



# In hot water: sustained ocean warming reduces survival of a low-latitude coral reef fish

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## Abstract

Tropical species are predicted to be particularly vulnerable to the impacts of climate change given the relatively narrow thermal range they naturally experience. Within the tropics, average temperature and thermal variation can differ among populations and consequently low-latitude populations may respond differently to increased temperatures than higher latitude tropical populations. In this study, we investigate the long-term effects of climate change relevant temperature increases on commonly measured condition metrics for a low-latitude population of damselfish (*Acanthochromis polyacanthus*). Adult fish were randomly assigned to one of the three seasonally cycling treatments: (1) current average ocean temperatures for the collection locations, (2) 1.5 °C, or (3) 3 °C higher than current average temperatures. Treatments were maintained for approximately 10 months. At the end of the experimental period, Fulton's *K* and hepatosomatic index were calculated for fish from each treatment group and critical thermal limit (CT<sub>Max</sub>) was measured for a subset of fish at control temperatures. Fish mortality was recorded throughout the experimental period, as well as at the end of the experimental period after the introduction of a secondary exercise stressor. No significant effect of temperature was observed on fish condition (Fulton's *K* and hepatosomatic index); however, significant mortality was observed for fish maintained at 3 °C higher than current average temperatures. When a secondary exercise stressor was introduced, significant mortality was also observed at 1.5 °C higher than current average temperatures. Acute exposure to higher temperatures (CT<sub>Max</sub>) suggested a much higher thermal tolerance for this population than long-term mortality, producing a thermal limit of 37.1 °C compared with a chronic thermal limit of 33 °C. Our results show that some basic measures of fish condition may not be capable of detecting lethal and sub-lethal effects of increased temperature. The results of this study are consistent with the hypothesis that low-latitude species are already living close to their thermal maximum.

## Abbreviations

AIMS Australian Institute of Marine Science  
CO<sub>2</sub> Carbon dioxide  
CT<sub>Max</sub> Critical thermal maxima  
GBR Great Barrier Reef

PNG Papua New Guinea  
SST Sea surface temperature

## Introduction

Tropical species are expected to be especially sensitive to projected future environmental warming, because they have evolved in a relatively stable thermal environment that now faces a high trajectory for temperature change when compared with other regions (Janzen 1967; Deutsch et al. 2008; Tewksbury et al. 2008; Burrows et al. 2011). An organism's physiological performance is often plotted as a function of temperature in what is commonly known as a thermal reaction norm (Angilletta 2009). The thermal reaction norm of species from tropical environments is generally observed to be narrower than for temperate species (Deutsch et al. 2008; Tewksbury et al. 2008; Sunday et al. 2011). Furthermore, numerous tropical organisms

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appear to currently live close to the warm edge of their thermal tolerance (Stillman 2003; Kellermann et al. 2012; Rummer et al. 2014) and, thus, may be unable to cope with even small increases in environmental temperature. However, a range of thermal environments exist within the tropics and, consequently, the thermal sensitivity of populations is likely to vary throughout tropical regions. In particular, lower latitude and equatorial populations could be more sensitive to elevated temperatures associated with climate change than populations from higher tropical latitudes, because the thermal range experienced is further restricted in these locations (Nguyen et al. 2011; Rummer et al. 2014; Bowden et al. 2014). Data testing these predictions is generally lacking, and the ability of equatorial and low-latitude populations of marine species to respond to ocean warming is largely unknown.

Ectotherms are predicted to be sensitive to future warming, as they have limited capacity for endogenous temperature regulation (Cossins 2012). Yet, many ectotherms span large geographic ranges, indicating that single species can function over wide thermal gradients. To achieve this, species may adopt a generalist thermal strategy, whereby individuals throughout the range are adapted to function over a wide range of temperatures (Huey and Hertz 1984; Angilletta 2009; Kellermann et al. 2012). Alternatively, populations throughout the range may be locally adapted and composed of thermal specialists who perform best within a narrow range of temperatures (Huey and Hertz 1984; Angilletta 2009). Evolutionary costs and benefits exist for each of these strategies and these will influence which strategy is favoured. In populations where there is a strong genotype  $\times$  environment interaction, in combination with limited gene flow between populations, local adaptation is likely to occur (Kawecki and Ebert 2004). Importantly, locally adapted populations may have reduced capacity to respond to environmental change and, consequently, may be at increased risk from a warming climate.

Research so far on the thermal sensitivity of coral reef fishes has focused largely on populations at latitudes greater than  $\sim 18^\circ$ . These studies show that elevated sea temperatures can markedly affect reproductive output, growth rates and physiological performance (Pankhurst and Munday 2011; Munday et al. 2012). Increased temperature has a significant effect on physiological performance measures, including aerobic scope, hypoxia tolerance and swimming speed (Nilsson et al. 2009, 2010; Johansen and Jones 2011). Elevated temperatures can have a significant effect on the timing or occurrence of reproduction (reviewed in Pankhurst and Munday 2011), and when increased temperature is combined with other potential effects of climate change such as reduced ocean productivity and consequently food availability, reproduction may cease completely (Hays et al. 2005; Harley et al. 2006; Donelson et al. 2010).

Growth in both adult and juvenile fish is also restricted at higher temperatures (Munday et al. 2008a).

Despite the well-described effects of increased ocean temperatures on higher latitude tropical reef species, we have only a limited understanding of how low-latitude populations may tolerate the higher water temperatures projected to occur over the next 100 years. In one of only two studies conducted to date (Bowden et al. 2014; Rummer et al. 2014), Rummer et al. (2014) showed that low-latitude fish populations are already living at or above their aerobic thermal optimum during summer. Low-latitude populations exhibit a decreased aerobic capacity at higher temperatures and significant mortality was observed for one species at  $34^\circ\text{C}$ . Although the study by Rummer et al. (2014) shows a significant effect of temperature on the performance of coral reef fish, the duration of exposure was only 14 days. Holding fish under elevated temperatures for longer periods is necessary if we are to gain a greater understanding of the effects of temperature on low latitude populations.

The purpose of the present study was to characterise the effects of climate change relevant temperature increases on physiological performance of a low-latitude population of a coral reef damselfish (*Acanthochromis polyacanthus*). Commonly tested metrics of physical condition and mortality were measured at current average ocean temperatures for the collection locations, and at 1.5, and  $3^\circ\text{C}$  higher than current average temperatures, with a longer period of temperature exposure than has previously been tested (approximately 10 months). Critical thermal limit ( $CT_{\text{Max}}$ ) was also tested for a subset of fish maintained at control temperatures. Condition metrics considered were Fulton's condition factor and hepatosomatic index. These basic measures provide an indication of the status of energy reserves in an animal and are commonly used as general indicators of fish condition throughout fisheries and general fish biology studies (e.g. Svåsand et al. 1996; Rätz and Lloret 2003; Lenhardt et al. 2009; Donelson et al. 2010, 2011, 2012). Fish were exposed to a secondary stress test (exercise) to determine their physiological resilience at the reared temperatures. Because of the stable thermal environment naturally experienced by this population we expected it to be composed of locally adapted thermal specialists who perform best within a narrow thermal range and, therefore, are likely to be particularly vulnerable to the effects of climate change relevant warming. Consequently, we predicted that increased temperatures would have a significant negative effect on the physiological condition and survival of this low-latitude population.

## Materials and methods

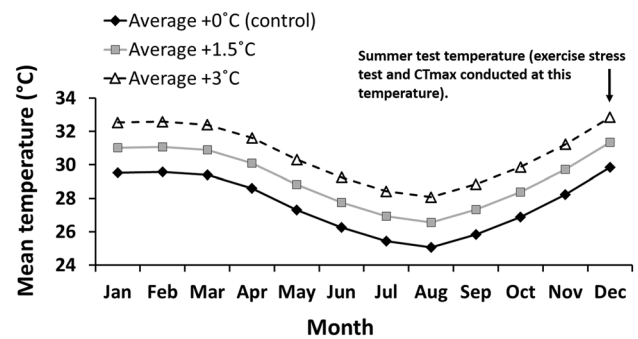
### Study species

Spiny chromis damselfish (*A. polyacanthus*) from Torres Strait (far northern Great Barrier Reef; GBR; 10°31'–10°46'S) were collected as adults and held in three seasonally cycling temperature treatments for 10 months (current average ocean temperatures, +1.5 and +3 °C) to test their long-term response to projected future increases in water temperature. *A. polyacanthus* is common across many reef locations on the GBR, is easily maintained in captivity and has been used in a number of previous studies. *A. polyacanthus* is a brooding tropical damselfish, with complete larval development occurring during embryogenesis (Doherty et al. 1994). This coral reef fish forms monogamous pairs and breeds primarily during the summer months (October–February; Robertson 1973; Thresher 1983). Offspring typically remain with their parents for approximately 30–45 days post-hatching (Kavanagh 2000). This restricts juvenile dispersal, causing the juvenile and parental environment to be the same, and resulting in genetically distinct sub-populations (Planes et al. 2001). As a result of this, it may be expected that *A. polyacanthus* is a good candidate for exhibiting local adaptation among populations throughout its range. *A. polyacanthus* is widely distributed in the Indo-Australasian region and has a large latitudinal range (15°N–26°S) from the equator to as far north as the Philippines and Indonesia, and to the southern Great Barrier Reef in Australia (Randall et al. 1997). This distribution makes *A. polyacanthus* an excellent species for latitudinal comparisons of thermal sensitivity. Across its range populations experience a total temperature span (average temperature inclusive of seasons) of approximately 20–31 °C. This range is comparable with a large number of other coral reef fish species (Munday et al. 2008b).

### Experimental design

Tropical sea surface temperatures (SSTs) are projected to increase by up to 3 °C by year 2100 if the current CO<sub>2</sub> emissions trajectory is maintained (Stocker et al. 2013). In the current study, we tested the long-term (10 month) response of a low-latitude population of a coral reef fish to future increases in water temperature under moderate and high warming scenarios. Light levels within the temperature controlled aquarium followed the natural season cycle at the collection location.

The three temperature treatments used in this study were: (1) current average ocean temperatures for the



**Fig. 1** Experimental design demonstrating seasonal water temperature at the Torres Strait fish collection location and in experimental treatments. The solid black line shows average monthly water temperature at the collection location based on 10 years of water temperature records from the Australian Institute of Marine Science weather station. Experimental treatment temperatures were: present day (+0 °C; solid black with closed diamonds), +1.5 °C (solid grey with closed squares) and +3 °C (black dashed with open triangles)

collection locations (+0 °C: 25.0–30.0 °C, seasonally cycling; Fig. 1a), (2) 1.5 °C higher than current average ocean temperatures (+1.5 °C: 26.5–31.5 °C), and (3) 3 °C higher than current average ocean temperatures (+3 °C: 28.0–33.0 °C). Seasonal  $\Delta T$  was maintained at 5 °C across all treatments. Water temperature was maintained within  $\pm 0.4$  °C of the set point using 3 kW electronic heaters with Carel IR33 temperature controllers (Control Distributions Pty Ltd, New South Wales, Australia). Temperature was monitored from a centralised environmental control system at the experimental aquarium facility and was also checked manually every second day. The summer temperature experienced by the +1.5 °C treatment (31.5 °C) is close to the maximum temperature experienced by wild populations of *A. polyacanthus* at the study location. The highest recorded average daily temperature at the study location over all the years used to calculate temperatures for this study (May 1998–March 2010) was 31.34 °C (Australian Institute of Marine Science (AIMS) SST database; <http://data.aims.gov.au/aimsrtids/datatool.xhtml?site=921&param=water%20temperature>). The +1.5 °C treatment (31.5 °C) is also directly comparable with similar studies conducted with more southern populations of *A. polyacanthus* where the +3 °C treatment has a maximum summer temperature of 31.5 °C (Donelson et al. 2010, 2011; Donelson and Munday 2012). The +3 °C treatment in this study has a summer maximum of 33 °C, which is greater than the population currently experiences, but would be the average summer temperature by 2100 under RCP 8.5 where emissions continue at their current rate.

## Fish collection and treatment allocation

Adult *A. polyacanthus* were collected from three reefs in the Southern Torres Strait, far Northern GBR during December 2011: Dugong Reef, Twin Cays and Kagar Reef (142°20'–142°35' and 10°31'–10°46'). All fish were collected from shallow patch reefs (< 10 m depth), characteristic of Torres Strait. Fish were transported to James Cook University Aquarium in Townsville, Australia where they were maintained in pairs in 60 L tanks in a recirculating system. Each tank contained a shelter site (half of a terracotta pot) and fish were fed ad libitum, 1–2 times per day using commercial fish pellets (INVE NRD G12). Conditions were initially maintained at the average water temperature for the collection location as determined from the AIMS SST database (described above).

In June 2012, male–female pairs were randomly assigned to one of the three temperature treatments described above (initial  $n = \sim 80$ ; 25–28 fish per treatment; fish from the three different reefs were mixed between treatments at random), with temperatures adjusted over a 7-day period. Fish were maintained in the treatment temperatures for 10 months (approximately 300 days) to test the chronic effects of increased water temperature. The 10-month experimental period was selected as fish were able to experience almost a full year within their assigned treatment group and consequently allowed for both summer and winter conditions to be experienced. Fish were weighed when they were first handled at day 90 of the experiment (for tests not presented here), and size between treatments was not significant [one-way ANOVA:  $F(2,59) = 1.29$ ,  $P = 0.28$ ]. The mean ( $\pm$  SE) weight of all individuals was  $23.0 \pm 5.02$  g, with a maximum size up to 39.8 g.

## Survival analysis

Throughout the experimental period, all fish deaths were recorded. A survival analysis was performed for the periods both before and after the introduction of a secondary exercise stressor. This allowed determination of the effect of temperature elevation alone on fish mortality, as well as the effect of temperature in combination with the secondary stressor. To test the effect of a secondary exercise stressor, commencing on experimental day 234, 45 days after summer temperatures were reached, fish were transferred to an upright circular swim chamber with a diameter of 145 mm. Due to high mortality, fish at +3 °C were stress tested and sampled earlier than the +1.5 °C treatment group. All testing was completed within 45 days and testing of the control group was spread throughout in order to help balance the experiment. Water current inside the cylinder was created using a magnetic stirring bar inside the chamber and stir-plate placed below the cylinder and water bath. The speed of the magnetic stir bar was increased

slowly until the fish could sustain a maximal swimming speed while maintaining its position in the water column. Fish were exercised for a period of 5 min. Secondary stress tests can be particularly useful indicators of an organism general level of stress, as individuals under stress are less able to physiologically cope with additional challenges. Such tests have been particularly useful for determining the effects of environmental stressors such as pollutants and thermal change associated with power plants (e.g. Adams et al. 1989; Sandhu et al. 2014).

Cox proportional hazards survival analyses were applied using S-Plus (TIBCO Software Inc., Palo Alto, USA) to identify significant differences in (1) fish mortality from the time that water temperature began to increase after the winter low (September 2012, day 90; timing of seasons artificially controlled and were offset slightly from actual seasonal temperature), and (2) for the 1 week period after exercise, or until the proportion of fish remaining reached 50% or below, to allow for the collection of tissues from the survivors. As fish were maintained in male–female pairs, any death that occurred due to within tank conflict or due to fish jumping from their tank was removed from the dataset and was not considered in the statistical test ( $n = 4$  at +0 °C, 0 at +1.5 °C and 2 at +3 °C), this contributed to some variation in the number of fish in each of the treatment groups. Seven fish were removed from the control treatment and two from the 1.5 °C treatment group before exercise testing to be used in additional treatments not presented in this manuscript. Fish standard length was included in the survival analysis as a covariate. For periods where mortality of the control group was zero, a dummy censored value was added to the final day of each of the three temperature treatments in order to deal with the effects of convergence and allow for interpretation of the Wald test. A likelihood ratio test was carried out to assess goodness of fit of the models and testing of the proportional hazards assumption showed no significant violation of the models ( $P = 0.28$  and 0.43 for the before and after exercise models, respectively). None of the individual values achieved significance for the proportional hazards assumption ( $P > 0.05$  in all cases). For statistical analysis, significance was accepted to a level of  $P < 0.05$ .

## Condition metrics

At the end of the experimental period, after exercise stress testing, the standard length (SL) and whole weight of all fish were measured. All fish were then euthanized by cervical dislocation and liver weight ( $W_{\text{Liver}}$ ) was recorded. Fulton's condition factor,  $K$ , was calculated from fish weight and length and provides an index of fish condition. The value of  $K$  was calculated following Froese (2006):

$$K = 100 \times \frac{W}{L^3},$$



where  $W$  is fish weight in g and  $L$  is fish SL in cm.  $N=6$ , 7 and 12 for control, +1.5 and +3 °C treatment groups, respectively.

Hepatosomatic index (HSI) is defined as the ratio of  $W_{\text{Liver}}$  in g to whole fish body weight. HSI provides an indication on status of energy reserve in an animal and was calculated following Htun-Han (1978):

$$\text{HSI} = \frac{W_{\text{Liver}}}{W} \times 100.$$

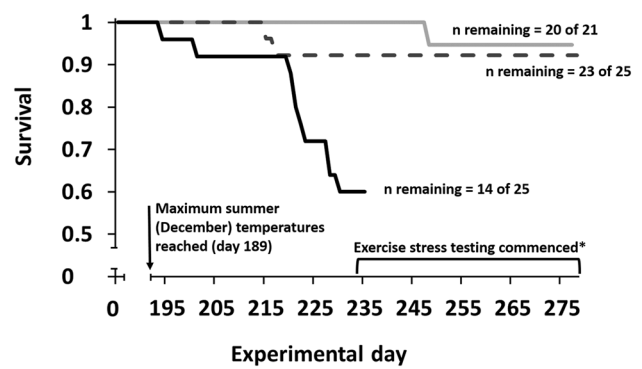
Here,  $n=6$ , 8 and 11 for control, +1.5 and +3 °C treatment groups, respectively.

Fulton's  $K$  and HSI were compared among temperature treatments using a one-factor analysis of variance (ANOVA). ANOVAs were carried out using SPSS (IBM, Armonk, USA), and assumptions of homoscedasticity and normality were examined with residual analysis prior to interpretation. For statistical analysis, significance was accepted to a level of  $P < 0.05$ .

Final measures of standard length, whole weight and liver weight were also compared among treatments, but are not presented here and did not produce significant differences.

### Determination of critical thermal limits

The critical thermal limit ( $CT_{\text{Max}}$ ) of *A. polyacanthus* in this study was calculated by rapid environmental temperature increase to estimate the temperature at which locomotory activity became disorganised independent of fish death (Cox 1974; Becker and Genoway 1979). Fish used in this experiment had experienced the average present-day Torres Strait thermal regime under lab conditions for approximately 10 months (June 2012–April 2013). Fish were transferred to individual testing tanks and given at least 1 h to recover from handling. On commencement of trials, water temperature was increased from the average summer temperature of 30 °C at a rate of 1 °C h<sup>-1</sup>. Pilot studies indicated that this rate was the fastest rate at which loss of equilibrium could be observed independently of the death of the fish, without a significant time lag in temperature increase. Water temperature was maintained within  $\pm 0.3$  °C of the desired temperature. Loss of equilibrium was defined as the point where the fish lost the ability to remain dorso-ventrally upright and could not regain this ability (Becker and Genoway 1979). As variance in  $CT_{\text{Max}}$  was low, we measured this trait for six replicate fish in order to avoid stressing more fish than necessary. The average  $CT_{\text{Max}}$  across the six replicates was calculated.



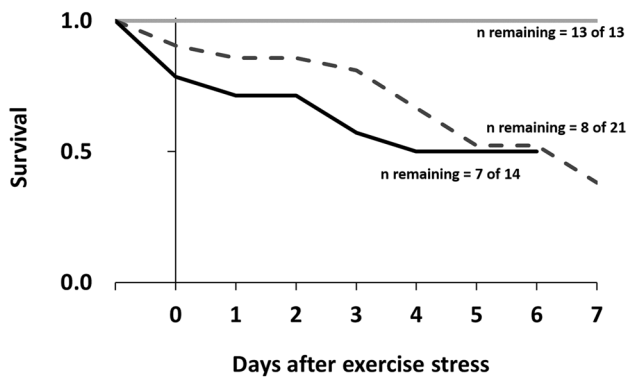
**Fig. 2** Proportion of fish remaining in each temperature treatment in relation to experimental day. The +0 °C treatment group is represented by the solid grey line, +1.5 °C by the broken black line and +3 °C by the solid black line. Data before summer temperatures were achieved are not shown on the graph as there were no deaths during this time. \* Due to high mortality, fish at +3 °C were stress tested and sampled on experimental days 236 and 241, earlier than the other two treatment groups. For this reason, they do not appear on the figure through to the end of the experimental period

## Results

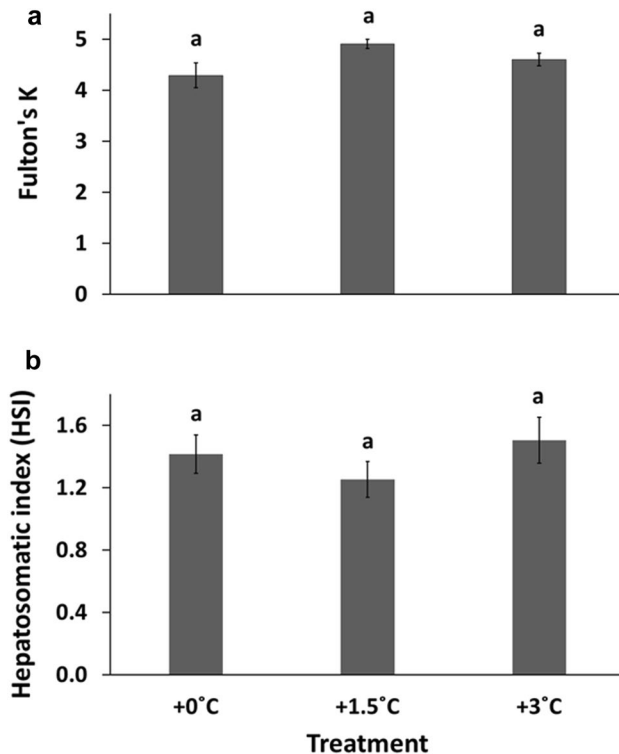
### Fish mortality in relation to temperature

No mortality was observed until experimental day 194, which was approximately 1 week after maximum summer temperatures were reached (Fig. 2). Significantly, higher mortality was observed for fish maintained at +3 °C, compared with fish at current-day +0 and +1.5 °C temperature treatments (Cox proportional analysis:  $Z=2.66$ ,  $P=0.01$ ). Mortality within the +1.5 °C temperature treatment was not significantly different from the current-day +0 °C control ( $Z=0.84$ ,  $P=0.40$ ). The majority of mortality occurred from experimental day 219 onwards, approximately 30 days after summer maximum temperature were achieved. Here, the number of fish remaining within the +3 °C treatment decreased from 23 to 16 (~30%) within a period of 10 days. The mortality of fish was significantly correlated to their standard length ( $Z=-2.33$ ,  $P=0.02$ ), with smaller fish surviving better.

Mortality during the week after exercise testing was significantly greater for fish in both the +1.5 and +3 °C treatments than the current-day +0 °C (Fig. 3;  $Z=2.67$ ,  $P=0.008$  and  $Z=2.32$ ,  $P=0.02$ , respectively). However, mortality between the +1.5 and +3 °C treatments was not significantly different ( $Z=0.51$ ,  $P=0.61$ ). Both elevated temperature treatments followed a similar trend of consistent mortality throughout the week after maximal exercise, with both elevated temperatures experiencing approximately 50% mortality by the end of the week. The number of fish in



**Fig. 3** Proportion of fish remaining in each temperature treatment in relation to the number of days post introduction of a secondary exercise stressor. The +0 °C treatment group is represented by the solid grey line, +1.5 °C by the broken black line and +3 °C by the solid black line. Day 0 is the day of exercise. Mortality was recorded for 1 week, or until the proportion of fish remaining reached 50% or below



**Fig. 4** Mean ( $\pm$ SE) **a** Fulton's condition factor ( $K$ ) and **b** hepatosomatic index (HSI) of *Acanthochromis polyacanthus* against temperature treatment. Temperature treatments tested were current average ocean temperatures (30.0 °C), +1.5 °C (31.5 °C) and +3 °C (33.0 °C). Letters indicate significant differences between treatment groups

treatment decreased from 21 to 8 in the +1.5 °C treatment

group, and from 14 to 7 in the +3 °C treatment. Fish standard length did not have an effect on mortality post-exercise testing ( $Z=0.98$ ,  $P=0.33$ ).

### Fish condition

Treatment temperature did not have a significant effect on Fulton's  $K$  [Fig. 4a;  $F(2,22)=3.23$ ,  $P=0.06$ ], despite fish that were maintained at control temperatures appearing to display a slightly lower index when compared with those maintained at the two higher temperature treatments. HSI was stable across all temperatures and statistical analysis showed no significant effect of temperature treatment on fish condition [Fig. 4b;  $F(2,22)=0.90$ ,  $P=0.42$ ].

### Critical thermal limit

The  $CT_{Max}$  of *A. polyacanthus* maintained at current-day +0 °C temperatures, ranged from 36.8 to 37.5 °C (mean = 37.07 °C,  $\pm$ SE = 0.11 °C). This is on average 7 °C higher than the average summer temperatures experienced by this population and 5.73 °C above the maximum temperature that Torres Strait reefs have experienced in the past 10 years.

### Discussion

Sustained elevated water temperature had little effect on fish body condition for this low-latitude population of *A. polyacanthus* after 10 months. Nonetheless, a significantly increased level of mortality was observed as water temperatures reached their summer maximum for fish maintained in the +3 °C above current ocean temperature treatment. In addition, substantial mortality occurred in both elevated temperature treatments (+1.5 and +3 °C) after the introduction of a secondary exercise stressor. Post-stressor mortality at +3 °C reached 50% within a period of less than 10 days at 33 °C. Given the significant effects of temperature on fish mortality, adults in this population of low-latitude *A. polyacanthus* may be extremely vulnerable to increasing water temperature projected with future climate change.

Based on mortality data, the population of *A. polyacanthus* tested in this study is currently living only 3 °C below their thermal limit (33 °C) for chronic exposure to elevated water temperature. Previous research on the same species has shown that short-term exposure (1–2 weeks) to water temperatures of 34 °C resulted in significant mortality in populations from PNG (Rummer et al. 2014) and the Central GBR (Zarco-Perelló et al. 2012), compared to 33 °C in the present study, despite differing thermal regimes (absolute and variation) between these locations (temperature variations span approximately 7 and 5 °C for

central GBR and low-latitude populations, respectively). These similarities suggest that the chronic thermal limit of this species is relatively stable between populations, irrespective of latitude. Previously, this lack of variation in thermal limits across latitude has only been observed in terrestrial ectotherms (Addo-Bediako et al. 2000; Deutsch et al. 2008). Consistency of thermal limits between populations is concerning for low-latitude populations as it means that they will be living closer to their thermal limits than higher latitude populations. While consistency in the warm thermal limit across populations has only been found for this one reef fish species, it could be common within coral reef fish, especially given that it was hypothesised that *A. polyacanthus* should be a strong candidate for local adaptation due to their lack of a pelagic larval phase.

Additional stress, caused by maximum exercise, resulted in a significant increase in the mortality rate of fish at +1.5 °C above current-day temperatures to a level not significantly different from the +3 °C treatment group. In the wild, a secondary stressor could include a vast range of biotic and abiotic factors. The effects of climate change are expected to be coincident with additional stressors, such as changes in ocean chemistry and food availability, as well as additional non climate-related anthropogenic effects (Harley et al. 2006). Fish health after a secondary stressor is likely to be more indicative of their capacity to deal with higher temperatures in nature as stressors do not tend to occur in isolation. For this reason, it is possible that studies which test the effects of increased temperature alone may provide a conservative estimate of the impacts of climate change on a population.

Fishes live under the ubiquitous threat of predation and escape can be both energetically expensive and stressful (Palacios et al. 2016). Exercise as a stressor represents an ecologically relevant way to test the effects of temperature on oxygen supply to tissues, and identify the mismatch in oxygen supply and demand that may occur at the limits of an organism's thermal tolerance (Pörtner 2010). There are, however, many possible alternative stressors and it is likely that impacts on survival would be variable. Other stressors that might reduce aerobic capacity when combined with warming include ocean acidification or pollution (Munday et al. 2009; Hicken et al. 2011). However, these stressors are likely to act over much longer timescales than the secondary stressor applied in this study. Exercise represents an acute or pulsed, rather than a chronic stressor. Other stressors that may occur over short time periods include changes in salinity, parasitism or disease (Varsamos et al. 2004; Marcogliese et al. 2005). The combination of systems that a stressor acts upon and the severity and time period over which it is applied will influence the effect of that stressor on fish mortality.

Interestingly, fish were able to maintain body condition across all temperature treatments despite a significant effect of temperature on fish mortality at both of the higher temperatures. The disparity between fish condition and the level of mortality reported in this study indicates that for the low-latitude population of *A. polyacanthus* examined, basic condition indices do not provide a reliable indicator of the ecological effects of sustained increases in temperature. It is possible that other indicators of fish condition not considered in this study may provide a more reliable indication of the lethal and sub-lethal effects of temperature increases; however, the most indicative measure may also vary between species, population or life stage. It is important to consider that this study may provide a conservative estimate of the effect of increased temperature on fish condition, because fish that were sampled for condition metrics included only those that survived the temperature treatments and the stress test, including those died immediately after or during the stress test. Fish that died during the 10-month temperature treatments were not sampled for condition. This potentially excludes the most susceptible fish from the analysis.

Maintaining body size at higher temperatures is likely to be a disadvantage for larger individuals as fish mortality was shown to be significantly correlated to their standard length, with smaller fish surviving better. There are likely to be physiological disadvantages of being too large in warmer conditions, as Clark et al. (2017) also found that smaller fish seem to deal better with higher temperatures during  $CT_{max}$  testing. Previous studies where higher latitude populations of *A. polyacanthus* were reared at elevated temperatures have observed a negative effect of increased temperature on fish condition; however, these studies considered fish that were exposed to higher temperatures during development (Donelson et al. 2010, 2011, 2012). Whilst HSI is commonly used as an indicator of fish condition, it is also possible that maintaining liver size may be an indication of change in other important stress-response processes such as the removal of waste products or increased immune defence (Bruslé and Anadon 1996), rather than energy storage. *A. polyacanthus* has been shown to store a large amount of energy in visceral fat (Donelson et al. 2010) and, thus, liver size relative to body size may not necessarily indicate a change in condition for this species. Regardless, in this study Fulton's *K* condition index was also similar across temperature treatments, suggesting that physical condition was maintained at higher temperatures in the fish tested.

Based on the mortality data for this study, low-latitude populations of *A. polyacanthus* are unlikely to persist past 33 °C (or even 31.5 °C with a secondary exercise stressor) and, consequently,  $CT_{Max}$  does not give a reliable indication of thermal sensitivity to future warming. The observed mortality due to chronic exposure to elevated water temperature occurred at 4 °C lower than the critical thermal maximum

(37.1 °C). Based on these measures, low-latitude populations will be far more vulnerable to the temperature changes associated with ocean warming than predicted by  $CT_{Max}$ . Studies that use  $CT_{Max}$  as their only method of predicting the effect of future increased temperatures (e.g. Mora and Ospina 2001) should be interpreted with caution. These findings differ from those of Sunday et al. (2012), who show that  $CT_{Max}$  may be used to accurately assess the potential latitudinal ranges of marine ectotherms. Instead, we believe that this method may provide an indication of an animal's short-term tolerance to thermal stress, akin to thermal tolerance under a transient heat-wave scenario. It is likely though to overestimate a species' ability to deal with long-term temperature stress associated with climate change (Pratchett et al. 2015). A comparison of  $CT_{Max}$  across temperature treatments could provide additional information relating to the overall thermal tolerance of the species.

Our study supports previous findings that tropical marine fishes have limited capacity to undergo reversible acclimation as adults (Nilsson et al. 2009, 2010; Gardiner et al. 2010; Rummer et al. 2014). Reversible acclimation involves short-term, regulated responses to environmental variation, often within a life stage, and is commonly associated with a response to diel or seasonal change by species that live in heterogeneous environments (Angilletta 2009; Sunday et al. 2014). Although the capacity for acclimation was not directly tested in this study, the increased mortality observed even after long periods of time at higher temperatures indicates that even if reversible acclimation is occurring at some level, possibly maintaining fish condition, it is unlikely to result in any significant ecological benefit. Compared with temperate species, tropical reef fishes are subjected to limited temperature variations (Sunday et al. 2011; Rummer et al. 2014) and, consequently, this may explain why reversible thermal acclimation is more commonly observed for temperate fishes (Fry and Hart 1948; Johnson and Bennett 1995; Wilson et al. 2007). Surprisingly, some polar fishes that also experience a narrow thermal range may have more capacity for reversible acclimation than tropical species, as they have been shown to acclimate to increased temperatures under relatively short time periods of less than 8 weeks (Seebacher et al. 2005, 2015; Podrabsky and Somero 2006; Franklin et al. 2007). At present, too few low-latitude fish species have been tested to know whether exceptions also exist in this environment, but at least for the species tested to date there is limited evidence.

This long-term study is consistent with the hypothesis that low-latitude species may be particularly vulnerable to higher temperatures associated with climate change. Future research should consider the extent to which the response seen in *A. polyacanthus* can be generalised to other low-latitude populations of coral reef fishes. In addition, given the vulnerability to higher temperatures seen here, our results

suggest that low-latitude populations of *A. polyacanthus* will need to adapt if they are to persist at the higher temperatures associated with climate change. Some individuals considered in this study were able to survive at elevated temperatures and displayed no change to body condition. This suggests that there might be genetic variation in thermal tolerance among individuals in this population that could provide scope for adaptation to warmer temperatures to occur (Munday et al. 2013). This of course depends upon the population being able to reproduce at higher temperatures and previous studies suggest that reproduction may be constrained at higher temperatures (Donelson et al. 2010, 2016). Genetic adaptation will also likely be constrained by the rapid pace of climate change compared with the multi-year generation time of these fish. Therefore, non-genetic mechanisms, such as developmental (within a generation) and trans-generational (across multiple generations) acclimation, may be particularly important in enabling low-latitude population to persist at higher temperatures, as they are capable of operating over climate change relevant time scales (Salinas and Munch 2012; Munday et al. 2013). Assessing the adaptive potential of low-latitude fish populations to climate change should be a priority for future research.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All applicable institutional and/or national guidelines for the care and use of animals were followed. This project was completed under JCU Ethics A1737.

**Availability of data and supporting materials** All data are available in the Tropical Data Hub (TDH) Research Data repository.

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