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Effects of Light Dynamics on Coral Spawning Synchrony

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Abstract. Synchrony of spawning in many hermatypic corals, typically a few nights after the full moon, is putatively dependent on solar and lunar light cycles in conjunction with other possible cues such as tides and temperature. We analyze here the contributions of separate components of light dynamics, because the effects of twilight and lunar skylight on coral spawning synchrony have previously been conflated and the alternative hypothesis that these components have differential contributions as proximate cues has not been tested. Moonlight-dependent changes in spectra during twilight, rates of decreasing twilight intensities, and changes in lunar photoperiod were experimentally decoupled using programmed light-emitting diodes and compared for their separate effects on spawning synchrony in *Acropora humilis*. Effects on synchrony under the control of synthetic lunar cues were greatest in response to changes in lunar photoperiod; changes in light intensities and spectra had lesser influence. No significant differences among treatment responses were found at the circa-diel time scale. We conclude that spawning synchrony on a particular lunar night and specific time of night is a threshold response to differential periods of darkness after twilight that is primarily influenced by lunar photoperiod and secondarily by discrete optical components of early nocturnal illumination.

Introduction

Spawning synchrony by gamete-broadcasting marine invertebrates is a complex phenomenon that is essential to maximize species-specific reproductive success, enhance larval recruitment (Knowlton *et al.,* 1997; Levitan *et al.,* 2004), and enhance the population fitness of clonal organisms through sexual reproduction and larval dispersion (reviewed by Jackson, 1986). Synchronized spawning events are relatively common in marine invertebrate systems (Korringa, 1947; Giese, 1959; Hauenschild, 1960). The most conspicuous example may be seen in tropical reef systems (*e.g.,* Great Barrier Reef) when as many as 100 species of reef-building corals display mass spawning behavior over a period of a week, each species typically spawning within a 4-h window on one or two nights within that week (Harrison *et al.,* 1984; Babcock *et al.,* 1986). These events likely involve coordination on three time scales—circa-annual, circa-lunar, and circa-diel—as well as immediate direct responses by individual colonies to dynamic environmental conditions. While reproduction based on solar insolation and energy-allocation strategies has been shown to approximately fix corals to a particular season or month of spawning, more recent studies show that a final trigger independent of seasonal drivers is probably required to initiate the tight synchrony observed on a particular lunar night and hour (Van Woesik *et al.,* 2006; Ananthasubramaniam *et al.,* 2010). Possible final triggers proposed include sea surface temperature (Orton, 1920), lunar irradiance (Harrison *et al.,* 1984; Jokiel *et al.,* 1985; Hunter, 1988), lunar photoperiod (Hauenschild, 1960), tidal levels (Babcock *et al.,* 1986), seasonal photoperiod (Babcock *et al.,* 1994), and twilight chromaticity (Sweeney *et al.,* 2011). Here, we focus on light effects, experimentally decoupling several parameters of the complex optical dynamics of circa-diel and circa-lunar light cycles and investigating their possible roles as proximate cues for coral spawning synchrony.

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Abbreviations: NAFM, nights after full moon.

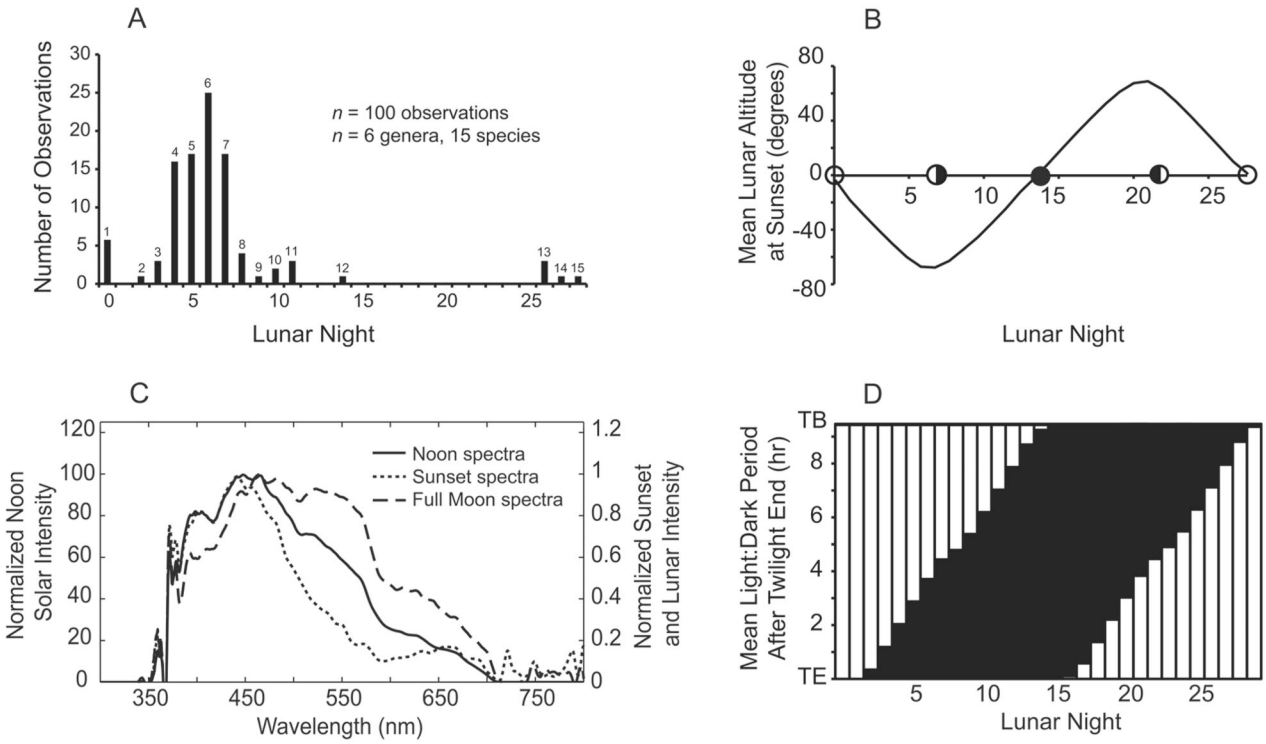

Figure 1. Coral spawning observations relative to the lunar cycle. (A) Compilation of *in situ* observations from the literature. Data from 6 genera and 15 coral species from both the Pacific and Caribbean basins at various latitudes and years show correlation with the lunar altitude cycle in panel (B). Numbers above bars in panel (A) refer to spawning reports from the following references: (1) Boch, pers. obs., (2) Hayashibara *et al.,* 1993, (3) Mundy and Green, 1999; Sweeney *et al.,* 2011, (4) Harrison *et al.,* 1984; Willis *et al.,* 1985; Mundy and Green, 1999; Levitan *et al.,* 2004; Boch, per. obs.; Sweeney *et al.,* 2011, (5) Harrison *et al.,* 1984; Mundy, 1999; Levitan *et al.,* 2004, (6) Willis *et al.,* 1985; Mundy and Green, 1999; Levitan *et al.,* 2004, (7) Willis *et al.,* 1985; Levitan *et al.,* 2004; Vize *et al.,* 2005, (8) Vize *et al.,* 2005; Rosser, 2005, (9) Vize *et al.,* 2005, (10) Babcock *et al.,* 1994; Vize *et al.,* 2005, (11) Hayashibara *et al.,* 1993; Babcock *et al.,* 1994, (12) Hayashibara *et al.,* 1993, (13) Richmond and Hunter, 1990; Hayashibara *et al.,* 1993, (14) Hayashibara *et al.,* 1993, (15) Hayashibara *et al.,* 1993. (B) Mean lunar altitude (black line) during sunset. (C) Example of peak normalized relative intensity of noon spectra (primary *y*-axis, black line), sunset spectra 4 nights after the full moon (secondary *y*-axis, short dashes), and full moon spectra only (secondary *y*-axis, long dashes). (D) Mean nightly sequence and duration of lunar photoperiod between astronomical twilight end (TE) and astronomical twilight beginning (TB) derived from 2006–2009 astronomical data for Palau. Black bars represent dark period and white bars represent lunar illumination. Astronomical and lunar altitude data from http://www.usno.navy.mil/USNO/astronomical-applications/data-services/rs-one-year-us and Stellarium freeware, ver. 0.10.2, respectively.

Published observations of *in situ* spawning are relatively sparse, but most of the documented spawnings occur in the period 4–7 nights after the full moon (NAFM), independent of the year or month (Fig. 1A). During this portion of the lunar cycle, the moon is low on the horizon when the sun sets and becomes progressively lower with each passing night (Fig. 1B). This correlation suggests that the synchrony of coral spawning may depend on some mechanism that has direct or indirect lunar dependence, such as light or tidal cycles. In the context of lunar light, Gorbunov and Falkowski (2002) suggested that the detection of the blue region of moonlight may cue the night of spawning: several species of corals were extremely sensitive to the blue region of the spectrum, and Levy and colleagues (2007) showed that cryptochrome expression correlated significantly with phases of moonlight. Additionally, increasing chromaticity in the blue region of the spectrum during twilight after the full moon night was found to correlate significantly with observations of spawning in coral and other marine reef invertebrates (Sweeney *et al.,* 2011). Previous empirical work suggested that coral spawning might be a direct response to failing light intensities at sunset or to the onset of

darkness after sunset (Babcock, 1984; Hunter, 1988; Van Veghel, 1994; Knowlton *et al.,* 1997; Brady *et al.,* 2009). Taken together, the correlation of *in situ* circa-lunar and circa-diel spawning observations with solar and lunar light cycles led us to hypothesize that coral and other invertebrate spawning responses depend on some aspect of light dynamics at twilight around the full moon. In contrast to previous empirical studies that have treated lunar light (Jokiel *et al.,* 1985; Hunter, 1988) and solar light (Babcock, 1984; Hunter, 1988; Knowlton *et al.,* 1997; Brady *et al.,* 2009) as independent cues, we investigated the possibility that each of these components could have differential contributions to a complex proximate cue for coral spawning synchrony, and we evaluated how spawning synchrony on a particular lunar night and specific time of night might occur as a function of light dynamics.

To elucidate which physical features of the complex twilight and lunar cycle process might contribute to spawning synchrony, we identified three major physical aspects of nightly light stimuli that are (a) unique to each night during the lunar nights when corals are likely to spawn and (b) also constitute physiologically plausible proximate spawning cues. (1) Skylight on each night of the lunar cycle undergoes major chromatic changes due to differing mixtures of sunlight and lunar light that shift the spectrum toward blue at twilight (Fig. 1C, short dashes). This blue shift is caused by increased absorption of long-wave visible light when the sun is below the horizon and red-shifted light reflected from the moon is absent (Fig. 1C, long dashes). (2) Each night during this period also has a unique rate of decreasing light intensities—also due to the changing position of the moon on the horizon. (3) The sequence and duration of illumination by the moon after sunset causes a unique lunar photoperiod during each night—this is distinct from photoperiod due to changing seasons of the solar cycle, which is particularly minimal at tropical latitudes (Fig. 1D). Although the above processes of twilight dynamics (Sweeney *et al.,* 2011) and lunar photoperiodicity (Hauenschild, 1960) have been described previously, empirical work decoupling these major optical components as proximate cues remained to be experimentally tested.

To investigate the effects of the above-described three physical aspects of light dynamics on spawning synchrony on a particular night of the lunar cycle, we decoupled these light components using calibrated light-emitting diode (LED) lightboards and displayed them to gravid corals during a month when spawning was likely to occur. More specifically, we programmed the lightboards to display the following aspects of light dynamics: (1) ambient twilight and lunar light dynamics on the reef around the full moon (treatment A), (2) a shift in the illuminating spectrum to the blue region of the spectrum, held at lunar intensity (treatment B), and (3) changes in the decreasing rates of twilight intensity (normalized to the spectrum observed at noon) combined with changes in lunar photoperiod (treatment C). We compared the spawning responses between these treatments and to spawning responses under treatments of 13:11 light/dark photoperiod conditions (treatment D) and spawning responses under changes in photoperiod from 24:0 to 13:11 light/dark cycle (treatment E).

We demonstrate that optical and photoperiod dynamics of the solar and lunar light cycles have differential contributions to the proximate cue for spawning synchrony of the coral *Acropora humilis* (Dana, 1864), a relatively widely distributed coral species of the Western Pacific. We show that thresholds achieved under lunar photoperiod cues are apparently the major driver of spawning synchrony on a given night of the lunar cycle and a specific time of night and that differences in spectral dynamics have secondary effects on spawning. This latter chromatic effect on coral spawning is reported for the first time.

Materials and Methods

Coral collection, experimental treatments, and spawning observations

Work was performed in the laboratory and nearshore shallow reefs of Palau (7°N latitude; 134°E longitude). We chose to work with *Acropora humilis*, colonies of which were abundant and gravid during the chosen month of our experiment. Gravidity, or readiness of gonads to spawn, can be determined by visual confirmation of dark pink coloration in gametes (Baird *et al.,* 2002). Experiments were conducted during the lunar cycle of April 2010 using replicated fragments from 12 mature *A. humilis* colonies. Four days before the full moon in April, we collected gravid fragments (12 fragments/treatment) from Ioul Lukes Reef (7°17.238′N; 134°30.204′E) and immediately placed one sample from each colony ($n = 12$ colonies) in each of six plastic grids to minimize any differential genetic response. Each grid was randomly placed in one of five light treatments to reduce bias; the sixth rack was exposed to the ambient sky at the laboratory. Because the donor colonies were widely spread (>10 m apart, 4–5 m in depth) across Ioul Lukes Reef, they were collected and temporarily relocated to 5 m depth (about 25 cm apart) in an open sandy patch on the reef for monitoring with gamete traps (cylindrical transparent plastic containers fitted over 5–6 branches). These gamete traps were checked every morning, and any gametes present were inferred to have been spawned during the previous night.

Once all coral fragments were collected and labeled, samples were immediately brought back to the *ex situ* aquarium system at the Palau International Coral Reef Center. Six experimental aquarium tanks were fed by seawater from the lagoon that was filtered through a sand filter (Jacuzzi Sandstorm sand filter) and a 75-μm mesh bag at the pipe inflow. Monitoring of seawater temperature showed

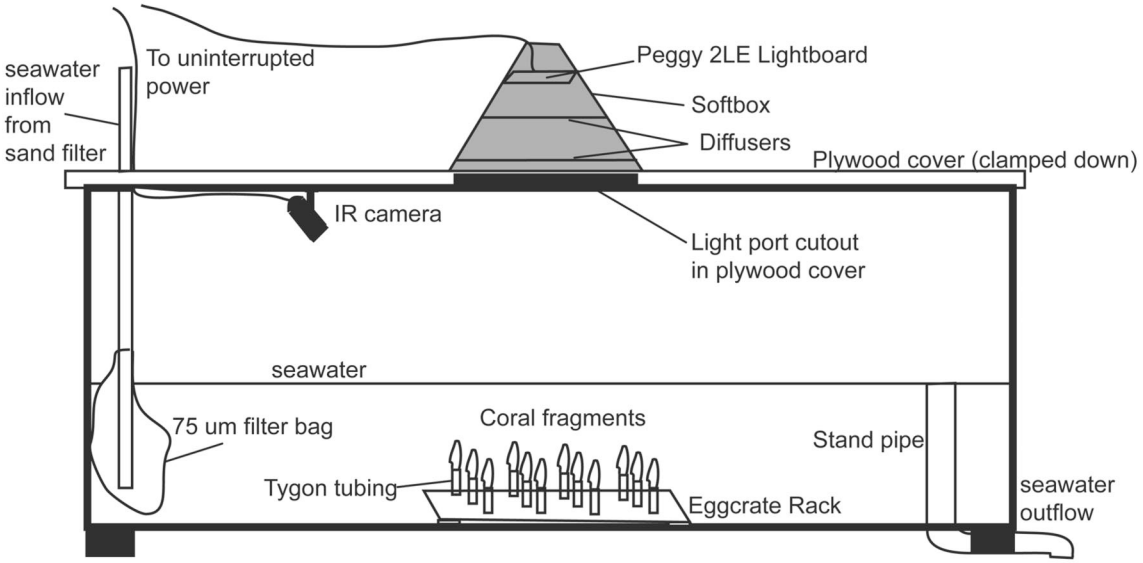

Figure 2. Diagram of *ex situ* experimental light treatment aquarium system.

that temperature in all tanks remained essentially equal as they were fed from the same source. The daily mean (±SE) temperature of the seawater in the tanks was 29.7 ± 0.7 °C. The flow and constant volume of seawater were kept at 4.5 l/s and 200 gallons (≈757 l) respectively (total volume of tank = 400 gal [≈1514 l]). Flow was turned off from 1830 to 2130 h each night of the experiment (and turned back on thereafter) to minimize any flow effects on spawning. Over these tanks, light-tight plywood covers were built to hold the LED displays and surveillance cameras. These covers were placed on top of the tanks at the –7° solar elevation each evening and removed at –7° solar elevation before sunrise the following day for daylight exposure and photosynthesis. The experimental set up is illustrated in Figure 2.

To monitor circa-lunar and circa-diel coral spawning in tanks under the plywood covers, infrared cameras (Defender Sentinel 3 surveillance system, Cheektowaga, NY) with built-in infrared LED illumination were turned on from 2030 to 2130 h, the range of spawning time determined for the subject species from the March lunar cycle. This restricted recording time made data sizes manageable in the field and also minimized any effect of the tail of far-red emission from the infrared camera LEDs. The digital video recorder for the infrared camera system recorded time-stamped videos of gamete release for later temporal analysis of spawning dynamics. From these records, we were able to determine the exact minute of initial egg bundle release from individual fragments. At the conclusion of the experiment, we cross-validated spawning of fragments by cracking open the experimental fragments and checking for the presence or absence of gametes within the coral skeleton.

Light treatments

To simulate relevant characteristics of light dynamics in our experiments, moon-independent sun spectra and sun-independent moon spectra were extracted from twilight sky irradiance spectra collected at the U.S. Virgin Islands (see Sweeney *et al.,* 2011, for details) and assembled into a "sun matrix" and a "moon matrix." For each night of our experiment in Palau, we reassembled the predicted twilight spectra for that night by adding the appropriate subsets of the solar and lunar twilight matrices to rates of sunset and moonrise altered to account for differences in solar and lunar elevation in Palau *versus* the USVI. To account for the fact that solar elevation in Palau was higher than in the USVI, once the moon reached an altitude greater than 45° at the Palau site, the spectrum was held constant at the brightest moon spectrum we measured in the USVI. When the moon set below 45° elevation, the moonrise data were displayed in reverse (moonset). Each light treatment is described in Table 1.

Peggy 2LE lightboards (Evil Mad Scientist Laboratories, Sunnyvale, CA) were specially constructed for this experiment to display the above sun and moon dynamics to gravid coral fragments. We characterized candidate LEDs using the same equipment we used for field measurements in a previous investigation (Sweeney *et al.,* 2011). Nine different LEDs with emission maxima at 423, 464, 507, 521, 568, 593, 630, 651, and 664 nm were selected that when combined provided reasonable fits to the range of spectra measured in the field. We next calculated the distribution and number of LEDs of each type required in each lightboard to

Table 1

Light treatments for coral spawning experiment

Tank	Light treatment
A	Simulate spectra, intensity, and lunar photoperiod characteristics of the sunset and moonrise on the reef starting from 4 nights before full moon to 9 nights after full moon.
B	Hold intensity constant but change the spectrum according to what was happening on the reef. The arbitrary irradiance level we chose was that of full moon. Holding the intensity constant required a lack of lunar photoperiod.
C	Hold color spectrum constant (spectrum at noon was arbitrarily chosen) but intensity and lunar photoperiod changed to match reef observation.
D	Cover the tank completely (each evening at −7° solar elevation) and remove (at −7° before dawn) to create constant dark conditions at night. About 13:11 L/D photoperiod.
E	Display broad-spectrum white light (LED rope light) to create constant light condition at night. After most of the spawning passes in other treatments, convert to treatment D conditions—*i.e.,* change from 24:0 to 13:11 L/D photoperiod
F	No cover: open to the sky with no light treatment to determine whether fragments could spawn in this *ex situ* system.

produce the output specified for each experimental light treatment (A, B, and C). These lightboards were programmed to turn on different numbers of LEDs of a given spectral channel for the appropriate length of time in order to match the shape and intensity of spectra for each night and treatment, as verified by our direct spectroscopic measurements. All programming and coding for each LED on the lightboards were done with Arduino software (Banzi *et al.,* 2009)—an open source development environment for microcontroller platforms. After an initial iteration of modeling the match between LED lightboard output and the recorded environmental spectrum, an empirical correction was applied to our fitting algorithm to account for small variations in individual LED output. LED lightboards were mounted in highly diffusing "softboxes" that are commonly used in photography applications (Interfit, Interfit Photographic Limited, UK). Additionally, when sea conditions permitted, natural twilight spectra at Ioul Lukes Reef were measured to validate LED simulations. Examples of Ioul Lukes Reef spectra and spectra from our programmed LED treatments A, B, and C are shown in the Appendix.

Statistical analyses

We used coefficient of variation (CV) to test for differences in relative spawning synchrony at both the circa-lunar and circa-diel scales. CV (standard deviation/mean) has been previously used as an index of relative synchrony (Spencer *et al.,* 1961; Liebold *et al.,* 2004; Lamontagne and Boutin, 2007) and can be used to test for differences in dispersion when means differ significantly. This measure of relative synchrony accounts only for individuals that spawned during the experimental period—non-spawning fragments are taken into account in logistic regression analysis (see below). These circa-lunar and circa-diel spawning responses were compared using permutation tests that are appropriate with limited data and do not require specific population shapes such as normality (Manly, 1997). Furthermore, to reduce Type I error, significance between treatments was limited to calculated P values that exceeded alpha levels adjusted by the Bonferroni correction (Manly, 1997). With all other parameters held equal in the seawater system, any differences in the observations of the spawning response were inferred to be caused by the light treatments. Because we could not control for artificial light coming from the nearby Palaun community on the lagoon or for the interference with natural lunar light dynamics from a rain-cover rooftop over the open tank used for treatment F, we did not evaluate comparisons with treatment F spawning results.

To supplement our understanding of the light processes contributing as possible proximate cues for synchronized spawning, we used logistic regression analysis of spawning data from the light treatment results. Logistic regression can be used to investigate the linear dependence of binary responses as a function of independent variables (x) (Pampel, 2000; Van Woesik *et al.,* 2006) and the inflection point ($p = 0.5$) of the regression can be evaluated as the critical threshold (Thomas, 1995; Butler *et al.,* 2004). Thus, the model for our initial investigation for this research is

$$y = \log\left(\frac{p}{1-p}\right) = \alpha + \beta x,$$

where y—the binary response—is the log odds ratio of the probability of spawning (p) and the probability of not spawning ($1–p$) during a particular light process. The Newton-Raphson method was used to estimate the maximum likelihood of the constant α and coefficient β. Chi-square and P values are reported to show goodness of fit. In this way, we were able to conservatively evaluate differences in relative spawning synchrony and infer whether synchronized spawning was a response to a particular feature of light dynamics.

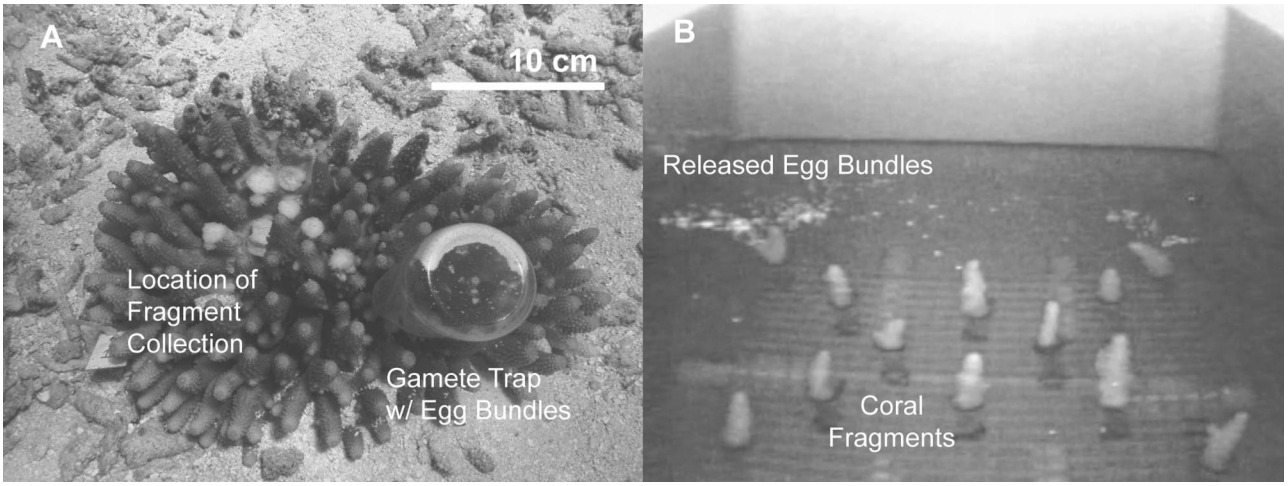

Figure 3. Spawning observations. (A) Example of *Acropora humilis* egg bundle release captured by a gamete trap on the night of 4 May 2010 at Ioul Lukes Reef. White bar = scale bar shown for reference. (B) An example of fragments spawning around 20:50 (Palau time) captured by infrared camera observations on the night of 4 May 2010 under light treatment. White specks are egg bundles on the surface of the water.

Results

In situ *spawning*

Monitoring by gamete traps revealed that 12 of 12 *Acropora humilis* donor colonies (*i.e.,* colonies from which experimental fragments were collected) spawned on the reef on the night of 4 May 2010—6 nights after full moon (NAFM). An example of the egg bundles captured by a gamete trap is shown in Figure 3A.

Spawning under light treatments

Using the infrared camera system, we clearly observed release of egg bundles from individual coral fragments under each light treatment (Fig. 3B). These results are illustrated in Figure 4A–F. Under treatments D (Fig. 4D) and E (Fig. 4E), 12 of 12 gravid fragments were observed to spawn. Additionally, three fragments spawned twice under treatment D and one fragment spawned twice under treatment E. In the other treatments, we observed 8 of 12 fragments spawning under treatment A and 11 of 12 fragments spawning under treatment C. Under treatment B, fragments were not observed to spawn. In the open-air tank with no light treatment (tank F), 11 of 12 gravid fragments were observed to spawn during the study period. At the conclusion of the light treatment experiments, cross-validation—by cracking open all spawning and non-spawning fragments—showed that fragments that spawned contained neither pigmented nor non-pigmented gametes; conversely, all fragments that were not observed to spawn contained pigmented gametes. The numbers of fragments with pigmented gametes at the conclusion of the study period were 4, 12, 1, 0, 0, and 1 for treatments A, B, C, D, E, and F respectively—which confirmed the total spawning observations viewed with the infrared camera system.

On the circa-diel time scale (Fig. 5A), the mean (HH:MM ± SE) initial times of egg bundle release for A, C, D, and E were 20:46 ± 0.0113, 20:43 ± 0.014, 20:47 ± 0.0096, and 20:38 ± 0.0083 respectively. On the circa-lunar time scale (Fig. 5C), the mean spawning night (±SE) during the experiment was 5.875 ± 0.1239 NAFM under treatment A and 5.9091 ± 0.0637 NAFM under treatment C. Under treatment D, the mean night of spawning occurred on 3.5833 ± 0.1793 NAFM. After converting to a 13:11 L/D cycle (treatment E), 50% of the fragments spawned on the first night of conversion and 50% of the fragments spawned on the second night. This latter pattern of delayed spawning response is similar to the results found by Hauenschild (1960) working with the effects of lunar periodicity on the palolo worm *Platynereis dumerilii.*

Spawning synchrony varies depending on lunar photoperiod and optical qualities of light

Comparative analysis at the circa-diel time scale among treatments (Table 2) showed that the relative spawning synchrony under treatment A and treatment C did not significantly differ relative to the synchronous response under photoperiod only (*i.e.,* treatments D and E). These results indicate that differential rates of decreasing light intensities at twilight are not essential for cueing synchronous response on the circa-diel time scale. That is, spawning synchrony on the circa-diel time scale is affected by the removal of light

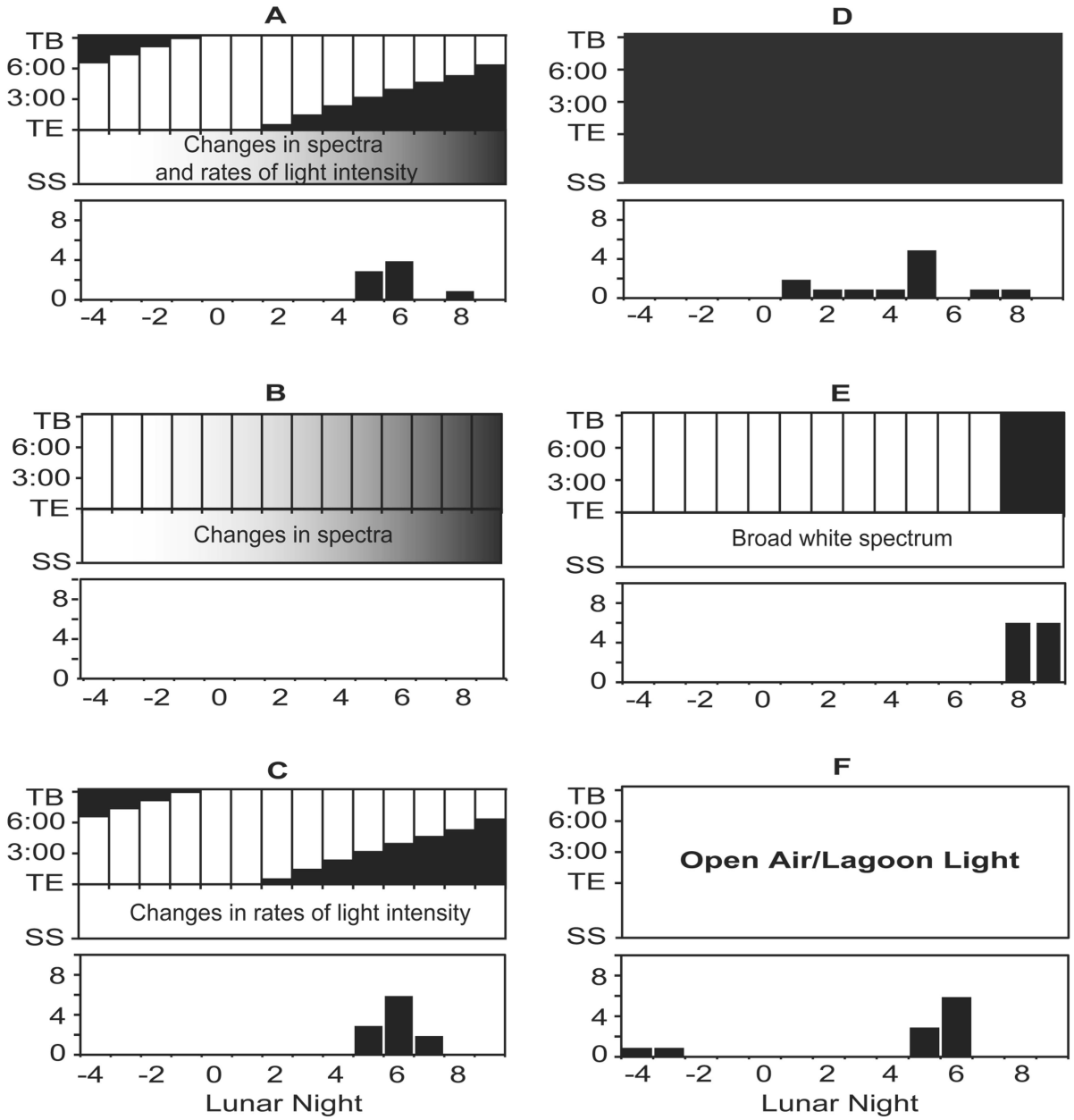

Figure 4. Spawning response of coral fragments under each treatment. (A–F) Upper panels: relative light treatments* described in the materials section (Table 1); bottom panels: spawning frequency observed during the experiment. Upper panel in (A): the period of changes in spectra and rates of decreasing light intensities from sunset (SS) to astronomical twilight end (TE) followed by the lunar photoperiod. Upper panel in (B): a shift in the spectrum (held at constant full moon intensity all night): darker shades represent greater number of photons from the blue wavelengths relative to the red wavelengths over each night starting at sunset. Upper panel in (C): a period of changes in the rates of decreasing light intensities from SS to TE followed by the lunar photoperiod. TE, astronomical twilight end; TB, astronomical twilight beginning of the next day. White bars indicate period of lunar illumination; black bars indicate periods of no light. Bottom panel: spawning frequency observed (*x*-axis = lunar night; *y*-axis = number of fragments spawned). *Due to scaling limitations, the changes in spectra and rates of decreasing light intensities from sunset (SS) to astronomical twilight end (TE) are not to scale in the upper panels of A, B, and C. 3:00 and 6:00 refers to hours after astronomical twilight end (TE).

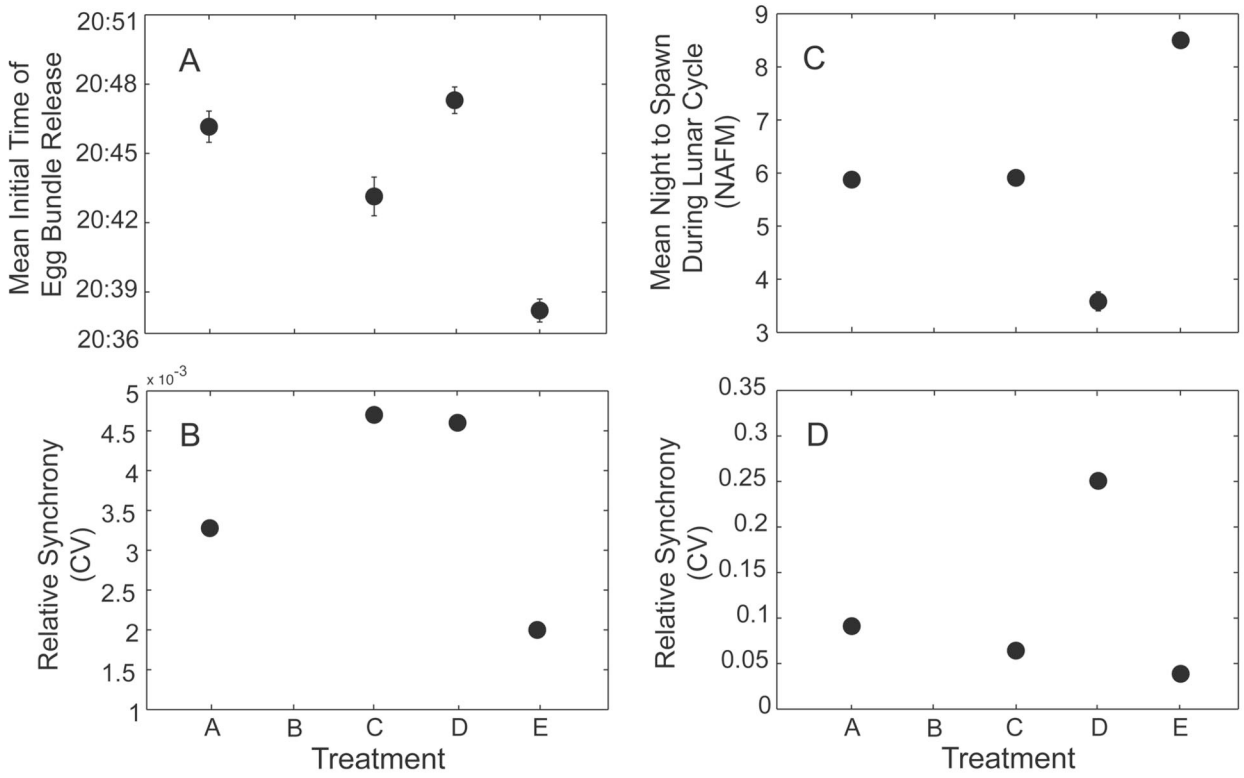

Figure 5. Spawning and relative synchrony under different light treatments. (A) Mean initial time (HH:MM) of egg bundle release for fragments. Error bars are standard error. (B) Coefficient of variation (CV) indicating relative spawning synchrony within treatments. 0 is perfect synchrony. (C) Mean night to spawn during 14 nights of experimentation. NAFM, nights after full moon with night zero as full moon night. Data do not include repeated spawning by fragments. Error bars are standard error and may be too small to be seen. (D) Coefficient of variation (CV) indicating relative lunar scale synchrony within treatments; 0 is perfect synchrony.

at sunset independent of differential rates of decreasing light intensity.

In contrast to spawning synchrony at the circa-diel time scale, the circa-lunar time scale showed significant differences relative to treatment D (Table 2). That is, relative spawning synchrony was significantly greater under treatments A, C, and E than under continuous 13:11 L/D photoperiod conditions (treatment D). In summary, for the coral fragments that spawned under each light treatment, the order of greatest to least relative synchrony was E > C and

Table 2

Permutation analysis results for treatment comparisons of relative spawning synchrony

Spawning response comparison	Absolute observed difference in circa-diel scale relative synchrony $\lvert CV_{treatment\ x}\text{-}CV_{treatment\ y}\rvert$	*P* value	Absolute observed difference in circa-lunar scale relative synchrony $\lvert CV_{treatment\ x}\text{-}CV_{treatment\ y}\rvert$	*P* value
A *vs.* C	1.50E-03	ns	2.69E-02	ns
A *vs.* D	1.40E-03	ns	1.60E-01	*
A *vs.* E	1.30E-03	ns	5.24E-02	*
C *vs.* D	1.00E-04	ns	1.86E-01	**
C *vs.* E	1.70E-03	ns	2.55E-02	ns
D *vs.* E	2.60E-03	ns	2.12E-01	***

CV = coefficient of variation.

Significance noted only for *P* values that exceeded alpha levels with Bonferroni corrections: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, ns = not significant ($n = 10000$ permutations, $n = 6$ comparisons).

Table 3

Logistic model fit to spawning response

Model fit to spawning response					Goodness of fit*
Coefficient	Estimate	Std. Error	*z* value	*P* value	χ^2
			Treatment A		
α	−3.94	8.73E-01	−4.509	<0.001	7.2634
β	0.01	3.64E-03	2.487	<0.05	
			Treatment C		
α	−5.29	1.27E+00	−4.15	<0.001	23.318
β	0.02	6.04E-03	3.435	<0.001	

Model fits of treatments A and C spawning data to length of the dark periods after twilight.
* Degrees of freedom = 1; $P < 0.001$.

A > D. These results suggest that spawning synchrony in this coral species is greater under changes in nightly photoperiod or with the delayed removal of lunar illumination after sunset.

We also observed a peak (5 NAFM) in the number of fragments spawning (*i.e.,* 6 of 12 fragments) under continuous 13:11 L/D cycle (Fig. 4D). Although all of the fragments under this treatment spawned, this peak in proportion of total fragments spawning on one night indicated a possible entrained endogenous response. Thus, in the absence of dynamic light signals, a degree of relative synchrony measured by the total proportion of fragments spawning on one night was observed on the circa-lunar time scale.

Spawning synchrony on particular lunar nights is a threshold response to length of dark period

As spawning by gravid fragments occurred only under some length of dark exposure, spawning responses were evaluated with the simplest component of light dynamics—that is, the length of dark period after the end of astronomical twilight on each night of the lunar cycle. The results show that the spawning response as a function of nightly increases in the length of dark period after astronomical twilight end is a significant fit for both treatment A and C spawning data (Table 3).

In treatment A, the critical threshold (*i.e.*, 0.5 probability of spawning) was determined to be about 436 minutes of dark period, or about 10–11 NAFM (Fig. 6A). In contrast, the critical threshold value of $p = 0.5$ for treatment C spawning response was evaluated to be 255 minutes of darkness or about 6–7 NAFM (Fig. 6B). This discrepancy in differential thresholds may be due to significant differences in the proportion of fragments spawning under treatments A (8/12) and C (11/12) (permutation test, $P < 0.05$). Therefore, while the relative synchrony of the fragments that spawned may be the same under both treatments (Table

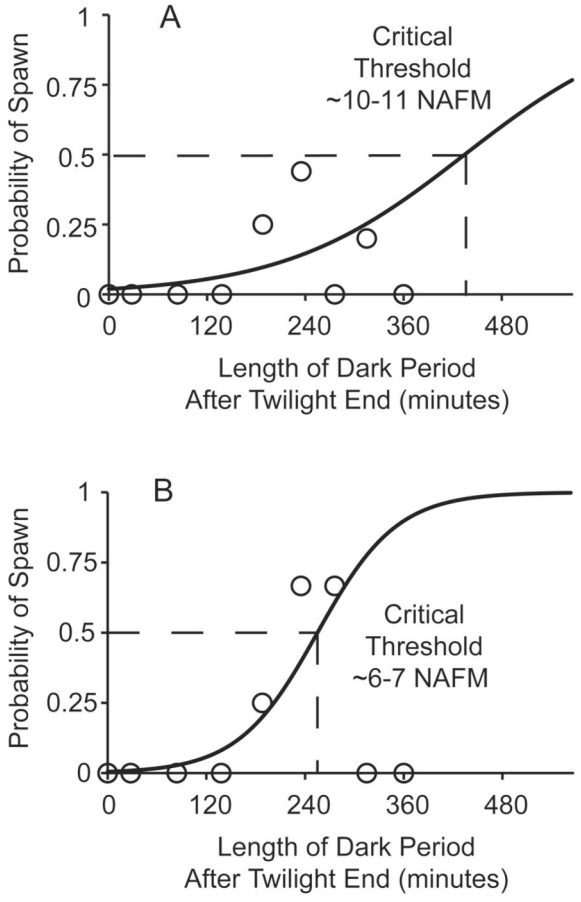

Figure 6. Time to critical threshold in treatment A *vs.* treatment C. (A) Predicted probability response (solid black line) as a function of lengths of dark period for treatment A spawning response. Open circles are observed probabilities from treatment A. $p = 0.5$ indicates a critical threshold point predicted to be reached at 10–11 nights after full moon (NAFM). (B) Predicted probability response (solid black line) as a function of lengths of dark period for treatment C spawning response. Open circles are observed probabilities from treatment C. $p = 0.5$ indicates a critical threshold point predicted to be reached at 6–7 NAFM.

2), the chromatic differences between these treatments may have had a significant effect on the proportion of total coral fragments spawning.

Spawning synchrony as a critical threshold response to lengths of dark period after twilight end (treatment C) is shown to be a reasonable estimate of the peak nights of spawning observed in natural systems (Fig. 1A). This result indicates that for spawning synchrony to occur on a particular night in the lunar cycle, *Acropora humilis* and possibly other coral species require thresholds to be met under certain light conditions. More specifically, for spawning to occur 6–7 NAFM, the length of the dark period after sunset must be long enough for the spawn-initiating process to come to completion for individual gravid corals; either exposure to lunar illumination or certain spectral features at twilight will inhibit the initiation and thus reduce the number of individuals reaching the critical threshold. This threshold response as a function of differential lengths of dark period after twilight and correlation with *in situ* observations of spawning synchrony on particular nights in the lunar cycle is reported for the first time.

Discussion

Although it has long been suspected that light dynamics play a role in the spawning synchrony of many hermatypic corals, the physical phenomena involved are complex. In data reported to date, the physical aspects involved (lunar photoperiod, rate of decreasing intensities, and spectral changes with the lunar cycle) have been conflated, making it difficult to specify the proximate physical cues to synchronous spawning. Therefore, we designed a study to decouple these physical aspects of light dynamics to determine their effects on the occurrence and synchrony of spawning in *Acropora humilis.* We recognize that our experimental design is limited in precision and power by the transferring and handling of the coral fragments and by the single batches of fragments exposed to each light treatment. However, the permutation analysis we used allowed us to apply a reasonable nonparametric test to determine if differences in the test statistic could have occurred by random chance in similarly handled corals (Manly, 1997), with the results revealing new insights into the effects of light dynamics on coral spawning and its synchrony. First, it is clear from this and other investigations that both circa-lunar (Hunter, 1988) and circa-diel (Knowlton *et al.,* 1997; Brady *et al.,* 2009) spawning synchrony are directly influenced by light after sunset. Second, our results show that the major driver of spawning on a given night of the lunar cycle appears to be a critical threshold determined by lunar photoperiod cues. Third, we report for the first time that there may also be a wavelength-dependence on this critical threshold.

Spawning synchrony as cued by lunar photoperiod could be achieved if a group of individuals are all inhibited from spawning for a length of time, followed by the removal of the inhibiting cue—in our case the removal of lunar illumination after sunset. Thus, if there is variation in the time that individuals require to reach a threshold to spawn (*i.e.,* variation in the accumulation of spawn-initiating factors to a critical level), prevention of all individuals from spawning could result in a greater proportion of individuals reaching the critical threshold at the same time. Then, once the inhibiting factor is removed, spawning synchrony would be observed. This hypothesis was supported by the results of changing the photoperiod conditions (treatment E). Under treatment E, spawning was inhibited during the 24:0 L/D cycle. However, when the light source was removed after sunset or changed to a 13:11 L/D cycle, we observed the greatest spawning synchrony relative to all the other light treatments. Additionally, if variations in individual responses to the time of removal of the inhibiting cue are produced by photoperiod dynamics or variations in spectra (treatments A and C), our results suggest that these components could have significant effects on spawning and its synchrony.

Twilight is a complex phenomenon involving strong shifts in the color as well as the intensity of skylight, and the pattern of these shifts is different for each night during the critical window of the lunar cycle when corals spawn. It had previously been hypothesized that these differences in spectrum within and between nights of the lunar cycle play a role in cueing mass spawning events (Sweeney *et al.,* 2011). Although the exact mechanism by which corals differentially respond to specific wavelengths of light remains to be determined, spectral differences at twilight in our experiment were shown to have some effects on spawning. When corals were exposed to the changing spectra observed on the reef (treatment A), relatively fewer corals spawned than in the treatment with a constant spectrum but decreasing intensity (treatment C). Because the net photon flux each night was the same in these treatments, the observed differences in spawning response could have been due to the spectral differences between the treatments. The differences between these treatments were greatest for exposures to the LEDs for which emissions peaked around 420, 525, and 595 nm. The LED at 420 nm was relatively brighter in the twilight component of the display and relatively dimmer in the moonlight component in treatment A compared to treatment C. The opposite pattern was displayed in the 525 and 595 nm channels, which were relatively dimmer in the twilight period and brighter during the moonlight period (Fig. 7). Testing the specific effects of individual spectral channels and combinations of channels was beyond the scope of our investigation, but the relative changes described suggest candidates for further investigation of chromatic effects on coral spawning.

Despite increasing evidence that the coral species studied

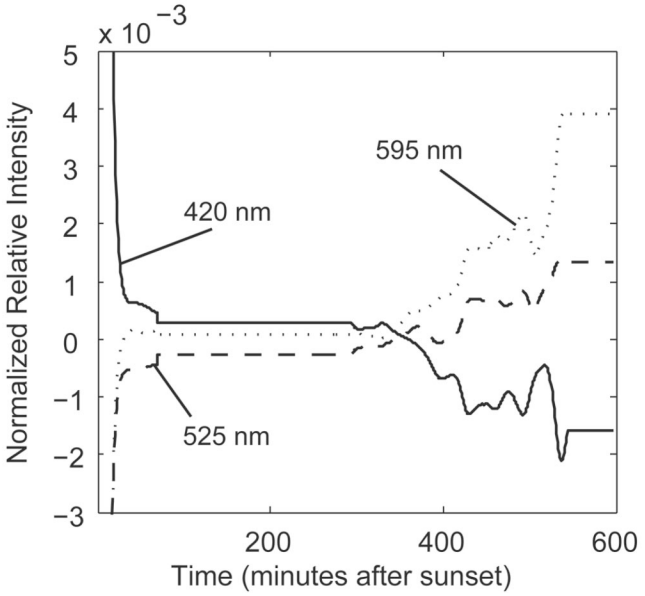

Figure 7. Chromaticity ratio of treatment A over treatment C on the main night of spawning (4 May 2010). At 420-nm wavelength (solid line), the normalized relative intensity decreases over time. At 525-nm (dashed line) and 595-nm (dotted line) wavelengths, the normalized relative intensity increases over time.

here and other organisms are indeed synchronized to the lunar cycle and influenced by light dynamics, it remained unclear what specifically induces these organisms to spawn on a particular night in the lunar cycle and how this process is related to the circa-diel time of spawning. In our experiment, egg bundles were released from 2035 to 2105 h (5–6 NAFM) in the A and C treatments. This result is similar to the *in situ* observations of the same species reported by Harrison and colleagues (1984) and to reports by Babcock *et al.* (1986) that other Pacific acroporids also spawn before 2100 on 5–6 NAFM. Other invertebrate systems such as polychaete worms (*Eunice viridis*) do not synchronously spawn until around midnight 7–10 NAFM (Caspers, 1984), while the worm *Odontosyllis luminosa* begins spawning about 1 h after sunset 0–4 NAFM (Gaston and Hall, 2000). This correlation for gamete release between time after sunset and the particular night in the lunar cycle seems to be a species-specific response that enhances reproductive isolation (Knowlton *et al.,* 1997; Levitan *et al.,* 2004). Our analysis of the spawning response in treatment C showed that the critical threshold value as a function of nightly lengths of dark period is a reasonable estimate of *in situ* observations (*i.e.,* 6–7 NAFM), and thus the length of dark period each day could explain the restriction of spawning behavior to specific nights of the lunar cycle. That is, species that spawn later in the night would spawn most frequently on the nights in the lunar cycle with longer periods of darkness. Spawning synchrony as a function of threshold response in coral systems has not been previously tested. Therefore, further investigations with other spawning schedules as a function of length of dark periods may reveal differential critical thresholds for different species, and a correlation to spawning on other nights in the lunar cycle.

Finally, although we showed that dynamic light processes are an important cue for spawning synchrony in corals in the laboratory, mass spawning of corals on 27 March 2010 (3 nights before the full moon) at Ioul Lukes Reef (*ca.* 5 miles from the laboratory location of the experiments reported here) indicated that other system-wide signals might advance or delay the spawning process. Other investigators (Babcock *et al.,* 1986; Hayashibara *et al.,* 1993) also reported rare occurrences of coral spawning before the full moon—for example, 2–3 days before the full moon by *Acropora digitifera* and *Acropora tenuis*. Thus, factors such as changes in sea surface temperature (Orton, 1920) and tidal cycles (Babcock *et al.,* 1986) could act synergistically or antagonistically in advancing or delaying normal synchronous spawning behavior. Furthermore, biological effects such as entrainment of endogenous rhythms (Vize, 2009) and hormones—for example, estradiol-17β (E_2) or other steroid byproducts (Atkinson and Atkinson, 1992)—released into the seawater during spawning are difficult to decouple from direct response to light signals at this time. In the former case, we found a degree of spawning synchrony under continuous 13:11 L/D dark treatments, indicating a possible entrained endogenous response. For the latter case, although coral fragments in this experiment did not display a spatially cascading behavior from the first spawning fragment to the last, hormonal cues may play a significant role at ecological scales. Full control of spawning synchrony through variation in lunar photoperiod and associated changes in skylight chromaticity should help elucidate how other environmental and biological variables can affect spawning and spawning synchrony in corals and other invertebrate systems.

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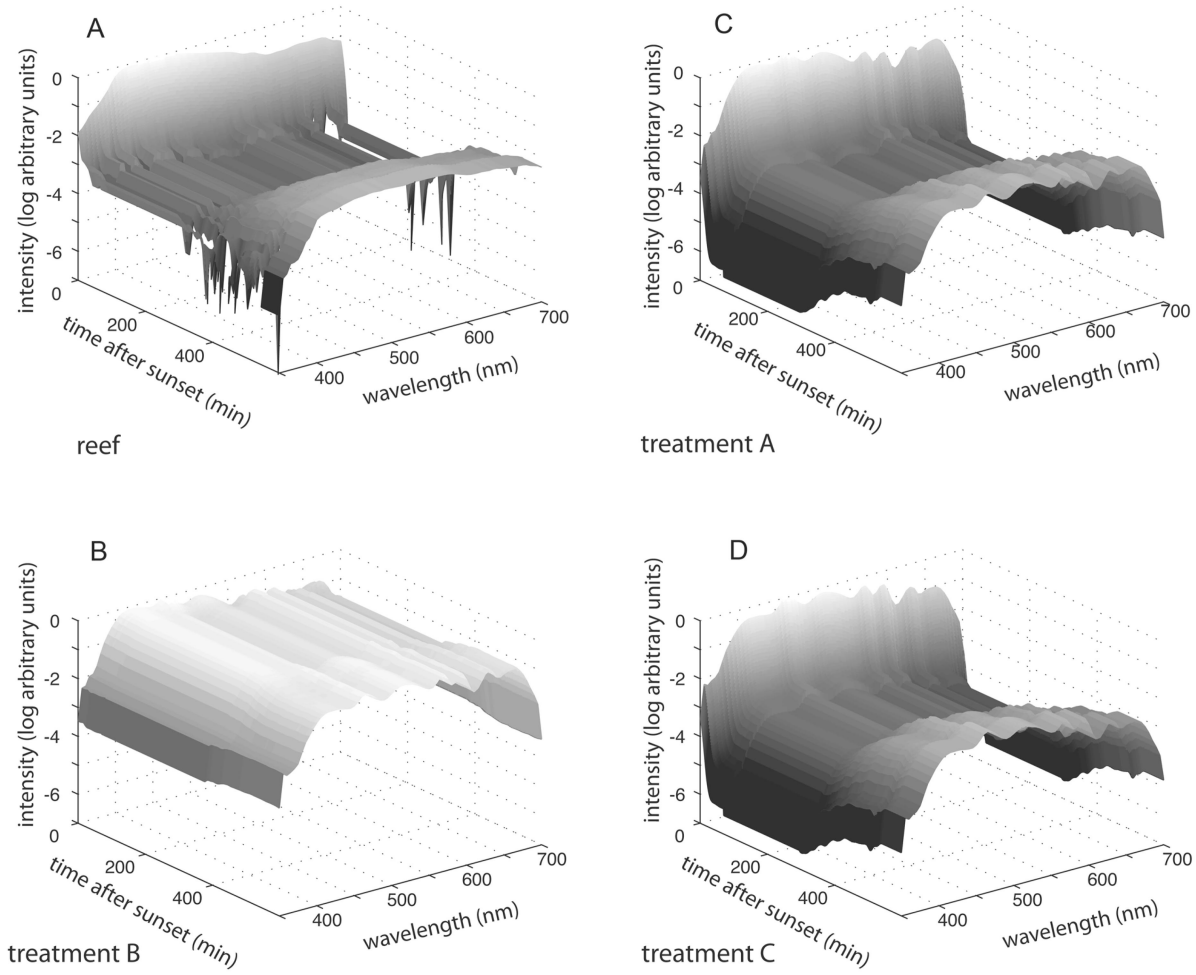
Appendix



Appendix Figure 1. Examples of log normalized light spectra over time in light treatments on the main night of spawning during the experiment—4 May 2010. (A) Example of the light dynamics model based on natural reef light dynamics at Ioul Lukes Reef. (B) Light treatment B—*i.e.,* irradiance held constant at lunar intensity but spectra blue-shifted with time. (C) Light treatment A, the best approximation of reef light dynamics. (D) Light treatment C in which the color spectrum was held constant (spectrum at noon was arbitrarily chosen) but intensity and lunar photoperiod were changed to match reef observations. For panels A, C, and D, the spectra progressively show twilight end, a period of darkness before moonrise, and light dynamics of the rising moon, all as a function of time after sunset.