# Introduction

The rate of increasing global temperatures are causing species to undergo range redistributions or rapidly adapt to new thermal conditions; otherwise decline 1–4. How species respond to warming temperatures will depend on the collective response of populations 5,6. Spatial variation in traits across populations represents genetic adaptation and phenotypic plasticity along geographic and environmental gradients 6–9. 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 plants 10,11, insects12,13, crustaceans7,14,15, and fish (see review by Conover *et al.,* 2009)16. However, optimal performance temperatures typically do not follow the trajectory of environmental gradients. Counter-gradient variation, whereby genetic and environmental influences on phenotypes are opposed, has been recorded in a number of taxa (fish17, lizards 18,19, and turtles 20) and appears more frequently in nature than co-gradient variation 16. Evidence of counter-gradient evidence suggests limited thermal plastic potential in low-latitude populations.

Low-latitude environments characterized by stable temperatures near physiological maximums favor specialized (narrow) thermal niche breadths that evolve primarily through genetic adaptation rather than phenotypic plasticity– Climate Variability Hypothesis (CVH) 21,22 (*but see* 23,24). Narrow thermal niche breadths, limited plasticity, and evidence of hard ceilings for upper thermal tolerance 25–27, suggest that low-latitude populations are more vulnerable to shifting temperatures than high-latitude conspecifics 28–32. Wider thermal niche breadths, present in high-latitude populations with greater levels of phenotypic plasticity, can increase thermal tolerance5; however, heat-tolerant phenotypes present in low-latitude populations may be unattainable within high-latitude populations (for example, ref 33). Populations that are locally adapted, therefore, may possess thermal niches that are narrower than the species as a whole34. Consequentially, population responses to warming temperatures may differ depending on occupied thermal niches 35.

Thermal variation between populations within marine systems has not received the same attention as terrestrial systems; despite marine organisms having greater confinement to thermal tolerance limits 31,36–38. 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 act as challenges to gene flow and promote local adaptation 36. To date studies that have addressed intraspecific variation in marine species have focused on invertebrates (see review Sanford and Kelly., 201136) including copepods 33,39,40, porcelain crabs 41, intertidal snails 7,15, and coral 42; few broach the topic among fish.

Studies on intraspecific variation in marine fish have often examined populations on the Great Barrier Reef (GBR), Australia, which represents a broad spatial and thermal gradient. Pratchett *et al.,* (2013)43 compared aerobic physiology and hematological metrics among low- and high-latitude populations of coral trout (*Plectropomus leopardus*), however, no significant differences were identified. Further analysis suggests there is little genetic variation between coral trout populations across the GBR44,45. Gardiner *et al.,* (2010)17 and Donelson and Munday (2012)46 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)17 found evidence that high-latitude populations maintained higher aerobic capacity than low-latitude populations at warmer temperatures – counter-gradient variation. Donelson and Munday (2012)46 reported that high-latitude populations displayed increased acclimation capacity (i.e., developmental plasticity) compared to low-latitude populations –supporting the CVH. Differences in intraspecific variation between coral trout and *A. polyacanthus*, are perhaps unsurprising considering ecological differences between species; in particularly, *A. polyacanthus’s* lack of a pelagic larval stage. Nonetheless, evidence of intraspecific variation within *A. polyacanthus* suggest the potential to explore intraspecific variation in a non-commercial marine fish species, a currently underexamined area of research that has important implications for the conservation of coral reef fish.

Robust genetic variation between *A. polyacanthus* populations 47–49 suggests that existing physiological studies provide a rudimentary understanding of the *A. polyacanthus’s* thermal landscape. Gardiner *et al.,* (2010) and Donelson and Munday (2012) both focused on a single high-latitude population, however, genetic analysis suggests high levels of genetic differentiation between populations throughout *A. polyacanthus’s* range; particularly within the southern region 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 of intraspecific variation is required. This study compares thermal performance curves of *A. polyacanthus* from three different populations in two regions, Cairns and Mackay, on the GBR with different thermal profiles. Thermal performance curves were used to compare physiological metrics including resting oxygen consumption (MO2rest), maximal oxygen consumption (MO2max), absolute aerobic scope (AAS), immune response, and enzyme activation, between regions. Hematocrit ratios were also compared at a single temperature. Testing temperatures 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)50. We tested the hypothesis for counter-gradient variation across a thermal gradient between northern and a novel southern region. Based on evidence of greater phenotypic plasticity among low latitude populations 46, populations from Mackay are expected to have increased thermal tolerance and performance at warmer temperatures than populations from the Cairns region. However, co-gradient represents an alternative hypothesis considering the limited amount of research available on the topic, and genetic differences between populations from the Mackay region and previously examined southern populations.

# Methods

**# metrics that we measured in this manuscprit (metabolic rate, pha, hematocrit, enzymes, genetics)**

## Sampling

The tropical damselfish, *Acanthochromis polyacanthus* [citation: Bleeker 19xx], ranges from the southern Great Barrier Reef (GBR) to the central Philippines (; lat-lon). *A. polyacanthus* populations are thought to have propagated the Indo-Pacific during glacial times [when is glacial times] when lowered sea levels would have provided shallow water channels that acted as dispersal corridors51. However, such dispersal opportunities would have eliminated as water levels began to rise and 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 habitat52 [citation: Planes -> Robertson 1973; Thresher 1983]. This unusual life history trait, among marine fish, coupled with *A. polyacanthus* inability to disperse between reefs separated by depths greater than 10-15m [citation needed], creates conditions that should promote local adaptation36; 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 XXX kilometers (spanning XX° 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 were 27°C, 28.5°C, 30°C, and 31.5°C. These temperatures are within the natural range both populations (27 and 28.5°C) or temperatures that occur infrequently a maximal temperature or with marine heatwaves (30 and 31.5°C), as well as being projected to occur by the end of the century (IPCC 2100 projected temperatures under project xxxxx citation).

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)53 [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 studies54,55 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:

[ENTER FORMULA HERE}

(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 AS 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 AS 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 AS differed between populations. Optimal AS for Cairns and Mackay populations was 30°C (10.31 MgO2 hr-1) and 31.5°C (8.57 MgO2 hr-1), respectively; +1.5°C above the average summer temperature in each region. Interestingly, Cairns region fish showed similar AS 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

## Limitations

Thermal tolerance can rapidly change via phenotypic plasticity, no understanding yet of genetic differentiation or adaptive potential

Current research occurs over short period a time from just a small subet of populations.

Did not test extremes which may have yielded crypto variation/stronger signal of local adaptation (i.e., x-pattern).

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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 pace56.

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 34. 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 34. 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** 57–59. 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 57,60–64. 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~~ ~~40~~~~.~~ Moreover, temperature-dependent clines (i.e. local adaptation) between populations represent diverging evolutionary histories that can elucidate how populations will respond to climate change 30,40,65.

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 65.

**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 16,40,66,67. However, local adaptation and genetic subdivision within metapopulations can also produce populations with narrow thermal breadths; increasing susceptibility to warming temperatures 34,68.

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) 69,70. 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 17,30,70,71. 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) 72.

~~Intraspecific variation within marine systems (outside of a few economically important species) have not received the same attention as terrestrial systems~~ ~~36~~~~. 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~~ ~~36~~~~. 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~~~~31,37,38~~~~.~~

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 50 have negative effects on sex ratios 73,74 , growth 75–77, reproduction 78,79, and aerobic capacity 17,46,80,81 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 33,36,65,82–84. 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 1,5,67,85. ~

~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~~ 5–7.

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 ubiquitous23,24.

Thermal tolerance of individuals can be used as a proxy to estimate a population’s ability to tolerate warmer temperatures 7.