**Algae Protocol**

To replenish one culture of algae at a time follow the steps below. Always remember to change gloves and clean the bench space with 70% ETOH between working with each culture. Only use materials labeled for use with copepods. Grow lights in the Algae Room will be OFF 10:30 am-4:00 pm.

1. RM 106: Collect one large cube, a 50 mL pipette tip, gloves, small glass pipette tip, section of plastic tubing, and at least 1.5 scoops of salt.
2. RM 009: Make 8L of 30 ppt FSW in a large cube, stirring with the 50 mL pipette.
3. RM 106: Retrieve a 1000 microliter micropipette and clean it with 70% ETOH.
4. RM 106: Pipette 300 microliters of F/2A and F/2B media for every 1L of FSW into the FSW cube (2400 ul for 8L FSW).
5. RM 106: TW (brown) ONLY: pipette 100 microliters of silicate media for every 1L of FSW (800 ul or 8L FSW).
6. RM 106: Transfer 900 mL of this solution to a stock bottle to create a stock solution. Label with culture and date.
7. RM 106: Transfer remaining solution to an autoclaved carboy. Label with culture and date.
8. RM 220: Locate the oldest stock bottle available for the given culture, screw on lid, shake to mix. Transfer 1000 microliters into the fresh stock and screw top on only 1 rotation.
9. RM 220: Empty remaining solution from the oldest stock into the new carboy of solution.
10. RM 220: Be sure the new carboy is being aerated using a small glass pipette and tubing.
11. RM 106, 324: Dispose of waste (pipette tips, gloves etc.) in cardboard waste bins, return cube and any extra salt to Cope Room (clean up!).

**Carboy Maintenance**

When a carboy that once contained algae is empty, follow the steps below to ensure proper sanitation.

1. Fill with boiling hot water from the sink in RM 106
2. Soak for at least 24 hours
3. Scrub and rinse with water
4. Autoclave