Use of Triploidy for Biocontrol of Invasive Species

Tillmann Benfey
University of New Brunswick (Canada)

International Symposium on Genetic Biocontrol of Invasive Fish
Minneapolis, June 21-24, 2010
Outline

• Brief background on triploid biology
• Production and identification of triploids
• Use of female triploids (the typical scenario)
• Use of male triploids (a novel application?)
• Questions / discussion
Biology of Triploids

• Three sets of homologous chromosomes
• Mitosis (somatic growth) unaffected
• Meiosis (germ cell development) disrupted
  – Results in reproductive sterility
• Used in aquaculture to …
  – Prevent spawning of escaped farmed fish
  – Eliminate early (pre-harvest) sexual maturation
  – Protect investments in novel genotypes
Biology of Triploids

• Sex-specific effects on gonadal development
• Affects …
  – Endocrinology
  – Secondary (external) sexual characteristics
  – Behaviour
• Females retain juvenile characteristics whereas males appear to mature
Production of Triploids

2n zygote

P = paternal genome
M = maternal genome
Production of Triploids

3n zygote

P = paternal genome
M = maternal genome
Production of Triploids

- Preferred method is pressure shock
  - Same treatment works for diversity of species:
    5 min at 8,500 – 9,500 psi
    (58.6 – 65.5 x 10³ kPa)
  - Only variable is time after fertilization
Identification of Triploids

- Preferred method is flow cytometry
  - Measure erythrocyte DNA content \((3n = 1.5 \times 2n)\)
  - Easy, fast and accurate

<table>
<thead>
<tr>
<th>Number of Cells</th>
<th>Channel Number (Relative DNA Content)</th>
<th>Modal Channel No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3n</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fish Number

- 0 10 20 30 40 50 60
Female Triploids

• The typical scenario
• Works *a priori*
• Requires all-female populations

3n female rainbow trout

2n

female cod

3n

female chinook salmon
Female Triploids

Standard procedure in trout aquaculture:
Mixed-sex (XX/XY) population + androgen

100% phenotypically male population
(still 50% XX, 50% XY)

F₁:  XX♂ x XX♀  ➡  100% XX♀
+ hydrostatic pressure  ➡  100% XXX♀
Female Triploids

• Easy, accepted technology
  – E.g., rainbow trout (Troutlodge)

• Option for control of new introductions
  – Does not avoid ecological impacts but these will be limited to lifetime of the individuals

• Of no use for control of established invasive species
Male Triploids

• Typically avoided in commercial aquaculture
  – Normal reproductive physiology
  – Normal spawning behaviour
  – Produce functional sperm
  – Offspring are not viable

• Candidate for “sterile male” approach to biocontrol
Male Triploids

2n male sperm & blood  
1n  1.5n  2n  3n male sperm & blood  
3n

male brook charr
Male Triploids

• Atlantic cod as example (Nat Feindel MSc)
Male Triploids

<table>
<thead>
<tr>
<th>Trio</th>
<th>Batch</th>
<th>n</th>
<th>% sired</th>
<th>% sired</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2n</td>
<td></td>
<td>3n</td>
</tr>
<tr>
<td>1</td>
<td>01.02.08</td>
<td>24</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>11.02.08</td>
<td>19</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>19.02.08</td>
<td>25</td>
<td>96</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>02.03.08</td>
<td>13</td>
<td>15</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>06.03.08</td>
<td>21</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>12.03.08</td>
<td>6</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>18.03.08</td>
<td>32</td>
<td>72</td>
<td>28</td>
</tr>
<tr>
<td>4</td>
<td>21.02.08</td>
<td>14</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Trio</th>
<th>Batch</th>
<th>n</th>
<th>% sired</th>
<th>% sired</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2n</td>
<td></td>
<td>3n</td>
</tr>
<tr>
<td>5</td>
<td>27.02.08</td>
<td>14</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>06.03.08</td>
<td>11</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>10.03.08</td>
<td>35</td>
<td>14</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>18.03.08</td>
<td>11</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>24.03.08</td>
<td>11</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>30.03.08</td>
<td>15</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>09.03.08</td>
<td>31</td>
<td>29</td>
<td>71</td>
</tr>
</tbody>
</table>

Atlantic cod (N. Feindel MSc)
Male Triploids

Fertilization Success (%)

Female

Atlantic cod (N. Feindel MSc)
Male Triploids

Hatching Success (%)

Female

Hatching Success (%)

Atlantic cod (N. Feindel MSc)
Male Triploids

Larval Survival (%) vs Days Post-hatch

- 2n Sired Larvae
- 3n Sired Larvae
Male Triploids

2n-sired larvae

3n-sired larvae
Male Triploids

• Suitability for “sterile male” approach to biocontrol will depend on …
  – Effectiveness of large-scale triploidy induction
    • Does it have to be 100% successful?
  – Production of all-male populations
    • Techniques only developed for a handful of species
  – Confirmation of competitive spawning ability
  – Ecological effects of mass release of 3n males
Thanks!

Some New Brunswick Exotics

Muskellunge

Smallmouth bass

Rainbow trout

http://pond.dnr.cornell.edu/nyfish/Esocidae/muskellunge.jpg
http://pond.dnr.cornell.edu/nyfish/Centrarchidae/smallmouth_bass.jpg
http://chazylakekings.files.wordpress.com/2008/02/rainbow_trout.jpg