**A tale of two corals: multi-omic stress signals and ecological outcomes diverge in two sympatric Hawaiian coral species**

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**Abstract and Rationale**

Abiotic stress poses significant risk to coral holobionts with thermal and pH fluctuation being two of the main drivers of reef damage under climate change. Here we used multi-omics methods to study the response to thermal and pH stress, both alone and in combination, over a 12-week period in two divergent Hawaiian coral species. One, *Montipora capitata*, is highly resilient, able to use thick tissue reserves and heterotrophic feeding to survive periods of bleaching, whereas the second, *Pocillopora acuta*, is stress sensitive, more dependent on algal symbiont photosynthesis-derived energy resources, with thin tissues that bleach readily. These corals offer, therefore, distinct models for understanding the nature and sources of stress signals in holobionts. We took two different approaches to this problem. The first was conventional, whereby we gathered physiological, eukaryotic gene expression, holobiont polar metabolomic, and algal symbiont composition data and used linear and network approaches to partition the data into the animal and algal components. These results allowed us to identify the major genes, metabolites, and gene-metabolite interactions that comprise the pre-bleaching, bleaching, and recovery phases in *M. capitata* and the stress-related gene expression “toolkit” in *P. acuta* under the single and multi-stressor condition. These data demonstrate...

Still to be worked out: The second approach considered the holobiont as a single cell (coral *holocell*) in which small polar metabolites were assumed to flow freely between the animal, algal, and microbiome components. We studied overall eukaryotic gene-holobiont metabolite interactions in *M. capitata* and *P. acuta* to gain a symbiosis-based perspective on the stress response. Corals have a relatively simple morphology and millions of algal cells, housed in symbiosome compartments, are packed tightly into the polyp. Therefore, we reasoned that stress pathways that are regulated by metabolites may strongly intersect and potentially be coordinated in the *holocell*. These data demonstrate…

The conventional and *holocell* approaches to the coral holobiont stress phenome showcase the extent to which natural selection drives the evolution of divergent survival strategies in sympatric species. What overall insights can we learn about the resilience strategies in different coral species? Details follow here...

What did we learn by using these two approaches and what are the next steps? Can we change how we think about corals to foster more realistic conservation strategies?

**Introduction**

Sessile marine organisms must evolve specialized reproductive strategies and cope with stress over the course of their lifespan (Strathmann 1990). For reef-building corals (Scleractinia), the inability to respond to sustained thermal stress or environmental insults can result in dysbiosis and the subsequent loss of the dinoflagellate algal endosymbionts (Symbiodiniaceae) that sustain them. This process, known as “bleaching” (due to the loss of endosymbiotic algae in corals that confer their color), may lead to coral mortality and widespread reef degradation (Muscatine and Porter 1977; National Academies of Sciences, Engineering, and Medicine 2019). Therefore, there is an urgent need to understand the response of the coral holobiont (cnidarian host, algal symbionts, prokaryote microbiome, viruses; Knowlton and Rohwer 2003; Bosch and McFall-Ngai 2011) to climate change-associated stressors, in particular, increased sea surface temperature and ocean acidification (Hoegh-Guldberg et al. 2007). A potentially illuminating model for studying coral stress responses is to compare sympatric species that, although sharing the same habitat and stressors, have diverged in their physiological strategies for survival. These differences are expected to impact genome-wide responses to stress that can be elucidated using multi-omics methods. Here we focus on such a model in Hawaiian waters. The targets of our study, the rice coral *Montipora capitata* and the lace coral *Pocillopora acuta*, are both rapidly growing, weedy species, yet they differ in their reproductive strategies (x) and ability to withstand stress (X). *P. acuta* is comprised of imperforate thin tissues and is a member of the robust coral clade. It is a brooding species that releases sexual (and likely, asexual) larvae, which settle within days to hours of release (Harii et al. 2002). The recruitment and early survival of *P. acuta* in Hawaii appears to not be negatively impacted by moderate levels of thermal (~2°C increase) and/or pH (~0.2 pH units) stress (Kuffner Jokiel) (Bahr et al. 2020). However, adults of this species are highly sensitive to warming waters, bleaching relatively rapidly ([Bahr et al. 2016)](https://paperpile.com/c/9pBnhg/4RFy) and showing lower control of intracellular pH [(Gibbin et al. 2015)](https://paperpile.com/c/9pBnhg/GHJS) leading to reduced tissue lipids and biomass (Wall et al. 2017). These, and other traits contribute to increased mortality rates under prolonged bleaching, that is exacerbated by the lack of effective (heterotrophic) feeding to meet energy shortfalls (Bahr et al. 2020). *P. acuta*, however, displays greater phenotypic plasticity and DNA methylation change in response to ocean acidification [(Putnam et al. 2016)](https://paperpile.com/c/9pBnhg/E4Ja). In contrast, *M. capitata*, a member of the complex coral clade, is a stress tolerant, dominant reef builder with perforate thick tissues. It is a mass spawning coral that can meet 100% of its daily metabolic energy requirements through feeding during periods of bleaching (Grottoli et al. 2006), explaining its stress resistance (Williams et al. 2021). NEED TO ADD SYMBOINT INFO UP HERE AS WELL.

Another fascinating aspect of our study is its location in Hawaii. Compared to hotspots of diversity such as the Coral Triangle, where more than 600 species exist (Veron et al. 2009), Hawaiian reefs are at the northern limits of coral distribution and dominated by only three genera (*Porites*, *Montipora*, and *Pocillopora*; <80 species in total). Despite this paucity of biodiversity, the isolated Hawaiian Archipelago is of interest because it may give rise to rapid evolution and adaptation of species that can provide novel insights into functional diversification, unavailable in ubiquitously distributed coral species (Olson 2004). As one of the most isolated subtropical regions in the Indo-Pacific (Simon 1987; Veron 1995), the Hawaiian Islands house many endemic coral species (Hughes et al. 2002; PNAS 2004). The fossil record provides evidence for genetic bottlenecks associated with Hawaiian corals that is explained by repeated invasions by marine species that have become extinct and been replaced by others (Kay and Palumbi 1987). Although corals can be transported long distances either as larval stages or on driftwood, the existing data suggest that these modes of dispersal have not broadly impacted Hawaiian coral genetic diversity (Jokiel 1987). Consistent with these ideas, extant populations of *M. capitata* in Hawaii diverged from each other only 0.6 (0.02–2.4) Mya (Cunha et al. 2019), whereas the split between *P. damicornis* and *P. acuta* occurred ~0.99 Mya (Johnston et al. 2017). The Hawaiian Islands, formed by a stationary volcanic hot spot, range in age from 5.1 My (Kauai) to 0.4 My for Hawaii, with Oahu having been formed 3.7 Mya (PNAS 2004, Clague). A parallel story to Hawaiian *M. capitata* appears to have developed in the remote Ulithi Atoll, Yap State, Federated States of Micronesia, where a novel *Montipora* sp. has become an “opportunistic” species (Crane et al. 2017). Like the Hawaiian congener, Ulithi *Montipora* sp. is highly stress resilient and dominates disturbed sites such as near villages and boat landings, where it may comprise 38-59% of coral cover (Crane et al. 2016).

Given these observations, our study addresses three major hypotheses about Hawaiian coral biology and evolution. First, regarding strategies for dealing with stress onset, we hypothesize that *P. acuta* and *M. capitata* will display divergent strategies for responding to thermal and pH stress that will be discernible in coral animal and holobiont-derived omics data. Second, regarding limits to survival under prolonged stress, we hypothesize that *M. capitata* will have the capacity recover from bleaching due to heterotrophic feeding, allowing us to identify resilience-related genes and metabolites in this species. In contrast, *P. acuta* will not up-regulate these markers but rather express cell death pathways. Third, host-algal symbiont interactions will differ widely in these two species, which we hypothesize can be divided into three different stages of bleaching (namely, early stress, late stress, and recovery [only in *M. capitata*]). These stages can be used to organize and analyze the omics datasets to build species-specific models of the coral stress phenome.

**Results and Discussion**

***Physiological data***

Exposure of *M. capitata* and *P. acuta* to increased temperature and reduced pH for nine weeks, followed by seven weeks of recovery under ambient conditions (Supplementary Materials) supported contrasting responses between the tolerant *M. capitata* and sensitive *P. acuta*. Both *M. capitata* and *P. acuta* significantly bleached in response to treatment through time (*M. capitata*: significant interactions; *P. acuta*: significant interactions) as shown by X% reduction in color score under high temperature. However, in *P. acuta* this led to substantial shift in multivariate physiology (Fig. 1C) and >80% mortality (Fig. 1E, p<0.0001), whereas in *M. capitata* there was no change in multivariate physiology (Fig. 1C), and relatively little mortality with no differences in survivorship by treatment (Fig. 1F, p=0.18).

**Figure 1. Exposure of corals to increased temperature and ocean acidification.**  (A, B) Color change of the corals indicating visual bleaching that was quantified using color standardized photographs. (C, D) Multivariate physiotype of corals in the four treatment conditions colored by temperature treatment. The values are significantly different for *M. capitata* and *P. acuta*, with the centroids plotted for each time point and the arrows as the termini. (E, F) Probability of survivorship under each treatment during the 16-week study period. The dashed lines in images (B) and (E, F) indicate the transition from treatment to recovery periods.

A picture containing graphical user interface

Description automatically generatedSpecifically, the physiology of *P. acuta* shifted to X (Supplementary Materials)

This was not due to a shift in Symbiodiniaceae through time (PERMANOVA P>0.05) or between treatments (PERMANOVA P>0.05). *P. acuta* was dominated by C1 related profiles, while *M. capitata* housed C17/C31 or D1, or a mix of these profiles (Supplementary Materials). ADD 16S RESULTS HERE

These results clearly indicate that *P. acuta* is a stress sensitive coral, which displays substantial bleaching (Fig 1A), shifts in physiology (Fig. 1C), and high mortality (Fig. 1E) due to high temperature alone and in combination with high pCO2, or low pH. At the molecular level, *P. acuta* is showing an enrichment of genes involved in protein catabolism pathways under thermal stress (Table Sx, Fig. X). This breakdown of amino acids also is supported by changes in the metabolite pools, specifically X, Y, Z. Further at the organismal level, we see a significant decline in protein per unit area in *P. acuta* under both high temperature treatments at timepoints X, Y, Z.

***Gene expression and metabolic data***

***Persistent features - Core Stress Phenome***

1. Questions

Here we contrasted the omic pathways in two species with starkly differing physiological and survivorship outcomes to assess strategies of sympatric species against thermal stress. We identify the core coral stress phenome and X, and Y

***Conventional approach***

Mapping for genes identified from the genome only analyzed with DESeq2

From any genes that are significantly differentially expressed in any pairwise at particular time points where we have metabolite data

2 species, 5 timepoints (), MAGI, concerted responses (regulated gene-metabolite interactions) between species

Secondary goal - all RNASeq timepoints (may be in supplement or in Emmas approach)

Identify the shared, conserved ortho-groups (OGs) in Mcap and Pacu and compare and contrast their gene expression patterns across the time points to understand if and how selection has shaped these two divergent strategies for the ancestral gene set. Is there a core stress response or can these diverge strongly in sympatric species?

Create “toy” models of different gene expression patterns (e.g., early up and then down, early up and stays up, late up as bleaching proceeds, etc... ) and determine which best fit the actual data for both species so that we can describe these results more accurately. Or, simply use standard supercluster analysis as Tim has done but then discriminate them quantitatively.

Partition the animal and gene expression data, as best as possible, and do separate MAGI analyses to identify the major components in each member, as a prelude for *holocell* analysis.

Compare and contrast the metabolic networks in the two species to identify divergent patterns.

Outcomes: Gain an understanding of the role of dark metabolites in the Mcap stress response. Can we connect known and unknown compounds in a meaningful fashion using network analysis?

***Hawaii effect***

It should be noted that beyond coral species, the marine habitat of the Hawaiian Archipelago displays some of the highest levels of endemism worldwide with regard to mesophotic coral ecosystems, with 100% fish species endemics reported from deep coral reefs in the northwestern islands (Kosaki et al. 2017). Many seaweed (e.g., *Ulva* and *Umbraulva*) and sponge endemics, many of the latter remain to characterized, have also been identified in this region (Spalding et al. 2019).

***Coral holocell approach***

Run MAGI blind to eukaryote gene origin and interpret data under *holocell* assumption. What does this tell us about the highest MAGI scoring reactions? How many duplicate stress pathways do we find and how many combine animal and algal pathways, potentially indicating shared co-regulation, putatively led by the animal? Think of the Mcap montiporic acid results.

Be restrictive at first to see how well this approach works and what it is telling us. Group discussion is needed to interpret the preliminary results.

**Analysis of synergism**

Using the RNA-seq and metabolomic data from Mcap, we should try to identify patterns of gene expression and metabolite accumulation that appear to be independent, additive, or synergistic under individual and combined thermal and pH stress. Can we identify genes that have dual roles or comprise distinct response pathways? This will admittedly be difficult because we expect the coral stress phenome to be very broad but this is worth trying to address I think, even if we have negative outcomes. For example, can the gene and metabolite networks be used to follow the fate of individual genes/metabolites that appear in similar or dissimilar positions under the single and double stressor regimes? Again, challenging, but worth a try. All of these approaches, work best in genetic model systems for which many/most genes and metabolic pathways are well-studied and their functions are understood under ambient and stress conditions. With corals, we will need to be careful in our interpretations.

RNASeq top modules

Host

Sym

Metabolomics Networks

MAGI analysis with the metabolites

Host

Sym

Interpret through multivariate physiology

**RNASeq and Metabolites involved in Reproduction**

Periodicity of sex hormones in P. acuta

Generate a graphic on timing of reproduction in Mcap and Pact relative to the experimental timeline? - Probably don’t need this, as spawning is finished by the time these corals were collected in the first week of September.