# Introduction

The response of species to climate change is determined by the collective response of populations (Bennett et al. 2019; McKenzie et al. 2020). Of interest is the variation in traits across populations, due to genetic adaptation and phenotypic plasticity, that occur along geographic and environmental gradients and that may influence the response to environmental change (Sorte et al. 2011; Des Roches et al. 2018; Bennett et al. 2019; Plumb et al. 2020). 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 diversity 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 including, 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).

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 (CVH) (Janzen 1967; Pörtner and Farrell 2008) (*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 environmental conditions that regularly fluctuate and sit below physiological limits, often retain greater benefits from maintaining high levels of phenotypic plasticity than low-latitude conspecifics (Donelson et al. 2019). Wider thermal niche breadths and plasticity capacity in high-latitude populations, can increase thermal tolerance (McKenzie et al. 2020); however, heat-tolerant phenotypes present in low-latitude populations may be unattainable within high-latitude populations (for example see (Kelly et al. 2012). Therefore, locally adapted populations may possess thermal niches that are narrower than the species as a whole (Kelly and Griffiths 2021). Consequentially, population responses to warming temperatures will likely differ depending on occupied thermal niches.

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). What we know from the terrestrial is... XXXX. 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 (Sanford and Kelly 2011). To date studies that have addressed intraspecific variation in marine species have focused on invertebrates (see review Sanford and Kelly., 2011) including copepods (Kelly et al. 2012; Pereira et al. 2017; Sasaki and Dam 2019), porcelain crabs (Stillman 2002), intertidal snails (Kuo and Sanford 2009; Sorte et al. 2011), and coral (van Oppen et al. 2014); few broach the topic among marine fish.

Thermal intraspecific variation patterns in marine fishes are variable depending on study species. In a common garden experiment (Marcil et al. 2006) 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 (Van Herwerden et al. 2009; Taboun et al. 2021). (Gardiner et al. 2010) and (Donelson and Munday 2012) compared thermal performance and acclimation capacity, respectively, between low- and high-latitude populations of a 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 – counter-gradient variation. (Donelson and Munday 2012) reported that high-latitude populations displayed increased acclimation capacity (i.e., developmental plasticity) compared to low-latitude populations – supporting the CVH. Findings from (Gardiner et al. 2010) and (Donelson and Munday 2012) suggests that *A. polyacanthus* provide an opportunity to understand how intraspecific variation will affect the responses to warming temperatures within a non-commercial coral reef fish; a topic that has received little attention to date.

Intraspecific thermal variation within *A.* polyacanthus is evident; however, robust genetic variation between *A. polyacanthus* populations (Doherty et al. 1994; Planes et al. 2001; Van Herwerden and Doherty 2006) suggests that existing physiological studies provide a coarse understanding of *A. polyacanthus’s* thermal landscape. (Gardiner et al. 2010) and (Donelson and Munday 2012) both focused on a single high-latitude population (Heron Island), however, genetic analysis suggests high levels of genetic differentiation between populations throughout *A. polyacanthus’s* range; particularly within the higher latitudes of their distribution. Therefore, to increase the resolution of *A. polyacanthus’s* thermal landscape and allude to a greater understanding of intraspecific variation within marine environments, further exploration is needed. This study compared thermal performance curves of key physiological traits within *A. polyacanthus* from three different populations among two regions of the GBR, Cairns (low-latitude) and Mackay (high-latitude), that experience different thermal profiles. We tested the hypothesis for counter-gradient variation across a thermal gradient between a low latitude and a novel high-latitude region. Based on evidence of greater phenotypic plasticity among low latitude populations(Donelson and Munday 2012), populations from the high latitude region are expected to have increased thermal tolerance and performance at warmer temperatures than populations from the low-latitude region. However, co-gradient variation represents a valid alternative hypothesis considering the limited amount of research available on the topic and observed genetic differences between the high latitude populations examined in this study and the high-latitude population examined by past research.

# Methods

## Sampling

The tropical damselfish, *Acanthochromis polyacanthus* (Bleeker 1855), ranges from the southern Great Barrier Reef (GBR) to the central Philippines (spanning 30° of latitude). *A. polyacanthus* populations are thought 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, eventually such dispersal corridors ceased to function as water levels began to reach present-day levels. *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 unusual life history trait, among marine fish, coupled with *A. polyacanthus* inability to disperse 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.

Adult *A. polyacanthus* were collected via professional collectors from June to December 2021 from six different reefs and two different regions (central GBR [Cairns] and southern GBR [Mackay]). Three reefs from locations around Cairns including, Tongue Reef ([-16.341, 145.773], *n =6*), Vlassof Cay ([-16.657, 145.990] *n =6*), and Sudbury Reef ([-16.996, 146.202] *n =9*), as well as from inshore islands and reefs in proximity to Mackay including: Cockermouth Island ([-20.772, 149.390] *n =8*), Keswick Island ([-20.908, 149.406] *n =4*), and Chauvel Reef ([southern; -20.863, 150.363] *n =5*; **Figure 1**). Cairns and Mackay collection regions are separated by ~400 kilometers (spanning ~5° in latitude). In total XX fish were sampled over the duration of the experiment (**STable 1**). Resting metabolic rate, maximum metabolic rate, aerobic scope, immunocompetence, maximal enzyme analysis, hematocrit samples, and genetic sequencing data were all collected for *n =38* fish in total, sampled from Tongue Reef (*n =6*), Sudbury Reef (*n =9*), Vlassof Cay (*n =6*), Cockermouth Island (*n =8*), Keswick Island (*n =4*), and Chauvel Reef (*n =5*). Additional samples were included for the respirometry and immunocompetence trials, however, not all fish survived the duration of the experiment. [Merge with paragraph above].

Adult fish were held in separate 60L opaque aquariums ([DIMENSIONS]) 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) at set experimental conditions (see below). Fish were transferred to the experiment room that was used for trials on May 25th, 2022. Respirometry trials occurred from June 6th, 2022 – August 17th, 2022.

## Thermal conditions

To understand local thermal conditions for reefs within Cairns and Mackay locations were examined using temperature data collected via AIMS Temperature Logger data series, at a of depth 10-15m, for a subset of reefs (**ST1**) from each region (citation for AIMS data; **SF1**). Experimental temperatures for repeated aerobic physiology and immune response testing included the approximate daily mean summer temperature for both Mackay (~27°C) and Cairns (~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 complete, fish were warmer to the next temperature of +1.5°C, at a rate of +0.5°C/day for three consecutive days. Fish were then provided 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.

## Aerobic physiology

Routine 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 (**SF2**). Experimental setup consisted of two sumps (volumeL), 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. XXXX watt heaters and temperature sensors were used to ensure that experimental temperatures remained within +/-0.3°C of experimental temperature set points. Minimal background respiration was achieved through UV filteration, 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. Trials were conducted in a fully lit room to eliminate metabolic costs associated with digestion and photoperiod [citation].

Maximum oxygen consumption (MO2max) was used as a proxy for maximum metabolic rate [citation]. 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 (**SV1**). 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 routine metabolic rate (MO2routine).

Fish were randomly placed in respirometry chambers for 3.5 – 6 h ( =4.67 h) to measure MO2routine. 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.MO2routine 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 net respirometer volume of chambers ranged from 1:116 to 1:36 ( = ; **SF2**) 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* [citation]. Absolute aerobic scope (AAS) was calculated by subtracting MO2routine 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 and T-cell proliferation)(Martin et al. 2006) [citation: Add lamonica paper in coral reefs and add the mosquito fish work to show it works in fish]. 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 (model xxx) 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 spinal cut? 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 were used to collect XX ul of blood from extracted blood samples. Collected blood samples were centrifuged at XXX rpm for XX seconds to separate red cells from blood plasma. The proportion of blood volume occupied by red blood cells (hematocrit) was recorded by usinga 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 it plays an important role in locomotion activities, compromises most of the body mass for *A. polyacanthus*, and is easily accessible (more information on why w. muscle tissue was used; citation). White tissue samples were extracted from fish immediately after fish had been euthanized, placed in liquid nitrogen, and then transferred to a -80°C freezer for storage.

The maximal enzyme activity method used here was adapted from previous studies(Thibault et al. 1997; Lang et al. 2021) Seebacher (2003), McClelland (2005). 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 6, Benchmark Scientific, Edison NJ – double check) 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 5430, 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 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 assay 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 (citation). Final maximal enzyme activity levels were calculated in units per milligram tissue (U mg-1 tissue) using the following formula:

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(Description of variables in formula).

## Statistical analysis

Generalized linear mixed effect models were used to test for differences in metabolic, immune, hematocrit, and enzyme activity, responses between Cairns and Mackay region fish to temperature. All aerobic metabolic models were run using a gaussian distribution. To model metabolic responses including MO2routine, MO2max, and MO2net, 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 MO2routine included the additional covariate of testing runtime. The same fixed variables, region and temperature, were used for modelling PHA immunocompetence response, and enzyme (LDH) activity. However, for the PHA swelling response model instead of a gaussian distribution, 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, but no significant differences were seen in MO2rest when comparing fish from Cairns and Mackay regions at 27°C, 28.5°C, 30°C, or 31.5°C. (**Figure 2**). At the lowest two temperatures, 27°C and 28.5°C, MO2routine was most similar between Cairns and Mackay (*p*27 =0.58, [CI: -0.45, 0.78]; *p*28.5 =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 region Mackay fish (*p*Leading27v30 <0.0022, [CI: -1.78, -0.29]; *p27–31.5* <0.0001, [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 Mackay region fish was observed between 28.5°C and 30°C. In the Cairns 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 <0.0001, [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 Cairns 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 among fish from Cairns and Mackay regions (**Figure 2b**). A positive relationship was seen between MO2max and temperature among fish from Cairns populations; steadily increasing between temperature intervals (27-28.5°C: 10%; 28.5-30°C: 6%; 30-31.5°C: 3%). Fish from Mackay populations differences between temperature intervals were <2%, producing a flat response, where MO2max values were constantly ~14.2 MgO2 hr-1. Cairns region fish had significantly higher MO2max compared to Mackay 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 fish from Cairns and Mackay 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]; **Figure2a**). This enhanced AAS possessed by Cairns 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 differed between populations. Optimal AAS for Cairns and Mackay 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, Cairns 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 Cairns and Mackay region fish (*p27* =0.76; *p28.5* =0.20).

## Immune response

Immune swelling response among Cairns and Mackay fish exhibited a curved response that peaked at 28.5°C (**Figure 3**), however, no significant differences were found between regions at any of the 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 75%, 60%, and 53% compared to 28.5°C (*p* <0.0001*,* [CI: 0.87, 1.88]), 27°C (*p* <0.0001*,* [CI: 0.43, 1.42]), 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 Cairns and Mackay region fish at 31.5°C (*p* =0.058). Packed red blood cells composed 22.4% and 25.9% of whole blood for Cairns and Mackay region fish, respectively.

## Enzyme analysis

Lactate dehydrogenase activity was positively correlated with temperature (*p* <0.0001, [CI: 1.81, 2.64], *R2 =*0.79; **Figure 4**), however, no significant differences were seen in LDH activity between Cairns and Mackay region populations at any of the tested experimental temperatures for lactate dehydrogenase activity: 20°C (*p* =0.14), 30°C (*p* =0.22), 40°C (*p* =0.064), and 50°C (*p* =0.28).

# Discussion

brief description of results

## Aerobic physiology

Fish from low-latitude populations demonstrated significantly higher aerobic physiology capacity at warmer temperatures than conspecifics from high-latitude populations – evidence of co-gradient variation. Furthermore, low-latitude populations showed had higher thermal optimal temperatures than high-latitude conspecifics. Differences in AAS between regions was driven by low-latitude population’s ability to increase their MO2max at warmer temperatures; offsetting increases in MO2rest. Fish from high latitude populations were unable to increase their MO2max at warmer temperatures and therefore experienced lower AAS as MO2rest increased.

Within aquatic ectotherms AAS serves as a proxy for the limits of oxygen demanding processes that can be performed simultaneously (Clark et al. 2013). The thermal breadth of fish is therefore suggested to be optimized to local conditions that maximize AAS (Stillman 2002; Sunday et al. 2011), although how oxygen is prioritized among processes remains unresolved (Clark et al. 2013; Farrell 2016). Nonetheless, improved capacity for oxygen, related processes such as locomotion, growth, and reproduction, suggests low-latitude populations are adapted to optimize AAS at higher temperatures, compared to high-latitude conspecifics.

Co-gradient variation

## Limitations and future research

The two 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 adaptive potential among populations from each region. 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 (Pilakouta et al. 2020). Results from this study identified optimal temperatures in aerobic physiology, however, the shape of the thermal performance curve between optimal temperatures and the onset of anaerobiosis remains unexplored. Such information may be necessary for detecting difference in adaptive potential between species (Pilakouta et al. 2020). Additional, the exploration of aerobic physiology at cooler temperatures may have better revealed ‘local vs. foreign’ criterion, outlined by (Kawecki and Ebert 2004), used to detect local adaptation.

Lastly, this study was unable to source fish from the equatorial (i.e., trailing edge) 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 on *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 how these populations will respond to warming conditions, compared to populations at lower latitudes.

## Conservation implications

## Conclusions

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**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). ~

~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).