Daphnia Cultivation & Maintenance Protocol

Dr. Tang's Plankton Ecology Lab

I. Water Quality Maintenance

1. Remove your 1000 mL cultivation beaker(s) from the incubator and place them on a clean benchtop.
2. Carefully remove approximately 200–400 mL of water from each beaker and dispose of it in a designated liquid waste receptacle.
3. Using a clean 25 mL graduated pipette, gently extract any visible detritus or organic waste from the bottom of the beaker.

   Note: Use a handled sieve to avoid removing healthy Daphnia.
4. Refill the beaker with filtered spring water (e.g., Ozarka) until it reaches a final volume of approximately 800 mL.

   Note: Ensure the water is at room temperature before use to avoid shocking the culture.

II. Algal Feeding

1. Remove algal cultures from the incubator, ensuring the caps are securely fastened after removal.

   Note: The caps will be loosely on the container whilst in the incubator.
2. Gently invert and shake the bottles to homogenize the cultures and evenly distribute the algae.
3. Place the algal container caps face-down on a clean sheet of paper to avoid contamination.
4. Pour approximately 20 mL of each algal culture (e.g., Algae-1 and Algae-2) into separate 50 mL beakers.
5. Using a clean 10 mL pipette, dispense 20 mL of each algal type into the cultivation beaker(s) that received a water change.
6. Replace the caps on the algal culture bottles, leaving them loosely secured.

   Note: Ensure the caps on the algal cultures are secured loosely for storage to allow gas exchange to occur.
7. Return the algal cultures to the incubator, making sure to place them on a different rack than the *Daphnia*.

III. Post-Maintenance Setup

1. Return the cultivation beaker(s) to the incubator.
2. Secure the air stone(s) at the top of each beaker using tape.

   Note: Failure to secure the air stone(s) at the top of the beaker will lead to the death of the Daphnia cultures.
3. Place a watch glass over the beaker(s) to ensure the tubing and stone remain in place and to reduce the risk of contamination.

IV. Cleaning Procedures

1. Dispose of the liquid waste from the cultivation beaker(s) down the sink.
2. Wash all used supplies (pipettes, beakers, etc.) with DI water and soap in the sink inside of the Nutrition Room.
3. Hand-dry all glassware and supplies using clean lab paper towels.
4. Allow remaining items to air-dry thoroughly on the benchtop before storage.

V. Documentation

1. Immediately after completing feeding and water changes, log details in the digital **Daphnia Cultivation & Maintenance Log Sheet**.