Introduction

Shallow-water coral reefs are some of the most biologically diverse, ecologically important, and monetarily valuable ecosystems on the planet. Despite the evident importance of coral reefs, it has been well established that anthropogenic activities threaten the persistence of these ecosystems, globally (Hughes et al., 2018). The continued use of fossil fuels over the last two centuries has increased the concentration of atmospheric carbon dioxide, leading to elevated sea surface temperature (SST) as well as ocean acidification. Although the effects of elevated SST and ocean acidification on coral reefs have been studied extensively, the nature of the coral stress response is still unknown. Metabolomics provides the opportunity to discover chemical responses at the cellular level to specific disturbances such as elevated temperature and reduced pH. Although metabolomics has been used widely in human health fields it is just recently being realized in coral biology as a way to assess physiological condition (Seguin et al., 2016). This study will be the first to use 1H NMR to provide insight into the effects of global climate change on the coral metabolome.

This project will occur in two parts with two different sets of samples: (1) Samples acquired from the Maug Shallow Hydrothermal Vent (MSHV) will be used to accomplish objective (1). The vent creates a natural pH gradient and coral nubbins were sampled at three different distances from the hydrothermal vent in order to collect from colonies living at different ambient pH values (Figure 1): Low pH (6.07 closest to the vent) to High pH (8.04 at a distance of 11 m). (2) Samples acquired from the Hawaiian Institute of Marine Biology (HIMB) where colonies of two major reef-building species have been exposed to different treatments of pH and temperature in a flow-through system, will be used to accomplish objective (2).

Objectives:

1) To assess the differences in the coral metabolome over a natural pH gradient using samples acquired from the Maug Shallow Hydrothermal Vent.

2) To compare the coral metabolome of colonies exposed to different treatments of pH (low and high) and temperature (high and low) as well as the combination of these two treatments, and to examine inter- and intra-species variation of two major reef building species (HIMB samples).

Methods

MSHV Sample Collection

Coral colonies were sampled at three different distances from the hydrothermal vent in order to collect from colonies living at different ambient pH values (Figure 1): • Low: 7.94 ± 0.051 • Mid: 7.98 ± 0.027 • Background: 8.04 ± 0.016 • Nine nubbins of each Porites rus, Porites lobata, and Pocillopora edmundsi were collected at each pH level using a hammer and chisel (total n=9).

The samples were then transported to the NIST Environmental Specimen Bank in Charleston, SC and stored in liquid nitrogen until metabolomic analysis via 1H NMR.

HIMB Experimental Design and Sample Collection

The laboratory exposure of coral colonies of Montipora capitata and Pocillopora compressa at HIMB includes four treatment regimes (Figure 2b) with 10 tanks per treatment. Within each species there are two phenotypes (tolerant and sensitive) and within each phenotype there are five different genotypes. One to three nubbins of each genotype will be collected. Triplicate samples will allow the examination of intra-genotype variation. The collected coral fragments will be submerged in liquid nitrogen to preserve the metabolic state, shipped to NIST Charleston, and stored at -80°C until metabolomic analysis via 1H NMR.

Metabolite Extraction

All frozen samples will be lyophilized and stored at -80°C until sample processing. A firm-bristled brush will be used to remove the soft tissue from the coral skeleton. The collected tissue powder will be homogenized and metabolites will be extracted using methanol, chloroform, and water in a 2:2:1.8 ratio (Bligh and Dyer 1959, Wu et al. 2008) creating a biphasic system, from which the metabolites can be isolated (Figure 3).

The resulting methanol and chloroform phases will be combined and rehydrated in a phosphate buffer (Figure 3). 1H NMR metabolomic measurements will be performed using the 700 MHz NMR with NIST at Hollings Marine Laboratory.

Figure 1. Maug is a ring of uninhabited islands, part of the Northern Mariana Island chain in the Philippine Sea (20°27'N, 145°13'E). The CO2 vent creates a natural pH gradient ranging from 6.07 (closest to the vent) to 8.04 (farthest from the vent). (C) and (D) show the location of the background, mid, and low pH sampling sites. Samples from Maug will be used to complete Objective (1). Figure modified from Enochs et al. 2015.

Figure 2. (A) An example of a coral mesocosm at HIMB, Oahu, HI used for Objective (2). (B) A representation of the four treatment or exposure groups. ‘Low temperature’ and ‘high pH’ are seasonal averages, so both will fluctuate throughout the year. High temperature is considered +2°C and low pH -0.2 units from those seasonal averages. The appropriate pH is maintained by aerating individual aquariums with a mix of CO2 gas and CO2-free air.

Figure 3. Workflow of metabolite extraction starting with lyophilized and homogenized coral tissue powder and ending with the analysis of the polar metabolites via 1H NMR.

Figure 4. Representative 1H NMR spectra of Porites compressa from Seguin et al. 2014.

Figure 5. Schematic diagram of metabolite extraction with metabolomic and proteomic analysis. A univariate approach will also be used for more targeted analyses of specific portions of the spectra. Statistical analysis will be performed using a principal components analysis (PCA) to reduce the dimensionality of the data by creating fewer, intelligent variables. This analysis will help identify general trends, outliers, and illustrate the variability within the data.

Methods Continued

Statistical Analysis

Spectrum-wide relative standard deviations (% RSD) are used as a measure of reproducibility of 1H NMR metabolomics datasets and will be employed to: • Ensure spectral quality and • Allow for the detection of technical variation throughout the experiment

Principal components analysis (PCA) will be used to reduce the dimensionality of the data by creating fewer, intelligent variables. Another univariate approach will also be used for more targeted analyses of specific portions of the spectra.

For confirmation, annotations will be matched to metabolite databases, like the human metabolome database (HMDB), the biological magnetic resonance bank (BMRB), and inhouse metabolite standards.

Significance

Metabolomics offers the opportunity to understand the coral stress response at the cellular level by providing a bridge between genotypic and phenotypic data, compared to other ‘omics’ methods. The knowledge gained from this project has many future applications that are diagnostic, predictive, and preventative. 1H NMR metabolomics is a powerful tool for the assessment of coral health. As more species are studied (in similar experiments, predictive models can be constructed that forecast the change in community structure over time. Reef replenishment activities can also utilize data collected from coral metabolomics studies to transplant more tolerant species or genotypes in order to combat coral cover loss. This work will lay the foundation for future research exploring different species’ responses and answering fundamental questions about how our reef systems will change as global climate change continues to alter their physical and chemical environments.

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References


