

STANDARD OPERATION PROCEDURE

Sampling for pathogen screening in
the lumpfish (*Cyclopterus lumpus*)





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Sampling for pathogen screening in the lumpfish (*Cyclopterus lumpus*) – Standard Operation Procedures 1, version1, Centre for Sustainable Aquatic Research, 6 pages.

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 with Atlantic salmon there is a risk of disease spread between both species.

Therefore there is a need to screen lumpfish broodstock for known pathogens to ensure disease free offspring.

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 pre-screening male broodstock and post-screening of females to ensure only disease free offspring are sent for deployment.

This SOP guides the user on the best practice methods for pre-screening male lumpfish and the post-screening of female lumpfish.

EQUIPMENT USED

- Disposable gloves, aprons & oversleeves
- 2-Phenoxyethanol & beaker
- 2 ml vials with ethanol & storage boxes
- 70% Ethanol
- Lab coat
- PatoGen sampling kit
- PCR sample record sheet
- Record sheets
- Cool box
- Endoscope and equipment for sampling
- Overshoes
- Eppendorfs
- Dissection kit
- Formalin

Sampling for pathogen screening process

Milt and ovarian fluid samples can be obtained from males and females during stripping of gametes, see *SOP – Lumpfish stripping and egg fertilisation*. Tissue samples need to be obtained once female fish are euthanised. Tissue samples from male fish can be obtained using endoscopy methods.

Milt and ovarian fluid samples for PatoGen

1. Using a sterile pipette, obtain a sample (approximately 0.5ml) of milt immediately after stripping from a male. Place in a labelled 1.0ml sample vial and store at <6.0°C.
2. Using a sterile pipette, obtain a sample (approximately 0.5ml) of ovarian fluid from females immediately after stripping. Place in a labelled sterile 1.0ml sample vial and store at <6.0°C. These samples have a limited lifespan and maintained at these conditions will need to be analysed within 5-7 days.
3. The female must be Schedule 1 euthanised after releasing eggs in order to obtain tissue samples.

Tissue samples for PatoGen

4. Dissect the euthanised individual and remove a 2mm² sample from the head kidney. Ensure the kidney is cleaned and dried beforehand to ensure there is no risk of contamination from other visceral fluids. Place sample in a labelled 1.0ml sample vial containing RNA later^{TM} .
5. Ensure apparatus is cleaned thoroughly before sampling another individual. This can be done by using bleach, a flame or commercially available DNA degrading applications.
6. Store samples in fridge at <6.0°C until they are ready to be sent for analysis.

Tissue sampling from live individuals using the endoscope

This procedure can only be performed by the Veterinary Surgeon

This procedure is currently performed on male fish that are to be maintained in the Quarantine facility for the future fertilisation of egg batches.

7. Anaesthetise the fish (See *SOP – Lumpfish stripping and egg fertilisation*).
8. The individual is placed on a fish surgical bench and sedation is maintained using an anaesthetic bath and a recirculating pump.
9. An incision is made in the side of the fish and the endoscope inserted into the cavity.
10. Once the head kidney and the gonads are located, biopsies are taken.
11. As described above these samples are stored in RNA^{later}TM until they are sent off for analysis.
12. Once the operation is complete the individual is allowed to recover from the anaesthetic and placed on a course of antibiotics.

Sending samples off for analysis

13. Place labelled samples (liquid and tissue) in one sealed bag for each individual and then place in a padded envelope.
14. Add an ice pack and the documentation containing information about each sample (PIT tag, sex etc).
15. Ensure samples are sent at the beginning of the week to ensure they are received before the weekend.

Biosecurity measures

Strict biosecurity measures must be followed when dealing with new arrivals. This needs to be maintained whilst they remain in the Quarantine facility and even if the results reveal that they are free from any notifiable diseases these measures should remain in place.

Please see the *SOP - Quarantine biosecurity plan for receiving wild lumpfish (Cyclopterus lumpus) broodstock* for more information.

This document was supported by SMARTAQUA: aquaculture beyond food.

SMARTAQUA is supported by the Welsh Government and the European Regional Development Fund

