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RESEARCH ARTICLE

Twilight spectral dynamics and the coral reef invertebrate spawning response

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SUMMARY

There are dramatic and physiologically relevant changes in both skylight color and intensity during evening twilight as the pathlength of direct sunlight through the atmosphere increases, ozone increasingly absorbs long wavelengths and skylight becomes increasingly blue shifted. The moon is above the horizon at sunset during the waxing phase of the lunar cycle, on the horizon at sunset on the night of the full moon and below the horizon during the waning phase. Moonlight is red shifted compared with daylight, so the presence, phase and position of the moon in the sky could modulate the blue shifts during twilight. Therefore, the influence of the moon on twilight color is likely to differ somewhat each night of the lunar cycle, and to vary especially rapidly around the full moon, as the moon transitions from above to below the horizon during twilight. Many important light-mediated biological processes occur during twilight, and this lunar effect may play a role. One particularly intriguing biological event tightly correlated with these twilight processes is the occurrence of mass spawning events on coral reefs. Therefore, we measured downwelling underwater hyperspectral irradiance on a coral reef during twilight for several nights before and after the full moon. We demonstrate that shifts in twilight color and intensity on nights both within and between evenings, immediately before and after the full moon, are correlated with the observed times of synchronized mass spawning, and that these optical phenomena are a biologically plausible cue for the synchronization of these mass spawning events.

Key words: color vision, irradiance, mass spawning, twilight.

INTRODUCTION

Johnsen and colleagues demonstrated that dramatic, physiologically relevant spectral changes in skylight irradiance occur every morning and evening during twilight, largely due to the increasing absorbance of long-wavelength light by the ozone layer as the path length of direct solar radiation through the atmosphere increases (Johnsen et al., 2006). Additionally, moonlight is long-wavelength-shifted relative to direct sunlight, and the full moon, when high in the sky, has an irradiance roughly equal to that of the beginning of nautical twilight. Therefore, in the absence of the moon, twilight is dramatically blue shifted compared with daytime illumination, but when the moon is present in the sky, its longer-wavelength illumination causes this twilight blue shift to be lessened or absent altogether.

The presence, phase and elevation of the moon during evening twilight varies over the lunar month (Fig. 1A), so we hypothesize that twilight spectral dynamics will also vary over the month. Because the evening moon is closest to the horizon when it is full, but the moon is below the horizon during the twilight of the following day, we expect that twilight irradiance spectra will undergo the greatest change between the few successive evenings before and after the full moon. As this crepuscular period is crucially important for visual behavior in many species, these lunar-mediated changes likely have biological relevance.

The synchronization of invertebrate mass spawnings may be one of the biological phenomena that has evolved in response to lunar-mediated spectral variations. Mass, synchronous free-spawning in marine invertebrates is a widespread phenomenon that has long fascinated biologists (Korringa, 1947; Giese, 1959; Hauenschild, 1960; Babcock et al., 1986; Darwin, 1860). The phenomenon of thousands of animals synchronizing gamete release within a brief temporal window on a few nights of the year indeed demands explanation. Synchronized spawning is observed in invertebrates as diverse as barnacles, polychaete worms, sponges, corals, ascidians and echinoderms (Fig. 2), but the phenomenon has attracted the most research in reef-building corals. This is in part because of their status as threatened keystone species in critically important tropical reef habitats, and in part because the physiological basis of massively synchronized behavior in animals without a central nervous system is mysterious. Spawning synchrony likely requires interactions between several different physiological clocks: annual, seasonal, monthly and daily. However, recent work has shown that although entrainment to these clocks could cue organisms to a particular night of spawning, a final, discrete trigger is probably needed to achieve the tight synchrony observed (Ananthasubramaniam et al., 2011).

Over the last century, many hypotheses have been advanced to explain seasonal spawning synchrony in invertebrates, particularly in corals. An early study concluded that temperature could be the major driver for most organisms (Orton, 1920), whereas other hypotheses include total solar radiation over the course of a season (van Woesik, 2006), photosynthate levels produced by symbiotic zooxanthellae (Muscatine et al., 1984) and cryptochrome cycling entrained by the lunar cycle (Levy et al., 2007; Shlesinger and Loya, 1985; Jokiel et al., 1985). Additionally, Hunter showed that lunar phase, seawater temperature and day length all impacted spawning periodicity in several species of corals (Hunter, 1988). Giese (Giese et al., 1959) demonstrated that photoperiod is important for several taxa of invertebrates. Experimental manipulation of light:dark

cycles for organisms such as the sponges *Halichondria panicea* (Amano, 1986) and *Callyspongia ramosa* (Amano, 1988), the holothurian *Isostichopus fuscus* (Mercier et al., 2007) and the hard corals *Goniastrea aspera* (Babcock, 1984) and *Acropora digitifera* (Hayashibara et al., 2004) all resulted in peak spawning occurring on or near the change from the light to dark phase of the photoperiod. Differential cryptochrome expression between full moon and new moon cycles has been invoked as a possible mechanism contributing to synchrony in *Acropora millepora*, although the evidence was tenuous (Levy et al., 2007).

Although each of these observations helps explain how organisms converge on a season and month for spawning, and thus address within-year or within-month synchrony, they do not address within-day synchrony. None of these ideas adequately explains the observed tight coupling of spawning behavior within a single day, or the spawning observed for some species synchronized to a single 20 min period following sunset on a few consecutive nights during the waning gibbous phase of the moon at latitudes where the day length varies by a few percent over the course of an entire year (Fig. 2). Our compilation of published data on spawning windows demonstrates that in all taxa with documented dates and times of spawning, mass spawnings are much more tightly constrained to a specific point in the 24 h circadian cycle (twilight) than within the monthly circalunar cycle (Fig. 2). This observed tight coupling to a feature of the 24 h cycle demands explanation, yet there have been few, if any, hypotheses advanced that can explain this tight circadian synchrony in addition to circalunar synchrony, or that explain why circadian synchrony is tighter than circalunar synchrony. We hypothesize that the 'blue pulse' that intensifies each night for a few nights around the full moon could be a mechanism cuing spawning and explaining patterns of both circalunar and circadian synchrony in mass-spawning organisms. Therefore, we quantified these spectral changes on a coral reef during a month and week when coral spawning was predicted, to determine whether they merit further experimental investigation as a plausible cue for mass-spawning behavior.

MATERIALS AND METHODS

We measured hyperspectral twilight irradiance using the methods described in Johnsen et al. (Johnsen et al., 2006), modified for measurements underwater. A splash-proof ruggedized laptop computer (Panasonic Toughbook model CF-30) and a miniature fiber-optic spectrometer (Ocean Optics USB2000+, Ocean Optics Inc., Dunedin, FL, USA), optimized for high sensitivity, were mounted in a waterproof case (Hardigg Storm Case model iM2400, Torrance, CA, USA). The laptop was used to run SpectraSuite spectrometer software for data acquisition (Ocean Optics). The case in turn was lashed to an inflated 18 inch tractor tire inner tube that was lashed to a 1 m^2 PVC raft with foam pipe insulation covering the PVC for additional stability and flotation. A custom-fabricated waterproof 10 m fiber-optic cable with a 1 mm light-conducting core (Ocean Optics) was run from the spectrometer down to the reef (at a depth of 2.5 m). The underwater end of the cable was inserted through a brass tube 15 cm in length mounted to a ring stand at a fixed angle and pointed at a horizontal metal plate painted with matte titanium-dioxide-based white paint. Reflectance measurements confirmed that the reflectance of the painted plate was that of a spectrally neutral and diffuse (Lambertian) reflector. In this case, the radiance of the plate (viewed by the end of the fiber) is linearly proportional to the downwelling irradiance striking it. Although Spectralon is a more commonly used as a Lambertian reflector, its foam structure makes it unsuitable for underwater work, particularly at depth.

The spectrometer raft was deployed over a reef habitat in 2.5 m of water near Yawzi Point in Great Lameshur Bay, St John, US Virgin Islands (18°19′1.25″N, 64°43′28.19″W). All of the *Acropora palmata* Lamarck 1816 colonies in this region were observed close to this depth, and there were colonies within 100 m of our site, making the optical properties of our site identical to those where the coral spawnings in this region occur.

Starting at 18:30 h, approximately sunset, we measured downwelling underwater irradiance at intervals of 3 min, increasing the spectrometer integration time as light levels decreased until we reached the software-determined maximum integration time of 60 s. After this point, we took one spectrum every minute until the moon reached its zenith. We measured spectra on 3, 4, 6, 7 and 8 August 2009 and checked local *A. palmata* colonies for spawning beginning at 21:00 h each night between 3 and 10 August 2009. For comparison and calibration, we also measured spectra in the same manner at the same location at sunset, when the moon was at its zenith (72 deg lunar elevation) and in the afternoon (25 deg solar elevation).

Raw spectrometer data were smoothed using a median filter and boxcar algorithm, corrected for the spectral sensitivity of the spectrometer as a function of wavelength (including attenuation of the optical fiber), and converted to relative quantal irradiance using a custom-written MatLab script (Mathworks Inc., Natick, MA, USA). We determined the optimal dichromatic color visual system for perceiving the changes in twilight color that we measured. We assumed the template of a typical opsin absorbance spectrum, a photoreceptor length of 30 μm and a fairly typical opsin absorption coefficient of 0.028 μm^{-1} (Warrant and Nilsson, 1998; Stavenga et al., 1993). We then calculated the differences in photon catch for each possible pair of hypothetical opsin pigments in the typical visible range (350 to 700 nm) discerning the spectra from –10 deg solar elevation from the nights of 3 and 8 August. We then determined the optimal pair of hypothetical pigments for discerning these stimuli to calculate neural opponencies using the difference between the photon catches of the two optimal pigments divided by the sum of those catches (Fig. 3) for the spectra that we measured:

$$\text{Opponency} = \frac{\text{Catch}_1 - \text{Catch}_2}{\text{Catch}_1 + \text{Catch}_2},$$

Therefore, opponency values near zero represent an equal photon count in both hypothesized pigments for a given irradiance, whereas increasingly positive or negative values result from a relatively greater photon count in one pigment *versus* the other.

RESULTS

We successfully measured twilight irradiance on 3, 4, 6, 7 and 8 August 2009 (no spectra were measured on 5 August due to logistical difficulties) and observed a significant impact of lunar elevation on underwater irradiance on the evenings around the full moon. Compared with the waxing gibbous phase, on the night of the full moon, the skylight irradiance spectrum demonstrated a significant blue shift during twilight, because this is the first night when the moon is close to or below the horizon at sunset. This shift became increasingly pronounced on evenings following the full moon (waning gibbous phase) because the moon rose later on each successive night (Fig. 1A,B and Fig. 3). On each successive night, twilight irradiance started bluer at the beginning of our study period and became bluer as twilight progressed until the moon rose and shifted irradiance toward the lunar spectrum (see Fig. 4). Although the raw spectral data for these nights may appear similar on first inspection, our analyses revealed significant differences that are illustrated explicitly in Figs 3 and 4.

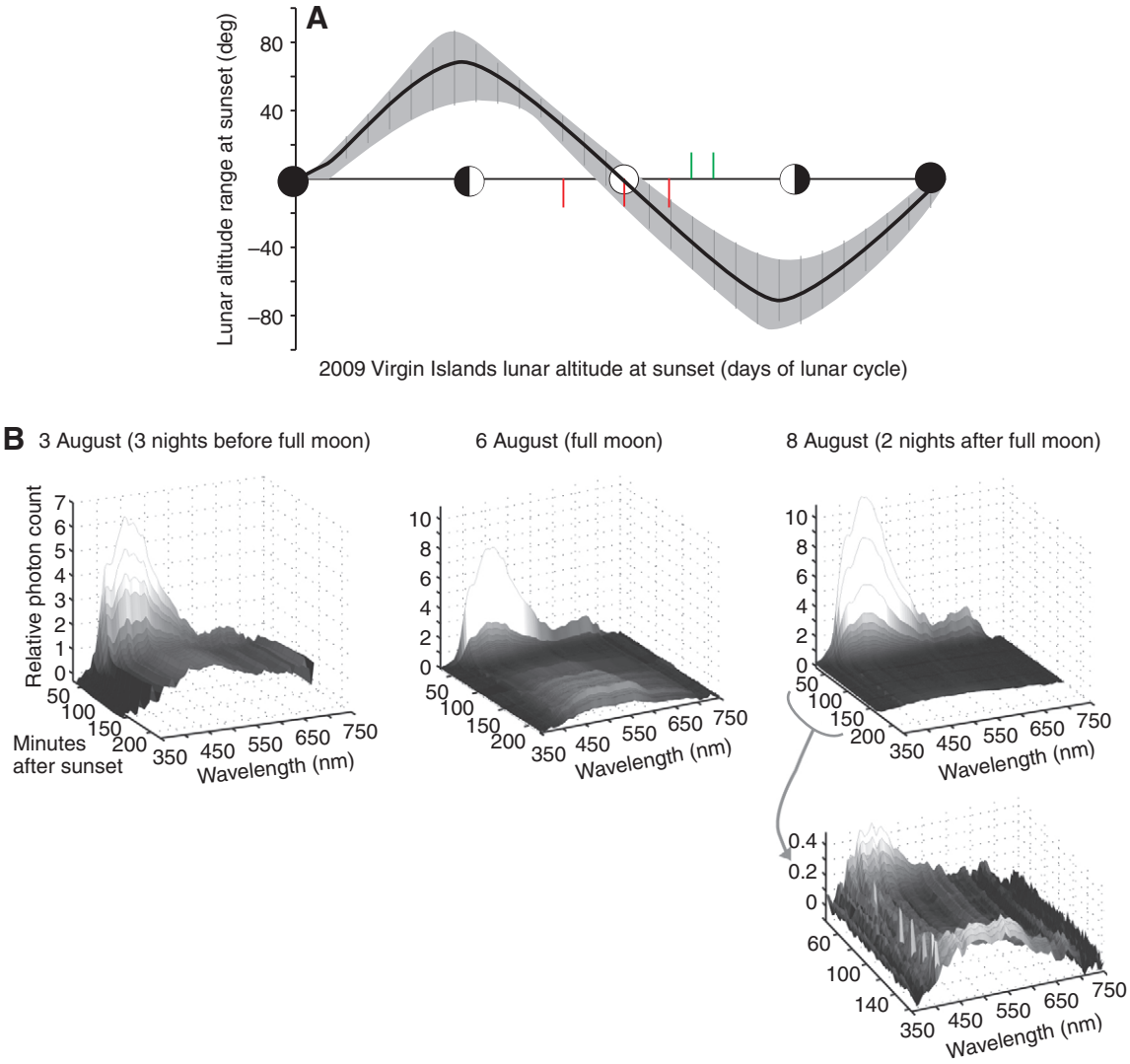
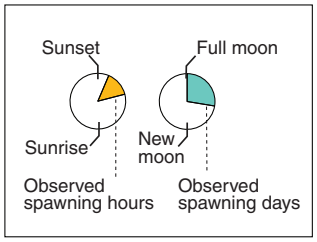


Fig. 1. Twilight spectral dynamics during waxing gibbous, full and waning gibbous moon phases. (A) Range of lunar altitudes at sunset over a year of lunar cycles in the US Virgin Islands. Red tick marks indicate nights of the lunar cycle for which data are shown; green tick marks indicate nights of the lunar cycle during which we observed spawning. (B) Twilight spectra taken every minute, beginning 45 min after sunset and continuing until moonrise for the nights of 3 August (waxing), 6 August (full moon) and 8 August (waning).

The differences in twilight spectra between the waxing gibbous and waning gibbous phases (i.e. the nights immediately preceding and following the full moon, respectively) are due to the varying mixture of blue-shifted skylight and the slightly red-shifted moonlight on each night. In the coral reef habitat we studied, the blue-shifted twilight spectrum with no lunar influence is relatively narrow and has a peak at 450 nm (Fig. 1B). The spectrum of moon alone on the reef, with no twilight influence, is much broader and redder than the twilight spectrum; it has a broad peak at 470 nm with a broad shoulder extending at nearly the same intensity to 575 nm (Fig. 1B). Intermediate spectra between these two extremes of twilight-only and moon-only occur on nights where the nearly full moon is above the horizon during twilight, with the lunar spectrum dominating when the moon is significantly above the horizon during twilight (Fig. 1B).

Visual system modeling

An analysis of all possible dichromatic visual systems showed that two opsins with peaks at 434 nm (P_{434}) and 546 nm (P_{546}) would best discriminate the twilight spectra from different days (Fig. 3). We examined the patterns of the opponency in these pigments for the spectra we measured each night of our experiment. Each night at approximately –10 deg solar elevation, the opponency value in our hypothetical visual system was near zero, indicating an equal photon catch in both hypothetical visual pigments, and eventually wound up near –0.55, the opponency of skylight dominated by the moon. However, the dynamics between 0 and –0.55 were quite different every night. On the night of 3 August, three nights before the full moon, this opponency immediately decreased from near zero to a minimum of –0.55 within an hour after sunset (approximately –12 deg solar elevation), indicating an increasing photon catch in P_{546} *vs* P_{434} over the course of the evening. On each subsequent night, the opponency value immediately increased slightly from zero and stayed positive (greater photon catch in P_{434} *vs* P_{546}) for a longer period of time before eventually decreasing to the negative opponency value obtained from the moon-dominated spectrum after moonrise. On the night of 8 August, two nights after the full moon, there was a dramatic shift in opponency dynamics from the



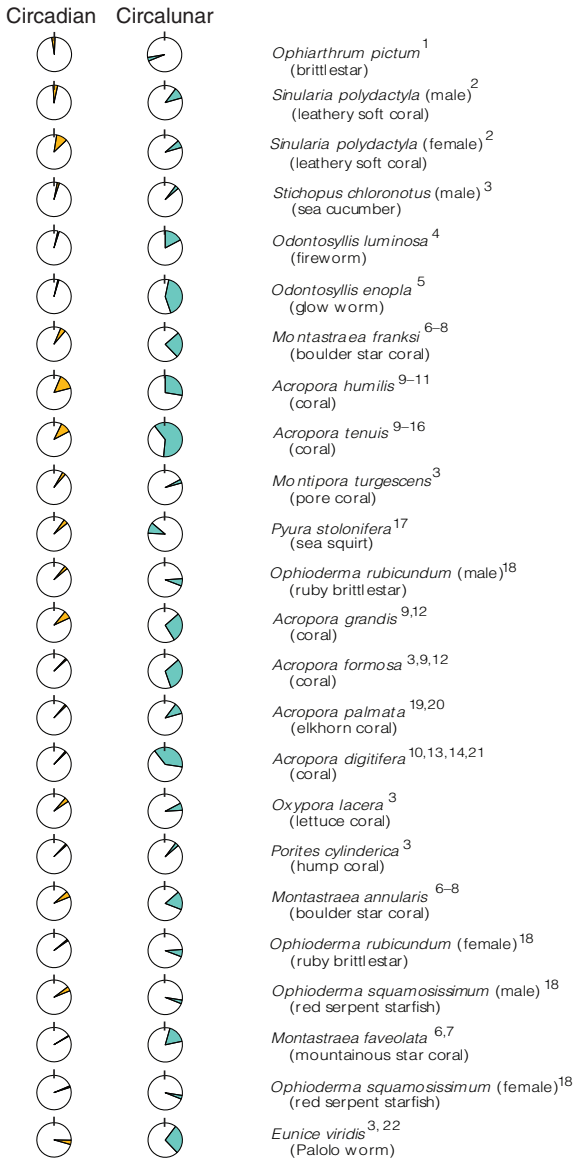


Fig. 2. Invertebrate spawning is more tightly coupled to the circadian cycle than to the circalunar cycle. Filled wedges the show period of a diurnal cycle in which a given species has been documented to spawn. A tick mark at the top of the circle indicates the relative position of the full moon. When spawning observations are shown in the context of the cycles in which they are contained and presumably triggered, it becomes evident that within-day spawning patterns are more tightly constrained than within-month spawning patterns, as shown by the fact that the wedges in the circadian cycle observations are smaller and move oriented than the wedges in the circalunar observations. Data were compiled from the literature as follows: [1]Hendler and Meyer, 1982; [2]Slattery et al., 1999; [3]Mundy and Green, 1999; [4]Gaston and Hall, 2000; [5]Markert et al., 1961; [6]Levitan et al., 2004; [7]Szmant et al., 1997; [8]Vize et al., 2005; [9]Babcock et al., 1986; [10]present study; [11]Harrison et al., 1984; [12]Babcock et al., 1994; [13]Hayashibara et al., 1993; [14]Richmond and Hunter 1990; [15]Rosser, 2005; [16]Willis et al., 1985; [17]Marshall, 2002; [18]Hagman and Vize, 2003; [19]present study; [20]Miller et al., 2009; [21]M. Omori, K. Iwao and M. Hatta, personal communication; [22]Caspers, 1984.

preceding nights: the opponency value increased immediately and abruptly from zero to a maximum of 0.45, stayed positive for a much longer period of time, past the irreversible commitment to spawning marked by bundle-setting in corals, and didn't decrease to the lunar value of –0.55 until almost 3 h after sunset (Fig. 4). Therefore, spectral dynamics of twilight on the evening of 8 August were significantly different from any other night we measured. Interestingly, the observation of bundle-setting behavior, when gamete bundles are moved out of the gonads and into the gastric cavity of coral polyps and spawning is inevitable, occurred just after the peak in opponency and when the opponency value was quite positive (higher photon catch in P_{434} than in P_{546}) even though actual spawning, or release of gamete bundles into the water column, occurs when opponency is dominated by moonlight an hour or two later. This difference in spectral dynamics was due to the relative positions of the moon during twilight, as the intensity of maximum lunar illumination due to changing lunar phase each of those nights did not significantly differ.

On the nights that we collected data, there were intermittent bands of clouds moving across the sky. When light clouds crossed the moon, the intensity of the spectrum dropped (Fig. 1), but the chromaticity remained relatively constant (Fig. 4). Therefore, our data suggest that late twilight color is unaltered by light or intermittent cloud cover, increasing its possible utility as a cue. During late twilight, sunlight is scattered from parts of the sky that are more than 40 km in altitude (Bohren and Clothiaux, 2006) whereas cloud cover is much lower. The overall effect at the sea surface of twilight under cloud cover should therefore be that of the usual blue-shifted twilight going through a diffuser, and our data seem to demonstrate this.

Reef observations

We observed two out of ten focal *A. palmata* colonies at Yawzi Point spawn on 9 August, three nights after the full moon, and again on 10 August, four nights after the full moon (Fig. 1). We observed the same two colonies spawn beginning at 21:30 h until spawning was complete at 21:50 h on 9 August again from 21:30 to 21:47 h on 10 August. On both nights, only partial spawning of the largest colonies >1 m diameter occurred. We were not on site to observe any spawning after 10 August.

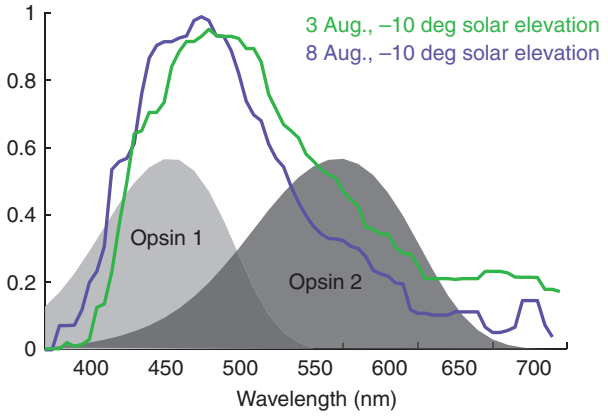


Fig. 3. Model of an optimal dichromatic visual system for perceiving twilight color changes. Hypothetical visual pigments and focal spectra. Blue curve shows spectral data from –10 deg solar elevation on 8 August (waxing gibbous moon). Green curve shows spectral data from –10 deg solar elevation on 8 August (waning gibbous moon). Grey filled curves show hypothetical opsin absorption optimized to detect the chromatic difference between sky color at –10 deg solar elevation during the waxing gibbous and waning gibbous phases of the moon.

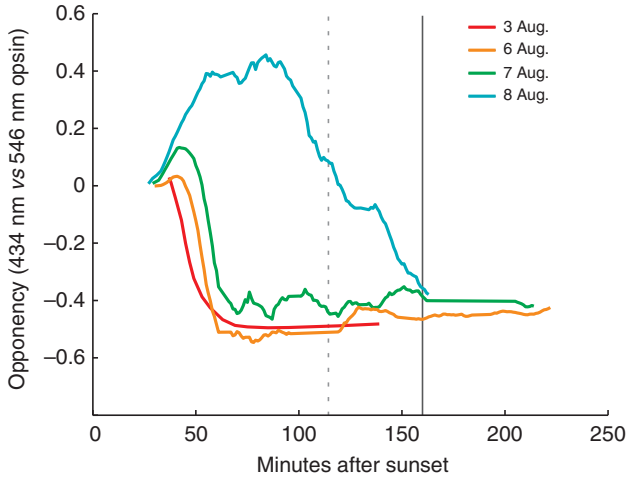


Fig. 4. Dichromatic opponencies of twilight spectra on a coral reef. Opponency values for the optimized opsin templates, calculated once per minute during twilight on the nights of 3, 6–8 August, *versus* the minute after sunset each opponency was calculated. Full moon occurred on 6 August. The dashed grey line indicates the timing of bundle setting, the marker of the irrevocable decision to spawn in corals. The solid grey line shows the timing of gamete release in corals.

DISCUSSION

It was previously demonstrated that physiologically relevant blue shifts in irradiance spectrum that occur during twilight are a result of the increasing absorption of long visible wavelengths by ozone as the pathlength of direct sunlight through the atmosphere increases (Johnsen et al., 2006; Hulbert, 1953). Johnsen and colleagues reported one spectrum of a full moon sky with the moon at an altitude of 70 deg on land, but did not quantify the impact of lunar phase or elevation on this twilight blue-shift effect or measure how these phenomena are altered underwater relative to on land (Johnsen et al., 2006). Because of the long-demonstrated importance of twilight and lunar phase on synchronized invertebrate reproductive behavior, we quantified changes in irradiance spectrum during twilight for sequential nights with changing lunar elevation around the full moon on a coral reef. As anticipated, the spectral dynamics in skylight irradiance that occur underwater on a coral reef are similar to the terrestrial spectral shifts previously observed (Johnsen et al., 2006). Additionally, we demonstrated that even underwater, the degree of the twilight blue shift depends on whether the moon is above or below the horizon.

Synchronized mass spawning is an especially intriguing correlate of the twilight phenomena we document here. There are several physical features of the twilight processes that could be robust, physiologically relevant cues for spawning behavior consistent with the short 20 min interval of spawning observed on reefs. These cues are more closely synchronized to actual observed times of spawning than the other previously hypothesized synchrony drivers discussed above, such as temperature. For instance, detecting a twilight spectrum in the absence of the moon, beyond a certain blue threshold or in a differing direction of opponency (Figs 5, 6) could cue bundle-setting behavior (i.e. the final stages of gamete maturation and extrusion prior to release), subsequently followed by gamete-release behavior cued by total skylight irradiance reaching values near zero. Alternatively, a unique rate of decrease of total irradiance characterizes each night in this portion of the lunar cycle and could constitute a cue.

The spawning times and dates of reef invertebrates documented in the literature (Fig. 2) are generally consistent with this hypothesis that chromatic changes during twilight contribute to spawning synchronicity. We compiled spawning data for all available species that have both dates and times of spawning documented. Of these 22 species, 17 always spawn within 2 h of sunset and during the waning gibbous phase of the moon, consistent with the two-pigment mechanism cued on the narrowing blue twilight spectrum we outlined above. Two of these taxa, *Pyura stolonifera* (a tunicate) and *Ophiarthrum pictum* (a brittlestar), clearly do not fit this particular hypothesis, because these species spawn during the waxing phase of the moon when the moon is small and consistently near its zenith in the twilight sky (Fig. 1A). If light cues play a role in cueing spawning in these species, these spawning observations are more consistent with more general lunar or solar photoperiod cues [as proposed in Levy et al. (Levy et al., 2007)] rather than particular characteristics of a given twilight, as the twilights observed during this phase of the moon should be fairly constant across evenings. Two other coral species, *Acropora tenuis* and *Acropora digitifera*, also occasionally spawn before the full moon, which is also inconsistent with our hypothesis of changing twilight cues. Therefore, if the chromatic and dynamic twilight phenomena we describe here play a role in cueing spawning in these taxa, they would more likely serve as secondary refiners of synchrony, rather than as primary cues.

In terrestrial invertebrate and vertebrate species, twilight shifts in irradiance spectrum have been shown to be physiologically relevant to visual activities such as foraging (Kelber et al., 2002; Roth and Kelber, 2004). For the species with documented spawning times and dates that closely fit the pattern discussed above, we hypothesize that opsin-transduced color vision coupled to a robust clock sense may be a mechanism that synchronizes and/or triggers invertebrate spawning occurring during evening twilight following the full moon. Opsins are a family of proteins that absorb photons with a carotenoid cofactor to initiate the photosensory transduction cascade. It is apparently not uncommon for the duplication and divergence of opsin genes to lead to the evolution of color vision,

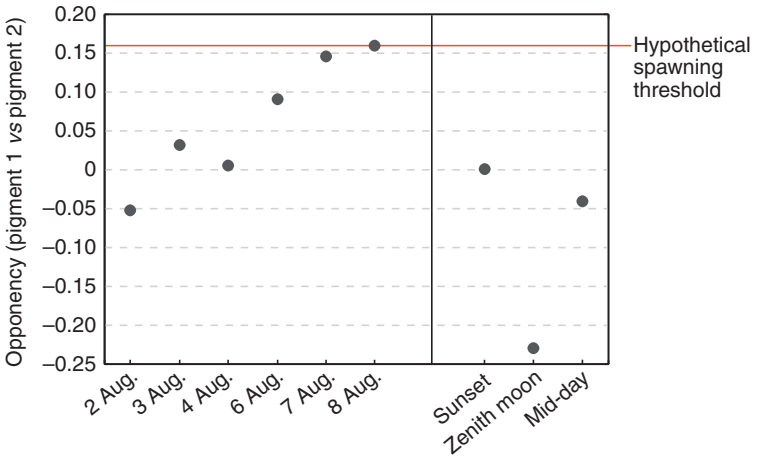


Fig. 5. Possible thresholding mechanism for spawning synchrony. Circles indicate opponency values observed at 19:30 h, or ~40 min after sunset for each night we recorded spectra, the time organisms are expected to begin to commit resources to spawning behavior. Also shown for comparison are opponency values for sunset, moon at the zenith and mid-day measured in the same location. The red line indicates a hypothetical neural threshold beyond which spawning may be initiated.

because individual opsin alleles can evolve differing wavelength sensitivities. This process has occurred frequently, and sometimes rapidly, in evolutionary time (Pohl et al., 2009; Gojobori and Innan, 2009; Trezise and Collin, 2005). The recent expansion in genomic data of invertebrates has shown that even animals lacking morphologically complex eyes are often rich in opsin genes. For instance, the sea urchin *Strongylocentrotus purpuatus* has at least six light-detecting opsins (Raible et al., 2006), and the tunicate *Ciona savignyi* has at least three (Kamesh et al., 2008). Our analysis of the larval *Acropora millepora* transcriptome identified at least four visual opsins (data not shown), whereas the fully sequenced genome of the cnidarian *Nematostella vectensis* revealed dozens of light-sensitive opsins (Putnam et al., 2007). Although the field has not yet progressed to experimental analysis of the action spectra of the encoded opsin proteins or behavioral experiments necessary to establish color vision, this observed opsin diversity provides plenty of genetic potential for the detection of subtle color shifts.

Vize and colleagues recently proposed that opsins and their associated downstream phosphorylations could be important transducers of whatever phenomenon ultimately triggers spawning (Vize et al., 2008). They noted that, in several coral species, there is differential protein expression between the day and night, although they do not report results for differential protein phosphorylation or possible differential expression of downstream messengers from opsins. A recent survey of the *Acropora* transcriptome showed that the core components of evolutionarily conserved metazoan circadian circuitry, including a likely melanopsin ortholog, are also present in *Acropora* (Vize, 2009). This result provides evidence that corals can likely perform entrainment to circadian and circalunar cycles (i.e. possess a robust diurnal clock). This clock sense is required for priming and response to a hypothesized proximate twilight trigger for broadcast spawning (Ananthasubramaniam et al., 2011; Nilsson, 2009).

Somewhat counterintuitively, no eyes are required to detect the spectral changes we describe here, and eyes probably reduce the ability of an organism to perceive low-intensity spectral changes like these. A broad, unobstructed sheet of photoreceptive tissue like that present in corals and sea urchins would actually be the optimal detector design for observing changes in the spectrum of low-intensity downwelling skylight. The apertures that define an eye increase angular resolution of the visual system at a steep cost to sensitivity in part because enclosing an eye to achieve spatial resolution by definition must block light from many angles. In addition, angular resolution actually impedes the ability of an organism to distinguish colors, because there is a trade-off between packing in more photoreceptors of a given spectral class to achieve angular resolution and packing in more photoreceptors of varying spectral classes to achieve detailed color perception (Land and Nilsson, 2002).

Because organisms that may be using these phenomena as cues must perceive the color of ambient light rather than the colors of objects in a scene, any organism taking advantage of these cues must lack a color constancy mechanism such as the one found in humans. That is, the visual system used for this task must lack an ‘auto white-balance’ function to account for changes in background illumination when perceiving colors. Interestingly, a visual system like this has already been postulated for organisms that maintain position in an isolume in the pelagic ocean, and would require many of the same characteristics we hypothesize for observing twilight color (Nilsson, 2009). There is one documented case of an opponency mechanism like this acting in a single photoreceptor cell (Solessio and Engebretson, 1993). In this instance, two opsins coexpressed in photoreceptor cells of the parietal eyes of lizards exert opposing effects on ion channels, resulting in an opponency mechanism similar to the one we postulate here (Su et al., 2006). Although the behavioral function of this mechanism in lizards is not known, it is especially intriguing that it is active in the parietal eye, an organ involved in mediating the organism’s responses to dawn and dusk (Solessio and Engebretson, 1993). It seems plausible that a similar mechanism operating in mass-spawning invertebrates with decentralized nervous systems could perceive and transduce the spawning-associated changes in twilight color we describe here.

Knowlton and colleagues, and Brady and colleagues, demonstrated that corals in the genus *Montastraea* can be manipulated to spawn earlier than control colonies by covering them to mimic an earlier sunset (Knowlton et al., 1997; Brady et al., 2009). However, these experiments also demonstrate an apparent loss of tight synchrony when twilight spectral dynamics are perturbed. The Brady study showed that untreated corals spawned over a 16 min interval whereas experimentally covered corals spawned over a 28 min interval. The Knowlton study produced a similar result: control corals spawned over a 60 min interval whereas experimentally covered corals spawned over a 90 min interval (Knowlton et al., 1997). Given that the tight synchrony achieved by natural spawning cues is thought to be critical for maximum fertilization rates (Levitan and Petersen, 1995), this elongation of spawning behavior is likely to reduce reproductive success. These results complement our findings, as these studies primarily attempted to advance spawning on a single night when control organisms also

spawned, and did not address how that particular night or point in the circadian cycle was chosen by the organisms for synchronous spawning. The sharpening blue pulse over a few nights before spawning could provide both a potential opsin-mediated mechanism for restricting spawning behavior to a short temporal window on a single night and an accelerating cue to specify the appropriate night of spawning. Even early in the evening after the full moon, 2h before actual spawning is observed, there are changes in twilight spectra correlated with observed spawning behavior (Fig. 5). The dynamics of the blue pulse as perceived in a two-pigment opponency system could provide a thresholding mechanism by which the night of spawning in addition to the timing of spawning within a night could be determined (Fig. 5).

Artificial light pollution is quite red-shifted compared with both moonlight and sunlight (Johnsen et al., 2006), and may therefore shift twilight spectral dynamics in densely populated areas. As many of the world's coral reefs are located in attractive travel destinations and coastal cities, if our hypotheses bear out, it will be important to investigate the effects of artificial light pollution on the invertebrate spawning response. Although this issue has not, to our knowledge, been rigorously studied, anecdotal reports from researchers studying coral spawning indicate that increased light levels may inhibit spawning. In contrast, mass spawnings are not reported to be much altered under cloudy skies, in accordance with our observations that light clouds do not alter spectral dynamics, but corals tend not to spawn during monsoon months in the tropics, when extremely heavy clouds and rain may disrupt twilight color more than normal cloudy skies (Mendes and Woodley, 2002). Therefore, the data shown here indicate that if lunar irradiance affects the spawning response, then light pollution may delay or inhibit spawning altogether, whereas routine cloud cover should not.

Although the work we have shown here is correlative, we are currently planning experiments to investigate the effects of spectral dynamics on the coral spawning response. Further studies will be required to assess any impact of this phenomenon on spawning behavior; however, the changes in spectral dynamics around the full moon that we report are an interesting environmental phenomenon with a strong correlation to mass-spawning behavior that has not previously been documented. We hypothesize that the rapidly changing spectrum at twilight specific to the nights immediately following the full moon may be a proximate trigger for invertebrate reproductive activity. In the case of coral spawning, it could potentially account for the tight synchrony of this behavior to an approximately 20 min period in deep twilight.

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