Collection and storage of *N. furzeri* embryos

Materials

- Weigh Boats - Large Standard (Fisher 08-732-115)
- Lapis Luster Sand (Monterey Bay Brand): silica, not aragonite sand.
- Colanders (OXO SteeL® 6-Inch Strainer)

http://www.bedbathandbeyond.com/store/product/oxo-steel-reg-6-inch-strainer/1011731597

- Transfer Pipets (Fisher #357524)
- 6 cm Dishes (standard item #NC9211252)

Embryo Collection

1. Remove weigh boat with sand from the tank and pour sand through a mesh colander. The mesh size should pass sand but not eggs/embryos.
2. Rest the colander in a small dish with RO water in the bottom
3. Use an end-cut transfer pipette to collect and transfer eggs/embryos to a clean 6cm dish
4. Before move on to the next tank, clean the colander with RO water and be sure that no eggs/embryos are left in the colander. This could be a potential place where strain contamination occurs.

Embryo Storage

1. Remove debris and dead/bad/unfertilized eggs under scopes. Wash with embryo solution or ringer's solution several times. Bad eggs usually show yolk sac deformities. Unfertilized eggs are without inter-vitelline space.
2. Transfer or keep good embryos in embryo solution at 27'C.

Fertilized embryos show clear Inter-vitelline space (yellow arrows)

Reagent Recipe

10X Yamamoto's Ringer Solution or Yamamoto's Embryo Solution
(AKA: 10X Yamamoto's isotonic BSS, Ringer's Solution)

- 7.5 g NaCl
- 0.2 g KCl
- 0.2 g CaCl2
- 0.02 g NaHCO3
- Dissolve in distilled water and bring to a final volume of 100 ml
- Published protocol adjusts pH to 6.7, but many labs find this step unnecessary
- For a 1X working solution add methylene blue (100ul/L)
- Solutions can be stored at room temperature

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