**Running title1:** Intraspecific thermal variation in a coral reef fish (*Acanthochromis polyacanthus*)

Potential journals:

1. Coral Reefs
2. Journal of Experimental Biology
3. Journal of Thermal Biology
4. Conservation physiology
5. Marine Biology
6. Marine Ecology Progress Series
7. Journal of Fish Biology

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# Abstract

How species respond to climate change will depend on the collective response of populations. Intraspecific variation in traits, evolved through genetic adaptation and phenotypic plasticity, can cause thermal performance curves to vary over species’ distributions. However, intraspecific variation within marine environments has received relatively little attention due to the belief that marine systems lack dispersal barriers strong enough to promote locally adapted traits. Here we show that intraspecific variation is present among low- and high-latitude populations of a coral reef damselfish (*Acanthochromis polyacanthus*). Co-gradient variation was observed when examining aerobic physiology across thermal gradient (i.e., 27°C, 28.5°C, 30°C, 31.5°C) that reflected mean summer temperatures of high- and low-latitude regions, as well as projected future ocean temperatures. However, not all traits displayed intraspecific variation; no significant differences were observed between high- and low-latitude regions when measuring immunocompetence, hematocrit, and enzyme activity. The presence of co-gradient variation suggests that dispersal limitations in marine systems can promote local adaptive responses, however, intraspecific variation may not be ubiquitous among traits.

# Introduction

The response of species to climate change is determined by the collective response of populations (Bennett et al. 2019; McKenzie et al. 2020). How populations respond to environmental change can vary along geographic and environmental gradients due to variation in traits that has evolved via genetic adaptation and phenotypic plasticity (Sorte et al. 2011; Des Roches et al. 2018; Bennett et al. 2019; Plumb et al. 2020). Temperature conditions, particularly among ectotherms, are hypothesized to produce macro-ecological patterns that reflect thermal constraints on organism’s biochemistry and physiology (Somero 2010; Pereira et al. 2017). Co-gradient variation across thermal clines, whereby genetic and environmental influences on phenotype are aligned (e.g., populations exposed to higher temperatures have high optimal performance temperatures), has been demonstrated in a variety of taxa (plants (Aitken and Bemmels 2016; Mahony et al. 2020), insects (Hoffmann et al. 2003; Barton et al. 2014), crustaceans (Kuo and Sanford 2009; Sorte et al. 2011; Yampolsky et al. 2014), and fish (see review by Conover et al. 2009)). However, optimal performance temperatures often do not follow the trajectory of environmental gradients (Conover et al. 2009). Counter-gradient variation, whereby genetic and environmental influences on phenotypes are opposed, occurs when phenotypic and genetic divergence are decoupled to maximize fitness (Schmid and Guillaume 2017; Stamp and Hadfield 2020). Counter-gradient variation has been recorded in several taxa (lizards [Angilletta et al. 2004; Hodgson and Schwanz 2019], turtles [Snover et al. 2015], and fish [Gardiner et al. 2010]); however, the extent to which phenotypic plasticity and genetic differentiation contribute to counter-gradient variation varies (Stamp and Hadfield 2020).

Population responses to warming temperatures will depend on their occupied thermal niche. Low-latitude environments characterized by stable temperatures near physiological maximums favor specialized (narrow) thermal niche breadths that primarily evolved through genetic adaptation (i.e., selection for particular phenotypes) rather than plasticity – Climate Variability Hypothesis (Janzen 1967; Stevens 1989; *but see* Overgaard et al. 2011; Chiono and Paul 2023). Narrow thermal niche breadths, limited plasticity, and evidence of hard ceilings for upper thermal tolerance (Gunderson and Stillman 2015; Sandblom et al. 2016; Morgan et al. 2020), suggest that low-latitude populations are more vulnerable to shifting temperatures than high-latitude conspecifics (Stillman 2003; Deutsch et al. 2008; Tewksbury et al. 2008; Somero 2010; Sunday et al. 2011). High-latitude populations, that experience variable environmental conditions, are predicted to retain greater benefits from phenotypic plasticity than low-latitude conspecifics (Janzen 1967; Stevens 1989); nonetheless, empirical evidence remains scarce (but see, Molina-Montenegro and Naya 2012; Naya et al. 2012; Donelson et al. 2019). Wider thermal niche breadths have been reported in high-latitude populations (Sunday et al. 2011; Shah et al. 2017; Stuart-Smith et al. 2017; McKenzie et al. 2020), however, heat-tolerant phenotypes present in low-latitude populations may be unattainable within high-latitude populations (Kelly et al. 2012). Individual populations may therefore possess thermal niches that are narrower than the species as a whole (Kelly and Griffiths 2021).

Intraspecific-variation in thermal performance between populations within marine systems has not received the same attention as terrestrial systems; despite marine organisms having greater confinement to thermal tolerance limits (Sanford and Kelly 2011; Sunday et al. 2011; Pinsky et al. 2019; Lenoir et al. 2020). Within terrestrial systems local adaptation is already being incorporated into conservation considerations to prepare organisms for projected climate change scenarios (Aitken and Whitlock 2013; Aitken and Bemmels 2016; Liepe et al. 2016; Bazzicalupo et al. 2023). Although, marine systems hereinto have been viewed as demographically well-connected networks where local adaptation would be overwhelmed by gene flow. However, a growing body of evidence suggests that oceanographic features, life history traits, and larval dispersal/establishment ability can act as challenges to gene flow and promote local adaptation (Jones et al. 1999; Swearer et al. 2002; Sanford and Kelly 2011). Evidence of local adaptation between distinct populations has been demonstrated among marine crustaceans (Stillman 2002; Kuo and Sanford 2009; Sorte et al. 2011; Kelly et al. 2012; Pereira et al. 2017; Sasaki and Dam 2019, see review Sanford and Kelly., 2011), and coral (van Oppen et al. 2014), further suggesting that marine systems are not connect ubiquitously; yet, few studies broach the topic among marine fish.

Thermal intraspecific variation in marine fishes varies depending on life-history traits and population connectivity, therefore, broadscale geographical patterns, such as the climate variability hypothesis and co-/counter-gradient variation, are unlikely to be universally applicable (Calosi et al. 2008; Sasaki and Dam 2019). A case study comparing low- and high-latitude populations of coral trout (*Plectropomus leopardus*), a species with a pelagic larval stage and high level of population connectivity (via spatial and temporal variation in larval recruitment (Van Herwerden et al. 2009; Taboun et al. 2021)), found no significant differences in physiological metrics between populations (Pratchett et al. 2013). However, patterns of counter-gradient variation, climate variability, and genetic distinctness have been identified among marine fish species with high- (*Gadus morhua*; Marcil et al. 2006) and low-dispersal (*Acanthochromis* polyacanthus; Gardiner et al. 2010; Donelson and Munday 2012) ability between populations. The lack of uniformity in broadscale geographic patterns among marine fish necessitates the examination of population-based responses (i.e., intraspecific variation).

Intraspecific thermal variation within the coral reef damselfish, *A. polyacanthus*, is evident; however, existing physiological studies provides a coarse understanding. For example, knowledge of high-latitude thermal performance comes from a single lagoonal population (Heron Island; Gardiner et al. 2010; Donelson and Munday 2012), that is genetical different from surrounding reefs (Miller-Sims et al. 2008) . To increase the resolution of *A. polyacanthus’s* thermal landscape and allude to a finer understanding of intraspecific variation within marine environments, this study compared thermal performance curves of key physiological traits within *A. polyacanthus* from three different populations among two regions of the Great Barrier Reef, low-latitude (~Cairns) and high-latitude (~Mackay) that experience different thermal profiles. We tested the hypothesis for counter-gradient variation across a thermal gradient between the low-latitude and a high-latitude region. Based on previous evidence, *A. polyacanthus* are expected to display counter-gradient variation (Gardiner et al. 2010; Donelson and Munday 2012). However, considering previously demonstrated genetic differentiation, lack of variability in studied populations, and the unique nature of the previously tested high-latitude population (i.e., lagoonal), co-gradient variation remains a valid alternative hypothesis.

# Methods

Study species

The tropical damselfish, *Acanthochromis polyacanthus* (Bleeker 1855), ranges from the southern Great Barrier Reef (GBR) to the central Philippines (spanning 30° of latitude; Allen 1991). *A. polyacanthus* populations are hypothesized to have propagated the Indo-Pacific proceeding the Pleistocene (2.6 Ma- 11.7 ka) as rising sea levels reestablished dispersal corridors between reefs (Van Herwerden and Doherty 2006; Ludt and Rocha 2015). However, such dispersal corridors ceased to function as water levels reached present-day conditions, as *A. polyacanthus* lacks a pelagic larval development period. *A. polyacanthus* perform parental care during embryonic and early life development, in socially monogamous pairs, where eggs are defended by both parents until fry are large enough to disperse into the surrounding habitat (Robertson 1973). Limited dispersal between reefs separated by depths greater than 10m (Miller-Sims et al. 2008), creates conditions that should promote local adaptation (Sanford and Kelly 2011); a broad geographic distribution across thermally variable environments, where gene flow is limited.

## Sampling

Adult *A. polyacanthus* were collected via professional collectors from June to December 2021 from six different reefs and two different regions (low- and high-latitude). Three reefs from low-latitude locations were sampled including, Tongue Reef (-16.341°, 145.773°; *n =8*), Vlassof Cay (-16.657°, 145.990°; *n =10*), and Sudbury Reef (-16.996°, 146.202°; *n =11*). High-latitude sites included Cockermouth Island (-20.772°, 149.390°; *n =10*), Keswick Island (-20.908°, 149.406°; *n =6*), and Chauvel Reef (southern; -20.863°, 150.363°; *n =10;* **Figure 1**). Low and high-latitude collection regions are separated by ~400 kilometers (spanning ~5° in latitude). In total 55 fish were sampled over the duration of the experiment (**Supplemental table 1**). Of the initial 55 fish, 38 completed all experimental assays including: resting metabolic rate, maximum metabolic rate, aerobic scope, immunocompetence, hematocrit, and enzyme activation analysis.

Adult fish were held in separate 60 L opaque aquariums (56 x 35 x 30 cm) inside an environmentally controlled aquarium room at the Marine and Aquaculture Research Facility at James Cook University (Townsville, Australia). Each aquarium contained a shelter (half a terra-cotta pot), constant aeration, and water flow (2 L min-1) at set experimental conditions (see below). Fish were transferred to the experiment room that was used for trials on May 25th, 2022. Respirometry and immunity trials occurred from June 6th – August 17th, 2022. Tissue (enzymes) and blood (hematocrit) samples were collect on September 1st, 2022, 2-weeks after immunity trails concluded.

## Thermal conditions

To understand local thermal conditions for reefs within low-latitude and high-latitude regions temperature data was collected via AIMS (Australian Institute of Marine Science) temperature Logger data series. Temperature data was collected from loggers at depths of 7-15m, for a subset of reefs (**Supplemental table 2**) from each region (Australian Institute of Marine Science (AIMS) 2020; **Supplemental figure 1**). Experimental temperatures for repeated aerobic physiology and immune response testing included the approximate daily mean summer temperature for both high-latitude (~27°C) and low-latitude (~28.5°C) regions, as well as 30°C (mid-2100 century; SSP2-4.5, SSP3-7.0, and SSP5-8.5), and 31.5°C (end of 2100 century; SSP2-4.5 and SSP5-8.5; Masson-Delmotte et al. 2021). Testing began at the coolest temperature of 27°C, and once aerobic physiology and immune response testing was completed, fish were warmed to the next temperature of +1.5°C, at a rate of +0.5°C Day-1 for three consecutive days. Fish were then provided with an additional five days to adjust to the new temperature treatment before the next sampling period began. This process was repeated for all testing temperatures. Final testing of XXX... Here I would also give the gaps between each testing at the same temps ??

## Aerobic physiology

Resting and maximum metabolic rate were determined via measuring the rate of oxygen consumption using intermittent flow respirometry. Chambers were 1.5 L in volume and custom built from PVC pipe and acrylic (**Supplemental figure 2**). The experimental setup consisted of two sumps (260 L), with continuous water exchange and aeration, each containing four submerged respirometry chambers placed in parallel. Chambers were opaque except for the lid, so that fish could not view each other. Each respirometry chamber unit contained an independent brushless DC recirculation pump (flow rate 240 L h-1), vinyl tubing (composing ~1% of the total water volume), and an inline oxygen sensor probe (multichannel FireSting-O2, PyroScience GmbH, Aachen, Germany). Oxygen sensor probes were calibrated to 0% air, using sodium sulphite (Na2SO3) saturated seawater, at the beginning of the experiment and when spot material was replaced. 100% air calibrations were conducted at the beginning of each trial. During flush periods a pump (AQUAPRO, AP750LV; 750 L h-1) was used to flush each set of four chambers simultaneously. Heaters (2 kilowatt) and temperature sensors (Semitec 103AT-11 IP67) were used to ensure that experimental temperatures remained within +/-0.3°C of experimental temperature set points. Minimal background respiration was achieved through UV filtration, particle filtration (100 µm bag filters), and daily cleaning of equipment (bleach diluted to 200 ppm with fresh water). Fish were deprived of food for 18-24 h before aerobic respiration trials began and trials were conducted in a fully lit room to eliminate metabolic costs associated with digestion and photoperiod.

Maximum oxygen consumption (MO2max) was used as a proxy for maximum metabolic rate (Norin and Clark 2016). To achieve maximum oxygen consumption fish were placed in a swim tunnel for 10 min. During the initial 5 min interval, the speed of water flow through swim tunnel was slowly increased until fish displayed a changed in gait swimming behavior, defined as a transitioning behavior from predominately pectoral swimming to body/tail undulations (**Supplemental video 1**). The speed of the swim tunnel that produced this intermediary transitional swimming behavior was maintained for the second 5 min interval. Immediately after the 10 min swimming period, fish were collected by hand, and transferred to a randomly selected respiration chamber. Pilot studies (unpublish data, Schmidt) determined that highest MO2max levels were achieved with the immediate transfer of fish from the swim tunnel to respiration chambers, rather than including an intermediary air exposure period. Therefore, no air exposure time was included prior to fish being transferred into respiration chambers. The time between fish being placed in respiration chambers and data being recorded (i.e., start of the wait period) was less than 10 s. MO2max was measured over 30 s intervals via rolling regressions within the *‘*auto\_rate’ function included in the R package ‘*respR’* (v2.0.1; Harianto et al. 2019). The steepest slope (highest oxygen consumption rate) with an *r2* threshold of 0.95 was used to determine MO2max. MO2max was measured prior to resting metabolic rate (MO2resting).

Fish were held in respirometry chambers for 3.5 – 6 h ( =4.67 h) to measure MO2resting. Oxygen consumption was measured continuously over cycles consisting of a 15 s wait, 225 s measurement, and 180 s flush period. Air percentage never dropped below 80% air saturation. Oxygen consumption rates were measured over a 220 s interval with an *r2* threshold of 0.95.MO2resting was measured by taking the mean of the lowest 3 oxygen consumption slopes. Background respiration was measured at the start of each trial by measuring oxygen consumption within empty chambers for at least three consecutive cycles. Background respiration levels typically accounted for <2% of measured oxygen usage rates and were therefore ignored. The mass of fish was measured at the end of all respiratory trials, after fish had been euthanized and patted dry with paper towel to avoid the inclusion of excess moisture. The mean fish-to-chamber volume ratio was 1:60 (**Supplemental figure 3**) but varied depending on the size of each fish. Oxygen consumption rates were converted from percent air saturation values to mg h-1 via the *‘convert\_rate’* function within the R package *respR* (Harianto et al. 2019). Absolute aerobic scope (AAS) was calculated by subtracting MO2resting fromMO2max.

## Immune response

To test immunocompetence subcutaneous phytohemagglutinin injections were used to produce a (localized) cell-mediated response *in vivo* (e.g., inflammation, T-cell proliferation, infiltration of immune cells; (Martin et al. 2006; LaMonica et al. 2021). Tissue swelling 24-hours post-injection is mediated via complex immunological cascade, however, is primarily driven via the congregation of leukocytes to the injection site (Martin et al. 2006). Fish were injected in the caudal peduncle with 0.03 mL of phytohemagglutinin (Phytohemaglutinin; L8754 Sigma-Aldrich, 45 ug 10 uL-1) dissolved in phosphate buffer saline (PBS), made to a ratio of 1 mg PHA to 1 mL PBS. The immunocompetence of fish was determined by measuring the injection area with pressure sensitive calipers (Mitutoyo ABS Digimatic; accuracy 0.1mm) pre-injection, and ~18-24 hours post-injection. The difference in localized swelling pre- and post-injection was used as a proxy for immunocompetence.

## Fish sampling

Whole blood and tissue samples (i.e., white muscle tissue) were collected 10 days after all aerobic physiology and immune responses trails were completed at the final testing temperature (31.5°C). Whole blood was collected from the caudal vein via heparin-coated 25-gauge surgical needles. Fish were then euthanized via cervical dislocation. White muscle tissue samples were dissected from tissue between the dorsal fine and lateral line; once obtained tissue samples were stored in liquid nitrogen and then transferred to a -80°C freezer.

## Hematocrit

Microcapillary tubes (75mm Drummond Hemata-clad plain) were used to centrifuge blood samples at 10,000 rpm for 60 seconds to separate red cells from blood plasma. The proportion of blood volume occupied by red blood cells (hematocrit) was recorded by using a ruler to first measure the space of the microcapillary tube that was occupied by the total blood volume (packed red blood cells and blood plasma), followed by measuring the space occupied by packed red blood cells. Hematocrit scores were calculated using the following formula:

## Enzyme activity

White muscle tissue was used to examine the maximal enzyme activity of lactate dehydrogenase (LDH) and citrate synthase (CS). Testing temperatures of 20°C, 30°C, 40°C, and 50°C were used to determine maximal enzyme activity and the associated thermal performance curve. White muscle tissue was used for the maximal enzyme activity analysis because, its anaerobic capacity has been shown to correlate to whole organism oxygen consumption, and it plays an important role in bursts of high-speed swimming (Sullivan and Somero 1980). Additionally, white muscle tissue compromises most of the body mass for *A. polyacanthus*.

The maximal enzyme activity method used was adapted from previous studies (Thibault et al. 1997; Seebacher et al. 2003; Lang et al. 2021). Samples were defrosted on ice. A sterile scalpel blade was used to extract a tissue sample (20-40 mg). Extracted tissue samples were homogenized via a microtube homogenizer (BeadBug 3, Benchmark Scientific, Model D1030-E) in a 1:10 dilution with a buffer consisting of 50 mmol L-1 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 1 mmol L-1 ethylenediaminetetraacetic acid (EDTA), 0.01% Triton X-100, and 99.99% Milli-Q water, and adjusted to pH 7.4 with sodium hydroxide (NaOH). A subset of homogenized tissue was extracted for LDH, and CS. Homogenized tissue samples used for the LDH assay were centrifuged (Eppendorf Centrifuge 5424, Hamburg, Germany) at 150 rpm for <3 s. Homogenized tissue samples used for the CS assay were not centrifuged to allow mitochondria to be retained within the supernatant.

Absorbance readings were measured with a spectrophotometer every 2 s, with 20 readings over 13 min (UV5, Mettler-Toledo, Columbus, OH). Testing temperatures were maintained with a Loop L100 circulation thermostat (Lauda, Lauda-Königshofen, Germany). All samples were measured in triplicate and included a blank control.

LDH was assayed at a final dilution of 1:200 in 0.5 mmol L-1 of β*-*nicotinamide adenine dinucleotide reduced disodium salt hydrate (NADH)-TRIS solution (pH 7.4). and 50 mmol L-1 of sodium-pyruvate-NADH-Tris solution (pH 7.4). NADH absorbance was measured at a wavelength of 340 nm (Seebacher 2003). CS was assayed at a final dilution of 1:100 in 2 mmol L-1 5,5’-dithobis-(2-nitronemzoic acid) (DTNB)-ethanol solution, 12 mmol L-1 acetyl coenzyme A-lithium salt-Milli-Q solution, and 50 mmol L-1 oxaloacetic acid-Tris solution (pH 8.0). DTNB absorbance was measured at a wavelength of 412 nm (Seebacher 2003; Blank 2004).

The mean slope was used to determine maximal enzyme activity. Background activity was subtracted from sample absorbance slopes when background activity exceeded 5% of sample absorption levels. Final maximal enzyme activity levels were calculated in units per milligram tissue (U mg-1 tissue) using the following formula where: represents the mean absorption of tested sample in triplicate, represents to the light path length (cm), represents the molar absorptivity/extinction coefficient (M-1 cm-1), represents tissue sample concentration (mg/ml), and represents volume.

## Statistical analysis

Generalized linear mixed effect models were used to test for differences in metabolic, immunocompetence, and enzyme activity, responses between low- and high-latitude populations to temperature (**Supplemental table 3**). A linear regression model was used to model hematocrit. All aerobic physiology models were run using a gaussian distribution. To model aerobic physiology, including MO2resting, MO2max, and MO2AAS, independent variables including, latitude and temperature were modelled as fixed factors with an interaction. Fish mass (centered) was used as a covariate. Individual identification codes for each fish were used as a random factor due to repeated measures. The model for MO2resting included the additional covariate of testing runtime. All oxygen consumption traits were modelled with temperature as a continuous second order polynomial.

PHA was modelled via an interaction term between latitude and temperature (3rd order polynomial). As a random effect individual fish identification codes were nested within known populations. Additionally, a gamma distribution (with a log-link function) was used instead of a gaussian distribution. Fish mass was not included as a co-variate within the PHA model. Hematocrit was modelled via a gaussian distribution as a linear regression with latitude as the only independent variable. No random factor was included within the hematocrit model.

When modelling enzyme activity LDH, CS, and LDH:CS were modelled using a gaussian distribution with an interaction between latitude and temperature, as well as sample tissue mass (centered) as a co-variate. Additionally, citrate synthase was modelled with a log-link function. Temperature was modelled as a continuous 3rd order polynomial for LDH, 2nd order polynomial for citrate synthase, and linearly for LDH:CS. Within all enzyme models individual identification codes for each fish were used as a random factor.

All statistical analysis was conducted in R (v 4.2.2). During the model selection analysis GLMs were run using the ‘glm’ function via the ‘*stats*’ (v.4.2.2) package. GLMMs were run using the ‘glmmTMB’ function within the ‘*glmmTMB’* (v.1.1.5). Model selection occurred using the function ‘AICc’ and ‘r.squaredGLMM’ (or ‘r.squaredLR’) via the *‘MuMin’* (v.1.47.1), and ‘BIC’ from ‘*stats*’. Visual and statistical performance of models was checked via both the ‘check\_model’ function in the *‘performance’* (v. 0.10.0) package and the ‘simulateRedisuals’ and ‘testResiduals’ functions in the ‘*DHARMa’* (v. 0.4.6) package. The *‘emmeans’* (v. 1.8.2) package was used to extract estimated marginal trends and means from models that were used to tested for statistical significance, as well as calculated effect sizes (Cohen’s *d*) for models that identified significant differences. All figures were made using the ‘*ggplot2*’ (v. 3.4.0) package.

# Results

## Aerobic physiology

MO2rest displayed a positive relationship with temperature (χ² =51.57, df =2, *p* <0.001), but no significant differences were seen in MO2rest when comparing the thermal performance curves of fish from low- and high-latitude regions (*p* =0.51, [CI: -0.21, 0.10]; **Figure 2a**). The largest increase in RMR (14%) between temperature intervals within high-latitude region fish was observed between 28.5°C and 30°C. Alternatively, the largest increase in RMR (14%) with low-latitude region fish was observed between 30°C and 31.5°C.

A positive relationship was seen between MO2max and temperature (χ² =16.28, df =2, *p* <0.001). MO2max among fish from low-latitude populations experienced a plateauing increasing between temperature intervals (27-28.5°C: 11%; 28.5-30°C: 7%; 30-31.5°C: 4%). Fish from high-latitude populations differences between temperature intervals were <2%, producing a flat response, where MO2max values were constantly ~14.1 MgO2 hr-1. MO2max and temperature displayed diverging patterns between low- and high-latitude regions. Low-latitude fish had significantly different thermal performance curve for MO2max compared to high-latitude region fish (*p* =0.0010, [CI: 0.26, 1.02], *d* =0.83; **Figure 2b**). The biggest divergence in MO2max values were observed at 30°C and 31.5°C, where low-latitude fish values were 15% (+2.21 MgO2 hr-1) and 21% (+2.92 MgO2 hr-1) higher than high-latitude fish, respectively.

Significant differences in AAS were seen between low- and high-latitude thermal performance curves (*p* =0.0010, [CI: 0.28, 1.10], *d* =0.94; **Figure 2c**). Enhanced AAS within low-latitude fish was primarily driven by their improved MO2max. Low-latitude fish displayed increased AAS at 30°C and 31.5°C compared to high-latitude fish and represented a difference of 33% (+2.64 MgO2 hr-1) and 38% (+2.79 MgO2 hr-1), respectively. At 28.5°C low-latitude fish only performed 18% (+1.53 MgO2 hr-1) better than high-latitude fish, and at 27°C low- and high-latitude fish performed equally.

## Immune response

Immune swelling response exhibited a thermal performance curved that was significant correlated with temperature (χ² =50.41, df =3, *p* <0.001) and peaked at 28.5°C in both low- and high-latitude populations; however, no significant differences were found when comparing latitudes (*p* <0.85*,* [CI: -0.57, 0.48]; **Figure 3**).

## Hematocrit

No significant difference was observed in hematocrit levels between low- and high-latitude populations at 31.5°C (*p* =0.058; **Supplemental figure 4**). Packed red blood cells composed 22.4% and 25.9% of whole blood for low- and high-latitude populations, respectively.

## Enzyme analysis

Lactate dehydrogenase activity was positively correlated with temperature (χ² =2297.23, df =3, *p* <0.001), however, no significant differences were seen in the LDH thermal performance curves of low- and high-latitude fish (*p* =0.98, [CI: -2.00, 1.94]; **Figure 4a**). Citrate synthases displayed similar results to LDH, a positive correlation with temperature (χ² =1364.86, df =2, *p* <0.001), but no significant difference between the low- and high-latitude thermal performance curves (*p* =0.14, [CI: -0.0097, -0.0014]; **Figure 4b**). LDH:CS ratio was also positively correlated with temperature (χ² =51.70, df =1, *p* <0.001), but no significant difference was observed between low- and high-latitude fish (*p* =0.91, [CI: -0.23, 0.25]; **Figure 4c)**.

# Discussion

How populations respond to climate change will depend on traits that are adapted to localized environmental conditions. Localized environment conditions can influence thermal preferences and limitation within populations via plastic and evolutionary mechanisms, creating complex adaptive landscape across species’ distributions (Huey et al. 2012; Valladares et al. 2014). Identifying existing intraspecific variation is therefore essential to accurately predicting populations’ (and therefore species’) responses to climate change. Our study found evidence of co-gradient variation (i.e., aligned environmental and genetic influences) within aerobic physiology traits, suggesting that these traits are adapted to localized environmental conditions. However, no intraspecific variation was found in several other traits including immunocompetence, hematocrit, and enzyme activity.

Evidence of co-gradient variation was observed in aerobic capacity. Low-latitude populations showed a higher thermal optimum for aerobic performance (MO2max and AAS) from 30-31.5°C, and higher capacity at this temperature range than high-latitude conspecifics. Fish from low-latitude exhibited rising MO2max and MO2Rest with warming, however, high-latitude populations displayed a plateaued MO2max across the testing temperature range and consequently reduced AAS, due to increasing MO2Rest. Improved aerobic capacity at higher temperatures suggests low-latitude populations are adapted to warmer temperatures, compared to high-latitude conspecifics. AAS can serve as a proxy for the limits of oxygen demanding processes (e.g., motor activity, reproductive output, growth) that can be performed simultaneously (Clark et al. 2013) and is expected to be a primary mechanism that determines how fish will respond to climate change (Pörtner and Knust 2007; Pörtner et al. 2017). Therefore, under future projected warming low-latitude fish are expected to have increased fitness over high-latitude populations.

All other traits investigated did not display differences (co- or counter-gradient) between low- and high-latitude populations. Immune response and enzymatic performance across the temperature range, as well as hematocrit at the warmest temperature of 31.5°C, were similar between latitudes, suggesting that natural selection on these phenotypic traits is not differing between tested locations. Immune response, enzyme performance, and hematocrit may therefore represent traits not placed under strong selection pressure by local thermal conditions, potentially due to the presence of a stronger selection pressure, and/or physiochemical limitations.

Considering the observed pattern in AAS, we might have expected latitudinal differences in hematocrit (proxy for oxygen carrying capacity) and aerobic enzyme performance if these were correlated to limited maximum oxygen consumption. In the case of the coral reef snapper (*Lutjanus carponotatus*), exposure to marine heatwave of 29.5 and 30.5C (+1-2C) conditions for 4-weeks, resulted in an increase in hematocrit to allow maintenance of aerobic capacity (McMahon *in review*). However, hematocrit was shown to be unresponsive in both the fusilier *Caesion cuning* and the cardinalfish *Cheilodipterus quinquelineatus* when exposed to elevated temperatures (+3.0°C above ambient temperature) for 5-weeks (Johansen et al. 2021). Similarly, the pattern of aerobic enzyme performance (CS) and a lack of significant difference between regions suggests that enzymatic performance does not limit aerobic capacity. Our findings instead support the theories that the heart and/or gills limit the ability to maintain oxygen delivery (Pörtner and Farrell 2008; Pauly 2019) and ultimately determine thermal, tolerances, local adaptation, and plasticity in fish. Consequently, enzymatic activity with the heart may be more relevant to whole organismal aerobic by limiting cardiac function (Farrell 2009; Ekström et al. 2017; Nyboer and Chapman 2018; Pichaud et al. 2019).

While there was no latitudinal difference in immune response, there was a dependence on temperature, with significantly reduced response at temperatures above current-day summer of 28.5°C. Interestingly for *A. polyacanthus* this finding shows that this species may be immunocompromised prior to impacts on aerobic capacity, especially in the low-latitude region. A similar response has been observed in another coral reef fish at a similar low latitude, the rabbitfish *Siganus doliatus*, where immune response was reduced to nothing at 31.5°C (LaMonica et al. 2021). While immunological research in fish is emerging and scarce compared to other taxa, within bird species PHA swelling responses have been shown to be less costly than other activities (e.g., molting, breeding; (Martin et al. 2006).

If similar conditions exist within fish, we expect energetic demanding behaviors, such as reproduction, to be reduced or cease at temperatures above 28.5°C. Evidence of such trade-offs have been previously demonstrated in *A. polyacanthus* where reproductive output (i.e., clutch size \* egg area) was reduced at temperatures above 28.5°C when fish were placed on a high food diet, and ceased when placed on a low food diet(Donelson et al. 2010).Our study adds to the growing evidence that supports the multiple performance – multiple optima hypothesis (Clark et al. 2013) and highlights the need to study a range of performance metrics that are associated with fitness. There is the potential that repeated PHA injections may allow for acquired immune response as previous research in blue-footed boobies (*Sula nebouxii*) detected an average increase of 90% between first and second PHA injections; attributing the increase to acquired T-mediated immunity (Santiago-Quesada et al. 2015). Thus, the increased swelling at 28.5°C compared to 27°C we observed may be indicating acquired immune system. However, this would make the substantial decline in immune response at 30°C and 31.5°C even more concerning in relation future ocean warming.

LDH and CS activity, as well as LDH:CS ratios were significantly positively correlated with temperature, however, neither enzyme showed significant differences between low- and high-latitude populations. LDH and CS are proxy representations for anaerobic glycolysis (Savoie et al. 2008) and aerobic capacity that can achieved via the citric acid cycle (Savoie et al. 2008; Pichaud et al. 2019), respectively. The positive relation between temperature and LDH:CS ratios suggest that as temperatures warm there is a greater reliance on anerobic metabolism, a pattern that is expected among ectotherms, and has previously been identified in crown-of-thorns sea starts (*Acanthaster spp.*; Lang et al. 2021). However, a lack of significant difference between regions suggests that enzymatic performance within white muscle of *Acanthochromis polyacanthus*, does not contribute to organismal differences that were demonstrated via AAS. Enzymatic activity relevant to whole organismal response may be more prevalent in mitochondrial-rich muscle tissue-types, such as heart tissue that is associated with cardiac function (Farrell 2009; Ekström et al. 2017; Nyboer and Chapman 2018; Pichaud et al. 2019). However, within small coral reef fish the lack of obtainable tissue mass can prove challenging.

Evidence of co-gradient variation in aerobic capacity suggests that for the populations examined genetic and environmental influences are aligned, however, counter-gradient variation in this trait and species has previously been observed Gardiner et al. (2010). The primary driver of counter-gradient variation is expected to be differences in phenotypic plasticity and therefore may be dependent on differences experienced at smaller scales due to biogeography including depth, water flow, and isolation, that may be more predictive of local thermal variability than latitude. Counter-gradient variation between *A. polyacanthus* populations was previously identified when comparing low-latitude (i.e., Lizard Island) and high-latitude (i.e., Heron Island) populations, which are both further north and south than the low- and high-latitude populations examined in this study. Gardiner et al. (2010) sampled juvenile fish from shallow lagoons, whereas fish in this study were older and collected from ~7-12 meters on coral reef slope. Reef flats and lagoons generally experience greater thermal variability (minimums, maximums, and magnitude of diurnal variation) via exposure to semidiurnal tidal oscillations compared to reef slopes that are exposed the open ocean and hence more thermally stable, and this is true for the lagoon sites at Heron Island (Brown et al. 2023). Additionally, *A.* polyacanthus from Heron Island have been shown to have high capacity for phenotypic plasticity (Donelson and Munday 2012; Ryu et al. 2018). This results in the potential for multiple patterns on variability and performance to occur when exploring across latitudes (Donelson et al. 2019), depending on the population in question, elucidating the importance of incorporating macro- and fine-scale biogeography in understanding intraspecific variation between populations.

Determining spatial patterns of thermal adaptation underlie the ability to predict population responses to climate change (Sorte et al. 2011; Moran et al. 2016). Climate envelope models frequently assign populations identical thermal ranges, however, such approaches risk inaccurately projecting species trajectories. Findings from this experiment demonstrated different aerobic physiology capacity among *A. polyacanthus* populations from low- and high-latitude regions as well as a decline in immune response and increased reliance on anaerobic pathways within both regions as temperatures warmed. Models that assume all *A. polyacanthus* populations occupy the same environmental niche, in regard to AAS, as low-latitude populations, risk underestimating the impact of elevated temperatures on high-latitude populations; vice-versa, models that assume that all *A. polyacanthus* populations occupy the environmental niche of high-latitude populations would risk underestimating the ability of low-latitude populations to response to climate change. Furthermore, when results from this study are examined concurrently with Gardiner et al. (2010), evidence suggests that fine scale biogeographic features can create pockets of adaptive heterogeneity. These findings suggest that the adaptive landscape of species within marine environments may resemble a heterogenous matrix of populations with varying levels of adaptability, and therefore, necessitate the sampling of populations from different environments to understand species’ adaptive landscape. Such an understanding would allow for more accurate predictive modelling as well as yield benefits for translocation-based conservation techniques, such as assisted gene flow, that rely on balancing the introduction of beneficial traits with outbreeding depression and genetic compatibility between populations.

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# Statements and declarations

Authors declare no competing interests.

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# Figures

**Fig.1:** Maps outlining A) low- (red) and high-latitudinal (blue) regions that fish were collected from across the Great Barrier Reef. Insert B) provides a zoomed in perspective of the low-latitude region which was made up of fish from three different reefs including Sudbury Reef, Tongue Reef, and Vlasoff Reef. Insert C) provides a zoomed in perspective of the high-latitude region that is made up of two inshore island, Cockermouth and Keswick Island, and one offshore reef, Chauvel Reef (southern).

**Fig.2:** Thermal performance curves of A) resting oxygen performance, B) maximum oxygen performance, and C) absolute aerobic scope of fish from low- (solid red lines) and high-latitudinal (dashed blue line) regions across four different temperatures (i.e., 27°C, 28.5°C, 30°C, 31.5°C). Ribbon represents 95% confidence intervals.

**Fig.3:** Thermal performance curve of swelling response of the caudal peduncle ~18-24 hours post injection of phytohemagglutinin across four experimental temperatures (i.e., 27°C, 28.5°C, 30°C, 31.5°C). Solid red lines represent low-latitude populations. Dashed blue line represents high-latitude populations. Ribbon represents 95% confidence intervals.

**Fig.4:** Thermal performance curve of maximal activity of A) lactate dehydrogenase (LDH), B) citrate synthase (CS), and C) LDH:CS ratio of low- (solid red line) and high-latitudinal (dashed blue line) populations across four experimental temperatures (i.e., 20°C, 30°C, 40°C, 50°C). Ribbons represent 95% confidence intervals.

# Supplemental Material

## Supplemental figures

**SFig.1:** Seasonal temperature profile for reefs within the low- and high-latitude region of the Great Barrier Reef (see **Stab.2** for list of reefs names). A) mean daily temperature and B) mean daily range are shown for both low- (solids red line) and high-latitudinal (dashed blue line) regions, as well as density plots identifying the most frequently experienced temperatures or ranges experienced. Data was obtained via the Australian Institute of Marine Science Temperature Logger (Australian Institute of Marine Science (AIMS) 2020) dataset.

**SFig.2:** Respirometry chambers that were made in-house and used to measure aerobic physiology traits.

**SFig.3:** Density plots displayed fish body size to chamber ratios. Fish that were sampled for aerobic physiology from the low-latitude region are represented in red; fish from the high-latitude region are represent in blue.

**SFig.4:** Comparison of hematocrit ratios, that were measured at 31.5°C, between low- (red) and high-latitudinal (blue) populations. No significant difference was observed between the different latitudes (*p* =0.058). Solid (low-latitude) and dashed (high-latitude) lines represent 95% confidence intervals.

## Supplemental tables

**STab.1:** Samples sizes of fish that were used from each population over the course of the experiment. *N*all measurements refers to fish that completed aerobic physiology and immunocompetence experiments at all testing temperatures (i.e., 27°C, 28.5°C, 30°C, 31.5°C), in addition to having tissue and blood samples collected at the conclusion of the experiment.

**Stab.2:** List of reefs from the Australian Institute of Marine Science Temperature Logger (Australian Institute of Marine Science (AIMS) 2020) dataset that were used to determine the thermal regime of low- and high-latitude regions. Only temperature loggers that were placed between 7-15m deep were sampled. Latitude and longitude are measured in decimal degrees.

**Stab.3:** Information pertaining to statistical models that were run to identify differences between low- and high-latitude reefs for aerobic physiology, enzyme, immunocompetence, and hematocrit metrics.

## Supplemental videos

**Svid.1:** Display of swimming gait-change behavior in *Acanthochromis polyacanthus*. Within the video the fish can be seen using predominately pectoral swimming behavior, however, when the fish starts to lose its position in the swim tunnel it temporally switches to body undulations (easily identified by the fanning of the tail) to restore its position. This type of swimming behavior forces the fish to maximize its reliance on aerobic energy processes.