

## Background

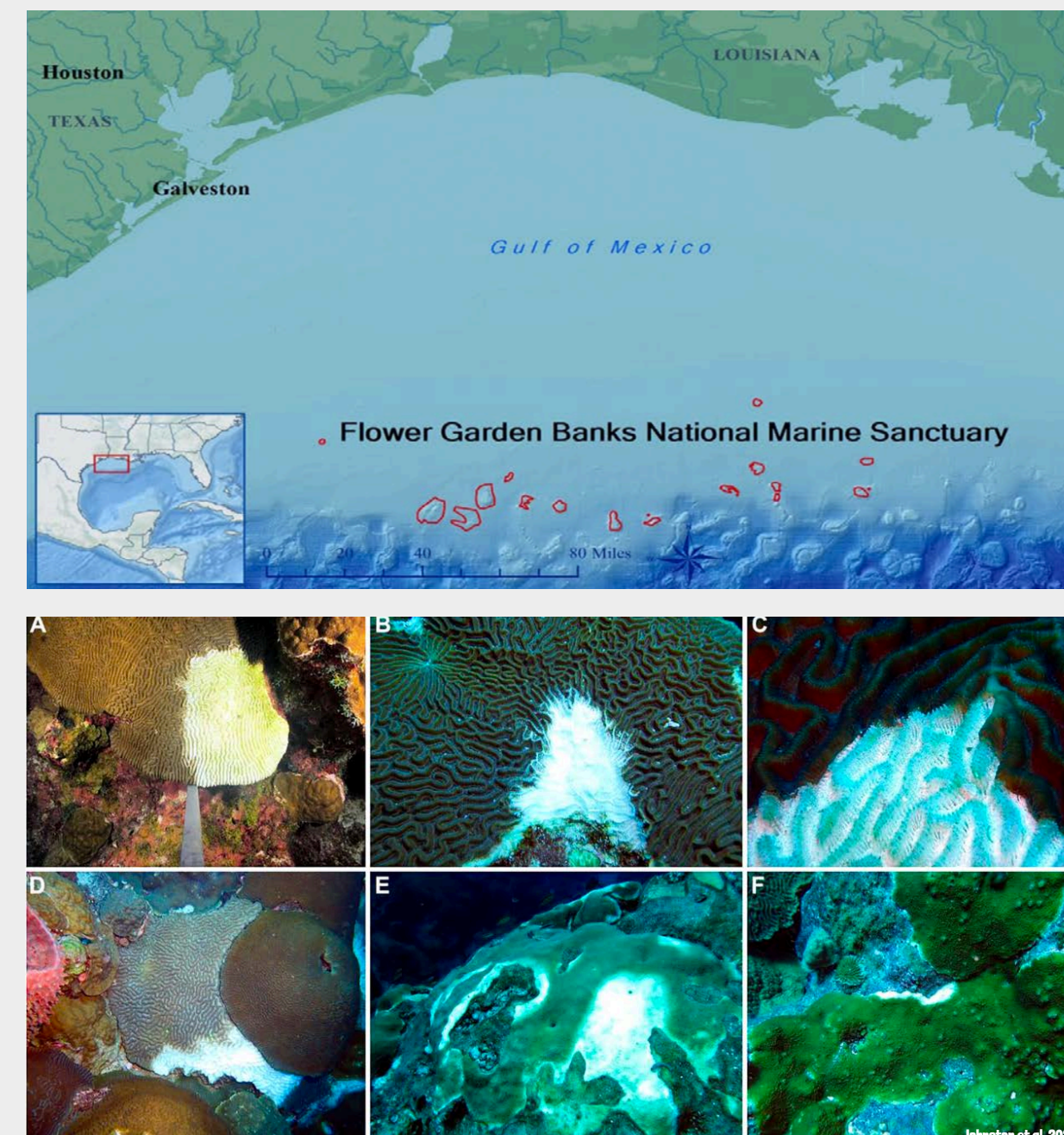
In August 2022, an unidentified white syndrome disease was observed at the East and West Banks of Flower Garden Banks National Marine Sanctuary [1]. In September 2022, samples of *Pseudodiploria strigosa* were collected.

Disease characteristics [1]:

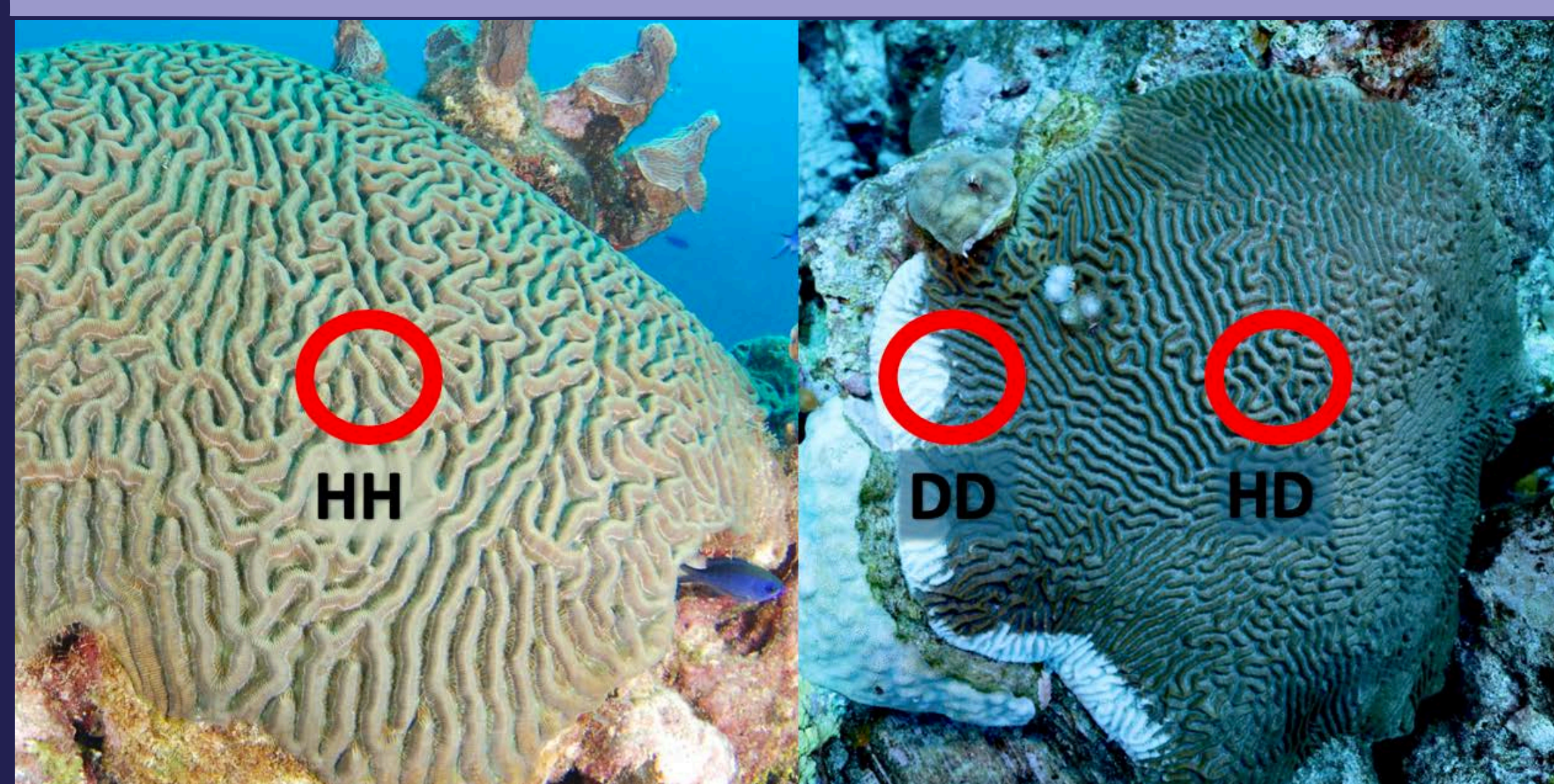
- Acute tissue loss
- Multi-focal lesions
- Lesions along colony margins

### Research Goal

Link genes to phenotypes of disease from various types of data

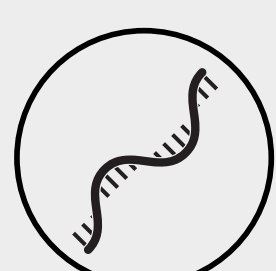


## Sample Collection



n = 15 *Pseudodiploria strigosa*  
5 of HH: healthy sample from a healthy colony  
5 of HD: healthy sample from a diseased colony  
5 of DD: diseased sample from a diseased colony

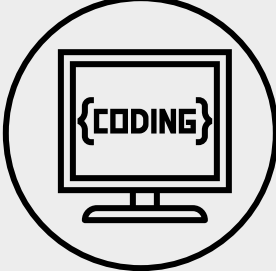
## Methodology



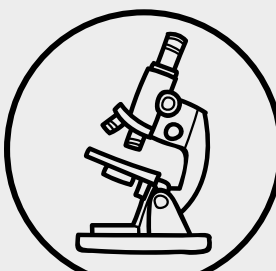
Total RNA extracted from flash frozen coral tissue. Kit: Invitrogen RNAqueous.



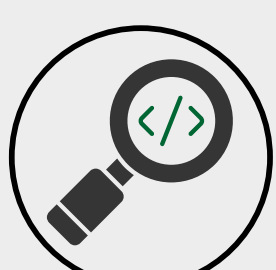
RNA sequencing: 150bp paired-end, poly-A trail enrichment on the Illumina Novaseq 6000 platform.



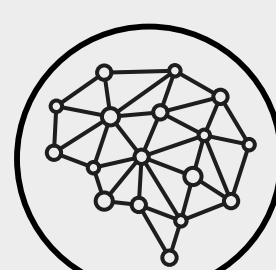
A *de novo* transcriptome was assembled and reads were mapped: FastP - Trinity - BUSCO - BBsplit - Salmon



Histology traits were measured across 5 slides per sample by Dr. Ashley Rossin.



DeSEQ2: Identified differentially expressed genes across health state comparisons.



Weight Gene Co-Expression Analysis (WGCNA) to identify networks of genes correlated to histology traits: average symbiont area and average vacuole area.

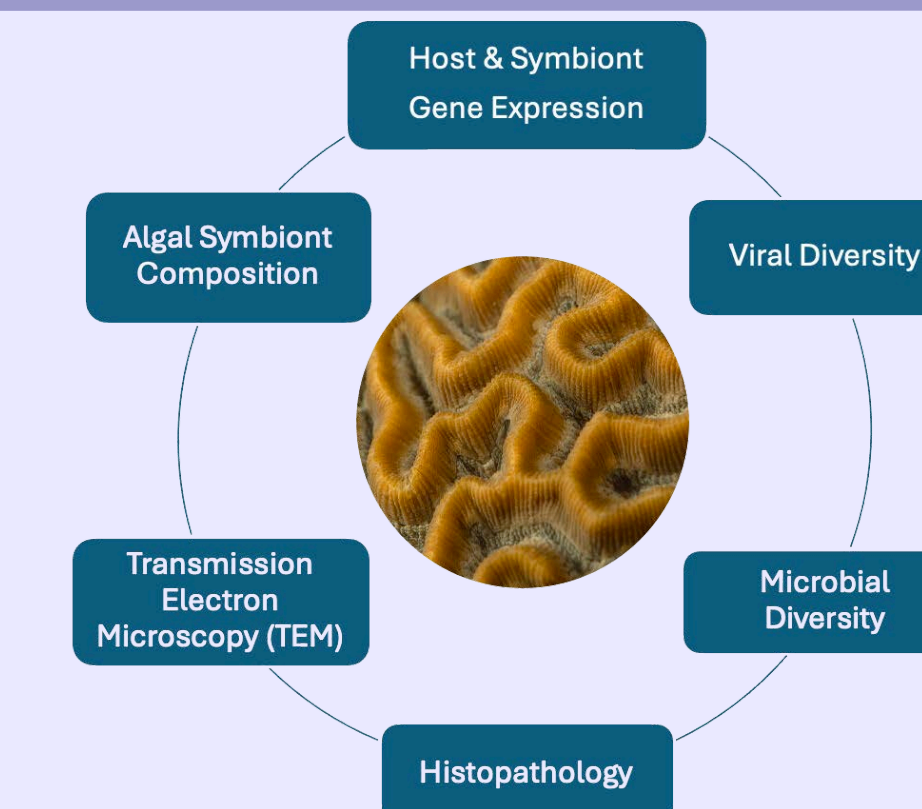


Gene Annotation & GO Enrichments: Transcripts are annotated using NCBI Uniprot Database. GO enrichment analysis was done using the STRING Database.

## An Integrative Framework to Study Coral Disease

### The Importance of Gene Expression Analysis

Genes will increase or decrease in expression in response to different stimuli such as disease, heat stress and other environmental variables. Understanding gene expression in both the coral host and its algal symbiont in response to disease—even an unidentified one—can offer valuable insights into mechanisms of the disease.



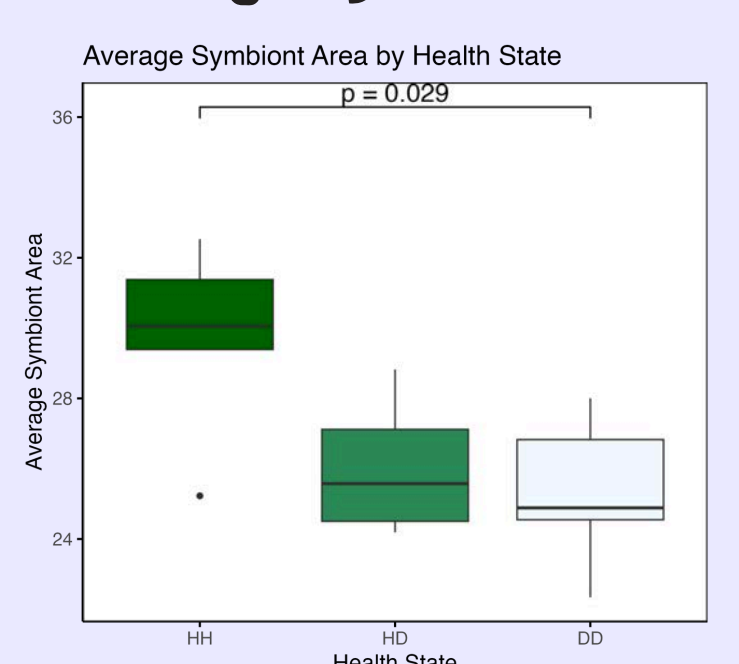
### The Importance of a Holistic Analysis

The coral holobiont is highly complex. To fully understand what occurs during a disease, it is essential to integrate all components of the holobiont—coral host, algal symbionts, and microbial and viral communities—into our analyses. Additionally, incorporating microscopy techniques, such as histology and transmission electron microscopy (TEM), provides tangible, observable phenotypes associated with disease that we can correlate to gene expression.

## Gene & Disease Trait Relationship

### Disease Traits

#### Average Symbiont Area



#### Average Vacuole Area

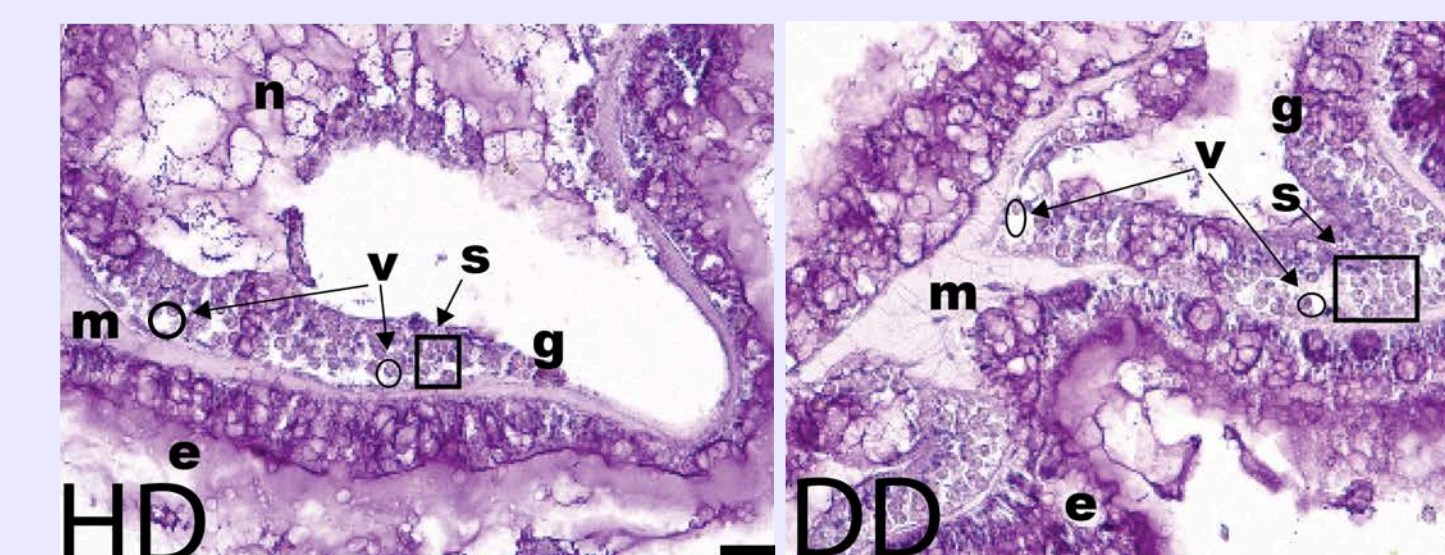
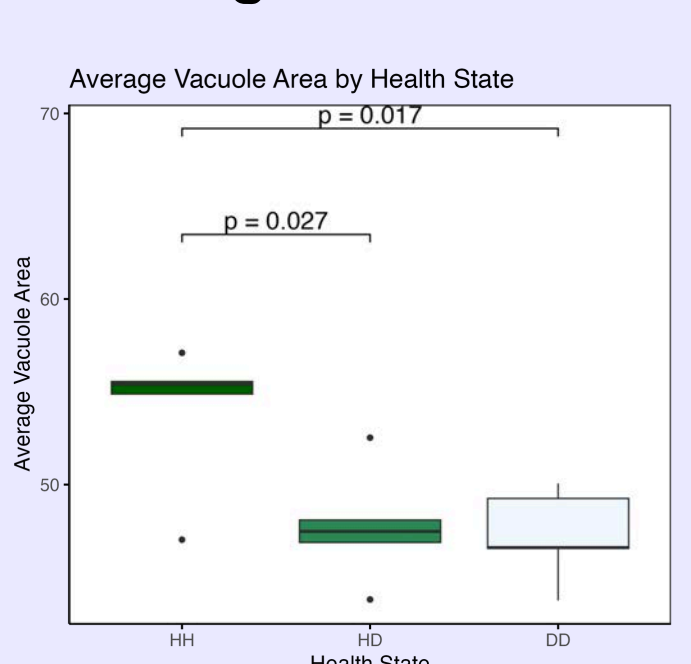
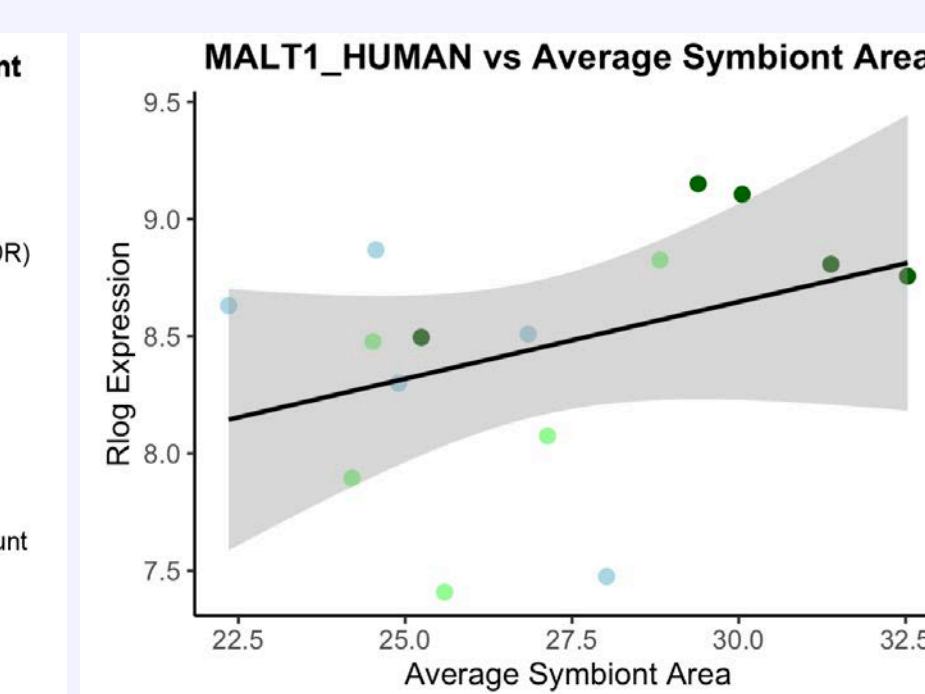
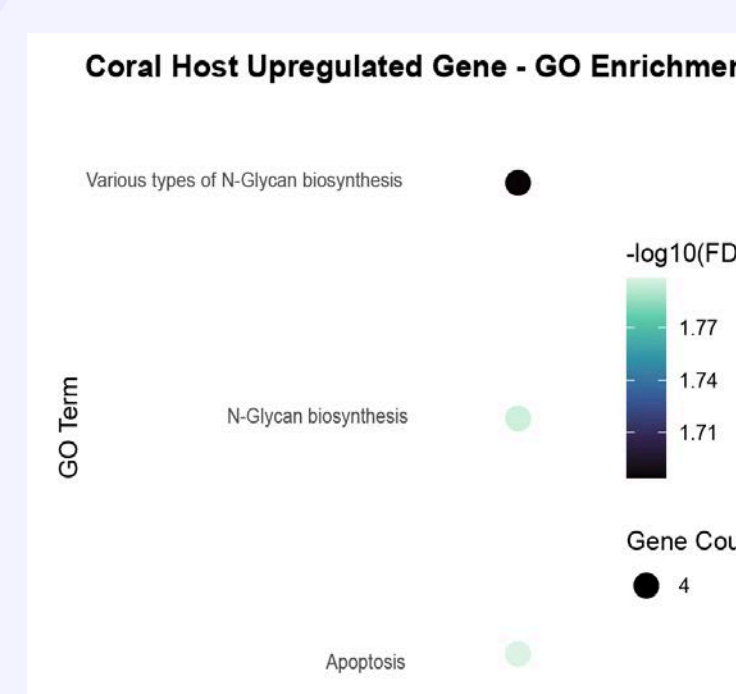


Figure 1 Symbols are denoted as follows: e: epidermis, m: mesogloia, g: gastrodermis, s: algal symbionts, square surrounds smaller and degraded symbionts, v: vacuolization, oval surrounds symbionts with larger vacuoles.

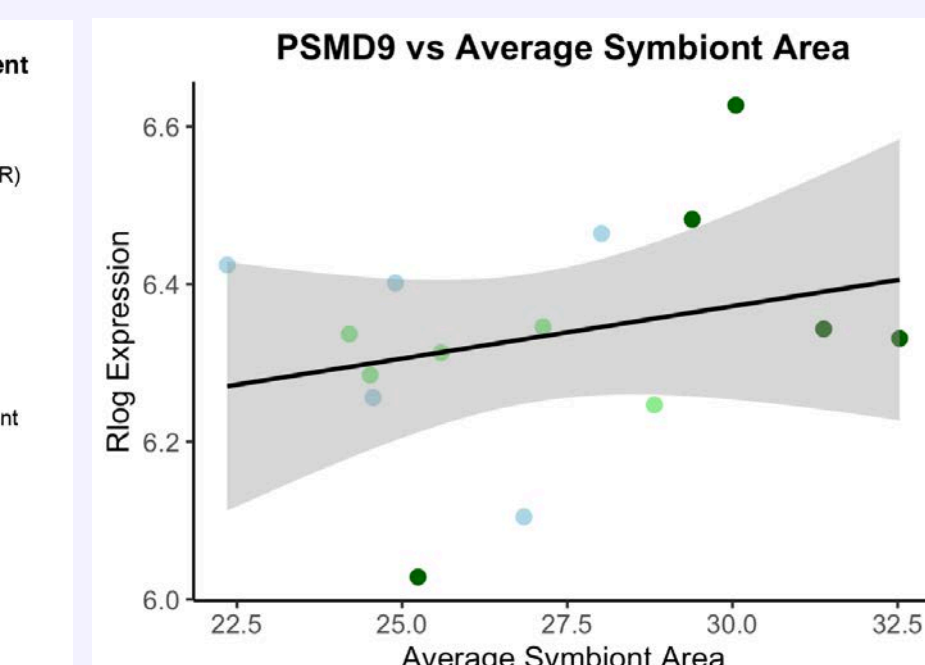
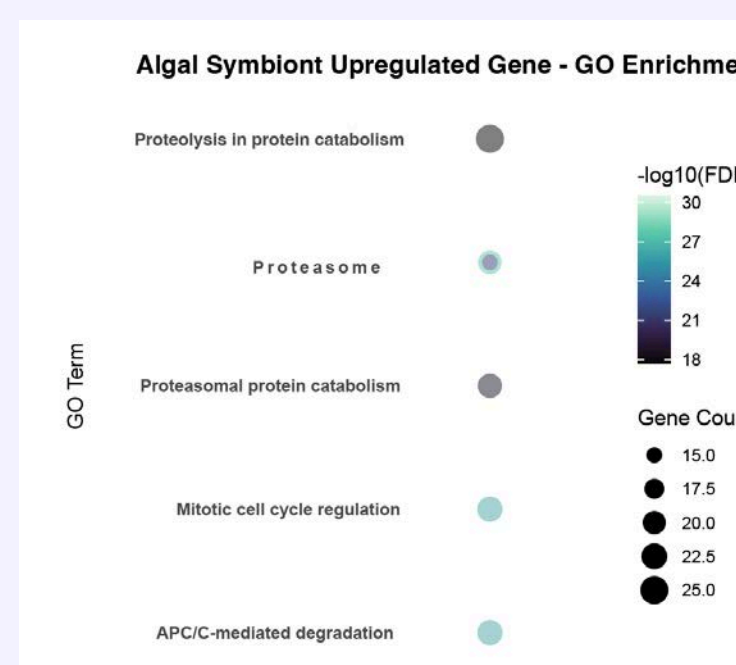
### Histological Characterization of Disease

- High intensity of necrosis across diseased samples (DD)
- Vacuolization of symbionts (symbiont to vacuole ratio) not significant in diseased samples.
- Vacuoles and algal symbionts are shrinking in diseased colonies (HD, DD)
- Disease does not follow a consistent pattern of vacuolization and exocytosis

### AVERAGE SYMBIONT AREA

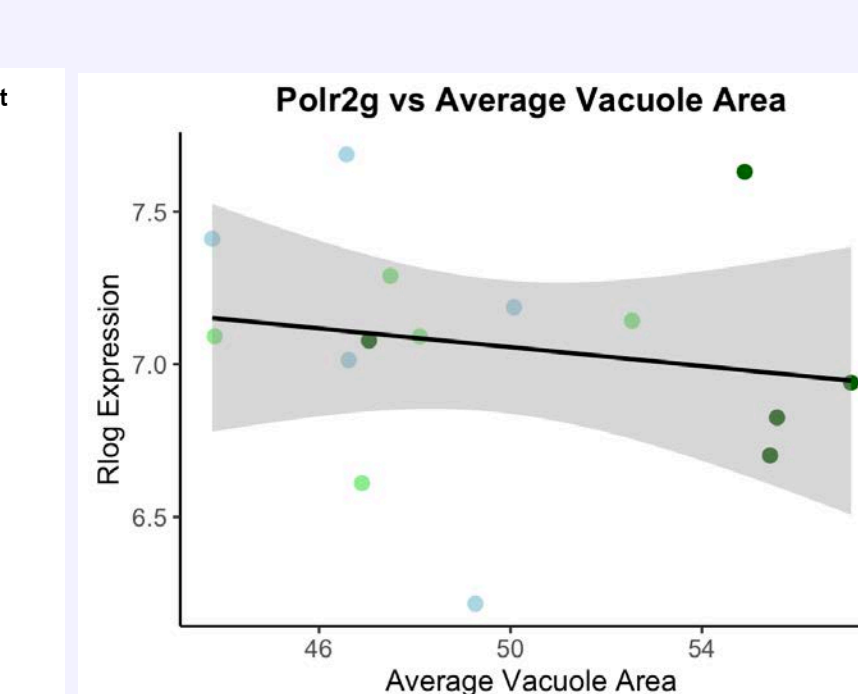
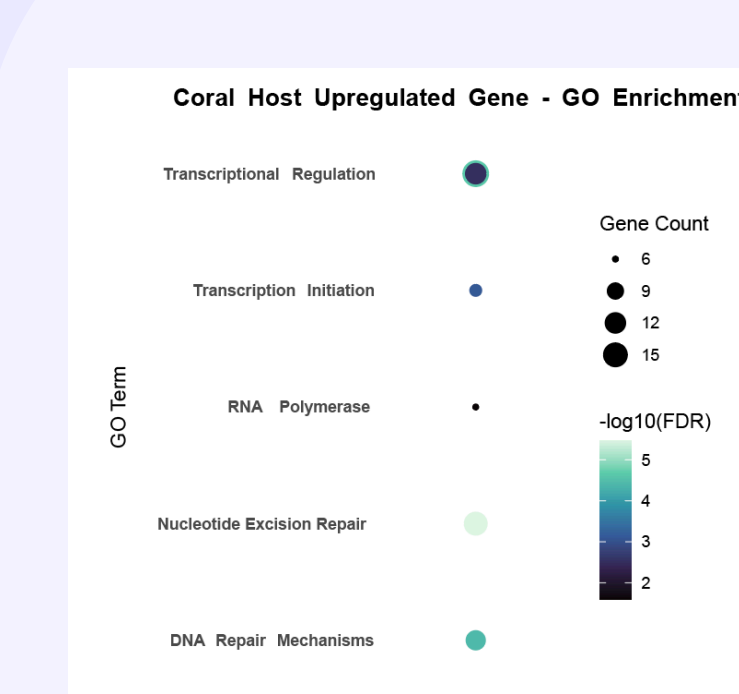


Mucosa-associated lymphoid tissue lymphoma translocation protein  
Function: Activates innate immunity and NF- $\kappa$ B signaling; linked to suppression of apoptosis  
HH = Supporting the coral's ability to maintain cellular integrity and health  
HD, DD = Actively promoting programmed cell death to prevent further spread of disease



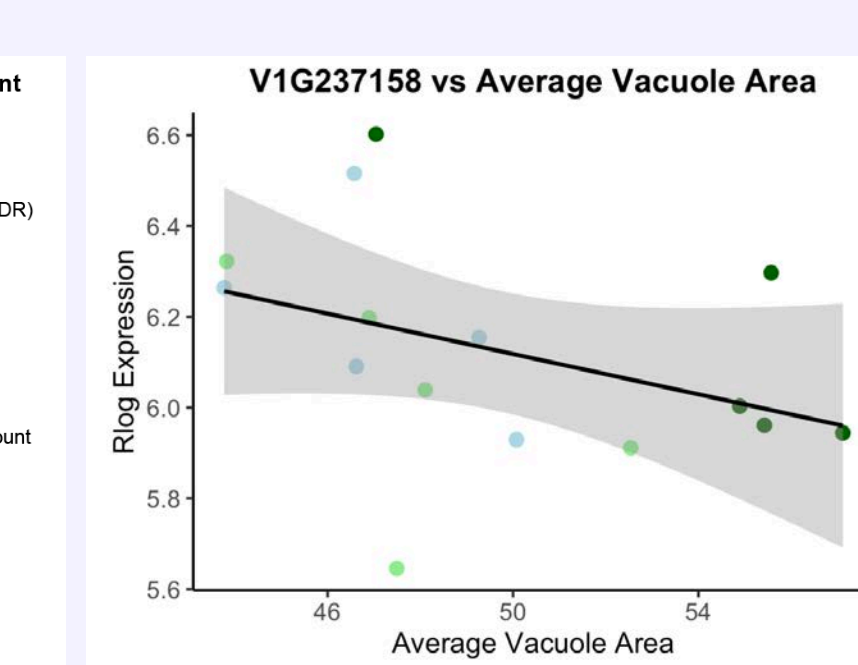
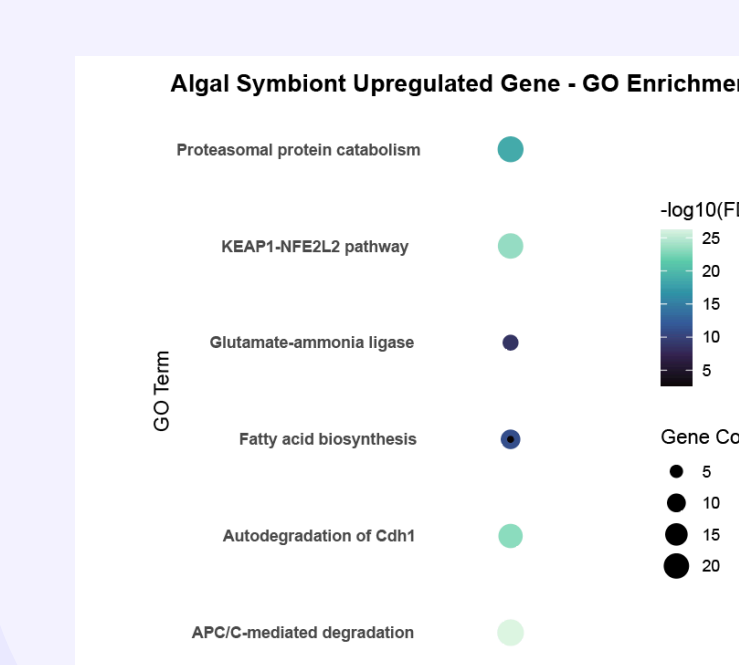
26S proteasome non-ATPase regulatory subunit 9  
Function: Regulates protein degradation as part of the 26S proteasome complex.  
HH = Supporting the coral's ability to maintain cellular integrity and health  
HD, DD = Actively promoting programmed cell death to prevent further spread of disease

### AVERAGE VACUOLE AREA



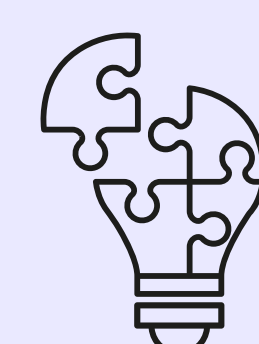
#### DNA-directed RNA polymerase II subunit RP87

Function: Supports transcription initiation and stabilizes the interaction between the polymerase and RNA during elongation.  
HH = Stable state with minimal need for transcriptional reprogramming.  
HD, DD = Indicates increased host transcriptional activity, likely reflecting stress, immune response, or breakdown of symbiosis.

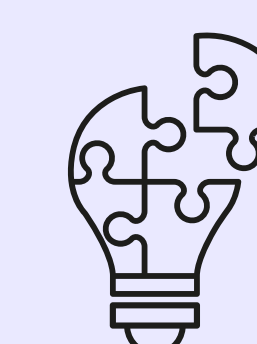


#### Ubiquitin-conjugating enzyme E2 S

Function: Tags proteins for degradation, especially during cell cycle control and stress.  
HH = Stable state with reduced need for proteasomal turnover.  
HD, DD = increased protein degradation activity, likely linked to stress, immune activation, or removal of damaged proteins.



As the algal symbiont and vacuole degrade with disease, the host immune response involves activation of apoptosis and elements of the innate immune response, and genes involved in recycling and elimination of degraded proteins and cells.



## Conclusions

The host immune response to the unidentified disease is characterized by elimination of degraded proteins, and apoptosis suggesting an intracellular pathogen and oxidative stress.

The HD portion of the colony is mounting the strongest response against the disease, creating the potential for recovery.

In March 2023, more samples (and species!) were collected. In May 2024, the majority of the above diseased colonies had recovered from the disease!

Additional Info

