Quick Care Sheet for *Nothobranchius furzeri*

Water Quality and Room Check Guidelines----------

Check Daily

Temperature	26°C
Light cycle	12hr/12hr
Aeration System	on
Filtration System	on (turnover rate 4 gph)

Check Twice a Week

pH	6.4-6.5
Ammonia (NH_3/NH_4)	0.0 ppm
Nitrite (NH_2^-)	0.0 ppm
Nitrate (NO_3^-)	0.0 – 10.0 ppm
Salinity	2000-3000 µS/cm; 1000-1500 ppm

System Water Recipes----------

Water should be mixed well and left to sit for at least 1 hour (preferably overnight) before adding to fish. System water should also be aerated.

For every 1-L deionized H_2O add:
1.4 g Instant Ocean Sea Salts
0.03g Seachem Acid Regulator*

Alternatively, for every 1-gallon deionized H_2O add:
5.3 g Instant Ocean Sea Salts
0.11 g Seachem Acid Regulator*

*Adjustments of Seachem Acid Regulator concentration will be necessary to correct for differences in starting pH of dH_2O.

Health Check----------

Embryos	Development of tissue (specifically eye coloration), pigmentation, and movement of circulatory system. Diapause embryos will have no development but should be clean of fungus.
Fry (0-4 weeks post-hatch)	Red belly coloration (from brine shrimp), ability to move, mostly upright on tank ground. Fry at least one week of age should be able to maintain buoyancy.
Juveniles and Adults	Movement of animal, ability to maintain buoyancy, physique of gills, scales, fins, and overall body structure, behavior of animal, any observable abnormalities.

Feeding Procedures----------

Embryos	None
Fry (0-4 weeks post-hatch)	Live brine shrimp; 2-3 times every day. Check to make sure live brine shrimp is always present and available. Brine shrimp does not need to be decapsulated; however, avoid introducing unhatched cysts or shells as they will quickly deteriorate water quality.
Juveniles and Adults	Otohime fish diet pellets C1; twice a day on weekdays, and once a day on weekends. Feeding should be consistent; weekend feedings or fish in breeding tanks should not be fed extra. Give 1/32 tsp for every two fish. For a rough estimate, over feeding is when there is more food than a fish can reasonably eat in two minutes.

Cleaning Procedures----------

Embryos	Check for fungal outbreaks and maintain moist conditions. Please read specific section below for *Egg Care.* Remove any infected eggs.
Fry (0-4 weeks post-hatch)	Clean away dead brine shrimp before each feeding; 2-3 times a day, every day. Conduct a 50-60% water change at least once a day if in a stand-alone system. Remove any dead fry immediately.
Juveniles and Adults	Fecal matter and uneaten food should be siphoned out of tanks once a day. Water quality conditions should be closely monitored, and water changes should be conducted relative to water quality conditions. This will depend on the density of fish. For a rough estimate, partial water changes should be done 1-2X a week.

Breeding, Egg Care, and Hatching----------

Breeding

1. Have a designated breeding tank, at least 6L for a breeding pair.

2. Place a large weigh dish of non-abrasive sand.
    a. Weigh dish: Fisher Scientific (08 732 115)
    b. Sand: Carib Sea Super Natural Moonlight

3. Leave fish overnight and collect eggs an hour after the lights turn on.
    a. The fish will continuously breed throughout the day.
    b. The first weeks' worth of eggs are typically not fertilized or healthy. They should be thrown out.

Egg Collection

4. Remove the dish of sand from tank. Using a fine mesh strainer, sift the eggs from the sand into a bucket using system water. This stage can damage the eggs, so be careful when sifting.
    a. Strainer: OXO Good Grips 6-inch mesh strainer

5. Turn the strainer upside-down over a dish and pour system water over strainer to displace eggs into dish. Alternatively, use a soft ended pipette to remove eggs from strainer.

6. Rinse eggs with normal system water. Then remove clean, undamaged eggs placing them into a secondary container with methylene blue and Ringer's solution.
    a. Methylene blue: Kordon
    b. Ringer's solution tab: Sigma-Aldrich (96724-100TAB)
    c. Solution concentration: For every 1L dH_2O, add 2 Ringer's Tabs and 90 microL of methylene blue.

Egg Care

7. From this container, collect 10-20 eggs and place into a small petri dish containing a fresh aliquot of the Ringer's solution described previously. Store eggs in incubator.
    a. 26^{o}C will give you a 50/50 hatch/diapause rate
    b. increasing temperature to 28^{o}C for a 90% hatch rate
    c. decreasing temperature to 24^{o}C for a 90% diapause rate

8. Eggs should be checked at 24hrs and 48hrs. Unfertilized, damaged, or fungus-infected eggs should be removed and tossed. During each checkpoint eggs should be rinsed with fresh Ringer's solution.

9. After 48hrs, move petri dishes into a sterile work area. Healthy eggs should be moved to a large petri dish. The petri dish should have two layers. A bottom layer with autoclaved coconut fiber that has been moistened with normal system water, until when clumped together it holds its shape. This layer should be packed down. A top layer of filter paper that has been moistened enough to stick to the coconut fiber. This layer allows the eggs to be easily visible, where the coconut fiber allows for moisture control.
    a. Coconut fiber: Zoo Meg Eco Earth Loose Bag
    b. Filter paper: Whatman

10. Eggs should be kept in this manner for the rest of their development. Dishes should be check once every day for fungal growth and moisture content. Eggs with fungal growth should be removed immediately.

Egg Hatching

11. Embryos with fully golden eyes that are orientated facing up should be removed and placed into an aerated dish of 4^{o}C hatching solution, at 26^{o}C. Meaning, the eggs should be placed in cold hatching solution, that is slowly rising to 26^{o}C. Moderate aeration is very important. Make sure fresh hatched brine shrimp is already on hand.
    a. Humic acid: Sigma-Aldrich (53680-50G)
    b. Hatching solution: To 1L of normal system water add 1g humic acid. Place in refrigerator overnight, or until it reaches 4^{o}C.
    c. Adding a bit of sand or coconut fiber to the hatching dish can help prevent the egg chorion from getting stuck on their heads (yes, this is an issue).

12. Embryos can hatch immediately or take up to 48 hours. Embryos that did not hatched can be placed back on the coconut fiber. Alternatively, embryos that are ready to hatch, can be delayed for up to a month. To delay the hatching of ready-to-hatch embryos, ensure that coconut fiber has a relatively lower humidity level.