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

by

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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. fotinalis*) 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.

Date	Sample ID	Fork Length (mm)	Capture Method	Location/Comments
8/31/07	07MF1	213	Hook & line	Near USFS north boundary
8/31/07	07MF2	177	Hook & line	Near USFS north boundary
8/31/07	07MF3	155	Hook & line	Near USFS north boundary
8/31/07	07MF4	180	Hook & line	Near USFS north boundary
8/31/07	07MF5	140	Hook & line	Near USFS north boundary
8/26/08	08IG6	96	electrofishing	0.5 mile beyond end of road
8/26/08	08IG7	78	electrofishing	0.5 mile beyond end of road
8/26/08	08IG8	119	electrofishing	0.5 mile beyond end of road
8/26/08	08IG9	110	electrofishing	0.5 mile beyond end of road
8/26/08	08IG10	177	electrofishing	0.5 mile beyond end of road
8/26/08	08IG11	94	electrofishing	culvert
8/26/08	08IG12	118	electrofishing	culvert
8/26/08	08IG13	98	electrofishing	culvert
8/26/08	08IG14	122	electrofishing	culvert
8/26/08	08IG15	110	electrofishing	0.5 mile upstream of culvert
8/26/08	08IG16	122	electrofishing	0.5 mile upstream of culvert
8/26/08	08IG17	128	electrofishing	0.5 mile upstream of culvert
8/26/08	08IG18	120	electrofishing	0.5 mile upstream of culvert
8/26/08	08IG19	80	electrofishing	0.5 mile upstream of culvert
8/26/08	08IG20	90	electrofishing	Campground with pond
8/26/08	08IG21	132	electrofishing	Campground with pond
8/26/08	08IG22	93	electrofishing	Campground with pond
8/26/08	08IG23	161	electrofishing	Campground with pond
8/26/08	08IG24	118	electrofishing	Campground with pond
8/28/08	08IG25	130	electrofishing	Forks
8/28/08	08IG26	88	electrofishing	Forks
8/28/08	08IG27	97	electrofishing	Forks
8/28/08	08IG28	185	electrofishing	Forks
8/28/08	08IG29	84	electrofishing	Forks
8/28/08	08IG30	107	electrofishing	Site 2
8/28/08	08IG31	116	electrofishing	Site 2
8/28/08	08IG32	182	electrofishing	Site 2
8/28/08	08IG33	150	electrofishing	Site 2

8/28/08	08IG34	137	electrofishing	Site 2
8/28/08	08IG35	134	electrofishing	Site 3
8/28/08	08IG36	152	electrofishing	Site 3
8/28/08	08IG37	162	electrofishing	Site 4
8/28/08	08IG38	162	electrofishing	Site 4
8/26/08	08IG39	162	electrofishing	culvert, questioned as brook/bull trout hybrid based on morphology

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).

Table 2. Mitochondrial COIII/ND3 region primers used for identifying *Oncorhynchus* sp., Atlantic salmon, brown trout, and brook trout/bull trout/Dolly Varden.

Primer Name
SpID-L10333 (F)
SpID-H10678 (R)
Ots-H10446 (R)
Ocl-H10382 (R)
One-H10576 (R)
Omy-H10637 (R)
Oke-H10425 (R)
Oki-H10676 (R)
Ogo-H10585 (R)
Ssa-H10653 (R)
Sal-H10469 (R)
Sfo-H10532 (R)
Sco-H10537 (R)

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 ^{o}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 ^{o}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 0.2µL 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

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 ^{o}C.

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

Locus	Dye label	Touch down	Reference
Sco-107	ned	4 cycles	Bettles et al. (2005)
Sco-102	vic	4 cycles	Bettles et al. (2005)
Omm-1128	vic	4 cycles	Rexroad et al. (2001)
Sco-215	pet	10 cycles	DeHaan & Ardren (2005)
Sco-202	6fam	10 cycles	DeHaan & Ardren (2005)

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.

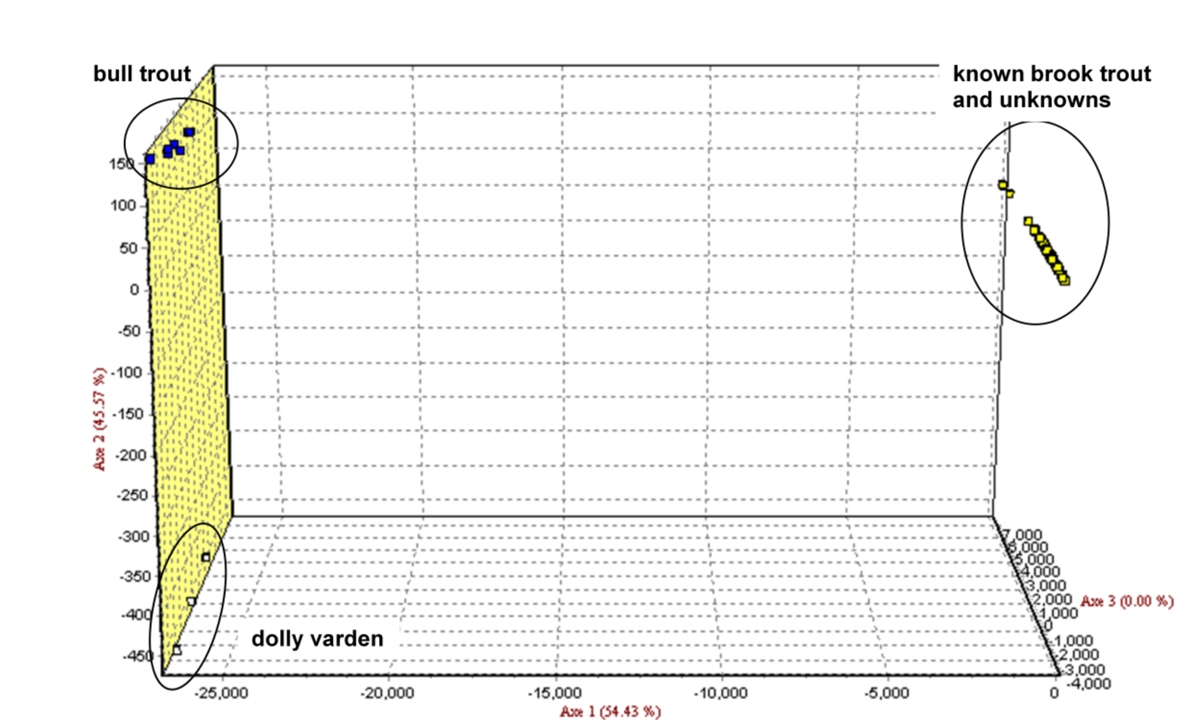
PCR products were visualized by electrophoresis on an ABI-3730 automated analyzer (Applied Biosystems), with alleles sized (to base pairs) and binned using an internal lane size standard (GS500Liz from Applied Biosystems) and GENEMAPPER 3.7 software (Applied Biosystems).

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

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