Testing the climate variability hypothesis in an aposymbiotic coral, *Astrangia poculata*

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Abstract

Astrangia poculata, the Northern Star coral, is a facultatively symbiotic stony coral found along the Atlantic Coast of the United States and the Gulf of Mexico. Although this species is widespread, much is still unknown about their phenotypic plasticity; specifically in regards to increasing temperatures. In order to better understand the ability of *A. poculata* to acclimate to increased temperatures, the thermal performance curves (TPC) of two different populations were studied. The two populations, Rhode Island and North Carolina, were chosen due to their differing annual temperature ranges, distal locations, and therefore the ability to test the climate variability hypothesis in *A. poculata*. Coral fragments were collected from both locations and acclimated for two weeks in 18$_{o}$C and 22$_{o}$C saltwater before being exposed to increasing heat stress (18-36$_{o}$C). Respiration in the increasing temperature range was measured for both populations to determine if the corals are locally adapted to their temperature environment, or if acclimation temperature correlates with performance in their environment. Corals acclimated to 18$_{o}$C had consistently higher respiration rates than those acclimated to 28$_{o}$C, with North Carolina corals almost always respiring more than Rhode Island corals. Corals acclimated at a lower temperature exhibited a higher respiration rate, most likely due to more acute stress than the 28$_{o}$C corals which experienced chronic stress during acclimation.

Introduction

Temperature is an important factor in determining species distribution patterns in ectothermic organisms (Angilletta 2009; Somero et al. 2017). As sea surface temperatures continue to increase, understanding how changing global temperatures may alter these patterns demands an understanding of physiological sensitivities to increased mean and maximum temperatures across the native range. For sessile organisms (e.g organisms that lack the ability of self-locomotion), increased thermal tolerance plasticity could be essential for the ability of an organism to survive rapid warming events (Calosi et al. 2007).

Two main factors determine a species' temperature sensitivity: its individual heat tolerance (CT_{max}) and its ability to compensate against increasing temperatures (plasticity; Angilletta 2009; Verberk et al. 2017). CT_{max} often refers to the ecologically significant thermal threshold after which an organism is unlikely to survive or survival is time-limited (Angilletta 2009). CT_{max} may be higher in environments that are thermally challenging, like the intertidal zone where temperature shifts vary greatly. In addition, differences in latitude may affect CT_{max} and these values may vary across populations within a species that are exposed to different annual temperature ranges.

Phenotypic plasticity refers to an individuals' ability to shape a response due to environmental factors and disturbances, such as warming temperatures. Phenotypic plasticity allows organisms to fit their environment, and is of the utmost importance to sessile organisms who are unable to relocate from unfavorable environments and are forced to adapt or acclimate (Calosi et al. 2007). Manipulation of abiotic factors in a lab experiment with controlled variables is typically used to quantify plasticity among individuals of

similar taxa, including ectotherms (Gunderson et al. 2015). Relationships between temperature and metabolic rates can be defined by measuring respiration at regular rates, and using these relationships, we can quantify the range of plasticity for thermal limits to predict which individuals may fare better as global temperature increases.

Understanding how differences in phenotypic plasticity within benthic communities will be impacted by rising sea surface temperature (SST) associated with projected climate trends is essential for predicting how these shifts will impact community structure. One way of understanding the survivorship of these benthic communities is to generate thermal performance curves (TPC), which predict the CT_{max} of local organisms. Tropical scleractinians, who have seen rising mortality events with increasing SST, are of particular interest for thermal optimum studies (McClanahan et al. 2007). However, studying thermal responses in tropical species can be difficult and impractical because species are limited to tropical latitudes. Thus, they are predicted to have narrow temperature thresholds, which is consistent with the latitudinal hypothesis. This hypothesis, also referred to as the climate variability hypothesis, predicts a pattern of increasing thermal tolerance plasticity as one moves from the equator to the poles due to increased variability in annual temperatures (Addo-Bediako et al. 2000, Bozinovic et al. 2011). In order to better our ability of understanding how changes in latitude can influence phenotypic plasticity in scleractinian corals, a species with a wide geographic distribution across latitudes is required.

Astrangia poculata, the Northern Star coral, is a small facultatively symbiotic coral and unlike the obligate relationships observed between tropical coral hosts and their algal symbionts, *A. poculata* is able to persist with or without its symbionts across its range. *A. poculata* reside in shallow coastal waters along the northwest Atlantic, reaching its northern limit around Cape Cod (Dimond and Carrington, 2007). With its wide range, *A. poculata* populations can be found both in temperate and subtropical latitudes within the Atlantic Ocean. Due to differences in latitudinal location, subpopulations are exposed to a wide range of different annual temperature fluctuations averaging 20 °C (Aichelman et al., 2019).

Differences in latitude have been shown to influence the thermal tolerance (CT_{max}) of different subpopulations of *A. poculata*, specifically by altering respiration curves (Aichelman et al. 2019). *A. poculata* is an ideal species for studying thermal tolerance thresholds because it has a wide range, which is expected to influence how individual populations respond under rapid change. If *A. poculata's* thermal physiology is consistent with the latitudinal hypothesis, one would expect to see greater thermal tolerance plasticity in *A. poculata* collected further from the equator when compared to a more subtropical population. This study will meet three objectives: 1) understand how respiration rates change with increasing temperatures in *A. poculata*, 2) compare the respiration curves of a subtropical and temperate *A. poculata* populations in response to increasing temperature, and 3) compare the capacities for phenotypic plasticity in a subtropical and temperate *A. poculata* population in response to acclimation temperature. We hypothesize that more northern populations of *A. poculata* will express greater thermal tolerance plasticity than their more southern counterparts following the predictions of the latitudinal hypothesis.

Methods

Study Sites

During May 2019 and September 2019, aposymbiotic colonies of the Northern Star Coral *(Astrangia poculata)* were collected from two different locations (Figure 1). Colonies were collected by SCUBA divers using a hammer and chisel and were separated by at least 0.5 m to avoid the collection of clones (Aichelman et al. 2019). A subtropical population of *A. poculata* was collected from the Outer Banks at the Triangle Wrecks (NC; May 2019) and a temperate population was sampled at Fort Wetherill (RI; September 2019). Sites were selected based on readily available wild populations of *A. poculata* and variation in natural temperature range. Triangle Wreck experiences an annual temperature range between 12-28˚C while Fort Wetherill experiences a temperature range of 0-27˚C. Wild corals were fragmented using a band saw (Chicago Pneumatic, Rock Hill, SC, USA) fitted with a diamond tip blade. Each genotype (n = 12 NC and n = 9 RI) collected from both the Triangle Wreck and Fort Wetherill were fragmented into at least two nubbins. Coral fragments were fixed to ceramic frag plugs using InstaCure ethyl cyanoacrylate gel (IC-Gel, Bob Smith Industries Inc., Atascadero, CA, USA) and designated to respective tank systems described below.

Coral Husbandry

Fragments were acclimated for two weeks in two recirculating saltwater aquarium tank systems: tank system 4 (4A, 4B, and 4C) and tank system 7 (7A, 7B, and 7C). Tank system 4 was maintained at 28°C and tank system 7 was maintained at 18°C. Water quality for both systems was measured twice daily to ensure salinity remained between 33-35 PSU and temperature did not deviate further than 0.1˚C from the set temperature. Salinity was measured using a YSI Probe (PRO 30) while temperature was monitored using NIST-calibrated glass thermometers. Light intensity was measured across each individual tank on a weekly basis via a Full Spectrum Quantum Sensor (model mq500) and light levels were maintained on a 5 hr light-dark cycle (lights on from 10am-3pm). Corals were spot-fed a diet of *Cyclops vicinus* three times a week during acclimation. Corals were not fed for a minimum of 48 hours leading up to their respiration trials. Coral frag plugs and individual tanks were cleaned of any debris of macroalgae growth as needed using soft-bristle toothbrushes prior to trials.

Coral Physical Measurements

Corals were individually measured for wet weight (g) using an Ohaus Scout Pro SP-401 portable balance scale. The volume of each coral fragment was quantified by measuring the volume of each respiration chamber with their respective frag subtracted by the volume of the control chamber. Each frag was photographed with Limostudio equipment from a bird's eye view to maximize visible live-tissue captured. Using ImageJ software, and a coral health chart to account for scale, all live tissue was manually traced across each photo multiple times and averaged to estimate surface area.

Coral Thermal Physiology

Respiration rate of each coral were determined by measuring changes in dissolved oxygen (O_2) concentration using a fiber-optic O_2 sensor connected to a 10-channel Fiber Optic Oxygen Transmitter (OXY-10 SMA, Pre-Sens Precision Sensing GmbH, Regensburg, Germany) in a custom-made acrylic ten-chamber respirometry system (fabricated by the Australia Institute of Marine Science). Each chamber had a volume of approximately 0.624 L. The OXY-10 SMA instrument was calibrated in accordance with the supplier's manual. Each

of the ten chambers were mounted in a Plexiglas base that was submerged in a water bath filled with artificial seawater. Chambers were positioned on a submersible magnetic stir plate, and a magnetic stir bar maintained ample water circulation in each chamber.

Temperature in the water bath was maintained at each temperature of the ramp by an APEX fusion system. The APEX controlled both a chiller (13hp; Aqualogic Delta Star In-line Water Chiller) and a 1000 W Titanium heater to prevent temperature fluctuations beyond 0.1°C. O_2 consumption was recorded in a control chamber (e.g. only containing seawater), during each experiment to account for any metabolic flux caused by microbes present and/or instrument drift. O_2 concentrations were recorded using the Pre-Sens Measurement Studio 2 software (version Oxy10v3_33fb) at 0.07 Hz and were automatically corrected for temperature and salinity by the software. O_2 consumption in the trials was recorded for 30 minutes at each of six temperatures (18°C, 22°C, 26°C, 30°C, 34°C, and 36°C). Respiration rate was calculated from O_2 concentrations and time-scales which were corrected in R (v3.6.1) after being corrected using the packages *stringr*, *tidyverse*, and *dplyr*. Respiration rate was expressed as nmol O_2 min^{-1} cm^{-2}. Figures were produced in R using the *ggplot2* package and maps were produced using *ggmap*.

Statistical Analysis

All statistical analyses were implemented in the R v3.6.1 statistical environment (https://www.r-project.org/).

To compare the effect of acclimation temperature and origin population, a repeated-measure ANOVA was used with fixed factors of acclimation temperature, target temperature, and population origin. Genotype was included as the repeated-measure term to account for natural variation between genotype and target temperature. Tukey's honestly significant difference (HSD) post hoc analyses were used to explore potential significant trends between main effects.

Results

The Effect of Acclimation Temperature

Regardless of population location (i.e. RI and NC), nubbins in the 18°C acclimation treatment experienced significantly higher respiration rates than nubbins acclimated in the 28°C treatment (Tukey's HSD, $p < 0.0001$). The elevated respiration rates observed in the 18°C acclimation were consistent throughout the duration of the respiration experiments between 18-34°C (Figure 2). Further, there was a significant influence on acclimation temperature and observed respiration rates (ANOVA, $p < 0.0001$).

The Effect of Population

Regardless of acclimation temperature (i.e. 18°C and 28°C), nubbins originating from NC experienced significantly higher respiration rates than nubbins originating from RI (Tukey's HSD, $p = 0.03$). This trend was consistent throughout at all exposed temperature measurements between 18-34°C (Figure 3). Further, there was nearly a significant influence on origin of population and observed respiration rates (ANOVA, $p = 0.055$).

Thermal Performance Curves (TPC)

When comparing the TPC's of between acclimation temperature and nubbin population, we see a trend where the NC nubbins respired more than the RI nubbins. This holds true for both acclimation temperatures (Figure 4). However, there was no significant difference between both acclimation temperature and population

origin at each measured temperature in the TPC (Tukey's HSD, $p > 0.05$).

Thermal Max

The thermal max of *A. poculata* was able to be identified as 36°C due to the deaths of cold population (RI) and warm population (NC) coral after the experiment.

Discussion

The Influence of Acclimation Temperature on Respiration Rate

A. poculata acclimated to 18°C always exhibited higher respiration rates than corals acclimated to 28°C. This trend is most likely the result of different durations and degrees of stress experienced by the nubbins both during acclimation and respiration trials.

The reduced respiration rates observed in the 28°C acclimation treatment may be the result of chronic stress experienced during through the duration of the acclimation period. The evidence for chronic stress during acclimation can be attributed to notable reductions in polyp extension by the nubbins acclimated in 28°C when compared to the nubbins acclimated in the 18°C system. Since the 28°C nubbins were already kept at a relatively high temperature for two weeks, reduced respiration rates may be an adaptive response to conserve energy as a result of chronic stress.

Unlike the 28°C treatment, the nubbins acclimated in the colder treatment (18°C) experienced an acute stress during the respiration trials, resulting in a trend of elevated respiration rates. During the acclimation period, nubbins in the 18°C system consistently had longer polyp extension than the corals acclimated in the 28°C suggesting the corals acclimated at a lower temperature experienced less stress than the higher temperature.

Unfortunately, we were unable to test if these responses in respiration rate were the result of differences in stress experienced both during acclimation and in the respiration trials. In order to explore this possibility in the future, we would recommend the incorporation of other physiological and metabolic measurements in the future.

The Influence of Population on Respiration Rate

Contrary to what we hypothesized, we found that corals from the North Carolina population exhibited greater respiration rates than those from the Rhode Island populations. This finding follows a pattern of cogradient variation, which predicts; populations from warmer sites should display an elevated phenotype relative to populations from colder sites at all temperatures. From our results, one might infer that our North Carolina corals exhibited a higher thermal optimum than those of the Rhode Island population. Unfortunately, our data proved to be inconclusive in that regard.

Temperature exerts strong selective pressures, often affecting phenotypes and distributions of species. It is possible that thermal adaptation to local environment explains the difference in respiration rate and presumably upper thermal limit across our populations. Consequently, we may see difference in physiology in the North Carolina corals that optimizes performance in warmer temperature if we were to analyze our fragments. However, the temperate *A. poculata* is generally able to withstand a vast seasonal temperature gradient. Therefore, a changing temperature may not have as strong of an effect on respiration as it may on a less thermally adaptive coral. Regardless, our results indicate that our two populations may represent a true warm and cold adapted set of populations

Respiration rates are often used as an indicator of metabolic rate. However, to state an effect of change in metabolism conclusively requires further methodological examination. With this in mind, we can only make inferences regarding differences in metabolic function across populations. We hypothesized that we would see a pattern of counter gradient variation, in line with what was found in Aichelman et al. Following the metabolic cold adaptation hypothesis, we expected that Rhode Island corals would exhibit higher metabolic rates as a compensatory mechanism of homeostasis maintenance(Addo-Bediako et al, 2002; Clarke, 1993). Instead, we found no such evidence of this trend. Perhaps *A. poculata* has a lower thermal optimum value than what conventional wisdom suggests which may explain a lack of metabolic compensation in temperature regulation in our treatments. However, it is more plausible that our observed pattern of cogradient variation simply suggests local adaptation.

Experimental Corrections and Future Work

Although we were able to correct respiration rate by the volume of the chamber and coral surface area, we were unable to correct rates with the blank. We observed that at high temperatures, the blank exhibited a thermal performance curve (TPC) similar to the experimental chambers. We had expected the blank to exhibit a TPC of zero, as the blanks only contained artificial seawater from the acclimation systems.

From the measure TPC in our blanks, we must consider if there was the possibility for gas exchange to-and-from our chambers. At the higher temperatures of our respiration trials (26-36°C), all of our chambers experienced the production of fine bubbles within the chambers. Bubbles were not observed to form on the probes, but, it is possible the formation of these bubbles had unforeseen implications for O_2 flux within the chambers.

In order to move forward, we must produce a solution for the TPC observed from our blank chambers. To do this, we recommend additional trial runs of chambers filled with artificial seawater in a well-lit area to allow for better observation of the chambers during a temperature ramp trial. Chambers should be monitored closely for any potential bubbles exiting the chambers, signifying leaks. If leaks can be located, modifications can be made to the chambers to ensure they are air-tight in future trials.

Although unlikely, the TPC produced by the blanks could have also been the result of microbial activity from microbes inhabiting the water column of the acclimation system. We were not able to test for the presence or abundance of these potential microbes, but as microbial metabolism increases with temperature (Giorgio, et al. 1997), this possibility should not be denounced.

In addition to the correction of the blank chambers, we suggest that the high-range acclimation temperature should be lowered. It is possible the chronic stress experienced by the nubbins in the 28°C acclimation suppressed metabolic rates during the experiment. Lowering the high-range acclimation temperature to 26°C could reduce stress prior to the experiment and result in respiration experiments which mimic acclimation conditions the species would face in their natural habitat. In addition, lowering the cold acclimation temperature to 16°C could provide the cold corals with a more accurate average temperature for their environment.

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Figures

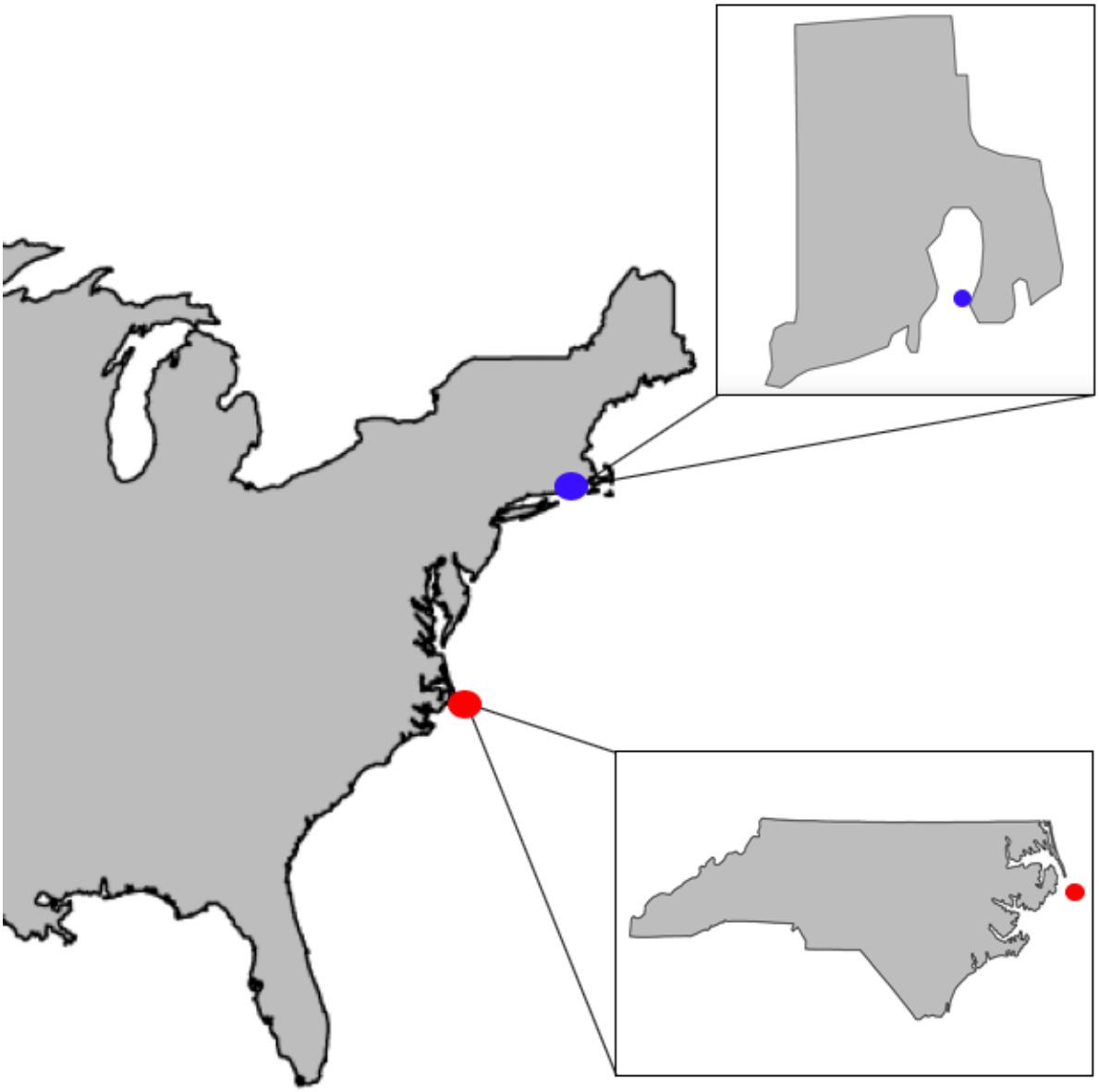

Figure 1. The locations of the *Astrangia poculata* collection sites. The collection site of the North Carolina population from the Outer Banks at the Triangle Wrecks (NC; May 2019) is represented in red while the Rhode Island population from Fort Wetherill (RI; September 2019) is in blue.

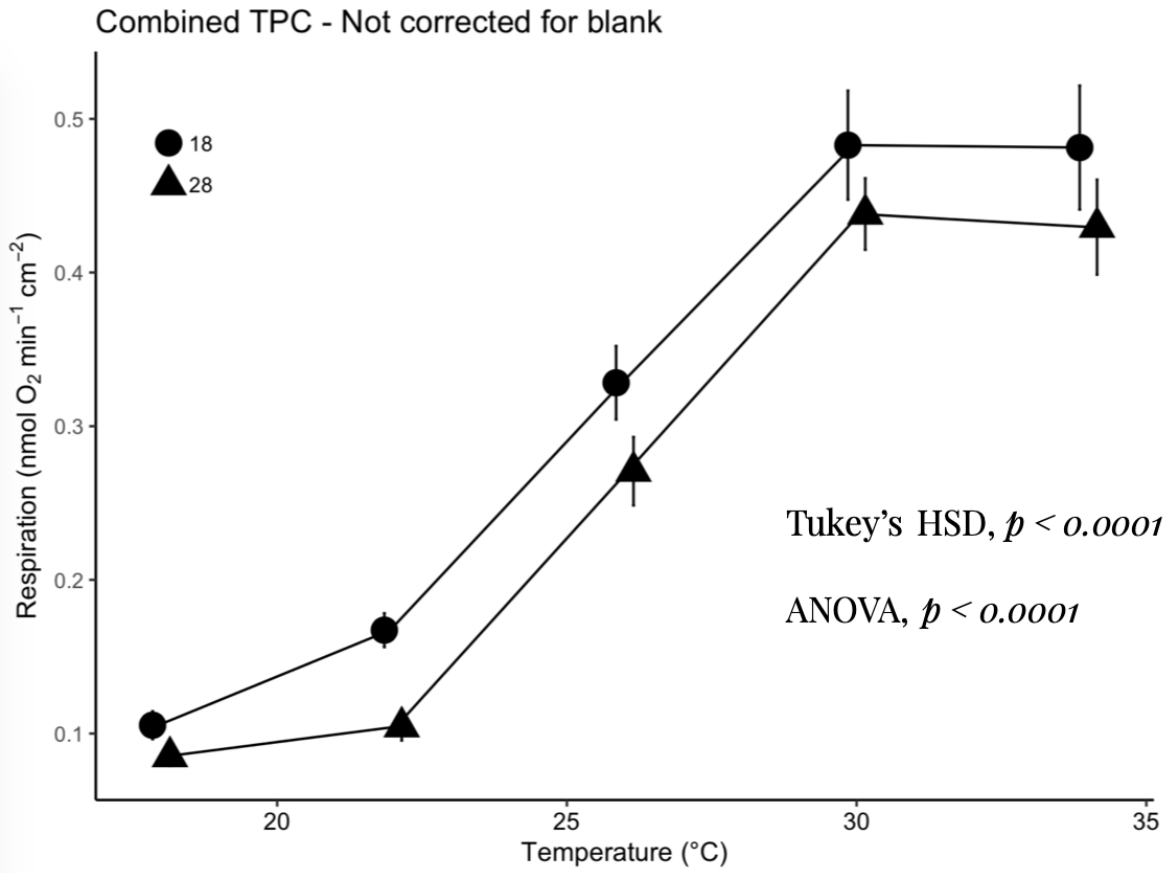

Figure 2: The thermal performance curves (TPC) of nubbins acclimated in 18ₒC (circles) and 28ₒC (triangle). Respiration rate (nmol O_2 min^{-1} cm^{-2}) was measured for all coral numbers during ramped exposure from 18-36ₒC. However, only data between 18-34ₒC is expressed above as data collected at 36ₒC was corrupted. Standard error is expressed at each temperature point by error bars.

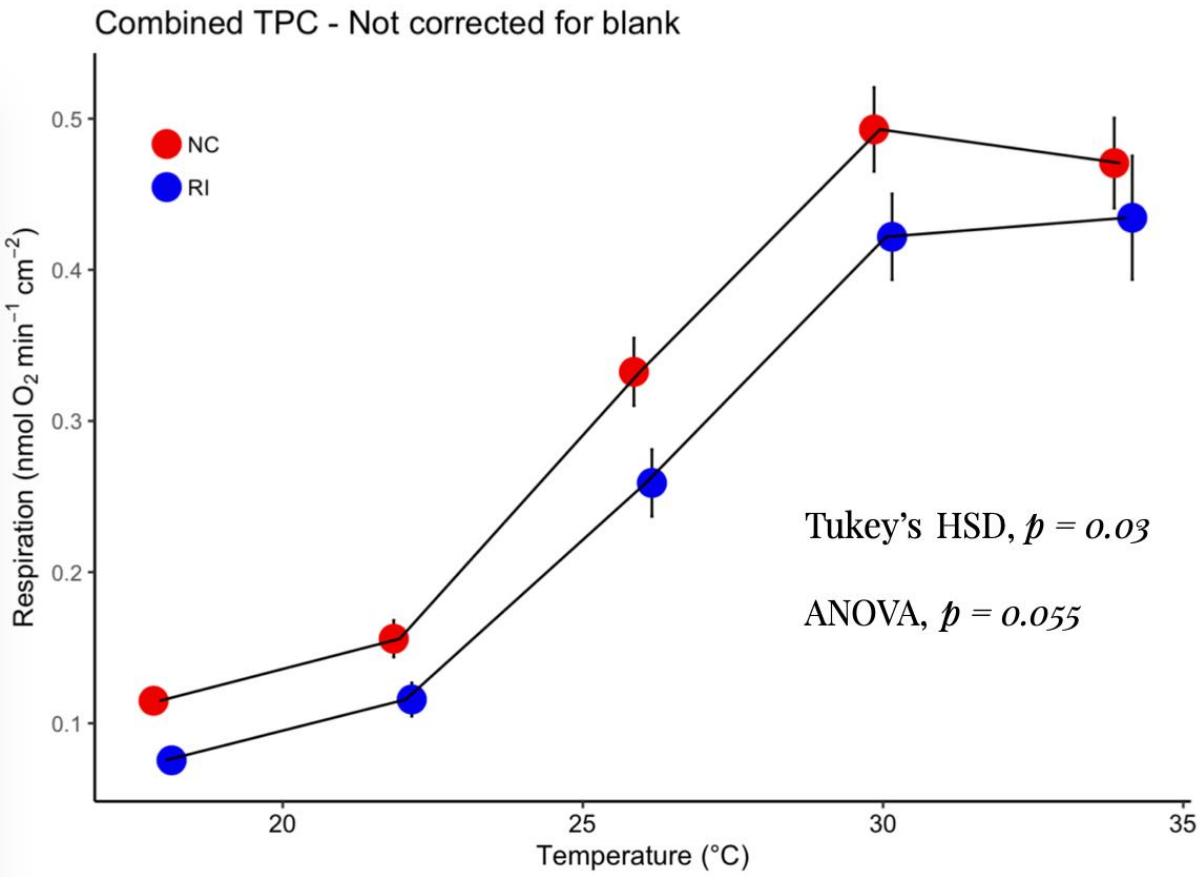

Figure 3: The thermal performance curves (TPC) of nubbins originating from NC (red) and RI (blue). Respiration rate (nmol O_2 min^{-1} cm^{-2}) was measured for all coral numbers during ramped exposure from 18-36°C. However, only data between 18-34°C is expressed above as data collected at 36°C was corrupted. Standard error is expressed at each temperature point by error bars.

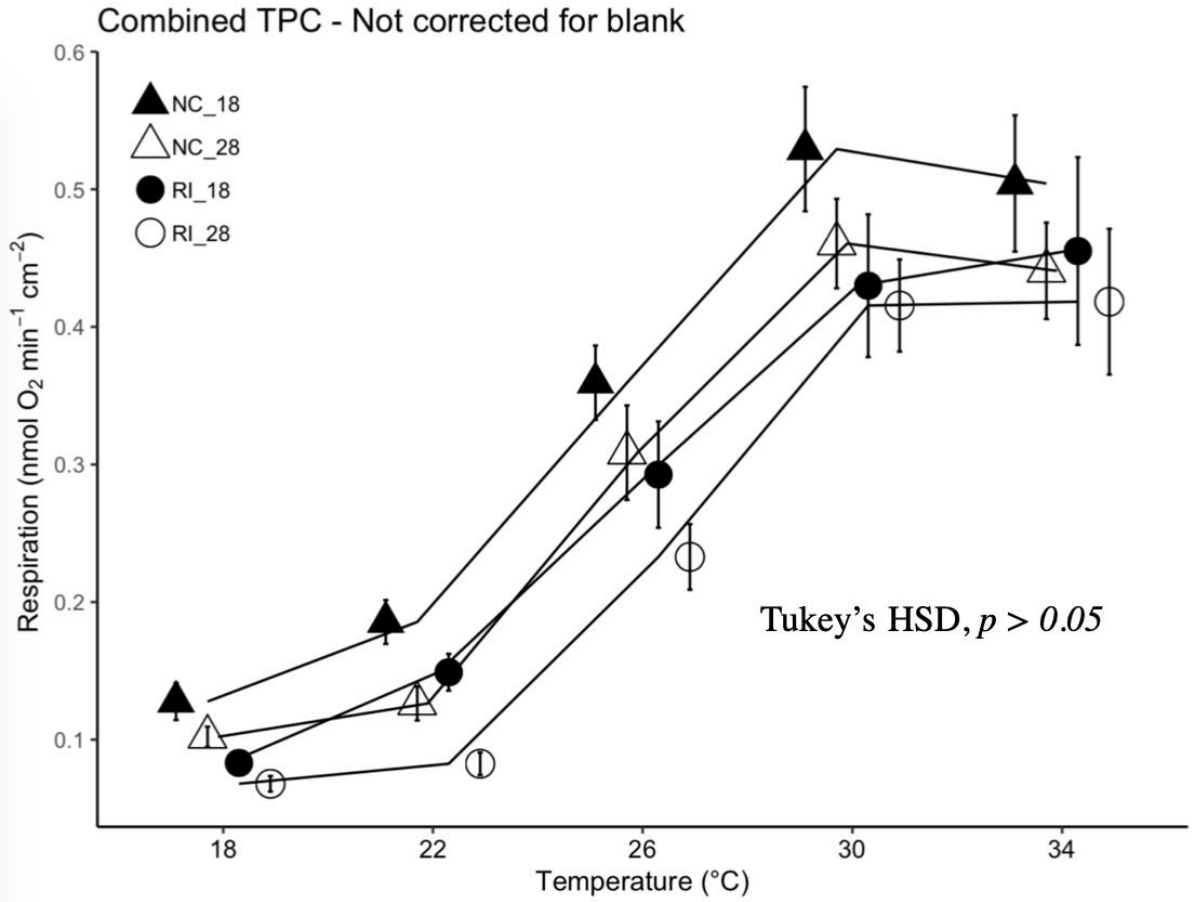

Figure 4: The thermal performance curves (TPC) as a result of both acclimation temperature (18₀C, filled shapes, 28₀C open shapes) and population location (circle RI, triangle NC). Respiration rate (nmol O_2 min^{-1} cm^{-2}) was measured for all coral numbers during ramped exposure from 18-36₀C. However, only data between 18-34₀C is expressed above as data collected at 36₀C was corrupted. Standard error is expressed at each temperature point by error bars.

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