This document describes protocols for production of 1) triploids and 2) neomales in European whitefish *Coregonus lavaretus* L.

1) Protocol for optimisation of triploid induction by hydrostatic pressure in European whitefish *Coregonus lavaretus*

Aim

The goal is to produce sterile fish. Sterile triploid fish are less prone to *Saprolegnia* infection because whitefish are most vulnerable to the disease during maturation. *Saprolegnia* oomycete infection causes serious economic losses and reduces fish health in aquaculture.

Approach to develop the protocol

The protocol is based on a set of three different experiments conducted in years 2020, 2021 and 2022. Hydrostatic pressure to which the fertilized eggs are exposed is one of the main determinants inducing triploidy. In the experiments, different levels of hydrostatic pressure was applied to groups of eggs post fertilisation. Ploidy level of eggs was determined by flow cytometric analysis of eyed-stage eggs. The results are summarized in Figure 1, and the protocol is based on the hydrostatic pressure of 9500 pound-force per square inch (PSI).

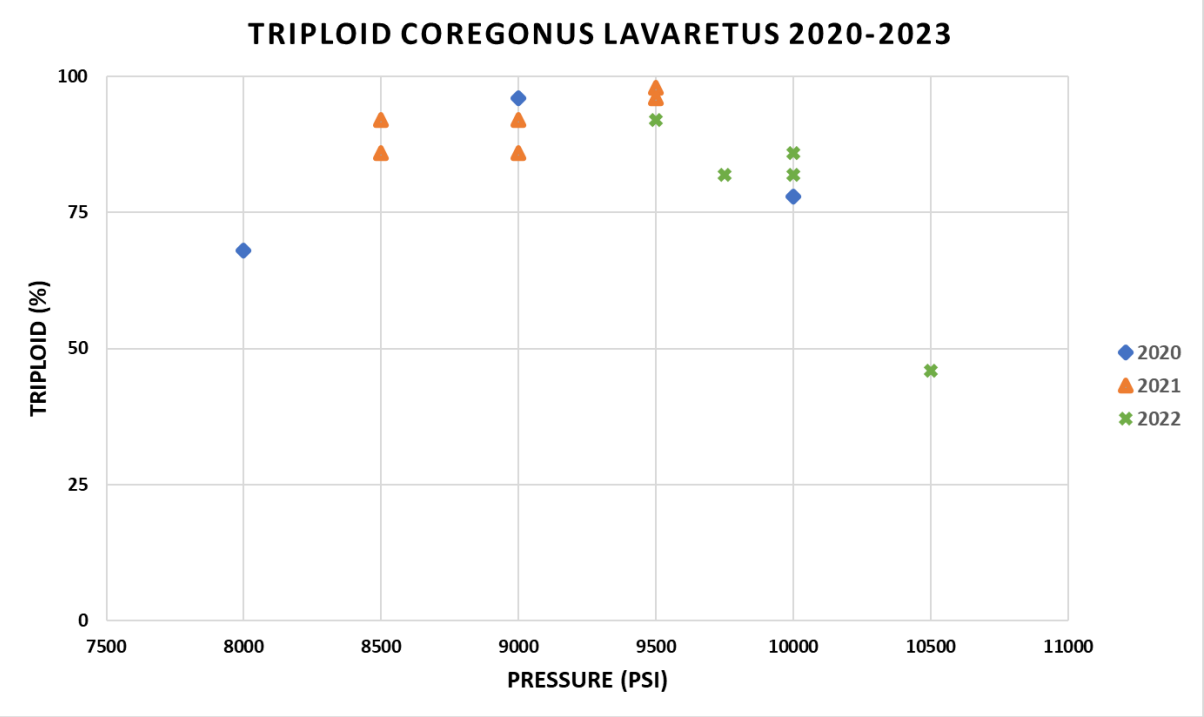

Figure 1. The percentage of triploid eggs as a function of hydrostatic pressure (pound-force per square inch, PSI) applied on fertilised eggs of European whitefish, *Coregonus lavaretus*.

The same protocol was applied also to muksun, *Coregonus muksun,* with three different pressure levels used in a single experiment. In all pressure treatments, ranging between 800-1000 PSI, close to 100% percentage of fish were triploid (Figure 2).

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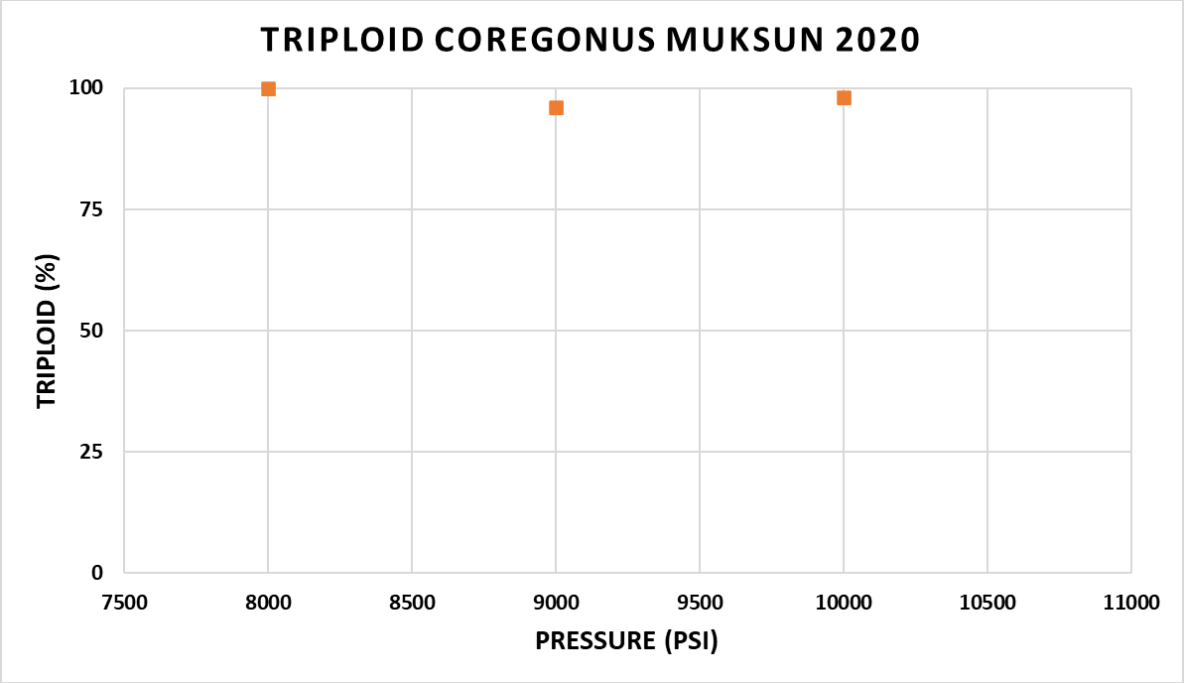

Figure 2. The percentage of triploid eggs as a function of hydrostatic pressure (pound-force per square inch, PSI) applied on fertilised eggs of muksun, *Coregonus muksun*.

General protocol

- Use 5°C water during the whole process including fertilisation, rinsing, swelling and triploidisation.
- Fertilise eggs with milt in a plastic container using tap water and fertilisation solution (e.g. Billard).
- Rinse and swell the fertilised eggs in a container filled with water before the pressure treatment.
- 10 minutes post fertilisation, place the eggs to the triploid cylinder filled with water.
- Using the hydrostatic pressure machine, apply hydrostatic pressure of 9500 PSI exactly 20 minutes post fertilisation for 5 min at 5°C water.
- Make sure all the joints are tightly closed to keep the pressure inside the container.
- Keep distance from the pressure container, given the high pressure is a risk factor.
- Collect the eggs and incubate in cool water.

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A nitrogen gas operated power unit attached to the pressure cylinder containing the eggs. The machine induces triploidy via hydrostatic pressure.

Remarks

The optimized protocol generated results between 92-98 % level of triploidy in European whitefish (Figure 1), and hence some diploid individuals are expected to occur in the treated stocks.

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2) Protocol for production of neomales in European whitefish *Coregonus lavaretus*

Note: This is very initial protocol based on a single experiment, and hence without any validation of the results. Further experiments are needed to validate the results and find the optimum treatment that produces solid repeatable results.

Aim

Neomales (XX-males) used in matings produce all-female offspring. In salmonids, real males typically have XY-genotype that determines their sex, while females have XX-genotype. Hence, neomales with XX-genotype mated to real females with XX-genotype produce all females offspring will XX-genotype. Typically all-females are preferred in aquaculture because females mature years later than males, and females produce valuable caviar. In addition all-female eggs combined to induced triploidy produces sterile fish that have multiple advantages in aquaculture.

Approach to develop the protocol

Sex reversed neomales were produced by hormonal induction of methyldihydrotestosterone (MDHT) during the initial feeding stages of the European whitefish. Several levels of hormone were used in an experiment with replicated treatments. Phenotypic sex of the fish was determined by histology. Phenotypic males include both the real males (XY-males) and sex-reversed females (XX-neomales). Increasing the level of hormone in a feed produced a maximum of 90% phenotypic males.

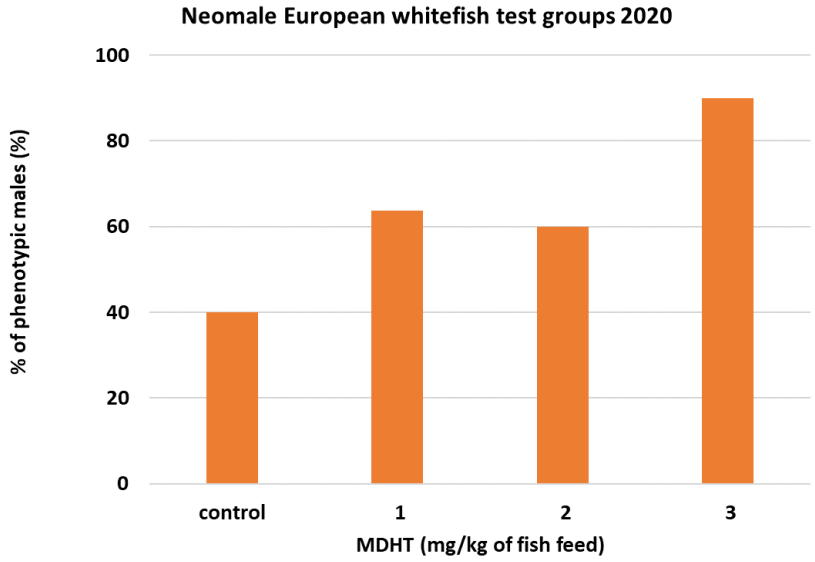

Figure 3. Percentage of phenotypic males as a function of 17α- methyldihydrotestosterone treatment in European whitefish, *Coregonus lavaretus*. Phenotypic males include both the real males (XY-males) and sex-reversed females (XX-males).

General protocol

- Dry chemical hormone 17α-methyldihydrotestosterone (MDHT).
- At 450 day degrees post hatching, the hormone feeding is initiated.
- Concentration of 3 mg hormone / kg start feed (e.g. start feed of size 0.3-0.5 mm, AgloNorse3®).
- The dry chemical hormone (MDHT) is first mixed together with 96 % ethanol to produce a stock solution. Using a desired volume of the stock solution and mixing it with small amount of pure 96 % ethanol, 1 kg of start feed is placed as a thin layer on piece of plastic etc. and hormone+alcohol solution

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is sprayed evenly to the feed. Feed has to be mixed several times during the spraying in order to produce feed with even hormone concentration in it. When all hormone+alcohol solution has been mixed to the feed by spraying, let the feed rest as a thin layer overnight resulting dry feed free from alcohol but containing 3 mg of hormone in 1 kg of start feed.

- Slight overfeeding / *ad libitum* while feeding fish with hormone feed (belt feeder) for 1000 day degrees.
- Normal on-growing with standard feed and husbandry until sexual maturation is reached.

Remarks

This is very initial protocol based on a single experiment, and hence without any validation of the results. Further experiments are needed to validate the results and find the optimum treatment that produces solid repeatable results.

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