Triploid Induction in cultured burbot (*Lota lota*) using thermal and hydrostatic shock

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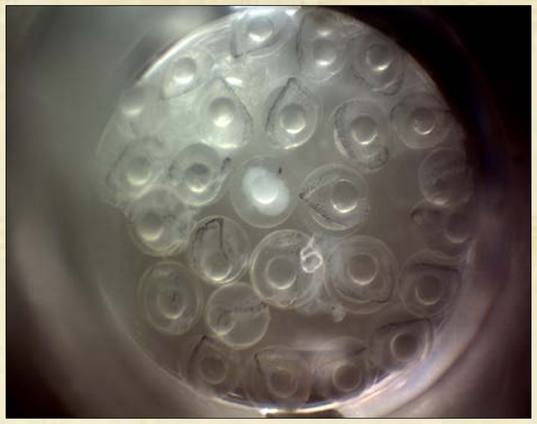

Introduction

The feasibility of triploid induction for burbot (*Lota lota*) was determined following a series of hydrostatic (pressure) and thermal (heat) shock treatments. Hydrostatic shock treatments were designed to test a range of variables including 1) duration of shock; 2) timing of shock (post-fertilization); and 3) shock pressure. Shock times post-fertilization and shock duration were varied by degree minutes (ºC minutes). Production of sterile burbot may increase opportunities for culturing burbot in areas where escapement may be a concern or when growth is inhibited due to reproductive maturation.

Methods

Fertilization was achieved by pipetting and mixing 60µl of milt into the 30ml glass vial containing aliquoted eggs, then activating the milt with 10ml of 2°C water. At 35 minutes post-fertilization incubation tubes were placed in a 2ºC 25ppm ovadine solution for disinfection. At 45 minutes post-fertilization, eggs in incubation vials were rinsed with 2ºC water to remove ovadine and placed back into a 2ºC static dechlorinated water bath until the administration of the shock treatment.

Flow Cytometry Analysis

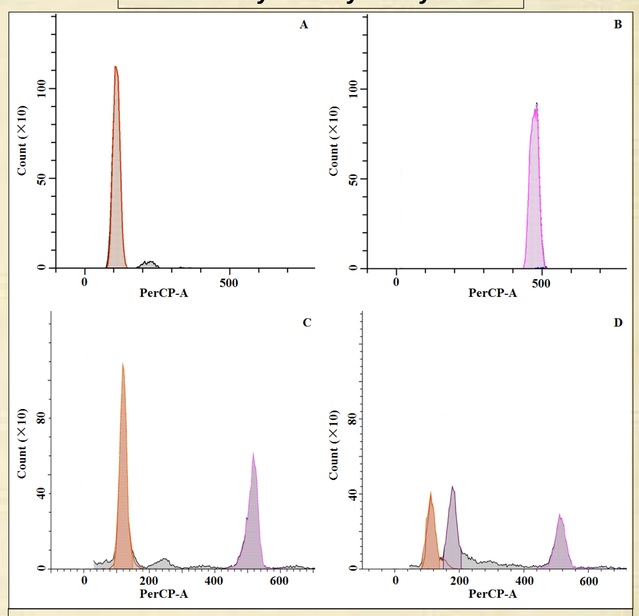

Figure 1. Illustration of flow cytometry analysis of nuclear DNA from burbot (*Lota lota*) and rainbow trout (*Oncorhynchus mykiss*) samples showing peak fluorescent intensity (PerCP-A) corresponding to either diploid or triploid cells. Nuclear DNA content values are reported in arbitrary units of fluorescence intensity (PerCP-A) along the x axis, and count of events along the y axis. Figures 1A and B represent the output readings from control diploid burbot and rainbow trout blood samples, respectively. Figure 1C illustrates an analysis and corresponding peaks from both internal burbot and rainbow trout controls plus a diploid larval sample. Figure 1D illustrates a sample containing internal diploid controls and a triploid burbot larvae sample.

Experiment	Shock type	Post-fertilization time (ºC·min)	Duration of shock (ºC·min)	Shock intensity / Temperature
I	Hydrostatic	180	10, 20, 30, 40	8,500 psi
II	Hydrostatic	90, 180, 270	30	8,500 psi
III	Hydrostatic	180	30	7,500, 8,500, 9,500 psi
IV	Thermal	90, 120, 150	400	16° C
V	Thermal	120	400	10, 14, 18, 22° C
VI	Thermal	120	300, 400, 500	16° C

Table 1. Summary of triploid induction experiments

Hydrostatic Shock Experiments

Experiment I: Shock of 8500 psi at 180°C minutes post fertilization with different duration of shock (p <0.05).

Duration of shock (ºC·min)	Relative pre-hatch larvae survival (%)	Triploid induction (%)
10	95.0 ^{a}	100^{m}
20	0.3^{b}	-^{n}
30	0.0 ^{b}	-^{n}
40	0.0 ^{b}	-^{n}
control	100^{a}	0.0 ^{n}

Experiment III: Shock at 180ºC minutes post fertilization for 30ºC minutes with different shock intensity (p <0.05).

Pressure shock intensity (psi)	Relative pre-hatch larvae survival (%)	Triploid induction (%)
7,500	66.0^{a}	100^{m}
8500	4.3 ^{b}	-^{n}
9500	0.0 ^{b}	-^{n}
control	100 ^{a}	0.0 ^{n}

Thermal Shock Experiments

Experiment VI. Shock of 16ºC at 120ºC minutes post fertilization with different duration of shock (p <0.05).

Shock duration (°C-min)	Relative pre-hatch larvae survival (%)	Triploid induction (%)
300	54.0^{a}	96.6^{m}
400	79.1 ^{a}	90.0 ^{m}
500	57.4 ^{a}	96.6 ^{m}
control	100 ^{a}	0.0^{n}

Experiment V: Shock at 120ºC minutes post fertilization for 400ºC minutes with different shock temperature (p <0.05).

Shock temperature (°C)	Relative pre-hatch larvae survival (%)	Triploid induction (%)
10	100.0^{a}	0.0 ^{n}
14	94.2^{a}	23.3 ^{n}
18	87.8^{a}	83.3^{m}
22	53.6 ^{b}	90.0^{m}
Control	100 ^{a}	0.0 ^{n}

Results

Hydrostatic

A hydrostatic shock of 8,500psi at 180ºC minutes post-fertilization for 10ºC minutes yielded the highest percent triploid induction (100%) and survival (95%) relative to the controls. Duration of pressure-shock longer than 10ºC minutes at 8,500psi and higher, resulted in 100% pre-hatch mortality. A reduced shock pressure (7,500psi) resulted in a high percent triploidy (100%), but pre-hatch larval survival was 65.5% at a shock duration of 30ºC minutes.

Thermal

. Triploid induction and survival were greatest following a thermal shock of 16ºC at 120ºC minutes post-fertilization for 500ºC minutes. This resulted in 96.6% triploidy and 57.4% survival relative to control groups. Shock temperatures above 16ºC generally resulted in a higher percent triploid induction but lower survival.

Summary

This study has demonstrated the successful induction of triploidy in burbot using both hydrostatic and thermal shocks to prevent extrusion of the second polar body after fertilization. The most effective hydrostatic protocol was found to be a shock of 8,500psi for 10ºC minutes, administered at 180ºC minutes post fertilization. This regimen resulted in high triploidy rates (100%) and relative survival (95%). Future research should follow on from this work and refine the hydrostatic shock protocol based on the results obtained from these experiments. The most effective thermal treatment was a shock of 16ºC for 500ºC minutes, 120ºC minutes post-fertilization. This regimen resulted in high triploidy rates (96.6%) and relative survival (57.4%). Further work is needed for optimization and to confirm scale up potential, survival dynamics, sterility of triploid burbot, and changes in growth performance. Production of sterile burbot may increase opportunities for culturing burbot in areas where escapement may be a concern or when growth is inhibited due to reproductive maturation.

Acknowledgements

WRAC Western Regional Aquaculture Center

USDA NIFA United States Department of Agriculture National Institute of Food and Agriculture

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