STANDARD OPERATION PROCEDURE

Lumpfish Milt Extraction and Storage

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 lumpfish are being cultured for deployment there is a need to be able to extract and store lumpfish milt successfully, in order to preserve its viability and ensure maximum larval yield at the fertilization stage.

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 extracting and storing lumpfish milt to be used in egg fertilization.

This SOP guides the user on the best practice methods for extracting and storing lumpfish milt.

EQUIPMENT USED

- Disposable gloves, aprons & oversleeves
- Blue roll
- Tea strainer
- Microscope slides and cover slips
- Lab coat
- Sieve
- Cool boxes
- 2 - phenoxyethanol
- Ice bucket
- Scissors/ scalpel & tweezers
- Weighing scale
- Sterile pipettes and Eppendorfs
- Gonadal grinder
- Innoculation loop
- Nunc flasks
- Aquaboost ® SpermCoat
- 250ml beaker
- Spatula

PROCEDURE

1. Ensure that all equipment listed above are available

2. See 'Prior to stripping/fertilization' section of SOP Lumpfish Stripping and Fertilisation for information on PPE and overdose bath.

3. Mature males will exhibit vibrant colouration (purple, orange, bright red).

4. Males should only be euthanized once it has been established that there is a female that has just been stripped or is ready to strip. This is minimizes time eggs and milt are spent 'in vitro' . A suitable number of males should be selected and killed with an overdose of anaesthetic (0.9 ml 2-phenoxyethanol per liter of water), followed by destruction of the brain.

5. **Ensure this procedure is carried out in the Quarantine plant room and ONLY by a member of staff listed on the Schedule 1 register.**

6. Dry off the fish abdomen with blue roll and place it on a cutting board. Place paper under the fish to avoid seawater/blood on the gonads.

7. Open the abdomen with a scalpel from the gonadal pore up to the sucker and cut out the gonad carefully. Carefully pinch the two gonads and cut them away from the guts. Be careful to avoid contact with seawater as this will activate the sperm. Move the gonads to a clean plastic container, using a separate container for each male.

8. Bring dissected gonads to the laboratory or clean working area. Place the gonads on a clean cutting board and cut away as much of the blood vessels and connecting tissue as possible. If a sample of milt is being taken for PatoGen it should be taken at this point (see SOP – Sampling for pathogen screening).

9. Grind the gonad through a gonadal grinder. Then sieve the milt through a sieve into a beaker, to get rid of debris. If some is being kept back for cryopreservation, place the beaker on ice or in the fridge.

10. Check the milt quality- pipette a drop of activating liquid (sea water) onto a microscope slide and add a loop of milt using an inoculation loop, place a cover slip on top. Examine under light microscope in dry lab and assess for motility. Scoring should be carried out by the same person. Ensure that milt quality and total volume of milt is recorded.

2	Majority of sperms that are active and 'fizzy'
1.5	Majority of sperms that are active but not showing the 'fizziness' of a grade 2 sample
1	Only a proportion of sperms are active
0	No sperm activity

A motility activity level of 1 or less would be considered to be inadequate for use as viable milt

11. Mix the milt 1:1 with a physiological solution (AquaBoost® SpermCoat from Cryogenetics). This dilution allows the sperm cells better access to oxygen during storage.

12. Transfer a 6ml of the diluted (1:1) milt to 25 cm2 cell culture flasks (Nunc flasks). It is very important that the milt form a thin layer to ensure that all sperm cells have access to oxygen during storage.

13. Mark the flasks with the volume of milt (ml) added, sperm density and date, and place them in a fridge (3-3.5°C). Place the flasks flat to get as large bottom surface as possible and move the flasks gently 2-3 times daily to ensure that all sperm cells get access to oxygen.

14. Use 3ml per batch of eggs, to continue see 'Egg Fertilisation and Incubation' section of SOP 3 – Lumpfish Stripping and Egg Fertilization.

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