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NOTE

Coral spawn timing is a direct response to solar light cycles and is not an entrained circadian response

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Abstract Broadcast spawning corals release gametes into the oceans with extraordinarily accurate timing. While the date of spawning is set by the lunar cycle, the hour/minute of spawning is set by the solar cycle. In this report, we describe experiments that test whether the time of spawning is regulated by an entrained biological clock or whether it is directly controlled by the solar cycle. *Montastraea franksi* samples were collected on the morning of the predicted spawning. Fragments from colonies were kept under three different lighting conditions and spawning monitored. The three conditions were sunset times of 0, 1 or 2 h earlier than normal. Fragments from the same colony spawned differently under these three conditions, with an early sunset causing a corresponding early shift in spawning. These results indicate that spawn timing is not controlled by a circadian rhythm and that it is directly controlled by local solar light cycle.

Keywords Broadcast spawning · Circadian rhythm · Biological clock · Mass spawning

Introduction

Broadcast spawning by corals is an amazingly precise temporal process. Every year, on just one or two evenings, many of the individuals of each broadcast spawning species release their gametes in a time window that is usually approximately 30 min long. With only a simple nerve net and no specialized light sensing organs to sense and interpret their local environment (Shelton 1975), these simple cnidarians somehow manage to tell the time of year, the time of the lunar month, and the time after sunset. Precise timing is critical in the ocean environment to achieve high fertilization rates. As corals generally cannot self-fertilize, gamete dilution and drift result in extremely low fertilization rates when corals spawn more than 2 h after their neighbors on the reef (Levitan et al. 2004). Each of the three different elements that control spawn timing season, moon phase and sunset time, must be read accurately to achieve the extraordinary accuracy of the broadcast. Although it has not yet been demonstrated, it is assumed that these three independent inputs somehow converge to select the exact moment of spawning (e.g., Harrison et al. 1984; Babcock et al. 1986; Hunter 1988; van Veghel 1993; Hagman et al. 1998; Mendes and Woodley 2002; van Woesik et al. 2006).

The month of spawning is set by local weather cycles. The key environmental variable correlated with the selection of the month of spawning is solar insolation cycles (Penland et al. 2004; van Woesik et al. 2006), but exactly how solar radiation and the weather patterns they drive are sensed or responded to by corals remains unknown. The night of spawning is set by the lunar cycle. Lunar cycles can be used to accurately predict the broadcast spawning night in many locations (e.g., Willis et al. 1985) and altering lunar irradiance cycles can change planula release cycles in brooding corals (Jokiel et al. 1985). Low levels of light such as moonlight can be perceived by corals and can result in changes in gene transcription (Levy et al. 2007). However, exactly how lunar light entrains broadcast spawning also remains unknown. A third key environmental component of broadcast spawn timing is sunset time, which sets the

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hour and minute of spawning and it is the aspect of spawn coordination that is focused on in this report. The importance of sunset time in establishing spawning time in broadcast spawners was determined by observing that gamete release times are very consistent for a species from year to year when measured against local sunset time (e.g., Babcock et al. 1986; Hagman et al. 1998). Predictions based on this assumption are very accurate and the start and end of spawning times for many species can be predicted within about 10 min from season to season in some locations (Vize et al. 2005). However, although this convincingly shows that sunset time is associated with spawning behavior, it does not show that sunset directly controls spawning. It is possible that the solar light cycle entrains a biological clock and that the clock controls spawning behavior. As biological clocks can free run for long periods of time after the entraining signal is removed (Dunlap et al. 2004), it is the clock, not the entraining signal that controls responses. An example of such a process in corals is circadian cycles of tentacle extension and retraction that continue to occur for many days after corals are placed in constant darkness (Sweeny 1976). The other possibility is that light/darkness directly regulates spawning behavior and that no biological clocks/circadian rhythms are involved (Wallace et al. 1986).

When corals have an artificial sunset time imposed for as little as two evenings prior to the evening of spawning, they spawn earlier than do control corals. This experiment was performed in Panama on fragments of *Montastraea annularis* by Knowlton et al. (1997). Four fragments were darkened 2 h ahead of normal for three evenings and four fragments were darkened 2 h early for two evenings. In both cases darkened colonies spawned earlier than did control colonies and colonies on the reef. Colonies darkened for three evenings spawned on average 2.4 h post actual sunset and colonies darkened for two evenings spawned on average 3.1 h post actual sunset, while controls spawned 3.5–4.5 h post-sunset and undisturbed corals on the reef spawned at 4–5 h post-sunset. As the time of spawning shifts depending on how many evenings corals are darkened for, the shift may be due to a biological clock becoming re-entrained. However, this would be unusually rapid re-entrainment and the difference between the two darkening treatments may not be statistically real as sample sizes were quite small (four samples each treatment). In reports by Babcock (1984) and Hunter (1988) on *Goniastrea* and *Montipora*, respectively, spawn timing was also shifted by changing light cycles, but once again, in both examples the cycle was changed for multiple days. Babcock (1984) changed light patterns for "several days" (details are not provided) while Hunter (1988) changed sunset time by 10 min per day for 12 days- both regimens could therefore have allowed re-entrainment of a biological clock. There is also anecdotal evidence that delaying sunset with artificial lighting correspondingly postpones the time of spawning, but details have not been published (Wallace et al. 1986). In sum, the published evidence to date on just three scleractinian species indicates that coral spawning time can be shifted by an early artificial sunset, but as the darkening was performed over multiple evenings and sample sizes were small it is not really possible to distinguish between an entrained circadian system and a direct response to light.

To directly address this question a similar experiment was performed on a larger sample size and, importantly, with only one modest experimental variable-sunset time shifted on only the night of spawning. It was observed that this single change accurately shifted spawn timing conclusively demonstrating that the time of coral broadcast spawning in this species is under direct environmental control.

Methods

Colony collection

The Flower Garden Banks are located approximately 180 km south of the Texas-Louisianna border at latitude 27°59′, longitude 93°35′. All operations were conducted from the deck of a chartered dive vessel. At 0830 h on August 23, 2008, 12 colonies of *Montastraea franksi* were collected from the east bank between mooring buoys 4 and 5 at 23 m depth. Using a hammer and chisel, a fragment of approximately 20 cm × 20 cm was removed from each colony and placed in a numbered ziplock bag. Immediately prior to collection, the colony was tagged, photographed and recorded for reattachment at the end of the experiment. Samples were taken to the surface and transferred into bins in a shaded flow-through system on the boat deck. Each collected colony was split into three smaller clonal fragments and was then left for the remainder of the day to acclimate to the bins and recover from the stress caused by collection.

Experimental design

Three bins were set up for the three treatments tested: (A) sunset 2 h earlier than normal; (B) sunset 1 h earlier than normal; and (C) normal sunset (control). Within each bin, one of the three clonal fragments of coral from each colony was placed into a smaller, pre-labeled container. A half-hour before the experiment began, these containers (filled with flow through seawater) were raised higher than the level of water in the bin, ensuring each colony was

separated from each other, but still cooled by the surrounding flow through seawater. Black plastic shades, made from thick black contractor bags, were used as the darkening cover for early sunset. These shades were placed directly on top of the bins, as well as around the sides of the bins, to ensure no natural light could enter. At 17:45 h CDT the first bin was covered, at 18:50 h CDT the second bin was covered and at 19:47 h CDT the third bin was covered. The third (control) bin was also covered to reduce any artificial light from boat lights entering the tank during the experiment. The experimental area was enclosed with similar shades, to reduce any natural light exposure.

Data collection

Beginning 1 h after artificial sunset, both experimental bins were monitored every 10 min for spawning activity. The control bin was also monitored every 10 min; however, observations began immediately after covering. Using a red-filtered flashlight, the cover was lifted briefly (less than 10 s) to determine if any spawning occurred. A red light was used to monitor spawning to minimize impact (Gorbunov and Falkowski 2002). Spawning was obvious with orange egg-sperm packets released and floating on top the water surface in the container (Fig. 1). The experiment continued until approximately 22:20 h, 20 min beyond when *M. franksi* corals were spawning on the reef below (Vize et al. 2005).

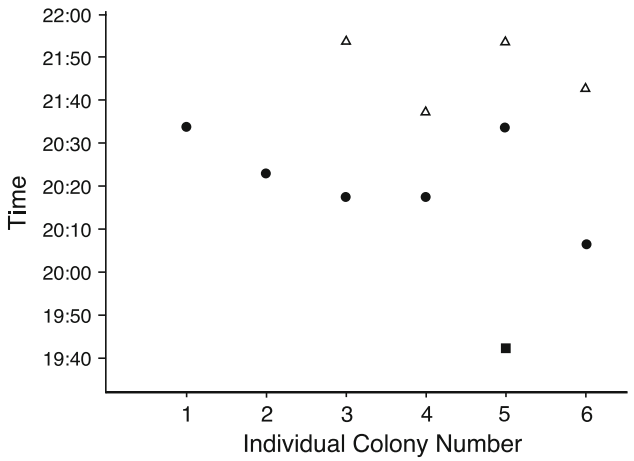

Fig. 1 Coral spawning response to alteration of sunset time. Colony number on the *X* axis refers to the individual clonal sample number. Squares indicate spawn time in a 2-h early sunset tank, circles indicate spawning by a sample in the 1-h early sunset tank, and a triangle indicates spawning by a coral in the control (normal sunset) tank. Three samples (3, 4, 6) spawned under two different sunset regimens, and one sample (5) spawned under all three regimens

Results and discussion

Approximately one half of *M. franksi* coral fragments removed from the reef and kept in flow through tanks on deck spawn in synchrony with corals on the reef (JD Hilton and PD Vize, pers.obs.). In this experiment in 2008, 12 colonies were sampled on the morning of the predicted spawn and clonal fragments of each transferred to three separate flow through tanks on the boat deck. Each tank had a different sunset time and spawning behavior was monitored as described above. Samples behaved differently under the three different sunset regimen. Both the number of individuals spawning and the time at which they spawned differed (Table 1 and Fig. 1). The most spawning was observed in samples with a sunset time of 18:50 h, followed by the control tank at 19:47 h, while of the corals set at the earliest sunset time, of 17:45 h, only one spawned. The scale of spawning per sample was qualitatively similar between the 18:50 h and 19:47 h sunset samples. The reason for such poor spawning when sunset time was shifted by 2 h is not known, but it is possible that gamete packets did not have sufficient time to reach the polyp mouth with the 2-h shift. This, or some other factor, may be an independently entrained process that needs to coincide with an appropriate sunset time to achieve successful spawning. As spawning was so poor in the 17:45 h sample set, the remainder of this report will focus on the two other treatments.

The average spawning time for corals with an 18:50 h sunset was 20:58 h (128 min post-sunset), while that of those with a 19:47 h sunset was 21:44 h (117 min post-sunset). An offset in sunset of 57 min resulted in an average shift of 46 min. The difference in spawn clock time from 20:58 to 21:44 h was highly significant (Mann–Whitney *U* test, $P = 0.013$) and the delay in spawning post-sunset between these two samples from 128 to 117 min was not significantly different (Mann–Whitney *U* test, $P = 0.331$). Thus, artificially changing sunset time by approximately 1 h caused an equal shift in spawn time. As the only variable between these two samples was illuminated by ambient light, this must be responsible for the shift in spawn timing. As there was no opportunity for a biological rhythm to be reset under these conditions, the time at which a coral spawns post-sunset must be under the direct control of light. These data also demonstrate that it is

Table 1 Number of colonies spawning under different light conditions

Time of sunset	17:45	18:50	19:47
Number of corals tested	12	12	12
Number of corals spawning	ND	6	4

sunset itself and not some earlier component of the solar cycle, such as peak illumination at midday, that is responsible for setting the spawning timer, as all corals were treated in an identical manner up until sunset.

Spawning on the reef at this location was modest in the 2008 season, with a limited number of individuals participating in the spawning event, slightly lower than we have observed in other seasons in on-deck tanks. However, although only a modest proportion of samples spawned in the experimental samples, control samples still spawned in the same time window as did those that spawned on the reef, where a spawning window of 105–137 min post-sunset was recorded for *M. franksi*. Spawning for *M. franksi* normally occurs over a 30-min time window (Vize et al. 2005), and experimental samples conformed to this range.

Corals express genes encoding multiple putative light receptors (Anctil et al. 2007; Vize 2009). Photoreceptors convert light into changes in intracellular levels of second messengers, typically cyclic nucleotides in mammals and calcium in invertebrates (Rayer et al. 1990). If second messenger levels directly control spawning time, one would expect that shifting sunset by an hour would result in a corresponding shift in messenger levels and a corresponding shift in spawn timing. We are currently exploring what second messengers are responsible and how changes in cytoplasmic messenger levels in turn regulate spawning behavior.

While these data show that in this species the time of spawning is a direct response to environment, it is possible that other components of spawn timing are under the control of biological clocks, namely the month and date of spawning. Excellent evidence exists for brooding corals planulating in response to an entrained circalunar clock (Jokiel et al. 1985). It is possible that broadcast spawners use a similar clock, and that this component needs to intersect with the direct environmental response demonstrated here to zero in on the correct time window and achieve precise temporal control over spawning.

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