thermal exposure on the tropical coral *A. millepora* and quantified a number of physiological traits to examine the underlying mechanisms underpinning these responses. Additionally, this study tested for potential trade-offs between hot and cold tolerance at the colony level by correlating performance under one treatment with performance under another. Based on previous studies showing that some taxa are capable of exhibiting high heat or cold tolerance but not both (Sorensen et al. 2001; Anderson et al. 2003), it was hypothesised that coral colonies which exhibited the maintenance of physiological condition in response to high temperatures would not do so under cold exposure, and as such, a trade-off would exist between heat and cold tolerance.

Materials and methods

Treatment selection

This study aimed to examine physiological responses at stressful, sub-lethal temperature levels. Experimental treatments were selected to represent likely, yet uncommon thermal scenarios occurring in the Palm Island group. To

Table 1 Physiological parameters investigated in this study and their relevance to ecology and thermal tolerance physiology studies

Level	Parameter	Rationale	References
Holobiont	Symbiont density	Related to the ability of symbionts to produce energy molecules for the coral host, changes in symbiont densities following temperature exposure impact the nutritional state of the coral. Can also influence holobiont thermal tolerance	Saxby et al. (2003) Roth et al. (2012)
	Buoyant weight gain	Non-invasive technique that allows for repeated sampling of the same fragment Changes in mass gain have been attributed to both heat and cold exposure as a response to decreased energy transfer from symbionts. Method makes no distinction between changes in skeletal or tissue masses	Bay and Palumbi (2015) Kemp et al. (2011)
Coral	Water-soluble protein content	Corals undergoing stress are likely to change their resource allocations, and as such, stress can be detected by a decrease in quantity of protein molecules	Roth and Deheyn (2013)
	Lipid content	Lipid content is an important predictor of coral health and one of the first impacted by thermal stress due to changes in coral nutritional state. Having a high lipid content during periods of thermal stress may aid in resilience	Yamashiro et al. (2005)
	Basal disc extension	The process of skeletal extension onto new surfaces. Important for corals to occupy and attach to new space	Howells et al. (2011) Baird and Marshall (2002)
	Skeletal density	The "quality" of the skeleton. A coral may increase rapidly through skeletal extension; however, if this new skeleton is highly porous, it is at risk of easy damage	Rocker et al. (2017) Madin et al. (2016)
	Catalase activity	This oxidative enzyme is an indicator of oxidative stress, associated with the production of ROS in response to thermal stress	Krueger et al. (2015)
Symbiont	Chl α content	Used widely as a direct indicator of bleaching, Chl α content reflects cellular changes in the photosynthetic symbionts in response to temperature exposure. Changes in pigment content influence coral nutritional state and survival. In response to heat, Chl α content is generally reduced to protect symbionts and coral from ROS. Report on effects of cold exposure is conflicting	Abrego et al. (2008) Higuchi et al. (2015)
	Photosynthesis rate	Describes the efficiency of the symbionts in producing oxygen. This is often reduced in response to temperature	Saxby et al. (2003) Higuchi et al. (2015)
	Symbiont type	The type of Symbiodiniaceae harboured plays a key role in the thermal tolerance of corals	Berkelmans and van Oppen (2006) Mieog et al. (2009) Fisher et al. (2012)

determine these temperatures, we calculated the monthly temperature averages over 17 yr (1998–2015) and plotted their frequency of occurrence (Suppl. Fig.1a) to obtain the distribution of mean monthly temperatures (MMTs) and identify the MMT during the cooler months (~ 23.5 °C June–August) and the warmer months (~ 29 °C December–February). We selected treatment temperatures slightly below (cooler) and above (hotter) the identified MMTs. To inform about the thermal history of the region, we calculated the rate of return for a 10-week thermal event at both 23 and 29.5 °C using temperature data from Pandora reef from 2007 to 2016.

Coral collections and experimental conditions

Fragments (< 10 cm Ø) of 23 individual *Acropora millepora* colonies were collected from an inshore reef (18.4898°S, 146.54283°E, GBRMPA permit # G12/35236.1) in the Palm Island Group of the central Great Barrier Reef (GBR) on 17–18 October 2016 from depths < 6 m. Colonies were held in flow-through unfiltered seawater for 2 days in transit and then housed at the National SeaSimulator at the Australian Institute of Marine Science (AIMS, Townsville, Australia) under ambient temperature (27.0 °C). The colonies were further fragmented (12 replicate branches) and attached with superglue onto aragonite plugs (n = 276). Fragments were acclimatised to control conditions (max 250 µmol photons m^{-2} s^{-1}, 27.0 °C) for 3 weeks before being haphazardly assigned to experimental tanks (n = 4 tanks per treatment, 1 fragment per colony in each tank).

Tanks (50 L) were supplied with filtered seawater (5 µM, FSW, 50 L h^{-1}). To achieve and reliably maintain the desired temperature treatments, two different sources of water were used. First, the different treatment temperatures of the seawater feeding into the experimental tanks were achieved by mixing two lines of GPA FSW of different temperatures at different ratios. This seawater was then fed

flow-through to each experimental tank separately and not re-circulated so that each tank acts independently in terms of the water it receives. Second, experimental tanks were placed in water baths to assist with maintaining temperature in tanks. To control the temperature of these baths, they were supplied with freshwater from sumps (one per treatment). This re-circulated water was never in contact with the corals. The tanks were equipped with pumps (Turbelle® nanostream® 6055, Tunze, Penzberg, Germany) to maintain circulation, exposed to artificial light similar to conditions experienced on a nearby reef (12:12-h light/dark cycle, 6-h ramping, max 250 µmol photons m^{-2} s^{-1}, Hydra, AquaIllumination®, 400–700 nm, C2 Development, Ames, Iowa, USA, http://data.aims.gov.au/aimsrtds/datatool.xhtml?from=2013-11-08&thru=2018-11-09&period=MONTH&aggregations=AVG&channels=148,8602), and the coral fragments were fed *Artemia* daily (1 *Artemia*: 5 ml FSW). Temperatures were ramped over 5 days (to 29.5 °C at 0.5° C d^{-1} and to 23.0 °C by 0.8 °C d^{-1}, respectively) and subsequently maintained for each treatment (± 0.1 °C) for the following 10 weeks.

Symbiodiniaceae responses

Instant net photosynthesis was quantified for a subset of coral fragments (2 per colony per treatment, $n = 131$) according to the method detailed in Strahl et al. (2015) and detailed in Supplementary Material. Oxygen production was standardised to surface area and incubation time and reported as mg O_2 cm^{-2} min^{-1}.

Coral tissue colour was quantified from photographs at 0 and 10 weeks with reference to the Coral Colour Reference Chart (Siebeck et al. 2006), and the same camera was used throughout the experiment. For each photograph, the mean grey value of the chart squares was measured (in triplicate) using ImageJ, and a standard colour curve created. The mean grey value was then calculated for each coral fragment per time point, and the standard curve was used to relate grey value to a colour score to standardise between time points. Changes in colour were calculated by subtracting initial colour score from final score.

Symbiodiniaceae cells were obtained by airbrushing and centrifugation (see Suppl.mat). Chlorophyll α pigments of the Symbiodiniaceae pellet were extracted and quantified following the method of Ritchie (2006, see Suppl.mat). Chlorophyll α was standardised to surface area of the coral fragment.

Coral holobiont physiological responses are known to depend on Symbiodiniaceae community composition (Little et al. 2004; Yuyama and Higuchi 2014). Therefore, we typed the coral colonies in the experiment. To identify the Symbiodiniaceae present within the coral fragments, PCRs followed by an RFLP (restriction fragment length polymorphism) were performed on the 23 colonies according to methods of van Oppen et al. (2001) after the conclusion of the experiment. This assay distinguishes between four genera (*Symbiodinium*, *Breviolum*, *Cladocopium* and *Durusdinium*) of Symbiodiniaceae along with some of the types (such as C1, C2, C3; see Supplementary Material for further details). Restriction digests were examined by gel electrophoresis (2 µl product, 1% agarose in TAE, 1 h, 80 V, 400 mA) stained with EtBr (ethidium bromide) and imaged on Fusion FX® Imager (Vilber Lourmat, Collégien, France) including three DNA standards that have been previously identified as C1, C2 and D to aid in identification. Three colonies out of 23 predominantly harboured both symbiont types D and C2, while 20 colonies predominantly harboured type C2, leading to an unbalanced design with regard to symbiont type. The three colonies harbouring a different community were excluded from data analysis to aid in interpretation of results presented.

Symbiodiniaceae densities were quantified according to the methods of Krediet et al. (2015) with the following modifications; each sample was resuspended in FSW according to pellet density (10 ml, 5 ml, 3 ml, or 2 ml, respectively). All counts were carried out under a 10 ×/0.25 objective (Olympus CX22LED), and density was standardised to surface area. See Supplementary Material for further details.

Coral host responses

Coral bulk mass gain during the experiment was quantified with the buoyant weighing method (Davies 1989) at 0 and 10 weeks and expressed as a percentage of initial mass. The size of coral fragment basal discs was quantified using planar projection photography (Naumann et al. 2009) with images (Nikon® D18, four Ikelite® strobes) taken at 0 and 10 weeks. Skeletal extension, measured by basal disc extension, was calculated as changes in arithmetic mean radius (AMR) according to Pratchett et al. (2015). While obtaining coral sizes through planar projection photography is known to yield low accuracy relative to other methods such as wax-dipping and newer 3D methods (Naumann et al. 2009), we needed a rapid, non-invasive method for quantifying changes in disc size during the experiment. Post-experiment, processing samples for energetic content analyses required that the samples were not exposed to repeated heating and cooling in order to ensure protein and lipid molecule integrity, thus eliminating methods such as wax-dipping. Skeletal density was calculated at the conclusion of the experiment for the respirometric fragments using the dry weight and volume of each fragment. Fragment volume was estimated by the formula for volume of a cylinder using radius (measured at

1/3 the length of fragment from the base) and height of fragments measured from photographs.

Assays to determine water-soluble protein content were carried out according to manufacturer's protocol (Bio-Rad *DC* Protein Assay). Sample preparation and modifications are outlined in Supplementary Material. Absorbance was read using the Synergy H4 Hybrid Reader® (BioTek, Winooski, VT, USA). Protein content (where coefficient of variation < 20% among technical replicates) was standardised to unit surface area of the coral fragment.

Catalase activity (U) was calculated as the change in H_2O_2 concentration over time. Aliquots of coral tissues were defrosted and homogenised (1 min, The Jitterbug model 13000). Reagents were loaded in three technical replicates into UV-transparent microplates (UV-Star®, 96 wells, Greiner Bio-One, Monroe, NC, USA) in the following order; 60 µl PBS (50 mM, pH 7.0), 30 µl coral tissue slurry (MilliQ H_2O for blanks), and 120 µl H_2O_2 (50 mM). Plates were immediately loaded onto the Synergy H4 Hybrid Reader®, and absorbance was read at 240 nm every 30 s for 15 min. Catalase activity (U) was calculated as the change in absorbance over the linear portion of the absorption curve and standardised to protein content (U min^{-1} mg^{-1} protein).

The lipid extraction procedure was modified from Folch et al. (1957, Supplementary Material for more information). The weight of extracted lipid was standardised to the quantity of freeze-dried material on which the extraction was performed (g dry weight).

Statistical analysis

Linear mixed effects models were used to assess the effect of temperature treatment on the physiological condition of *A. millepora*. To assess the effect of temperature treatment, the fixed effects of temperature (levels; cold, ambient, heat) were modelled using the *lme* command of the *nlme* package (Pinheiro et al. 2017). Coral colony was included as a random effect to account for variations among individual colonies. Tank was also designated as a random effect to account for differences arising between tanks not due to temperature treatment.

All models were checked for normality of residuals and homoscedasticity. Where required, data were transformed to satisfy assumptions and back-transformed for graphical representation. Differences between treatments were examined using Tukey's HSD tests, applying a correction for multiple tests. Correlations between physiological traits were examined by Spearman's rank correlation where normality was not met as a nonparametric alternative to Pearson's correlations (Whitlock and Schluter 2009). Traits were averaged across tanks such that $n = 1$ per colony per treatment. All analyses were performed using R version 3.3.2 (R Core Team 2017).

Results

Thermal history

The amount of time field-collected corals experienced below 23.0 °C and above 29.5 °C was determined for the full calendar year 2015 (Suppl. Fig.1b). The corals were at or below the cold treatment (23.0 °C) for 59 of 362 days (16.3% of the year), while the time spent at or above the heat treatment (29.5 °C) was similar at 50 days (13.8%). Sustained hot events (≥ 10 weeks at or above 29.5 °C) were found to only have occurred once in 10 yr (2007, 13 weeks). Sustained cold events (≥ 10 weeks at or below 23.0 °C) were recorded five times in the last 10 yr (2008, 2011, 2012, 2013, 2014).

Changes in symbiosis and photosynthesis

The dominant type(s) of Symbiodiniaceae hosted varied between colonies. Three out of 23 hosted a mix of types D and C2, while the remaining hosted only Symbiodiniaceae type C2. Symbiodiniaceae densities varied between temperature treatments (Wald's test, $df = 2$, $F = 65.86$, $p < 0.0001$, Fig. 1a). Cold-treated corals showed a significant increase in symbiont density relative to ambient corals (Tukey's HSD < 0.001, $1.96 \pm 0.12 \times 10^6$ cm^{-2} and $1.15 \pm 0.08 \times 10^6$ cm^{-2}, cold-treated and ambient, respectively), while heat-treated corals showed a significant reduction in symbiont density (Tukey's HSD < 0.001, $0.63 \pm 0.07 \times 10^6$ cm^{-2}).

The colour of coral fragments was impacted by temperature treatment (Wald's test, $df = 2$, $F = 170.23.14$, $p < 0.0001$, Fig. 1b). Over time, corals at ambient conditions did not show any change in tissue colour, while the cold-treated corals experienced a darkening in colour and the heat-treated corals had paled (-0.004 ± 0.05, 0.39 ± 0.05, -1.12 ± 0.08, respectively). At the end of the experiment, heat-treated corals were significantly lighter in colour by 1.16 units compared to ambient corals (4.29 ± 0.09 units and 5.45 ± 0.08 units, Tukey's HSD < 0.0001), while cold-treated corals were significantly darker by 0.4 units than ambient corals (5.89 ± 0.05 units, Tukey's HSD < 0.0001, Suppl. Fig.2).

Chlorophyll α content was significantly influenced by temperature treatment (Wald's test, $df = 2$, $F = 82.52$, $p < 0.0001$, Fig. 1c). Heat-treated corals had the lowest chlorophyll α concentration, whereas cold-treated corals had 40% more chlorophyll α (0.67 ± 0.04 µg cm^{-2} and 1.64 ± 0.07 µg cm^{-2}, respectively). Both heat- and cold-

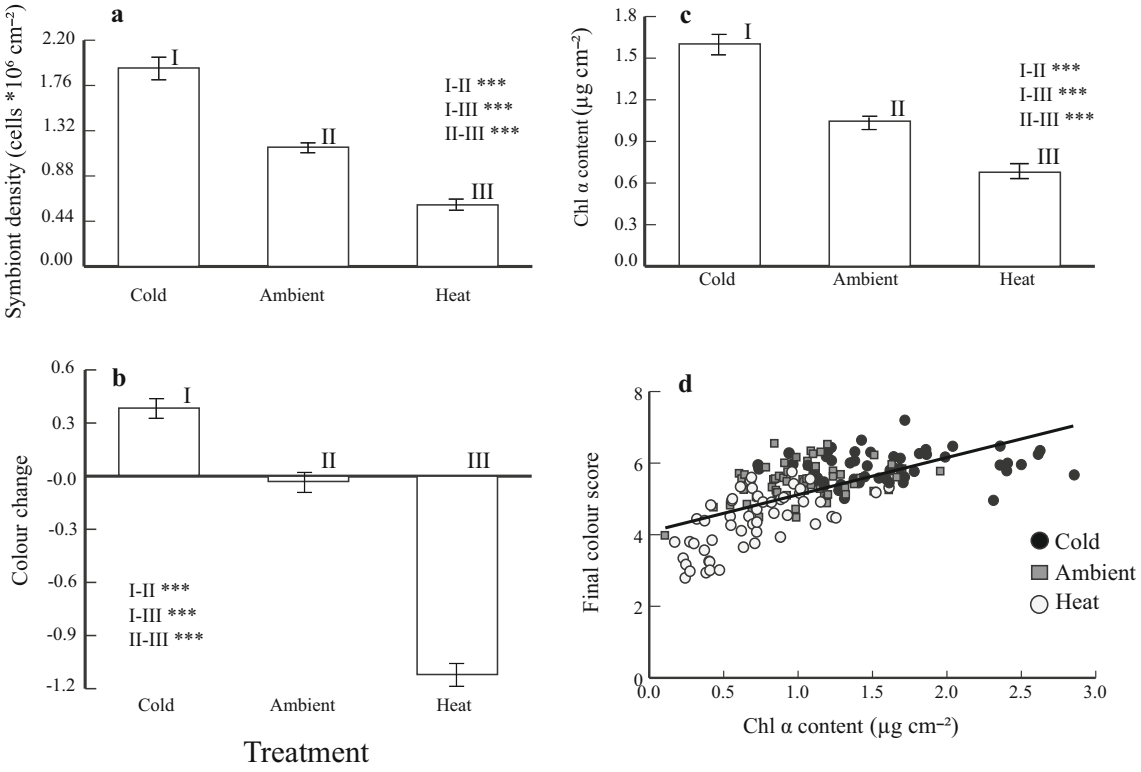

Fig. 1 Symbiont responses to experimental treatments; **a** Symbiodiniaceae density (mean ± CI cells × 10^6 cm^{-2}), **b** absolute colour change (mean ± CI colour change unit) and **c** chlorophyll α content (mean ± CI µg cm^{-2}) at the completion of the experiment (± 95% confidence intervals are given as error bars. Roman numerals indicate statistical groupings, and asterisks *indicate the post hoc Tukey's significance level; *0.01; **0.001; ***< 0.001). **d** Correlation between the final colour score (colour unit) and chlorophyll α content (µg cm^{-2})

treated corals were significantly different to the ambient group, which contained 1.06 ± 0.05 µg cm^{-2} chlorophyll (Tukey's HSD < 0.0001 for all comparisons, Fig. 1c). Overall colour score (week 10) displayed a strong, positive correlation with chlorophyll α content (µg cm^{-2}) with clear treatment effects (Spearman's rank correlation, S = 184,340, $p < 0.0001$, $\rho = 0.67$). Additionally, there was a strong, positive correlation between Symbiodiniaceae density and chlorophyll content (Spearman's rank correlation, S = 103,180, $p < 0.001$, $\rho = 0.779$). Chlorophyll content per cell varied significantly between temperature treatments (Wald's test, $df = 2$, $F = 8.88$, $p < 0.001$). The symbiont cells of heat-treated corals (2.07 ± 0.28 × 10^{-6} µg $cell^{-1}$ cm^{-2}) contained significantly more chlorophyll than the ambient (2.07 ± 0.28 × 10^{-6} µg $cell^{-1}$ cm^{-2}, Tukey's HSD = 0.0014) and cold-treated corals (1.05 ± 0.097 × 10^{-6} µg $cell^{-1}$ cm^{-2}, Tukey's HSD = 0.000298).

The activity of catalase did not vary significantly among treatments, although it tended towards an increase in the heat-treated corals with the lowest activity recorded in the cold-treated corals (8.09 ± 1.79 × 10^{-3} and 4.46 ± 0.7 × 10^{-3} U min^{-1} mg^{-1} protein, respectively). Photosynthetic output was not significantly affected by treatment.

Growth

Coral bulk mass gain was significantly affected by treatment (Wald's test, $df = 2$, $F = 5.36$, $p = 0.0054$, Fig. 2a). Despite large variation in the initial bulk mass of the experimental coral fragments (mean = 3.03 g; standard deviation = 1.31 g), there was no correlation between starting mass and overall bulk mass gained (Spearman's rank correlation test; S = 2,788,900, $p = 0.1423$, $\rho = 0.09$). Heat-treated corals gained significantly less bulk mass than cold- and ambient corals (4.55 ± 0.37% 10 $week^{-1}$, 5.68 ± 0.21% 10 $week^{-1}$, and 5.49 ± 0.19% 10 $week^{-1}$, heat and cold Tukey's HSD = 0.0058, heat and ambient Tukey's HSD = 0.029), while there was no difference in mass gained between the cold and ambient treatments (cold and ambient Tukey's HSD = 0.86).

Although not significant, basal disc extension was lower in both the heat and cold treatments relative to the ambient treatment (0.89 ± 0.05 cm AMR, 0.83 ± 0.05 cm AMR, and 0.97 ± 0.06 cm AMR, respectively).

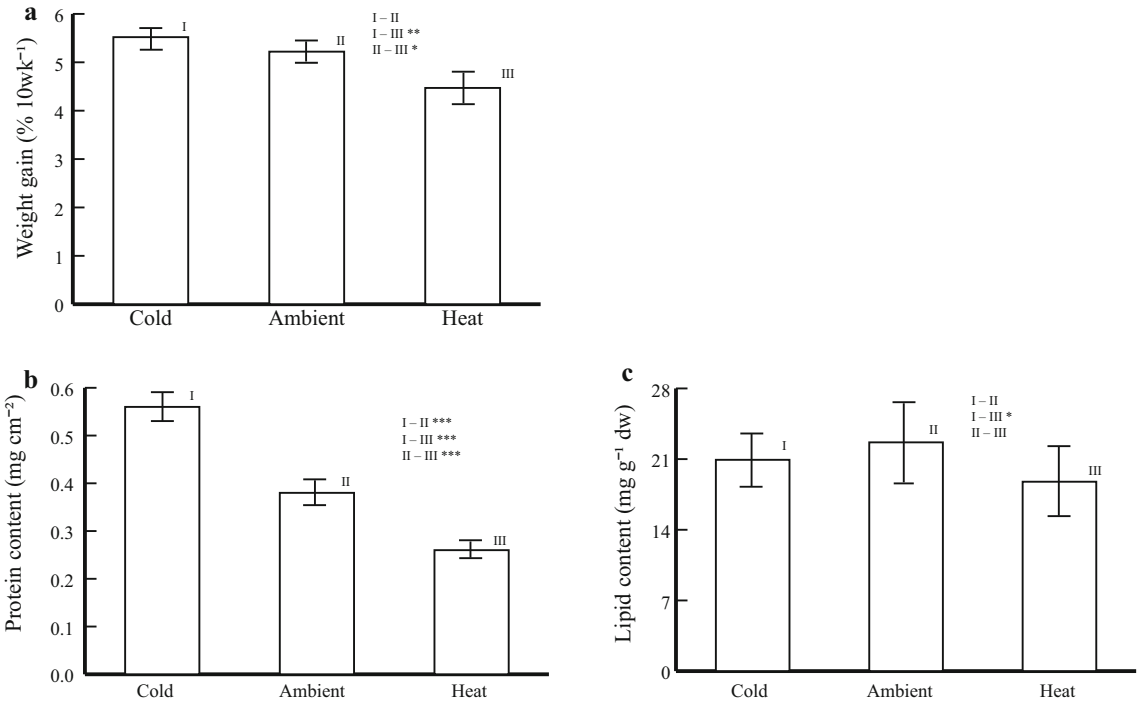

Fig. 2 Growth and energetic condition of corals in response to temperature; **a** absolute weight changes (mean ± CI%) in response to treatment. Energetic responses to temperature treatments of **b** total protein content (mean ± CI mg cm^{-2}) and **c** total lipid content (mean ± CI mg g^{-1} dw). 95% confidence intervals are given as error bars. Roman numerals indicate statistical groupings, and asterisks * indicate the post hoc Tukey's significance level; *0.01; **0.001; ***< 0.001

Skeletal density varied significantly between treatments (Wald's test; $df = 2$, $F = 4.85$, $p = 0.0106$). Skeletal densities of ambient-treated corals averaged 2.95 ± 0.21 g cm^{-3}, while those recorded in the heat treatment were significantly lower (2.19 ± 0.18 g cm^{-3}, Tukey's HSD = 0.005). There was no difference in skeletal densities between the cold and heat treatments (2.55 ± 0.15 g cm^{-3}, Tukey's HSD = 0.33).

Energetic condition

Treatment significantly affected protein content (Wald's test, $df = 2$, $F = 29.44$, $p < 0.0001$, Fig. 2b). Cold-treated corals contained 49% more protein than heat-treated corals (0.55 ± 0.03 mg cm^{-2} and 0.27 ± 0.02 mg cm^{-2}, Tukey's HSD < 0.001) and 33% more protein than ambient corals (0.37 ± 0.03 mg cm^{-2}, Tukey's HSD < 0.001, Fig. 2b).

Lipid content varied significantly with treatment (Wald's test, $df = 2$, $F = 3.93$, $p = 0.0217$, Fig. 2c). Corals exposed to cold conditions contained 20% more lipid than heat-treated corals (20.94 ± 2.58 mg g^{-1} dw and 16.57 ± 2.83 mg g^{-1} dw, respectively. Tukey's HSD = 0.015, Fig. 2c).

Tolerance trade-offs and resource allocation shifts

A significant negative correlation was found between cold and heat lipid contents (%, Spearman's rank correlation, $S = 34{,}114$, $p = 0.028$, $\rho = -0.3$). Cold and heat basal disc extension showed a strong, significant correlation (Spearman's rank correlation, $S = 344$, $\rho = 0.74$, $p = 0.00028$). There were no significant correlations observed neither between bulk mass gain (%) nor protein content under cold and heat conditions.

A slight positive, significant relationship was observed between the absolute changes in bulk mass changes (%) and basal disc extension (AMR, Spearman's rank correlation, $S = 2{,}400{,}900$, $p < 0.001$, $\rho = 0.18$). A strong negative and significant correlation was detected between basal disc extension and lipid content (%, Spearman's rank correlation, $S = 1{,}102{,}100$, $p < 0.001$, $\rho = -0.37$). Overall, there was no correlation between basal disc extension and skeletal density (Spearman's rank correlation, $S = 143{,}890$, $p = 0.3$, $\rho = -0.11$). When examined individually within treatments, a slight correlation was detected between basal disc extension and skeletal density in the cold treatment (Spearman's rank correlation, $S = 43{,}300$, $p = 0.043$, $\rho = -0.27$). These correlations were not detected within the heat or ambient treatments

(Spearman's rank correlations; $S = 4348$, $p = 0.71$, $\rho = -0.071$:$S = 5352$, $p = 0.92$, $\rho = 0.019$, respectively).

Discussion

Physiological responses to thermal stress influence the ability of organisms to cope successfully with environmental perturbations. As a result of climate change, corals are increasingly exposed to larger temperature ranges, and projected increases in severity and frequency of extreme cold and heat events threaten their continued existence (Vavrus et al. 2006; Lirman et al. 2011; Hughes et al. 2017). Results presented here demonstrate that heat tolerance does not confer a physiological disadvantage at cold temperatures.

It is well documented that heat stress causes severe disruptions to an organism's metabolism (Gillooly et al. 2001; Pörtner 2002). As such, heat stress influences the performance of an organism to a larger extent than cold exposure (Pörtner 2002; Roth et al. 2012). We selected our treatment temperatures for this laboratory-based examination of differential physiological effects of heat and cold exposure in *A. millepora* samples with this in mind: the magnitude of the cold treatment was nearly twice that of the heat (+ 2.5 °C vs. − 4.0 °C on ambient, respectively). We also considered the range of temperatures these corals would have experienced in situ: our exposures represented approximately the top and bottom 15% of the normal temperature range for Pandora Reef. Despite this similarity in total time spent at treatment temperatures per year, this region experiences frequent cold events (every 2 yr) similar to the cold treatment employed here. Therefore, it was perhaps not surprising that corals exposed to the cold treatment showed an improved physiological condition relative to heat-treated ones. We also found evidence of resource allocation shifts within corals in response to treatment which highlights that both experimental treatments did indeed elicit physiological responses. However, no physiological trade-offs between treatments were observed in a series of within colony-level trait correlations. This may suggest that potential reef restoration and adaptation efforts that incorporate *A. millepora* are not at risk of losing cold tolerance while promoting more heat-tolerant genotypes through assisted gene flow or ex situ selective breeding.

Differential physiological effects of cold and heat exposure

The physiological responses of animals to heat and cold exposure are known to vary in response to both temperature and the duration of the exposure (Pörtner 2002). Previous studies of heat and cold exposure on tropical corals have not agreed on their respective effects, potentially due to a difference in exposure times. Thermal exposure times of 10 weeks or above are rare in the literature, and this hinders the comparison of the results obtained in the present study. Roth et al. (2012) showed that heat stress was ultimately more deleterious than cold stress (22 °C) as *Acropora yongei* corals eventually acclimated in the cold treatment following 20 days of exposure. In contrast, working on four species of corals in Hawaii, Jokiel and Coles (1977) found the effects of severe cold stress to impact coral health and survival more negatively than severe heat stress when experiments exceeded 1-week duration. Our results generally support the former, with deteriorated conditions observed in response to heat but not cooling.

Temperature treatment affected the three growth metrics (bulk mass gain, basal disc extension and bulk density) differently. Mass gain varied significantly between treatments with heat-treated corals gaining less weight than the ambient and cold-treated corals. These results support previous findings of decreased mass gain in response to heat stress across species and source populations (Bay and Palumbi 2015; Lohr and Patterson 2017). Based on previous studies in which reduced bulk mass gain was documented in response to cold thermal stress (Kemp et al. 2011; Roth et al. 2012; Higuchi et al. 2015), we expected to see a similar response to the cold treatment applied here, but none was observed. Surprisingly, there was no treatment effect on coral basal disc extension despite prior studies demonstrating reduced skeletal extension in response to heat and cold stress in both short- and long-term experiments (Jokiel and Coles 1977; Baird and Marshall 2002; Roth et al. 2012; Higuchi et al. 2015). Cold-treated corals increased in bulk mass, but this was not reflected in size increases in AMR. An increase in mass in the absence of an increase in size could be explained by an increase in density (Lohr and Patterson 2017; Rocker et al. 2017); however, this was not the case in this study. At the conclusion of the experiment, heat-treated corals exhibited reduced density relative to the ambient group. Although not assessed in the present study, the low bulk density recorded in the heat-treated corals could indicate an increase in skeletal porosity which would affect the quality of the coral skeleton.

The bleaching indicators of tissue colour, chlorophyll α content, and symbiont densities all differed between treatments. Visibly paler, heat-treated corals had 46% less chlorophyll α content compared to the ambient treatment. This is similar to previous results for *Acropora tenuis* from overlapping locations (Abrego et al. 2008). Heat-treated corals also hosted less Symbiodiniaceae relative to both ambient and cold corals. Rodríguez-Troncoso et al. (2014)

reported an increase in symbiont densities in Eastern Pacific *Pocillopora* spp. following short-term (4 days) cold exposure which further corroborates the trends observed in this study. However, the effect of cold exposure on Symbiodiniaceae densities appears to be highly species specific. Gates et al. (1992) and Higuchi et al. (2015) reported reductions in symbiont densities in response to cold treatments in three different species (*Pocillopora damicornis*, *Acropora pruinosa and A. hyacinthus*). The observed increase in chlorophyll α in the cold treatment correlated with the increased symbiont density recorded for these corals. Observed changes in chlorophyll α may not be exclusive due to observed changes in symbiont density. They could also result from changes in pigment content per symbiont cell, but this was not investigated here. In this study, pigment content per cell was greater in the heat-treated corals than in those exposed to ambient and cold conditions. This result is surprising as decreases in chlorophyll α content within symbiont cells has been documented previously in response to heat (Hoegh-Guldberg and Smith 1989; Gates et al. 1992) but has been less extensively observed in response to cold stress (Howells et al. 2013; Rodríguez-Troncoso et al. 2014). Colour scores and chlorophyll α content showed a strong, positive correlation, indicating that coral tissue colour is an appropriate, non-invasive proxy of pigment concentration changes in *A. millepora* (Winters et al. 2009).

Potential shifts in resource allocation but no evidence of thermal tolerance trade-off

This study found evidence of resource allocation shifts in response to temperature treatment. Potential shifts in resource allocations were identified by negative correlations among some physiology traits. Lipid content was negatively correlated with basal disc extension (skeletal extension), suggesting that corals which laid down more skeleton did not have sufficient energetics to maintain lipid content. Skeletal extension also showed a negative relationship with skeletal density in the cold treatment. This indicates that the corals exposed to cold conditions shifted energy into skeletal extension rather than maintaining a high skeletal density (Ward 1995; Rocker et al. 2017). Previous studies have discussed energy allocation into growth and maintenance by proxies of linear extension and skeletal density, respectively (Anthony et al. 2002; Leuzinger et al. 2012; Rocker et al. 2017). Our demonstration of resource allocation shifts in response to experimental treatments is well supported in the existing literature (Anthony et al. 2002; Clarke 2003), but it was surprising not to identify more correlations between heat and cold performance. Previous research has found organisms that can maintain physiological condition under one extreme and do not tend to do so under another (Anderson et al. 2003; Clarke 2003). Hence, our expectation was to find a negative correlation. One possible explanation for this is that corals are still better adapted to the colder conditions represented by our cold treatment than to the warmer scenarios as suggested by Dove et al. (2013).

Previous studies have shown that heat-resistant organisms are often more susceptible to cold exposure (Sorensen et al. 2001; Anderson et al. 2003), and hence, trade-offs in traits such as growth and mortality can be observed (Roth et al. 2012). The results mainly showed no relationship between cold and heat responses except from a positive relationship observed in basal disc (skeletal) extension between the heat and cold treatments. Corals which exhibited large skeletal extension in the heat treatment also did so in the cold treatment. Thus, in terms of skeletal extension, selective breeding of heat-tolerant individuals should not result in the loss of skeletal extension during cold events. This provides some evidence that some coral colonies may have a greater thermal range, making them ideal candidates for translocation restoration efforts.

The results presented in this study highlight the potential for phenotypic plasticity in response to temperature exposure. The results demonstrate that corals from populations characterised by a heterogeneous thermal environment could make ideal candidates for coral restoration initiatives relying on assisted migration or breeding of *A. millepora*. In addition, this study highlights that corals are less susceptible to cold stress relative to heat stress which could be useful for future translocation/assisted migration restoration efforts in which corals from warm locations are relocated to colder locations in an attempt to increase population thermal tolerance. In such a case, it is vital that the translocated colonies are capable of coping with the seasonal temperature fluctuations, both hot and cold, in the new location. Interestingly, this study provides evidence that cold thermal tolerance is not necessarily lost in more heat-tolerant individuals. For future studies, it would be of interest to investigate the lower thermal threshold in *A. millepora* in populations across the GBR to determine suitable transplantation origins and destinations.

Acknowledgements The authors wish to thank the staff at the National SeaSimulator Precinct at the Australian Institute of Marine Science for their generous help and expertise offered during the course of the experiment. This research was funded by internal funds from the Australian Institute of Marine Science. Student support and transport to AIMS was provided by AIMS@JCU.

Compliance with ethical standards

Conflict of interest The authors have declared no conflicts of interests.

References

Abrego D, Ulstrup KE, Willis BL, van Oppen MJ (2008) Species-specific interactions between algal endosymbionts and coral hosts define their bleaching response to heat and light stress. Proc R Soc B Biol Sci 275:2273–2282

Ainsworth TD, Heron SF, Ortiz JC, Mumby PJ, Grech A, Ogawa D, Eakin CM, Leggat W (2016) Climate change disables coral bleaching protection on the Great Barrier Reef. Science 352:338–342

Anderson AR, Collinge JE, Hoffmann AA, Kellett M, McKechnie SW (2003) Thermal tolerance trade-offs associated with the right arm of chromosome 3 and marked by the hsr-omega gene in *Drosophila melanogaster*. Heredity (Edinb) 90:195–202

Anthony KRN, Connolly SR, Willis BL (2002) Comparative analysis of energy allocation to tissue and skeletal growth in corals. Limnology 47:1417–1429

Baird AH, Marshall PA (2002) Mortality, growth and reproduction in scleractinian corals following bleaching on the Great Barrier Reef. Mar Ecol Prog Ser 237:133–141

Baker AC, Glynn PW, Riegl B (2008) Climate change and coral reef bleaching: An ecological assessment of long-term impacts, recovery trends and future outlook. Estuar Coast Shelf Sci 80:435–471

Bay RA, Palumbi SR (2015) Rapid acclimation ability mediated by transcriptome changes in reef-building corals. Genome Biol Evol 7:1602–1612

Berkelmans R, van Oppen MJH (2006) The role of zooxanthellae in the thermal tolerance of corals: a "nugget of hope" for coral reefs in an era of climate change. Proc Biol Sci 273:2305–2312

Berkelmans R, Willis BL (1999) Seasonal and local spatial patterns in the upper thermal limits of corals on the inshore Central Great Barrier Reef. Coral Reefs 18:219–228

Cai W, Wang G, Santoso A, McPhaden MJ, Wu L, Jin F-F, Timmermann A, Collins M, Vecchi G, Lengaigne M, England MH, Dommenget D, Takahashi K, Guilyardi E (2015) Increased frequency of extreme La Niña events under greenhouse warming. Nat Clim Chang 5:132–137

Clarke A (2003) Costs and consequences of evolutionary temperature adaptation. Trends Ecol Evol 18:573–581

Davies PS (1989) Short-tern growth measurements of corals using an accurate buoyant weighing technique. Mar Biol 101:389–395

De'ath G, Fabricius KE, Sweatman H, Puotinen M (2012) The 27 year-decline of coral cover on the Great Barrier Reef and its causes. PNAS 109:17995–17999

Dove SG, Kline DI, Pantos O, Angly FE, Tyson GW, Hoegh-Guldberg O (2013) Future reef decalcification under a business-as-usual CO2 emission scenario. PNAS 110:15342–15347

Eakin CM, Lough JM, Heron SF (2009) Climate variability and change: Monitoring data and evidence for increased coral bleaching stress. In: van Oppen MJH, Lough JM (eds) Coral Bleaching. Patterns, Processes, Causes and Consequences. Springer, Berlin, pp 41–67

Fisher PL, Malme MK, Dove S (2012) The effect of temperature stress on coral-*Symbiodinium* associations containing distinct symbiont types. Coral Reefs 31:473–485

Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chemsitry 226:497–509

Gates RD, Baghdasarian G, Muscatine L (1992) Temperature stress causes host-cell detachment in symbiotic Cnidarians: implications for coral bleaching. Biol Bull 182:324–332

Gillooly JF, Brown JH, West GB, Savage VM, Charnow EL (2001) Effects of Size and Temperature on Metabolic Rate. Science 293:2248–2251

Higuchi T, Agostini S, Casareto BE, Suzuki Y, Yuyama I (2015) The northern limit of corals of the genus Acropora in temperate zones is determined by their resilience to cold bleaching. Sci Rep 5:18467

Hoegh-Guldberg O, Smith GJ (1989) The effect of sudden changes in temperature, light and salinity on the population density and export of zooxanthallae from the reef corals *Stylophora pistillata* (Esper) and *Seriatopora hystrix* (Dana). Jounral Exp Mar Biol Ecol 129:279–303

Howells EJ, Beltran VH, Larsen NW, Bay LK, Willis BL, van Oppen MJH (2011) Coral thermal tolerance shaped by local adaptation of photosymbionts. Nat Clim Chang 2:116–120

Howells EJ, Berkelmans R, Van Oppen MJH, Willis BL, Bay LK (2013) Historical thermal regimes define limits to coral acclimatization. Ecology 94:1078–1088

Hughes TP, Kerry JT, Álvarez-Noriega M, Álvarez-Romero JG, Anderson KD, Baird AH, Babcock RC, Beger M, Bellwood DR, Berkelmans R, Bridge TC, Butler IR, Byrne M, Cantin NE, Comeau S, Connolly SR, Cumming GS, Dalton SJ, Diaz-Pulido G, Eakin CM, Figueira WF, Gilmour JP, Harrison HB, Heron SF, Hoey AS, Hobbs J-PA, Hoogenboom MO, Kennedy EV, Kuo C-Y, Lough JM, Lowe RJ, Liu G, McCulloch MT, Malcolm HA, McWilliam MJ, Pandolfi JM, Pears RJ, Pratchett MS, Schoepf V, Simpson T, Skirving WJ, Sommer B, Torda G, Wachenfeld DR, Willis BL, Wilson SK (2017) Global warming and recurrent mass bleaching of corals. Nature 543:373–377

Jokiel PL, Coles SL (1977) Effects of Temperature on the Mortality and Growth of Hawaiian Reef Corals. Mar Biol 43:201–208

Kemp DW, Oakley CA, Thornhill DJ, Newcomb LA, Schmidt GW, Fitt WK (2011) Catastrophic mortality on inshore coral reefs of the Florida Keys due to severe low-temperature stress. Glob Chang Biol 17:3468–3477

Krediet CJ, DeNofrio JC, Caruso C, Burriesci MS, Cella K, Pringle JR (2015) Rapid, Precise, and Accurate Counts of *Symbiodinium* Cells Using the Guava Flow Cytometer, and a Comparison to Other Methods. PLoS One 10:e0135725

Krueger T, Hawkins TD, Becker S, Pontasch S, Dove S, Hoegh-Guldberg O, Leggat W, Fisher PL, Davy SK (2015) Differential coral bleaching - contrasting the activity and response of enzymatic antioxidants in symbiotic partners under thermal stress. Comp Biochem Physiol A Mol Integr Physiol 190:15–25

Leuzinger S, Willis BL, Anthony KRN (2012) Energy allocation in a reef coral under varying resource availability. Mar Biol 159:177–186

Lirman D, Schopmeyer S, Manzello D, Gramer LJ, Precht WF, Muller-Karger F, Banks K, Barnes B, Bartels E, Bourque A, Byrne J, Donahue S, Duquesnel J, Fisher L, Gilliam D, Hendee J, Johnson M, Maxwell K, McDevitt E, Monty J, Rueda D, Ruzicka R, Thanner S (2011) Severe 2010 cold-water event caused unprecedented mortality to corals of the Florida reef tract and reversed previous survivorship patterns. PLoS One 6. https://doi.org/10.1371/journal.pone.0023047

Little AF, van Oppen MJH, Willis BL (2004) Flexibility in Algal Endosymbioses Shapes Growth in Reef Corals. Science 304:1492–1495

Lohr KE, Patterson JT (2017) Intraspecific variation in phenotype among nursery-reared staghorn coral *Acropora cervicornis (Lamarck, 1816)*. J Exp Mar Bio Ecol 486:87–92

Madin JS, Hoogenboom MO, Connolly SR, Darlin ES, Falster DS, Huang D, Keith SA, Mizerek T, Pandolfi JM, Putnam HM, Baird AH (2016) A trait-based approach to advance coral reef science. Trends Ecol Evol 31:419–428

Mieog JC, Olsen JL, Berkelmans R, Bleuler-Martinez SA, Willis BL, van Oppen MJH (2009) The roles and interactions of symbiont, host and environment in defining coral fitness. PLoS One 4:e6364. https://doi.org/10.1371/journal.pone.0006364

Naumann MS, Niggl W, Laforsch C, Glaser C, Wild C (2009) Coral surface area quantification-evaluation of established techniques by comparison with computer tomography. Coral Reefs 28:109–117

Oliver TA, Palumbi SR (2011) Do fluctuating temperature environments elevate coral thermal tolerance? Coral Reefs 30:429–440

Paz-García DA, Balart EF, García-de-Léon FJ (2012) Cold water bleaching of *Pocillopora* in the Gulf of California. Proc 12th Int Coral Reef Symp 9–13

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2017) nlme: Linear and Nonlinear Mixed Effects Models

Pontasch S, Fisher PL, Krueger T, Dove S, Hoegh-Guldberg O, Leggat W, Davy SK (2016) Photoacclimatory and photoprotective responses to cold. J Phycol. https://doi.org/10.1111/jpy.12492-15-123

Pörtner HO (2002) Climate variations and the physiological basis of temperature dependent biogeography: Systemic to molecular hierarchy of thermal tolerance in animals. Comp Biochem Physiol - A Mol Integr Physiol 132:739–761

Pratchett MS, Anderson KD, Hoogenboom MO, Widman E, Baird AH, Pandolfi JM, Edmunds PJ, Lough JM (2015) Spatial, temporal and taxonomic variation in coral growth - implications for the structure and function of coral reef ecosystems. Oceanogr Mar Biol An Annu Rev 53:215–296

R Core Team (2017) R: A language and environment for statistical computing

Ritchie RJ (2006) Consistent sets of spectrophotometric chlorophyll equations for acetone, methanol and ethanol solvents. Photosynth Res 89:27–41

Rocker MM, Francis D, Fabricius KE, Willis BL, Bay LK (2017) Variation in the health and biochemical condition of the coral *Acropora tenuis* along two water quality gradients on the Great Barrier Reef. Mar Pollut Bull, Australia. https://doi.org/10.1016/j.marpolbul.2017.03.066

Rodolfo-Metalpa R, Peirano A, Houlbrèque F, Abbate M, Ferrier-Pagès C (2008) Effects of temperature, light and heterotrophy on the growth rate and budding of the temperate coral *Cladocora caespitosa*. Coral Reefs 27:17–25

Rodríguez-Troncoso AP, Carpizo-Ituarte E, Pettay DT, Warner ME, Cupul-Magaña AL (2014) The effects of an abnormal decrease in temperature on the Eastern Pacific reef-building coral *Pocillopora verrucosa*. Mar Biol 161:131–139

Roth MS, Deheyn DD (2013) Effects of cold stress and heat stress on coral fluorescence in reef-building corals. Sci Rep 3:1421

Roth MS, Goericke R, Deheyn DD (2012) Cold induces acute stress but heat is ultimately more deleterious for the reef-building coral *Acropora yongei*. Sci Rep 2:240

Saxby T, Dennison WC, Hoegh-Guldberg O (2003) Photosynthetic responses of the coral *Montipora digitata* to cold temperature stress. Mar Ecol Prog Ser 248:85–97

Siebeck UE, Marshall NJ, Klüter A, Hoegh-Guldberg O (2006) Monitoring coral bleaching using a colour reference card. Coral Reefs 25:453–460

Sorensen JG, Dahlgaard J, Loeschcke V (2001) Genetic Variation in Thermal Tolerance among Natural Populations of Drosophila buzzatii: Down Regulation of Hsp70 Expression and Variation in Heat Stress Resistance Traits. Funct Ecol 15:289–296

Strahl J, Stolz I, Uthicke S, Vogel N, Noonan SHC, Fabricius KE (2015) Physiological and ecological performance differs in four coral taxa at a volcanic carbon dioxide seep. Comp Biochem Physiol -Part A Mol Integr Physiol 184:179–186

van Oppen MJH, Palstra FP, Piquet AMT, Miller DJ (2001) Patterns of coral-dinoflagellate associations in *Acropora*: significance of local availability and physiology of *Symbiodinium* strains and host-symbiont selectivity. Proc R Soc B Biol Sci 268:1759–1767

Vavrus S, Walsh JE, Chapman WL, Portis D (2006) The behavior of extreme cold air outbreaks under greenhouse warming. Int J Climatol 26:1133–1147

Visram S, Douglas AE (2007) Resilience and acclimation to bleaching stressors in the scleractinian coral *Porites cylindrica*. J Exp Mar Biol Ecol 349:35–44

Ward S (1995) Two patterns of energy allocation for growth, reproduction and lipid storage in the scleractinian coral *Pocillopora damicornis*. Coral Reefs 14:87–90

Weis V (2008) Cellular mechanisms of Cnidarian bleaching: stress cause the collapse of symbiosis. J Exp Biol 3059–3066

Whitlock MC, Schluter D (2009) The Analysis of Biological Data. Roberts and Comapny Publishers, Greenwod Village

Winters G, Holzman R, Blekhman A, Beer S, Loya Y (2009) Photographic assessment of coral chlorophyll contents: Implications for ecophysiological studies and coral monitoring. J Exp Mar Bio Ecol 380:25–35

Yamashiro H, Oku H, Onaga K (2005) Effect of bleaching on lipid content and composition of Okinawan corals. Fisheries Science 71:448–453

Yuyama I, Higuchi T (2014) Comparing the effects of symbiotic algae *(Symbiodinium)* clades C1 and D on early growth stages of *Acropora tenuis*. PLoS One 9:1–8