

## **Cold-shock Protocol to Bleach *Aiptasia***

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The best way to get apos is to cold shock them:

- Cool seawater without the anemones to 4°C.
- Drain the room temperature seawater (I keep them in glass bowls) and replace with the cold water.
- Place them in the fridge (at 4°C) in the dark for 4 hours.
- Drain the cold water and replace again with room temp water. Keep them in the DARK (this is critical!) at room temp.
- It is better if the anemones have not fed for at least a week before shocking them.
- Change their water every day as they spit out algae.
- As they start to open again and look 'happier' you may want to repeat the procedure to get more of the remaining algae out.

Sometimes it takes a while to get 100% of the algae out (i.e. check under the dissecting scope with good magnification).

- To get the last few algae out I have experimented by incubating them for 30 min in 50µM of 3-(3,4-Dichlorophenyl)-1-1 dimethylurea “DCMU” (Sigma D2425; see below for prep instructions) in the dark, then blasting them with the strongest lights you have for another 30min (watch out overheating them)

Once 'stabilized', keep them in the dark and feed them 1-3 times a week with freshly hatched brine shrimp.

### **DCMU recipe:**

To make stock first make 0.0313 M solution in 95% ethanol, or 0.0073g DCMU into 50ml 95%EtOH. To get 50µM add 32µl of stock to 200ml of seawater.