

## **Protocol for egg incubation**

### ***Planning for incubating the spotted wolffish eggs***

All equipment for incubation should be procured before the spawning season approaches.

Here is how to plan for egg incubation:

- Prepare the incubation room, preferably a cool room to maintain a stable low temperature (for example, the cooling room at our faculty is set to 7.0 °C)
- Family hatchers (Figure 1) can be used to incubate the eggs
- Make sure that you have procured the required equipment to care for the eggs during the incubation period:
  - Red light headlamps (and batteries)
  - Disposable gloves
  - Coffee spoons (for water surface cleaning in the incubators)
  - Egg pipettes (6 or 7 mm)
  - Disinfectant (Buffodine) and related equipment (see disinfection protocol)



*Figure 1: Family hatching chamber that can be used to incubate fertilized eggs from a single fish.*

### ***General guidelines for egg incubation***

- Disinfect all equipment before, in between, and after every operation throughout the incubation period
- Salinity of water should be 33-34 ‰ (we employed sea water pumped from 250 meter)
- Should use cold water filtered through 6 µm Faivre drum filters
- Optimum temperature is 5.0 °C, max 6.0 °C (our target was 5.5 °C)

- Preferred waterflow through the incubators is 160 liters/hour (flow rate can be increased, but the eggs should remain stationary)
- Oxygen saturation should be close to 100%
- **The eggs are sensitive to light! Light exposure leads to increased mortality!**  
Incubate in a dark, cold room
- Use red light while you tend the eggs or when you inspect their viability
- If possible, the eggs should be left undisturbed during the first 14-30 days after fertilization
- It should be noted that if you pick dead eggs during this period, you risk disturbing healthy eggs
- After the first period and if the eggs are of good quality, dead eggs should be removed only once in 2 weeks
- Check the eggs at least once a week and carefully remove the surface film from the water
- The eggs should be disinfected with buffodine once a week, until approximately 700 degree days
- Eggs should not be disinfected after 700 degree days to prevent premature hatching
- At approximately 280 degree days, the eggs would have developed visible eyes and would be more capable of tolerating handling stress
- If possible do not disturb the eggs as it can affect the survival rate

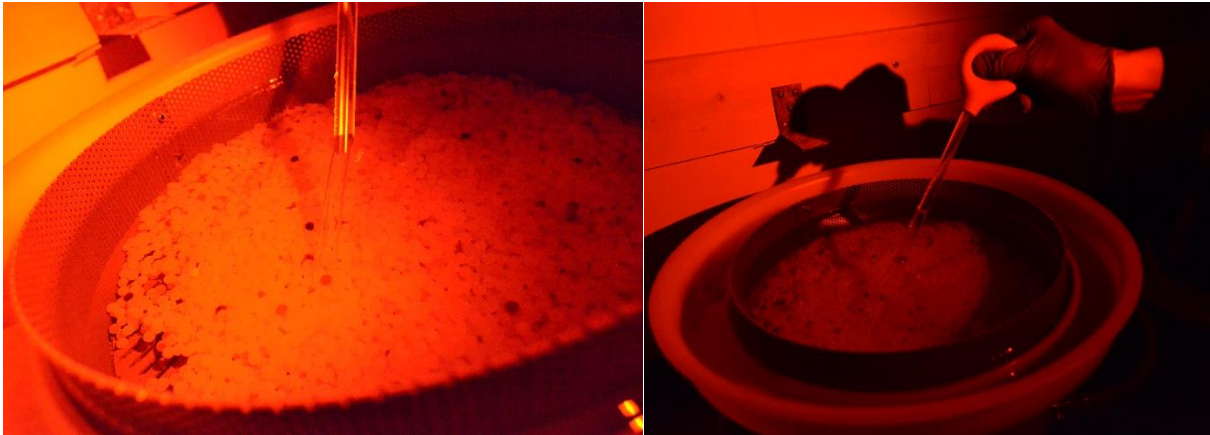
#### ***Transferring the eggs from the fertilization buckets into the family hatches/ incubators***

- Be careful while you transfer the eggs from the fertilization bucket to the incubator
- Transfer the semen-egg mixture carefully and slowly, and the transfer should be done just above the water surface to avoid any drop
- Divide the eggs into batches, with a maximum of 1.5 L of eggs in each incubator
- After all eggs are transferred to the incubator, use either a utensil (no sharp edges), or your hand (use gloves) to distribute the eggs carefully as a thin layer in the incubator
- The eggs must not be disinfected until at least 12 hours post-fertilization

#### ***Identifying and discarding dead eggs***

- Avoid disturbing healthy eggs while you remove the dead eggs
- Use an egg pipette to carefully remove loose dead/contaminated eggs from the incubated egg batch (Figure 2)

- If the dead/contaminated egg is not loose and cannot be removed easily, leave it for later picking in a few days
- Dead eggs should be discarded in an ensilage system



*Figure 2: Removing dead eggs with a wide-diameter pipette.*

### ***Disinfecting the eggs***

See the disinfecting protocol

***Hatching*** - See the hatching protocol

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