

Langerhans Lab Protocols

NC STATE UNIVERSITY

Water Treatment and Aging

For *Gambusia* and other blue hole fish:

1. Make sure the inside of a 5 gallon bucket has been scrubbed with the green plastic scrubber or the coarse side of the sponge and thoroughly rinsed.
2. For *Gambusia hubbsi* and other blue hole fish, add:
 - a. 2.5 mL Amquel Plus.
 - b. 2.5 mL Stress Coat.
 - c. 4 average-sized crystals of sodium thiosulfate.
 - d. $\frac{3}{4}$ tsp (~3.750 g) of sodium bicarbonate (baking soda).
 - e. 1 tablespoon (4.5 g) Marine Mix or Instant Ocean for ~0.7 ppt salinity.
3. Fill the bucket to the top with tap water.
 - a. Watch the flow for unwanted debris.
 - i. You may need to run the water for a few seconds to flush out debris.
 - ii. If you notice water quality issues, pour the water out and start over.
4. Use the aged water log sheet on the wall in each room to record a description of the bucket (e.g. white or orange; flagging or no flagging) and the date and time the water is ready for use (i.e. current date/time plus 24 hours)
 - a. The water may be used after aging for 18 hours if absolutely necessary, but age the water for 24 hours as normal procedure.

For North Carolina creek Chub:

- Prepare the water as above excluding the salt and use only $\frac{1}{4}$ teaspoon of baking soda.

Procedure notes:

- Re-fill any water buckets you empty, so that plenty of aged water will be available for use at any time.
- The last liter of water in the bucket often contains debris and should not be used.
- If the aged water stands more than 48 hours, it will start to grow pink slime.
 - You may be able to feel it on the inside of the buckets.
 - The water may also get cloudy.
 - Do not add pink slime or cloudy water to the fish tanks.
- The white grains on the bottom of the water buckets are undissolved salt or sodium bicarbonate.
 - Do not add these undissolved particles to the fish tanks.