STANDARD OPERATING PROCEDURE

Lumpfish stripping and egg fertilisation

Centre for Sustainable Aquatic Research
CSAR

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NEED

Parasitic sea-lice are the biggest threat facing sustainable salmon production in the world with an annual cost to industry of £500 million.

Using lumpfish as cleaner fish offers an attractive alternative to the use of chemicals or medicines.

As a new species to aquaculture there is yet a lot to be understood about the artificial culture of lumpfish, including best practice for fertilizing eggs.

NOVELTY

CSAR are the only facility in the UK involved in the collection of lumpfish broodstock to harvest fresh milt and eggs for incubation. Therefore, no SOP exists which can guide the growing industry on best practice for successfully selecting male and female broodstock for a selective breeding programmes.

This SOP guides the user on selecting broodstock, harvesting eggs and milt and best practice for the successful fertilization of eggs.

EQUIPMENT USED

- ☐ Disposable gloves
- ☐ Weighing scales
- ☐ Transport bags
- ☐ Apron(s)
- ☐ Oversleeves
- ☐ Plastic sieve
- ☐ Vial & ethanol
- ☐ Airline
- ☐ Spoon/beaker
- ☐ Scissors/Scalpel & tweezers
- ☐ Plastic bowl
- ☐ Record sheets
- ☐ Cool boxes
- ☐ Blue roll/Paper towel
- ☐ Ice bucket
- ☐ 2-Phenoxyethanol

Please ensure that the following work is carried out in quarantine upon arrival or as soon as females are gravid. Use only material belonging to the Quarantine facility.

Prior to stripping/fertilisation

- Ensure that all equipment listed above are available.
- Wear appropriate PPE (quarantine specific wellies, gloves, oversleeves and apron).
- Ensure that the animals have been passed through a freshwater bath for removing lice.
- Prepare an anesthetic bath. Fill a cool box with 30 litres of system water of a similar temperature to that of the transport water. Add 9 ml of 2-phenoxyethanol (0.3ml/litre) to a beaker of the same water and stir well to ensure it is well mixed. Add this to the cool box and aerate the water.
- Prepare an overdose bath. Fill a cool box with 30 litres of system water of a similar temperature to that of the transport water. Add 27 ml of 2-phenoxyethanol (0.9 ml/litre) to a beaker of the same water and stir well to ensure it is well mixed. Add this to the cool box and aerate the water.
- Prepare a recovery bath with system water in a cool box (appropriate to the size of the animal), for fish that do not produce eggs.

Selection of females

1. See SOP for receiving lumpfish broodstock.
2. During the above SOP monitor the development of the gonadal pore in all females and choose fish with a swollen, often red gonadal pore.
3. Any females that are ready should be stripped at this point whilst still under anesthesia.

4. If not already under anesthesia, place the fish into the pre-prepared anesthetic bath. Check the fish is appropriately anaesthetised by gently tilting it on its side. If it is slow to right itself it is ready to be worked with.
5. Female is to be weighed and length measured before stripping. Record on the data sheet.
6. Dry the gonadal pore with blue roll / paper towel to prevent seawater coming into contact with the eggs.
7. It may be necessary to apply gentle pressure to the gonadal opening. **Do not apply extreme force.**
8. Apply **gentle** pressure along the flanks of the fish starting from underneath the sucker downwards conveying the pressure towards the gonadal opening and allow the eggs to run into the net over the plastic bowl to separate ovarian fluid from the egg mass. If the fish does not immediately produce eggs then it may be necessary to attempt gently breaking the gonadal membrane with a finger. If the fish does not produce eggs after this then place into recovery bath. Once the fish is able to hold its own body position and is clearly pumping water through its gills it can be returned to the appropriate quarantine tank.
9. Weigh the batch of eggs collected and record on the data sheet (if over 1kg the eggs will need to later be split between two incubators).Weigh the ovarian fluid and record on the data sheet. Additionally take a sample of ovarian fluid (approximately 0.5 ml) to be sent to PatoGen for disease screening (*see SOP Sampling for pathogen screening*).
10. The abdomen will be completely empty after stripping and will appear flaccid. Only the skin and a thin layer of muscles will cover the abdominal cavity.
11. Weigh the female after stripping and record on the data sheet.
12. The fish should then be placed in the overdose bath. Leave for at least 10 minutes and when no signs of life remain Schedule 1 and transfer the female to the dry lab for sampling. Once samples have been taken the fish should be placed into a labelled bag and into an appropriate freezer for storage.

Selection of males and Schedule 1

13. *See SOP for Receiving Lumpfish Broodstock and SOP for Lumpfish Milt Extraction and Storage.*

Egg fertilization and incubation – Egg shed

14. Ensure that the water temperature in the Egg Shed (where the incubation will take place) is around 8°C.
15. Whilst in Quarantine, pour a combined minimum of 3 ml of milt from the males into the plastic bowl containing the eggs from one female.
16. Mix the milt and eggs gently, then add ~400 ml seawater and continue mixing for approx. four minutes (or until eggs start to clump together). Ensure the water used is the same temperature as in the Egg Shed.

17. Pour surface water away from the milt and egg mixture to remove dead cells. Add sufficient seawater (again ~400 ml) to rinse the fertilized eggs, repeat this a couple of times.
18. Pour the eggs (up to 1kg) into a transport bag with **14l** of water in it. Using an airline hose bubble air into the egg mass for 5 seconds. Ensure that the blowing of air is sufficient to disperse eggs before they harden (1 short sharp blow)
19. Tie up the bag and leave for up to 20 minutes while the egg mass hardens.
20. Take the egg mass into the Egg Shed. Each incubator can hold approximately 1kg of eggs. If a female has produced more than this quantity of eggs then more than one incubator will need to be used.
21. Flow rate within the incubators should be roughly 20 L min-1 and aeration should be significant but not excessive.
22. Leave to incubate for 20 days. Once eggs have been certified disease free, they should be treated with Buffodine (*see Treating Lumpfish Eggs with Buffodine SOP*) before being moved into RAS B.
23. Record all details in the appropriate daily sheets which are attached to the bench directly underneath the incubation tanks.

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