STANDARD OPERATION PROCEDURE

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

Centre for Sustainable Aquatic Research

CSAR

Leigh Biddiscombe, Rob Smith, Josella Hunt, Rebecca Stringwell, Paul Howes, 26/02/2020

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-screeninig 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 $2mm^2$ 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*™.
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*™ 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

STANDARD OPERATION PROCEDURE

Reception and Traceability of Lumpfish Broodstock

Centre for Sustainable Aquatic Research
CSAR

Authors, Josella Hunt, Robert Smith, Rebecca Stringwell

Reception and Traceability of Lumpfish Broodstock– Standard Operation Procedures 7, Version 4, 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 it is important that robust quarantine procedures are in place. Additionally it is important that broodstock can be traced throughout stripping and egg production, and that the parents of resultant larvae are known.

Therefore there is a need to receive lumpfish into a quarantine facility, and ensure that broodstock are traceable throughout the season.

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 receiving lumpfish broodstock into a facililty and tracing individual broodstock throughout a season.

This SOP guides the user on the best practice methods for receiving lumpfish into a quarantine facility, and ensuring traceability broodstock during the season.

EQUIPMENT USED

- ☐ Disposable gloves, aprons & oversleeves
- ☐ 2-Phenoxyethanol & beaker
- ☐ PIT tags, implanter and reader
- ☐ Sharp scissors and tweezers
- ☐ 2 ml vials with ethanol & storage boxes
- ☐ 70% Ethanol
- ☐ Lab coat
- ☐ Record sheets
- ☐ Measuring board
- ☐ Cool boxes
- ☐ Weighing scale
- ☐ Overshoes

PROCEDURE

Please ensure that all the following work is carried out in quarantine upon arrival and before fish are stripped or transferred to the tanks.

Prior to arrival

- Ensure that all equipment listed above is available.
- Ensure a freshwater bath is prepared for removing lice from new arrivals. The temperature of this bath needs to match that of the transport water.
- Prepare an anaesthetic bath. Fill a cool box (or suitably sized container) with 30 litres of system water of a similar temperature to that of the transport water. Add 9ml 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 a recovery bath. Fill a cool box (or suitably sized container) with 30 litres of system water of a similar temperature to that of the transport water.

Arrival

1. Check temperature of transport water is within 2°C above or below that of the Quarantine room to avoid thermal shock.
2. Place one fish at a time into the freshwater bath and leave for two minutes. Gently brush lice off the fish with a gloved hand. Lice may need to be physically picked off with forceps.
3. Transfer the fish to the anaesthetic bath and monitor throughout.
4. 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 measured.

Measuring and sampling for traceability

5. Lift fish from the anaesthetic bath and allow excess water to run off the fish.
6. Place on measuring board on a tared balance and record total length (to the tip of the tail (mm), weight (g) and sex (based on external characters).
7. Select a PIT tag and check it works with the scanner.

8. Insert the PIT tag into the individual in the marked X on the diagram on the left flank. **Please note that the applicator needle should be inserted at an angle of ~25 degrees.**
9. Check the tag has been secured inside and that the tag can be identified by the reader. The tag number should be noted and recorded on the record sheet.
10. Half fill a screw-cap 2ml vial with ethanol and ensure it is labelled with the **correct tag number (use self-adhesive barcode label that comes with PIT tag)**.
11. A fin clip (with sharp scissors) should be removed from the top end of the caudal fin (marked on the diagram \). The clip should be 2 – 3 mm wide and 5 -10 mm long, cutting across 1 fin ray and surrounding material.
12. If the caudal fin is not present, a fin clip can be taken from the dorsal or anal fin; ensure to note where the fin clip has been taken on the record sheet.
13. Place fin clip in the tube containing ethanol and place in the storage box.
14. If females are gravid they should be stripped of eggs at this point, **see SOP – Lumpfish stripping and fertilisation**. If not stripped proceed to step 14.
15. Place fish in a darkened recovery tank for 10 – 15 minutes to recover.
16. Once the fish is able to hold its own body position and is clearly pumping water through its gills it can be stocked into the appropriate quarantine tank (Q1 – Q5) and record the destination tank number to complete the record sheet.

    NOTE: any fish that are already present in Quarantine and have tested negative for any notifiable diseases should be kept separate from any new unscreened arrivals.

17. All fin clip samples should be stored in the designated lumpfish freezer.

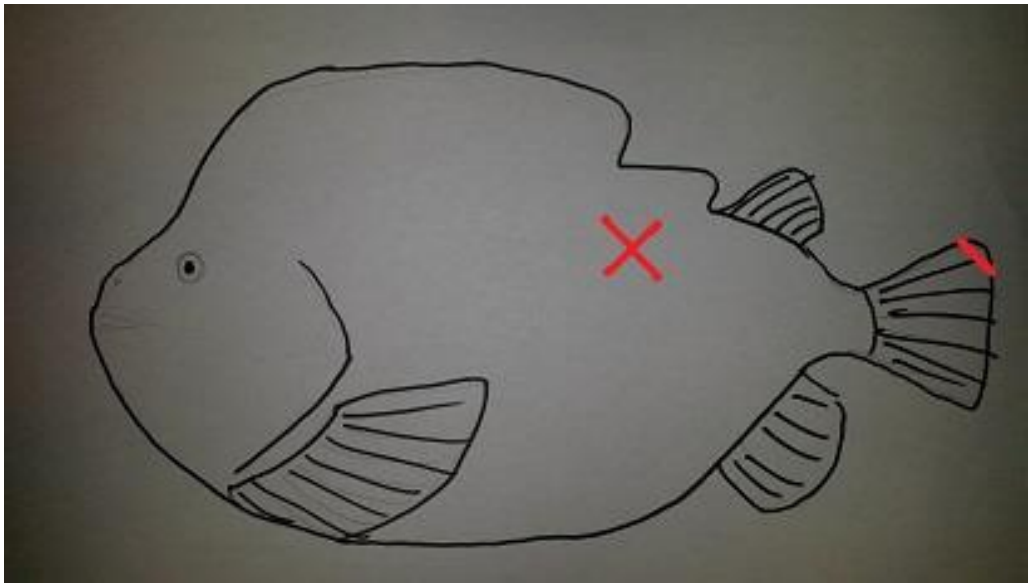

Diagram showing positions for inserting the tag and taking a fin clip.

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 – Lumpfish Broodstock Biosecurity**

This document was supported by SMARTAQUA: aquaculture beyond food.

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

STANDARD OPERATING PROCEDURE

Lumpfish stripping and egg fertilisation

Centre for Sustainable Aquatic Research
CSAR

Craig Pooley, Robert Smith, Josella Hunt, Leigh Biddiscombe, Rebecca Stringwell, Paul Howes 26/02/2019

Lumpfish stripping and egg fertilisation– Standard Operating Procedures 3, Version 3, 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 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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STANDARD OPERATION PROCEDURE

Lumpfish Milt Extraction and Storage

Centre for Sustainable Aquatic Research
CSAR

Authors, Craig Pooley, Josella Hunt, Robert Smith, Rebecca Stringwell

Lumpfish Milt Extraction and Storage – Standard Operation Procedures 8, Version 2, 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 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.

This document was supported by SMARTAQUA: aquaculture beyond food.

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STANDARD OPERATION PROCEDURE

Treatment of Lumpfish Eggs with Buffodine®

Centre for Sustainable Aquatic Research
CSAR

Authors, Josella Hunt, Robert Smith, Rebecca Stringwell

Treatment of Lumpfish Eggs with Buffodine® – Standard Operation Procedures 9, Version 1, Centre for Sustainable Aquatic Research, 5 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 within a recirculating system at CSAR it is important to prevent foreign mirco- and macroscopic organisms from entering the system. It is also important to prevent vertical transmission of disease from wild broodstock to the egg mass.

Therefore once the broodstock are certified disease free, there is a need to treat lumpfish eggs with Buffodine® to ensure the surface of the eggs are disinfected before moving them into the main CSAR building.

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 disinfecting and transferring eggs which have been in contact with wild broodstock parents into a main ongrowing facility.

This SOP guides the user on the best practice methods treating lumpfish eggs with Buffodine® and transferring them into RAS B.

EQUIPMENT USED

- Disposable gloves, aprons & oversleeves
- Buffodine®
- 10 L bucket
- Filter baskets
- Measuring beaker
- Safety goggles
- Mesh
- Elastic bands
- Nets / sieves

PROCEDURE

Eggs should be treated with Buffodine® after the results for disease screening have come back clear, before moving them over to RAS B.

Treating with Buffodine®

1. Fill a bucket with 10 litres of system water from the egg shed
2. Add Buffodine® to the water. Dilution is 1:100 parts water (100 ml Buffodine® per 10 litres of water).
3. Place two filter baskets containing mesh into the bucket
4. Carefully lift a batch of eggs from their hopper and place into one of the filter baskets. Gather up any loose eggs from the same batch with a net or sieve and also place into the respective filter basket. It should be possible to treat two batches at the same time unless the batch is very large, then it should be split.
5. Leave the eggs in the Buffodine solution for 10 minutes.
6. Rinse eggs well (at least four or five times) with system water from RAS B and transfer into the appropriate RAS B egg hopper. Move egg record sheet over with eggs.

This document was supported by SMARTAQUA: aquaculture beyond food.

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

STANDARD OPERATION PROCEDURE

Lumpfish weaning

Centre for Sustainable Aquatic Research
CSAR

Authors, Rob Smith

Lumpfish weaning – Standard Operation Procedures 22, version 1, Centre for Sustainable Aquatic Research, 4 pages.

NEED

Lumpfish larvae are offered live *Artemia* for the first 8 weeks post hatch.

However, the nutritional requirements of larvae cannot be feasibly sustained through live feeding alone. To maintain the provision of enough sustenance it is essential that larvae are weaned on to formulated feed.

NOVELTY

CSAR undertakes a prolonged period of weaning compared with examples from industry. Feeding live and formulated feeds in tandem for 6-7 weeks before reducing live feeding.

It is proposed that this decreases mortality over the weaning period.

EQUIPMENT USED

- Artemia culture equipment (see SOP 21)
- Record sheets
- Belt feeders
- Marine Larvae formulated diet:
  - 75um - 250um (Otohime 'A')
  - 250um – 360um (Otoheime 'B1')
  - 360um – 650um (Otoheime 'B2')
- 50ml beakers

Weaning

- Artemia are immediately offered after larval introduction to hatchery tanks (1-3 days post hatch (dph)) (For artemia culture and feeding procedure refer to SOP 21).
- Otohime grade A can also be offered at this time.
- From 7 dph a small amount of 'A/B1' should be offered to each tank, roughly **5-10**% of the estimated biomass per day.
- The feeding should be done by hand and ideally on an hourly basis over the working day.
- Alternatively dry feed can be offered between live feeds eg: 1st AM (before live feed prep), Lunch time (after live feed 1), Last PM (after live feed 2).
- Feed can be mixed with 'B2' after 14dph, phasing out B1 in the following weeks
- At around 56 dph feed can be dispensed using a belt feeder with the days feed evenly spread along the belt.
- Feed at **5**% 'B2'. This can be mixed with a larger grade (e.g Otoheime C1) if required.
- At the same time (56 dph) artemia should be withdrawn, this should be done by reducing the amount introduced to the tanks during each feed by 10-20% per day over the following week.
- It is highly likely that mortalities will significantly increase for a couple of weeks following this period but should soon settle.

This document was supported by SMARTAQUA: aquaculture beyond food. *SMARTAQUA is supported by the Welsh Government and the European Regional Development Fund*

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STANDARD OPERATION PROCEDURE

Lumpfish larvae transport

Centre for Sustainable Aquatic Research
CSAR

Craig Pooley, Robert Smith, Josella Hunt, Rebecca Stringwell, Paul Howes 26/02/2019

Lumpfish larvae transport– Standard Operating Procedures 2, version2, Centre for Sustainable Aquatic Research, 5 pages.

NEED

The safe transport of lumpfish larvae is a legal requirement in the UK.

Anyone involved with the transport of live fish must be aware of their obligations to protect animals during transport (See the Aquatic Animal Health (England & Wales) Regulations 2009).

NOVELTY

As lumpfish stick to surfaces, transporting larvae in a transport container can cause more harm to the fish due to difficulties of removing fish on arrival.

Therefore, CSAR has developed a SOP for the safe transport of lumpfish larvae within bags and insulated boxes.

EQUIPMENT USED

- ☐ Oxygen bottles
- ☐ Plastic bags
- ☐ Elastic bands
- ☐ Insulated polystyrene boxes
- ☐ Cardboard boxes
- ☐
- ☐
- ☐ Tally counter
- ☐ 2L jugs
- ☐ Soft brush
- ☐ Transport documents
- ☐ Ice packs
- ☐
- ☐

Transport trial

1. Ensure that numbers of larvae to be transported per bag have been pre-agreed with the receiving party.
2. Carry out a transport trial (by monitoring oxygen levels) to ensure that the density of larvae to be stocked in each bag can survive the transport time between CSAR and the receiving facility. Should DO levels drop below 5mg/l stop the trial and reassess densities.

Preparation

1. Prepare the polystyrene boxes with fish transport bags (doubled). Wrap the edges of the plastic bags over the box and leave the lids off.
2. Fill up to 10 of the fish transport boxes with 14 L of system water (avoid filling more than 10 to prevent the water heating up).
3. Start to fish/siphon the larvae out of the tanks into a 2L jug/mesh tub.

Counting

4. Count desired number of larvae to be transported per bag into a 2L jug of system water.
5. Use this sample as a reference for density of fish per transport bag.
6. Alternatively, older fish can be counted and weighed, and then the weight used as a reference for each transport bag.

Bagging

7. Add desired number of larvae to each transport bag.
8. Use the soft brush to gently remove lumpfish from the side of the jug.
9. Top up the transport bags with the remaining volume of water required for transport.
10. Wrap the excess of the plastic bag twisting it for two turns.

11. Add oxygen directly into the water to a maximum of 120%
12. Add oxygen to the bags [1/3rd H2O: 2/3rds O2] (Ensure to keep hand tight around the neck of the bag and the oxygen hose while doing this).
13. Remove the oxygen hose and then twist and tighten the bag until you are able to wrap it with the elastic bands.
14. If transport vehicle has no means of keeping the boxes cool, wrap ice packs in paper towel and place on top of the bags.
15. Close the box with the polystyrene lid
16. Close the cardboard box lid.
17. Tape the lid shut
18. Provide transport documents to driver.

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STANDARD OPERATION PROCEDURE

PIT Tagging of Lumpfish

Authors, Robert Smith, Josella Hunt, Rebecca Stringwell

PIT Tagging of Lumpfish – Standard Operation Procedures 10, Version 2, 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.

For certain studies involving the lumpfish and for broodstrock tracing it is important to be able to identify individual fish over a period of time.

Therefore there is a need to PIT tag lumpfish on occasion in order to be able to repeatedly identify individuals.

NOVELTY

CSAR are the only facility in the UK involved in the collection of lumpfish broodstock to harvest fresh milt and eggs for incubation. This specialisation results in the need for disease screening, which makes the identification of individual fish essential. In addition CSAR is focusing on Scottish fish for Scottish salmon farms. This unique focus lends itself to study, in which the identification of individuals may be of particular importance.

This SOP guides the user on the best practice methods for PIT tagging lumpfish.

EQUIPMENT USED

- Disposable gloves
- 2-Phenoxyethanol & beaker
- PIT tags, implanters and reader
- Cool boxes
- Measuring board
- Weighing scale
- Record sheets

PROCEDURE

Please ensure that all the following work is carried out in RAS B.

Prior to tagging

- Ensure that all equipment listed above are available.
- Prepare an anaesthetic bath. Fill a cool box (or suitably sized container) with 30 litres of system water. Add 9ml 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 a recovery bath. Fill a cool box (or suitably sized container) with 30 litres of system water.

Anaesthetic

1. Transfer the fish to the anaesthetic bath and monitor throughout.
2. 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 measured.

Measuring and sampling

3. Gently remove excess water from fish (with damp cloth or paper roll),
4. Place on measuring board on tared balance and record total length (to the tip of the tail (mm), weight (g) and sex (based on external characters).
5. Select a PIT tag and check it works with the scanner.
6. Insert the PIT tag into the individual in the marked X on the diagram on the left flank. **Please note that the applicator needle should be inserted at an angle of ~25 degrees.**
7. Check the tag has been secured inside and that the tag can be read by the reader. The tag number should be noted and recorded on the record sheet.

Recovery

8. Place fish in a darkened recovery tank for 10 – 15 minutes to recover.
9. Once the fish is able to hold its own body position and is clearly pumping water through its gills it can be stocked into the appropriate tank and record the destination tank number to complete the record sheet

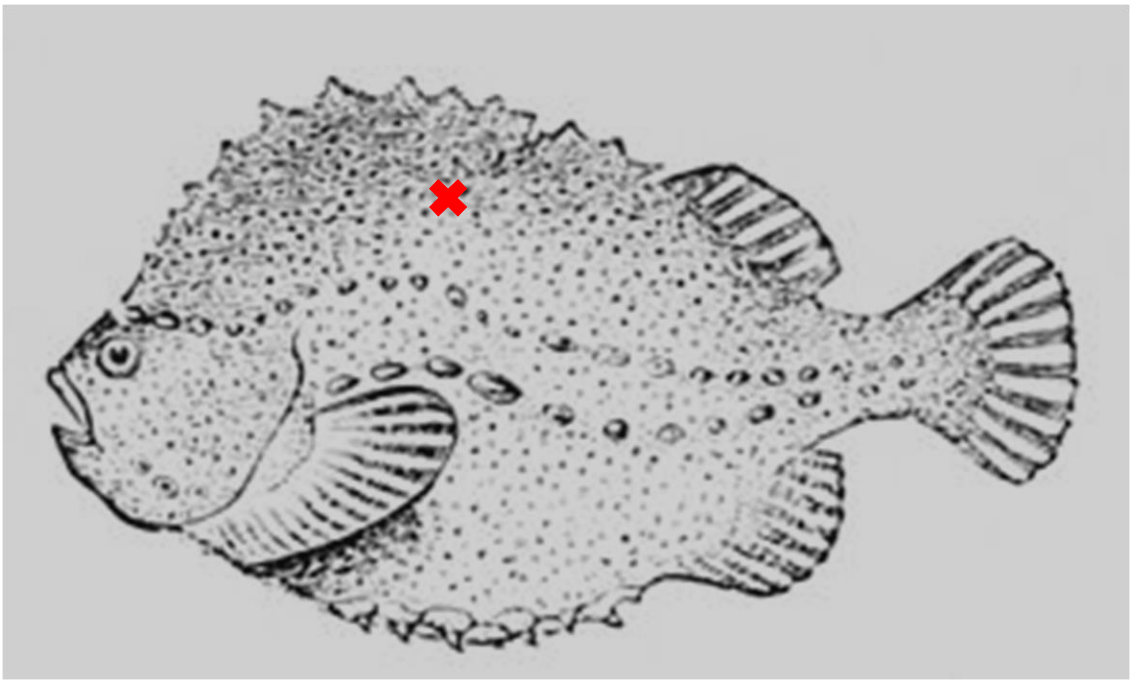

Diagram showing position for inserting the tag.

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STANDARD OPERATION PROCEDURE

Lumpfish Vaccination

Centre for Sustainable Aquatic Research
CSAR

Authors, Chloe Davies

Lumpfish Vaccination – Standard Operation Procedures, Version 2, 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. There is therefore a need to ensure that any lumpfish deployed to salmon cages are disease free.

Vaccinating lumpfish prior to deployment ensures that they are immune to several diseases that could otherwise be contracted in the cages and passed between the two species.

NOVELTY

Lumpfish vaccinations have previously been carried out using vaccination baths, which require a large amount of vaccine and only provide a low level of protection and duration of immunity. However, the vaccination delivery method for lumpfish reared at CSAR has now changed to intraperitoneal vaccination injection by hand.

This method is highly efficient in generating both humoral (antibody) and cellular cytotoxic response and requires a much smaller dose of the vaccine.

This SOP guides the user on the best practice method for vaccinating lumpfish.

EQUIPMENT USED

- Disposable gloves
- 2 x 500 ml vaccine bottle with tubing
- Vaccination table
- 2 submersible pumps and piping
- Waterproof box
- Extension lead
- Oxygen & airlines
- Dissolved oxygen meter
- 100/150mm flexible ducting
- 1 x 400L white sump
- 1 x 100L white sump
- 2 x orange baskets
- 2 x metal/plastic pole (1m min length)
- Large and small nets
- Headtorches
- Weighing Scales
- System water

PROCEDURE

1.0 Preparation

1.1 Ensure that all tanks to be vaccinated have an average weight of over 10 g and have been starved for 24 hrs.
1.2 Ensure that there are enough empty tanks to transfer the fish and make a plan of which tank(s) the vaccinated fish will be transferred into.
1.3 Set up the vaccination table, ensuring that the pumps and piping are connected correctly and securely, and that the electricals are safely contained in a waterproof box.
1.4 Position the 100 L sump next to the inflow end of the table and the 400L sump next to the outflow end of the table.
1.5 Place one submersible pump into each of the sumps, fill the tubs with system water, and turn the pumps on to ensure that the table is running correctly.
1.6 Ensure the oxygen bottle and blue oxygen tubing are ready for use.
1.7 Connect two airstones to the blue oxygen tubing, place one in the 400 L white sump (keep oxygen off until it is required).
1.8 Connect another airstone to the main air supply and place into the sump to aerate the water.
1.9 Fill 2 10 L buckets with system water and place either side of the vaccination table ready for any fish that are rejected on the table.

2.0 Vaccination

2.1 Ensure that the vaccine bottle has been brought up to room temperature
2.2 Provide vaccine to vaccinators and set up vaccine table as they prepare the vaccine
2.3 Place one orange basket into the 400 L sump, balancing it over the edges with the pole (slotted through the handles on either side) to ensure that it is not entirely submerged
2.4 Ensure that the fish shoots are flowing into the basket
2.5 Once vaccinators are ready, begin netting fish onto the vaccine table

2.6 Ensure that the vaccination table is not over-loaded at any one time (based on judgement -always consider fish welfare).
2.7 Keep an eye on the number of fish on the table and continue to add nets of fish as and when needed.
2.8 Once a basket is full of fish (roughly 2 nets – do not overfill) lift the basket from the vaccination sump and move fish into their new tank. Replace full basket with empty basket.
2.9 Continue this process until the tank is empty.
2.10 Ensure that the vaccination table (including both sumps) is thoroughly searched for any fish that may have been missed/escaped.
2.11 Ensure that the vaccinators provide the number of vaccinated fish once the tank is empty.
2.12 The water in the sumps should be replaced after every 2 tanks that are vaccinated.
2.13 Once all tanks are complete, the table should be disassembled and all equipment hosed down.

Considerations:

2.14 Keep an eye on the flow rate for the table inflow – ensure that the fish are fully submerged while on the table, but the water should not overflow. If the table does overflow, reduce the flow rate using the valve on the submersible pump in the 100 L sump. Ensure that this sump is kept topped up throughout the vaccination process.
2.15 Regularly check the oxygen level in the vaccination sump, use oxygen from cylinder to boost oxygen level as and when needed (when O_2 conc. Drops below 80%).
2.16 Regularly check the floor for any fish that may have fallen off the table.
2.17 Keep an eye on the number of fish in the 'reject' buckets – if they become crowded, it may be necessary to empty them before the tank is complete. Ensure that you note the number of fish that have been returned to a non-vaccinated tank, and note the tank number.
2.18 Keep an eye on the level of the RAS B sump – particularly when tanks are being drained/filled.
2.19 Fish may be rejected at the table due to deformities. Any fish that have been rejected should be checked for deformities and euthanized if necessary.

3.0 Post-vaccination monitoring

3.1 Following vaccination, there may be mortalities due to stress/injury. Tanks should therefore be checked daily for mortalities
3.2 If mortalities are found, they should be removed from the tank, counted, and the number recorded on the tank sheet. Mortalities due to vaccinations mostly occur within 72 hrs.

PHOTOS OF TABLE SET UP?

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