



# Impacts of increased ocean temperatures on a low-latitude coral reef fish – Processes related to oxygen uptake and delivery

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## ABSTRACT

Increasing temperatures are expected to significantly affect the physiological performance of ectotherms, particularly in tropical locations. The shape of an organism's thermal reaction norm can provide important information on its capacity to persist under climate change scenarios; however, difficulty lies in choosing a measurable trait that best depicts physiological performance. This study investigated the effects of elevated temperatures on processes related to oxygen uptake and delivery, including oxygen consumption, haematology, and tissue health for a low-latitude population of coral reef damselfish, *Acanthochromis polyacanthus* were collected from the Torres Strait (10°31'–46'S, 142°20'–35'E) and maintained at current average ocean temperatures (+0 °C; seasonally cycling), +1.5 °C and +3 °C higher than present day temperatures for 10 months. Aerobic performance indicated a limit to metabolic function at +3 °C (33 °C), following an increase in aerobic capacity at +1.5 °C (31.5 °C). Neither haematological parameters nor gill morphology showed the same improvement in performance at +1.5 °C. Gill histopathology provided the first indicator of a decline in organism health, which corresponded with mortality observations from previous research. Findings from this study suggest thermal specialisation in this low-latitude population as well as variation in thermal sensitivity, depending on the physiological trait.

## 1. Introduction

Temperature increases associated with ocean warming are projected to be greater toward the poles and less toward the tropics (Stocker et al., 2013). Despite this, tropical species are expected to be highly sensitive to projected ocean warming due to the higher trajectory of change in these environments and the thermally stable climate in which organisms have evolved (Tewksbury et al., 2011; Deutsch et al., 2008; Janzen, 1967; Burrows et al., 2008). Climate change is expected to impose significant effects on behaviour, ecology and physiology of organisms (Walther et al., 2002). Due to the lack of internal thermal regulation in ectotherms, the effect of environmental temperature on whole organism performance as a proxy for fitness can be represented using a thermal reaction norm (Angilletta, 2009). The optimal thermal window is located at the peak of this curve, and as temperatures deviate from this window performance is expected to decrease. The thermal

reaction norm is also often left skewed, so performance may deteriorate relatively quickly once temperatures increase above an organism's thermal optimum (Angilletta, 2009). The thermal reaction norm for tropical species is narrower than that of temperate species, again reflective of their naturally narrower thermal range (Deutsch et al., 2008; Tewksbury et al., 2008; Sunday et al., 2011). As a result, some tropical organisms appear to live at the edge of their thermal tolerance, even at present day temperatures (Stillman, 2003; Kellermann et al., 2012; Rummer et al., 2014a), and may be unable to cope with even small temperature increases in the future. Within the tropics different thermal environments exist, and lower latitude populations are expected to be more sensitive to elevated temperatures than populations from higher tropical latitudes (Nguyen et al., 2011; Rummer et al., 2014a; Payne et al., 2016).

The shape of an organism's thermal reaction norm can provide important information on the likely persistence of that population under

*List of abbreviations:* AIMS, Australian Institute of Marine Science; ANOVA, Analysis of variance; EtOH, Ethanol; GBR, Great Barrier Reef; H and E, Haematoxylin and Eosin; Hb, Haemoglobin; Hct, Haematocrit; MCHC, Mean corpuscular or cell haemoglobin concentration;  $\dot{M}O_2$  Max, Maximum oxygen consumption rate;  $\dot{M}O_2$  Routine, Routine oxygen consumption rate; PBF, Phosphate buffered formaldehyde; RBC, red blood cells; SL, Standard length

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climate change scenarios (Angilletta, 2009). Difficulty in making such predictions lies with selecting the performance measure or traits that are most related to whole organism performance and therefore individual and even biological fitness. One hypothesis is that there is no single optimal temperature for organism function, but instead, different physiological processes may exhibit differences in thermal performance, even at the same temperature (Hadfield, 1966; Bustard, 1967; Du et al., 2000; Clark et al., 2013). As well as determining direct causes of organism mortality, considering multiple fitness traits could have the added advantage of identifying a range of indirect, interacting or sub-lethal effects of a stressor on organism health, which may be equally important to the organism's functional niche.

The oxygen transport and delivery system is a common area of study for those wanting to better understand thermal performance in marine ectotherms (Pörtner, 2001; Farrell, 2002; Sollid and Nilsson, 2006; Nilsson et al., 2010; Drost et al., 2016). Even within the limitations of oxygen uptake and transport, there are many measures that could be examined which may help to determine an organism's thermal range. Aerobic scope is among the most commonly considered performance metrics for these types of studies. Aerobic scope describes the oxygen available – above basic maintenance of the organism – for important ecological activities such as reproduction, foraging, and predator avoidance (Pörtner and Knust, 2007). For ectotherms, restriction of whole-animal aerobic scope may provide an early indicator of a negative effect on organism health associated with temperatures outside the optimal thermal range. This is due to insufficient uptake, transport, and delivery of oxygen (Pörtner, 2001; Pörtner and Farrell, 2008).

The gills are likely to play a critical role in responding to temperature stress and subsequent oxygen deficiencies because they are the respiratory organ primarily responsible for oxygen uptake (Tzaneva et al., 2011). As a result, changes to gill morphology may be expected with temperature stress. Past studies have described significant changes in gill structure in response to hypoxia and temperature stress in a number of fish species (Sollid et al., 2005; Sollid and Nilsson, 2006; Nilsson, 2007; Mitrovic et al., 2009; Tzaneva et al., 2011). Sollid and Nilsson (2006) hypothesised that, in highly plastic species, gill remodelling is likely to occur in response to a changing oxygen demand based on the levels of variability in the availability of oxygen in the environment. Species such as the crucian carp (*Carassius carassius*) experience seasonal hypoxia and the capacity to change the area of the gill surface is a major evolutionary advantage (Sollid and Nilsson, 2006).

Other ways to modulate oxygen transport during stress events, such as those caused by elevated temperatures can occur at the level of the blood. Haemoglobin [Hb], which is encapsulated within the red blood cells (RBCs), is responsible for transporting 98–99% of the oxygen in blood. In times of thermal stress, oxygen demand within an organism increases, and this can subsequently result in hypoxemia if the oxygen transport system is not able to sufficiently meet increased demands (Cocking, 1959; Brett, 1971). Compounding this problem, the oxygen content of water also decreases with increasing temperature and this can reduce oxygen uptake by the blood at the gills (Farrell, 2002). Oxygen availability in water can be much more variable than in air, and so between species both the oxygen-carrying capacity of the blood (e.g., Hb concentration) and the oxygen affinity of Hb itself may be adapted to suit life-history (Wells et al., 1989). There is also evidence that these characteristics can vary within a species in response to short-term changes in the environment (Weber, 1982; Wells et al., 1989).

The above are, of course, only a limited selection of measures that could be considered in response to temperature stress, but due to their critical role in oxygen transport, they were selected for analysis in this study. Here, we compare the effect of climate change relevant increases in ocean temperatures on a range of physiological measures in a low-latitude population of a coral reef fish that has previously been shown to experience significant mortality at increased temperatures. The population examined is the spiny chromis damselfish (*Acanthochromis*

*polyacanthus*) from the Torres Strait (far northern Great Barrier Reef; GBR). Average temperatures for this location range from approximately 25 °C in winter to 30 °C in summer; a seasonal variation of only 5 °C. Whole animal metabolic performance, haematological parameters (including spleen-somatic index), and tissue health were compared for fish maintained at average temperatures for the collection location, as well as + 1.5 °C and + 3 °C above a seasonally cycling average for a period of 10 months. We hypothesised that there would be variation in response to increased temperatures among physiological traits tested. By comparing the thermal performance for each trait, we were able to determine the thermal sensitivity of this low latitude population to increased temperatures and establish the level of variation in sensitivity between several closely related traits. We consider how the performance of tested traits may effect whole organism performance and suggest possible directions for further research.

## 2. Methodology

### 2.1. Fish collection and temperature treatments

Adult spiny chromis damselfish were collected from three reef locations – Dugong Reef, Twin Cays, and Kagar Reef (10°31'–46'S, 142°20'–35'E) – in Southern Torres Strait, the northernmost part of the GBR, during December 2011. The three reef locations are in close proximity and are subject to similar thermal conditions. Fish were transported by plane to aquarium facilities at James Cook University (Townsville, Australia) where they were maintained as pairs, each in 60 L tanks containing a shelter (half of a terracotta pot) and connected to a system providing recirculating filtered, UV-sterilized seawater. The mean ( $\pm$  SE) mass of *A. polyacanthus* was 26.54  $\pm$  0.80 g, with a maximum size of 45.32 g. Pairs were fed ad libitum, one to two times per day using commercial fish pellets (INVE NRD G12). All pairs were initially maintained at the average summer water temperature for the collection location (30 °C). From December 2011 to June 2012 temperatures followed a simulated seasonal cycle (Australian Institute of Marine Science (AIMS) sea surface temperature database, Thursday Island reef slope; <http://data.aims.gov.au/aimsrtids/datatool.xhtml?site=921&param=water%20temperature>).

In June 2012, pairs were randomly assigned to one of three temperature treatments (initial  $n = \sim 80$ ; 25–28 fish per treatment; fish from the three different reefs were mixed between treatments at random). Treatment temperatures were: 1) current average ocean temperatures for the collection locations (control; +0 °C; 25.0–30.0 °C, seasonally cycling), 2) 1.5 °C above current average ocean temperatures (+1.5 °C), and 3) 3 °C above current average ocean temperatures (+3 °C). Treatment temperatures were adjusted over a 7-day period (< 0.5 °C per day) until target temperatures were reached. 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). Fish were maintained within their treatment temperature for 10 months to test the chronic effects of increased water temperature. The 10 month experimental period was selected as fish were able to experience the full thermal range of their temperature treatment, after almost a year within their assigned treatment group. Measurement of physiological metrics was carried out at summer temperatures (+0 °C = 30.0 °C; +1.5 °C = 31.5 °C and +3 °C = 33.0 °C), as previous work has shown that this is when temperature has the most significant impact on organism health (based on mortality) for this population (Rodgers et al., 2018).

### 2.2. Whole animal oxygen consumption rates

To determine the effects of temperature on aerobic performance of *A. polyacanthus*, routine oxygen consumption ( $\dot{M}O_{2\text{Routine}}$ ) and maximum oxygen consumption ( $\dot{M}O_{2\text{Max}}$ ) were measured for all fish ( $n = 22, 18$  and 14 for treatments +0, +1.5 and +3 °C, respectively).

All measures of oxygen consumption were carried out at the fish's experimental mean summer (February/March 2013) treatment temperature. Measurements were then used to calculate absolute aerobic scope ( $\dot{M}O_{2\text{Max}} - \dot{M}O_{2\text{Routine}}$ ) for each fish. The sampling design was unbalanced due to differences in mortality between the temperature treatments (Rodgers et al., 2018).

Food was withheld for 24 h prior to metabolic testing, so that specific dynamic action increases in  $O_2$  consumption associated with digestion would not affect the results. On commencement of metabolic testing, each fish was gently corralled into a 3.33 L respirometer, which was submerged for 1 h in a water bath in order to allow the fish to habituate to the chamber. All fish were tested at their treatment temperature. A pilot study showed that, for the fish used in this study, 1 h is sufficient to recover from any handling stress that may have elevated  $\dot{M}O_2$  (One-Way ANOVA;  $F_{5,23} = 2.50$ ,  $P > 0.05$ ). This short habituation time has also been shown to be sufficient to achieve routine measurements in other trials for this species when fish are not netted (Supplementary methods; Fig. S1). During the habituation period, chambers were flushed with clean, well-oxygenated, temperature-controlled sea water. This allowed for habituation to occur whilst preventing the accumulation of carbon dioxide and other metabolites, as well as excretory products, which may influence oxygen consumption (Steffensen, 1989). Following acclimatisation, the chamber was sealed and oxygen concentrations were monitored for 30–40 min to determine  $\dot{M}O_{2\text{Routine}}$ . Oxygen concentration never fell below 75% air saturation during this time. Although it is generally recommended that all respirometry systems should include a mixing device, previous work has shown that for *A. polyacanthus* this type of respirometry can provide reliable results in which trends in the data are not distorted (Rodgers et al., 2016; Supplementary methods; Fig. S1). This is likely due to natural constant pectoral fin movement exhibited by this species. Oxygen concentrations were monitored using a Firesting contactless oxygen system (Pryoscience).

Blank (empty) chambers were measured during the above mentioned pilot study and showed that microbial (background) respiration was negligible in this system until approximately 7 h after commencement of measurements. For this reason no microbial oxygen consumption was subtracted from fish  $\dot{M}O_2$ .

Fish were given at least 1 h after  $\dot{M}O_{2\text{Routine}}$  estimation before  $\dot{M}O_{2\text{Max}}$  measurements were taken. To determine  $\dot{M}O_{2\text{Max}}$ , fish were transferred to an upright circular swim chamber with a diameter of 145 mm (Nilsson et al., 2007; Donelson and Munday, 2012; Seebacher et al., 2014). 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 and without making (presumably anaerobic) lunge movements. During this time, the oxygen concentration in the water was measured every second for a minimum of 5 min. Directly after  $\dot{M}O_{2\text{Max}}$  measurements were taken, the wet mass of each fish was measured to the nearest mg.  $\dot{M}O_{2\text{Max}}$  and  $\dot{M}O_{2\text{Routine}}$  ( $\text{mg } O_2 \text{ consumed kg}^{-1} \text{ h}^{-1}$ ) were calculated for each fish using the recorded fall in oxygen and fish wet mass.

### 2.3. Blood collection and haematology

One week following respirometry trials, fish were terminally sampled. In order to minimise the effects of handling time and associated stress response in the fish, blood was sampled immediately upon netting a fish from its holding tank (< 10 s). Blood was drawn from the caudal artery/vein using a 25 G hypodermic needle into a 1 ml heparinized syringe. Blood haemoglobin [Hb] (oxygen-transport protein) was determined using the HemoCue® (Hb 201 System, Australia Pty Ltd) with 10  $\mu\text{l}$  of whole blood and was reported as grams per 100 ml using a calibration curve according to Clark et al. (2008) and corrected for tropical reef species (Rummer et al., 2013). Haematocrit (ratio of

packed red blood cells to whole blood volume; Hct) was determined by centrifuging 60  $\mu\text{l}$  of whole blood in heparinized microcapillary tubes for 3 min at 17,000 g and calculated as the ratio of packed red blood cells to total blood volume (reported as a percentage). Both [Hb] and Hct were used to calculate the mean corpuscular or cell haemoglobin concentration (MCHC;  $n = 6$  fish per treatment except for [Hb] where  $n$  was 6, 8 and 7 for treatments +0, +1.5 and +3 °C, respectively).

### 2.4. Tissue samples and preservation

Immediately after blood was drawn fish were euthanized by cervical dislocation prior to tissue sampling. The wet mass (nearest 0.01 g) and standard length (SL, in mm) of each fish was then recorded. Then, the second and third gill arches were removed and preserved in 4% phosphate buffered formaldehyde (PBF) and subsequently transferred to ethanol after ~48 h for histological analyses. The spleen was also removed, weighed, and then snap frozen in liquid nitrogen. The teleost spleen contains erythrocytes (red blood cells) sequestered from circulation, as well as erythropoietic tissue involved in the synthesis of new erythrocytes (Wells and Weber, 1990). The spleen is a major storage organ for blood cells and can contract during acute stress (Wells et al., 1989). Spleen mass was used to calculate spleen-somatic index, which provides a measure of spleen mass relative to whole fish mass.

### 2.5. Histology

Gill samples were grouped into cassettes according to treatment temperatures. Samples ( $n = 5$ –10 fish per treatment) were dehydrated through a series of graded ethanol (EtOH) concentrations (Shandon Southern Duplex Processor BS5), embedded in paraffin wax blocks (Shandon Histocentre 3, Thermo Electron Corporation) and sectioned using a microtome (Microm HM 325) into 4  $\mu\text{m}$  sections. Then, all tissue samples were stained with standard Haematoxylin and Eosin (H and E).

Gill tissue was cut parallel to the long axis of the filament and as the gill block orientation was not consistent and thus the number of observable primary lamellae varied, a standardised subsample was quantified for each sample. To do this, sections from each gill arch were randomly selected and digitally photographed (Olympus BX43 and Olympus camera DP27) up to a maximum of five times (less if total preserved area was captured in a lower number of images) at 200 $\times$  magnification. Gill aneurisms and dilations were noted and if present, counted (average number per standardised section of gill arch), and measured (widest section). Common stress response indicators such as cell proliferations, mucus, or inflammation were also recorded when present.

### 2.6. Calculations and statistical analyses

Prior to statistical analysis measures of  $\dot{M}O_{2\text{Routine}}$ ,  $\dot{M}O_{2\text{Max}}$  and aerobic scope for each fish was log-transformed and a homogeneity of slopes model was examined to ensure that the relationship between fish metabolism and fish mass was consistent across treatments. All measures were then standardised to the average fish body mass using a residuals plot of log metabolic rate ( $\text{mg } O_2 \text{ h}^{-1}$ ) vs log fish mass (g). Differences in aerobic measures between the three temperature treatments were compared for the adjusted values using a one-way analysis of variance (ANOVA).

Differences in haematological parameters, spleen-somatic index, and gill morphology among the three temperature treatments were compared using a one-factor ANOVA with temperature treatment (+0, +1.5 and +3 °C) as the fixed factor.

All statistical analyses were carried out using Statistica (StatSoft Inc., Tulsa, USA), and all assumptions were examined with residual analysis and transformed when necessary to meet the assumptions of normality and homogeneity of variance. Where necessary, Tukey's post-

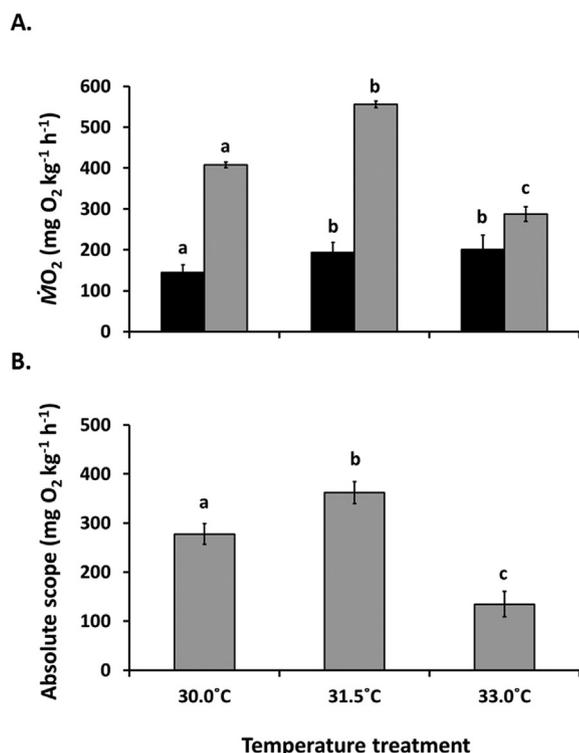


Fig. 1. Routine (black columns) and maximum (grey columns) oxygen consumption rates (a) and absolute aerobic scope (b) (means  $\pm$  SE) for *A. polyacanthus* during the austral summer. Temperature treatments included average ocean temperature (30 °C) for the collection site as well as + 1.5 °C (31.5 °C) and + 3 °C (33.0 °C). Letters indicate significant differences between treatment groups within a measurement.

hoc means comparisons were used to identify the nature of significant effects found by ANOVA.

### 3. Results

#### 3.1. Whole animal oxygen consumption

$\dot{M}O_{2\text{Routine}}$  ( $\text{mg O}_2 \text{ h}^{-1}$ ) of fish differed significantly between temperature treatments ( $F_{2,46} = 12.65$ ,  $P < 0.001$ ; Fig. 1A). There was a significant increase in  $\dot{M}O_{2\text{Routine}}$  between the current-day control treatment and the + 1.5 °C treatment group (post-hoc:  $P < 0.001$ ), however no significant difference in  $\dot{M}O_{2\text{Routine}}$  was observed between the + 1.5 °C and + 3 °C treatment groups (Post hoc:  $P > 0.05$ ).

$\dot{M}O_{2\text{Max}}$  also differed between treatments for *A. polyacanthus* ( $F_{2,44} = 23.92$ ,  $P < 0.001$ ; Fig. 1A).  $\dot{M}O_{2\text{Max}}$  increased significantly from 30 °C (+0 °C, control) to 31.5 °C (+1.5 °C; post-hoc:  $P < 0.001$ ), but then fell sharply from 31.5 to 33 °C (+3 °C;  $P < 0.001$ ) to a level that was significantly lower than  $\dot{M}O_{2\text{Max}}$  values measured in fish at the control ( $P < 0.001$ ; Fig. 1A).

Changes in aerobic scope were largely driven by changes in  $\dot{M}O_{2\text{Max}}$  and again depended on temperature treatment ( $F_{2,38} = 21.27$ ,  $P < 0.001$ ; Fig. 1B). Aerobic scope increased in fish from 30 °C to peak at 31.5 °C (post-hoc:  $P < 0.05$ ), which appeared to be the thermal optimum for the fish in this study. Aerobic scope then significantly decreased in fish from 31.5 to 33 °C (post-hoc:  $P < 0.001$ ) to a level that was just 35.39% of the control (30 °C) aerobic scope and 27.12% of the aerobic scope for fish maintained at 31.5 °C.

#### 3.2. Haematology

Both [Hb] and Hct followed the same trend with increasing

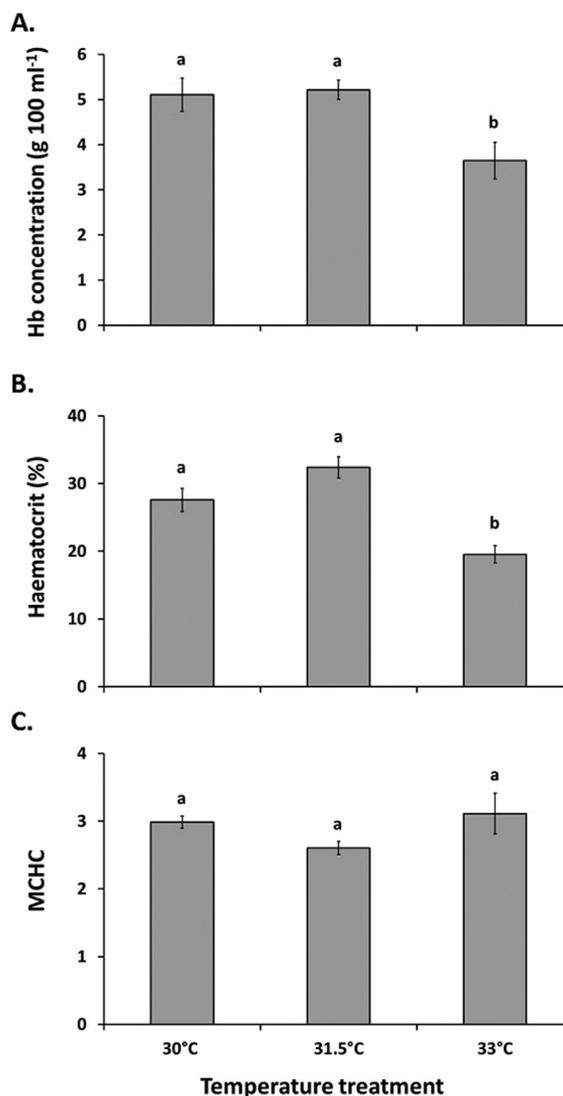


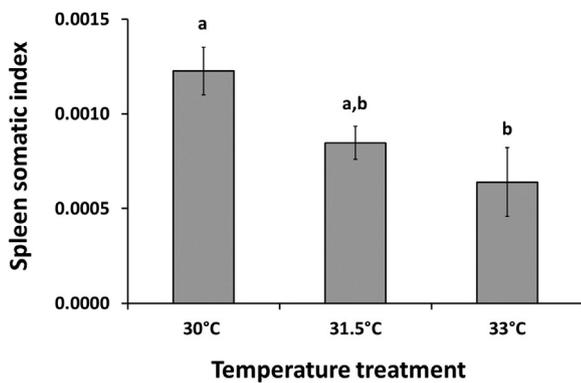
Fig. 2. Haematological parameters for *A. polyacanthus* sampled during the summer months at current average ocean temperatures (30 °C), + 1.5 °C (31.5 °C) and + 3 °C (33.0 °C): (a) Haemoglobin concentration ( $\text{g } 100 \text{ ml}^{-1}$ ), (b) haematocrit (% packed red blood cells to total blood volume), and (c) mean cell haemoglobin concentration (MCHC,  $\text{g } 100 \text{ ml}^{-1}$ ). Letters indicate significant differences among treatment groups.

temperatures as aerobic scope (i.e., decreasing at 33 °C;  $F_{2,20} = 7.23$ ,  $P < 0.01$  and  $F_{2,17} = 18.06$ ,  $P < 0.001$ , respectively; Fig. 2). There was no significant difference between values from the control fish (30 °C) and the + 1.5 °C fish (31.5 °C) for either measure (post-hoc:  $P > 0.05$ ), but both values significantly declined from the + 1.5 °C when compared to fish from the + 3 °C (33 °C) treatment group ( $P < 0.05$ ). There was no significant difference in MCHC between any of the treatments ( $F_{2,17} = 1.95$ ,  $P = 0.18$ ).

There was a significant decrease in spleen-somatic index with temperature (SSI;  $F_{2,13} = 5.10$ ,  $P = 0.02$ ; Fig. 3). SSI was significantly lower at + 3 °C when compared to fish from the control (30 °C; post-hoc:  $P < 0.05$ ), indicating a smaller spleen size relative to fish mass at this temperature.

#### 3.3. Histological analysis

Aneurysmal dilations were the most striking feature in the gill tissue, particularly at higher temperatures (Fig. 4), and the number and diameter of aneurysms increased with increased water temperatures.



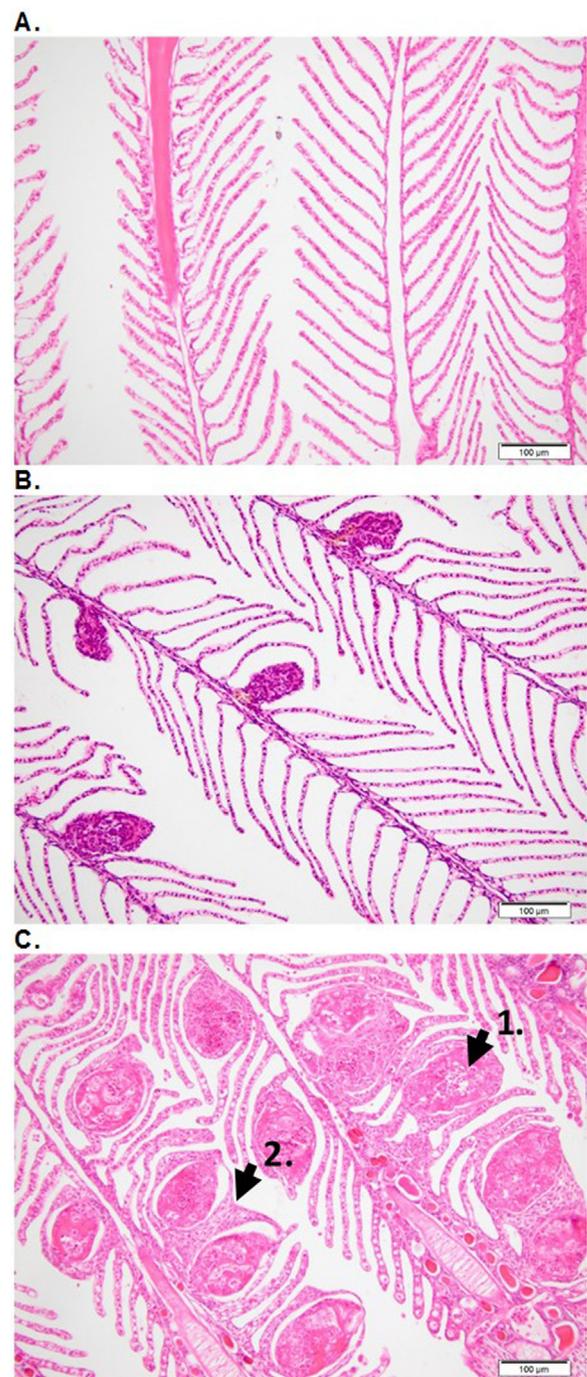
**Fig. 3.** Spleen-somatic index (means  $\pm$  SE) for *A. polyacanthus* sampled during the summer months at current average ocean temperatures (30 °C; n = 5), + 1.5 °C (31.5 °C; n = 8) and + 3 °C (33.0 °C; n = 3). Letters indicate significant differences among treatment groups.

This observation was significant for the number of aneurisms per standardised section of gill arch ( $F_{2,20} = 4.67$ ,  $P < 0.05$ ; Fig. 5A), as there were more aneurisms per gill arch in fish from the warmest treatment temperature (+3.0 °C/33 °C) when compared to control fish (post-hoc:  $P < 0.05$ ). The number of aneurisms in the gills from fish maintained 31.5 °C (+1.5 °C) was highly variable, ranging from between 2 and 178 aneurisms per gill arch, and the mean was not significantly different than that of either the control temperature or 33 °C treated fish (post-hoc:  $P > 0.05$  in both cases). Although not significant ( $F_{2,17} = 3.50$ ,  $P = 0.056$ ; Fig. 5B), there was a strong trend for the mean width of the aneurisms to increase with increasing temperature. In addition to the quantified data, it was apparent that fusion of dilations and concentric fibrin formation encircling the endothelium (recanalization) were common in fish reared at 33 °C, which is indicative of chronicity. Other commonly noted gill abnormalities such as specific cell proliferations, mucus, or inflammation, however, were not apparent in any of the samples.

#### 4. Discussion

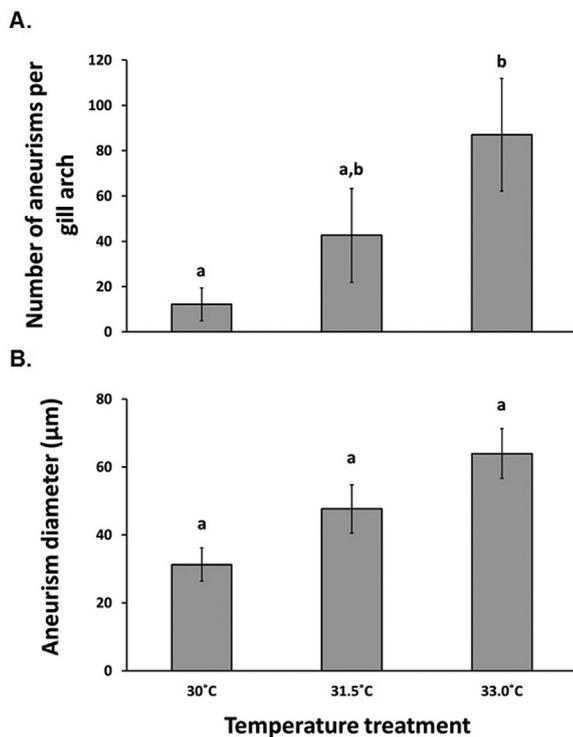
Elevated temperatures affected each physiological and morphological trait considered in this study, but differences in performance were not consistent across all indicators of fitness. While both aerobic scope and haematological measures revealed a decline in performance after temperatures exceeded 31.5 °C, gill histopathology began to show a trend toward declining performance at this temperature. Thresholds for reduced performance in aerobic capacity and haematological parameters were similar to mortality thresholds observed in previous research (Rummer et al., 2014a; Rodgers et al., 2018). Results from histopathology, aligned more closely with mortality thresholds observed when a secondary stressor (e.g. maximal exercise) was introduced in previous studies of *A. polyacanthus* from this low-latitude location (Rodgers et al., 2018). A significant increase in mortality at 31.5 °C post-exercise was observed here. Of the measures examined, morphological changes in the gills appeared to provide the first indication of declining fish health and subsequently the best gauge of thermal tolerance among the traits considered for this low-latitude population.

Elevated temperatures of 33 °C resulted in a significant decline in the aerobic scope of *A. polyacanthus*. Aerobic scope was maximised at 31.5 °C, which is 1.5 °C above the average summer temperature experienced by this population. When temperatures were increased by 3 °C above the average summer temperature (to 33 °C), aerobic scope was significantly reduced to the lowest level observed over all testing temperatures. Previous work on this population found significant (> 50%) mortality at 3 °C above average summer temperatures (Rodgers et al., 2018). Based on measurements of aerobic scope alone, it appears that fish performance was maximised at 31.5 °C. However,



**Fig. 4.** Micrographs of *A. polyacanthus* gill sections following prolonged exposure to (a) current average ocean temperatures (+0 °C; seasonally cycling), (b) + 1.5 °C and (c) + 3 °C higher than present day temperatures. Images are 200 $\times$  magnification, and scale bars in the lower right corner of each image are 100  $\mu$ m. Fibrous remodelling/re-canalization of aneurisms in the + 3 °C treatment group are indicated by arrow 1. Enlargement and fusion of secondary lamellae are indicated by arrow 2.

this may be a best-case scenario for this population as Rodgers and colleagues (2018) also showed that when a secondary stressor (e.g. short periods of maximal exercise) was imposed on fish living at 31.5 °C, mortality rates significantly increased. Additionally, gill histopathology in this study showed gill health of some individuals at + 1.5 °C was reduced and similar to the + 3 °C temperature treatment. This suggests that there may be other more sensitive physiological attributes that respond to elevated temperatures before a decrease in



**Fig. 5.** Frequency (a) and size (b) of gill aneurysms (means  $\pm$  SE) for *A. polyacanthus* sampled during the summer months at average ocean temperatures (30 °C; n = 6) for where fish were collected, + 1.5 °C (31.5 °C; n = 7) and + 3 °C (33.0 °C; n = 7). Letters indicate significant differences among treatment groups.

aerobic scope is observed, and these could provide an earlier indication of temperature stress.

Gill histopathology revealed some surprising trends. Typically, when analysing damage to gill tissue, gill health will be ranked based on a predetermined set of stages (e.g., Flores-Lopes and Thomaz, 2011; Salamat et al., 2013), but these stages of degradation were not found in the current study. Primary and secondary lamellae did not show common, less severe histopathological changes, such as specific cell proliferations, mucus, or inflammation; however, aneurysmal dilations were present. An aneurysm is an irreversible change, so any gills with this feature would ordinarily be classified as in an advanced stage of damage. This is the first time to our knowledge that these types of changes to the gill have been observed in response to temperatures above a species' optimum. Aneurysmal injuries are common in response to pollutants such as pesticides or heavy metals (van den Heuvel et al., 2000; Cengiz and Ünlü, 2002; Simonato et al., 2008; Oliva et al., 2009), which is unsurprising, as the gills are a first point of contact with pollutants. One review suggests that an increase in plasma cortisol and catecholamines in response to stress may contribute to gill tissue degradation in some instances of pollutant exposure (Wendelaar Bonga, 1997). Whilst this may be a contributing factor, we predict that oxygen deficiency and related increases in blood pressure may be the primary cause of aneurysms observed here.

Although there has been no previous evidence of aneurysmal injury to gill tissue in response to temperature stress, past research has described significant changes in gill structure (namely surface area) in response to hypoxia and temperature stress for a number of fish species (Sollid et al., 2005; Sollid and Nilsson, 2006; Nilsson, 2007; Mitrovic et al., 2009; Tzaneva et al., 2011). The population of *A. polyacanthus* examined in the present study are from an environment with no evidence for changes in oxygen availability, thus based on this, morphological gill remodelling may not be expected. Bowden et al. (2014) tested capacity for gill remodelling of the secondary lamellae in another

low-latitude population of *A. polyacanthus*, as well as four other damselfish species. Fish were exposed to increased temperatures for periods of 12–14 days, and no evidence for changes in gill morphology in response to increased temperatures were observed. Remodelling is energetically costly, and so it could be expected that many species show limited plasticity in this trait, especially over short exposure times. No capacity to remodel the gills in order to optimise oxygen uptake may mean that fish are unable to cope with extended periods of temperature stress, possibly leading to the gill damage observed in the present study. Evidence for repair (recanalization) of aneurysms was indicative of chronicity, suggesting that poor gill health had been an ongoing problem at higher temperatures.

Similar to measures of aerobic scope, exposure to 33 °C resulted in a significant decline in [Hb] and Hct for the *A. polyacanthus* population considered in this study. Decreases in [Hb] and Hct at 33 °C indicate that there was either a decrease in the number of RBCs in circulation, a decrease in the size of the RBCs, or possibly a decrease in the Hb concentration within each cell. The former is supported because there was no change in MCHC between treatments. There are at least four possible causes for this decrease in [Hb] and Hct. The first is that RBCs were being reabsorbed by the spleen. This seems unlikely, as it would restrict the transportation and uptake of oxygen throughout the fish; a response that would be counterproductive during stress. The spleen also showed a significant decline in relative mass with increasing temperatures, indicating that it is more likely that the spleen released more RBC into circulation, rather than reabsorbing them (Wells et al., 1989). The second possible explanation is that an increase in the volume of the primary circulation occurred, possibly through water being pushed into circulation from muscle tissue. This scenario is also thought unlikely as marine fish typically dehydrate during stress (Wendelaar Bonga, 1997). Higher temperature reduces the viscosity of the blood and so potentially reduces pumping costs (Graham and Fletcher, 1983; Randall and Brauner, 1991). Reductions in Hct and Hb could therefore have been the result of a temperature-driven viscosity effect, however again the decline in SSI does not support this proposition either, as spleen relative mass should not be affected by a change in blood viscosity. The final possibility, and the hypothesis considered most likely in this study, is that RBCs were being re-distributed into the secondary vascular system, which under non-stressful conditions contains only plasma (Kampmeier, 1969).

The secondary vascular system was initially thought to function in a similar way to the mammalian lymphatic system (Kampmeier, 1969; Yaniv et al., 2006; Isogai et al., 2009), however further studies have challenged this hypothesis, providing evidence of a connection to the arterial system and thus describing the vessels as a secondary vascular system in form and function (Vogel, 1981; Vogel and Claviez, 1981; Steffensen and Lomholt, 1992; Jensen et al., 2009; Rummer et al., 2014b). Jensen et al. (2009) and Rummer et al. (2014b) both show that during exercise and in some cases hypoxia, RBCs are able to pass from the primary vascular system to the secondary vascular system. Normally, the input vessels from the primary vascular system to the secondary vascular system are too small to allow RBC to pass through under resting conditions, but Rummer et al. (2014b) suggest that the anastomoses open during stressful conditions – potentially via adrenergic pathways – thus permitting RBCs to flow into the secondary vascular system. The current study is the first to suggest that elevated temperatures may also trigger this pathway. Benefits of allowing RBCs into the secondary vascular system may include reducing pressure on the heart, buffering ionic or osmotic changes in the primary vascular system, or enhancing oxygen uptake across the skin (cutaneous respiration); all of which would assist in the event of thermal stress (Rummer et al., 2014b).

Each of the measures examined in this study relate to the uptake and transport of oxygen, and all were found to display a response to increased temperature which indicates an attempt to increase the level of oxygen in circulation. In addition to their individual effects on fish

health, it is likely that these measures interact, potentially resulting in changed, more, or less significant effects on whole organism health than if considered in isolation. For example, it is possible that in this study, an inability of haematological responses to compensate for lower aerobic capacity at higher temperatures put greater pressure on the gills, leading to or exacerbating the observed tissue damage. Changes to one system may then go on to have implications for another (Barton, 2002). For example, additional energy requirements to maintain respiratory pathways may lead to a reduction in immunocompetence or reproductive capacity. It is possible that the effects of increased temperatures on different systems are likely to feed back into each other, creating an even more complex pathway. Untangling the interactive effects of multiple measures is a difficult task and would benefit from further research.

Of the measures tested, no one was able to provide a definitive causal mechanism for the fish mortality at higher temperatures previously reported for this population by Rodgers et al. (2018). In some fish that died prior to metabolic testing, rupturing of gill aneurysms was predicted to be the mechanism driving mortality because, in several cases, bleeding from the gills was observed at time of death. This symptom was not seen for all fish however and so was thought to provide cause of death in only some instances. It is possible that another mechanism, not examined in this study, could provide a more definitive early indication of declining fish health under temperature stress. A growing body of evidence suggests that this mechanism may be heart function (Farrell, 2002, 2009; Muñoz et al., 2014). Limitation of maximum heart rate, resulting in ischemia injury through deficit of oxygen to the myocardial tissue (myocardial hypoxia) is commonly cited as the mechanism driving heart failure (Farrell, 2002, 2009; Muñoz et al., 2014). Heart rate fails to meet oxygen demand in the tissues when a higher volume of oxygen is extracted from the venous blood supply by the skeletal muscle before it can reach the heart, subsequently causing an oxygen deficit leading to arrhythmia and bradycardia (Farrell, 2002). Myocardial hypoxia could be further exacerbated or preceded by cardiac mitochondrial dysfunction, as mitochondrial cells in the heart have also been shown to suffer damage due to heat stress, ischemic damage, and oxidative stress, particularly in species from a thermally stable environment (Iftikar and Hickey, 2013; Iftikar et al., 2014). A cascade of physiological symptoms could result from this central problem of heart failure, including some of those reported in this study.

Changes in blood chemistry and in aerobic performance can be observed as a short term response to increased temperatures (Tun and Houston, 1986; Roche and Bogé, 1996; Farrell, 2002; Pörtner and Knust, 2007), whereas the changes in gill morphology observed in this study are likely to have occurred over much longer time scales. This is potentially why the changes in gill morphology seen here have not been observed in previous studies. It is difficult to predict whether the physiological responses to higher temperatures observed in this study would have been different over a shorter exposure period, as the stress response can change and even become a maladaptive with increasing length of exposure to a stressor (Barton, 2002). For aerobic scope at least, a similar trend has been observed for *A. polyacanthus* over much shorter time periods in Papua New Guinea (exposure time 12–14 days; Rummer et al., 2014a). The lack of difference in short and long term response not only indicates consistency in the stress response of that trait over time, but also suggests a limited capacity for acclimation of these traits to higher temperatures. This hypothesis is supported by previous work which has already suggested a low capacity for reversible acclimation both in this population specifically, and in other populations of coral reef fishes (Gardiner et al., 2010; Rummer et al., 2014a; Rodgers et al., 2018). Further studies should examine potential acclimation capacity over multiple generations.

## 5. Conclusions

Findings from this study align with predictions that thermal

specialisation and a limited capacity for acclimation are characteristic of low-latitude marine and terrestrial ectotherm populations (Janzen, 1967; Deutsch et al., 2008; Tewksbury et al., 2008; Angilletta, 2009; Burrows et al., 2011). Despite the low capacity for acclimation observed in this and other studies, some ability to acclimate and/or adapt over multiple generations will be essential for these populations to persist under future climate change scenarios. It is important to note that different life stages may be affected differently by thermal stress, and so impacts over various ontogenetic stages should also be considered. Our study shows that regardless of the causal mechanism of death, slight differences in thermal performance could be observed across the measures examined for this low-latitude population of coral reef fish. The gills were the first of the organs to display a response to increased temperature. Considering multiple fitness measures and how they interact to influence whole organism health is a complex approach, and the field would clearly benefit from further research. Our study supports the idea that by expanding the number of measures used to quantify the physiological impacts of elevated temperatures, a much better understanding of how an organism may respond to a stressor can be obtained.

## CRedit authorship contribution statement

**G.G. Rodgers:** Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing - original draft, Writing - review & editing. **J.L. Rummer:** Methodology, Investigation, Visualization, Writing - review & editing. **L.K. Johnson:** Methodology, Investigation, Visualization, Writing - review & editing. **M.I. McCormick:** Conceptualization, Methodology, Visualization, Writing - review & editing.

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## Data availability

All data is available in the Tropical Data Hub (TDH) Research Data repository at doi: [10.25903/5c0eff685e349](https://doi.org/10.25903/5c0eff685e349).

## 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.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:[10.1016/j.jtherbio.2018.12.008](https://doi.org/10.1016/j.jtherbio.2018.12.008)

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