

Light sensing and the coordination of coral broadcast spawning behavior

P.D. Vize, J.D. Hilton, A.K. Brady, S.W. Davies

Department of Biological Sciences, University of Calgary, 2500 University Drive NW, Calgary, Alberta, T2N1N4, Canada

Abstract. In the Caribbean and Gulf of Mexico hermatypic corals undergo mass spawning on just one or two nights per year, peaking on the eighth evening after the August full moon. This event occurs with extraordinary consistency and is predictable to within a few minutes from year to year. Corals use the moon to determine the date of spawning and sunset time to set the hour and minute of gamete release. We have been exploring whether these two parameters entrain biological rhythms or whether corals respond directly to environmental signals. The literature supports a potential role for entrained circalunar systems setting the spawn date and for non-entrained direct responses to light setting the spawn time but neither has been conclusively demonstrated to date. We propose that G-protein coupled photoreceptors modulate second messenger levels in the cytoplasm and that these in turn lead to changes in protein abundance or protein phosphorylation patterns which then control spawning behavior. In support of this hypothesis we have identified dozens of differences in the proteome of *Montastraea cavernosa* tissue dependent on light. We are using these molecular markers to dissect the cellular basis of lunar and solar light responses.

Key words: circadian rhythms, lunar periodicity, broadcast spawning, mass spawning, scleractinian

Introduction

Broadcast spawn timing is regulated by multiple environmental cycles- seasonal, lunar and solar. The first of these, seasonal, sets the month of spawning in each locale. How exactly corals synchronize spawning with seasonal cycles of gonad maturation remains unknown, but it is associated with solar insolation patterns (van Woesik et al. 2006) and periods of calm weather (Hagman et al. 1998). The second environmental parameter important in spawn timing is the lunar cycle, which sets the date of spawning (Babcock et al. 1986). This is clearly illustrated by the accuracy with which spawning dates can be predicted using the lunar calendar. The third parameter is the solar cycle, which sets the hour and minute of spawning (Levitan et al. 2004; Vize et al. 2005). This is clear from the observation that spawn time changes with the time of year and changes in day length and can be predicted to within minutes relative to sunset time from year to year.

Each of these cycles- seasonal, lunar and solar- could directly regulate cellular processes in corals or could act by entraining biological rhythms with annual, lunar or daily periodicities, and the entrained rhythms would then regulate the processes. As little is known about how the month of spawning is set, we will focus on the date and time of broadcast spawning for the remainder of this report. Excellent evidence

exists for the presence on circalunar rhythms in reproductive behavior in brooding corals. Planula release by brooders can continue for many months even when the lunar input is blocked or is held constant (Jokiel et al., 1985). Furthermore, monthly cycles of planula release takes months to be reestablished when placed in a lunar phase shifted regimen, once again strong evidence that entrainment is involved (Jokiel et al., 1985). An entrained system would have considerable advantages in keeping spawning dates consistent from year to year as fluctuations in weather, cloud cover etc. would have minimal effects on timing.

While corals display some circadian rhythms with a periodicity of around 24 hours (Sweeney, 1976) these do not appear to regulate the time of spawn release. When sunset time is artificially moved forward on the day of spawning, spawn release occurs in a correspondingly early manner (Knowlton et al., 1997; Levitan et al., 2004), which would not happen if an entrained system regulated this process. This indicates that either light directly inhibits or darkness directly promotes spawning behavior. Species species that spawn in different time windows must have different thresholds for a common light regulated factor (e.g., a second messenger) or the factor changes at different rates in different species. By determining what signal transduction pathways

are involved and what second messengers they use will allow us to uncover how corals perceive light and to understand the molecular control of coral spawning behavior.

Material and Methods

Spawning observations

The documentation of spawning times at the Flower Garden Banks, northwest Gulf of Mexico, have been made by our research group for the past 12 seasons. The detailed observations from 1997-2003 were compiled in a recent report (Vize et al., 2005) and are also summarized below. Common species at the FGB that were surveyed include: *Colpophyllia natans* (6%), *Diploria strigosa* (13%), *Montastraea annularis* (<6%), *M. cavernosa* (13%), *M. faveolata* (9%), *M. franksi* (37%) and *Stephanocoenia intersepta* (<6%), where the percentage of live coral cover indicated in brackets if it is known (Pattengill-Semens and Gittings, 2003).

Tissue sampling

Fragments were collected from a single *M. cavernosa* colony at noon and kept on deck in a bucket of seawater along with an air-stone and illuminated by ambient light. Tissue was collected from coral fragments by scraping the surface layer into a test tube containing a commercial protein preservation buffer (phosphosafe- Novagen). A second sample was treated in an identical manner but harvested 4.25 hours post-sunset (midnight).

Protein analysis

Proteins were visualized by 2D electrophoresis followed by silver staining using standard protocols (Marengo et al., 2008). The first dimension was a pH 3 – 10 IEF gradient and the second dimension by molecular weight (10% polyacrylamide).

Results

Spawn timing

Observations of spawn timing were made by the same group of divers each year. This was found to greatly increase the consistency of spawn timing reports (Vize et al., 2005). Spawn times for each of the six common species that broadcast spawn on the eighth evening after the August full moon are shown in Fig.1. Spawning windows for each species are approximately 30 minutes in length except for *D. strigosa*, which is due to a slow tailing off in spawning activity in this coral. However, like other species, the peak of *D. strigosa* spawning also lasts for around 30 minutes. Fig.1 also highlights the unique time windows in which each species spawns, once again with the single notable exception of *D. strigosa*.

In both gonochoric species surveyed the males colonies begin to release sperm approximately

20 minutes prior to the time at which females begin to expel eggs. We have hypothesized that while females may be primed and ready to release via similar timing processes as males, sperm may act as the ultimate trigger of egg release (Hagman et al., 1998). In the case of the common and large species, *M. cavernosa*, females spawn for approximately 30 minutes. For the less common and much smaller species *S. intersepta*, spawning is much briefer and females all release within a window lasting only about 10 minutes.

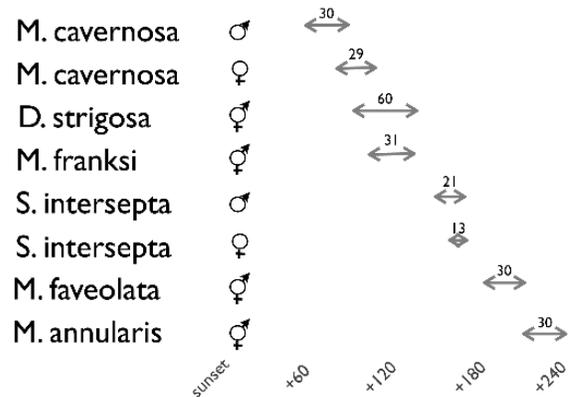


Figure1: FGB spawn times relative to sunset. The number above the arrow indicates the length of the spawning window in minutes. Time after sunset is indicated on the X axis.

This pattern is extremely consistent from season to season. Figure 2 shows the average start and stop times for each species, averaged over eight spawning events, plus the variance over the eight seasons. Of the 16 times noted, 10 are consistent to within 15 minutes over all eight seasons, and 8 consistent to less than 10 minutes.

		start	+/-	stop	+/-
<i>M. cavernosa</i>	♂	1:01	4	1:31	13
<i>M. cavernosa</i>	♀	1:27	23	1:56	9
<i>D. strigosa</i>	♀	1:29	21	2:29	21
<i>M. franksi</i>	♀	1:51	16	2:20	19
<i>S. intersepta</i>	♂	2:38	13	2:59	7
<i>S. intersepta</i>	♀	2:46	10	2:59	7
<i>M. faveolata</i>	♀	3:13	7	3:43	7
<i>M. annularis</i>	♀	3:42	6	4:12	6

Figure 2: Seasonal consistency in coral spawning times. The average time at which coral spawning begins, along with the variation from the average for each species/sex is indicated.

The number of individual corals spawning in any particular season varies quite widely at this location.

Light sensing

Other invertebrates sense light via opsin and rhodopsin visual photoreceptors and non-visual melanopsins (Terakita et al., 2005). In all cases the photoreceptors act via trimeric G-proteins and regulate levels of a soluble second messenger within the cytoplasm of the light-responsive cell (Rayer et al., 1990). If the temporal precision of coral spawn timing is regulated by the same system, as it is likely to be, light may act by upregulating a second messenger that is an inhibitor of spawning or darkness may allow the accumulation of a second messenger that activates spawning. In both scenarios the altered second messenger levels will lead to changes in protein phosphorylation patterns and the changes in protein activity caused by these changes could trigger spawning. Similar universal systems regulate cellular processes in examples ranging from bacterial chemotaxis to human neurobiology and are direct cellular responses to environmental stimuli (Bourne, 2006). An alternative to this class of processes is entrained biological rhythms (Fig. 3)(Dunlap et al., 2004). While light still acts on cells in the same manner and causes changes in second messengers and protein phosphorylation, these changes entrain biological clocks over a period of time. An entrained clock could then accurately regulate cellular processes even when the entraining stimulus is removed (Dunlap et al., 2004).

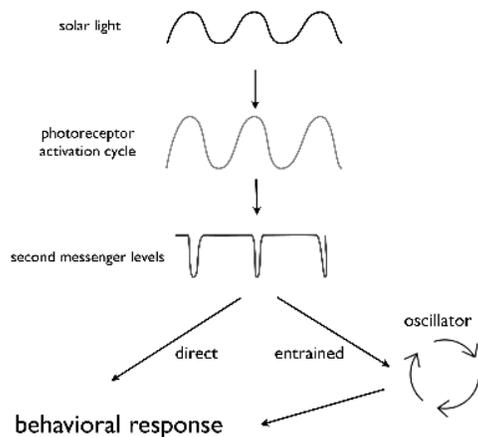
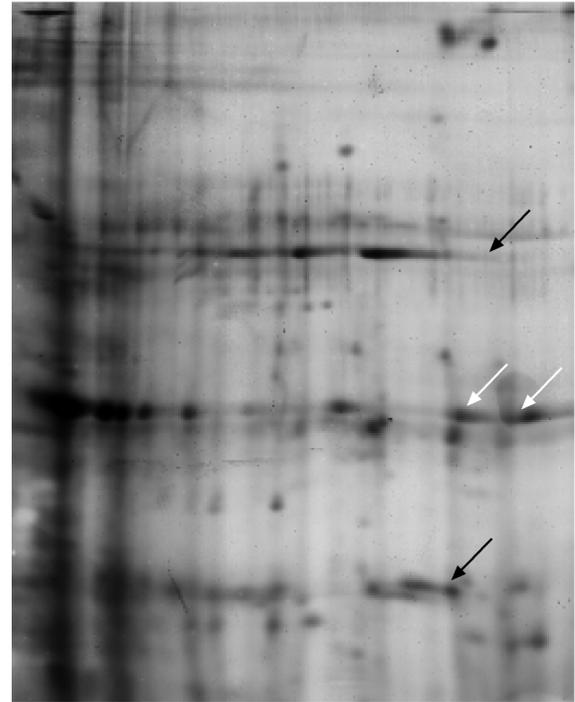


Figure 3: Coral timing may be directly controlled by environmental signals or it may be indirectly regulated by an entrained biological rhythm.

If the spawning response is direct, changes in second messenger levels will control spawn timing while if an entrained clock regulates spawning the key molecules may be regulated at the transcriptional or protein stability levels. There is considerable overlap between these options, but in each case a different set of mediators is actually regulating the biological process.

We have initiated an analysis of changes in protein abundance associated with changes in

Day



Night

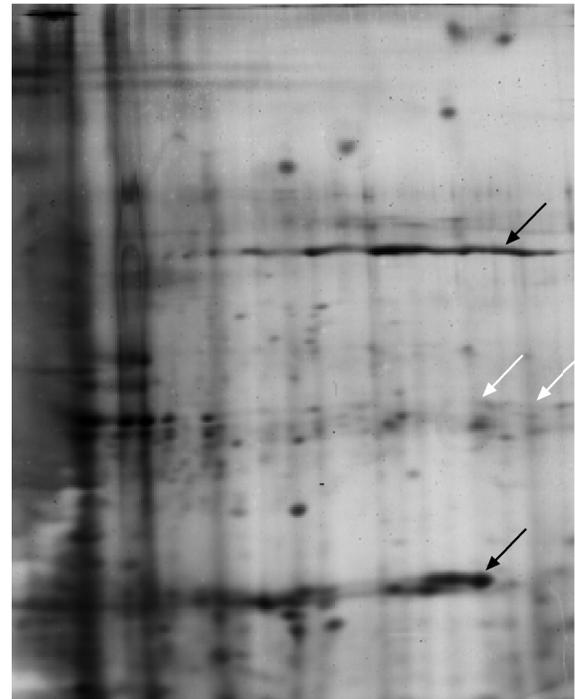


Figure 4: Proteomic analysis of coral tissue harvested in the day or at night. White arrows indicate examples of proteins more abundant in the day samples and black arrows indicate proteins more abundant in darkness. IEF in dimension 1 was from 3 (left) to 10 (right). Molecular weight runs from high (top) to low (bottom).

coral illumination using 2D electrophoresis. Preliminary analysis indicates that dozens of proteins differ in the proteome in *M. cavernosa* under different lighting conditions (Fig. 4).

Discussion

Caribbean corals display extraordinarily consistent spawning behavior, varying by only a few minutes in the time at which they begin and end spawning from season to season. Evidence in the literature implicates circalunar systems as playing a central role in regulating the date of spawning (Jokiel et al., 1985), but the system controlling the time of spawning appears to be a direct response to the environment (Knowlton et al., 1997). However, definitive evidence for direct versus entrained regulation of spawning time and date has not been generated.

Analysis of proteins from the same individual coral collected at midday or midnight indicates that there are many differences in the proteome over this time. As this analysis was performed on whole tissue homogenates containing both coral cells and zooxanthellae, identified proteins could belong to either organism, or to other symbiotic microorganisms. However, as the vast bulk of the protein is from coral cells these are the most likely to be visualized by silver staining.

Although the identity of the proteins that change in abundance from day to night is not known, these can be used to test many aspects of coral responses to light. For example, if a protein is present in the day sample but not the night sample, we can test whether this change still occurs if coral tissue is kept under constant illumination or constant darkness. If it continues to cycle when environmental parameters are constant this must be due to an entrained biological clock, while if it remains constant under constant conditions, it must be directly regulated. We can also test whether pharmacological agents that stimulate or block specific signal transduction pathways can convert light type protein patterns to dark type, and visa versa. Finally, protein gels such as these can be transferred to membranes and probed with phosphospecific antibodies to study changes in the phosphoproteome, determine if these are entrained or direct, and whether they respond to signal transduction pathway changes. This course of experiments will allow us to determine the biochemical basis of the temporal regulation of coral spawning behavior.

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