

Protocol for egg- and sperm-collection, and fertilization of eggs

Plan beforehand

Preparations for stripping of spotted wolffish has to be done as the spawning season approaches. Following materials are required for the task.

- Anaesthetic - Metacain (MS-222, Tricaine methanesulfonate), remember HSE
- Tags (if not already marked) to identify the fish easily (Figure 1).
- Stripping table (Figure 2, 3)
- Adequate numbers of non-transparent 5-liter buckets (with lid) and disposable pipettes cut in half (see Figure 4).
- Towels



Figure 1: standard ID-tag attached to dorsal fin of the fish.

Monitoring of the female broodstock fish for signs of spawning

Here we describe the careful monitoring that should be done before egg collection. Based on our experience, the average time from the first visual signs of egg build-up in females until the release of eggs is 10-14 days, but sometimes this range can be a bit wider. Most females will release their eggs shortly after the first light stimulus, and therefore broodstock monitoring should start 1-2 hours prior to turning on the lights. A 70 – 80 % batch return could be achieved by adopting such a strategy. Familiarity with each individual female fish and experience in broodstock monitoring are huge advantages because the females will exhibit several small, often subtle signs of spawning as the spawning period approaches.

Here are the spawning signs to look for in females:

- Periods of 10-15 seconds of rapid trembling

- Mating behaviour (lying on her side with her tail underneath and the ovarian opening lifted)
- Other unusual behaviour
- Just prior to ovulation the female will appear inflated (like a balloon).
- During ovulation there will be a transformation from rounded to elongated shape, i.e., as the eggs are released inside the abdominal cavity, causing the lower belly to swell
- The ovarian opening will widen, to a 0.5-1.5 cm round or oval opening

After ovulation, the female will release the eggs into the rearing water within 6-10 hours.

Precautions, careful judgement and collection of eggs

As for the females in captivity, the eggs need to be collected by hand stripping before they are released into the rearing water. It should be noted that internal fertilization occurs in the wild. If the eggs come in contact with water, the micropyle closes within a few seconds, and the eggs cannot be fertilized. However, it is important to wait as long as possible before stripping to allow complete ovulation. If possible, wait until slime (the mucus plug) can be seen seeping out of the ovarian opening, and also the first eggs are on their way out.



Figure 2: A broodstock on a wet towel-covered stripping table. The head of the female fish was covered before stripping.

The precautions and procedures to be adopted to collect eggs:

- Room light, preferably dimmed to the minimum brightness
- Red light headlamps for dark room vision
- Cover the stripping table (Figure 2, 3) with some wet towels

- Place the fish on the towel to avoid skin injury
- A washed, disinfected, clean and completely dry bucket should be kept ready to collect the eggs
- Collect a female from the rearing tank with a net and anaesthetize it employing MS-222 (140 mg/litre SW)
- After anesthetizing, place the fish on the table with the ovarian opening facing the bucket and a wet towel covering the eyes
- The aforementioned disinfected bucket should be dried first and care should be taken not to spill any water into the bucket, prior to or during stripping.
- The stripping process is based on procedures described by Le François and Archer (2007):
 - The fish should be starved during the spawning period to avoid any internal injuries caused by hand stripping
 - Gently massage the eggs out of the ovarian opening and into the bucket by softly pressing the abdominal area
 - The eggs should run out smoothly, and the mucus plug (slime) should “act as a funnel” for the eggs and the ovarian fluid to run down into the bucket (Figure 3)
 - Continue the gentle massage and pressing on the abdominal area until the female has emptied all her eggs
 - The egg-filled buckets (with a lid) should be placed in a refrigerator (+ 4°C) immediately after stripping
 - This precaution is to avoid temperature change while the milt (sperm) is being collected from males for fertilization
 - The eggs should be kept in the dark because they are sensitive to light
 - Carefully return the female into the rearing tank
 - Monitor the fish until it recovers from anaesthesia



Figure 3: Collecting eggs from a female spotted wolffish. The released eggs should flow freely into a clean, dry bucket.

Precautions and collection of sperms from male fish

- Room light, preferably dimmed to the minimum brightness
- Red light headlamps for dark room vision
- Cover the stripping table (Figure 2, 3) with some wet towels
- Place the fish on the towel to avoid skin injury
- Keep a bucket ready to collect the sperm
- Collect a male from the rearing tank with a net and anaesthetize it employing MS-222 (140 mg/litre SW)
- After anesthetizing, place the fish on the edge of the table with the urogenital opening facing you and a wet towel covering the eyes of the fish
- The stripping process is based on the procedures described by Le François and Archer (2007):
 - Massage the abdominal area to release most of the urine and faeces before semen collection
 - Insert a disposable pipette into the urogenital tract (Figure 4). The vacuum created will force the sperm into the pipette
 - Massage the lateral area to press the testis region to retract the semen
 - Semen samples should be stored on ice in a Styrofoam box with lid, to always protect from light and to maintain a constant low temperature

- The motility of the sperm samples that are used for fertilization should be greater than 60%
- Samples that show a yellowish colour (contaminated with urine, feces, urine crystals, blood) should not be used to fertilize the eggs



Figure 4: Collecting sperm from a male spotted wolffish. The cut pipette is attached over the opening of the urogenital tract using the vacuum made by the pipette.

Egg fertilization procedure

- Fertilization should be conducted in a dark room
- Use red-light headlamp for dark room vision
- Wolffish eggs can be stored for some time (<12 hours) before they are fertilized (Le François and Archer, 2007)
- Only egg batches of good quality should be fertilized and incubated
- For fertilization, the ratio of sperm (of sufficient motility, > 60 %) to eggs should be 2 mL:1 L
- The eggs can be fertilized following the procedure described by Le François and Archer (2007):
 - The collected semen should be mixed (in 2 rounds) with the eggs
 - Eggs should be transferred (5-6 times) gently from one bucket to another (two, 5 litre buckets)
 - The eggs and semen should be swirled around each bucket, in both directions

- For good fertilization rate, the eggs are to be swirled, as described above, every 30 minutes for at least 2 hours before incubating them
- Batches of 50 eggs should be incubated separately in SW at ambient temperature in a 100 ml plastic vial with lid so that the fertilization rates can be assessed properly
- For poor-quality semen (>50% motility), the contact time should be more (>4 hours) to obtain the best possible fertilization rate

Incubation of eggs

- See the protocol for egg incubation.

References

(Le Francois and Archer, 2007)

Le Francois, N. R. & Archer, B. 2007. L'élevage du loup tacheté: Reproduction et incubation. *Les publications de la Direction de l'innovation et des technologies*, 7.

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