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

Potential journals:

1. Coral Reefs
2. Marine Biology
3. Marine Ecology Progress Series
4. Conservation physiology
5. PLoS One?
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# Abstract

# 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 will likely 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 wide 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 likely differ depending on occupied thermal niches. Low-latitude environments characterized by stable temperatures near physiological maximums favor specialized (narrow) thermal niche breadths that primarily evolve 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). Marine systems have previously been viewed as demographically open networks with minimal dispersal barriers. 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). Additionally, the absence of dispersal barriers and presence of gene flow does not exclude the potential for local adaptation. 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 patterns in marine fishes vary 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) among marine species.

Intraspecific thermal variation within the coral reef damselfish, *A.* polyacanthus, is evident; however, existing physiological studies provides a coarse understanding of intraspecific variation. 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 than surrounding reefs (Miller-Sims et al. 2008) . To increase the resolution of *A. polyacanthus’s* thermal landscape and allude to a greater 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 GBR, 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.

Allen GR (1991) Damselfishes of the World. Mergus Publishers, Melle, W. Germany.

# 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 thought to have propagated the Indo-Pacific proceeding Pleistocene (2.6 Ma- 11.7 ka) bottlenecks 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 began to reach present-day levels as *A. polyacanthus* lacks a pelgic 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). This 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°), Vlassof Cay (-16.657°, 145.990°), and Sudbury Reef (-16.996°, 146.202°). High-latitude sites included Cockermouth Island (-20.772°, 149.390°), Keswick Island (-20.908°, 149.406°), and Chauvel Reef (southern; -20.863°, 150.363°; **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**). Fish were sampled from Tongue Reef (*n =8*), Sudbury Reef (*n =11*), Vlassof Cay (*n =10*), Cockermouth Island (*n =10*), Keswick Island (*n =6*), and Chauvel Reef (*n =10*). However, not all fish survived the duration of the experimental testing. 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 were examined using temperature data collected via AIMS (Australian Institute of Marine Science) temperature Logger data series, at a of depth 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 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 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 respiration chambers. Pilot studies (unpublish data, Schmidt) determined that highest MO2max levels were achieved with the immediate transfer of 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 the of 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). 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 randomly placed 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 second wait, 225 second measurement, and 180 flush period. Air percentage never dropped below 80% air saturation. Oxygen consumption rates were measured over a 220 min 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 were 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 the sensitivity of the immune system, subcutaneous phytohemagglutinin injections were used to produce a (localized) cell-mediated response (e.g., inflammation, T-cell proliferation, infiltration of immune cells; (Martin et al. 2006; LaMonica et al. 2021). PHA swelling response provides useful information on individual’s immune system status *in vivo*, while limiting additional stress other than that derived from handling (Merino et al. 1999). 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 just 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 here 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, immune, hematocrit, and enzyme activity, responses between low- and high-latitude populations to temperature. All aerobic metabolic models were run using a gaussian distribution. To model metabolic responses including MO2resting, MO2max, and MO2AAS, independent variables including, region 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. The same fixed variables, latitude, and temperature, were used for modelling PHA immunocompetence response, and enzyme (LDH and CS?) activity. However, for the PHA swelling response model a gamma distribution was used with an inverse link. For the enzyme analysis for lactate dehydrogenase model tissue mass (centered) was used instead of fish mass. To model the (combined region) correlation between lactate dehydrogenase activity and temperature, temperature was modelled as a continuous numerical variable and third order polynomial, tissue mass (centered; fixed), and individual fish identification codes as a random factor. Hematocrit was modelled as a linear regression with percent packed blood cells as the dependent factor and region as an independent variable.

All statistical analysis was conducted in R (v 4.2.2). GLMMs were run using the ‘glmmTMB’ function within the ‘*glmmTMB’* (v.1.1.5). Model selection occurred using the function ‘AICc’ via the *‘MuMin’* (v.1.47.1). 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 means from models that were used to tested for statistical significance. All figures were made using the ‘*ggplot2*’ (v. 3.4.0) package.

# Results

## Aerobic physiology

MO2rest displayed a positive relationship with temperature (Fx,y = , p...), but no significant differences were seen in MO2rest when comparing fish from low- and high-latitude regions (Fx,y = , p...) across the temperatue range 27°C to 31.5°C. (**Figure 2a**). At the lowest two temperatures, 27°C and 28.5°C, MO2resting was most similar between regions (27°C: *p* =0.58, [CI: -0.45, 0.78]; 28.5°C: *p*=0.90, [CI: -0.67, 0.59]). MO2Rest was significantly higher at 30°C and 31.5°C, than at 27°C and 28.5°C for fish from high-latitude populations (*p*Leading27v30 <0.0022, [CI: -1.78, -0.29]; *p27–31.5* <0.001, [CI*27–31.5*: -2.17, -0.66]; *p*Leading28.5v30 =0.035, [CI: -1.53, -0.039]; *p*Leading28.5v31.5 =0.0006, [CI*28.5–31.5*: -1.91, -0.40]) region. The largest increase in RMR (14%) between temperatures within high-latitude region fish was observed between 28.5°C and 30°C. In the low-latitude region MO2Rest similar differences were seen (*p*Core27v30 =0.0077, [CI: -1.50, -0.17]; *pCore27v31.5* <0.0001, [CI: -2.07, -0.66]; *p*Core28.5v30 <0.001, [CI: -1.99, -0.65]), however there was no significant difference between 28.5°C and 30°C. The largest increase in RMR (14%) with low-latitude region fish was observed between 30°C and 31.5°C (*pCore30v31.5 <*0.01, [CI: -1.50, -0.17]).

MO2max and temperature displayed diverging patterns between low- and high-latitude regions (**Figure 2b**). A positive relationship was seen between MO2max and temperature among fish from low-latitude populations; steadily increasing between temperature intervals (27-28.5°C: 10%; 28.5-30°C: 6%; 30-31.5°C: 3%). Fish from high-latitude populations differences between temperature intervals were <2%, producing a flat response, where MO2max values were constantly ~14.2 MgO2 hr-1. Low-latitude fish had significantly higher MO2max compared to high-latitude region fish at 30°C (*p* <0.05, [CI: 0.030, 3.62]; 13% increase; 1.90 MgO2 hr-1) and 31.5°C (*p* <0.05, [CI: 0.22, 3.86]; 15% increase; 2.10 MgO2 hr-1).

Significant differences in AAS were seen between regions at warmer temperatures 30°C (*p* <0.01*,* [CI: 0.56, 4.01]) and 31.5°C (*p* <0.05*,* [CI: 0.28, 3.78]; **Figure 2c**). This enhanced AAS possessed by low-latitude region fish by a difference of 2.28 MgO2 hr-1 at 30°C and 2.03 MgO2 hr-1 31.5°C represented a difference of 28% and 27%, respectively. Optimal AAS for low- and high-latitude populations was 30°C (10.31 MgO2 hr-1) and 28.5°C (8.57 MgO2 hr-1), respectively; +1.5°C above the average summer temperature in each region. Interestingly, low-latitude region fish showed similar AAS values at 28.5°C (9.63 MgO2 hr-1) and 31.5°C (9.58 MgO2 hr-1). At lower temperatures, 27°C and 28.5°C, no significant differences were observed between regions (*p27* =0.76; *p28.5* =0.20).

## Immune response

Immune swelling response exhibited a curved response in both low- and high-latitude populations that peaked at 28.5°C (**Figure 3**), however, no significant differences were found between regions at tested temperatures (*p27* =0.19; *p28.5* =0.62; *p30* =0.59; *p31.5* =0.80). Combined results between regions showed that immune response was lowest at 31.5°C, showing a decrease of 60%, 75%, and 53% compared to 27°C (*p* <0.001*,* [CI: 0.43, 1.42]), 28.5°C (*p* <0.001*,* [CI: 0.87, 1.88]), and 30 °C (*p* <0.01*,* [CI: 0.23, 1.30]), respectively. At 28.5°C immune response was also significantly higher than responses produced at 27°C (*p* <0.05*,* [CI: -0.90, -0.0016]) and 30°C (*p* <0.01*,* [CI: 0.12, 1.10]).

## 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 (*p* <0.001, [CI: 1.94, 2.45], *R2 =*0.79; **Figure 4a**), however, no significant differences were seen in LDH activity between regions at any of the tested experimental temperatures for lactate dehydrogenase activity: 20°C (*p* =0.54), 30°C (*p* =0.48), 40°C (*p* =0.42), and 50°C (*p* =0.53). Citrate synthases was negatively correlated with temperature (*p* <0.001, [CI: -144, -73]; **Figure 4b**). Similar to LDH, no significant differences were identified between CS activity between regions at tested experimental temperatures: 20°C (*p* =0.90), 30°C (*p* =0.76), 40°C (*p* =0.72), and 50°C (*p* =0.33).

# Discussion

How populations will respond to climate change will depend on experienced local environmental conditions. If environmental and genetic influence are aligned (co-gradient variation) low-latitude populations living in warmer conditions are expected to respond to warming temperatures more adeptly than high-latitude populations. However, high-latitude populations that may experience greater environmental variability may be able to compensate performance at warmer conditions via greater investment in phenotypic plasticity (CVH; counter-gradient variation; Janzen 1967; Stevens 1989). Results from this study detected co-gradient variation when comparing AAS between low- and high-latitude *A. polyacanthus* populations. Immune response and hematocrit were similar between populations. Findings suggest that AAS is adapted to local regional conditions, and therefore, intraspecific variation in thermal performance needs to be accounted for when modelling responses to climate change.

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 warmer adapted, 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, with 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, or physiochemical limitations. Such conditions would support the multiple performance – multiple optima hypothesis proposed by Clark *et al.* (Clark et al. 2013), whereby different physiological process possess different thermal optima.

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 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 were significant correlated with temperature, positive and negatively, respectively; however, neither enzyme showed significant differences between low- and high-latitude populations. LDH and CS are proxy representation for anerobic glycolysis [citation] and aerobic capacity that can achieved via the citric acid cycle [citation], respectively. The transition from aerobic to anaerobic process is expected among ectotherms that experience warming thermal conditions, and has been previously 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. The anaerobic capacity of white muscle tissue 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). However, 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, that has been previously shown to be a central determining mechanism for thermal, tolerances, local adaptation, and plasticity in fish (Farrell 2009; Ekström et al. 2017; Nyboer and Chapman 2018; Pichaud et al. 2019). Heart tissue may be more ideal for future enzymatic analysis, 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 etc, 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.

While experimental temperatures in this study were chosen based on regional mean summer average temperatures as well as mid- and end-of-century predicted future ocean warming temperatures, a greater understanding about potential adaptive differences would be gained by exploring responses at cooler temperatures (Kawecki and Ebert 2004). Additionally, physiological traits were only measured here between populations at a single time point, however, thermal breadths are not static (Kelly et al. 2012). For example, measurement of performance outside of normal thermal ranges can reveal cryptic variation and local adaptation that may be otherwise unnoticed (Shama 2017; Pilakouta et al. 2020) . Genetic adaptation and phenotypic plasticity in combination will impact how populations will respond to environmental changes via shifts in thermal performance. This current study was limited to current thermal tolerances; however, future research should explore genetic and plastic differences between populations to determine future adaptive potential among populations from each region via multi-generational experiments. Previous studies on *A. polyacanthus*, have detected genetic (Doherty et al. 1994; Planes et al. 2001; Van Herwerden and Doherty 2006) and plastic (Donelson and Munday 2012; Ryu et al. 2018) differences between populations, suggesting that adaptive potential between examined regions are unlikely to be analogous.

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 all 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 within both regions as temperatures exceed 28.5°C. 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.

## Limitations and future research

Main limitations of this study reside in the 1) ever-changing nature of traits that are subjected to evolutionary processes, 2) researchers’ decision to focus on warmer, rather than cooler, temperatures to detect local adaptation, and 3) absence of equatorial populations. In this study physiological traits were measured between low- and high-latitude populations at a single time point, however, thermal breadths are not static (Kelly et al. 2012). Genetic adaptation and phenotypic plasticity will both impact how populations will respond to environmental changes via shifts in thermal performance. This current study was limited to current thermal tolerances; however, future research should explore genetic and plastic differences between populations to determine future adaptive potential among populations from each region via multi-generational experiments. Previous studies on *A. polyacanthus*, have detected genetic (Doherty et al. 1994; Planes et al. 2001; Van Herwerden and Doherty 2006) and plastic (Donelson and Munday 2012) differences between populations, suggesting that adaptive potential between examined regions are unlikely to be analogous.

Experimental temperatures in this study were chosen based on regional mean summer average temperatures as well as mid- and end-of-century predicted future ocean warming temperatures; physiological responses at cooler temperatures were not explored. Projected future ocean warming temperatures were chosen to explore population responses to future conditions, however, previous research on sticklebacks (*Gasterosteus aculeatus*) demonstrated that measuring aerobic physiology outside of thermal norms can reveal cryptic variation and local adaptation that may be otherwise unnoticed at intermediate temperatures (Pilakouta et al. 2020). Additionally, the exploration of aerobic physiology at cooler temperatures may have better revealed ‘local vs. foreign’ as well as ‘home and away’ criterion, outlined by Kawecki and Ebert (2004), used to detect local adaptation.

Lastly, this study was unable to source fish from known equatorial populations for *A. polyacanthus’s* range. Trailing edge populations are suggested to be living closest to their thermal limits and therefore possess greatest thermal tolerance as well as sensitivity to change in temperature; however, to date no *A. polyacanthus* have not explored comparisons between equatorial, low-latitude, and high-latitude populations. Future research should aim to include trailing edge populations within experiments to understand the extent of thermal tolerance within *A. polyacanthus*, and adaptive populations within populations that are predicted to be most sensitive to climate change.

## Conclusions

Population’s ability to respond to climate change will depend on experienced environmental conditions. Results from this study identify co-gradient variation between low- and high-latitude populations when examining AAS among populations found within habitats that possess similar fine-scale biogeographic features. However, previous *A.* polyacanthus research has identified counter-gradient variation, suggesting that *A. polyacanthus* populations represent a heterogenous matrix influenced by broad and fine-scale biogeographical features; further highlighting the need to understand intra-population variation within marine species. Assuming all populations will respond similarly to climate change will lead to inaccurate modelling and estimations of species vulnerability, and risk resource managers failing to provide effective conservation management solutions.

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# Competing Interests

Authors declare no competing interests.

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# Figures

# Table

# Supplemental Material

## Supplemental figures

## Supplemental tables

**Climate change has begun to shift environmental conditions away from historic thermal regimes that populations evolved under**. As climate continues to shift species may struggle to keep pace(Jump and Peñuelas 2005).

Evolutionary processes have previously been ignored when projecting species responses to climate change due to the thought that they were too slow to influence measurable demographic effects (Kelly and Griffiths 2021). However, species may overcome this obstacle via large effective population sizes and fecundity rates, whereby (if the necessary genetic variation is available) strong selection pressures could produce sizeable changes in allele frequency within a single generation/cohort (Kelly and Griffiths 2021). Therefore, important to consider evolutionary process that will influence past and future populations responses to climate change. **This information should come later maybe even just have in discussion??**

**Local adaptation occurs within metapopulations when native genotypes are better adapted to local environment conditions than foreign genotypes** (Linhart and Grant 1996; Kawecki and Ebert 2004; Hereford 2009). Through gene x environment interactions, local adaptation may arise in spatially heterogenous environments if divergent selection can overcome the homogenizing effects of gene flow and temporal instability in selective forces (Endler 1977; Bradshaw 1984; García-Ramos and Kirkpatrick 1997; Hendry 2001; Kawecki and Ebert 2004; Richardson et al. 2014). Isolated populations are particularly susceptible to local adaptation... Metapopulations may therefore be comprised of a mosaic of locally adapted populations that have evolved optimized traits suited to local environments.

**Thermal conditions across latitudinal gradients can shaped the fitness landscape via locally adapted traits.** ~~Local adaptation typically thought of \_\_\_\_\_, but can also be in the form of thermal tolerances (Aitkens and Travis 2010). The pervasive nature of temperature at various biological levels (e.g. cellular biochemistry, physiological processes), particularly among ectotherms, suggests that it can impose strong divergent selection pressures on populations (Pereira et al. 2017).~~ Moreover, temperature-dependent clines (i.e. local adaptation) between populations represent diverging evolutionary histories that can elucidate how populations will respond to climate change (Somero 2010; Hoffmann and Sgró 2011; Pereira et al. 2017).

Local adaptation, phenotypic plasticity, and genetic arhectiture represent threes components that much be analysed together to understand future responses.

However, recent evidence suggests that the rapid pace of climate change can disrupt local adaptation processes via shifting selection pressures (Hoffmann and Sgró 2011).

**The ability to response to shifting selection pressure will depend on the genetic architecture and demographic processes found within different populations.**

* Need to consider both populations:
  + Physiological traits and underlying:
  + CVH hypothesis and other one

**Broad range species may not always have increased adaptive potential/Genetic architecture to overcome changes in selection pressures caused by climate change. Isolated populations across large ranges may all be affected, therefore entire species affected (see (Jump 2005)).**

* Thus, making it important to consider regional influences within species ranges…
* Long lived species can rapidly change allele frequencies within generation due to number of offspring produced

**Species regions (trailing/core/leading edge)**

**Apoly/Research objectives and aims**

Metapopulations that exist over large geographical distributions and thermal gradients contain locally adapted populations that can help species buffer against extinction (Conover et al. 2006, 2009; Munday et al. 2008a; Pereira et al. 2017). However, local adaptation and genetic subdivision within metapopulations can also produce populations with narrow thermal breadths; increasing susceptibility to warming temperatures (Atkins and Travis 2010; Kelly and Griffiths 2021).

However, to accurately predict potential species responses to warming temperatures, intraspecific variation between populations must be accounted for.

Locally adapted optimums and phenotypes can be identified via thermal performance curves (i.e., TPCs; physiological metrics measured across temperatures) (Eliason et al. 2011; Jayasundara and Somero 2013). When used to understand key mechanisms that affect organisms’ performance, such as aerobic capacity, TPCs can begin to identify physiological limits and how populations will respond to thermal changes (Pörtner and Knust 2007; Gardiner et al. 2010; Somero 2010; Eliason et al. 2011). However, caution is warranted when extrapolating results from TPC experiments. Life stage (e.g., hatchling, juvenile, adult), and physiological state (e.g., reproductively active, food deprived) can alter an individual’s thermal performance; additionally, different physiological traits and functions (e.g., oxygen uptake, reproduction, immunity) may possess different thermal optima (multiple performance – multiple optima hypothesis) (Clark et al. 2013).

~~Intraspecific variation within marine systems (outside of a few economically important species) have not received the same attention as terrestrial systems (Sanford and Kelly 2011). Marine systems have previously been viewed as demographically open networks with minimal dispersal barriers. However, a growing body of evidence suggests that oceanographic features, life history traits, and larval dispersal ability act as challenges to gene flow; including the inability for few successful migrants to overcome localized selection pressures (Sanford and Kelly 2011). Evidence of greater confinement to organismal thermal tolerance limits suggests that marine species and their populations are locally adapted to thermal conditions and can be more sensitive to warming temperatures than terrestrial species(Sunday et al. 2011; Pinsky et al. 2019; Lenoir et al. 2020).~~

Intraspecific variation with *A.* polyacanthus populations suggests the presence of varying thermal tolerances and adaptive potential across different populations. Previous research on low-latitude populations have demonstrated that projected end of century temperature projects of +2-3°C (Masson-Delmotte et al. 2021) have negative effects on sex ratios (Donelson and Munday 2015; Rodgers et al. 2017) , growth (Munday et al. 2008b; Zarco-Perello et al. 2012; Spinks et al. 2019), reproduction (Donelson et al. 2010; Pankhurst and Munday 2011), and aerobic capacity (Nilsson et al. 2009; Gardiner et al. 2010; Donelson et al. 2011; Donelson and Munday 2012) among low-latitude populations. While there is limited research on southern populations, evidence from Gardiner *et al.,* (2010) and Donelson and Munday (2012) suggest that models for this species that assume a constant thermal niche across populations, would risk inaccurately projecting geographical persistence, and potential for evolutionary change (Hampe and Petit 2005; Hoffmann and Sgró 2011; Sanford and Kelly 2011; Kelly et al. 2012; O’Brien et al. 2017; Moffett et al. 2018). However, intraspecific variation between northern and southern populations of *A. polyacanthus* remains underexplored, with Gardiner *et al.,* (2010) and Donelson and Munday (2012), both examining the same southern populations (Heron Island). The lack of diversity in explored locations suggests the intraspecific variation within the region remains underexamined.

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When

~Fish are sensitive to temperature. Although they may not be living at their thermal maximums temperature shifts of a dew degrees can impact important fitness functions~.~The future distribution of marine fish species will be determined by the relationship between organisms’ biochemical and physiological constraints, and temperature (Munday et al. 2008a; McKenzie et al. 2020; Lefevre et al. 2021; Wu and Seebacher 2022). ~

#Lastly, Gardiner et al. (2010) and Donelson and Munday (2012) compared thermal performance and #acclimation capacity, respectively, between low- and high-latitude populations of a dispersal limited #tropical coral reef damselfish, *Acanthochromis polyacanthus*. Gardiner et al. (2010) found evidence #that high-latitude populations maintained higher aerobic capacity than low-latitude populations at #warmer temperatures. (Donelson and Munday 2012) reported that high-latitude populations #displayed increased acclimation capacity (i.e., developmental plasticity) compared to low-latitude #populations. Both studies provide evidence supporting the CVH within *A. polyacanthus*. However, #previous genetic analysis on *A. polyacanthus* has demonstrated strong differentiation between #populations, suggesting that not all populations may respond similarly.

~however, variation between fish populations has been largely ignored and restricted to few locations.~

~as temperatures warm it becomes increasingly more important to focus on marine species that are expected to witness +3c by the end of the century~

~~Irrespective of the evolutionary mechanisms at play, understanding thermal tolerance across populations is necessary for estimating species level response to warming temperatures~~ (Sorte et al. 2011; Bennett et al. 2019; McKenzie et al. 2020).

One of the leading hypotheses for predicting intraspecific spatial variation is the climatic variability hypothesis (CVH). Under the CVH, thermal conditions at low-latitudes, warmer temperatures and less variation, are hypothesized to favor genetic adaptation; whereas, high-latitudes conditions, cooler temperatures with more variation, are expected favor phenotypic plasticity. However, the evidence supporting the CVH is not ubiquitous(Overgaard et al. 2011; Chiono and Paul 2023).

Thermal tolerance of individuals can be used as a proxy to estimate a population’s ability to tolerate warmer temperatures (Sorte et al. 2011).

#as species with little separation can be represented bia since TPC, while other patterns suggest a #greater insigh into populations variation is essential for effective conservation management. #Populations may represent a heterogenous matrix of environmental variables that are typically #dismissed in marine fihs research.

#In a common garden experiment found differences in morphological traits between two different #Atlantic cod (*Gadus morhua*) populations that experience different thermal environments during #early life stages; intraspecific variation patterns followed a counter-gradient variation pattern across #a large (<1000 km) spatial scale.

#Whereas, Pratchett et al. (2013) compared aerobic physiology metrics among low- and high-latitude #populations of coral trout (*Plectropomus leopardus*), and found no significant differences between #populations. Further analysis found little genetic variation between coral trout populations across #the Great Barrier Reef (GBR) owing to spatial and temporal variation in larval recruitment.