

# Metadata

## Title

Local adaptation across a latitudinal gradient within a coral reef fish

## Description

To understand the evolutionary trajectory of a species under climate change it is important to quantify the magnitude of local adaptation, and genetic differentiation in populations throughout its range. This project aims to understand how local adaptation may vary across a latitudinal gradient in a reef fish (spiny chromis damselfish; *Acanthochromis polyacanthus*). This research project will 1) examine local adaptation in reproductive, metabolic, enzyme, and thermal performance traits between fish from three different latitudinal regions of the Great Barrier Reef (trailing edge, core, leading edge); 2) obtain shallow whole-genome sequences to gain an understanding of the genetic mechanisms underpinning local adaptation within metapopulations. Findings from this research aim to determine how evolutionary perspectives can be incorporated into wildlife conservation to achieve more effective outcomes.

## Contributors

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## Category

Project

## Affiliated institutions

*No affiliated institutions*

## License

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## Subjects

- Genetics and Genomics
- Genetics
- Biology
- Ecology and Evolutionary Biology
- Life Sciences
- Evolution

- Population Biology

- Marine Biology

## Tags

*No tags*

# Study Information

## Hypotheses

The hypothesis tested within this research project is: 1) *Acanthochromis polyacanthus* populations are phenotypically and genetically locally adapted to experienced thermal regimes (i.e. thermal gradient)

# Design Plan

## Study type

Experiment - A researcher randomly assigns treatments to study subjects, this includes field or lab experiments. This is also known as an intervention experiment and includes randomized controlled trials.  
Blinding

- No blinding is involved in this study.  
Is there any additional blinding in this study?

n/a

## Study design

To explore local adaptation three latitudinal regions within the range of the spiny chromis (*Acanthochromis polyacanthus*) will be targeted: 1) trailing edge (reefs around Port Stewart; [~13.81°S, 143.94°E], QLD, Australia), 2) core (reefs around Cairns, QLD, Australia; [16.67°S, 146.05°E]), and the 3) leading edge (reefs within the Capricorn-Bunker group; [23.45°S, 151.95°E]). Adult fish collected from approximately three different populations within each region during the austral winter of 2021 will be held in a laboratory setting at the Marine and Aquaculture Research Facility at James Cook University, QLD, AU. Fish from each region will be placed within a common garden; fish from each region will be held in temperatures that reflect ocean temperatures within the trailing (30°C), core (28°), and leading edge (26°C) of the range of Spiny Chromis. Within each temperature treatment temperatures will simulate seasonal temperature cycles, as well as diurnal temperature change of 0.6°C throughout a 24-hour period. Light cycles will simulate natural conditions within the core region. Fish will be maintained in breeding pairs (one male: one female) that reflect the natural mating system for this species. Reproductive performance, aerobic physiology, and morphometric traits will be investigated among each temperature treatment. Temperature preference, critical thermal maximum, and enzymatic performance will be measured using samples from the coolest temperature treatment (i.e. leading edge; 26°C) to minimize stress applied at fish from the starting point of the experiment (fish within each region are expected to have experienced ocean temperatures of 26°C previously). Reproductive performance traits will be measured during the breeding season (October-February). The remaining traits will be measured after the breeding season. Within each treatment group we expected to have a fifteen successful breeding pairs (although more than fifteen breeding pairs will be set up, as we only expected two-thirds of breeding pairs to be successful). Population will be included as a random factor in a cross design to be considered within this experiment.

- [Methods Local Adaptation.png](#)

## Randomization

We will use block randomization, where breeding pairs from each population will be randomly assigned to one of three similarly sized, predetermined blocks (i.e. temperature treatments). The random number list used to create these four blocks will be created using the web applications available at <http://random.org>.

## Sampling Plan

### Existing Data

Registration prior to creation of data

Explanation of existing data

As of July 7th, 2021 no data has been collected for this experiment.

Data collection procedures

Fish will be collected from the wild via professional collectors and then transferred to the Marine and Aquaculture Research Facility at James Cook University. Adult fish will be collected from three latitudinal regions within the range of the spiny chromis (*Acanthochromis polyacanthus*) will be targeted: 1) trailing edge (reefs around Port Stewart; [ $\sim 13.81^{\circ}\text{S}$ ,  $143.94^{\circ}\text{E}$ ], QLD, Australia), 2) core (reefs around Cairns, QLD, Australia; [ $16.67^{\circ}\text{S}$ ,  $146.05^{\circ}\text{E}$ ]), and the 3) leading edge (reefs within the Capricorn-Bunker group; [ $23.45^{\circ}\text{S}$ ,  $151.95^{\circ}\text{E}$ ]). Trait measurement data will be collected using the method described below:

**Reproductive traits:** Traits measured will include timing of reproduction; clutch size; egg size; offspring at hatching; yolk amount; and reproductive output (mean egg area  $\times$  clutch size). Fish tanks will be checked twice daily for the presence of eggs. For each clutch after clutch size is counted (photographed and counted using imageJ), 10 eggs will be randomly selected for reproductive output and yolk amount measurements. After hatching 20 juveniles per clutch will be sampled for morphometric measurements. The body condition of juveniles will also be measured at hatching, 1-month and 3-months post hatching date to provide a measurement for maternal investment.

**Morphometric traits (physical condition):** Standard length of adults will be acquired by taking a photograph of individuals, then measuring then using imageJ software to measure length. Wet weight will be recorded on a standard scale. Morphometric traits of adults will be measured when initially transferred to the facilities at James Cook University, as well as after the breeding season.

**Aerobic physiology:** Aerobic physiology will be measured after the breeding season using an intermittent-flow respirometer across the temperature range  $26^{\circ}\text{C}$  -  $32^{\circ}\text{C}$ . This temperature range includes the mean monthly maximums of all three regions. Prior to metabolic attributes being measured, fish will be starved for 12 - 24hrs to ensure that measurements are not affected by additional metabolic function such as digestion and given 1-3hr to acclimatize with the respirometer with constant water flow. Resting ( $\text{MO}_2\text{rest}$ ) and maximum oxygen ( $\text{MO}_2\text{max}$ ) consumption will be measured directly and then used to calculate factorial ( $\text{MO}_2\text{max} / \text{MO}_2\text{rest}$ ) and net ( $\text{MO}_2\text{max} - \text{MO}_2\text{rest}$ ).  $\text{MO}_2\text{max}$  will be induced in a swimming apparatus by stimulating maximal aerobic swimming speeds. Including both factorial and net aerobic scope will provide more robust results because factorial and net aerobic scope can often yield different results.

**Temperature preference:** Thermal preference will be tested with a 'shuttle-box' (Loligo) that consists of two interconnected choice chambers, allowing fish to choose their preferred temperature. Choice chambers will have a  $2^{\circ}\text{C}$  temperature differential. The exact temperature within each chamber will depend on which region the fish originated from.

**Critical thermal maximum:** To measure critical thermal maximum ( $\text{CT}_{\text{max}}$ ) fish will be transferred to an experimental tank where the temperature will be increased at a rate of  $1^{\circ}\text{C h}^{-1}$  (no more than  $0.5^{\circ}\text{C}$  in a 30 min period, after an initial 1-2hr adjustment period), until individuals can no longer maintain dorso-ventral posture.

**Enzymatic performance:** Enzyme activity will be explored for key enzymes across a temperature range of  $15-45^{\circ}\text{C}$  (i.e. thermal performance curve) in vitro with a spectrophotometer. Enzyme thermal performance curves will also be compared with metabolic thermal performances curves to determine how temperature influences different physiological processes within *A. polyacanthus*.

**Sequencing and genomic analysis:** The novel approach of shallow whole genome sequencing (sWGS) will allow genome-wide associations with phenotypic traits to be detected. Reduced genetic diversity in areas of the genome where an allele is under selection, distinct patterns of haplotype structure, and linkage disequilibrium will be explored to determine the genetic changes that underpin local adaptation within different regions. The phenotype-genomic integrated approach will allow for an increased understanding of the evolutionary events that occurred surrounding local adaptation within a widespread species.

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Sample size

Each experiment treatment (three different regions x three different temperature treatments = nine experimental groups) is expected to contain fifteen breeding pairs. In total for this experiment approximately 135 breeding pairs will be assessed for reproductive traits. Each individual (270) will then be measured for traits relating to morphometric measurements and aerobic physiology. A subset of ten individuals from each treatment will be used to measure temperature preference, critical thermal maximum, and enzymatic performance.

Sample size rationale

The decision making process in determining the sample size for this project related to logistics involved with spacing fish in aquariums, time needed to measure traits, and adhering to animal care protocols to ensure that an excess of fish are not stressed unnecessarily. Aerobic performance and temperature preference can be particularly time consuming and therefore it may be unrealistic to measure more than fish than stated. The sample sizes suggested have been used in previous experiments are expected to yield reliable results. Additionally, the enzymatic performance analysis involves lethal dissection and was set a level that would yield reliable results while limiting animal suffering.

Stopping rule

Data collection for this experiment may stop if we are unable to collect fish from at least two of our three requested regions. Data collection would stop if unable to successfully form enough breeding pairs to produce a reliable sample size for each experimental group.

## Variables

Manipulated variables

We manipulated temperature that fish were exposed to upon entering the lab. The three levels of this categorical variable are: 26C, 28, and 30C.

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Measured variables

Reproductive measurements: clutch size; egg size (area); yolk amount; size at hatching Morphometrics: standard length; wet mass Metabolic attributes: rest metabolic rate; maximum metabolic rate Enzymatic performance (activity across temperature gradient): Citrate synthase; Lactate dehydrogenase; cytochrome c oxidase Temperature preference; Critical thermal maximum Genetic analysis: Heterozygosity; genetic diversity; signs of adaptive sweeps;

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Indices

Reproductive output will be measured using the following formula: Reproductive output = number of eggs in a clutch X mean area of eggs. Body condition (morphometric) will be measured by calculating Fulton's K:  $Fulton's\ K = 100 * \frac{wet\ mass\ (g)}{Standard\ length\ (cm)^3}$  Aerobic and factorial scope will be measured using the following formulas, respectively: Net aerobic scope = Maximum metabolic rate - Minimum metabolic rate Factorial aerobic scope =  $\frac{Maximum\ metabolic\ rate}{Minimum\ metabolic\ rate}$

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## Analysis Plan

Statistical models

Most of the statistical analysis that will be run within this study will be generalized linear mixed effect models. Models that aim to determine differences between regions will be set up as shown in the example below:  $clutch\ size \sim region * treatment + (1 | population:region)$  Models may also be run to examine the relationship between variables and environmental data, as shown below:  $clutch\ size \sim monthly\_mean + monthly\_max + monthly\_min$  To explore local adaptation the following formula outlined by Hereford (2009) may also be used. Note W presents the mean trait performance of either a population or a location (site):  $Level\ of\ local\ adaptation = \frac{W_{population1} - W_{population2}}{W_{site1}}$  As of yet it is undetermined whether the analysis will be used via a frequentist or Bayesian approach.

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Transformations

Inference criteria

*No response*

Either frequentist or a Bayesian approach will be taken - undecided.

Data exclusion

*No response*

Missing data

*No response*

Exploratory analysis

*No response*

## **Other**

Other

*No response*