Aiptasia spawning and embryo/larvae handling - Weis Lab

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Abstract

This protocol outlines the steps taken to support sexual reproduction in Aiptasia lab cultures. It also provides steps for handling embryos and larvae, and basic husbandry to keep them alive. Spawning protocol based on the methods from Grawunder et al. 2015.

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Protocol

Pre-spawning

Step 1.

Aiptasia polyps are kept at low-density cultures to help maximize growth. Typically no more than 3 or 4 polyps are kept in plastic food containers (Cambro 92CW, 17.6 x 10.8 cm). These animals are kept at 25°C under a 12 hour light and dark cycle (standard culture conditions), with white light between 15-20 µmol photons /mm^2s.

Pre-spawning

Step 2.

Polyps are fed dense cultures of brine shrimp at least 5 days a week and the water is changed at least every other day. Under this feeding regime, polyps should grow rapidly. Once polyps have grown a sufficient size, i.e. 0.5 cm oral disk diameter, they are placed into a different incubator used for spawning ('spawning incubator'). Polyps continue to be fed brine shrimp at least 5 days a week, with the water changed at least every other day.

Pre-spawning

Step 3.

Male and female crosses can be setup in containers, especially if the timing of fertilization is not important and larvae are desired. To control the timing of fertilization, separate sexes are kept in different containers. The light and dark cycles in the spawning incubators can be setup for convenient spawning times. In the lab, Aiptasia typically release gametes 6 hours after the onset of darkness.

Pre-spawning

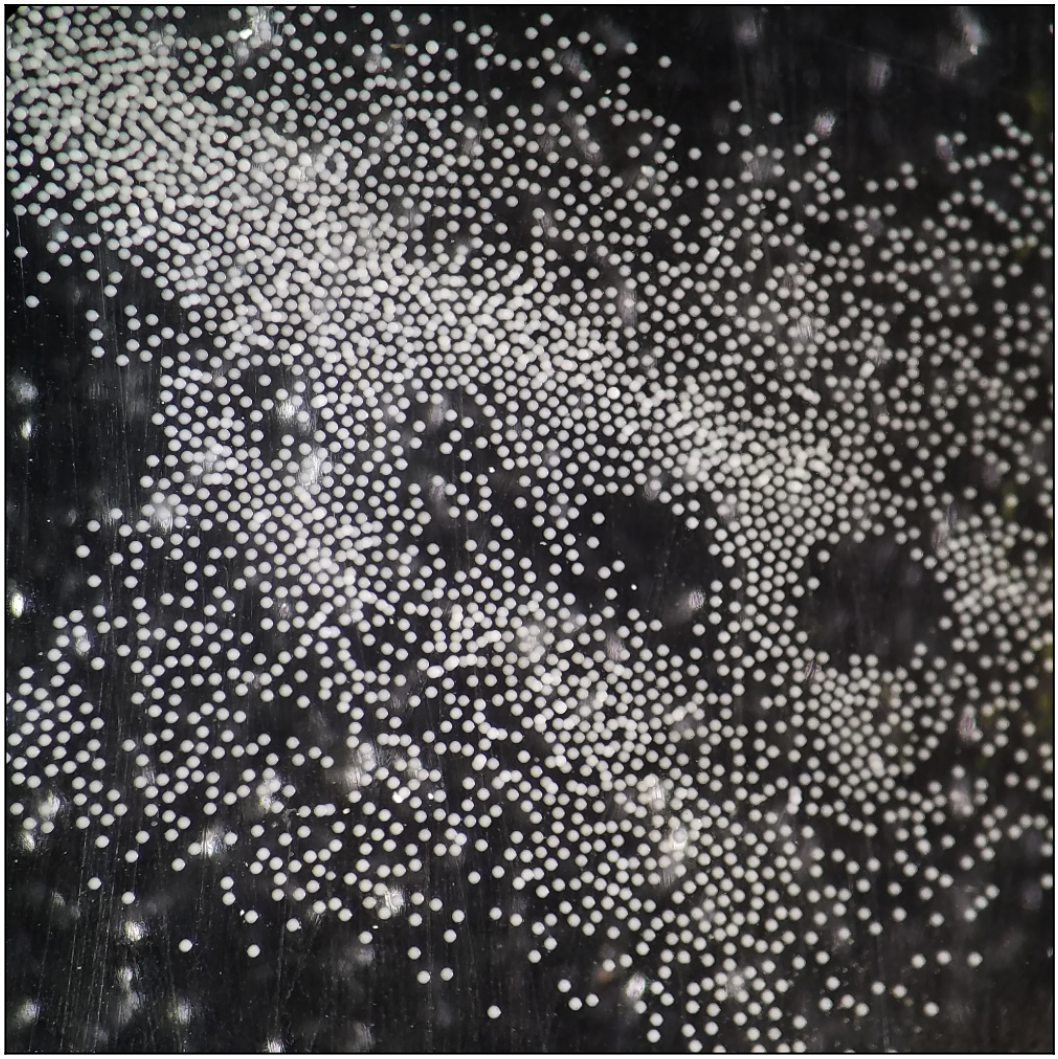
Step 4.

The spawning incubators are setup to mimic the lunar cycle by providing blue light (peak wavelength at 450 nm, intensity 10µmol photons /mm^2s) every 28 days. The blue light is turned on during the 12 hour dark period for the first 5 days of the 28-day cycle. If well-fed and healthy, polyps will likely spawn within the first two 28-day cycles. Spawning typically occurs between days 13 - 20.

Gamete collection and fertilization

Step 5.

Eggs sink to the bottom of the dish and are uniformly round, white, and opaque. Eggs are approximately 80 µm in diameter and are easily identifiable under a standard stereomicroscope (See image). Sperm is a bit more challenging to observe, but if enough is released the water will appear cloudy.



Gamete collection and fertilization

Step 6.

Different sizes of mesh screen (e.g. Nitex or cell strainers) can be used to collect gametes. For example, water containing sperm from male-only tanks can be poured through a small mesh screen to filter out large debris. Similarly eggs can be passed through a larger mesh screen (e.g. 100 um) to filter out large debris.

Gamete collection and fertilization

Step 7.

The eggs and sperm are then mixed in a 1,500 mL (8-inch) glass culture dish and placed at 25°C. Fertilization should take place within 10-20 minutes, depending on sperm concentration.

NOTES

Culture dishes are from Carolina Biological Supply Company (https://www.carolina.com/lab-dishes/carolina-culture-dishes/FAM_740996.pr).

Embryo/larvae handling

Step 8.

Embryos and/or larvae can be concentrated from larger dishes by pouring them through a filter small enough to capture eggs, roughly < 80 um (e.g., a 40 µm cell strainer).

Embryo/larvae handling

Step 9.

The captured embryos/larvae can then be placed into a smaller culture dish filled with filtered seawater (FSW). This is a good method to clean the water since ciliates will pass through the filter. Take care to not let the embryos/larvae be exposed to air. Once all the water containing the embryos/larvae has been poured through the filter, immediately immerse the filter in the new culture dish that is filled with FSW. Invert the filter to free the embryos/larvae.

Embryo/larvae handling

Step 10.

As noted in (Bucher et al. 2016), early cleavage stages take place a few hours post fertilization (hpf). Embryos reach the blastula stage by 12-14 hpf, and by 24 hpf have undergone gastrulation.
Embryos and larvae should be kept at 25°C in 4-inch glass culture dishes (or larger). Changing the water at least once a day should keep the larvae healthy.

Embryo/larvae handling

Step 11.

Cilia lining the ectoderm develops and begins to beat by 24 hpf, causing the embryos to “swim” around the bottom of the dish.

Embryo/larvae handling

Step 12.

By 48 hpf, planula larvae can be observed actively swimming through the water.