Egg Bleaching Procedure

Note: If fish are arriving from outside your facility, acclimate them to your fish room temperature for 2 hours prior to bleaching.

1. Using a spray bottle, spray and wipe down the counter with 95% alcohol (EtOH) or Disinfectant.
2. Set up beaker solutions (Note: All solutions should be adjusted to a pH of 7.0):
    a. **Beaker 1**: make bleach solution, 0.1 ml of 5.25% sodium hypochlorite (bleach) into 170ml of autoclaved distilled water or RO (Reverse Osmosis) water .
    b. **Beaker 2**: fill a second beaker with 200ml autoclaved distilled/RO water or sodium thiosulfate solution (0.5 g/1L autoclaved water)
    c. **Beaker 3**: fill with 200ml 0.5X E2 embryo medium
3. Bleach Eggs: first remove dead eggs (VERY IMPORTANT); next, rinse eggs thoroughly with fish system water, then pipette (being careful not to splatter) 0-100 eggs into **Beaker 1**.
4. Swirl the beaker/eggs periodically to separate the eggs and allow the entire chorion to contact the bleach solution.
5. Remove the eggs using a clean sterile pipette after 5 minutes and place them into **Beaker 2**.
6. Swirl eggs for 1 minute.
7. Remove eggs using a clean sterile pipette and place into **Beaker 3**.
8. Swirl eggs for 1 minute.
9. After final rinse in beaker 3, using a clean sterile pipette place the eggs into a clean petri dish containing methylene blue working solution (recipe below) and label appropriately.
10. Bleaching can cause the chorion to become tough enabling the embryos to hatch. This will result in the need to dechorionate (physically or use pronase) the embryos.
11. Wash all utensils (beakers, pipettes, etc.) after use.

Large Volume Working Solutions

Bleach Solution – 1.18ml 5.25% sodium hypochlorite (chemical grade bleach) into 2 Liters autoclaved distilled or RO (Reverse Osmosis) water. **Note:** Fresh bleach solution should be made daily.

Sodium Thiosulfate Solution – 1 gram sodium thiosulfate into 2 Liters autoclaved distilled or RO water

Final Rinse Solution – 0.5X E2 embryo medium

Methylene Blue Stock (Concentrate) Solution – 1 gram Methylene Blue powder into 1 Liter RO

Methylene Blue Working/Rearing Solution – 10ml methylene blue stock solution into 20L 0.5X E2 Embryo Medium