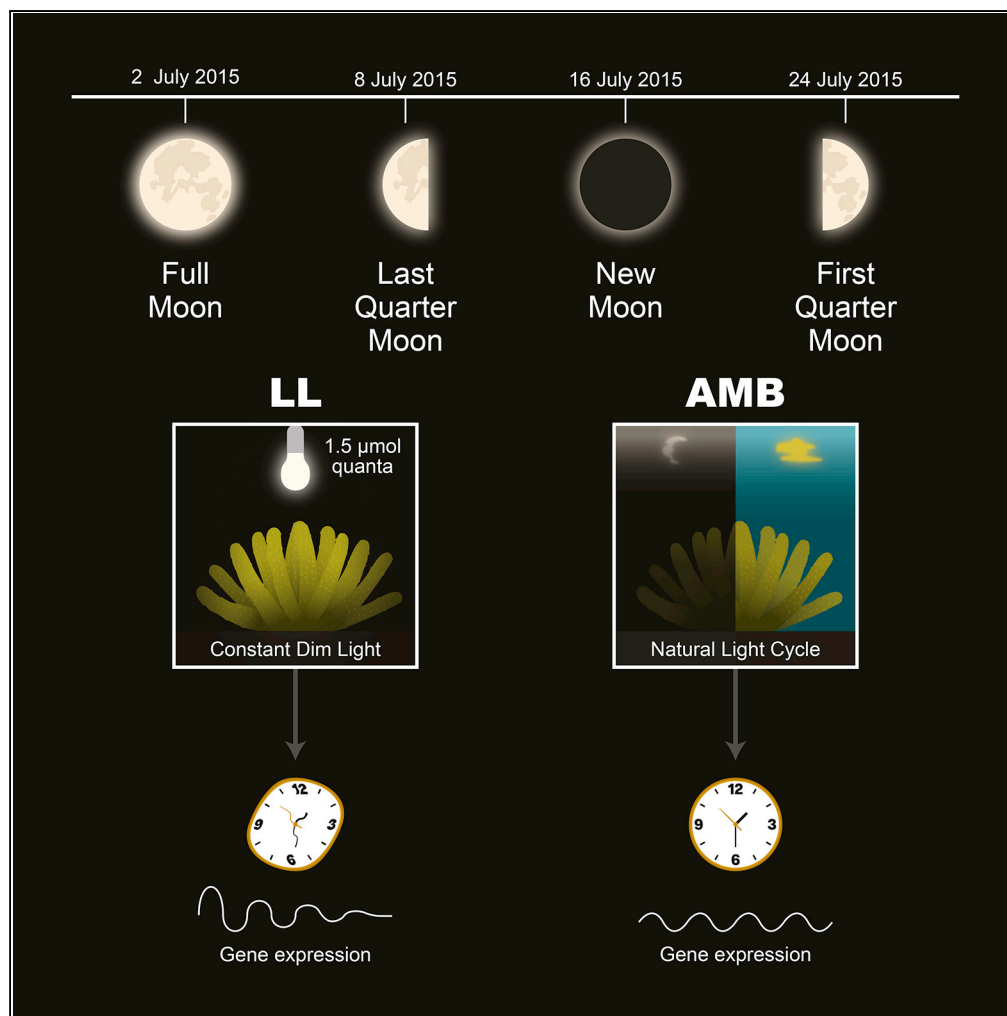**Article**

# Demystifying Circalunar and Diel Rhythmicity in *Acropora digitifera* under Constant Dim Light

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**HIGHLIGHTS**

Light entrains many biological processes governed by the endogenous clock

Constant dim light overrides the biological clock of *A. digitifera* corals

Artificial light impacts the processes that allow corals to thrive in our oceans

The increase of artificial light in coastal areas is a growing threat to coral reefs

## Article

# Demystifying Circalunar and Diel Rhythmicity in *Acropora digitifera* under Constant Dim Light

Yael Rosenberg,[1,3,*] Tirza Doniger,[1] Saki Harii,[2] Frederic Sinniger,[2] and Oren Levy[1]

## SUMMARY

**Life on earth has evolved under constant environmental changes; in response to these changes, most organisms have developed an endogenous clock that allows them to anticipate daily and seasonal changes and adapt their biology accordingly. Light cycles synchronize biological rhythms and are controlled by an endogenous clock that is entrained by environmental cues. Light is known to play a key role in the biology of symbiotic corals as they exhibit many biological processes entrained by daily light patterns. In this study, we aimed at determining the effect of constant dim light on coral's perception of diel and monthly cycles. Our results show that under constant dim light corals display a loss of rhythmic processes and constant stimuli by light, which initiates signal transduction that results in an abnormal cell cycle, cell proliferation, and protein synthesis. The results emphasize how constant dim light can mask the biological clock of *Acropora digitifera*.**

## INTRODUCTION

Most organisms, from cyanobacteria to mammals, exhibit biological rhythms by synchronizing their behavioral and physiological activities with cyclic changes in the environment (Aschoff, 1960). Among these rhythms of varying lengths, twenty-four-hour periodicity is widely observed in most organisms (Raible et al., 2017). In the absence of environmental cues, the periodicity drifts away from the natural phase and is referred to as "free running." For this reason, synchronization by environmental time cues is required (Harmer et al., 2001). Studies attempting to understand the mechanisms of endogenous clocks have focused mainly on circadian clocks showing it is based on positive and negative molecules interacting in feedback loops (Raible et al., 2017; Roenneberg and Merrow, 2005; Zantke et al., 2013). However, many organisms exhibit biological rhythms with longer (infradian) periodicity. One of these longer rhythms is the circalunar clock that oscillates with a period of approximately 1 month (~29.5 days) (Aschoff, 1981). External environmental cues (light, temperature) are used to entrain the oscillators of other biological clocks, with longer and shorter periodicities, in adaptation to the local conditions (Dupré and Loudon, 2007; Franke, 1985; Kaiser et al., 2011; Naylor, 2010; Schnytzer et al., 2018).

The lunar cycle refers to the 29.5 days (lunar month) required for the moon to orbit around the Earth and the 24.8 h (lunar day) required for the moon to reach the same spot on the Earth. Several recurring events are linked to the position of the moon relative to the Earth and the sun. Among these events, there are a few causing environmental changes, such as the tidal forces causing changes in water level and current, moonlight intensity, and time of moonrise (Gibson, 1992; Schnytzer et al., 2018).

Solar light is a well-known synchronizer of the circadian clock; yet, moonlight, which is incident sunlight reflected from the lunar surface, is known to affect periodic activities occurring in a variety of animals (Bentley et al., 2001; Kaniewska et al., 2015; Kronfeld-Schor et al., 2013; Rosenberg et al., 2017; Schnytzer et al., 2018; Takemura et al., 2004).

One of the earliest phenomena to be tied to the lunar cycle is the reproduction of marine invertebrates. In many species, including reef corals (Richmond and Hunter, 1990), fishes (Takemura et al., 2004), echinoderms (Mercier and Hamel, 2014), mollusks (Counihan et al., 2001), and crustaceans (Skov et al., 2005), the annual breeding events are synchronized with the lunar phase. This synchronization is commonly thought to be mediated by external cues that may act directly or indirectly to reset or maintain the biological clocks. Successful fertilization of marine invertebrates relies on the tight synchronization between

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the release of male and female germ products. The moon cycle provides these organisms with a predictable time frame that can be used for synchronization even across wide-spread populations (Babcock et al., 1986).

Coral reproductive timing is characterized by strong lunar rhythmicity (Boch et al., 2011; Hanafy et al., 2010; Jokiel et al., 1985; Kaniewska et al., 2015; Richmond and Hunter, 1990; Shlesinger and Loya, 1985). A common example is the mass spawning in the Great Barrier Reef, where changes in moonlight intensity can act as a final cue triggering the spawning of many different species of scleractinian corals as well as hundreds of other invertebrates, over a couple of nights (Harrison et al., 1984).

Light plays a key role in the growth, reproduction, and physiology of scleractinian corals that host phototrophic symbionts and reside in shallow water (Cohen et al., 2016; Kaniewska et al., 2015; Levy et al., 2007; Rosenberg et al., 2017; Wijgerde et al., 2014). The action spectra of coral photoreception reveal a maximum sensitivity in the blue region, at 480 nm (Gorbunov and Falkowski, 2002), which is also known to entrain the circadian clocks of insects and mammals via cryptochromes (CRYs), which are DNA photolyase-like photoreceptor proteins (Falciatore and Bowler, 2005). This makes corals capable of sensing low levels of blue light consistent with the detection of lunar irradiation (Gorbunov and Falkowski, 2002; Levy et al., 2007; Wuitchik et al., 2019).

Corals lack specialized light-sensing organs, yet display photosensitive behavior (Babcock et al., 1986; Gorbunov and Falkowski, 2002; Harrison et al., 1984; Jokiel et al., 1985; Levy et al., 2003, 2001; Oldach et al., 2017). A survey of *Acropora digitifera* photoreceptors revealed seven *opsin* and three *cryptochrome* genes (Shoguchi et al., 2013). A previous study on the coral *Acropora millepora* showed that the known light receptor and core clock gene cryptochromes displayed rhythmic expression in response to light:dark treatment and are influenced by lunar light (Levy et al., 2007). Photoreceptors are also responsible for the photoentrainment of circadian rhythms, and their threshold of photoreception sensitivity is low enough for corals to sense natural moonlight (Gorbunov and Falkowski, 2002; Levy et al., 2007; Reitzel et al., 2010). Photoentrainment of rhythmic processes in corals is highly important as it regulates metabolism, photosynthesis, synchronized spawning, and calcification (Sorek et al., 2014).

Here, we explore the complexities of gene expression according to the lunar phase changes in a reef-building coral, *A. digitifera* (Figure 1), using RNA sequencing (RNA-seq) analysis.

## RESULTS

### Photosynthetic Yield

By measuring photosynthetic yield at the beginning and end of the experiment for each treatment (AMB and LL), we confirmed that coral colonies had no significant decrease in maximal quantum yield; median values of maximal quantum yield (Fv/Fm) were 0.71 for all colonies at the start of the experiment and 0.70 and 0.71 for LL and AMB colonies, respectively, at the end of the experiment (Figure 1B). Thus, we can conclude that coral bleaching and/or damage did not have an impact on the results reported here.

### Differential Expression and Pathway Analysis

To discover biologically important changes in gene expression between conditions, we performed differential expression analysis of our RNA-seq data. Each of the 48 samples sent for RNA sequencing yielded an average of 16,062,693 paired-end sequences (Table S1 Sequencing metrics). Samples were aligned to the *A. digitifera* transcriptome with an average of 40% sequences from each sample mapped to the known transcriptome (Supplemental Information) and 23,642 gene models were found. We started with comparing all AMB with LL samples and arraying them by sampling time and moon phase to understand how the time of day and the moon phase causes differences in expression level between the experimental groups (Figures 2A and 2B). Our results showed a substantial difference in the expression levels of differentially expressed (DE) genes between the experimental groups; LL samples had 4,472 genes up-regulated (19% of total genes counts) and 4,691 genes down-regulated (20% of total genes counts) when compared with the AMB samples. In the AMB samples, we could recognize gene patterns responding to both moon phase and time of day with levels changing accordingly, with a specific pattern for the day time and night time samples (Figure 2A). As opposed to the AMB samples, the LL samples showed different expression levels at each sampling point (Figure 2B). The LL full moon samples (first sampling day) showed strong variation in expression levels between day and night that did not repeat in the following sampling points. Interestingly,
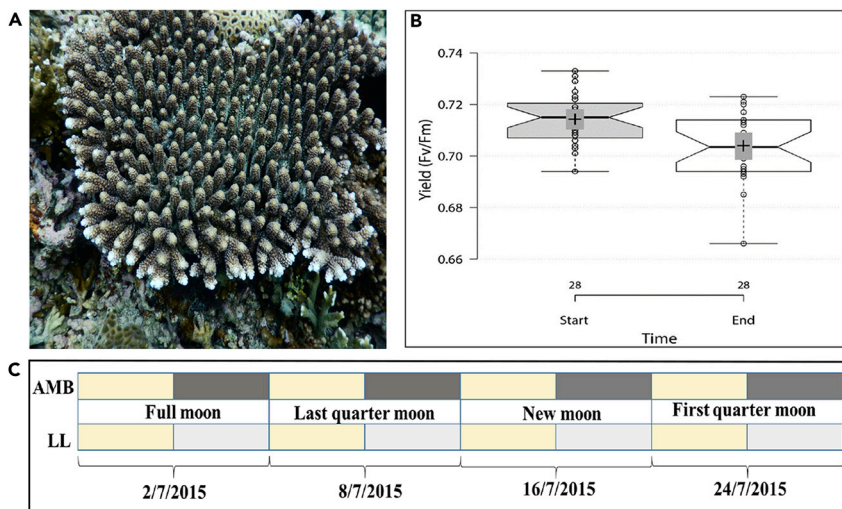
**Figure 1. Experimental Setup**

(A) *Acropora digitifera* colony prior to collection from Sesoko Island Reef, Okinawa, Japan.

(B) Box plot showing the maximum quantum yield (Fv/Fm) for LL colonies (n = 5) from the first sampling day (Start) and at the last sampling day (End). Centerlines show the medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend to a minimum and maximum values; crosses represent sample means; bars indicate 95% confidence intervals of the means. Median values are 0.71 and 0.70 at the start and end, respectively.

(C) Sampling regime indicating the two light treatments, dates of sampling, and moon phases. AMB, Ambient conditions; LL, Light: Light treatment, constant light. Yellow bars represent day time, black bars represent objective night time, and gray bars represent subjective night.

after almost 3 weeks of sampling and 4 weeks under LL conditions, the new moon samples showed a strong variation between day and night time samples. Although we observed the strong peak in expression during the new moon day time samples, the patterns had different expression than the AMB new moon day time samples. We then continued with the MetaCycle analysis to evaluate periodicity in the expressed transcripts in both conditions. AMB samples showed 58 synexpression clusters that were rhythmic across all sampling time points (Figure 3A) and had specific gene networks for every time point as well as unique genes that corresponded with only one sampling point (Figure 3B). The LL samples did not show any rhythmic gene clusters across the different sampling points and had more gene overlaps and less unique genes for specific time points than the AMB samples (Figure 3C).

We were able to identify the different clusters that are DE in each moon phase and arrange them according to specific pathways (Figure 4). Many of the pathways show variation across the moon phases in both groups but have the opposite expression pattern. For example, ERK/MAPK, Integrin, NGF, and CREB signaling in the LL samples are up-regulated or not DE in the different moon phases, whereas the same signaling pathways are down-regulated or not DE in the AMB samples. The main pathways that were down-regulated in the LL samples and up-regulated in the AMB samples include the D-myo-inositol (3,4,5,6)-tetrakisphosphate Biosynthesis, D-myo-inositol (1,4,5,6)-Tetrakisphosphate Biosynthesis, 3-phosphoinositide Degradation, G Beta Gamma Signaling, Cell Cycle Regulation by BTG Family Proteins, p53 Signaling, Role of p14/p19ARF in Tumor Suppression, NRF2-Mediated Oxidative Stress Response, and PPARα/RXRα Activation (Figure 4). We could also find pathways that are DE between the moon phases only in the LL samples and not in the AMB samples such as CD40 and GP6 signaling pathways. Some of the pathways found to be DE under AMB conditions and not under LL conditions include Rac, Dopamine, and IL-8 signaling. The AMB pathway cluster represents the natural variance coral express throughout the lunar phase of a single month.

## Cyclic Gene Expression

Next, the expression levels of known clock-related genes of *A. digitifera* in the different lunar phases under the two light conditions were established. In the AMB samples three of the genes, *cry1*, *npas2*, and *pdp*, showed the synexpression pattern (Figures 5A, 5B, and 5D), high levels during day time sampling and lower
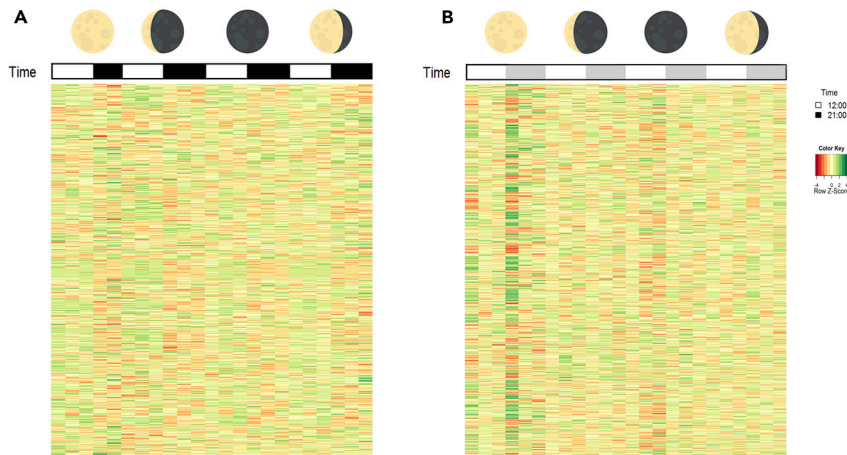
**Figure 2. Gene Expression across the Moon Phases**

(A) Corals under Ambient conditions (AMB).

(B) Corals under constant light conditions (LL). Moon phases are presented by chronological sampling days: starting from the left side: full moon, third quarter, new moon, and first quarter. White bars indicate 12:00 p.m. sampling; black and gray bars indicate 21:00 p.m. sampling. All bars represent three samples; AMB night sample from the full moon represents two samples. Gene expression values are represented by Z score.

levels during night time with a slight difference between the moon phases. The *clock* gene showed the opposite expression pattern (Figure 5C) with high levels during the night and lower levels during the day. All of the above clock-related genes expressed in the LL samples showed no significant variance between night and day and moon phases.

Last, we tested another group of genes in relation to light exposer, the Fibroblast growth factors (FGFs). Three annotated FGF genes in the *A. digitifera* transcriptome were found, FGF1R, FGF3R, and FGF1, that were highly expressed in the LL samples during all moon phases as opposed to the AMB samples where levels were constantly lower (Figures 6A–6C). In addition, no significant variance between day and night was observed.

By comparing all AMB genes with each other we were interested in genes that react solely to the lunar phase and not to the time of day. We found a group of DE genes (n = 23 genes with annotations) that are involved in initiating many signaling pathways via GPCRs activation and resulting in DNA binding and RNA transcription. Additional pathways that were enriched in response to lunar phase are microtubule binding and extracellular matrix organization.

## DISCUSSION

Only a few studies up to date have focused on the coral response to lunar cycle (Jokiel et al., 1985; Kaniewska et al., 2015; Levy et al., 2007; Rosenberg et al., 2017) and coral circadian gene expression across the lunar phase (Brady et al., 2016; Levy et al., 2007); yet, none of them have addressed the aspect of low dim light on the complete gene expression of a candidate coral over an entire lunar cycle. Continuous and intermittent low lighting both have been shown to have effects; short pulses of light during the night are sufficient to disrupt the circadian clocks. Artificial light may be difficult to distinguish from natural changes in light intensity and duration and could override the endogenous clock (Dominoni, 2015; Gaston et al., 2013). This study demonstrates the potential impacts that modern lighting can have on coral reefs.

Our results show that corals under ambient conditions, perceiving natural light:dark and lunar cycles, express diel and monthly variation in gene expression (Figure 2A) as a response to the environmental changes. Our AMB samples present the natural variation in gene expression that occurs in *A. digitifera* during the month of July and was used as a control reference in the analysis. In the LL samples, there was no continuous cyclic variation in gene expression in response to the time of day or the lunar phase (Figure 2B). In many organisms, rhythmicity loss as a result of constant conditions is well described for both gene
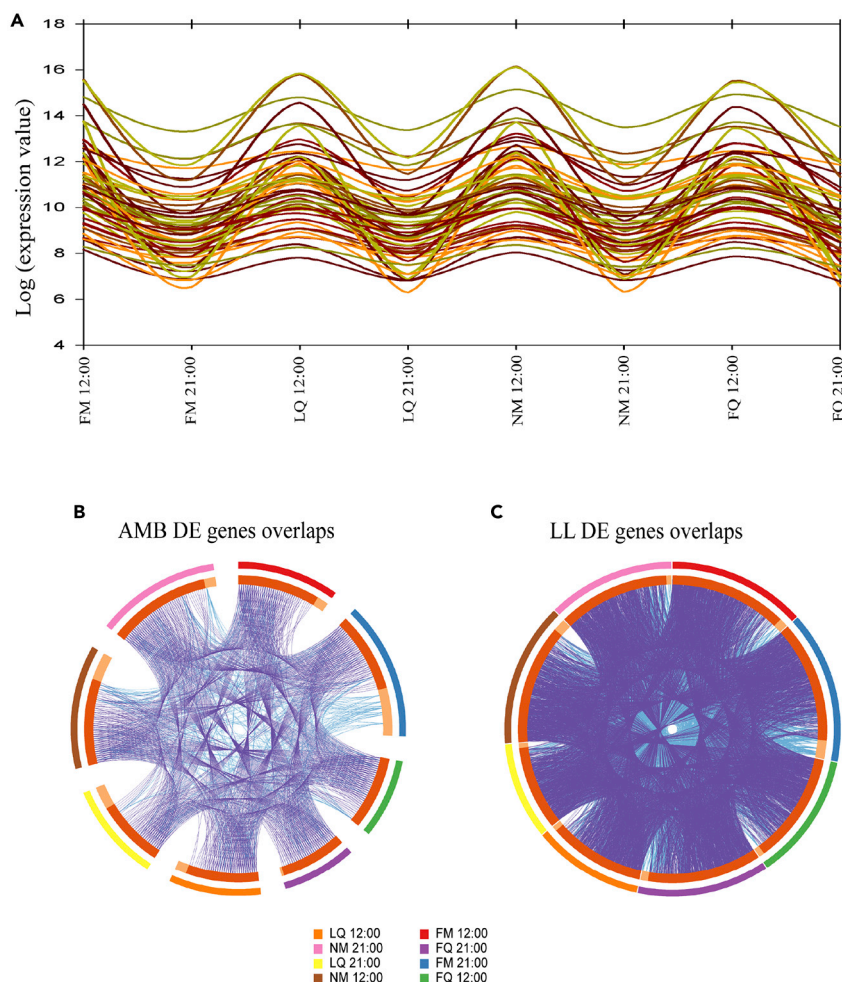
**Figure 3. Rhythmic Analysis and Gene Overlap**

(A) AMB sample rhythmic gene expression of top 58 gene clusters across all sampling points.

(B) Circos plot showing the overlaps of DE genes of AMB samples between all sampling points.

(C) Circos plot showing the overlaps of DE genes of LL samples between all sampling points. Outer circles represent sample time, inner circles show in light orange the unique genes and dark orange shared genes, purple lines are gene overlap, and blue lines are shared enriched terms. FM, full moon; LQ, last quarter moon; NM, new moon; FQ, first quarter moon.

expression and behavior, specifically under constant dim light (Granados-Fuentes, 2004; Njus et al., 1977; Steinlechner et al., 2002). The absence of entrainment cues causes the loss of these rhythmic patterns. Previous studies have shown that, in case of returning to natural light and dark conditions, the rhythmicity will be restored (Granados-Fuentes, 2004; Njus et al., 1977; Steinlechner et al., 2002). LL samples collected on the day of the full moon, after a few days of constant dim light, did have a significant difference in gene expression between the day and the night. We postulate that these differences are natural fluctuations in gene expression, which were still under the regulation of an endogenous clock that was able to overcome the masking effect of the continuous dim light. Many studies have shown that the biological clock of an organism can maintain a rhythm in gene expression or behavior after being kept in constant conditions for a few days (Chabot et al., 2016; Griffin et al., 1999; Levy et al., 2011; Oren et al., 2015; Peres et al., 2014; Sorek et al., 2013; Sorek and Levy, 2012). Therefore, we assume the oscillations in gene expression under LL conditions at the first sampling day are still governed by the clock machinery. Daytime new moon samples under LL conditions expressed down-regulation of gene clusters that are surprising not only in appearance but also in expression pattern when compared with the equivalent AMB samples. Observing the DE genes temporal pattern during the four moon phases under natural conditions shows
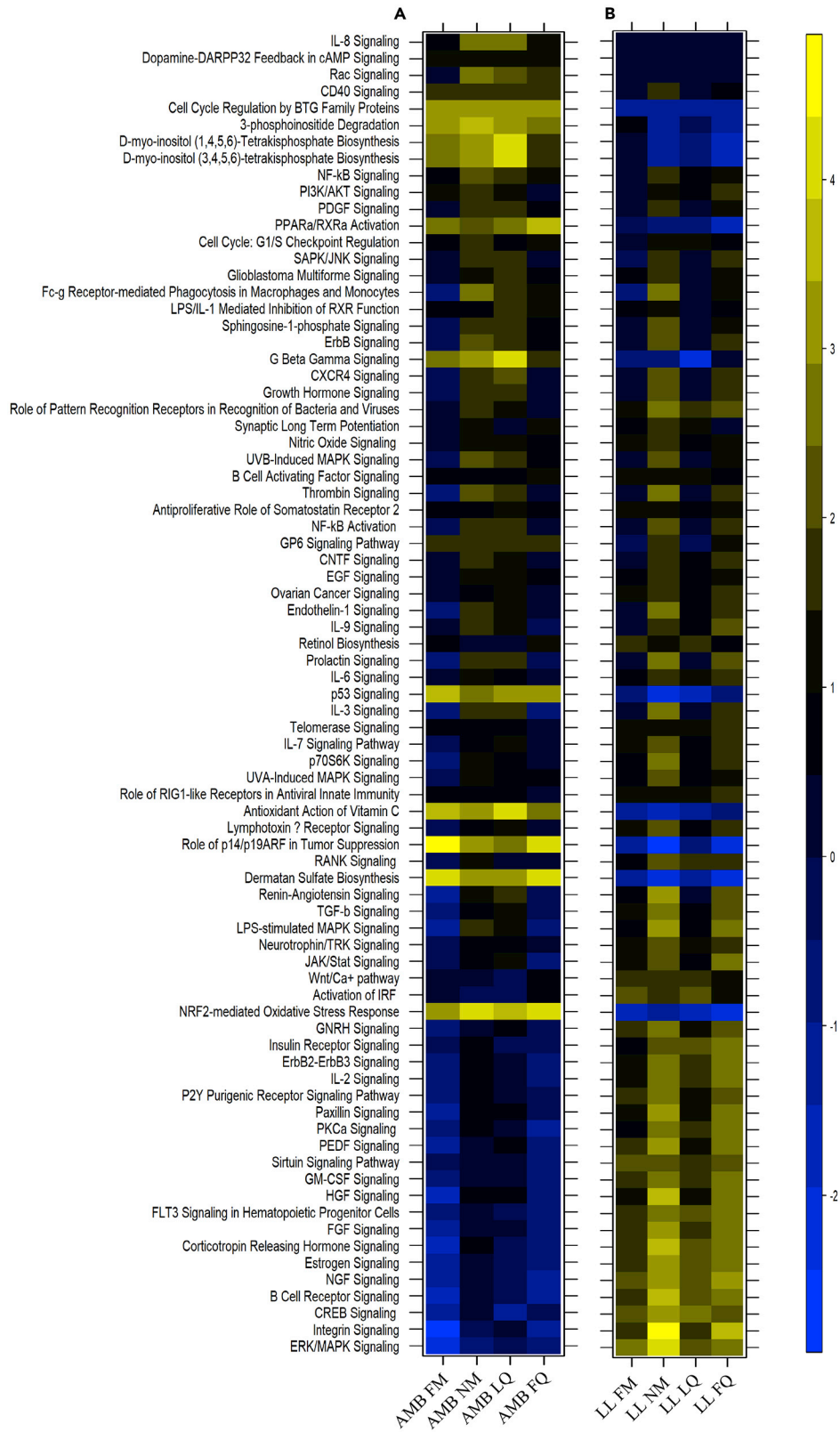
**Figure 4. Gene Ontology Enrichment Analysis of Differentially Expressed (DE) Genes**

(A) Canonical pathways oscillation enriched in corals under Ambient conditions (AMB).

(B) Canonical pathways oscillation enriched in corals under constant light conditions (LL). The color scale indicates the process enrichment based on $Z$ score. Moon phases are indicated at the bottom. FM, full moon; LQ, last quarter moon; NM, new moon; FQ, first quarter moon.

that corals present diel and monthly variations in gene expression, whereas after a few days under constant conditions rhythmicity is lost.

When focusing on enriched pathways constituted by the DE genes, we found many pathways that act in contrary mode between the AMB and LL samples (Figures 4A and 4B). The ERK/MAPK signaling pathway causes signal transduction from a receptor on the cell's surface to the cell's nucleus (Seger and Krebs, 1995) and was found to be up-regulated in the LL samples (Figure 4B) and down-regulated in the AMB samples (Figure 4A). This signaling pathway initiates cellular processes such as proliferation, differentiation, and development as a result of an external signal (Seger and Krebs, 1995). The ERK/MAPK signaling pathway together with the Integrin and NGF signaling pathways, which were also up-regulated in the LL samples, regulate the cell cycle, cell proliferation, and cell growth (Howe et al., 1998, 2001; Howe and Mobley, 2004; Niederhauser et al., 2000; Schwartz, 2001). We propose that constant dim light can cause a continuous signal for the initiation of these pathways and therefore known rhythmic processes, such as cell cycle, are no longer under tight regulation that is probably masked (Armbrust et al., 1989; Hunt and Sassone-Corsi, 2007; Jacquet et al., 2001; Matsuo et al., 2003).

An additional up-regulated pathway that was enriched in the LL samples, while down-regulated in the AMB samples, is the CREB signaling pathway. CREB is a transcription factor, which binds to DNA sequences called cAMP response elements (CRE) and causes an increase or decrease in the transcription of these genes (Bourtchuladze et al., 1994; Purves, 2004). Circadian rhythm entrainment by light and clock gene transcription is partly under CREB phosphorylation regulation (Riccio et al., 2006; Tischkau et al., 2003; Zhang et al., 1996). The CREB signaling pathway reacts to light and provides information essential for normal clock function as described previously (Obrietan et al., 1999). Therefore, it is not surprising that the CREB signaling pathway was up-regulated under dim light as it reacted to the constant stimulation of light. Other pathways enriched in the LL samples such as CD40 and GP6 signaling pathways are linked to cell regulation process, immune system, and phosphorylation (Faris, 1994, p. 40; Jones et al., 2007; Sokol et al., 2012). Pathways that were only DE under AMB conditions can all be found to have circadian regulation or modulating rhythmic processes by dynamically regulating the concentration of intracellular second messengers. These pathways include the Rac, Dopamine, and IL-8 signaling pathways (Beaulieu and Gainetdinov, 2011; Duffield et al., 2002; Ghasemi et al., 2011; Hermann et al., 2006; Hwang et al., 2013; Norman et al., 2005; Yujnovsky et al., 2006). Finding these rhythmic processes to be enriched only in the AMB samples gives an additional proof that under LL conditions the rhythmicity is lost and most known genes that have a diel or monthly fluctuation in expression would not be enriched in the LL samples.

To determine the loss of rhythmicity at the mechanism level and not only on gene expression level, we compared the gene expression of known clock genes of *A. digitifera* between the two light conditions (Figure 5). All four clock-related genes, *cry1*, *npas2*, *clock*, and *pdp*, exhibited diel variation in expression in AMB samples across all moon phases, whereas under LL conditions there was no significant diel variation in gene expression and levels were constantly lower than in the AMB samples. Loss of expression, "dampening," in related clock genes is a known phenomenon after a few days under constant conditions with no entrainment cue (Chabot et al., 2016; Dominoni, 2015; Dupré and Loudon, 2007; Griffin et al., 1999; Raible et al., 2017; Reitzel et al., 2010; Roenneberg and Merrow, 2005; Sorek et al., 2014, 2013). The coordination of many biological processes is facilitated by the biological clock; therefore, disruptions in cueing or masking the clock machinery have the potential to influence a range of downstream pathways (Ceriani et al., 2002; Kondratov et al., 2006; Somers et al., 1998; Takahashi et al., 2008; Zheng et al., 2001).

When looking at an organism under constant light it is important to examine the effect not only on rhythmic biological processes but also on cells that perceive light, photoreceptors. Studies attempting to understand this effect of light on photoreceptors have found that constant light causes photoreceptor degradation and upregulation of Fibroblast Growth Factors (FGFs) (Campochiaro et al., 1996; Hicks and Courtois, 1992, 1988; Hisatomi et al., 2002; Hochmann et al., 2012; Qin et al., 2011). FGFs are the largest family of growth factors involved in soft-tissue growth and regeneration (Basilico and Moscatelli, 1992). It was found
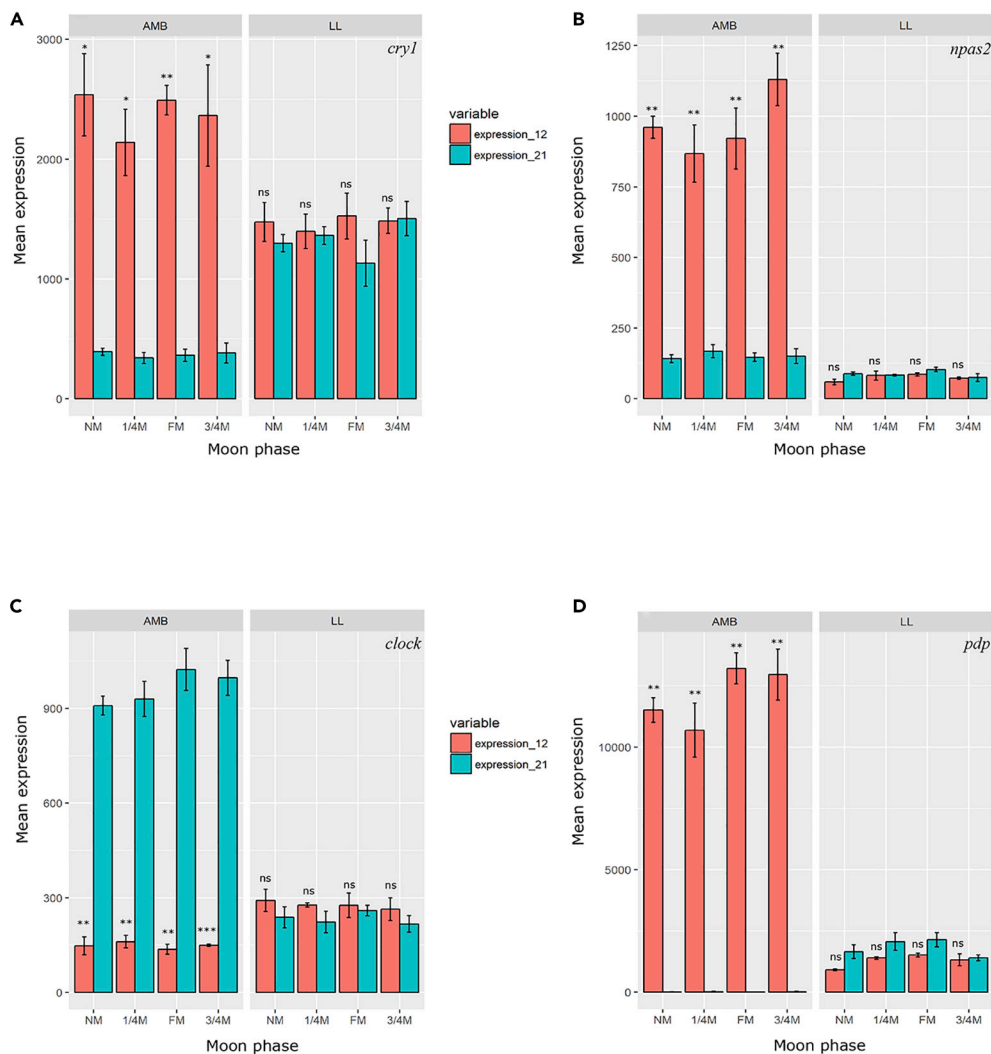
**Figure 5. Mean RNA Expression of Clock-Related Genes in the Coral *Acropora digitifera* along the Moon Phases under Natural and Constant Light Conditions**

(A) *cry1*; (B) *npas2*; (C) *clock*; (D) *pdp*. NM, new moon; 1/4M, first quarter moon; FM, full moon; 3/4M, third quarter moon. The left panel in every graph represents ambient conditions corals (AMB); the right panel represents corals under constant light conditions (LL). Red bars represent gene expression at 12:00 p.m., light blue bars represent gene expression at 21:00 p.m. Represented p value is for comparison between AMB of day and night samples and LL samples of day and night. *p < 0.05, **p < 0.01, ***p < 0.001, ns, p > 0.05.

that, under constant light, FGFs cause regeneration of photoreceptors that were degraded owing to the effect of the light treatment (Campochiaro et al., 1996; Hisatomi et al., 2002; Hochmann et al., 2012; Qin et al., 2011). In our data, we found that three FGFs were upregulated in the LL samples compared with the AMB samples (Figures 6A–6C). These results correspond with previous studies showing that FGFs protect photoreceptors from the damaging effect of constant light (Gao and Hollyfield, 1996; LaVail et al., 1992).

Genes that were found to be responsive to the lunar cycle and not to the diel cycle revealed pathways altered between the lunar phases. Most of these genes use signaling pathways initiated by GPCRs, pathways known to be environmentally responsive, mainly to light. Many genes show changes in expression levels based on the lunar phase (Brady et al., 2016; Fukushiro et al., 2011; Levy et al., 2007; Numata and Helm, 2014), but most of them show changes between day and night time. The observed genes create
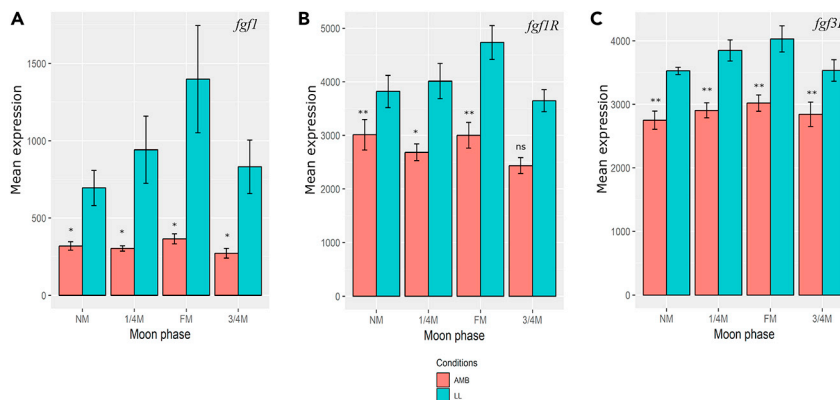
**Figure 6. Mean Expression of Fibroblast Growth Factors (FGFs) in the Coral *Acropora digitifera* along the Moon Phases under Natural and Constant Light Conditions**

(A) *fgf1*; (B) *fgf1r*; (C) *fgf3r*. NM, new moon; 1/4M, first quarter moon; FM, full moon; 3/4M, third-quarter moon; AMB, corals under ambient conditions; LL, corals under constant light conditions. Red bars represent gene expression in AMB samples; light blue bars represent gene expression in LL samples. The represented p value is for comparison between AMB samples and LL samples. *p < 0.05, **p < 0.01, ns, p > 0.05.

specific pathways that react solely to the lunar cycle and are most likely responsive to the moonlight since they are connected to the GPCR signaling pathway. These pathways result in DNA binding and RNA transcription, and we postulate the moon phase has a role in determining gene expression, which regulates the organization and formation of extracellular matrix assisted by microtubules.

To conclude, this study shows that constant dim light conditions could cause loss of rhythmic processes in the coral *A. digitifera*. The results demonstrate that even low levels of illumination could override the changes in the moon phase and cause loss of circadian and circalunar regulation. The diel light cycle controls many vital processes; the connection between artificial light and clock disorders is well known, and difficulties with adjusting the circadian clock in corals could impact the natural processes that allow them to thrive and populate the oceans. Since the lunar phase controls oogenesis in many corals, the masking of moonlight could affect the synchronized spawning phenomenon and cause reproduction failure in heavily lit areas. Therefore, the increase of artificial light in coastal areas is a growing threat to coral reefs around the world.

## Limitations of the Study

From a clock point of view, one of the challenging tasks is to plan the sampling regime according to the turnover of the RNA molecules to understand the daily and monthly oscillations of gene expression. Future studies should focus, at a molecular level, on the obstruction of constant light on a proposed circalunar oscillator, by diel sampling across an entire lunar month, to test whether the above processes are controlled by one circadian clock or by two separate circadian and circalunar clocks.

## METHODS

All methods can be found in the accompanying Transparent Methods supplemental file.

## DATA AND CODE AVAILABILITY

The sequencing data reported in this study have been deposited to the Sequence Read Archive "SRA, PRJNA526391": Characterizing circalunar and diel rhythmicity in the model coral *Acropora digitifera*, a molecular perspective. Correspondence and request for materials should be addressed to Y.R. yaelirose@gmail.com or O.L. oren.levy@biu.ac.il.

## SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2019.11.040.

## AUTHOR CONTRIBUTIONS

Y.R. and O.L. designed the research; the experiment was carried out by Y.R., O.L., S.H., and F.S. T.D. performed data analysis. Y.R. and O.L. wrote the first draft of the manuscript. All authors edited the manuscript. All authors read and approved the final manuscript.

## DECLARATION OF INTERESTS

The authors declare they have no competing interests.

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# Supplemental Information

# Demystifying Circalunar and Diel

# Rhythmicity in *Acropora digitifera*

# under Constant Dim Light

Yael Rosenberg, Tirza Doniger, Saki Harii, Frederic Sinniger, and Oren Levy

# Supplemental information

**Table S1.** Sequencing metrics for all libraries related to figures 2-4.

| Sample # | Lane | Sample ID | Bar code | colony | date | # of paired-end sequences | Light | Moon | Sampling Time | Total # of Sequences | # of sequences mapping to digitifera | % mapped to digitifera |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | L008 | Sample_21 | ATCACG | 1 | 8.7.2015 | 16236391 | LL | 3/4 moon | 12:00 | 32472782 | 12276611 | 37.80585 |
| 2 | L008 | Sample_22 | CGATGT | 2 | 8.7.2015 | 16632835 | LL | 3/4 moon | 12:00 | 33265670 | 13388338 | 40.24671 |
| 3 | L008 | Sample_23 | TTAGGC | 3 | 8.7.2015 | 14276673 | LL | 3/4 moon | 12:00 | 28553346 | 11804114 | 41.34056 |
| 25 | L001 | Sample_27 | ATCACG | 14 | 8.7.2015 | 14385269 | AMB | 3/4 moon | 12:00 | 28770538 | 12274604 | 42.6638 |
| 26 | L001 | Sample_29 | CGATGT | 15 | 8.7.2015 | 15560673 | AMB | 3/4 moon | 12:00 | 31121346 | 13058112 | 41.9587 |
| 27 | L001 | Sample_30 | TTAGGC | 16 | 8.7.2015 | 14517925 | AMB | 3/4 moon | 12:00 | 29035850 | 11722620 | 40.37292 |
| 4 | L008 | Sample_31 | TGACCA | 1 | 8.7.2015 | 17183513 | LL | 3/4 moon | 21:00 | 34367026 | 12426184 | 36.15729 |
| 5 | L008 | Sample_32 | ACAGTG | 2 | 8.7.2015 | 15559643 | LL | 3/4 moon | 21:00 | 31119286 | 12745452 | 40.95676 |
| 6 | L008 | Sample_33 | GCCAAT | 3 | 8.7.2015 | 12544791 | LL | 3/4 moon | 21:00 | 25089582 | 10399938 | 41.45122 |
| 28 | L001 | Sample_37 | TGACCA | 14 | 8.7.2015 | 17236053 | AMB | 3/4 moon | 21:00 | 34472106 | 14337497 | 41.59159 |
| 29 | L001 | Sample_39 | ACAGTG | 15 | 8.7.2015 | 15821903 | AMB | 3/4 moon | 21:00 | 31643806 | 12594369 | 39.80042 |
| 30 | L001 | Sample_40 | GCCAAT | 16 | 8.7.2015 | 15906877 | AMB | 3/4 moon | 21:00 | 31813754 | 12966533 | 40.75763 |
| 7 | L008 | Sample_51 | CAGATC | 1 | 16.7.2015 | 16090028 | LL | New moon | 12:00 | 32180056 | 13778139 | 42.81577 |
| 8 | L002 | Sample_52 | ACTTGA | 2 | 16.7.2015 | 12242751 | LL | New moon | 12:00 | 24485502 | 12020773 | 49.09343 |
| 9 | L008 | Sample_53 | GATCAG | 3 | 16.7.2015 | 13085116 | LL | New moon | 12:00 | 26170232 | 11623635 | 44.41548 |
| 31 | L001 | Sample_57 | CAGATC | 14 | 16.7.2015 | 14299523 | AMB | New moon | 12:00 | 28599046 | 12544530 | 43.86346 |
| 32 | L001 | Sample_59 | ACTTGA | 15 | 16.7.2015 | 13079087 | AMB | New moon | 12:00 | 26158174 | 10481332 | 40.06905 |
| 33 | L001 | Sample_60 | GATCAG | 16 | 16.7.2015 | 13585629 | AMB | New moon | 12:00 | 27171258 | 11694503 | 43.03998 |
| 10 | L008 | Sample_71 | TAGCTT | 1 | 16.7.2015 | 16987047 | LL | New moon | 21:00 | 33974094 | 12787638 | 37.63938 |
| 11 | L008 | Sample_72 | GGCTAC | 2 | 16.7.2015 | 17103958 | LL | New moon | 21:00 | 34207916 | 16880595 | 49.34704 |
| 12 | L008 | Sample_73 | CTTGTA | 3 | 16.7.2015 | 18284695 | LL | New moon | 21:00 | 36569390 | 17218762 | 47.08518 |
| 34 | L002 | Sample_77 | TAGCTT | 14 | 16.7.2015 | 15798055 | AMB | New moon | 21:00 | 31596110 | 13727250 | 43.44601 |
| 35 | L002 | Sample_79 | GGCTAC | 15 | 16.7.2015 | 15914986 | AMB | New moon | 21:00 | 31829972 | 14259830 | 44.80001 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 36 | L002 | Sample_80 | CTTGTA | 16 | 16.7.2015 | 17464018 | AMB | New moon | 21:00 | 34928036 | 14905394 | 42.67458 |
| 13 | L008 | Sample_81 | AGTCAA | 1 | 24.7.2015 | 16716182 | LL | 1/4 moon | 12:00 | 33432364 | 15194864 | 45.44956 |
| 14 | L008 | Sample_82 | AGTTCC | 2 | 24.7.2015 | 16886106 | LL | 1/4 moon | 12:00 | 33772212 | 16407474 | 48.58276 |
| 15 | L008 | Sample_83 | ATGTCA | 3 | 24.7.2015 | 17487139 | LL | 1/4 moon | 12:00 | 34974278 | 14452602 | 41.32352 |
| 37 | L002 | Sample_87 | AGTCAA | 14 | 24.7.2015 | 18173103 | AMB | 1/4 moon | 12:00 | 36346206 | 14828248 | 40.79724 |
| 38 | L002 | Sample_89 | AGTTCC | 15 | 24.7.2015 | 16952811 | AMB | 1/4 moon | 12:00 | 33905622 | 14542684 | 42.89166 |
| 39 | L002 | Sample_90 | ATGTCA | 16 | 24.7.2015 | 18660511 | AMB | 1/4 moon | 12:00 | 37321022 | 16563228 | 44.38042 |
| 16 | L008 | Sample_91 | CCGTCC | 1 | 24.7.2015 | 14349978 | LL | 1/4 moon | 21:00 | 28699956 | 11691428 | 40.73675 |
| 17 | L008 | Sample_92 | GTCCGC | 2 | 24.7.2015 | 13969186 | LL | 1/4 moon | 21:00 | 27938372 | 12576297 | 45.01442 |
| 18 | L001 | Sample_93 | GTGAAA | 3 | 24.7.2015 | 13999156 | LL | 1/4 moon | 21:00 | 27998312 | 11738970 | 41.92742 |
| 40 | L002 | Sample_97 | CCGTCC | 14 | 24.7.2015 | 14432991 | AMB | 1/4 moon | 21:00 | 28865982 | 12203170 | 42.27526 |
| 41 | L002 | Sample_99 | GTCCGC | 15 | 24.7.2015 | 14437812 | AMB | 1/4 moon | 21:00 | 28875624 | 13184525 | 45.65971 |
| 42 | L002 | Sample_100 | GTGAAA | 16 | 24.7.2015 | 20685286 | AMB | 1/4 moon | 21:00 | 41370572 | 16714820 | 40.40268 |
| 19 | L001 | Sample_111 | GTGGCC | 1 | 2.7.2015 | 14804533 | LL | Full moon | 12:00 | 29609066 | 14887847 | 50.28138 |
| 20 | L001 | Sample_112 | GTTTCG | 2 | 2.7.2015 | 14840507 | LL | Full moon | 12:00 | 29681014 | 14031780 | 47.27527 |
| 21 | L001 | Sample_113 | CGTACG | 3 | 2.7.2015 | 14329146 | LL | Full moon | 12:00 | 28658292 | 13158883 | 45.91649 |
| 43 | L002 | Sample_117 | GTGGCC | 14 | 2.7.2015 | 19376927 | AMB | Full moon | 12:00 | 38753854 | 16031988 | 41.36876 |
| 44 | L002 | Sample_119 | GTTTCG | 15 | 2.7.2015 | 20878736 | AMB | Full moon | 12:00 | 41757472 | 17644060 | 42.25366 |
| 45 | L002 | Sample_120 | CGTACG | 16 | 2.7.2015 | 19373664 | AMB | Full moon | 12:00 | 38747328 | 16807492 | 43.37716 |
| 22 | L001 | Sample_131 | GAGTGG | 1 | 2.7.2015 | 14585590 | LL | Full moon | 21:00 | 29171180 | 13824040 | 47.38938 |
| 23 | L001 | Sample_132 | ACTGAT | 2 | 2.7.2015 | 14905877 | LL | Full moon | 21:00 | 29811754 | 13277772 | 44.53871 |
| 24 | L001 | Sample_133 | ATTCCT | 3 | 2.7.2015 | 15766052 | LL | Full moon | 21:00 | 31532104 | 14189906 | 45.00146 |
| 46 | L002 | Sample_137 | GAGTGG | 14 | 2.7.2015 | 21224377 | AMB | Full moon | 21:00 | 42448754 | 17557731 | 41.36218 |
| 47 | L002 | Sample_139 | ACTGAT | 15 | 2.7.2015 | 19263395 | AMB | Full moon | 21:00 | 38526790 | 15851645 | 41.14447 |
| 48 | L002 | Sample_140 | ATTCCT | 16 | 2.7.2015 | 15112793 | AMB | Full moon | 21:00 | 30225586 | 13549077 | 44.82652 |

# Transparent Methods

**Detailed methods of this paper include the following:**

**- Contact for resource sharing**

**- METHOD DETAILS-**

**-+ Experimental design and sampling regime**

**-+ Pulse-Amplitude-Modulated (PAM) fluorometer measurements**

**- + RNA Extraction**

**- + Next-generation sequencing**

**- + Transcriptome annotation**

**- + Differential expression**

**- DATA AND SOFTWARE AVAILABILITY**

**- Contact for resource sharing**

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**- METHOD DETAILS**

**-+ Experimental design and sampling regime**

Ten mature colonies of Acropora digitifera (measuring >50 cm in diameter) were collected from Sesoko Island, Okinawa, Japan (26°38028″ N, 127°51035″ E) on June 25th, 2015 (Fig 1A). Coral colonies were kept in direct flow-through seawater, pumped directly from the reef flat where colonies were originally sourced. Cross-calibrated temperature loggers (HOBO Pendant® UA-002-64 or UA-001-64, Onset Corporation, Bourne, MA, USA) were placed in each aquarium to monitor temperature throughout the experiment and ensure consistency across treatments. Five colonies were exposed to natural cycles of sunlight and moon phases (ambient conditions, AMB). The remaining five colonies were not exposed to natural light cycles and moon phases and were under constant dim light (light/light conditions, LL) treatment from a fluorescent lamp mounted above the aquarium (1.5-2 μmol quanta m-2 s-1 at coral height, the light was measured using a LI-COR underwater quantum sensor LI-193). The specific light intensity was chosen based on Rosenberg et al., 2019 and Tamir et al., 2017 (Rosenberg et al., 2019; Tamir et al., 2017) as it is known to be dim light that affects coral photo-physiology. All colonies were acclimated for a week prior to sampling which started on July 2nd, the day of the full moon. Sampling of all colonies (n=10) started at 12:00 p.m. followed by a second sampling at 21:00 p.m. Sampling continued throughout the month of July, returning every week according to the moon phase, sampling twice a day (Fig. 1C). The two dial sampling points represent the midday with the highest light intensity and photosynthetic yield (12:00 p.m) and the moonrise time (21:00 p.m) that correlated with the spawning time of this Acropora. At each sampling time a small branch from each colony, measuring an average of 5 cm in length, using pliers, was sampled and snap-frozen in liquid nitrogen. In sum, we had four sampling days (full moon, third-quarter moon, new moon and first quarter moon) and eight

sampling points (12:00 p.m. and 21:00 p.m. on each day) from both experimental conditions. At each point, we sampled all colonies (n=10) giving a total of 80 coral fragments.


**-+ Pulse-Amplitude-Modulated (PAM) fluorometer measurements**

As corals were maintained in experimental conditions for a month we wanted to ensure there was no physiological degradation that could impact our results. We performed a measurement of photosymbiont output, used to indirectly assess holobiont health. Immediately after retrieving coral colonies from the reef, all ten colonies were dark acclimated for ten minutes and photosystem II (PSII) maximal quantum yield (Fv /Fm) was measured for each colony, using a diving pulse-amplitude-modulated (PAM) fluorometer (Walz GmbH, Germany). Maximum quantum yield, frequently termed Fv/Fm, where Fm is the maximum fluorescence yield in dark-adapted algae following a short period of saturating light and Fv is the variable fluorescence (Fv = Fm - F0; F0 is the minimum steady-state fluorescence yield measured in close-to-darkness). After one month, at the end of the experiment, all colonies (n=5 for each treatment) were evaluated again. The tip of the instrument's main optical fiber was placed five mm away from, and perpendicular to, the coral branch.


**- + RNA Extraction**

Total RNA was extracted using TRIzol reagent (Invitrogen) and a modified version of the manufacturer's protocol that included an additional chloroform extraction and magnesium chloride precipitation overnight. A small branch was cut off and placed in pre-cooled aluminum foil. The branch was crushed into a fine powder with a hammer, while the foil packet was occasionally dipped in liquid nitrogen to keep it frozen. The aluminum foil content was transferred to a 15 ml flacon pre-filled with 2 µl of TRIzol and left at room temperature for 5 minutes. The tubes were then centrifuged at 7500g for 5 minutes at 4°C to remove the skeleton powder. 1500 µl of the remaining supernatant from each sample was transferred to a 2 ml tube with 300 µl of chloroform, shaken vigorously and kept at room temperature for 10 minutes, followed by centrifuging at 12,000g for 15 minutes at 4°C. After this centrifugation, there were two visible phases. 800 µl of the aquatic phase was transferred to a 2 ml tube containing 600 µl of chilled isopropanol. Following a 10-minute incubation at room temperature, the tubes were centrifuged at 12,000g for 10 minutes at 4°C. The remaining supernatant was removed and the visible pellet was washed with 1 ml of 75% ethanol and then centrifuged at 7500g for 5 minutes at 4°C. The final step of the ethanol wash was performed a second time and was followed by removal of the ethanol and drying of the tubes in a clean chemical hood. The dry pellets were covered in 500 µl RNAse free water and incubated at 57°C for about 5 minutes until the pellet dissolved. 500 µl of 5M lithium chloride was added to the tube, gently mixed and stored in a -20 freezer overnight. The following morning, samples were defrosted on ice and centrifuged at 15,000g for 30 minutes at 4°C. The supernatant was removed without touching the pellet and 1 ml of 75% ethanol was added. Samples were centrifuged at 7500g for 5 minutes at 4°C and the supernatant was removed. The final step was again repeated, for a total of three ethanol washes. After the last wash, the ethanol was removed and the tubes were dried in a clean chemical hood. The dry pellets were covered in 40 µl RNase free water and incubated at 57°C for about 5 minutes until the pellet dissolved. Purified RNA samples were analyzed using a NanoDrop 1000

spectrophotometer (ThermoScientific) to assess RNA quantity and a 2100 Bioanalyzer (Agilent) to assess RNA quality (RIN >8.5).

**- + Next-generation sequencing**

1.5 µg RNA from each sample (three samples from each sampling time point from both groups, n=48) was sent for sequencing. RNA samples were prepared using the Illumina TruSeq RNA Library Preparation Kit v2, according to manufacturer's protocol. Libraries from each sampling point were run on lanes of an Illumina HiSeq2000 machine using the multiplexing strategy of the TruSeq protocol. The protocol starts with poly A selection that results in RNA selection only. Paired-end reads were obtained for each sample; the reads were 100 bases long. All sequencing libraries were trimmed using TrimeGalore version 0.4.0 (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) to remove adapters, primers and low-quality bases.

**- + Transcriptome annotation**

The sequenced reads were aligned to Acropora digitifera cDNA sequences downloaded from the Okinawa Institute of Science and Technology (OIST) website (http://marinegenomics.oist.jp) (Shinzato et al., 2011), according to default settings of the Bowtie2 program (v2.2.5) (Langmead and Salzberg, 2012). Using the published transcriptome and aligning the reads accordingly, removed all Symbiodinium contamination. Previously published annotations were used (OIST and ZoophyteBase). We used the raw read counts for DESeq (v1.18.0) (Anders and Huber, 2010), an R (Bioconductor) package, in order to detect differential gene expression between the different moon phases, time of day and light conditions.

**- + Differential expression**

 Genes with an adjusted P-value (Benjamini–Hochberg) of less than or equal to 0.05 and a minimum 1.5-fold change were considered differentially expressed (DE). We started with comparing the expression profile of genes within each experimental group (LL and AMB) in order to find gene groups that were DE during each moon phase and time of day regardless of the experimental light condition. We continued with comparing between the two experimental groups looking for different genes, for example: (i) comparing between LL samples and AMB samples during each moon phase regardless of the time of day in order to find DE genes responding to the moon phase, (ii) comparing between LL samples and AMB samples during the sampling times along the day regardless of the moon phase in order to find DE genes responding to the duration of the day, (iii) comparing all LL samples to all AMB samples in order to find specific pathways that are different between the two light regimes. Using the normalized data from the DESeq comparisons, we performed hierarchical clustering and generated heat maps of all the significantly expressed genes using the heatmap.2 function from the R BIOCONDUCTOR package GPLOTS (v2.17.0). We used meta2d, a function of the R package MetaCycle, to evaluate periodicity in the expressed transcripts (Wu et al., 2016). Transcripts were considered to be rhythmically expressed when the p-value was <=0.001. The MetaCycle analysis will allow us to identify periodic signals in the time-series data along the day and the month.

## - DATA AND SOFTWARE AVAILABILITY

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