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Title: Diurnal and nocturnal transcriptomic variation in the Caribbean staghorn coral, *Acropora cervicornis*

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Abstract

Reef-building corals experience large diel shifts in their environment, both externally due to changes in light intensity, predator activity and prey availability, and internally as a result of diel fluctuations in photosynthesis by their endosymbiotic algae, *Symbiodinium*. Diel patterns of tentacle

behavior, skeletal growth, and gene expression indicate reactions of the coral animal in response to light and through circadian regulation. Some corals, such as the Caribbean *Acropora*s, have strong within-colony division of labor, including specialized fast-growing apical polyps, accompanied by large gene expression differences. Here we use RNA-seq to evaluate how diel changes in gene expression vary within the branching Caribbean staghorn coral, *Acropora cervicornis*, between branch tips and branch bases. Multifactor generalized linear model analysis indicated that 6% (3005) of transcripts were differentially expressed between branch tips and bases, while 1% (441) of transcripts were differentially expressed between day and night. The gene expression patterns of 220 transcripts were affected by both time of day and location within the colony. In particular, photoreceptors, putative circadian genes, stress response genes and metabolic genes were differentially expressed between day and night, and some of these, including *Amcry1*, *tef* and *hebp2*, exhibited location-specific regulation within the coral colony as well. These findings indicate that the genetic response of the coral to day and night conditions varies within the colony. Both time of day and location within the colony are factors that should be considered in future coral gene expression experiments.

Introduction

The daily rotation of the earth subjects organisms to predictable oscillations of abiotic factors, such as light, temperature and tides, and biotic factors, such as predation and prey availability. To moderate the effects of these diel changes, organisms often alter their behavior and physiology by responding directly to environmental cues (e.g. light/dark) or through circadian regulation (Reitzel *et al.* 2013). A significant portion an organism's transcriptome can exhibit daily rhythms; for example, more than 20% of gene transcripts show daily fluctuations in intertidal

mussels (Connor & Gracey 2011) and up to a third of expressed genes are under circadian control in plants (Covington *et al.* 2008).

Hermatypic (or reef-building) corals inhabit tropical marine waters and live in symbiosis with dinoflagellate algae (*Symbiodinium*, aka zooxanthellae) that provide 40% to 100% of the coral's energy requirements (Falkowski *et al.* 1984; Muscatine *et al.* 1984). Corals experience large diel changes in their physiology between day and night to deal with the shift from acquiring food from their photosynthetic endosymbionts during the day to being heterotrophic at night, when they feed on demersal zooplankton (Heidelberg *et al.* 2004). Daytime stresses include high UV radiation (UVR) and light exposure and the production of reactive oxygen species (ROS) by *Symbiodinium* during photosynthesis (Lesser 1996; 1997), while nighttime stress results from respiration by coral and *Symbiodinium* which can lead to hypoxic conditions and decreased pH within the coral tissues (Kühl *et al.* 1995).

As sessile organisms, coral behavioral changes include polyp and tentacle extension and retraction, which often display diel patterns (Levy *et al.* 2006). Tentacle behaviors, which may help the animal avoid predation while maximizing photosynthesis, heterotrophic feeding and gas exchange (Levy *et al.* 2006), appear to be light-responsive, but may also have a circadian component of regulation (Tsang *et al.* 1997; Levy 2003). In some corals, such as the Caribbean *Acropora* corals, tentacle behavior varies between different parts of the colony (E. Hemond & S. Vollmer, personal observation), suggesting complex regulation beyond uniform light-response. Tentacles of *Symbiodinium*-rich radial polyps throughout most of the colony are extended during the day to capture light and remain extended at night, while axial polyps at the tip of the branch, which contain few *Symbiodinium*, are retracted during the day and only extended at night for feeding on active zooplankton.

Diel gene expression patterns have been observed in anthozoans, including corals, in response to both light and circadian regulation (Levy *et al.* 2007; Reitzel *et al.* 2010; Brady *et al.* 2011; Levy *et al.* 2011). Alteration of gene expression and subsequent protein production at regular intervals based on time of day is probably necessary for the coral to cope with cyclic patterns of stress (UV and ROS), facilitate digestion of molecules obtained from *Symbiodinium* or heterotrophy, and manage algal densities. Corals and other anthozoans possess a number of circadian genes (Vize 2009; Reitzel *et al.* 2010) as well as photoreceptors, such as cryptochromes (*crys*) and opsins, that may also interact with circadian genes (Levy *et al.* 2007; Shoguchi *et al.* 2013). It is not yet understood what coral functions may be under circadian regulation (Sorek *et al.* 2014); however, some stress response chaperone proteins, specifically heat shock proteins, are expressed with circadian rhythmicity (Levy *et al.* 2011). Coral photoreceptors are suspected to be involved in a number of light-responsive functions, such as growth (Kaniewska *et al.* 2009), polyp expansion or contraction (Levy 2003), and spawning (Levy *et al.* 2007; Brady *et al.* 2009).

In many corals, calcification increases in the light in a phenomenon now referred to as 'light-enhanced calcification' (LEC) (Goreau & Goreau 1959), which is related to light availability and *Symbiodinium* photosynthetic activity (Moya 2006). The Caribbean staghorn coral, *Acropora cervicornis*, is a good model for studying coral growth processes because of its rapid growth rate, its branching morphology, and a polyp-based division of labor (DOL) within the colony. *A. cervicornis* has dimorphic polyps: 1) radial polyps that contain a high concentration of *Symbiodinium* along the branch and are the site of gamete production, and 2) axial polyps at the branch tip that are the location of linear skeletal growth and that contain lower *Symbiodinium* concentrations. Although *A. cervicornis* branch tips contain fewer *Symbiodinium,* they have the highest calcification rates (Goreau & Goreau 1959), probably due to transfer of *Symbiodinium*-produced photosynthate

among polyps (Pearse & Muscatine 1971). Calcification in *A. cervicornis* exhibits a diel pattern in the quality of skeleton deposited at the axial corallite (Gladfelter 1983a) and predictable changes in the density of skeleton at different regions along the colony branch (Gladfelter 1982).

The DOL within Caribbean *Acropora* coral colonies represents substantial differences in function. For example, gamete production occurs only in mature radial polyps at least 2 cm from the branch tip (Szmant 1986), and the metabolic rate in branch tips is higher than the rest of the colony (Gladfelter *et al.* 1989). Differences in gene expression between branch bases and tips have been found to represent a substantial fraction, approximately 10%, of the transcriptome, including functions such as developmental signaling, metabolism and calcification (Hemond *et al.* 2014). In corals with DOL and variation in the density of *Symbiodinium* within the colony, such as *Acropora*s, there may also be location-specific, diel changes in gene expression. While studies of Pacific *Acropora* corals have indicated gene expression differences between day and night, or light and dark, with particular interest in circadian regulation or LEC (Levy *et al.* 2007; Brady *et al.* 2011; Levy *et al.* 2011), no study has yet compared full transcriptome differences between day and night in functionally distinct parts of the colony.

To identify diel and within-colony gene expression differences, we compared transcriptome-wide gene expression patterns of branch tips and bases in the Caribbean staghorn coral, *A. cervicornis,* in its natural reef environment at mid-day versus mid-night using RNA sequencing (RNA-seq). Full transcriptome data was analyzed with a multi-factor generalized linear model (GLM) to identify transcripts whose expression differs between day and night and within the coral colony (branch tips vs. bases), as well as whose expression depends on both factors

(significant for both factors or having a significant interaction between factors). We hypothesized that transcripts involved in LEC, phototropism or polyp behavior should show differences in expression between day and night in tips, while transcripts related to the coral's response to the symbiont's photosynthetic activity and population growth should display differences between day and night in bases, where symbiont densities are highest. Transcripts with a significant interaction effect may also be involved in these processes. Genes expressed differentially between day and night throughout the colony may be involved in stress response, circadian rhythms and other functions that may be shared among all polyps.

Materials and Methods

Sample collection & RNA extraction

Acropora cervicornis samples were collected from three large coral colonies located at least 10 meters apart at 5-6 meters depth in Crawl Cay, Bocas del Toro, Panama (09 15.517N 082 07.625W). Paired samples were taken from individual branches, with the base sample taken at 25-30 cm from the tip of the branch, and the tip sample being the top 1 cm of the branch, including the axial polyp. For each colony, one branch was sampled at 12:00-13:00 (~6 hrs post-sunrise) on August 2 (sunrise 6:09, sunset 18:39, moonrise 23:46, third quarter), and one branch was sampled at midnight-00:30 (~5.5 hrs past sunset) on August 3, 2010. At the time of the nighttime sampling, corals had been exposed to little, if any, moonlight; however, some exposure to dive lights was unavoidable while collecting. Temperature data was collected from a permanent data logger near Crawl Cay (Cayo Agua; data publicly available at http://biogeodb.stri.si.edu/physical_monitoring/research/bocas). Seawater temperature was 29.7-29.8°C during daytime sampling (12:00-13:00) and 29.6°C during nighttime sampling (24:00-

01:00). Coral samples were transported to the boat in covered plastic containers, then wrapped in aluminum foil, immediately preserved in liquid nitrogen, and stored at -80°C.

Illumina RNA-seq library preparation

Total RNA extraction was performed using Tri-Reagent (Molecular Research Center, Inc. Cincinnati, OH) following the manufacturer's protocol, with an additional 75% ethanol wash step. Total RNA quality was assessed using an Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA) and RNA Pico Chips, and only extractions with distinct 18s and 28S ribosomal RNA peaks were used (RIN values 4.9-8.0). mRNA was isolated using Dynabeads® oligo(dT) mRNA isolation beads (Life Technologies, Grand Island, NY) to exclude non-protein coding RNAs and non-eukaryotic mRNAs from the samples. cDNA was produced using random hexamer primers and SuperScript® II reverse transcriptase (Life Technologies) for first strand synthesis and DNA polymerase I (New England BioLabs, Ipswich, MA) for second strand synthesis. RNA-seq libraries were prepared by fragmenting the double stranded cDNA with dsDNA fragmentase (NEB) for 30 minutes at 37°C, followed by library preparation using NEBNext® next-generation sequencing modules. This included end-repair, A-tailing, and ligation of custom 4bp barcoded adapters. The cDNA transcripts with barcoded adapters were then size selected at 250 bp by gel extraction and amplified with 15 rounds of PCR. RNA-seq libraries were sequenced (6 samples per lane) using single-end 50 bp sequencing on the Illumina Hi Seq 2000 platform (Illumina, Inc., San Diego, CA, USA) at the FAS Center for System Biology at Harvard University.

Bioinformatics

Read quality control and barcode removal were conducted with custom Perl scripts in the FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/). RNA-seq reads were mapped against our published *de novo* transcriptome for *A. cervicornis* and *A. palmata* (Libro *et al.* 2013; Hemond *et al.* 2014) using CLC Genomics Workbench (CLC bio, Aarhus, Denmark). Reference transcriptome annotation was conducted using translated nucleotide queries against the curated UniProt/Swiss-Prot database and a threshold E-value of 10^{-5}. Coral genes were identified by aligning the de novo reference transcriptome contigs to the genomes of congeners *A. digitifera* (Shinzato *et al.* 2011) and *A. millepora* (D. Miller, unpublished). Transcripts with a significant BLAST hit (E-value < 10^{-10}) were identified as coral and retained for the analyses. Putative non-coral transcripts were removed.

Statistical analysis of differential expression

Using the DESeq package v. 1.14.0 (Anders & Huber 2010) in R v. 3.0.2 (R Development Core Team, 2012), the RNA-seq read count data were normalized by sample library size. Non-metric multidimensional scaling (nMDS; isoMDS) and PERMANOVA (Adonis) were conducted on the normalized data using the MASS v. 7.3.29 and vegan v. 2.0.10 packages (Venables & Ripley 2002; Oksanen *et al.*) in R to examine transcriptome-wide differences in gene expression. Differentially expressed (DE) gene transcripts were then identified using a three-factor negative binomial Wald test (design = ~ colony + position * time) in DESeq2 v 1.2.10 (Anders & Huber 2010; Love *et al.* 2014) with colony position (base vs. tip), time (day vs. night) and colony (genet) as the factors. An interaction effect between position and time factors was also tested. Colony (genet) was included as a factor only to control for inter-colony variation. An adjusted p-value < 0.05 was used to evaluate significance (Benjamini & Hochberg 1995). For the colony position factor, gene expression values in

bases were assigned as the baseline for comparison, so DE transcripts are considered 'up-regulated' or 'down-regulated' in tips, relative to bases. For the time of day factor, gene expression values in night samples were assigned as the baseline, so DE transcripts are considered 'up-regulated' or 'down-regulated' during the day. The moderated $\log_2$(fold change) values provided are maximum *a posteriori* estimations based on data-driven prior distributions calculated in DESeq2 (Love *et al.* 2014).

Functional genetic pathway analysis

Using UniProt accession IDs, contigs were annotated with Gene Ontology (GO) terms (www.geneontology.org). Transcripts of interest were determined based on GO terms and a review of the literature for transcripts that were DE between day and night, DE for both factors or that had a significant interaction between the two primary factors. Heat maps of gene expression data were created using log-transformed normalized count data in GENE-E (Gould). For the cryptochrome genes, an additional annotation step was performed using nucleotide BLAST (blastn) through NCBIs reference databases to more precisely discuss their known roles as putative circadian and light-response genes in cnidarians.

Results & Discussion

For the twelve samples (six sets of paired samples) RNA-seq libraries ranged in size from 8.4 to 22.5 million reads (16.6 million ± 1.2 million, mean ± SE) (Supplementary File S1), with 4.1 to 15.8 million reads (9.2 million ± 0.9 million) mapping to coral transcripts. PERMANOVA analysis revealed significant differences in transcriptome-wide gene expression patterns between branch bases and tips (d.f. = 1, Pseudo-F = 5.9631, P = 0.001), but not between day and night (d.f. = 1, Pseudo-F = 1.9308, P = 0.072) or among colonies (d.f. = 1, Pseudo-F = 1.9992, P = 0.08). The

interaction between colony position and time of day was also not significant (d.f. = 1, Pseudo-F = 1.1943, P = 0.261). The difference in gene expression profiles between tips and bases can be seen in the nMDS plot (Figure 1), in which transcription profiles cluster according to colony position along the x-axis.

Of the 47,688 transcripts in the analysis, a greater number of DE transcripts were observed between tips and bases of the *A. cervicornis* colony (3005 transcripts) than between day and night (441 transcripts) (Figure 2A). This indicates strong within-colony division of labor (DOL) between branch tips and bases in addition to moderate diel variation. The 231 transcripts that differed by colony position and time of day or had a significant interaction effect demonstrate that gene expression patterns underlying the within-colony DOL include a diel component. Approximately 55% of the DE transcripts had strong protein annotations (Figure 2B; Supplementary File S2).

While corals have previously been shown to exhibit clear diel patterns of calcification (Barnes & Crossland 1980; Gladfelter 1983a; Moya 2006; Schneider *et al.* 2009) and tentacle behavior (Levy 2003; Levy *et al.* 2006), as well as light-responsive and circadian patterns of gene expression (Levy *et al.* 2007; Brady *et al.* 2011; Levy *et al.* 2011; Sorek *et al.* 2014), our results suggest that diel shifts in gene expression between mid-day and midnight occur at less than 1% of *A. cervicornis*'s transcriptome, and thus diel variation may be relatively modest compared to that observed in some other marine organisms, like mussels, where over 20% of the transcriptome shows diel oscillations (Connor & Gracey 2011). In *A. cervicornis*, genes that differed significantly between day and night were involved in a variety of processes including regulating circadian rhythm and photoreception, mediating stress responses and immunity, as well as nitrogen, lipid,

and carbohydrate metabolism and carbohydrate transport. Selected DE transcript expression patterns are shown in figure 3.

Circadian rhythms & Photoreception

Eight transcripts representing regulatory and response components of the animal circadian clock (circadian locomoter output cycles protein kaput, *clock*; neuronal PAS domain-containing protein 2, *npas2*; cryptochromes, *cry* & *Amcry*; d site-binding protein, *dbp;* and thyrotroph embryonic factor, *tef*) were DE by time of day (Table 1; Figure 4). Two transcripts of *Amcry1* and a transcript of *tef,* had a significant interaction effect in which each exhibited a much higher (doubled) fold change up-regulation in day in tips compared to bases. In animals, the core circadian oscillation of genes occurs through positive and negative transcriptional feedback loops. Our results indicate that some circadian genes are differentially expressed between day and night; however, further studies sampling at multiple time points and observing rhythmicity of gene expression under manipulated light conditions would be required to determine endogenous circadian regulation of these genes.

We observed down-regulation of the circadian transcription factor *clock* during the day, which is consistent with previous findings of down-regulation of *clock* in light in *A. millepora* adult colonies; although larvae were previously found to up-regulate *clock* in light (Brady et al. 2011). Interestingly, *npas2*, a *clock* paralog that acts as a distinct circadian regulator in some mammalian tissues (Reick 2001), and which has not previously been studied in cnidarians, was up-regulated during the day, indicating that Clock and NPAS2 may function independently of one another in cnidarians. The other circadian gene down-regulated during the day, *dbp,* is a circadian output PAR

bZip transcription factor that may enhance production of enzymes involved in detoxification and metabolism important for processing food (Gachon *et al.* 2006). Corals would have a greater requirement for such enzymes at night when a greater abundance of demersal planktonic prey are available for consumption (Heidelberg *et al.* 2004).

Cryptochromes (Crys) are blue light sensitive photolyase-like proteins that act as photoreceptors to entrain the circadian clock in insects, but not in mammals, where they act in a light-independent part of the circadian oscillator feedback loop (Lin & Todo 2005). Cnidarian *crys* are more closely related to their mammalian counterparts, yet cnidarian *cry* gene expression appears to be light-induced (Levy *et al.* 2007; Reitzel *et al.* 2010) with only *Amcry2* showing some circadian periodicity (Brady *et al.* 2011). Correlation of *Amcry2* with lunar light exposure may be involved in regulation of coral spawning behavior (Levy *et al.* 2007). In this dataset, *Amcry2* and a transcript annotating to a mammalian-type *cry2*, were up-regulated during the day; whereas two *Amcry1* transcripts were up-regulated during the day and had a significant interaction effect due to greater fold change in tips, similar to the circadian output transcription factor *tef*. This within-colony variation observed in the expression of *Amcry1* and *tef* genes might reflect an effect of *Symbiodinium* on coral circadian gene expression, possibly through photosynthetic activity which strongly alters the oxygen content of the polyp tissue (Kühl *et al.* 1995). In a previous study of symbiont-containing adult versus aposymbiotic *A. millepora* larvae, a higher fold change of *Amcry1* between light and dark was found for larva than adults (Brady *et al.* 2011). It is possible that *Amcry1* expression change may be affected in cells containing or located near a high concentration of *Symbiodinium* through the effect of redox state on the circadian clock (Stangherlin & Reddy 2013). In *A. cervicornis* during the day, polyps located in the branch bases having a high density of *Symbiodinium* are likely to experience higher levels of ROS, including hydrogen peroxide (H_2O_2),

compared to polyps in branch tips with a lower density of algal cells. H_2O_2 is able to diffuse through cell membranes (Lesser 2006), and could therefore move freely between the endoderm where *Symbiodinium* are located and ectoderm where cryptochrome proteins have been found (Levy *et al.* 2007). A link may exist between *tef* expression and ROS due to the potential circadian regulation of DNA repair and ROS stress response proteins by *tef*, which has been observed in zebrafish (Gavriouchkina *et al.* 2010). A possible connection between ROS stress and regulation of circadian genes in corals warrants further investigation.

An alternate hypothesis for a greater magnitude change in expression of *Amcry1* in tips is a potential role outside of circadian regulation, specifically in the blue-light-activated phototropic growth observed in branching *Acropora.* In *Acropora*, the production and direction of growth of new axial corallites respond to blue light (Kaniewska *et al.* 2009), but the mechanism for this is not known. Crys, which are known to modulate phototropic growth in plants (Goyal *et al.* 2013) and may play a phototaxic role in sponges (Rivera *et al.* 2012), should also be considered as a potential candidate for phototropism in corals.

Genes involved in production of components of rhodopsin-like photoreceptors were found to be DE both by time of day and colony position. Rhodopsin, comprised of a transmembrane opsin apoprotein with photosensitive retinal chromophore, is a low-light photoreceptor that allows nighttime vision in animals. The production of retinal from retinol (vitamin A) is catalyzed by the enzyme Retinol dehydrogenase (Rdh). One rhodopsin-like *opsin* was down-regulated during the day and three transcripts of *rdh* were observed with differing patterns of expression. *rdh3* was most highly expressed in tips at night, *rdh7* was most highly expressed in tips during the day, and

rdh8 was down-regulated in tips. In addition to its role in producing retinal, Rdh can also produce retinaldehyde, which can be further oxidized to retinoic acid, an important signaling molecule used in embryonic patterning as well as circadian gene regulation (Shirai *et al.* 2006). The multiple gene expression patterns found for *rdh* genes suggest that Rdhs are involved in multiple functional pathways. Up-regulation of *rdh7* in tips during the day suggests it may be involved in phototropic growth.

Stress response & immunity

Predictably, UV and ROS stress response genes were mostly up-regulated during the day, but interestingly the degree of up-regulation for some genes varied within the colony. The UV stress response gene *deoxyribodipyrimidine photo-lyase* (*phr*), which codes for a light-induced DNA-repair enzyme, was up-regulated both during the day and also in branch tips. Photoreactivation, the light-induced repair of UV-damaged DNA through correction of pyrimidine dimers, is the primary DNA-repair mechanism in coral larvae (Reef *et al.* 2009), and it appears to be important for mature colonies as well, particularly in the growing tips. Growing tips may be more vulnerable to UV-induced DNA damage due to the production of new polyps through asexual reproduction, the high rate of mitosis, and the lower concentration of *Symbiodinium*, which absorb and block UVR through shading and mycosporine-like amino acids (MAAs; Dunlap & Shick 1998).

Additionally, while three transcripts of GFP-like fluorescent chromoproteins (FP486 and FP506) were up-regulated in tips only, one *GFP-like non-fluorescent chromoprotein* (*gtCP*), was up-regulated both in tips and during the day. Elevated expression of GFP-like proteins in growing regions of corals, has previously been observed in *Acropora* and other species (Bay *et al.* 2009;

D'Angelo *et al.* 2012), and up-regulation during the day is consistent with the observed increase in transcription and production of GFP-like proteins in response to blue light (D'Angelo *et al.* 2008). Our results indicate that different chromoproteins may be functioning differently within the colony. Numerous roles have been suggested for GFP-like proteins, including photoprotection, stress/redox response, symbiont interactions and a potential role in growth (D'Angelo *et al.* 2008). Higher expression of both known and putative UV stress genes in branch tips suggests that this region of the colony must cope with elevated UV stress compared to branch bases.

Excessive oxidative stress resulting from ROS produced by *Symbiodinium* and coral under high light and temperature conditions is believed to be the primary cause of mass coral bleaching episodes (Weis 2008). Multiple oxidative stress response genes have been proposed to be important in maintaining the symbiotic partnership (Downs *et al.* 2002; DeSalvo *et al.* 2008; Sunagawa *et al.* 2009). Three of these genes showed both diel and within-colony differential expression. These include *thioredoxin, glutathione S-transferase* (*gst*), and *extracellular superoxide dismutase* (*sod*). Thioredoxin is a small redox protein found in all organisms that can participate in redox signaling or antioxidant activity by reducing other proteins by cysteine thiol-disulfide exchange. GST is an enzyme that inactivates the oxidizing activity of substrates by conjugation with the antioxidant glutathione. Both *thioredoxin* and *gst* were up-regulated during the day, particularly in branch tips. Higher ROS during the day resulting from increased symbiont photosynthesis is expected to increase oxidative pressure on the host and trigger elevated expression of oxidative stress response proteins. However, these redox proteins were also up-regulated in tips, which due to a lower concentration of photosymbionts are expected to have lower ROS stress. One explanation for up-regulation in tips may be that UVR itself can induce ROS in animal tissues (Pourzand & Tyrrell 1999). Elevated UVR exposure in branch tips, which are shallower than bases and probably

benefit less from symbiont-produced photo-protective MAAs, may account for the observed elevated oxidative stress response. Alternatively, production of antioxidants by the symbiont itself may alleviate the burden of oxidative stress in symbiont-rich regions of the coral (Rodriguez-Lanetty *et al.* 2006). Interestingly, although counterintuitive based on the expectation of ROS generation by algal symbionts (Lesser 1997; 2006), an inverse relationship between *Symbiodinium* and ROS response genes, including *gst* and *sod*, has been observed in multiple studies comparing symbiotic and non-symbiotic cnidarians (Rodriguez-Lanetty *et al.* 2006; Ganot *et al.* 2011; Lehnert *et al.* 2014). One transcript of *sod* had a significant interaction effect with the lowest expression during the day in tips and highest at night in tips. SOD is an antioxidant enzyme that catalyzes the dismutation of the free radical superoxide ($\bullet O_2^-$) into hydrogen peroxide (H_2O_2). The pattern of expression observed for *sod* suggests that it has a function other than (or in addition to) coping with *Symbiodinium*-produced ROS, possibly related to ROS produced during metabolism of heterotrophically obtained food. As mentioned earlier, transcription of stress response genes may also be directly or indirectly linked to circadian rhythm. For example, in mammals *gst* transcription may be stimulated by *tef* and/or *dbp* transcription factors (Gachon *et al.* 2006).

Three iron homeostasis genes with putative roles in oxidative stress response, *heme-binding protein 2* (*hebp2*), *soma ferritin* (*sof*), and *mitoferrin-2,* were up-regulated during the day, indicating differential use of iron between day and night. The reaction of ferrous iron (Fe^{2+}) with H_2O_2 produces the highly reactive hydroxyl radical ($\bullet OH$); therefore, proteins that chelate iron or bind heme can act as antioxidants (Yu 1994). In this dataset, *hebp2* had a similar expression pattern with *Amcry1* and *tef*, being up-regulated during the day with a 2-fold greater change in tips than bases. *hebp2* was previously observed to follow a synexpression pattern with *α-CA*, *cry*1 and *cry2* in

A. millepora, indicating a potential link between redox state, *hebp2* and circadian expression (Levy *et al.* 2011).

Regulation of Symbiodinium by the coral host

The breakdown of the coral-algal symbiosis during coral bleaching under thermal and UV stress is considered one of the most serious threats to the future of coral reefs (Hoegh-Guldberg 1999). The healthy coral-algal symbiosis depends on the coral regulating the population biomass of algae through three proposed mechanisms: expulsion or degradation of excess symbionts or inhibition of symbiont growth and division (Davy *et al.* 2012). We identified two candidate genes that may be involved in such regulation: a nitrogen metabolism gene, *glutamine synthetase (gs)*, and an immunity-related gene, *hemagglutinin/amebocyte aggregation factor* (*haaf*). In addition to *gs*, other glutamine/glutamate pathway genes (Figure 5) had diel and within-colony differences, indicating that the coral may use transcriptional regulation of this nitrogen metabolic pathway to regulate its symbiont population.

Nitrogen metabolism may play an important role in regulating *Symbiodinium* population growth by restricting the available nitrogen (Rees 1986). Glutamine synthetase (GS), which is required for the assimilation of nitrogen from ammonium into glutamine (Figure 5), has been suggested as a mechanism of symbiont control (Rees 1986). The pattern of *gs* expression, which was higher in branch bases than branch tips during the day, with a significant interaction effect, supports the hypothesis of nitrogen control in symbiont-rich base polyps during the day when *Symbiodinium* growth would be at its height (Wang *et al.* 2008). However, it should be noted that GS protein levels may also be regulated post-translationally by the level of glutamine present (Labow

et al. 2001). Interestingly, the highest expression of *gs* was in symbiont-poor tips at night, which may be related to processing nitrogen waste derived from heterotrophy. *Glutamate synthase [NADH] (gogat),* which breaks down glutamine to glutamate, was elevated at night, particularly in branch bases. GOGAT produces two molecules of glutamate from glutamine and 2-oxoglutarate, without producing ammonia. Glutamate is an excitatory amino acid that may be used in signaling and to synthesize other amino acids. In a study of symbiotic and aposymbiotic *Aiptasia*, Lehnert et al. (2014) observed increased *gs* and *gogat* expression in symbiotic anemones, supporting the idea that host cnidarians alter their nitrogen metabolism in the presence of *Symbiodinium*. Genes for carbamoyl-phosphate synthase I (CPS1), which catalyzes the synthesis of carbamoyl phosphate from glutamine-derived ammonia and bicarbonate, and a putative glutamine amidotransferase (GATase), which transfers the ammonia group from glutamine to other substrates, were up-regulated during the day and had the lowest expression in bases at night. The expression patterns of these genes suggest that nitrogen cycling is elevated in bases during the day, where there is a higher concentration of symbionts, and that nitrogen availability for *Symbiodinium* may be controlled by conversion to glutamine followed by transfer of the ammonia group to carbamoyl phosphate and ultimately to urea or to other molecules.

The daytime up-regulation of the immune response gene *haaf* in bases suggests enhanced amebocyte activity to promote phagocytic clearance of dysfunctional or damaged *Symbiodinium*. Mobile amebocytes are part of the coral's innate immune response and remove infecting pathogens or necrotic tissue via phagocytosis (Libro *et al.* 2013), but *haaf* and amebocytes have also been found to respond to elevated heat stress in soft corals (octocorallia) (Woo *et al.* 2006; Mydlarz *et al.* 2008). It has been suggested that amebocytes may be activated under heat stressed conditions to dispose of damaged *Symbiodinium* cells (Strychar *et al.* 2004). Phagocytosis is one potential

mechanism of coral bleaching, but may also be a way for corals to manage symbiont populations when subjected to stressful (e.g. high light and ROS) conditions, even when bleaching is not the ultimate outcome (Stat *et al.* 2006). Because no sign of disease, bleaching or wounding was observed on these corals prior to collection and there was no difference in the way the corals were collected, the location and timing of elevated expression of an amebocyte-related gene suggests a role for these cells in regulating *Symbiodinium* populations under high light conditions.

Light-enhanced calcification

In *Acropora spp.* quantity and quality of $CaCO_3$ deposited into the skeleton as well as mitotic activity differ between day and night. At night the linear branch extension rate and mitotic activity are higher and $CaCO_3$ crystals accumulate in random orientation at the growing edge of the skeleton (Barnes & Crossland 1977; Gladfelter 1983a; Gladfelter *et al.* 1989). During the day, a larger quantity of $CaCO_3$ is incorporated to reinforce the skeleton (Barnes & Crossland 1977; Gladfelter 1983a). Functionally, this suggests that nighttime conditions promote abiotic precipitation of $CaCO_3$, while in the daytime coral-regulated activity requiring energetic inputs, such as skeletal organic matrix (SOM), encourages directed skeleton formation. We have identified two genes with putative roles in LEC, *carbonic anhydrase 2* (*CA2)* and *mucin-4,* and multiple genes that may provide the energy for LEC through metabolic functions or transport.

Two transcripts of *CA2*, an α-CA, were up-regulated in tips and also up-regulated during the day; however another transcript of *CA2* was up-regulated in bases but did not show differential expression between day and night. *Carbonic anhydrase* (CA), including *CA2*, was previously identified as highly up-regulated in the growing tips of *Acropora* corals (Hemond *et al.* 2014) and is

known to be involved in coral skeleton growth (Goreau 1959; Tambutté *et al.* 2006). CA, which catalyzes the interconversion of carbon dioxide and water to bicarbonate and protons, may participate in coral chemistry by providing bicarbonate for $CaCO_3$ production and by regulating pH, but it may also provide CO_2 for symbiont photosynthesis (Bertucci *et al.* 2013). Increased expression of CA during the day in colonies of *A. millepora* (Levy *et al.* 2011) supports a possible role of CA in LEC.

A potential SOM protein, *mucin-4*, that was up-regulated during the day in tips is similar to a protein extracted from *A. millepora* SOM (Ramos-Silva *et al.* 2013) and may play a role in LEC. Mucins, which are the primary functional protein component of the gel-like mucus secreted by corals to protect against stresses and trap food particles, may also have been some of the first molecules that evolved to control biomineralization (Marin *et al.* 2000). Examples of mucin-like calcification-related proteins have previously been found in corals, galaxin (Reyes-Bermudez *et al.* 2009), and molluscs, mucoperlin (Marin *et al.* 2000).

Carbohydrate and lipid metabolism and transport

Carbon metabolic processes, particularly mitochondrial respiration, were largely up-regulated during the day, consistent with a previous study in *A. millepora* (Brady *et al.* 2011). This should correspond to the increased production of photosynthetically-fixed carbon by *Symbiodinium*. Elevated respiration in tips indicates a high amount of energy consumption involved with growth during the day, which may permit the increase in mitotic activity at night previously observed in *A. cervicornis* tips (Gladfelter 1983b).

The elevated expression of lipid synthesis genes in branch bases (e.g. *fatty acid synthase*) observed in this study confirms a similar finding previously observed for *Acropora* corals (Hemond *et al.* 2014). Lipid synthesis also appears to be elevated during the day (*acyl-coA desaturase*), which may be a result of increased effort to store energy produced by symbiont photosynthesis. Up-regulation of lipid synthesis, storage, and catabolism genes has previously been associated with the symbiotic state (Ganot *et al.* 2011; Lehnert *et al.* 2014). However, lipid synthesis may also be increased in branch bases to support the high lipid requirements of gamete (egg) production (Arai *et al.* 1993) in bases, an activity suggested by elevated expression of the egg yolk protein gene *vitellogenin*.

Lipid metabolism genes that were up-regulated in tips have putative roles in arachidonic acid metabolism and eicosanoid production. These include *delta(5) fatty acid desaturase*, which was also up-regulated during the day, and *phospholipase A2*, which was also down-regulated during the day. Eicosanoids are thought to function as part of the coral's innate immune system (Libro *et al.* 2013). Interestingly, *arachidonate 5-lipoxygenase*, involved in production of leukotriene eicosanoids, and seven transcripts of *allene oxide synthase-lipoxygenase*, which are involved in biosynthesis of allene oxide from arachidonic acid, were also up-regulated in tips. Increased phenoloxidase activity in coral growth zones observed by D'Angelo *et al*. (2012) indicates that multiple immune pathways may be up-regulated in growing tissues of corals. Another interpretation can be that immune response is down-regulated in mature polyps to facilitate the symbiosis with *Symbiodinium* (Lehnert *et al.* 2014). The highest expression of *phospholipase A2* at night in tips, however, suggests that the protein may act as a toxin in the nematocyst complex (Nevalainen *et al.* 2004).

Transport of carbohydrates across organelle membranes and among polyps is critical for coral growth. *Symbiodinium*, the coral's primary source of carbohydrates, are isolated within symbiosomes, vacuoles comprised of both coral-derived and symbiont-derived membranes (Wakefield *et al.* 2000). In *Acroporas*, carbohydrates must also be transported from radial polyps to the growing tip of the branch (Pearse & Muscatine 1971). The three carbohydrate transport genes that were up-regulated during the day (*slc5a2*, *slc5a10*, *slc5a11*) are sodium/glucose and sodium/myo-inositol transporters, which have previously been observed to be up-regulated in symbiotic compared to aposymbiotic anemones (Lehnert *et al.* 2014). These are strong candidates for involvement in transfer of fixed carbon involved in LEC (Pearse & Muscatine 1971).

Conclusion

Though conditions within the coral animal have previously been shown to fluctuate dramatically between day and nighttime conditions, the coral response via gene expression is relatively modest. Transcriptomic differences between day and night reflect putative circadian clock functions, response to light and oxidative stress, lipid and nitrogen metabolic functions and carbohydrate transport. By exploring the effect of location within the colony (branch bases versus branch tips) on diurnal versus nocturnal gene expression, we have identified genes that show within-colony temporal differences and provide candidates for further exploration into LEC, phototaxis and the regulation of *Symbiodinium* by the host coral. These results support roles for carbonate ion regulation (*CA2*) and a mucin SOM protein in LEC, and two photoreceptor-related genes, a retinol dehydrogenase and *Amcry1*, in blue-light phototaxis. Furthermore, these results support the hypothesis that corals control *Symbiodinium* population densities by limiting nitrogen availability through uptake of ammonia by Glutamine synthetase activity, and additionally suggest removal of dysfunctional symbionts by phagocytotic amebocytes. Symbionts may also affect the

coral circadian rhythms through an effect of oxidative stress on circadian gene regulation. These findings add to a growing body of knowledge about coral growth and coral host-symbiont interactions.

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References

Anders S, Huber W (2010) Differential expression analysis for sequence count data. *Genome Biology*, **11**, R106.

Arai I, Kato M, Heyward A *et al.* (1993) Lipid composition of positively buoyant eggs of reef building corals. *Coral Reefs*, **12**, 71–75.

Barnes DJ, Crossland CJ (1977) Diurnal productivity and apparent 14C-calcification in the staghorn coral, *Acropora acuminata*. *Comparative biochemistry and physiology. A, Comparative physiology*, **59**, 133–138.

Barnes DJ, Crossland CJ (1980) Diurnal and seasonal-variations in the growth of a staghorn coral measured by time-lapse photography. *Limnology and Oceanography*, **25**, 1113–1117.

Bay LK, Nielsen HB, Jarmer H, Seneca F, van Oppen MJH (2009) Transcriptomic variation in a coral reveals pathways of clonal organisation. *Marine Genomics*, **2**, 119–125.

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society, Series B*, **57**, 289–300.

Bertucci A, Moya A, Tambutté S *et al.* (2013) Carbonic anhydrases in anthozoan corals - A review. *Bioorganic & Medicinal Chemistry*, **21**, 1437–1450.

Brady AK, Hilton JD, Vize PD (2009) Coral spawn timing is a direct response to solar light cycles and is not an entrained circadian response. *Coral Reefs*, **28**, 677–680.

Brady AK, Snyder KA, Vize PD (2011) Circadian cycles of gene expression in the coral, *Acropora millepora*. *PLoS ONE*, **6**, e25072.

Connor KM, Gracey AY (2011) Circadian cycles are the dominant transcriptional rhythm in the intertidal mussel *Mytilus californianus*. *Proceedings of the National Academy of Sciences USA*, **108**, 16110–16115.

Covington MF, Maloof JN, Straume M, Kay SA, Harmer SL (2008) Global transcriptome analysis reveals circadian regulation of key pathways in plant growth and development. *Genome Biology*, **9**, R130.

D'Angelo C, Denzel A, Vogt A *et al.* (2008) Blue light regulation of host pigment in reef-building corals. *Marine Ecology Progress Series*, **364**, 97–106.

D'Angelo C, Smith EG, Oswald F *et al.* (2012) Locally accelerated growth is part of the innate immune response and repair mechanisms in reef-building corals as detected by green fluorescent protein (GFP)-like pigments. *Coral Reefs*, **31**, 1045–1056.

Davy SK, Allemand D, Weis VM (2012) Cell biology of cnidarian-dinoflagellate symbiosis. *Microbiology and Molecular Biology Reviews*, **76**, 229–261.

DeSalvo MK, Voolstra CR, Sunagawa S *et al.* (2008) Differential gene expression during thermal stress and bleaching in the Caribbean coral *Montastraea faveolata*. *Molecular Ecology*, **17**, 3952–3971.

Downs CA, Fauth JE, Halas JC *et al.* (2002) Oxidative stress and seasonal coral bleaching. *Free Radical Biology and Medicine*, **33**, 533–543.

Dunlap WC, Shick JM (1998) Ultraviolet radiation-absorbing mycosporine-like amino acids in coral reef organisms: a biochemical and environmental perspective. *Journal of Phycology*, **34**, 418–430.

Duong HA, Robles MS, Knutti D, Weitz CJ (2011) A molecular mechanism for circadian clock negative feedback. *Science*, **332**, 1436–1439.

Falkowski PG, Dubinsky Z, Muscatine L, Porter JW (1984) Light and the bioenergetics of a symbiotic coral. *Bioscience*, **34**, 705–709.

Gachon F, Olela FF, Schaad O, Descombes P, Schibler U (2006) The circadian PAR-domain basic leucine zipper transcription factors DBP, TEF, and HLF modulate basal and inducible xenobiotic detoxification. *Cell Metabolism*, **4**, 25–36.

Ganot P, Moya A, Magnone V *et al.* (2011) Adaptations to endosymbiosis in a cnidarian-dinoflagellate association: differential gene expression and specific gene duplications. *PLoS Genetics*, **7**, e1002187.

Gavriouchkina D, Fischer S, Ivacevic T *et al.* (2010) Thyrotroph embryonic factor regulates light-induced transcription of repair genes in zebrafish embryonic cells. *PLoS ONE*, **5**, e12542.

Gekakis N (1998) Role of the CLOCK protein in the mammalian circadian mechanism. *Science*, **280**, 1564–1569.

Gladfelter EH (1982) Skeletal development in *Acropora cervicornis*: I. Patterns of calcium carbonate accretion in the axial corallite. *Coral Reefs*, **1**, 45–51.

Gladfelter EH (1983a) Skeletal development in *Acropora cervicornis* II: Diel patterns of calcium carbonate accretion. *Coral Reefs*, **2**, 91–100.

Gladfelter EH (1983b) Spatial and temporal patterns of mitosis in the cells of the axial polyp of the reef coral *Acropora cervicornis*. *Biological Bulletin*, **165**, 811–815.

Gladfelter EH, Michel G, Sanfelici A (1989) Metabolic gradients along a branch of the reef coral *Acropora palmata*. *Bulletin of Marine Science*, **44**, 1166–1173.

Goreau TF (1959) The physiology of skeleton formation in corals. I. A method for measuring the rate of calcium deposition by corals under different conditions. *Biological Bulletin*, **116**, 59–75.
Goreau TF, Goreau N (1959) The physiology of skeleton formation in corals. II. Calcium deposition by hermatypic corals under various conditions in the reef. *Biological Bulletin*, **117**, 239–250.
Gould J. *GENE-E*. http://www.broadinstitute.org/cancer/software/GENE-E/
Goyal A, Szarzynska B, Fankhauser C (2013) Phototropism: at the crossroads of light-signaling pathways. *Trends in Plant Science*, **18**, 393–401.
Heidelberg KB, Sebens KP, Purcell JE (2004) Composition and sources of near reef zooplankton on a Jamaican forereef along with implications for coral feeding. *Coral Reefs*, **23**, 263–276.
Hemond EM, Kaluziak ST, Vollmer SV (2014) The genetics of colony form and function in Caribbean *Acropora* corals. *BMC Genomics*, **15**, 1133.
Hoegh-Guldberg O (1999) Climate change, coral bleaching and the future of the world's coral reefs. *Marine and Freshwater Research*, **50**, 839.
Kaniewska P, Campbell PR, Fine M, Hoegh-Guldberg O (2009) Phototropic growth in a reef flat acroporid branching coral species. *Journal of Experimental Biology*, **212**, 662–667.
Kühl M, Cohen Y, Dalsgaard T, Jorgensen BB, Revsbech NP (1995) Microenvironment and photosynthesis of zooxanthellae in scleractinian corals studied with microsensors for O_2, pH and light. *Marine Ecology Progress Series*, **117**, 159–172.
Labow BI, Souba WW, Abcouwer SF (2001) Mechanisms governing the expression of the enzymes of glutamine metabolism - glutaminase and glutamine synthetase. *The Journal of Nutrition*, **131,** 2467S-2474S.
Lehnert EM, Mouchka ME, Burriesci MS *et al.* (2014) Extensive differences in gene expression between symbiotic and aposymbiotic cnidarians. *G3*, **4,** 277-295.
Lesser MP (1996) Elevated temperatures and ultraviolet radiation cause oxidative stress and inhibit photosynthesis in symbiotic dinoflagellates. *Limnology and Oceanography*, **41**, 271–283.
Lesser MP (1997) Oxidative stress causes coral bleaching during exposure to elevated temperatures. *Coral Reefs*, **16**, 187–192.
Lesser MP (2006) Oxidative stress in marine environments: Biochemistry and physiological ecology. *Annual Review of Physiology*, **68**, 253–278.
Levy O (2003) Photobehavior of stony corals: responses to light spectra and intensity. *Journal of Experimental Biology*, **206**, 4041–4049.
Levy O, Appelbaum L, Leggat W *et al.* (2007) Light-responsive cryptochromes from a simple multicellular animal, the coral *Acropora millepora*. *Science*, **318**, 467–470.
Levy O, Dubinsky Z, Achituv Y, Erez J (2006) Diurnal polyp expansion behavior in stony corals may enhance carbon availability for symbionts photosynthesis. *Journal of Experimental Marine Biology and Ecology*, **333**, 1–11.
Levy O, Kaniewska P, Alon S *et al.* (2011) Complex diel cycles of gene expression in coral-algal symbiosis. *Science*, **331**, 175–175.
Libro S, Kaluziak ST, Vollmer SV (2013) RNA-seq profiles of immune related genes in the staghorn coral *Acropora cervicornis* infected with white band disease. *PLoS ONE*, **8**, e81821.
Lin C, Todo T (2005) The cryptochromes. *Genome Biology*, **6**, 220.
Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-Seq data with DESeq2. *Genome Biology*, **5,** 550.

Marin F, Corstjens P, de Gaulejac B, de Vrind-De Jong E, Westbroek P (2000) Mucins and molluscan calcification: molecular characterization of mucoperlin, a novel mucin-like protein from the nacreous shell layer of the fan mussel *Pinna nobilis* (Bivalvia, Pteriomorphia). *The Journal of Biological Chemistry,* **275,** 20667-20675.

Moya A (2006) Study of calcification during a daily cycle of the coral *Stylophora pistillata*: implications for `light-enhanced calcification'. *Journal of Experimental Biology*, **209**, 3413–3419.

Muscatine L, Falkowski PG, Porter JW, Dubinsky Z (1984) Fate of photosynthetic fixed carbon in light- and shade-adapted colonies of the symbiotic coral *Stylophora pistillata*. In:, pp. 181–202.

Mydlarz LD, Holthouse SF, Peters EC, Harvell CD (2008) Cellular responses in sea fan corals: granular amoebocytes react to pathogen and climate stressors (RC May, Ed,). *PLoS ONE*, **3**, e1811.

Nevalainen TJ, Peuravuori HJ, Quinn RJ *et al.* (2004) Phospholipase A2 in Cnidaria. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, **139**, 731–735.

Oksanen J, Blanchet FG, Kindt R *et al.* (2013) vegan: community ecology package.

Pearse VB, Muscatine L (1971) Role of symbiotic algae (zooxanthellae) in coral calcification. *Biological Bulletin*, **141**, 350–363.

Pourzand C, Tyrrell RM (1999) Apoptosis, the role of oxidative stress and the example of solar UV radiation. *Photochemistry and Photobiology*, **70,** 380-390.

Ramos-Silva P, Kaandorp JA, Huisman L *et al.* (2013) The skeletal proteome of the coral *Acropora millepora*: the evolution of calcification by co-option and domain shuffling. *Molecular Biology and Evolution*, **30**, 2099–2112.

Reef R, Dunn S, Levy O *et al.* (2009) Photoreactivation is the main repair pathway for UV-induced DNA damage in coral planulae. *Journal of Experimental Biology*, **212**, 2760–2766.

Rees TAV (1986) The green hydra symbiosis and ammonium I. the role of the host in ammonium assimilation and its possible regulatory significance. *Proceedings of the Royal Society B: Biological Sciences*, **229**, 299–314.

Reick M (2001) NPAS2: An analog of clock operative in the mammalian forebrain. *Science*, **293**, 506–509.

Reitzel AM, Behrendt L, Tarrant AM (2010) Light entrained rhythmic gene expression in the sea anemone *Nematostella vectensis*: the evolution of the animal circadian clock. *PLoS ONE*, **5**, e12805.

Reitzel AM, Tarrant AM, Levy O (2013) Circadian clocks in the cnidaria: environmental entrainment, molecular regulation, and organismal outputs. *Integrative and Comparative Biology*, **53**, 118–130.

Reyes-Bermudez A, Lin Z, Hayward DC, Miller DJ, Ball EE (2009) Differential expression of three galaxin-related genes during settlement and metamorphosis in the scleractinian coral *Acropora millepora*. *BMC Evolutionary Biology*, **9,** 178.

Rivera AS, Ozturk N, Fahey B *et al.* (2012) Blue-light-receptive cryptochrome is expressed in a sponge eye lacking neurons and opsin. *Journal of Experimental Biology*, **215**, 1278–1286.

Rodriguez-Lanetty M, Phillips WS, Weis VM (2006) Transcriptome analysis of a cnidarian-dinoflagellate mutualism reveals complex modulation of host gene expression. *BMC Genomics*, **7**, 23.

Schneider K, Levy O, Dubinsky Z, Erez J (2009) In-situ diel cycles of photosynthesis and calcification

in hermatypic corals. *Limnology and Oceanography*, **54**, 1995.
Shinzato C, Shoguchi E, Kawashima T *et al.* (2011) Using the *Acropora digitifera* genome to understand coral responses to environmental change. *Nature*, **476**, 320–323.
Shirai H, Oishi K, Ishida N (2006) Bidirectional CLOCK/BMAL1-dependent circadian gene regulation by retinoic acid *in vitro*. *Biochemical and Biophysical Research Communications*, **351**, 387–391.
Shoguchi E, Tanaka M, Shinzato C, Kawashima T, Satoh N (2013) A genome-wide survey of photoreceptor and circadian genes in the coral, *Acropora digitifera*. *Gene*, **515**, 426–431.
Sorek M, Diaz-Almeyda EM, Medina M, Levy O (2014) Circadian clocks in symbiotic corals: The duet between *Symbiodinium* algae and their coral host. *Marine Genomics*, **14**, 47–57.
Stanewsky R, Kaneko M, Emery P *et al.* (1998) The cryb mutation identifies cryptochrome as a circadian photoreceptor in *Drosophila*. *Cell*, **95**, 681–692.
Stangherlin A, Reddy AB (2013) Regulation of circadian clocks by redox homeostasis. *Journal of Biological Chemistry*, **288**, 26505–26511.
Stat M, Carter D, Hoegh-Guldberg O (2006) The evolutionary history of *Symbiodinium* and scleractinian hosts—Symbiosis, diversity, and the effect of climate change. *Perspectives in Plant Ecology, Evolution and Systematics*, **8**, 23–43.
Strychar KB, Coates M, Sammarco PW, Piva TJ (2004) Bleaching as a pathogenic response in scleractinian corals, evidenced by high concentrations of apoptotic and necrotic zooxanthellae. *Journal of Experimental Marine Biology and Ecology*, **304**, 99–121.
Sunagawa S, Wilson EC, Thaler M *et al.* (2009) Generation and analysis of transcriptomic resources for a model system on the rise: the sea anemone *Aiptasia pallida* and its dinoflagellate endosymbiont. *BMC Genomics*, **10**, 258.
Szmant AM (1986) Reproductive ecology of Caribbean reef corals. *Coral Reefs*, **5**, 43–53.
Tambutté S, Tambutté E, Zoccola D *et al.* (2006) Characterization and role of carbonic anhydrase in the calcification process of the azooxanthellate coral *Tubastrea aurea*. *Marine Biology*, **151**, 71–83.
Tsang WH, McGaughey NJ, Wong YH, Wong J (1997) Melatonin and 5-methoxytryptamine induced muscular contraction in sea anemones. *Journal of Experimental Zoology Part A: Comparative Experimental Biology*, **279**, 201–207.
Venables WN, Ripley BD (2002) *Modern Applied Statistics with S*. Fourth Edition. Springer, New York.
Vize PD (2009) Transcriptome analysis of the circadian regulatory network in the coral *Acropora millepora*. *Biological Bulletin*, **216**, 131–137.
Wakefield TS, Farmer MA, Kempf SC (2000) Revised description of the fine structure of in situ "zooxanthellae" genus *Symbiodinium*. *Biological Bulletin*, **199**, 76–84.
Wang LH, Liu YH, Ju YM *et al.* (2008) Cell cycle propagation is driven by light–dark stimulation in a cultured symbiotic dinoflagellate isolated from corals. *Coral Reefs*, **27**, 823–835.
Weis VM (2008) Cellular mechanisms of cnidarian bleaching: stress causes the collapse of symbiosis. *Journal of Experimental Biology*, **211**, 3059–3066.
Woo S, Yum S, Kim YT *et al.* (2006) Thermal and organic chemical stress responsive genes in soft coral, *Scleronephthya gracillimum*. *Molecular & Cellular Toxicology*, **2**, 170–175.
Yu BP (1994) Cellular defenses against damage from reactive oxygen species. *Physiological reviews*, **74**, 139–162.

Supporting Information

Supplementary File S1. Mean and standard error of RNA-seq library sizes and number of mapped reads for each treatment group of samples.

Supplementary File S2. Data set of annotated (UniProt/Swiss-Prot E-val < 10^{-5}) coral transcripts. Includes adjusted P and moderated $\log_2$(fold change) values, GO terms and putative functions of genes discussed in the manuscript and/or included in Table 1. * Indicates annotation from GenBank.

Data Accessibility

The reference transcriptome sequences are available on Bioproject [accession number PRJNA222758]. Demultiplexed Illumina read data (.fastq) and normalized read count data (.csv) are available on Dryad doi:10.5061/dryad.7qq74.

Author Contributions

EMH and SVV conceived and designed the experiment and wrote the manuscript. EMH generated and analyzed the data.

Tables

Table 1. Number of differentially expressed transcripts for functional categories of interest. For transcripts with a significant interaction, *indicates also DE by colony position, †indicates also DE by time of day, §indicates DE for both factors.

Function	Total DE	Colony Position only	Time of Day only	Both Factors	Interaction
Circadian/photoreception					
Clock	1		1		
Cryptochrome	4		2		†2
D site-binding protein	1		1		
Melatonin receptor	1	1			
Melanopsin	1	1			
Npas2	1		1		
Nuclear receptor	2		1	1	
Other vision related	4	4			
Retinol dehydrogenase (3,7,8)	3	1		1	1
Rhodopsin-like opsin	1		1		
Thyrotroph embryonic factor	1				†1
Stress					
Chaperone (HSP, sacsin)	22	21	1		
Oxidative (thioredoxin, glutathione pathway, SOD, iron homeostasis)	23	17	2	2	*1, †1
UV (photolyase, GFP-like)	6	4		1	§1
Immune					
Hemagglutinin/amebocyte aggregation factor	4	3		1	
Glutamate/glutamine pathway					
Carbamoyl-phosphate synthetase	1		1		
Glutamine amidotransferase	1		1		
Glutamate synthase	1		1		
Glutamine synthetase	1				1
Lipid metabolism					
Carnitine synth. & transp.	3	2			1
Cytochrome P450	3	1	1		1
Fatty acid desaturase	4	2		1	†1
Fatty acid synthase	12	7		1	*3, §1
Mitochondrial metabolism					
ATP synthase	4	4			
Electron Transport Chain	10	4		6	
Carbohydrate transport					
Sodium/glucose transporter	2		2		
Sodium/myo-inositol cotransporter	1		1		
All annotated coral transcripts	1902	1593	176	42	29, *46, †11, §5
All coral transcripts	3428	2856	341	60	53, *78, †29, §11

Figure Legends

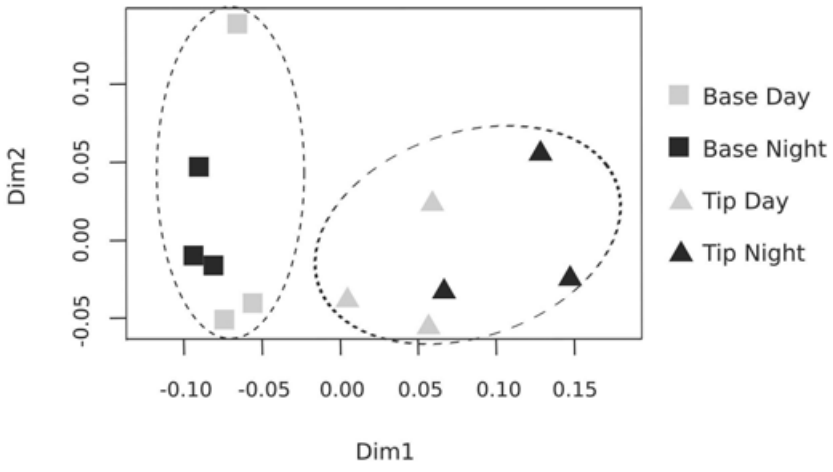
Figure 1. nMDS plot of transcription profiles for tips (triangles) and bases (squares), day (gray) and night (black). Clustering of base and tip samples indicated by dashed lines.

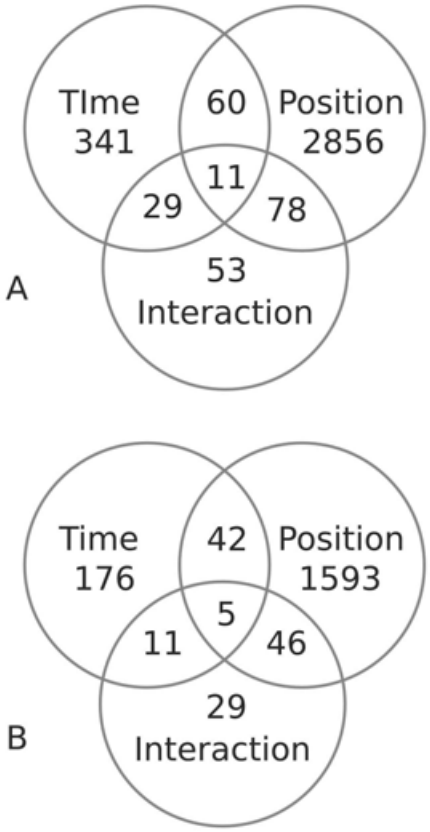
Figure 2. Number of DE genes for model factors (colony position and time) and their interaction considering all coral transcripts (A) and annotated transcripts (B).

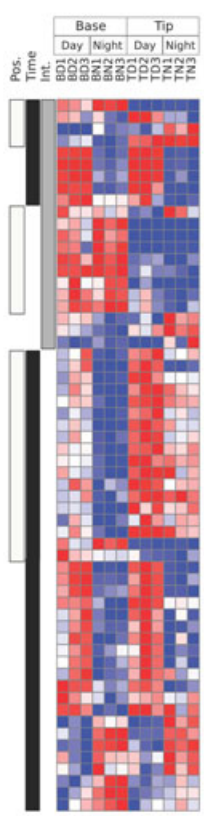
Figure 3. Heat map of selected annotated DE transcripts. Expression data is log-transformed. Significance of GLM tests indicated by bands to the left of expression data: time of day (black), colony position (light gray), interaction (dark gray). Moderated log2(fold change) for position (POS) and time of day (TIME) provided where significant (ns = not significant). Annotation ID for cryptochromes based on blastn results.

Figure 4. Possible circadian and light-responsive gene interactions and roles in diel processes in corals. Genes DE by time of day or showing an interaction between factors were up-regulated in day (left) or night (right). § indicates a significant interaction effect. Dashed lines indicate hypothetical relationships. Rdh enzymes (purple) may either produce retinal or retinaldehyde; retinal interacts with the opsin apoprotein (purple) to form rhodopsin, while retinaldehyde may be further reduced to retinoic acid. Amcrys and phr (gray), are in the photo-lyase family of light-responsive proteins. Cryptochrome expression may regulate the circadian clock, Clock and NPAS2 activity in a light-dependent manner. Clock and NPAS2 proteins (red) are two of the primary bHLH-PAS transcription factors that, when bound to BMAL1 as a heterodimer, direct the circadian rhythm of animals and activate expression of Crys. TEF and DBP PAR bZip transcription factors (green) activate transcription of circadian-regulated genes.

Figure 5. Glutamate and glutamine biosynthesis pathways including enzymes (boxes) with genes DE by time of day or with an interaction (black text) and present but non-DE enzymes (gray text). §Indicates significant interaction effect. Enzymes are GLDH, Glutamate dehydrogenase; GOGAT, Glutamate synthase; GLN, Glutaminase; GS, Glutamine synthetase; GATase, Glutamine amidotransferase; and CPSI, Carbamoyl phosphate synthetase I.







Description	Uniprot ID	log_POS	log_TIME
Fatty acid synthase	P12276	-3.2	-1.5
GFP-like non-fluorescent chromoprotein	Q95P04	1.7	1.4
Cytosolic phospholipase A2	Q7T0T9	1.0	-1.2
VWFA and cache domain-containing protein 1	Q6PDJ1	1.3	0.7
Thyrotroph embryonic factor	Q96LC6	ns	5.3
A. millepora cryptochrome-1	GB:EF202589	ns	5.2
A. millepora cryptochrome-1	GB:EF202589	ns	4.8
Heme-binding protein 2	Q9Y5Z4	ns	4.3
Acyl-CoA desaturase	Q92038	ns	1.7
Extracellular superoxide dismutase [Cu-Zn]	P34461	-1.0	ns
Fatty acid synthase	P36189	-2.4	ns
Fatty acid synthase	P12276	-3.2	ns
Fatty acid synthase	P12785	-2.4	ns
Vitellogenin-6	P18948	-3.3	ns
Casein kinase I	Q6QNM1	-2.9	ns
Hemicentin-1	Q9GRW7	-2.6	ns
Collagen triple helix repeat-containing protein 1	Q8CG08	-1.9	ns
Collagen alpha-1(8) chain	P28481	-2.0	ns
Glutamine synthetase	P51121	ns	ns
Mitochondrial carnitine/acylcarnitine carrier protein	Q9ZJZ6	ns	ns
Retinol dehydrogenase 3	P50169	ns	ns
Excitatory amino acid transporter 1	O57321	1.1	1.8
Nuclear receptor subfamily 2 group F member 5	Q06726	0.8	1.6
Retinol dehydrogenase 7	O88451	1.4	1.6
Electron transfer flavoprotein subunit alpha	Q99LC5	0.7	1.0
Cytochrome c1 heme protein	P00125	0.6	0.8
Succinate dehydrogenase [ubiquinone] flavoprotein subunit	Q7ZVF3	1.0	0.6
Succinate dehydrogenase [ubiquinone] iron-sulfur subunit	Q9YHT2	1.0	1.1
NADH dehydrogenase [ubiquinone] flavoprotein 1	Q8HXQ9	0.7	0.7
NADH dehydrogenase [ubiquinone] flavoprotein 1	Q0MQH4	0.8	0.9
Deoxyribodipyrimidine photo-lyase	P34205	1.1	0.9
Thioredoxin	O96952	0.8	0.7
Glutathione S-transferase 1	P46436	0.6	0.8
Carbonic anhydrase 2	P00920	1.6	1.1
Carbonic anhydrase 2	P00918	0.9	0.8
Mucin-4	Q99102	0.9	0.9
Delta(5) fatty acid desaturase	O74212	0.6	0.7
Fatty acid synthase	P49327	-2.6	-1.4
Hemagglutinin/amebocyte aggregation factor	Q01528	-1.9	1.2
Neuronal PAS domain-containing protein 2	Q99743	ns	3.4
A. millepora cryptochrome-2	GB:EF202590	ns	2.6
Mammalian cryptochrome-2	XM_007946529	ns	2.1
Mitogen-activated protein kinase 6	Q61532	ns	1.3
Mitogen-activated protein kinase 6	P27704	ns	0.9
Nuclear receptor subfamily 2 group C member 1	Q54RC5	ns	1.0
Sodium/glucose cotransporter 2	P53792	ns	1.1
Sodium/glucose cotransporter 5	Q6R4Q5	ns	0.9
Sodium/myo-inositol cotransporter 2	Q28728	ns	1.2
Soma ferritin	P42577	ns	0.9
Mitoferrin-2	Q7T292	ns	0.5
Carbamoyl-phosphate synthase [ammonia]	P31327	ns	0.9
Putative glutamine amidotransferase YLR126C	Q12288	ns	0.6
Circadian locomoter output cycles protein kaput	Q5RA48	ns	-0.9
D site-binding protein	P16443	ns	-2.8
Rhodopsin	Q17292	ns	-2.0
Chloride intracellular channel protein 2	Q5M883	ns	-1.4
Chloride intracellular channel protein 3	Q9D7F7	ns	-1.1
Voltage-dependent calcium channel subunit alpha-2/delta-2	Q6PHS9	ns	-1.0
Glutamate synthase	Q03460	ns	-1.1
Polycystic kidney disease protein 1-like 1	Q8TD30	ns	-0.7