A new method to confirm triploidy rates in salmonids

Diploid

Triploid
Outline

• Current FFSBC production and sampling

• Difference of Imaging software (ImageJ) and Flow Cytometry

• Example of imaging software sample analysis
FFSBC Production and Sampling

• Currently release 3 million triploid fish throughout the province of British Columbia

• Combination of hydrostatic pressure and heat shock since 1995

• Sampling to confirm triploid success at 0.05% of total release size
### Imaging Software

<table>
<thead>
<tr>
<th>Pro’s</th>
<th>Con’s</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Basic Lab equipment</td>
<td>• New unknown process</td>
</tr>
<tr>
<td>• No fish transfer</td>
<td>• Sample Quality</td>
</tr>
<tr>
<td>• Timeline extended</td>
<td></td>
</tr>
<tr>
<td>• Cost less per sample</td>
<td></td>
</tr>
</tbody>
</table>

*Source: Freshwater Fisheries Society of BC*
Imaging Software

- Blood smear
- Picture 200x magnification
- Upload pictures into software and run
- Output into datasheet
Flow Cytometry

Pro’s
• Proven method
• Familiar with process

Con’s
• Time sensitive
• Shipping fish
• More expensive
• Chemical disposal
• Mutagen / Carcinogen
Flow Cytometry

• Hold fish onsite, lethal sample
• Apply Nuclear Stain, Preserve
• Send to contractor
• Chemical disposal
Comparison of Methods

<table>
<thead>
<tr>
<th>Species</th>
<th>Percent 2n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RB</td>
<td>100 (FC)</td>
</tr>
<tr>
<td>CT</td>
<td>97 (FC)</td>
</tr>
<tr>
<td>EB</td>
<td>100 (FC)</td>
</tr>
<tr>
<td>KO</td>
<td>100 (FC)</td>
</tr>
</tbody>
</table>

Legend:
- FC
- IMG

Species:
- RB
- CT
- EB
- KO

Freshwater Fisheries Society of BC
gofishbc.com
Comparison of Methods

Imaging

- 88% Labour
- 10% Equipment/Reagents
- 2% Transport
- 0% Contract
Comparison of Methods

Flow Cytometry

- Contract: 54%
- Transport: 8%
- Labour: 32%
- Equipment/Reagents: 6%
Costing

• Based on 1900 samples

• Imaging Cost $3.29 / sample

• Flow Cytometry $6.40 / sample
Imaging Software

• Parameters

• Manipulation, measurement

• Organizing output, testing
Parameters

- Cell area, major/minor axis, circularity
ImageJ Manipulation
### ImageJ Manipulation

- Output into spreadsheet
- Sort by major axis identify outliers
- Check individual cell measurements

<table>
<thead>
<tr>
<th>YEAR</th>
<th>CELLID</th>
<th>STRAIN$</th>
<th>AREA</th>
<th>MAJOR</th>
<th>MINOR</th>
<th>CIRC</th>
<th>FISHNO</th>
<th>PLOIDY$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011</td>
<td>1</td>
<td>BW</td>
<td>187.282</td>
<td>19.064</td>
<td>12.508</td>
<td>0.846</td>
<td>1</td>
<td>3n</td>
</tr>
<tr>
<td>2011</td>
<td>2</td>
<td>BW</td>
<td>190.641</td>
<td>18.669</td>
<td>13.002</td>
<td>0.814</td>
<td>1</td>
<td>3n</td>
</tr>
<tr>
<td>2011</td>
<td>3</td>
<td>BW</td>
<td>191.313</td>
<td>19.418</td>
<td>12.545</td>
<td>0.82</td>
<td>1</td>
<td>3n</td>
</tr>
<tr>
<td>2011</td>
<td>4</td>
<td>BW</td>
<td>207.438</td>
<td>19.94</td>
<td>13.246</td>
<td>0.825</td>
<td>1</td>
<td>3n</td>
</tr>
</tbody>
</table>
Analyzing

• Import data from both genotypes

• Sort by major axis identify outliers

• Categorizes the sample based on the major axis measurement, 2 cluster analysis
Analysis

- 2 cluster analysis using the long axis

<table>
<thead>
<tr>
<th>Fish #</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Freq</td>
<td>Percent</td>
<td>Freq</td>
</tr>
<tr>
<td>Cluster</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0.00%</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>56</td>
<td>100.00%</td>
<td>22</td>
</tr>
<tr>
<td>Combined</td>
<td>56</td>
<td>100.00%</td>
<td>22</td>
</tr>
</tbody>
</table>
Flowchart

Blood sample

<20 measurements

Nuclei Measurements

2n/3n

>20 measurements

Software to classify

Error or 2n classification then use Nuclei measurement

2n/3n
Questions
Freshwater Fisheries Society of BC
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