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 (post-fertilization); and 3) shock pressure. Shock times post-fertilization and shock duration 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 2ºC 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.

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.

Table 1. Summary of triploid induction experiments

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Shock type</th>
<th>Post-fertilization time (ºC·min)</th>
<th>Duration of shock (ºC·min)</th>
<th>Shock intensity / Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Hydrostatic</td>
<td>180</td>
<td>10, 20, 30, 40</td>
<td>8,500 psi</td>
</tr>
<tr>
<td>II</td>
<td>Hydrostatic</td>
<td>90, 180, 270</td>
<td>30</td>
<td>8,500 psi</td>
</tr>
<tr>
<td>III</td>
<td>Hydrostatic</td>
<td>180</td>
<td>30</td>
<td>7,500, 8,500, 9,500 psi</td>
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<tr>
<td>IV</td>
<td>Thermal</td>
<td>90, 120, 150</td>
<td>400</td>
<td>16 ºC</td>
</tr>
<tr>
<td>V</td>
<td>Thermal</td>
<td>120</td>
<td>400</td>
<td>10, 14, 18, 22 ºC</td>
</tr>
<tr>
<td>VI</td>
<td>Thermal</td>
<td>120</td>
<td>300, 400, 500, 160</td>
<td>16 ºC</td>
</tr>
</tbody>
</table>

Acknowledgements

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 larval sample.

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