

# 🔗 General Aiptasia husbandry - Pringle lab

## Pringle Lab

### Abstract

This protocol describes the basic steps necessary to maintain and propagate Aiptasia as they are done in the Pringle lab.

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## Guidelines

If working with symbiotic anemones hosting different *Symbiodinium* strains make sure to keep all working surfaces clean and wash/sanitize your hands regularly.

Keep different strain combinations as separated as possible (e.g. different shelves or incubator compartments). Do water changes and tank cleaning for one host-symbiont combination at a time. Sanitize hands and surfaces after finishing one batch of host-symbiont combination.

Aiptasia is a very robust organism. Especially the symbiotic animals are more sensitive to impaired water quality than to short-term starvation (1-2 weeks).

The bigger the anemones the more feeding they need to keep their body mass.

## Materials

✓ BRINE SHRIMP NET, Aquatic/Commercial  
Quality Brine Shrimp Mesh Net {measures 4 x 6} - by Bubblemac Industries, Inc. FAN4x6 by  
Contributed by users

## Protocol

### Basic Needs

#### Step 1.

#### Light Levels:

- Symbiotic Aiptasia: 25  $\mu\text{mol photons/mm}^2/\text{s}$
- Aposymbiotic Aiptasia: 0  $\mu\text{mol photons/mm}^2/\text{s}$  (kept in black containers; can be brought out in the light for feeding and cleaning)

#### 📌 NOTES

We use Philips F25T8/TL841 fluorescent lamps in our incubators which have a color temperature of 4100°K

## Basic Needs

### Step 2.

#### Artificial seawater (ASW):

- Artificial seawater is mixed in the lab. Advantages of using artificial seawater are that the salinity can be easily controlled and the water can be easily kept free of harmful microbes.
- Salinity between 32 and 34 ppt (as read on a refractometer) and a pH of 8.0 is desired.
- Seawater is mixed according to manufacturer's instructions. We mix big batches of seawater in a 70-gallon (265 l) barrel with a pump for mixing the water over night. Salinity is checked the next day and adjusted by either adding RO water or some more salt. Then, the water gets pumped into a second (storage) 70-gallon barrel via a pipe connection. This second container is connected pipes leading to the valves for retrieving ready-to-use ASW. Between the barrel and the valves sits a filtration system (5  $\mu$ m, 1  $\mu$ m and 0.35  $\mu$ m) to filter out particles and microorganisms.
- If possible use RO/DI water for mixing the ASW. Tap water usually contains heavy metals that are harmful to marine invertebrate

#### 📌 NOTES

We use Red Sea Coral Pro Salt mix to make our ASW:  
<https://www.redseafish.com/red-sea-salts/coral-pro-salt/>

## Basic Needs

### Step 3.

#### Temperature:

- Stocks are generally kept at 27°C.
- Tanks for growing up spawning animals are kept at room temperature (22-27°C)

## Basic Needs

### Step 4.

#### Storage:

- Small plastic food containers are good for maintaining low numbers of different clonal lines or experimental groups. Don't overcrowd them. We use the small plastic food storage containers from Cambro; they have a large variety of container sizes as well as black ones that can be used to keep the light out for the aposymbiotic anemones.

#### 📌 NOTES

Cambro Camwear® line of plastic storage containers  
[https://www.cambro.com/Products/Food\\_Pans\\_and\\_Lids/10737419403/1033.aspx](https://www.cambro.com/Products/Food_Pans_and_Lids/10737419403/1033.aspx)

## Basic Needs

### Step 5.

#### Preparing brine shrimps for feeding:

- The best food source to use, with minimal upkeep and low costs, are brine shrimp. These can be obtained through online vendors, we purchase QUALITY BRINE SHRIMP EGGS from [aquacave.com](http://aquacave.com).
- HOW TO HATCH BRINE SHRIMP:

1. Fill a large hatching cone ( $\geq 6$  l usable volume) with a drain valve at the bottom with ASW, insert a small tube connected to an air pump so it reaches the bottom of the cone.
  2. Add brine shrimp eggs (15 mLs of decapsulated eggs or 20 mLs of non-decapsulated eggs)
  3. Turn on air line and light. *We use a standard 100W 120V T3 halogen bulb in a desk lamp as light source. Keeping the light on for the first 24 hours will increase the hatching rate, the bubbling air at the bottom is for both, good aeration and preventing eggs from accumulating on the ground.*
  4. Monitor and rinse sides with ASW as necessary to remove build up of eggs at water level.
  5. Decapsulated eggs (see below) will hatch in 24-48 hours, non-decapsulated eggs will take longer.
  6. When the eggs have hatched, turn off air and allow non-hatched egg shells to settle and/or float. *If using decapsulated eggs, there should be no floating shells.*
  7. Open the drain valve and collect shrimps in a large shrimp net. *We use this net from amazon.com: [https://www.amazon.com/Aquatic-Commercial-measures-Bubblemac-Industries/dp/B0165HFFQ0/ref=sr\\_1\\_9?ie=UTF8&keywords=brine%20shrimp%20net&qid=1490996532&sr=8-9](https://www.amazon.com/Aquatic-Commercial-measures-Bubblemac-Industries/dp/B0165HFFQ0/ref=sr_1_9?ie=UTF8&keywords=brine%20shrimp%20net&qid=1490996532&sr=8-9)*
  8. Quickly rinse shrimp with fresh ASW.
  9. Transfer to small cone (1 l usable capacity) filled with fresh ASW.
  10. Rinse cone with RO water and scrub down the inside walls with brush and rinsing again with DI water. Bleach at the end of the week.
- HOW TO DECAPSULATE BRINE SHRIMP EGGS:
    1. Fill a glass beaker with 600 mLs of DI water.
    2. Add 15 mLs (one full 60×15 mm petri dish bottom) of brine shrimp eggs.
    3. Aerate for 1-1 ½ hours. *Check often to make sure airline hasn't fallen out and to swirl the eggs that build up along the bottom edges of beaker.*
    4. Add 300 mLs of chlorine bleach.
    5. Continue to aerate for 3 to 3 ½ min, swirling the airline the whole time. The eggs should turn brownish orange. Be careful not to go over 4 min.
    6. Strain eggs through a shrimp net and rinse with DI water for 5 min or until you can't smell bleach.
    7. The eggs are now ready to be added to the hatching cone or stored in at 4°C for later use.

## Basic Needs

### Step 6.

#### Feeding:

- Feed anemones at least 2 times a week. To maximize feeding, the container of anemones can be saturated with brine shrimp.
- Be careful not to allow old food to stay in the containers, as this will quickly foul the water (especially in the smaller containers).
- Increasing the number of days a week of feeding should increase the growth rate of the anemones.

## Basic Needs

### Step 7.

#### Cleaning:

- Clean after feeding (wait at least 1 hr, but preferably > 3 hr, and no longer than 8 hr), with additional water changes if necessary.
- Water can simply be dumped, just look out for any detached anemones and try not to lose them.
- Use cotton swabs to clean algae off the surfaces of containers. Avoid disturbing anemones and pedal

lacerates. Sometimes swabbing isn't necessary at all (especially for apos), and you can simply dump and replace the water.

#### NOTES

If you see any dying/unhealthy anemones, isolate container and clean water. Causes can be hypoxia, salinity, temperature, bleach contamination, or accumulation of microbes on waste food or dying anemones.

### Bleaching containers

#### **Step 8.**

- Clean used containers thoroughly with detergent and a brush until no biofilm/dirt is visible, rinse with RO water..
- Let the containers soak in a 1% bleach solution for  $\geq 12$  h.
- Rinse with DI water.
- Clean containers in a commercial dish washer.
- Rinse with DI water and air-dry.

### Propagating Aiptasia

#### **Step 9.**

**There are two possibilities for propagating Aiptasia:**

1. Aiptasia can be propagated by simply collecting pedal lacerates and let them develop into full polyps (usually a few days, not longer than a week for transition).

*This works well for strains which produce a lot of pedal lacerates in a short time like H2.*

2. Aiptasia can be cut into halves (quarters with some practice and large anemones) with a razor blade or scalpel on a vertical axis through their oral disc.

It is important to use clean/sterile blades for cutting to not introduce any pathogens or transmit microorganisms/symbionts between different tanks.

Transfer the cut animals into a container with fresh ASW. Let them rest for 24-48 h (until they attach to the surface again), then feed them daily.

They should be fully regenerated after 1 week. *The newly grown tentacles will need some more weeks to reach the size of the old ones.*