Genetic Identification of Brook Trout or Brook/Bull Trout Hybrids in Pataha Creek, WA

by

Cheryl A. Dean and Todd W. Kassler

Washington Department of Fish and Wildlife
Molecular Genetics Laboratory
600 Capitol Way N
Olympia, WA 98501

and

Glen Mendel

Washington Department of Fish and Wildlife
Fish Management – SE WA
529 W Main St.
Dayton, WA 99328

April 2010
Introduction

In 1998, at the time of listing under the Endangered Species Act (ESA), bull trout (*Salvelinus confluentus*) distribution was believed to include the Pataha Creek drainage (USFWS 2002/04, pg 8). Anecdotal information from local residents had suggested that bull trout historically existed in upper Pataha Creek, but their status was uncertain during listing and through draft recovery planning (i.e. 2001-2004). Brook trout (*S. fontinalis*) are known to have been introduced into Pataha Creek by the Washington Department of Game multiple times in 1951, and once in 1968. Brook trout are now well established in Pataha Creek from near Columbia Center upstream into the headwaters in the Umatilla National Forest (Mendel 1999; Schuck et al. 1988). Rainbow trout or steelhead (*Oncorhynchus mykiss*) also exist in this portion of Pataha Creek, as well as downstream. Washington Department of Fish and Wildlife and US Forest Service staff collaborated in an effort to capture brook trout to determine if genetic evidence could confirm a prior presence of bull trout that may have hybridized with brook trout. This information would be important for management decisions regarding ESA recovery planning and implementation.

Methods

In 2007, WDFW and the USFS collected caudal fin clips from five brook trout (WDFW code 07MF) caught with hook and line in Pataha Creek near the northern boundary of the Umatilla National Forest. Staff from WDFW returned to Pataha Creek in August 2008 and electrofished the northern boundary area of the National Forest as well as upstream to the area around the forks of upper Pataha Creek and collected 35 samples (WDFW code 08IG) for genetic analysis. Tissues that were sampled from suspected brook trout from Pataha Creek ranged in size from 78 to 185 mm fork length (Table 1). Sample 08IG 39, captured near the upper end of the road near river mile 50, appeared to have physical characteristics of both brook trout and bull trout when examined in the field.
Table 1. Brook trout samples collected in Pataha Creek for genetic analyses.

<table>
<thead>
<tr>
<th>Date</th>
<th>Sample ID</th>
<th>Fork Length (mm)</th>
<th>Capture Method</th>
<th>Location/Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>8/31/07</td>
<td>07MF1</td>
<td>213</td>
<td>Hook &amp; line</td>
<td>Near USFS north boundary</td>
</tr>
<tr>
<td>8/31/07</td>
<td>07MF2</td>
<td>177</td>
<td>Hook &amp; line</td>
<td>Near USFS north boundary</td>
</tr>
<tr>
<td>8/31/07</td>
<td>07MF3</td>
<td>155</td>
<td>Hook &amp; line</td>
<td>Near USFS north boundary</td>
</tr>
<tr>
<td>8/31/07</td>
<td>07MF4</td>
<td>180</td>
<td>Hook &amp; line</td>
<td>Near USFS north boundary</td>
</tr>
<tr>
<td>8/31/07</td>
<td>07MF5</td>
<td>140</td>
<td>Hook &amp; line</td>
<td>Near USFS north boundary</td>
</tr>
<tr>
<td>8/26/08</td>
<td>08IG6</td>
<td>96</td>
<td>electrofishing</td>
<td>0.5 mile beyond end of road</td>
</tr>
<tr>
<td>8/26/08</td>
<td>08IG7</td>
<td>78</td>
<td>electrofishing</td>
<td>0.5 mile beyond end of road</td>
</tr>
<tr>
<td>8/26/08</td>
<td>08IG8</td>
<td>119</td>
<td>electrofishing</td>
<td>0.5 mile beyond end of road</td>
</tr>
<tr>
<td>8/26/08</td>
<td>08IG9</td>
<td>110</td>
<td>electrofishing</td>
<td>0.5 mile beyond end of road</td>
</tr>
<tr>
<td>8/26/08</td>
<td>08IG10</td>
<td>177</td>
<td>electrofishing</td>
<td>0.5 mile beyond end of road</td>
</tr>
<tr>
<td>8/26/08</td>
<td>08IG11</td>
<td>94</td>
<td>electrofishing</td>
<td>culvert</td>
</tr>
<tr>
<td>8/26/08</td>
<td>08IG12</td>
<td>118</td>
<td>electrofishing</td>
<td>culvert</td>
</tr>
<tr>
<td>8/26/08</td>
<td>08IG13</td>
<td>98</td>
<td>electrofishing</td>
<td>culvert</td>
</tr>
<tr>
<td>8/26/08</td>
<td>08IG14</td>
<td>122</td>
<td>electrofishing</td>
<td>culvert</td>
</tr>
<tr>
<td>8/26/08</td>
<td>08IG15</td>
<td>110</td>
<td>electrofishing</td>
<td>0.5 mile upstream of culvert</td>
</tr>
<tr>
<td>8/26/08</td>
<td>08IG16</td>
<td>122</td>
<td>electrofishing</td>
<td>0.5 mile upstream of culvert</td>
</tr>
<tr>
<td>8/26/08</td>
<td>08IG17</td>
<td>128</td>
<td>electrofishing</td>
<td>0.5 mile upstream of culvert</td>
</tr>
<tr>
<td>8/26/08</td>
<td>08IG18</td>
<td>120</td>
<td>electrofishing</td>
<td>0.5 mile upstream of culvert</td>
</tr>
<tr>
<td>8/26/08</td>
<td>08IG19</td>
<td>80</td>
<td>electrofishing</td>
<td>0.5 mile upstream of culvert</td>
</tr>
<tr>
<td>8/26/08</td>
<td>08IG20</td>
<td>90</td>
<td>electrofishing</td>
<td>Campground with pond</td>
</tr>
<tr>
<td>8/26/08</td>
<td>08IG21</td>
<td>132</td>
<td>electrofishing</td>
<td>Campground with pond</td>
</tr>
<tr>
<td>8/26/08</td>
<td>08IG22</td>
<td>93</td>
<td>electrofishing</td>
<td>Campground with pond</td>
</tr>
<tr>
<td>8/26/08</td>
<td>08IG23</td>
<td>161</td>
<td>electrofishing</td>
<td>Campground with pond</td>
</tr>
<tr>
<td>8/26/08</td>
<td>08IG24</td>
<td>118</td>
<td>electrofishing</td>
<td>Campground with pond</td>
</tr>
<tr>
<td>8/28/08</td>
<td>08IG25</td>
<td>130</td>
<td>electrofishing</td>
<td>Campground with pond</td>
</tr>
<tr>
<td>8/28/08</td>
<td>08IG26</td>
<td>88</td>
<td>electrofishing</td>
<td>Forks</td>
</tr>
<tr>
<td>8/28/08</td>
<td>08IG27</td>
<td>97</td>
<td>electrofishing</td>
<td>Forks</td>
</tr>
<tr>
<td>8/28/08</td>
<td>08IG28</td>
<td>185</td>
<td>electrofishing</td>
<td>Forks</td>
</tr>
<tr>
<td>8/28/08</td>
<td>08IG29</td>
<td>84</td>
<td>electrofishing</td>
<td>Forks</td>
</tr>
<tr>
<td>8/28/08</td>
<td>08IG30</td>
<td>107</td>
<td>electrofishing</td>
<td>Site 2</td>
</tr>
<tr>
<td>8/28/08</td>
<td>08IG31</td>
<td>116</td>
<td>electrofishing</td>
<td>Site 2</td>
</tr>
<tr>
<td>8/28/08</td>
<td>08IG32</td>
<td>182</td>
<td>electrofishing</td>
<td>Site 2</td>
</tr>
<tr>
<td>8/28/08</td>
<td>08IG33</td>
<td>150</td>
<td>electrofishing</td>
<td>Site 2</td>
</tr>
</tbody>
</table>
Genomic DNA was extracted by digesting a small piece of fin tissue using silica membrane based kits, NucleoSpin® 96 Tissue kit (Macherey-Nagel Bethlehem, PA, USA), following the manufacturer’s recommendations.

Samples were identified to species using a portion of the mitochondrial cytochrome-b gene sequence. This sequence is unique to each of the *Oncorhynchus* sp., Atlantic salmon, brown trout or brook trout/bull trout/Dolly Varden complex (WDFW unpublished) and allows for the identification to one of the species listed above. A suite of microsatellite loci were then used to distinguish brook trout from bull trout and Dolly Varden (Bettles et al. 2005).

Samples were identified to species using a two-step process: 1) mitochondrial COIII/ND3 region was used to identify samples to species); 2) microsatellite markers were then used to distinguish brook trout from bull trout and Dolly Varden. Both processes use polymerase chain reaction (PCR) based fragment analysis to visualize genetic markers.

The COIII/ND3 region spans a 368-nucleotide segment across the cytochrome oxidase subunit III gene, tRNA-Gly gene, and NADH subunit 3 gene, and contains 10 single nucleotide polymorphisms. PCR using 14 allele specific primers produces DNA fragments of different lengths that are diagnostic for identifying salmonids species (Table 2).
PCR reactions were conducted with a thermal profile as follows: an initial denaturation step of 3 min at 94°C, 30 cycles of denaturation at 94°C for 15s, annealing at 55°C for 30s, and 1 min at 72°C, plus a final extension at 72°C for 30 min and final holding step at 10°C. PCR reaction volumes were 10 μL, and consisted of 1.0 μL 10X PCR buffer (Promega), 0.60 μL MgCl2 (1.5 mM final) (Promega), 1.0 μL 10mM dNTP mix (Promega), 0.10 μL (0.05 mM final) Taq DNA polymerase (Promega) and 0.2 μL 2M solution of each primer.

PCR for microsatellite based evaluation to distinguish brook trout from bull trout and Dolly Varden was performed using five fluorescently end-labeled microsatellite loci, Omm-1128, Sco-202, Sco-215, Sco-102 and Sco-107 (Table 3). These loci are known to distinguish brook trout from bull trout with fixed allelic differences (Bettles et al 2005). PCR reactions were conducted with a thermal profile known as “touch down”. Touch down PCR begins with an initial annealing temperature, which decreases by one degree with each cycle. Touch down profiles were specific to each locus (Table 2). General PCR conditions: initial denaturation step of 2 min at 94°C, 4 or more cycles of

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>SpID-L10333 (F)</th>
<th>SpID-H10678 (R)</th>
<th>Ots-H10446 (R)</th>
<th>Ocl-H10382 (R)</th>
<th>One-H10576 (R)</th>
<th>Omy-H10637 (R)</th>
<th>Oke-H10425 (R)</th>
<th>Oki-H10676 (R)</th>
<th>Ogo-H10585 (R)</th>
<th>Ssa-H10653 (R)</th>
<th>Sal-H10469 (R)</th>
<th>Sfo-H10532 (R)</th>
<th>Sco-H10537 (R)</th>
</tr>
</thead>
</table>
denaturation at 94°C for 30s, annealing for 30s at 60°C with decreasing temperature each cycle, and 1 min at 72°C, followed by 30 or more cycles of denaturation at 94°C for 30s, annealing for 30s at 50°C, and 1 min at 72°C, plus a final extension at 72°C for 10 min and final holding step at 10 °C.

Table 3. Microsatellite loci used to distinguish brook trout from bull trout and Dolly Varden

<table>
<thead>
<tr>
<th>Locus</th>
<th>Dye label</th>
<th>Touch down</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sco-107</td>
<td>ned</td>
<td>4 cycles</td>
<td>Bettles et al. (2005)</td>
</tr>
<tr>
<td>Sco-102</td>
<td>vic</td>
<td>4 cycles</td>
<td>Bettles et al. (2005)</td>
</tr>
<tr>
<td>Omm-1128</td>
<td>vic</td>
<td>4 cycles</td>
<td>Rexroad et al. (2001)</td>
</tr>
<tr>
<td>Sco-215</td>
<td>pet</td>
<td>10 cycles</td>
<td>DeHaan &amp; Ardren (2005)</td>
</tr>
<tr>
<td>Sco-202</td>
<td>6fam</td>
<td>10 cycles</td>
<td>DeHaan &amp; Ardren (2005)</td>
</tr>
</tbody>
</table>

Reverse primers were redesigned to include a seven-nucleotide base extension (GTTTCTT) to their 5’ end to promote the incorporation of a nontemplated adenosine (+a) to the 3’ end of the PCR product.

PCR products were visualized by electrophoresis on an ABI-3730 automated analyzer (Applied Biosystems), with alleles sized (to base pairs) and binned using an internal lane size standard (GS500Liz from Applied Biosystems) and GENEMAPPER 3.7 software (Applied Biosystems).

GENETIX (version 4.03, Belkhir et al. 2001) was used for a factorial correspondence analysis and a graphical representation of the genetic variation among all individual samples in multi-dimensional space. Genotypic data for an individual sample is transformed into a value and plotted. The multi-dimensional data space represents all the individual values. Each axis (three-dimensional in this case) is derived from the individual values that correspond to percent of total chi-square distance, with chi square measuring the association between individual genotypes (weighted by the collection centroid when “sur populations” is selected for the analysis) and allele frequencies.
Results/Discussion

The mitochondrial analysis identified all samples to be brook trout/bull trout/Dolly Varden. The second analysis revealed that samples were brook trout and not bull trout or Dolly Varden. Genotypes were plotted in a factorial correspondence plot to illustrate the differences between brook trout, bull trout and Dolly Varden and how the unknown fish clustered with known brook trout (Figure 1).

Figure 1. Factorial correspondence plot of known brook trout, bull trout, dolly varden, and the unknown samples. The unknown samples are in the polygon with the known brook trout.

Acknowledgements

The Umatilla National Forest and WA State General Funds provided financial support for this project.
Literature Cited


