

Comparative transcriptomics reveals altered species interaction between the bioeroding sponge *Cliona varians* and the coral *Porites furcata* under ocean acidification

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Abstract

Bioeroding sponges interact and compete with corals on tropical reefs. Experimental studies have shown global change alters this biotic interaction, often in favour of the sponge. Ocean acidification in particular increases sponge bioerosion and reduces coral calcification, yet little is known about the molecular basis of these changes. We used RNA-Seq data to understand how acidification impacts the interaction between the bioeroding sponge, *Cliona varians*, and the coral, *Porites furcata*, at the transcriptomic level. Replicate sponge and coral genets were exposed to ambient (8.1 pH) and acidified (7.6 pH) conditions in isolation and in treatments where they were joined for 48 h. The coral had a small gene expression response (tens of transcripts) to the sponge, suggesting it does little at the transcriptomic level to deter sponge overgrowth. By contrast, the sponge differentially expressed 7320 transcripts in response to the coral under ambient conditions and 3707 transcripts in response to acidification. Overlap in the responses to acidification and the coral, 2500 transcripts expressed under both treatments, suggests a similar physiological response to both cues. The sponge expressed 50× fewer transcripts in response to the coral under acidification, suggesting energetic costs of bioerosion, and other cellular processes, are lower for sponges under acidification. Our results suggest how acidification drives ecosystem-level changes in the accretion/bioerosion balance on coral reefs. This shift is not only the result of changes to the thermodynamic balance of these chemical reactions but also the result of active physiological responses of organisms to each other and their abiotic environment.

KEYWORDS

Bocas Del Toro, Caribbean, gene expression, Panama, pH, RNA-Seq

1 | INTRODUCTION

Atmospheric carbon dioxide (CO₂) levels have risen at an unprecedented rate since the Industrial Revolution (Doney & Schimel, 2007) and are directly impacting marine carbonate chemistry by elevating dissolved seawater CO₂ and decreasing ocean pH and CaCO₃ saturation (Stocker et al., 2013). Extensive research has demonstrated

the effects of ocean acidification (OA) on a broad range of marine taxa (Kroeker et al., 2010, 2013), particularly those that use CaCO₃ to form skeletons or shells (Hofmann et al., 2010; Orr et al., 2005), because decreasing ocean pH limits the availability of carbonate ions (Bates et al., 2014). In corals, OA has deleterious effects on several life stages and biological processes including fertilization, larval settlement and metamorphosis, metabolism, growth, calcification, and

survival (Albright & Langdon, 2011; Albright et al., 2010; Cohen et al., 2009; Doropoulos et al., 2012; Nakamura et al., 2011; Webster et al., 2013) and simulations predict reduced resilience of coral reefs under ocean change (Anthony et al., 2011). In many cases, the negative effects of OA are exacerbated by other factors like increased temperature, pollution, and overfishing (Kroeker et al., 2013; Rodolfo-Metalpa et al., 2011).

While global change affects individuals, it also acts at higher levels, altering species abundance and distribution, biodiversity, and ecosystem function (Fabry et al., 2008; Hale et al., 2011; Walther et al., 2002). Global change can dramatically alter biotic interactions between species, whether mutualistic or antagonistic (Gilman et al., 2010; Kroeker et al., 2013; Tylanakis et al., 2008; Urban et al., 2012), particularly when interspecific variation in the response to change (Kroeker et al., 2010) leads one partner to disproportionately respond (Bell et al., 2013; Sanford et al., 2014). Information about species interactions can improve the explanatory and predictive power of bioclimatic models (Araújo & Luoto, 2007; Van der Putten et al., 2010), highlighting the importance of considering such interactions in global change research.

A species interaction that plays a large role in physical and community structure in the Caribbean is the interaction between bioeroding sponges (Family Clionidae) and hermatypic corals. Hermatypic corals often create the complex physical structure required for coral reef ecosystem function (Alvarez-Filip et al., 2009; Goreau, 1959). Clionid sponges also play a critical, yet opposing, role on coral reefs by bioeroding carbonate substrata such as coral skeletons, mollusc shells, and coralline algae (Rützler, 1975, 2002; Schönberg & Wilkinson, 2001), thereby facilitating CaCO_3 cycling through the system. Bioeroding sponges and corals compete for space and sponges are often repelled by unstressed corals (Rützler, 2002; Schönberg & Wilkinson, 2001). However, in the Caribbean, bioeroding sponges are thought to be increasing in abundance and their effects on coral reefs are leading to decreased physical stability and long-term changes in community structure (López-Victoria et al., 2006; Williams et al., 1999). Furthermore, evidence suggests that conditions considered stressful to corals promote excavation in clionid sponges, and have little negative impact on sponge performance (Fang et al., 2013; Stubler et al., 2014, 2015; Wisshak et al., 2012, 2014). Growing evidence suggests that the effects of OA on rates of bioerosion and accretion are shifting the balance toward bioerosion (Enochs et al., 2016; Schönberg et al., 2017) with serious implications for the future of coral reef ecosystems (Bell et al., 2013, 2018; Enochs et al., 2015).

Previous work evaluating the interactions between Caribbean corals and bioeroding sponges under increased temperature and acidification focused on the broader ecological implications of a net change in carbonate accretion/removal and has shown that bioerosion of the coral, *Porites furcata*, by the sponge, *Cliona varians*, is positively related to increases in temperature and concentration of carbon dioxide ($p\text{CO}_2$) (Stubler et al., 2014, 2015). However, the mechanisms underlying increased bioerosion in the sponge and the response of the coral to bioerosion are unknown. The physical

effects of OA may stem from alterations to the thermodynamic balance of the chemical reactions involved in calcification and erosion or, alternatively, organisms may be responding physiologically to the altered conditions by up- or downregulating pathways involved in calcification, bioerosion, and/or defence.

Here, we collected gene expression data to understand how OA changes the species interaction between *C. varians* and *P. furcata* at the transcriptomic level. We tested whether the sponge maintains or modifies its bioerosion strategy under acidification and whether the coral modifies its calcification and defence strategy against sponge bioerosion under acidification.

2 | MATERIALS AND METHODS

2.1 | Study species

We sampled *P. furcata* coral colonies and massive *C. varians* sponges (i.e., gamma-stage (López-Victoria et al., 2004), Figures S1A–C) ~30 cm² in surface area from the reef system on Isla Pastores, Bocas del Toro, Panama (9° 13.5510 N, 82° 19.5380 W). We collected individuals at least 5 m apart to reduce the chance of taking multiple samples from the same genet. Upon collection, sponges and corals were placed into large Ziploc bags underwater, and transferred into 5 gallon buckets of seawater without exposure to air. Onshore, we placed corals and sponges in unfiltered, flow-through seawater tables. From healthy corals we excised the growing tips to create smaller fragments between 3–6 cm in length (Figure 1a). Any coral fragments with signs of necrotic tissue, bleaching, disease, or visible infestation of bioeroders were excluded. We attached upright coral fragments to labeled glass microscope slides using CorAffix ethyl cyanoacrylate glue. Using a sterile razor blade, sponges were excised into fragments ~4 cm³. Each sponge fragment contained a portion of pinacoderm, in which *Symbiodinium* photosymbiotic algae reside, and a portion of choanosome. In total, we fractioned four colonies of sponges and corals into four genetic replicate fragments from each individual (i.e., 16 sponge and 16 coral fragments total). All sponge and coral fragments were acclimated in flow-through seawater tables for a minimum of 24 h before being placed into their respective experimental treatments (Figure 1b). The recovery period was determined based on previous experiments (Stubler et al., 2014, 2015; Wisshak et al., 2012) that showed after 24 h sponges had healed over their incisions, as indicated by a fresh cell layer and functioning ostia/oscula, and coral fragments had extended their polyps, which retained their colour (i.e., were not bleached) and were moving normally.

2.2 | Flow-through system and treatments

The experiments were performed using the outdoor, unfiltered seawater system at the Smithsonian Tropical Research Institute Bocas del Toro Station in summer 2013. The flow-through,

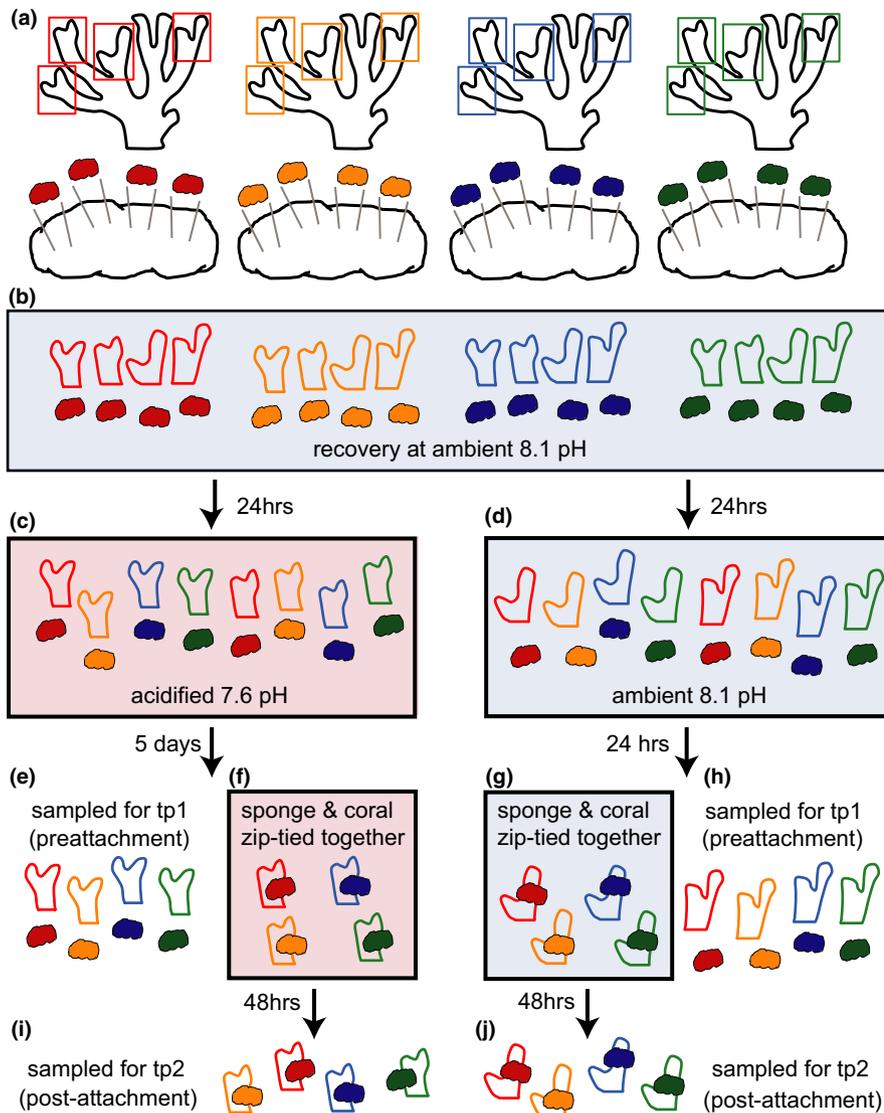


FIGURE 1 Diagram of the sampling strategy. Bold colours represent genetically identical replicate fragments taken from one individual. Light blue and red shaded boxes represent ambient and acidified conditions, respectively. Four *Porites furcata* coral and *Cliona varians* sponge individuals were sampled from the reef off Isla Pastores, Bocas del Toro, Panama. Four genetically identical replicate fragments were created from each individual sampled (a). Fragments were allowed to recover for 24 h at ambient pH (b). After recovery, eight coral and eight sponge fragments were placed into the acidified treatment (c) and eight coral and eight sponge fragments were placed into the ambient treatment (d). After being placed in their respective ambient and acidified treatments, one genetic replicate from each individual was removed and tissue from the fragment was sampled for time point 1 (preattachment) (e, h). The remaining sponge fragments were gently joined to the remaining coral fragments with a zip-tie (f, g). Then, 48 h after joining the sponge and coral fragments, tissue from each sponge and coral fragment was sampled for time point 2 (post-attachment) (i, j) [Colour figure can be viewed at wileyonlinelibrary.com]

pH-stat system described in detail in Stubler et al. (2015) was augmented with additional aquaria for this experiment. In brief, we exposed animals to one of two treatments with target pH values (NBS scale) of 8.1 (ambient) and 7.6 (acidified). The acidification treatment roughly corresponded to $p\text{CO}_2$ predicted for the year 2300 under the IPCC A1FI/RCP8.5 emissions scenario and models by Caldeira and Wickett (2003). Seawater was pumped into two 200 L reservoirs that gravity-fed either ambient or acidified seawater to 7.5 L aquaria housing the sponge and coral fragments. In the acidified treatment, pH was modified in the 200 L reservoir using a pH controller (Reef Fanatic) connected to a CO_2 regulator (Milwaukee MA957) to bubble CO_2 gas on demand when pH exceeded the setpoint. The pH of the water in the ambient treatment reservoir was not manipulated and reflected natural diurnal pH variation (full water chemistry reported in Stubler et al., 2015 and Table S1). After 24 h of acclimation, sponge and coral fragments were placed into their assigned ambient or acidification treatment aquaria. Fragments were elevated off the bottom of the aquaria using plastic grating ("egg-crate" material) and we siphoned sediment or algal growth as needed. We recorded daily

measurements of pH, temperature, salinity, and dissolved oxygen within each aquarium.

2.3 | Experimental design

Across all time points and treatments, tissue samples were only collected if both the sponge and coral fragments visibly appeared healthy. Four genetically identical replicate fragments were created for each individual taken from the field (Figure 1a). After the initial 24 h recovery period (Figure 1b), we placed sponge and coral fragments into their assigned treatments (Figure 1c,d) with one tank for the ambient treatment and one tank for the acidified treatment. Sponges and corals in the acidified treatment remained in low pH for 5 days (Figure 1c) before sampling to ensure we captured a baseline transcriptomic state after acclimation to acidification (Brothers et al., 2016). After the acclimation period, we removed four sponge and coral fragments (one fragment per genetic individual for both sponges and corals) and sampled tissue from each to represent time point 1 ("preattachment", Figure 1e). We then gently joined

the remaining sponge fragments to the remaining coral fragments using a zip-tie (Figure 1f, Figure S1D). This process was repeated for the ambient treatment with the exception that the time point 1 sponge and coral fragments were sampled 24 h after being placed into their ambient pH water treatment (Figure 1h). Although there was a difference between time point 1 sampling in ambient and acidified treatments (1 vs. 5 days, respectively), the animals stayed visibly healthy, therefore, there should be little difference between baseline signals of expression on day 1 vs. day 5 in the ambient samples. After sampling individuals for time point 1 (preattachment), the remaining sponge fragments were attached to the remaining coral fragments using a zip-tie (Figure 1f,g). Two days after the sponge and coral fragments were attached to each other, we took tissue samples of the remaining sponge/coral joined pairs to represent time point 2 (post-attachment, Figure 1i,j).

2.4 | Tissue sampling for transcriptome sequencing

We excised tissue only from visibly healthy individuals (i.e., no tissue necrosis or bleaching) using a new razor blade each time and immediately placed the sample in RNAlater buffer (Invitrogen). For time point 1, where corals and sponges were kept separate, we removed a ~8 mm³ piece of sponge from the choanosome (i.e., the inner layer of the sponge that is the site of bioerosion upon colonization of a new substrate and does not host photosymbiotic algae) and we scraped ~8 mm² of coral tissue from the skeleton. For species interaction treatments where the sponge and coral were physically attached, we gently pried the sponge and coral apart immediately prior to collecting tissue. For sponges, we sampled a thin layer of choanosome (~2 mm³) that had been in direct contact with the coral. Given the colonial growth of corals (i.e., many connected polyps that together form the whole animal), to capture the widest range of transcriptomic response across the coral, we scraped polyps from the surface that was in contact with the sponge, the area just around the sponge contact zone, and from the tip of the coral branch (which was within 1–2 cm from the sponge attachment site). Tissue samples were refrigerated for 24 h in RNAlater then frozen until processing.

2.5 | RNA extraction and sequencing

We extracted total RNA using the Qiagen RNeasy Plus Mini Kit. We isolated mRNA using the NEBNext Poly(A) mRNA Magnetic Isolation kit and prepared libraries using the NEBNext Ultra RNA library prep kit for Illumina and the AxyPrep Mag PCR Clean-up kit. We verified the distribution of fragment sizes by running libraries on a Bioanalyser high sensitivity DNA chip and quantified library concentration with qPCR using the NEBNext Library Quant Kit for Illumina. We pooled the barcoded libraries in equimolar concentrations. 160 bp reads were sequenced in four lanes (11 libraries per lane) on the HiSeq2500 platform using a TruSeq SBS sequencing kit v4 at the University of Illinois at Urbana-Champaign Roy J. Carver

Biotechnology Center. We generated two lanes of paired-end reads from sponge and coral libraries across experimental treatments and time points for transcriptome assembly. Single-end reads were sequenced in the other two lanes (Table S2). Fastq files were generated and demultiplexed with the BCL2FASTQ v1.8.4 Conversion Software (Illumina).

2.6 | Quality control, transcriptome assembly

We trimmed adapter sequences and low quality bases using the wrapper script TRIM GALORE v0.4.1 (Krueger, 2015) with CUTADAPT v1.9.1 (Martin, 2011) and FASTQC v0.11.5 (Andrews, 2010). We trimmed the first 15 bp of the paired end reads and for all reads discarded reads <40 bp long after trimming bases with a quality score below 20 and reads containing more than one N.

Because *C. varians* and *P. furcata* harbour intracellular algal photosymbionts, host and symbiont DNA was present in all tissue samples and therefore the RNA-Seq reads contain sequences from host and symbiont. We assembled holobiont (host + symbiont) transcriptomes for the sponge and coral and bioinformatically filtered the assemblies into separate host and symbiont contigs following Davies et al. (2016) and Rivest et al. (2018).

We assembled holobiont transcriptomes in TRINITY v2.1.1 (Haas et al., 2013) using commands to normalize reads and set the minimum count for k-mers to be assembled by Inchworm to 2. As input, for each host species we used six paired-end libraries representing different genetic individuals, different time points, and both ambient and acidified treatments (Table S2). We used BOWTIE 2 v2.2.9 (Langmead & Salzberg, 2012) to map all reads back to the transcriptomes. Libraries for one coral individual (ambient sample 4) had low alignment rates back to the *Porites* host transcriptome (see Results, Table S2). Therefore, we assembled a separated transcriptome for this sample and mapped reads back to its assembly as described above. We then concatenated the transcriptome for ambient coral sample 4 with the full *Porites* holobiont transcriptome assembly.

We used CD-HIT v4.6 (Fu et al., 2012) to collapse contigs in the holobiont coral and sponge transcriptomes using a 0.98 cutoff and then removed contigs <500 bp long. We performed a series of BLAST (Camacho et al., 2009) searches against databases created from sponge, cnidarian, and *Symbiodinium* genome and transcriptome sequences publically available as of July 2016. The databases contained i/ii) sequences from cnidarians ($n = 27$)/sponges ($n = 19$) that may contain *Symbiodinium* contamination, iii/iv) sequences from cnidarians ($n = 9$)/sponges ($n = 17$) known to be free of *Symbiodinium*, v/vi) sequences from *Symbiodinium* that may contain cnidarian ($n = 19$)/sponge contamination ($n = 19$), and vii/viii) sequences from *Symbiodinium* known to be free of cnidarian ($n = 13$)/sponge contamination ($n = 13$). The symbiont-free cnidarian and sponge databases contained sequences obtained from aposymbiotic larvae and adult individuals (e.g., deep sea corals). The cnidarian and sponge-free *Symbiodinium* databases contained sequences from free-living species or from cultured lines. A list of assemblies used to construct the

blast databases is available in Table S3. We indexed the databases and performed BLAST searches on the coral holobiont transcriptome against databases i, iii, v, and vii and on the sponge holobiont transcriptome against databases ii, iv, vi, and viii. If a transcript from our coral or sponge holobiont transcriptome had an amino acid overlap >100 bp with 80% identity cutoff to any cnidarian or sponge sequence, respectively, we considered it a host transcript. However, if the same transcript had a 100 bp amino acid overlap with 80% identity to a clean *Symbiodinium* sequence, we removed it from our host transcriptome. We identified *Symbiodinium* contigs in the same manner as described above, removing any transcripts that were assigned to aposymbiotic cnidarian or sponge sequences with 100 bp amino acid overlap and 80% identity from our *Symbiodinium* transcriptome. After filtering, we removed any transcripts that were present in both the host and symbiont transcriptomes, an approach that probably removed transcripts from genes conserved between host and symbiont, but ensured no unique symbiont transcripts were retained. Finally, any host transcript with an annotation for a chloroplast-associated gene was removed. We assessed the completeness of the holobiont and host transcriptome assemblies by searching against the eukaryote database in BUSCO v2/3 (Simão et al., 2015) as implemented in GVOLANTE v1.2.0 (Nishimura et al., 2017).

2.7 | Differential gene expression

We used RSEM v1.2.19 (Li & Dewey, 2011) to align sponge and coral reads at least 20 bp long from each library to the sponge host and coral host transcriptomes, respectively, using the transcript-to-gene-map option to obtain read counts at the gene level. We constructed a matrix of read counts using the perl script abundance_estimates_to_matrix.pl available in the Trinity package. We converted raw read counts to counts per million (cpm) to account for any size differences in libraries among treatments. We filtered the count data so that transcripts with one cpm in at least four libraries were retained and transcripts falling below this threshold were removed. We used DESEQ2 v1.30.0 (Love et al., 2014) to test for differential gene expression in the following comparisons for the sponge and for the coral: (i) time point 1 (preattachment) vs. time point 2 (post-attachment) under ambient conditions, (ii) time point 1 vs. time point 2 under acidification conditions, and (iii) time point 1 under ambient conditions vs. time point 1 under acidification conditions. Comparison (i) indicates how the sponge and coral respond to each other under ambient conditions, comparison (ii) indicates how the sponge and coral respond to each other under acidification conditions, and comparison (iii) indicates how the sponge and coral respond to acidification alone, in the absence of the species interaction (i.e., no bioerosion). We used a false discovery rate (FDR) threshold of 0.05 to correct for multiple testing (Benjamini & Hochberg, 1995).

We assessed overall patterns of expression by plotting a two-dimensional principal component analysis (PCA) of log-transformed counts in DESEQ2. To understand how patterns of gene regulation changed under different conditions, we plotted the log₂-fold

change in gene expression for differentially expressed transcripts (FDR < 0.05) before and after the sponge and coral were joined under ambient conditions and compared their expression pattern to the log₂-fold change of those same transcripts in three other contrasts.

We generated heatmaps to visualize expression differences among replicates, treatments, and time points. We filtered the raw count matrix to retain transcripts that had at least five reads in all replicates. We used variance stabilizing to transform the read counts using the R function vst. We filtered the transformed matrix to include transcripts that were significantly differentially expressed (FDR < 0.05) and had a log₂-fold change >3. We used GGPLOT2 and the geom_raster() function to create a heatmaps clustered by transcript and replicate.

2.8 | Carbonic anhydrases

Given the importance of carbonic anhydrases in sponge bioerosion and in coral calcification, we determined whether transcripts of these genes differed in expression across treatments. For the sponge, we downloaded the *Amphimedon queenslandica* amino acid sequences for carbonic anhydrase 1, 2, and 3 from uniprot.org and searched them against the sponge host transcriptome with TBLASTN (Altschul et al., 1997), which yielded six putative *C. varians* carbonic anhydrase transcripts (Table S4). We searched these six transcripts against the nonredundant NCBI protein database with BLASTX and five of the six had a top hit to a sponge carbonic anhydrase. We then searched the DESEQ2 output for these transcripts to examine their patterns of differential expression.

For the coral, we downloaded 21 carbonic anhydrase protein sequences from 13 coral species from NCBI and searched them against the *P. furcata* host transcriptome with TBLASTN, which yielded 12 putative *P. furcata* carbonic anhydrase transcripts (Table S4). We searched these 12 transcripts against the nonredundant NCBI protein database with BLASTX and eight had a top hit to a coral carbonic anhydrase. We then searched the DESEQ2 output for these eight transcripts to examine their patterns of differential expression.

2.9 | Annotation and functional enrichment analysis

We translated the sponge and coral host transcriptomes into amino acid sequences in TRANSDCODER v3.0.0 (Haas & Papanicolaou, 2015). We identified open reading frames with homology to known proteins by running BLASTP (e-value 1e-5) and HMMSCAN v3.1b2 (<http://hmm.org/>) searches against the SWISSPROT and PFAM-A databases (downloaded November 2016), respectively. We included the results of the homology searches in the final prediction step of TRANSDCODER. We retrieved gene ontology (GO) terms for the translated sponge and coral host transcriptomes using INTERPROSCAN v5.22-61.0 (Quevillon et al., 2005) and used the "weight01" option in the R

package TOPGO (Alexa & Rahnenführer, 2021) to test for enrichment of functional categories among transcripts significantly up- or down-regulated in the sponge and coral between time point 1 (preattachment) vs. time point 2 (post-attachment) under ambient conditions (comparisons [i] from above), time point 1 (preattachment) vs. time point 2 (post-attachment) under acidification conditions (comparison [ii]), and time point 1 under ambient conditions vs. time point 1 under acidification conditions (comparison [iii]).

3 | RESULTS

3.1 | RNAseq data and transcriptome assembly

Sequencing produced 139 million paired-end reads and 201 million single-end reads for the sponge and 206 million paired-end reads and 159 million single-end reads for the coral (Table S2). The number of reads per library ranged from 16.7 to 27.6 million with an average of 21.0 million (Table S2). Per-base quality scores were above 30.

The sponge holobiont and host transcriptomes contained 104,864 and 41,687 contigs, respectively. The N50 values for the sponge holobiont and host transcriptomes were 1473 and 1875, respectively. Average mapping of reads was 90.79% for the holobiont transcriptome and 53.62% to the sponge host transcriptome (Table S2). The BUSCO completeness scores indicated both sponge holobiont (98.68%) and host (94.06%) transcriptomes were highly complete, with few core sponge transcripts lost during bioinformatic symbiont filtering (Table S5).

The coral holobiont and host transcriptomes contained 144,850 and 24,924 contigs, respectively. The N50 scores for the coral holobiont and host transcriptomes were 883 and 972, respectively. Average mapping of reads was 84.46% for the holobiont transcriptome and 58.42% to the coral host transcriptome (Table S2). The BUSCO completeness scores for the coral holobiont (90.8%) and the host (61.4%) transcriptome (Table S5) suggest that coral transcripts were lost when filtering out symbiont sequences, that many core coral transcripts were not expressed at the time of tissue sampling, and/or that core coral transcripts were not captured by library prep or sequencing.

3.2 | Gene expression analyses

The principal component (PC) analysis for the sponge showed a large transcriptomic response to the coral (time point 1 vs. time point 2) under ambient and acidification conditions (Figure 2). In seven of the eight replicates, time point 1 replicates separated along PC axis 2, with the shift in gene in response to the coral occurring in the same direction, regardless of pH treatment. In contrast to the sponge, the coral showed very little change in response to the sponge (time point 1 vs. time point 2) under both ambient and acidification conditions (Figure 2). The ambient and acidification treatments separated along PC axis 2 and the acidification treatments clustered at the bottom of

the PCA plot but the direction of expression change between time points 1 and 2 in PC space was not consistent within or between ambient and acidification treatments. One ambient coral and two ambient sponge individuals showed variation in their overall gene expression patterns, clustering away from the other samples in PC space, but still changing in the same direction.

In the sponge, 7320 transcripts were differentially expressed in response to the coral under ambient conditions while only 145 transcripts were differentially expressed in response to the coral under acidified conditions (Figure S2). In response to acidification alone (i.e., in isolation from the coral), the sponge differentially expressed 3707 transcripts (Figure S2). There was substantial overlap between the acidification and coral responses with 2500 transcripts differentially expressed by the sponge in response to the coral under ambient conditions and in response to acidification (Figure S2). The coral differentially expressed 35 transcripts in response to the sponge under ambient conditions and four transcripts in response to the sponge under acidification conditions (Figure S2). The coral differentially expressed 28 transcripts in response to acidification alone.

In the sponge heatmap, time point 2 replicates clustered together according to pH treatment, while time point 1 replicates did not (Figure 3). Qualitatively, time point 1 replicates had higher transcript counts than time point 2 replicates. For the coral, replicates clustered in two groups that largely corresponded to time point (with the exception of a time point 1 replicate in the time point 2 cluster) but with no clear correspondence to pH treatment (Figure 3).

To understand patterns of sponge gene regulation, we plotted the log₂-fold change in expression for 7320 transcripts differentially expressed (FDR < 0.05) before and after being joined to the coral under ambient conditions and compared their expression pattern to the log₂-fold change of those same transcripts in three other contrasts (Figure 4). In these plots, each point represents the log₂-fold change of a transcript in two comparisons; points that fall on the 1:1 line represent transcripts with an equal expression change in both comparisons. Figure 4b compares the gene expression response of the sponge to the coral under ambient (x-axis) and acidified conditions (y-axis), and shows that the sponge's response to the coral under both conditions is a qualitatively similar, but reduced, under acidification, with 99.1% of transcripts (1850+643+308+4452/7320) showing a smaller expression change in acidified conditions. In other words, transcripts that are upregulated by the sponge in response to the coral are more highly upregulated in ambient conditions than in acidified conditions. This pattern also applies to downregulated transcripts. Figure 4c compares the response to the coral under ambient conditions (x-axis) and the response to acidification (in the absence of the coral) (y-axis). These two patterns were also broadly similar (i.e., transcripts that were upregulated in response to the coral under ambient conditions were also upregulated in response to acidification and those downregulated in response to the coral under ambient conditions were also downregulated in response to acidification), but different in magnitude, with 88.2% of transcripts (1777+96+4465+118/7320) showing a smaller change in

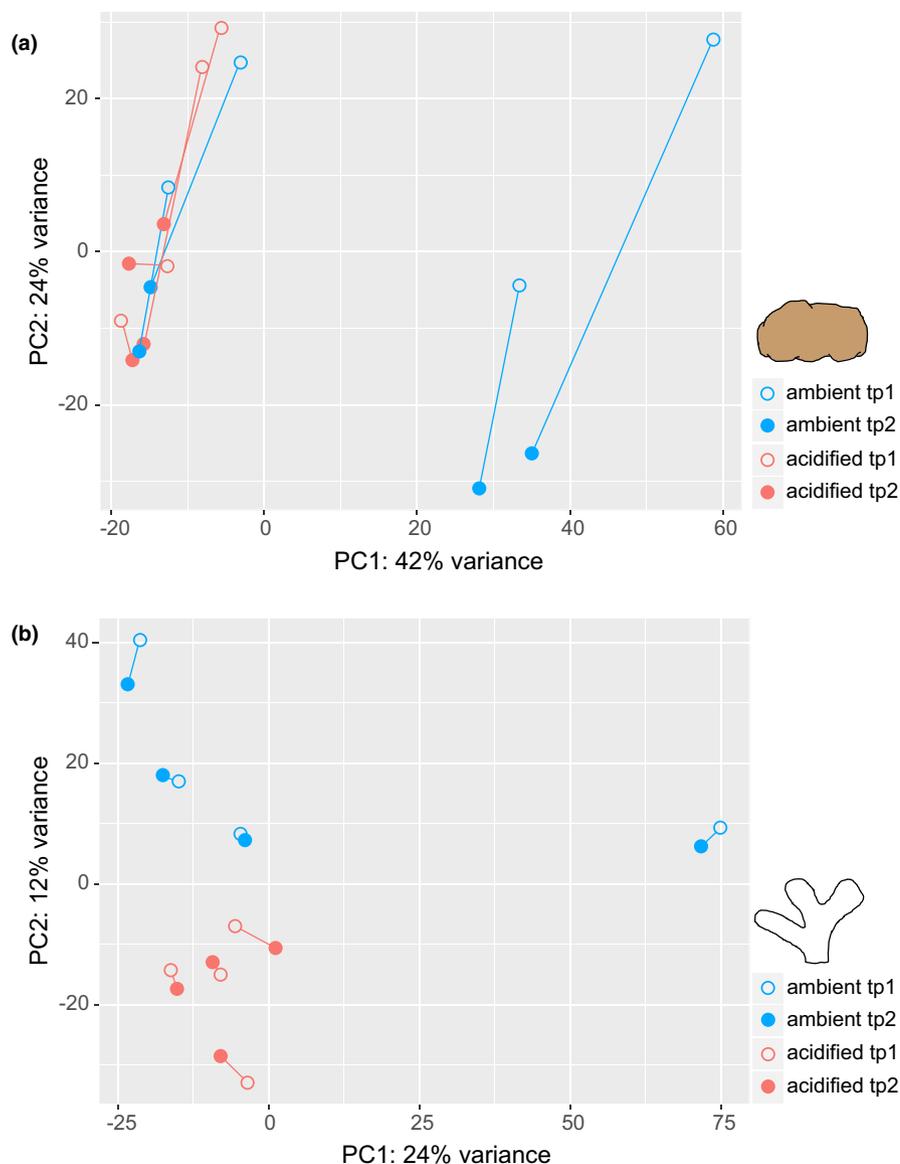


FIGURE 2 Plot of the principal component analysis for (a) the sponge and (b) the coral under ambient (blue) and acidified (red) conditions. Open circles represent time point 1 (tp1, preattachment) and closed circles represent time point 2 (tp2, post-attachment). The solid lines connect pre- and post-attachment time points sampled from the same individual (i.e., genetic replicate) [Colour figure can be viewed at wileyonlinelibrary.com]

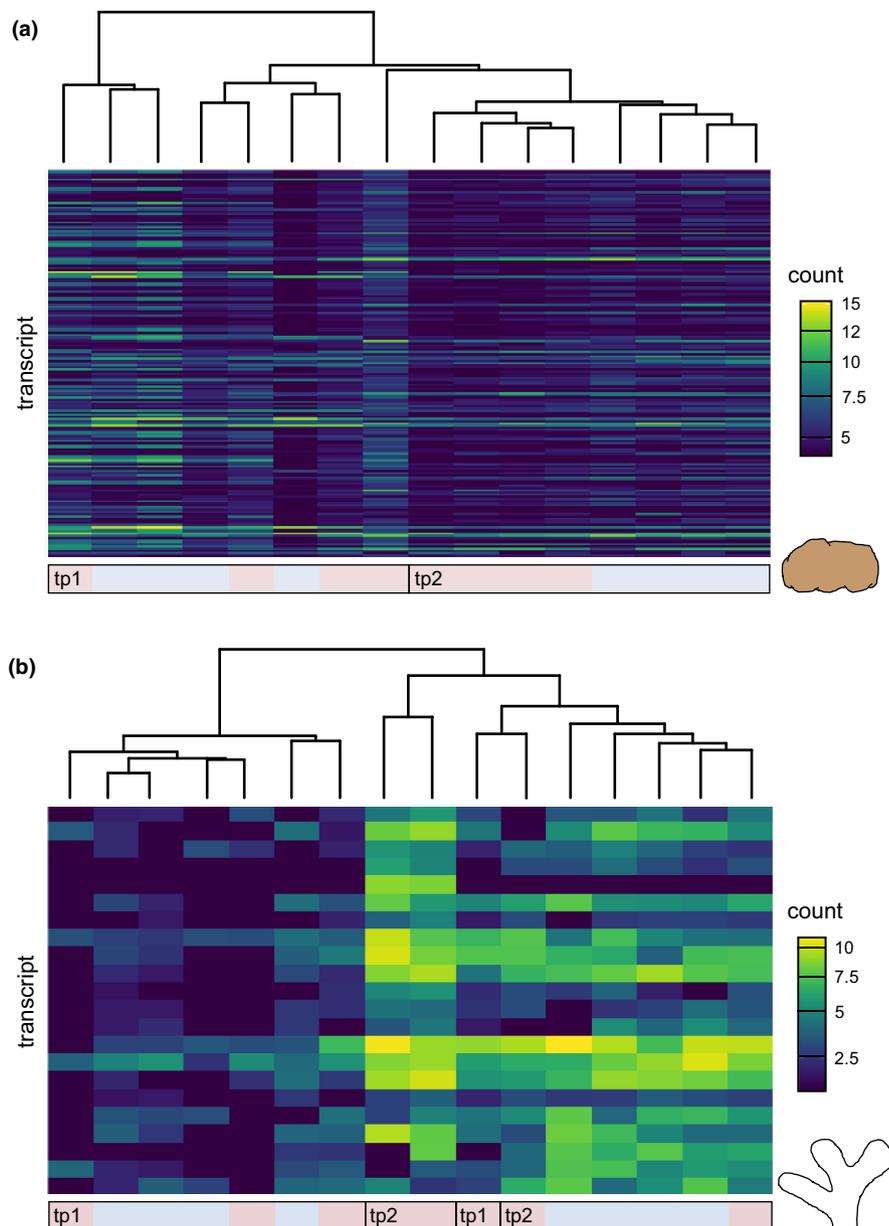
response to acidification than in response to the coral. [Figure 4d](#) compares the response to the coral under ambient conditions (x-axis) to the response to two stimuli combined- acidification alone and the presence of the coral (y-axis). These two patterns of differential expression are the most similar (i.e., points fall close to the 1-1 line), although the response to coral plus acidification is lower than the response to coral under ambient conditions, with 43.3% of transcripts ($(1253+28+1828+61)/7320$) showing a smaller change in expression. Taken together, it appears the response to the coral under acidified conditions in [Figure 4b](#) is more muted in part because many of the same transcripts used by the sponge to respond to the coral alone have already been partially up or downregulated in response to acidification alone, in the absence of the coral. When the response to acidification alone and the response to the coral in acidified conditions are considered together, the responses to the coral under acidified and ambient conditions are quite similar, as shown in [Figure 4c](#). In [Figure S3](#), we compare transcripts differentially expressed by the coral in response to the

sponge under ambient and acidified conditions. Transcripts that were differentially expressed in response to the sponge were more highly upregulated under ambient than acidified conditions.

3.3 | Carbonic anhydrases

We identified three carbonic anhydrase transcripts in the sponge host transcriptome. When we searched for these transcripts in our DESEQ2 results, we found two were upregulated (4.1 and 1.8 log₂-fold change) in response to the coral under ambient conditions and also upregulated (3.7 and 1.6 log₂-fold change) in response to acidification alone ([Table 1](#)), mirroring the broad scale responses shown in [Figure 4b,c](#). Intriguingly, these two transcripts and a third were not differentially expressed in response to the coral under acidification. None of the 10 carbonic anhydrase transcripts in the coral host transcriptome were differentially expressed in any comparisons ([Table S6](#)).

FIGURE 3 Heatmaps showing change the expression of transcripts (y-axis) in (a) sponge and (b) coral replicates across time points (tp1 preattachment and tp2 post-attachment) and pH conditions (x-axis) with light blue shading indicating replicates from ambient treatments and red shading indicating replicates from acidified treatments. Yellow shading indicates higher transcript expression while darker blue represents lower transcript expression. Read counts were transformed using variance stabilizing and filtered to retain significantly differentially expressed transcripts (FDR < 0.05) with a log₂-fold change greater than 3. The dendrogram at the top of each heatmap shows clustering of replicates based on similarities in expression patterns [Colour figure can be viewed at wileyonlinelibrary.com]



3.4 | Annotation and functional enrichment analysis

A comprehensive list of significantly enriched functional terms and a list of significant transcripts for each enriched functional term is reported in Table S7. For clarity, in Figure 5 we report results for specialized “child” terms in the GO hierarchy and terms with fewer than 1000 differentially expressed transcripts. Twenty-two categories were enriched in transcripts upregulated by the sponge in response to the coral under ambient conditions and nine categories were enriched in transcripts downregulated by the sponge in response to the coral under ambient conditions (Figure 5, Table S7). Categories with upregulated transcripts included ion binding, ion and transmembrane transport, and ion channel activity. Categories with downregulated transcripts included actin and unfolded protein

binding, vesicle mediated transport, and intracellular protein transport. Fourteen categories enriched in transcripts downregulated by the sponge in response to acidification included vesicle mediated, vacuolar, and intracellular protein transport and GTPase activity and GTP and actin binding (Figure 5, Table S7). Of the top 25% of transcripts upregulated by the sponge in response to acidification (534), 136 transcripts had a functional category assigned and only one of these was a stress response category (Table S7, GO:0006979 response to oxidative stress). Two categories were enriched in transcripts upregulated by the sponge in response to acidification alone (ion transport and transmembrane transport, Figure 5, Table S7). No categories were enriched in the transcripts differentially expressed by the sponge in response to the coral under acidification.

In the coral, the categories “negative regulation of circadian rhythm” and “negative regulation of transcription DNA-templated”

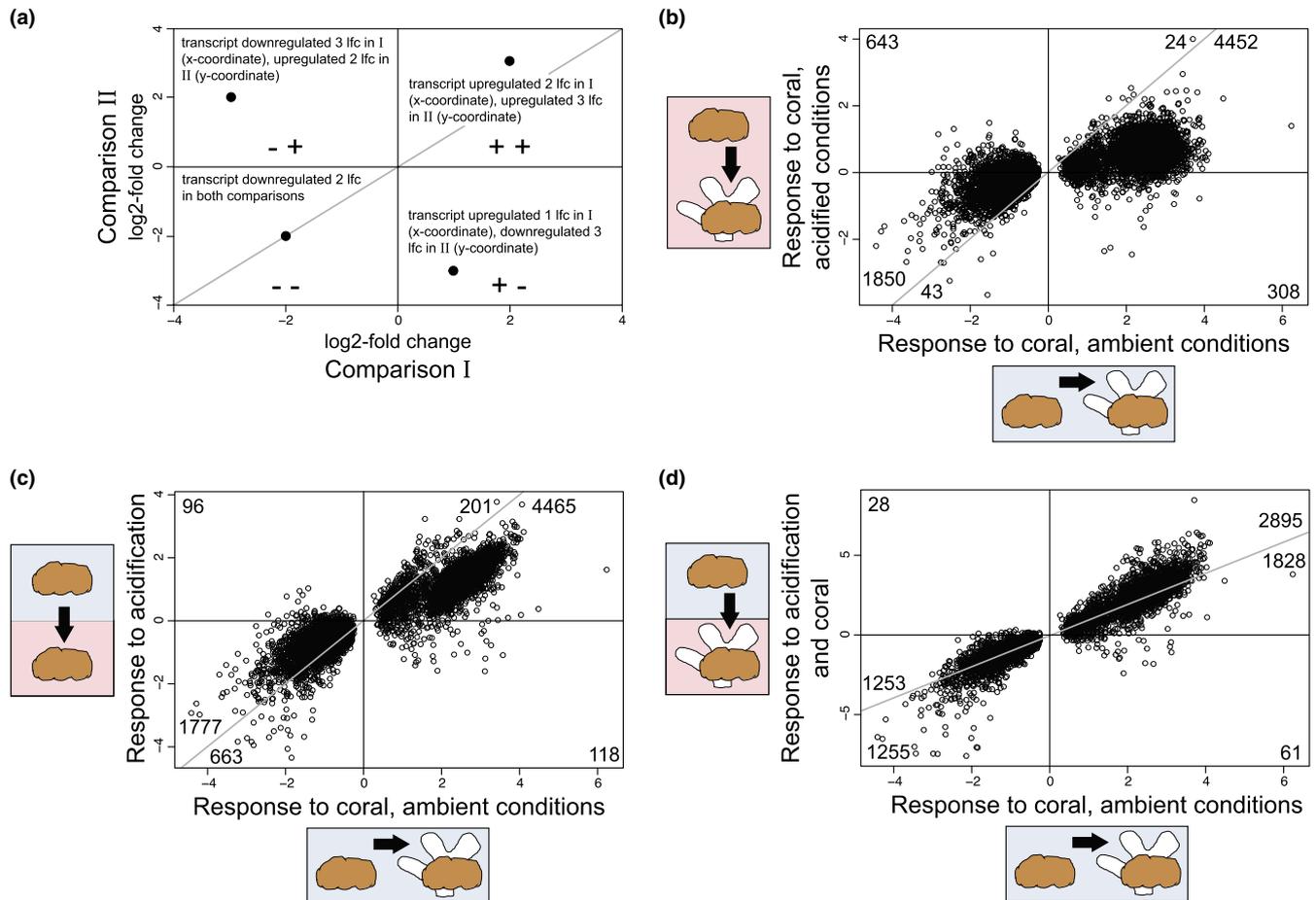


FIGURE 4 Scatterplots showing the log₂-fold change (lfc) for 7320 significantly differentially expressed (FDR < 0.05) transcripts, each represented by a point, across two comparisons. (a) Example plot showing lfc for four transcripts in two hypothetical comparisons, I and II, with log₂-fold change for comparison I on the x-axis and log₂-fold change for comparison II on the y-axis. Points that fall in the top left quadrant of the plot represent transcripts that were downregulated in comparison I and upregulated in comparison II. Points that fall in the top right quadrant of the plot represent transcripts that were upregulated in both comparisons. Points that fall in the lower left quadrant were downregulated in both comparisons. Points that fall in the lower right quadrant were upregulated in comparison I and downregulated in comparison II. The plus and minus signs in each quadrant also represents the direction of log₂-fold change for transcripts in each comparisons according to their x-y-coordinates, for example, - + indicates transcripts downregulated in comparison I (x-axis) and upregulated in comparison II (y-axis). Points on the grey 1:1 line represent transcripts with equal log₂-fold change between comparisons. In b, c, and d, the number of transcripts plotted in each quadrant is indicated with the number of transcripts falling above and below the 1:1 line reported for the lower left and upper right quadrants. Sponge and coral cartoon diagrams indicate which comparison is plotted on the x- and y-axes, with blue shading representing the ambient treatment and red shading representing the acidified treatment. (b) Expression changes in response to the coral under ambient conditions (x-axis) compared to expression changes in response to the coral under acidified conditions (y-axis). (c) Expression changes in response to the coral under ambient conditions (x-axis) compared to expression changes in response acidified conditions in isolation from the coral (y-axis). (d) Expression change in response to the coral under ambient conditions (x-axis) compared to the response to acidification and the presence of the coral (i.e., ambient time point 1 vs. acidified time point 2) (y-axis) [Colour figure can be viewed at wileyonlinelibrary.com]

were enriched in transcripts upregulated by the coral under acidification (Table S7). Only one category, “negative regulation of circadian rhythm” was enriched in transcripts upregulated by the coral in response to the sponge under ambient conditions (Table S7). Because the statistical test of enrichment gave little insight into the functional response of the coral, we created a list of GO term annotations for transcripts that were differentially expressed by the coral in response to the sponge under ambient and under increased acidification and under increased acidification in isolation from the sponge (Table S7).

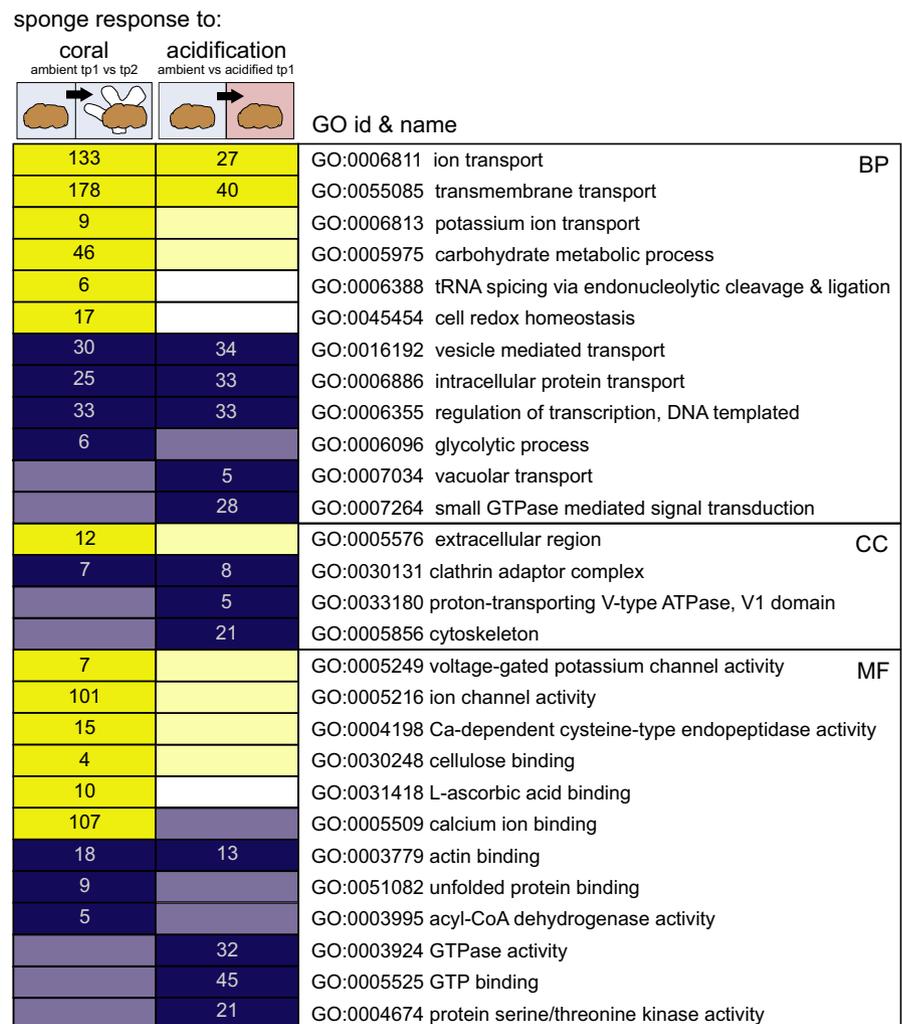
4 | DISCUSSION

Bioerosion is an ecologically important process on carbonate reefs. Given the influence of anthropogenic change on organisms that create and those that bioerode the reef, understanding the interaction between species is increasingly important for predicting how reef ecosystems will persist in the future. We used gene expression data to examine bioerosion in sponges and calcification in corals when these taxa interact under ambient conditions and acidified conditions expected in the future. Our results provide insight into the

TABLE 1 Differential gene expression in carbonic anhydrase transcripts in *Cliona varians*. Values in bold indicate transcripts that are significantly differentially expressed

<i>Cliona varians</i> sponge			
	Transcript	Log2-fold change	Adjusted p-value
Response to coral, ambient tp1 vs. tp2	DN128439_c0_g1	4.063518597	6.95E-08
	DN135279_c0_g1	1.805031066	0.000595181
Response to coral, acidification tp1 vs. tp2	DN128439_c0_g1	0.369063933	0.863067711
	DN135279_c0_g1	0.823859589	0.488793565
	DN75380_c0_g2	0.275241904	0.716343435
Response to acidification, ambient tp1 vs. acidified tp1	DN128439_c0_g1	3.692228675	2.16E-06
	DN135279_c0_g1	1.631387022	0.006450733
	DN75380_c0_g2	-0.172310811	0.687595311

FIGURE 5 Functional enrichment of transcripts differentially expressed by the sponge in response to the coral under ambient conditions and in response to acidification in isolation from the coral. Sponge cartoon diagrams indicate each comparison, with light blue shading representing the ambient treatment and red shading representing the acidified treatment. Gene ontology terms are grouped according to their subontologies (BP, biological process; CC, cellular component; MF, molecular function). Numbers in each box indicate the number of differentially expressed transcripts in a given category. Bold colours represent significant terms (corrected p -values $< .05$) and light colours represent nonsignificant terms. Yellow shading represents upregulated categories and blue boxes represent downregulated categories. White boxes indicate nonsignificant terms with no differentially expressed transcripts or an equal number of up- and downregulated transcripts [Colour figure can be viewed at wileyonlinelibrary.com]



genomic mechanisms of sponge bioerosion, how sponges and corals respond to the abiotic stress of OA, and the effect of global change on this species interaction.

4.1 | Genomic mechanisms of sponge bioerosion

Sponge bioerosion is powered in part by the photosynthetic activity of *Symbiodinium* algal symbionts (Achlati et al., 2019). Using

stable isotope labelling, transmission electron microscopy, and mass spectrometry, Achlati et al. (2018) showed ammonium and bicarbonate were passed from *Symbiodinium* cells to *Cliona orientalis* sponge cells. Here, when the sponge was exposed to the coral, we found upregulation of gene products associated with ammonium transport (ammonium transporter, ammonium transporter AmtB-like, ammonium transporter conserved site, ammonium/urea transporter) and bicarbonate transport within the enriched ion transport functional category (Table S7; GO:0006811),

providing molecular evidence for nutrient transfer. Our molecular results align with other studies indicating clonoid sponges and their symbionts are metabolically integrated (Weisz et al., 2010) and that sponge bioerosion is supported by *Symbiodinium* photosynthesis.

Sponge bioerosion is accomplished in two steps. In chemical bioerosion, the sponge produces acid to lower the pH at the tissue-substrate interface (Pomponi, 1980). Specialized sponge etching cells extend filipodia filled with low pH vesicles to the bioerosion site and deliver an acidic fluid that dissolves the calcium carbonate substrate. Webb et al. (2019) hypothesized that chemical bioerosion would require the continuous pumping of protons to the etching site and that the rate of CaCO_3 dissolution would be determined by the rate that bicarbonate ions are transported away from the etching site, which requires active transport across membranes (Cordat & Casey, 2009). In the mechanical step of bioerosion, "sponge chips," pieces of CaCO_3 ~10–100 μm created during chemical dissolution (Zundelevich et al., 2007), are removed by fusiform myocyte-like cells that form a reticulated matrix and appear to act as a conveyor to transport sponge chips away from the excavation site (Webb et al., 2019).

Carbonic anhydrases are long thought to be involved in chemical bioerosion in sponges and in other taxa including annelids and molluscs (Tresguerres et al., 2013; Webb & Saleuddin, 1977), speeding up the otherwise slow reaction of carbon dioxide and water to bicarbonate and hydrogen ions (Sueltemeyer et al., 1993). Hatch (1980) demonstrated that sponge carbonic anhydrases could shift the CaCO_3 equilibrium and further proposed that carbonic anhydrases could move hydrogen ions across cell membranes, lowering the pH through the exchange of hydrogen ions for bicarbonate ions, thereby facilitating substrate dissolution. We found that in response to the coral, the sponge upregulated carbonic anhydrase transcripts and gene products for several solute carriers, anion transporters, and anion exchangers (bicarbonate transporter C-terminal, SLC26A/SulP transporter, STAS domain, sodium/calcium exchanger, solute carrier family 13) within the enriched ion transport functional category (Figure 5 and Table S7; GO:0006811). Chloride/bicarbonate ion exchangers bind to carbonic anhydrases to form a membrane complex that accelerates the flux of bicarbonate across membranes (Sterling et al., 2002), a molecular mechanism possibly invoked by the sponge to initiate bioerosion. Solute carriers have been shown to be critical to the acidification of vacuoles used for phagocytosis (Sedlyarov et al., 2018), and perhaps in sponges, these carriers are used to produce the acid-filled vesicles in the filipodia that sponges extend to the bioerosion site. Interestingly, vesicle and vacuolar transport categories were downregulated (Figure 5) in response to the coral, the opposite of what we would expect during chemical bioerosion. One explanation is that at time point 2 (48 h post-attachment), the sponge had not exhausted its current cache of vesicles and it was not yet necessary to upregulate these pathways to produce more.

4.2 | Sponge response to acidification

Our use of an acclimation period for sponges in the acidified treatment (Figure 1c) has important ramifications for how we interpret gene expression between different comparisons. While using an acclimation period before taking the first tissue sample (i.e., time point 1, preattachment) meant we lost information about how gene expression changed over time in response to acidification, without prior acclimation, it would not be possible to determine whether differential expression at time point 2 (post-attachment) was due to the initial reaction to acidification or due to the species interaction. For the sponge, based on our experimental design and data collected (3707 differentially expressed genes in ambient vs. acidified conditions, Figure S2), two outcomes are possible: (i) the sponge had a large gene expression response to acidification that was maintained across the 5-day window; or (ii) the sponge had a large gene expression response that attenuated over time but was still elevated at day 5. While we cannot distinguish between these two hypotheses, the data show that sponges significantly change their gene expression when exposed to acidified conditions.

Much evidence suggests that sponges are relatively unimpacted by ocean change, including increased temperature (Guzman & Conaco, 2016), nitrification (Luter et al., 2020), and acidification (Achlati et al., 2017; Bell et al., 2013; Duckworth et al., 2012; Kroeker et al., 2010; Stubler et al., 2014, 2015). Stubler et al. (2014, 2015) observed 100% survival in 150 and 720 C. *varians* sponge replicates exposed to 51 and 140 days of acidification, respectively. We found here that the sponge differentially expressed 3707 transcripts in response to acidification (Figure 3a). Given the high survival, increased boring capability of *C. varians* under acidified conditions (Stubler et al., 2014, 2015), and lack of stress response categories in transcripts expressed under acidification (Table S7), this large transcriptomic change does not appear to represent a stress response. Moreover, 67% of the sponge transcripts differentially expressed in response to acidification were also differentially expressed by the sponge in response to the coral under ambient conditions (2500 transcripts total, Figure S2), which indicates a shared transcriptomic response to different stimuli, one biotic (the coral) and one abiotic (acidification). In other words, expression of these 2500 transcripts changes during bioerosion, which involves a localized reduction in pH at the excavation site (Pomponi, 1980), or when the sponge is exposed to acidification in the larger environment. This shared response was also evident in the pattern of functionally enriched GO categories (Figure 5). With few exceptions, categories enriched in transcripts upregulated by the sponge in response to the coral under ambient conditions were also enriched in transcripts upregulated by the sponge in response to acidification. The same is true of categories enriched in downregulated transcripts (Figure 5). A possible explanation for a shared response to different stimuli is that it represents an adaptation of the sponge to daily environmental pH changes. Mangroves typically experience large diel fluctuations in $p\text{CO}_2$ due to shallow water depths and decomposition of

organic matter in their sediments (Borges et al., 2003, mean $p\text{CO}_2$: 390–720 μatm , Zablocki et al., 2011, mean $p\text{CO}_2$: 390–2841 μatm). Because many coral reefs in Bocas del Toro, including those from which we sampled, are adjacent (<10 m) to mangrove habitats, they are exposed to low pH water that moves out from the mangroves. As $p\text{CO}_2$ fluctuates on a regular diel cycle on the reef, a drop in pH might act as signal to the sponge that conditions are energetically favourable for bioerosion, and to prepare for increased bioerosion by expressing transcripts involved in the bioerosion pathway. While we cannot rule out the possibility that acidification alters the expression of carbonic anhydrases used in cellular processes other than bioerosion, the hypothesis that acidification primes the sponge to bioerode is supported by the sponge upregulating carbonic anhydrase transcripts while under acidified conditions but in isolation from the coral (Table 1).

Although under some scenarios sponges might be global change “winners” (Bell et al., 2013, 2018), organismal responses to global change are likely to be variable and complex. While we found *C. varians* to be resilient to acidification, we did not test how sponges might respond to other environmental stressors or to multiple simultaneous stressors, which is how organisms will experience stress in the future. For example, the bioeroding sponge *Cliona orientalis* fared poorly in temperatures above 32°C in situ on the Great Barrier Reef, and in the laboratory, sponges bleached (Achlati et al., 2017) and failed to regain symbionts, even after one month at reduced temperatures (Ramsby et al., 2018). It is possible that any bioerosion gains by *C. varians* under acidification will be negated by poor performance under other simultaneous stressors, such as temperature and/or bleaching. Future studies should include multiple stressors to build upon the current results and gain a more complete understanding organismal response to global change.

4.3 | Coral resilience to biotic and abiotic stress

Across all treatments, the coral had a small transcriptomic response, differentially expressing tens of transcripts. One concern is that these low numbers reflect technical problems. The high number of sequencing reads for each coral library (average over 20 million, Table S2) suggests the tissue sampling and library prep were successful. However, lower read mapping for the coral host vs. holobiont transcriptome (Table S2) suggests our RNA-Seq libraries contained a higher proportion of *Symbiodinium* RNA compared to coral RNA and lower BUSCO scores for the coral host vs. holobiont transcriptome (Table S5) might reflect overly aggressive filtering of coral transcripts, particularly for core genes conserved across algal symbionts and the coral host. Both factors reduce our ability to measure full transcriptomic responses in the coral. Using the BUSCO scores as a rough proxy, we conclude that our results represent ~60% of the coral transcriptomic response.

As with the sponge, the 5-day acclimation period to low pH (Figure 1c) has biological implications for how we interpret results for the coral. Based on the experimental design, two possible

scenarios explain the coral's low response to acidification (28 differentially expressed transcripts): (i) the coral had a large transcriptomic response upon exposure to acidification followed by a return to baseline over the acclimation period, or (ii) the coral had little transcriptomic response to acidification at any time over the acclimation period, either because the pH (7.6) was not stressful or because the pH was so stressful that the coral could not mount any transcriptomic response (the latter of which is less likely since the coral remained visually healthy and other studies have shown *P. furcata* can survive under long-term exposure to low pH (Stubler et al., 2014, 2015)). While we cannot distinguish between these scenarios, our results show the transcriptomic response of the coral on day five of acidification was small.

While the clustering of replicates more closely by time point than pH condition qualitatively suggests interaction with the sponge caused upregulation of transcription in the coral (Figure 3), the low number of significantly differentially expressed transcripts (Figure S2) suggests the coral did not mount a substantial molecular defence response to the sponge. One explanation is that the 48 h interaction period with the sponge was too short to trigger a large transcriptomic response, although other studies have shown corals do react to short-term abiotic stimuli (Rivest et al., 2018, 24 h exposure to temperature stress, Lin et al., 2018, 3 h exposure to acidification). Studies with finer-scale, multireplicate time point sampling are ultimately required to quantify the exact timing of transcriptomic changes in corals (and in other organisms). A second explanation is that the sponge fragments we used were not large enough (i.e., stressful enough) to elicit a large transcriptomic change from the coral. Evidence from the laboratory and the field suggest that the size of the sponge determines, in part, the success of sponge overgrowth and perhaps the response of the coral. Fang et al. (2017) showed that *Cliona orientalis* sponge fragments joined to corals had decreased growth rates and lost area to the coral over a 90-day period, indicating a defensive coral response to the presence of the sponge. In the field, Schönberg and Wilkinson (2001) observed that upon removing *C. orientalis* sponge tissue from coral heads, smaller patches of remaining sponge had lower survival rates than larger patches.

The response of the coral to acidification was low and similar in magnitude to the response of the coral to the sponge under ambient conditions (28 and 35 differentially expressed transcripts, respectively), although few transcripts (5) were differentially expressed in response to both treatments. Phenotypic data for *P. furcata* and other coral species suggest the muted transcriptomic response is indicative of coral resilience to acidification. Acidification had no effect on *P. furcata* survival over 51 days (Stubler et al., 2014) or calcification over 140 days (Stubler et al., 2015). Calcification in a massive *Porites* sp. from Moorea, French Polynesia was also unaffected by acidification after 19 days (Brown & Edmunds, 2016). Species-specific differences in calcification suggest *Porites* species may expend less energy to calcify in low pH environments (McCulloch et al., 2012), thus obviating the need for a large transcriptomic reaction. Davies et al. (2016) showed net calcification rates in the

coral *Siderastrea siderea* were more negatively affected by increased temperature than acidification and gene expression data suggested *S. siderea* could acclimate to acidification over 95 days. *Pocillopora damicornis* coral larvae exposed to increased acidification for 24 h had no transcriptomic response and did not differ significantly from ambient treatments in larval quality metrics (size, protein content, *Symbiodinium* density) (Rivest et al., 2018), suggesting no deleterious effects of acidification at this life stage for this species. Adult *Poc. damicornis* also maintained constant calcification rates under pH conditions predicted for the end of the century (DeCarlo et al., 2018). Although the timescales of these studies are not directly comparable with our sampling point, and we did not consider the effects of concurrent abiotic stressors (Kroeker et al., 2013), these results suggest acidification levels predicted for the year 2300 might not be sufficiently stressful to elicit a large transcriptomic response from *P. furcata*. Alternatively, the coral's resilience to acidification might be due to adaptation to the aforementioned low pH conditions that occur diurnally in mangrove-adjacent reefs (Borges et al., 2003; Zablocki et al., 2011).

4.4 | Global change alters sponge-coral species interactions

Accurately predicting global change outcomes requires we understand both how changes in temperature and pH will affect individual species and how these abiotic stressors will alter biotic species interactions (Araújo & Luoto, 2007; Kroeker et al., 2013). Previous studies have shown that acidification changes sponge-coral interactions by allowing sponges to bioerode more coral substrate (Fang et al., 2013; Stubler et al., 2014, 2015, but see Achlatis et al., 2017). While important, these studies lacked the ability to determine whether the increased bioerosion rate observed was a result of (i) sponges performing "business as usual" under conditions (i.e., acidification) that made bioerosion easier, or (ii) sponges actively modifying their physiological bioerosion strategy under increased acidification.

Our data support the latter hypothesis that sponges actively capitalize on acidification conditions. Patterns of expression in the heatmap, wherein time point 2 (post-attachment) replicates cluster according to pH condition compared to no clustering of time point 1 replicates (preattachment), suggest sponges modify their transcriptomic response to the coral under acidification. We found that the sponge had a smaller transcriptomic response to the coral under acidification, expressing 50 times fewer transcripts than under ambient conditions. While it is possible that front-loading during acclimation (Figure 1d) led to some reduction in the transcriptomic response, these results suggest that under acidification sponges expend less energy during bioerosion, supporting previous hypotheses that acidification reduces the energetic costs of bioerosion for sponges (Bell et al., 2013; Fang et al., 2013; Wisshak et al., 2012).

Ocean change has the potential to alter the overall energetic cost of bioerosion, and how the sponge invests energy in chemical vs. mechanical bioerosion pathways. Higher ratios of chemical bioerosion

were measured in sponges in naturally more acidic habitats (c. pH 7.9, *Cliona vermifera*, 29% and *C. flavifodina*, 12%, Nava & Carballo, 2008) than in sponges in less acidic habitats (c. pH 8.1, *Cliona cf. celata*, <10%, Warburton, 1958, *Pione lampa*, 2%–3%, Rützler & Rieger, 1973). Under experimentally high acidification, chemical bioerosion rates were higher than mechanical bioerosion rates in *C. orientalis* (Fang et al., 2013; Meyer et al., 2019; Wisshak et al., 2012). Our findings that sponges have an overall lower transcriptomic response and do not upregulate carbonic anhydrase transcripts in response to the coral under acidification suggest increased chemical bioerosion rates are not the result of higher investment in chemical bioerosion by the sponge. In contrast, these results suggest that instead of investing energy in generating the acidic fluid and specialized etching cells required for chemical bioerosion, the sponge might skip, or scale back, that part of the pipeline and take advantage of the low pH conditions favourable for chemical bioerosion. While it is possible we were unable to detect significant expression of carbonic anhydrase transcripts after the sponge came into contact with the coral because these transcripts were front-loaded during the acclimation period (Figure 1d) for cellular processes unrelated to bioerosion, our results generate intriguing hypotheses for future investigations of the transcriptomics of chemical and mechanical bioerosion strategies.

In all comparisons the coral had a low transcriptomic response, which reduces our ability to draw conclusions. Qualitatively, acidification appears to alter the coral's interactions with the sponge. *Porites furcata* differentially expressed an order of magnitude fewer transcripts in response to the combination of sponge presence under acidification conditions (4) compared to acidification alone (28) and the response to sponge bioerosion under ambient conditions (35). We examined the ontology of the transcripts differentially expressed by the coral under each treatment (Table S7). In response to the sponge under ambient conditions, 30 categories were represented including those associated with a stress response (e.g., unfolded protein binding, response to oxidative stress) and those associated with the maintenance of normal cellular and molecular function. In contrast, in response to the sponge under acidification, only five categories were represented. These results suggest acidification reduces the magnitude of the coral's transcriptomic response to the sponge, which might limit coral resilience to sponge bioerosion under future environmental conditions.

4.5 | Conclusions

Here we show that OA alters the species interactions between a bioeroding sponge, *C. varians*, and the coral, *P. furcata*, at the transcriptomic level, complementing physical evidence of this alteration under ocean change (Stubler et al., 2014, 2015). Our study showed that when acidification was high, sponges bioeroded calcium carbonate substrate using a reduced transcriptomic response and, presumably, with reduced energetic costs for cellular processes, including bioerosion. Corals exhibited a muted transcriptomic response to the sponge when acidification was high, suggesting a reduction in the

coral's reaction to sponge colonization under conditions predicted for the future. The present study offers transcriptomic insight into the interaction occurring between these two species and suggests a mechanism through which sponges increase erosion of carbonate substrate under future acidification scenarios.

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AUTHOR CONTRIBUTIONS

Melissa B. DeBiase and Amber D. Stubler conceived the project, Melissa B. DeBiase and Amber D. Stubler performed the experiments, Morgan W. Kelly provided reagents, Melissa B. DeBiase, Amber D. Stubler, and Morgan W. Kelly performed the analyses, Melissa B. DeBiase, Amber D. Stubler, and Morgan W. Kelly wrote and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

Raw RNA-Seq data have been made available at the European Nucleotide Archive under study accession number [PRJEB44093](https://doi.org/10.6084/m9.figshare.14357225.v2). Custom scripts, command lines, and files used in these analyses are available at <https://doi.org/10.6084/m9.figshare.14357225.v2>

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