Advances in Lumpfish Broodstock Management

Akvaplan niva

BEST BROOD
Improving Fish Reproduction

1.- Introduction

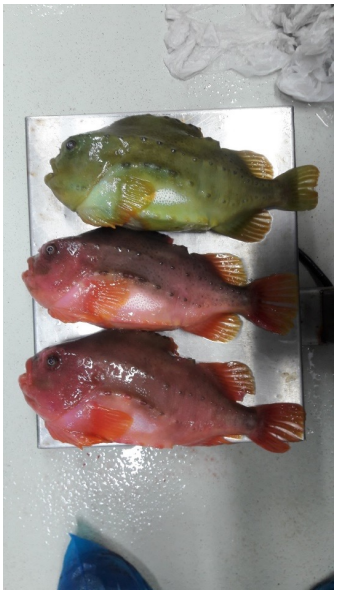

Figure 1. Mature male lumpfish

Lumpfish is Norway's 3rd most valuable farmed species, after salmon and trout. In 2018, 32 million individuals were farmed, at a value of 60 million Euros. Lumpfish is produced solely for the purpose of eating lice of salmon in sea-cages and have little value otherwise as it is not a common species in any market. Commercial production of lumpfish is still a very new industry, but has increased tremendously in salmon-producing countries, and especially in Norway where costs associated with salmon lice treatments amounted to more than 500 mill. Euros in 2018. Today, between 25-30 companies actively produce lumpfish (Directorate of Fisheries, 2019), but around 80-90 % of the eggs delivered to the industry comes from only two egg producers, and close to 100% of all eggs produced stem from wild-caught fish. For this reason, little effort has been put into broodstock optimization. Several projects have been, and are, determined to increase the sustainability of lumpfish as an aquaculture species to ensure that the farmed fish that are put into the sea-cages are robust and healthy. A vital part of such a work includes optimizing the processes in the hatcheries.

The BestBrood project is an international consortium funded by the ERA-NET Cofund BlueBio initiative. Significant achievements in sperm conservation methods, specifically in the areas of chilling and cryopreservation, and the use of antioxidants to preserve sperm quality have been done. These advancements, combined with the upscaling of cryopreservation methods, is a step forward for the industry to produce larvae and manage sperm banks. This guide will present the advances in broodstock management, sperm collection, evaluation and cryopreservation.

Reference

Directorate of Fisheries (2019). Cleanerfish statistics. https://www.fiskeridir.no/Akvakultur (In Norwegian).

2. Housing and handling procedures

Common practice for culture purposes is to collect fish from the wild that display signs of maturation and gonad build-up and keep them until ready to be stripped.

- **Transport.** Wild-caught fish are transported in on-board tanks with running water and carefully netted into transport tanks for a quality inspection. Fish that are immature or have already spawned are released at sea, and fish with wounds or deformities are slaughtered according to standard procedures.

- **Photoperiod.** The fish are held at a natural photoperiod (LDN) for the latidude/longitude in question.

- **Stocking density**. Should be moderate (2-5 kg m^{-3}) in circular or rectangular tanks withat least 1m height and simple hiding/resting spots.

- **Inspections**. Several daily inspections to observe near-spawning behaviour. Main cues for ready-to-be-stripped fish are:
  - Courtship towards males
  - Swelling of genital opening in females

  Females are pushy, courting males when ready to spawn. If this behaviour is observed a dip-net can be used to turn the fish upside down to observe the genital opening. If swollen, the female may be removed from the tank and stripped manually.

Figure 2. Mature male (top) and female (bottom) lumpfish. Photo: L.O. Sparboe

3. Sperm collection

Figure 3. Sieving and filtering lumpfish milt + extender into a vial for short-term storage.

3.1 General considerations

Testis Anatomy

The male gonads are normally white when fully mature, but milt with pink/red colour has also been observed, particularly in individuals displaying a strong external colour pattern. In farmed fish, such a milt colour is typically observed in fish fed broodstock feeds rich in astaxanthin. Lumpfish testis belongs to the unrestricted type, with spermatogonia distributed throughout the seminiferous lobules. Following spermatogenesis, spermatozoa are collected and stored in the efferent duct system.

Contamination

The males are killed by an overdose of anaesthetics before opening the abdomen. Gonads are thereafter removed. During the surgical removal of the gonads care should be taken not to puncture the gonads or any other organs. The gonads are placed in a sieve and rinsed with distilled water to remove blood and mucus and thereafter placed on a paper towel to absorb the distilled water.

Collecting fresh sperm

1. Clean gonads are ground, and the liquid is collected in a beaker. Add an identical amount of extender as the amount produced from grounding. Extender may be added through the grinder to collect more sperm.
2. Place a filtered cloth on top of a second beaker and use a fine-masked sieve to filter the sperm and extender into the second beaker. Stir gently in the sieve to speed the filtration. Save the liquid fraction.
3. Pour the liquid fraction into a Nunc tube. Mark tube with date, fish no. and volume. Place tube on a stirrer for constant oxygen supply awaiting PCR-analysis. The milt is thereafter stored at 3-4°C and stirred regularly.

4. Sperm storage

From experimental testing on fresh sperm, it is expected a fertilization rate >90% up to 14 days after preparation of the milt (pers. comm. Thor Arne Hangstad, Akvaplan-niva, Norway). During testing, eggs from different females were fertilized with cold-stored milt every second day. Only eggs from three out of 14 females had less than 90% fertilization, and there was no correlation between storing time and fertilization. Below we precent procedures for leong-term storage of lumpfish sperm.

Procedure

1

Prepare extender as described by Kime and Tveiten (2002). Add all salts (first 4 ingredients) to 1 litre of distilled fresh water, swirl to dissolve and filter through 0.22um filter paper via vacuum unit into a sterile flask.

Add glucose and BSA on day of use and swirl to dissolve. Prepare sperm with 1:1 extender:sperm ratio.

Chemical	Conc.	Weight (g) per L
NaCl	154 mM	9 g
$CaCl_2$	4.55 mM	0.669 g
$MgSO_4$	2.37 mM	0.58 g
$KHCO_3$	4.83 mM	0.484 g
Glucose	1.0 mM	0.180 g
BSA		1.0 g

Recipe for extender (Kime and Tveiten, 2002).

2

Freezing method:

Prepare 2.5 ml straws with stainless steel balls for plugs.

Prepare 2.5 cm floating rack on liquid nitrogen (LN).

Add 10% DMSO to final sperm:extender volume (keep above 19 °C), gently mix and start timer.

Load straws within 10 minutes and place on floating rack.

Leave for 10 minutes on floating rack, then tip directly into LN.

3

Thawing method:

Prepare a 5°C water bath.

Drop in straws for 1 minute, then cut and use. Beware of exploding straws-use a full-face mask.

Post thaw, sperm can be stored for up to 4 hours at 4 °C without significant loss of quality.

Reference

Kime, D.E. and Tveiten, H. (2002) Unusual motility characteristics of sperm of spotted wolffish. J. Fish. Biol. 61, 1549-1559.

5. Stripping and fertilization

During stripping, a registration sheet should be available, and the following data should be recorded: Date and time of stripping, running number of females, length and weight, volume of eggs, the Incubator number, sperm number, initials of the cutter and fertilizer, barcode number for kidney/liver/ovarian fluid samples, and any other comments that may be of relevance i.e pit tag code if relevant. Always use gloves and an apron.

Stripping

1. Dry the underside of the female. Insert a finger to break the membrane surrounding the eggs. Gently squeeze the belly to strip all eggs. Avoid including urine and faeces.
2. Mix the eggs by pouring them between two beakers 5-10 times. Use a clean pipette to sample ovarian fluid, add RNA later and freeze. Let eggs settle for 5 min. and estimate volume.
3. Prepare incubators, one for each egg beaker. If egg volume exceeds 0.7 l, mix and divide in two equal portions (two incubators) before fertilization. Use gloves.

Fertilization

1. Use two extenders (2 x 2 ml sperm per 0.5 l eggs). Add the required amount of extender from each flask using separate pipettes. Put flasks back in the fridge immediately after. Avoid any waterspill to avoid activating the sperm in the extenders.
2. Add both extenders to the egg beaker. Take a second beaker and add 20-40 ml of salt water. Add this to the egg/sperm beaker to activate the sperm cells. Mix thoroughly by pouring the eggs from beaker to beaker 10-20 times. Be careful to avoid lumps.
3. Pour eggs into the incubator inside a steel ring. Spread evenly using a finger.Leave for 2-5 min. Gently pat the eggs down to form a robust "cake". Leave for 5-10 min before gently adding a small waterflow. Remove the steel ring after a few minutes of flowing water.

6. Incubation

Key egg and sperm quality determinants

There are no scientifically based standards for determining quality in eggs and sperm, simply guidelines obtained from experience. When females are stripped, the eggs are inspected visually for blood residue in the egg/ovarian fluid solution and discarded if there is too much blood present. Eggs are checked approximately 50 day-degrees post fertilization to determine fertilization percentage. If below 50%, the eggs are discarded. The sperm quality/activity is checked prior to fertilization by inspecting a drop of extender-milt with a drop of seawater added under the microscope. If the quality of the milt is good, sperm activity will be visible.

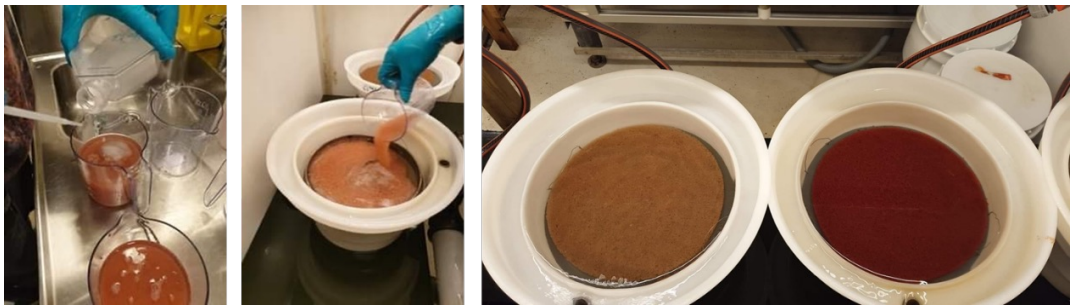

Figure 4. Fertilization, moulding and egg-hardening of lumpfish eggs

Egg disinfection protocols

Eggs are susceptible for disinfection between 5-20 day-degrees and after 50 day-degrees. If eggs are disinfected after 250 day-degrees, premature hatching is a risk. Eggs are disinfected using Buffodine (1 ml Buffodine/1 l seawater). The whole grate carrying the eggs is removed and placed in disinfection, left there for 10 min and then moved to a new, clean incubation unit. Gloves should be used at all times during disinfection.

7. Conclusions

- Optimal housing and handling procedures for lumpfish broodstock was reported
- Methodology to collect, evaluate and cryopreserve sperm was described. Methodology includes euthanasia for male broodfish and ground gonads for optimal sperm collection
- Sperm can be cryopreserved and stored in straws, which increases storage capacity without compromising safety. Fresh sperm also has a long shelf-life when stored with extenders in a constant (cold) temperature.
- Procedures for fertilization are described. Always use sperm from two males to obtain high fertilization success.
- A protocol for incubation and disinfection was presented. Eggs are only susceptible for disinfection at given points (no. of day-degrees).
- Egg batches with poor fertilization are discarded. Evaluation after approximately 50 day-degrees