Preregistration

Preregistration for LDP Test Project

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22. September 2024

Study Information

Title

Temperature drives changes in the composition of the microbial community in the non-native sea anemone *Diadumene lineata*.

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Description

The estuarine anemone *Diadumene lineata* is one of the most widely introduced anemones in the world. It is found predominately in estuarine conditions on hard substrates, however it exhibits an exceptionally broad tolerance for a wide range of temperature and salinity. Environmental tolerances are affected by the genetics and physiology of the organism, however the microbial community can also play a role. To identify which bacterial taxa contribute to temperature resilience, we sequenced the community across five temperatures between 20C to 30C, which is at the upper limit of their temperature tolerance.

Hypotheses This more of an exploratory experiment without a definitive hypothesis, however given what we know from coral research there are certain bacterial taxa we expect to be associated with higher temperatures.

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.

Study design This is a between subjects design with 1 factor five levels of temperature treatment. We will also be sequencing the microbial community of the water for comparison.

Randomization The anemones will not be randomly selected for treatments. There is a variety of sizes collected and an attempt will be made to ensure a variety of sizes are present within each treatment. Each anemone also must be checked for damage and removed if there are signs of disintegration.

Sampling Plan

Anemones will be collected from the docks shortly before the experiment begins to reduce stress. They will be transferred into fresh sterile artificial seawater water until they are completely cleaned of mud/debris and stored in individual 15mL tubes until the experiment begins.

Existing data Not applicable.

Explanation of existing data	A small pilot study was conducted ahead of this experiment to verify that this species would survive within the expected temperature ranges of the study.
Data collection procedures	After collection and cleaning, anemones will be fed ahead of the experiment and get a fresh water change. They will then be placed into a beaker together for 3 days to acclimate before being seperated for the temperature treatment (which will be for 3 more days). Seperating the anemones is important because if any die due to heat stress, it rapidly fouls the water. At the end of the trial, the anemones will be macerated in DNA/RNA shield. The water will be centrifuged and then the pellet will also be preserved in DNA/RNA shield until the DNA can be extracted.
Sample size	Each treatment will have a sample size of n=10, however only 5 will be selected for sequencing. This is to account for unexpected mortality that can be caused if the anemone is damaged during collection, but the damage is not noticeable.
Sample size rationale	Sample size (n=5) for each treatment is primarily constrained by sequencing costs, but is in-line with other host-microbe studies.
Stopping rule	Not applicable.

Variables

16S rRNA amplicon sequencing data for the microbial community Temperature (c) Wet weight of the anemone (mg)

Manipulated variables	We manipulated the temperature from 20-30 degrees C in 2.5 degree increments. There are five levels: 20, 22.5, 25, 27.5, and 30. The temperatures of the growth chambers were verified for accuracy with HOBO dataloggers.

Measured variables	Anemone wet weight was measured after the temperature experiment, but before maceration, in case larger anemones tend to host a larger diversity of microbiota simply because there is more habitat available.
Indices	Shannon's Biodiversity Index Beta Dispersion Non-Metric Multi-Dimensional Scaling (NMDS)

Analysis Plan

Exploratory analysis.

Statistical models	PERMANOVA PERMDISP
Transformations	16S rRNA data must be normalized to account for unpredicatable differences in sequencing depth between samples. Both cumulative sum scaling and rarefaction will be used (seperately, comparing results between the two). There is currently substantial debate around when to normalize and what normalization method to use.
Inference criteria	We will use a p-value of < 0.05 as the primary criteria for determining significance. Any tests requiring corrections for multiple comparisons will use the Benjamini-Hochberg correction.
Data exclusion	A cut-off of 1000 sequences will be used to remove samples that were poorly sequenced. This is to prevent losing too much information when normalizing sequencing depth. There can be a huge range in sequencing depth between samples that is difficult to predict ahead of time if the host species has not been sequenced previously. The only other samples that might be removed is if there is clear evidence of contamination (ex. finding a high relative abundance of human-associated bacteria in marine samples).

Missing data	Not applicable.
Exploratory analyses (optional)	Changes in the patterns of different bacterial taxa and their associatins with different temperatures will be used to generate new hypotheses that must be tested empirically.There are many genera that are poorly describe, particularly within the context of host-microbe intereactions.

Other

Other (Optional)	None.