This is a dual-author paper written by a biology undergraduate student with her faculty mentor, Professor Cawa Tran. This is a typical pathway for publication in the sciences. The student carries out research in the lab under the direction of faculty, and together they write up the description, analysis, and conclusions. This paper used the Council of Science Editors (CSE) Style Manual, which is typical for papers in this discipline.

Asexual Reproduction of the Sea Anemone *Exaiptasia Pallida* Under Artificial Moonlight

Cora Piper and Cawa Tran

Abstract

Many cnidarians live in symbiosis with intracellular algae (dinoflagellates from the family Symbiodiniaceae), and are provided nutrition through photosynthesis; in exchange, cnidarians provide their algal symbionts with protection from predation. In the sea anemone *Exaiptasia pallida* (commonly referred to as 'Aiptasia'), a model system for coral-symbiosis studies, planula larvae produced via sexual reproduction are initially aposymbiotic (without algae) and must obtain new algal symbionts each generation. In asexual reproduction, it is possible for pedal lacerates to acquire their algal symbionts from the parent. A previous study demonstrated that artificial moonlight can affect the rate of asexual reproduction in symbiotic Aiptasia, but it is unknown how asexual reproduction in aposymbiotic Aiptasia may differ. In this study, we hypothesized that the presence of algal symbionts and artificial moonlight together may enhance asexual reproduction in Aiptasia. We found that the rate of asexual reproduction in symbiotic anemones increased under blue light (artificial moonlight) but did not change under white light and no light. Meanwhile, the rate of asexual reproduction in aposymbiotic anemones did not considerably vary under the different light conditions. This study demonstrates how host reproduction is determined by the ability of photosynthetic endosymbionts to respond to light.

Key words: Aiptasia, *Exaiptasia pallida*, anemone, coral, asexual reproduction, pedal laceration, development, blue light, animal propagation, symbiosis

Sea anemones have an increased rate of asexual and sexual reproduction following a full moon by detecting the presence of blue light through ocelli (Tritt et al. 2017). In asexual reproduction, the anemone segments its body either through binary fission, pedal laceration, or budding, resulting ingenetically identical individuals (Chia 1976). Pedal laceration allows for the formation of a lacerate either through constriction or tearing of the pedal disk by contraction and extension of the body (Chia 1976). In sexual reproduction, a sperm and ovum, released from a male and female sea anemone respectively, come together in the water column to form a zygote, which develops into a larva (Schlesinger et al. 2010). The larva then settles on a surface and proceeds to grow into an adult polyp (Bucher et al. 2016). Even though sexual reproduction is evolutionarily important (Bocharova and Kosevic 2011), asexual reproduction is faster and more efficient. Asexual reproduction through pedal laceration is the reproductive strategy used by the tropical sea anemone *Exaiptasia pallida* ('Aiptasia') in the absence of a sexual partner (Clayton 1985).

Aiptasia CC7 clonal line (Sunagawa et al. 2009), hosting its endogenous *Symbiodinium* ITS2 type A4 strain SSA01 (Grawunder et al. 2015), was used in this study. The experiment consisted of six tanks of twelve anemones each, observed over 25 days. Two clear tanks, one containing symbiotic and the other containing aposymbiotic Aiptasia, were placed in an incubator with 453 nm actinic blue LED lights (cat. no. 1663, TrueLumen Lunar Lights, Current USA, Inc.), serving as artificial moonlight. Additionally, two clear tanks (one with symbiotic and the other with aposymbiotic anemones) were placed in an incubator with 12K white LED lights (cat. no. 1644, TrueLumen Lunar Lights, Current USA, Inc.), along with two dark tanks (also one with symbiotic and the other with aposymbiotic anemones) receiving no light (Figure 1). Each tank was partitioned into 15 cells (each cell 25.4 mm × 31.8 mm × 6.35 mm) to track single anemones and their asexual reproduction (i.e., the number of pedal lacerates and juveniles to develop from the single adult polyp). Additionally, this design allowed anemones (both parent and offspring) and brine shrimp (their food) to remain healthy with plenty of oxygen from artificial seawater (Coral Pro Salt, Red Sea Aquatics Ltd., Houston, USA) at 32 to 34 ppt salinity. All incubators were on a 12:12 h light:dark photoperiod at a light intensity of 25 μmol photons m^{-2} s^{-1} and maintained at 27°C.

During the 25-day duration of the experiment, the number of pedal lacerates, juvenile anemones, and adult anemones were counted three times per week prior to water changes and photographed under a stereo microscope (Leica M165FC). Three independent trials of this experiment were conducted. Following the experiment, a t-test was performed comparing the slopes (i.e.,rate of mean number of individuals) of (i) blue light to white light and (ii) blue light to the dark, for both symbiotic and aposymbiotic anemones (Figure 2). There was a significant difference between symbiotic anemones exposed to blue and white light (t-test, $P<.001$, Figure 2A), and blue light and the dark (t-test, $P<.001$, Figure 2A). Although there was also a significant difference between aposymbiotic anemones exposed to blue and white light (t-test, $P<.001$, Figure 2B), the rate of asexual reproduction under blue light was slower than that under white light and the dark, suggesting that blue light did not benefit aposymbiotic anemones at all. Taken together, these results suggest that symbiotic anemones benefit from blue-light exposure. Moreover, among symbiotic anemonesexposed to blue light, there was a greater proportion of juveniles over time (Figure 2C), indicating that pedal lacerates were ableto grow and develop normally into juveniles.

These findings demonstrate that exposing symbiotic Aiptasia to artificial moonlight enhances asexual reproduction. Given there was an increase in the reproduction of only symbiotic but not aposymbiotic Aiptasia in response to blue light, blue light is likely being detected by algal cells within the host and, in turn, potentially changing host behavior. Symbiodiniaceae have photoreceptors, such as phytochromes, cryptochromes, and phototropins that detect various wavelengths of light (Xiang et al. 2015). As observed in a previous study with Aiptasia, the detection of blue light by algal symbionts induced a sustained phototropism in symbiotic, but not aposymbiotic, anemones (Foo et al. 2020). Light detection by Symbiodiniaceae may also alter thecircadian clocks of their anemone host. In a separate study investigating the influence of algal symbionts on host rhythmic behavior (Sorek et al. 2018), the average periodicity of extension and contraction of the anemone body in symbiotic Aiptasia was twice that of its aposymbiotic counterpart. However, the reintroduction of algal cells intoaposymbiotic Aiptasia restored that periodicity comparable to that of symbiotic Aiptasia, demonstrating the key role of Symbiodiniaceae in controlling the timing of body extension and contraction in the host (Sorek et al. 2018). Therefore, if algal symbionts can detect blue light and mediate Aiptasia's body extension and contraction, itmay be possible that the algae are, in some capacity, signaling the host to extend a portion of its foot to gradually dissociate andform a pedal lacerate. This may be a probable method algal symbionts are using to affect host asexual reproduction, as they subsequently benefit and promote their own growth and division in a new individual animal, though precise mechanisms of host-algal signaling for this to occur are still unknown.

The effect of algal symbionts on anemone nutritional state may also play a role. We attempted to minimize the effects of variable nutrition by utilizing similar-sized anemones at the start of this experiment and providing each individual anemone with a consistent amount of brine shrimp as food, but symbiotic anemones are inevitably receiving extra nutrition in the form of photosynthate provided by their algal partners (Davy et al. 2012). In a previous study, symbiotic and aposymbiotic Aiptasia fed brine shrimp three times per week for 30 days produced a similar number of pedal lacerates; however, when both anemone groups were starved for 30 days, there were significantly more pedal lacerates produced by symbiotic than aposymbiotic anemones, suggesting that the presence of algal symbionts only mattered when anemones were lacking their food source. In our experiment, we maintained the food source but exposed both anemone groups to blue light and observed a significant difference in the number of symbiotic anemones over time. Therefore, we can conclude that blue light and the presence of algal symbionts are indeed inducing a higher rate of lacerate production, whereas the presence or absence of algal symbionts do not appear to matter when anemones were exposed to white light or the dark. Further development of those lacerates into juvenile anemones under blue light is also likely enhanced by the presence of algal symbionts, providing additional metabolites to the host to continue to support its growth and survival.

From a practical standpoint, this study uncovers a method to significantly improve laboratory propagation of Aiptasia, an important model organism for symbiosis research. In future experiments, the size of the tanks will be increased to a larger size (17.6 cm × 16.2 cm × 6.35 cm) typically used in normal laboratory maintenance of animals, with twelve anemones placed into each tank to verify that this form of propagation can be used on a larger scale. Through this experiment, we have explored some of the intricacies in how blue-light detection by algal symbionts can alter host growth and development. By supplying additional nutrition to the host and/or manipulating the host's body extensions and contractions, algal symbionts are either directly or indirectly improving the host's ability to asexually reproduce with the assistance of blue light as a source of artificial moonlight. This study provides a platform for further investigations into the mechanisms by which algal symbionts signal the host to induce pedal laceration. The cnidarian-algal symbiosis remains complex, as additional benefits previously unknown but exchanged by both partners continue to come to light.

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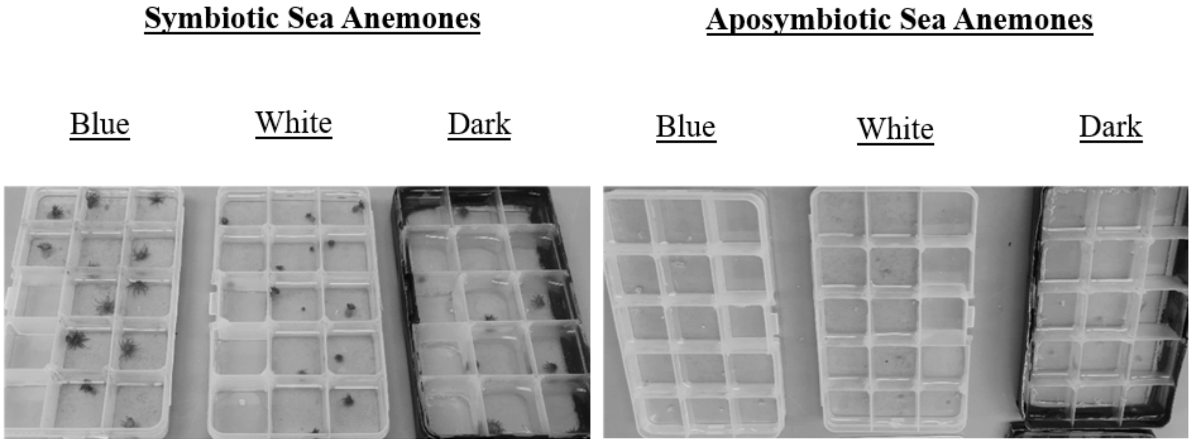

Figure 1. Asexual reproduction of symbiotic and aposymbiotic Aiptasia exposed to different types of light were observed. Each independent trial (of three trials total) consisted of two clear tanks exposed to blue light (mimicking artificial moonlight), two clear tanks exposed to white light, and two black tanks exposed to no light (i.e., completely dark). Black tanks included black lids and a black floor slip that tanks were placed on top of. The left three tanks contained symbiotic anemones (with algal symbionts) and the right three tanks contained aposymbiotic anemones (without algal symbionts). All tanks were maintained at a normal temperature of 27°C on a 12:12 h light:dark photoperiod. No light and white light served as controls to test the effects of blue light on asexual reproduction. Each anemone was fed 2 g of brine shrimp twice per week. Seawater was changed eight hours after each feeding and on one additional non-feeding day. Development and asexual reproduction were normal (generated new oral disks, stalks, and tentacles) in anemones exposed to all conditions.

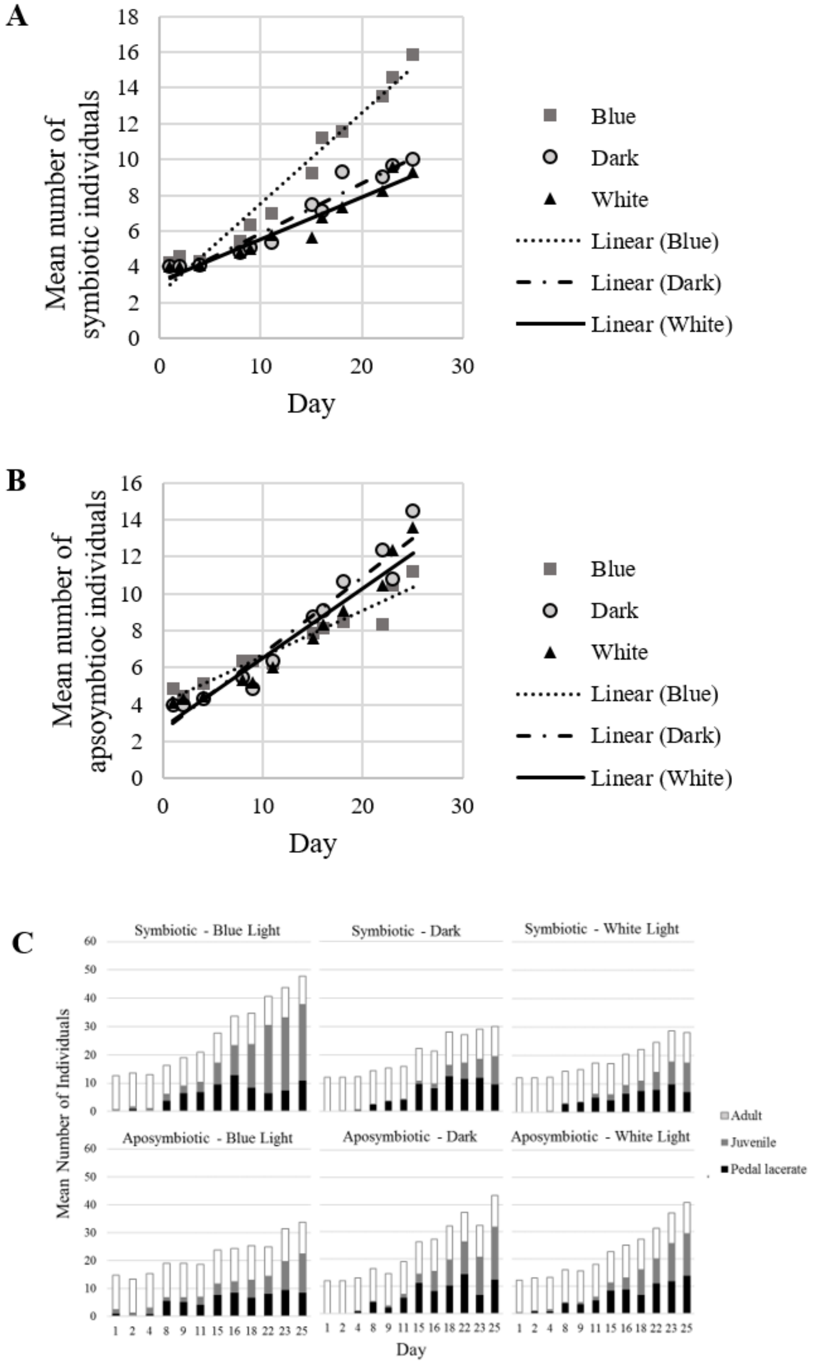

Figure 2. Asexual reproduction of symbiotic Aiptasia increased in response to blue light. (A) Mean number of symbiotic individuals across all developmental stages (adults, juveniles, and pedal lacerates) exposed to blue light significantly increased in number at a greater rate than those exposed to white light (t-test, *P*<.001) and the dark (t-test, *P*<.001). (B) Mean number of aposymbiotic anemones across all developmental stages (adults, juveniles, and pedal lacerates) exposed to blue light increased at a slower rate than those exposed to white light (t-test, *P*<.001) and the dark (t-test, *P*=0.39). (C) Among symbiotic anemones, there was a greater proportion of juveniles over time under blue light,

suggesting a more rapid rate of asexual reproduction and growth, in contrast to other light treatments. Among aposymbiotic anemones, there was no apparent difference in distribution of developmental stages among the light treatments. Adult and juvenile anemones had a mean body-column diameter of 16.25 and 2.35 mm, respectively, and pedal lacerates had a mean diameter of 1.55 mm. *n*=3 independent trials.