Hatching *N. furzeri* embryos

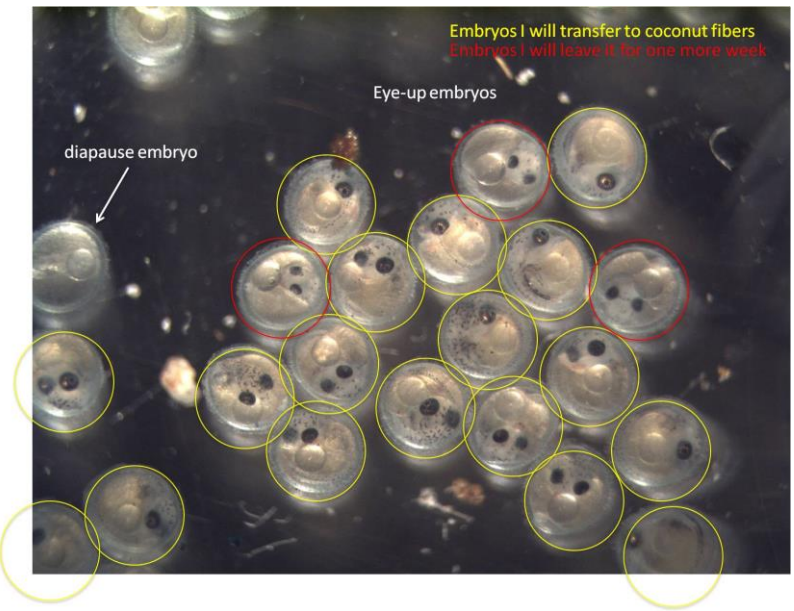
Materials

- Coconut Fiber (ZooMed Item # EE-8, 8 Dry quarts, loose coconut fiber substrate)
- Peat Moss (EB Stone Organics 8 Dry quarts, 8.8L
  http://www.ebstone.org/12_peat.php
- Humic acid (Sigma 53680)
  http://www.sigmaaldrich.com/catalog/product/aldrich/53680?lang=en&region=US
- Oxygen Tablets (Pemble-Halversion, Inc.)
  http://otabs.com/
  http://www.amazon.com/OTABS-Oxygen-tablets-2per-Card/dp/B0007RP7HW

Transfer non- or post-diapause embryos to dry media

N. fur embryos in natural habitat experience wet and dry seasons. Embryos are laid in water during wet season and buried in mud during dry season. Having embryos developing through dry condition could help and synchronize the hatching event in the population.

1. Press prepared coconut fiber into a clean 6 cm dish, filling the dish half full.
2. Transfer non- or post-diapause embryos from wet media (embryo solution) to dry media (coconut fiber plate) by glass pipette. Post-diapause embryos should show pigment in eyes ('eye-up'). One useful routine is selecting only eye-up embryos that are "1-week away" from ready to hatch, to avoid accumulation of dry plates with time.
3. Place the coconut fiber plates at 27 'C



Transfer ready-to-hatch embryos from dry media to hatching media

N.fur embryos hatch in sync with its natural habitat when ponds refilled with water. Factors in mud and water trigger the hatching process.

1. Hatching media can be autoclaved peat moss extract, or 1 g/L humic acid in fish/system water, stored at 4'C.
2. Using the tail of forceps scrape an oxygen tablet ~10 times and transfer the loose powder into the hatching containers (empty tip boxes or plates).
3. Pour cold hatching buffer into the hatching containers.
4. Transfer ready-to-hatch embryos into the hatching containers. Make sure the embryos have been completely submerged in the hatching buffer. Ready-to-hatch embryos should have big golden eyes, with clear and broad white rings in eyes.
5. On the next day, most or some embryos should be hatched already. Fill the hatching containers with fish/system water. Start feeding brine shrimp.
6. On the third day, newly hatched fry are ready to move on to fry system with brine shrimp feedings.

v1.0: Chi-Kuo Hu, 2021/09/01