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 30-31.5C, and higher capacity at this temperature range than high-latitude conspecifics. While, fish from low-latitude exhibited rising MO2max and MO2Rest with warming, fish from high-latitude populations displayed a plateaued MO2max across the testing temperature range and consequently reduced AAS due to the 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.

Contrastingly, 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 31.5C, were similar between both regions, suggests that natural selection on these phenotypic traits is not differing between latitudes. What does this mean....  evolution does this mean something for how they relate to fitness?

Considering the observed pattern in AAS, we might have expected latitudinal differences in hematocrit (proxy or oxygen carrying capacity) and aerobic enzyme performance if these were correlated to the limited maximum oxygen consumption (). In the case of the snapper XXXX, experience of a 4-week marine heatwave of 29.5 and 30.5C (+1-2C) resulted in an increase in hematocrit to allow maintenance of aerobic capacity (McMahon in review). However, hematocrit was shown to be unresponsive in both theusillier *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 likely limit aerobic capacity. Overall our findings instead support the theories that it is the likely the heart and/or gills that are limited the ability to maintain oxygen delivery () and ultimately determining thermal, tolerances, local adaptation, and plasticity in fish (Farrell 2009; Ekström et al. 2017; Nyboer and Chapman 2018; Pichaud et al. 2019). Consequently, enzymatic activity with the heart may be more relevant to whole organismal aerobic by limiting cardiac function (ref for where this has been seen).

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 28.5C. 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 is 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 would expect more energetic behaviors, such as reproduction, will be reduced and cease at temperatures above 28.5C. This has been found to occur for  *A. polyacanthus* … XXX (refsDonelson et al 2010…). Our study adds to the growing evidence that different physiological functions possess different optimal temperatures (i.e., multiple optima hypothesis Clark et al. (2013) and highlights the need to study a range of performance metrics and ideally those 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 , which . Counter-gradient variation between *A. polyacanthus* populations was identified when comparing low-latitude Lizard Island and a high-latitude Heron Island, which are both further north and south than the low and high-latitude populations examined in this study. In Gardiner et al (2010), juvenile fish were sampled from shallow lagoons, whereas fish in this study were older and collected from ~6-12 meters on coral reef slope. Reef flats and lagoons generally experience greater thermal variability (minimum, maximum, magnitude of diel 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; Vielleux et al). This results in the potential for multiple patterns on variability and performance to occur when exploring across latitudes (REF paper on leading, trailing expectations), 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 (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) differences between populations, suggesting that adaptive potential between examined regions are unlikely to be analogous.

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~~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.~~

Determining spatial patterns of thermal adaptation underlie the ability to predict population responses to climate change (Sorte et al. 2011; Moran et al. 2016). Species distribution models frequently assign all populations identical thermal ranges, however, such approaches risk inaccurately projecting species trajectories under climate change scenarios. 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 that contribute to experience environmental variability can create pockets of adaptive heterogeneity. While large scale latitudinal patterns, such as co-gradient variation, may be present among populations that experience similar climatic variability, neighboring populations that experience difference environmental conditions (e.g., shallow lagoons) may display alternative responses to climate change different than responses predicted by broad scale biogeographical (e.g. latitude) patterns (also see Pallarés et al. 2023). 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, the necessity to sample numerous populations in different environments to understand species’ adaptive landscape. Such an understanding would not only allow for more accurate predictive modelling but would also yield benefits for translocation-based conservation techniques such as assisted gene flow that rely on the introduction of beneficial traits as well as genetic compatibility between populations.