Effects of an Artificial Lunar Cycle on Reproduction in the sea anemone *Aiptasia sp.*

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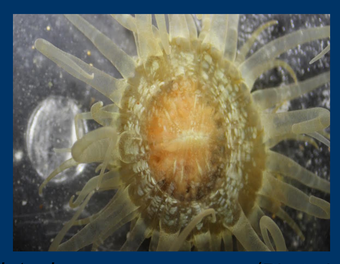

Aiptasia sp sea anemone (Photo by C. Crowder)

INTRODUCTION

The tropical sea anemone, *Aiptasia sp.,* is a model organism within the phylum cnidaria, a group that includes globally threatened corals which are the bio-engineers of coral reefs[5,6]. *Aiptasia* reproduce both sexually, in a process known as spawning, and asexually, mainly through a process known as pedal laceration[1,4]. During spawning, either visible egg bundles or microscopic sperm are released into the water column. During pedal laceration, small pieces of pedal disc separate from the body column and form new anemones. Many cnidarians, particularly corals, show lunar periodicity in their spawning behavior, by spawning often after a full moon[2,3].

In this experiment five individual *Aiptasia* genotypic strains (Table 1.) were subjected to an artificial sunlight (white light) and moonlight (LED light) cycle for one year and anemones were examined regularly to determine the timing and amount of sexual and asexual reproduction. Over the course of one year, pedal laceration counts, oral disc diameter measurements, and the number and length of spawning events were measured. Results concluded that *Aiptasia* did not reproduce on a monthly basis and showed weak synchrony of spawning events.

STUDY OBJECTIVES

- Determine sex of individual strains
- Document spawning events and measure rates of asexual reproduction
- Measure growth of parent individuals by measuring oral disc diameter
- Compare timing (days post “moonlight” treatment) of spawning events between strains to determine if there is synchrony in gamete release between strains

MATERIALS AND METHODS

- Five genotypic strains of *Aiptasia* were placed in individual containers and incubated at 25° C
- Anemones were subjected to a specific light regime (25 day cycle of 12 hours of light followed by 12 hours of dark with a 15 minutes dusk and dawn (LED light) and 5 days of a specific LED “moonlight” treatment (16 hours light with 8 hours just LED, no dark)
- *Aiptasia* were fed *Artemia sp*. brine shrimp and containers were cleaned and refreshed with artificial sea water three times a week
- 1 ml water samples were collected prior to water changes for each strain to check for the presence of sperm
- A dissection microscope was used to check for eggs
- Water samples were stained with Coomassie Blue dye (to stain gametes) and examined with a compound microscope to check for the presence of gametes.
- Weekly counts were taken of newly formed pedal lacerates and weekly measurements of oral disc diameter were collected to measure individual growth

Strain	Origin	Sex
VW3	Hawaii	Male
VW9	Hawaii	Male
GM15	Bermuda	NA
GM16	Bermuda	NA
H2	Hawaii	Female

Table 1. *Aiptasia* genotypic strain names, location of collection, and clonal sex.

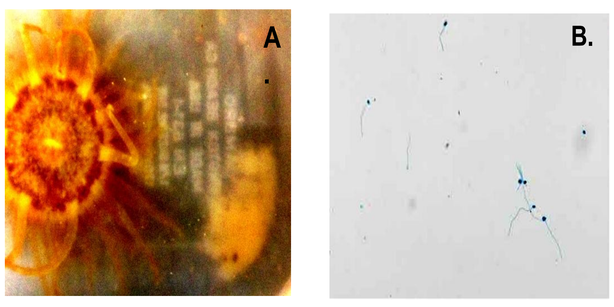

Figure 1. Egg mass released by H2 strain (A.) and microscopic sperm released by VW9 strain (40x objective)(B.).

RESULTS

Figure 2. Total number of spawning events per year.

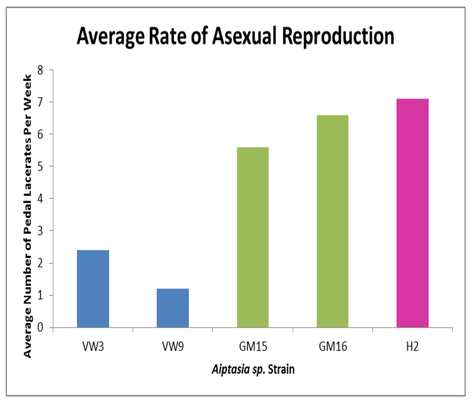

Figure 3. Graph depicting the average rate of asexual reproduction per strain. Asexual reproduction rate averages were measured as the total number of pedal lacerates produced per week.

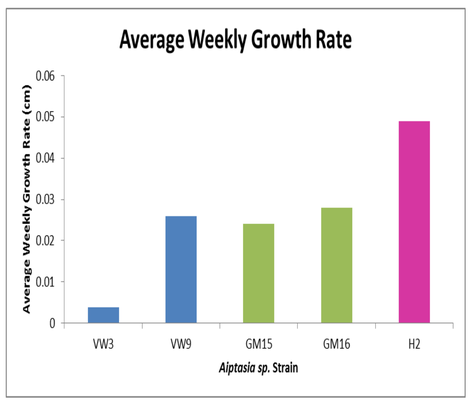

Figure 4. Growth rates measured as diameter of oral disc (cm) taken weekly and averaged over 50 weeks. Blue bars represent male strains, pink represents female strains, and green represents unknown strains.

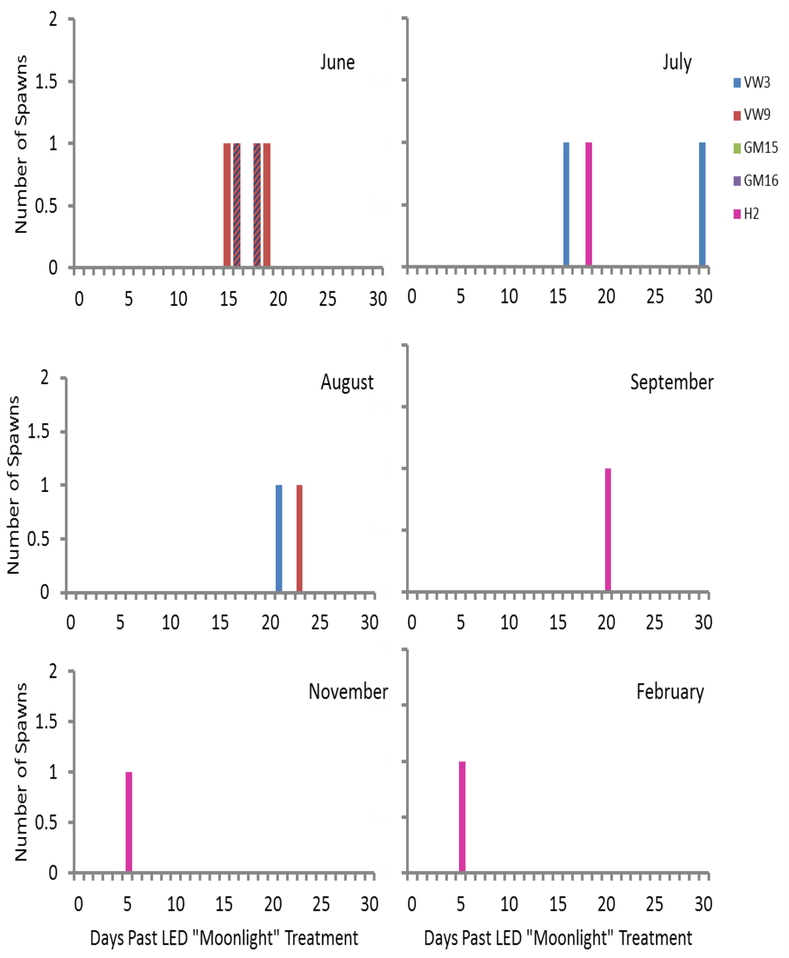

Figure 5. Synchrony of spawning events of *Aiptasia* strains. Graphs included represent all months that spawning occurred. Diagonal lines represent two strains spawning on the same day.

FINDINGS

- Three out of five strains spawned gametes (VW3 & VW9 released sperm and H2 released eggs)
- GM15 and GM16 had no spawning events
- H2 had the most spawning events (4 months) and highest rate of asexual reproduction (~ 7 pedal lacerates/week)
- H2 displayed the highest average weekly growth rate, while VW3 had the lowest average weekly growth rate.
- There appears to be weak to no synchrony in spawning events
- The number of spawning events varied between strains and did not occur on a monthly basis

DISCUSSION

All three spawning anemones (H2, VW3, and VW9) had a high number of spawning events with VW3 and VW9 spawning sperm 3 and 2 months of the year, respectively, and H2 spawning eggs 4 months out of the year. VW3 had low average weekly growth rates, which could suggest that the anemone expended energy towards sexual reproduction, leaving little energy for growth. It also appears that spawning female gametes may require less energy than spawning sperm, as H2, the only known female in the study, had high rates of oral disc growth and sexual and asexual reproduction. The GM15 and GM16 strains did not reproduce sexually, but had high average rates of asexual reproduction, with GM16 having one of the highest individual average growth rates in terms of oral disc measurement.

Overall, H2 seemed to have the highest fitness, as it had both high rates of asexual reproduction (7 pedal lacerates per week), sexual reproduction (4 monthly spawning events), and oral disc growth (0.05 cm per week). Based on our findings, it appears that artificial moonlight inspires spawning events in *Aiptasia* with differences in timing between genotypes. Most spawning occurred between Lunar Day 15 and Lunar Day 25, two to three weeks after the increased moonlight cycle. Future analysis will include statistical analyses to determine differences in reproductive phenotypes and oral disc growth between strains.

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