DNA-Based Parentage Assignments of Chinook Salmon from the Cle Elum Spawning Channel in 2005

Todd W. Kassler, WDFW Molecular Genetics Laboratory

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Background

- Joint project between WA Dept. of Fish and Wildlife (WDFW) and Yakama Nation (YN)
- Project objective is to assess parentage of Chinook salmon in a closed access spawning channel at the Cle Elum Hatchery
- There are difficulties assessing parentage of naturally spawning fish by observation
- Genetic analysis using microsatellite DNA loci provides a means to quantify individual spawners' reproductive output after spawning has occurred

Methodology

- DNA based parentage analysis has been conducted on spawning populations of spring Chinook in the Cle Elum spawning channel from 2001 - 2005

- Adult males and females, jacks, and precocious male Chinook were sampled for DNA analysis before they were stocked into Cle Elum experimental spawning channel

- Only natural-origin Chinook were used in 2001 while hatchery and natural-origin Chinook were used in 2002 - 2005

- The spawning channel was divided into two sections in 2001 – 2003, and remained open as one section in 2004 and 2005

- Fry and precocious males (not initially sampled) were recovered daily from the spawning channel in the spring and sampled for genetic analysis

Laboratory Methods

- DNA was extracted from fin tissue
- PCR amplification was performed using microsatellite loci
- Amplified products were run through an ABI-3730 Genetic Analyzer
- Electropherograms were scored using Genemapper software v.3.0
- Data was exported and binned using Microsatellite Binner v.1.h (available from S.F. Young, WDFW)

Jessica setting up DNA extraction

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Cheryl setting up thermalcycler

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Norm loading the ABI-3100

Laboratory Methods

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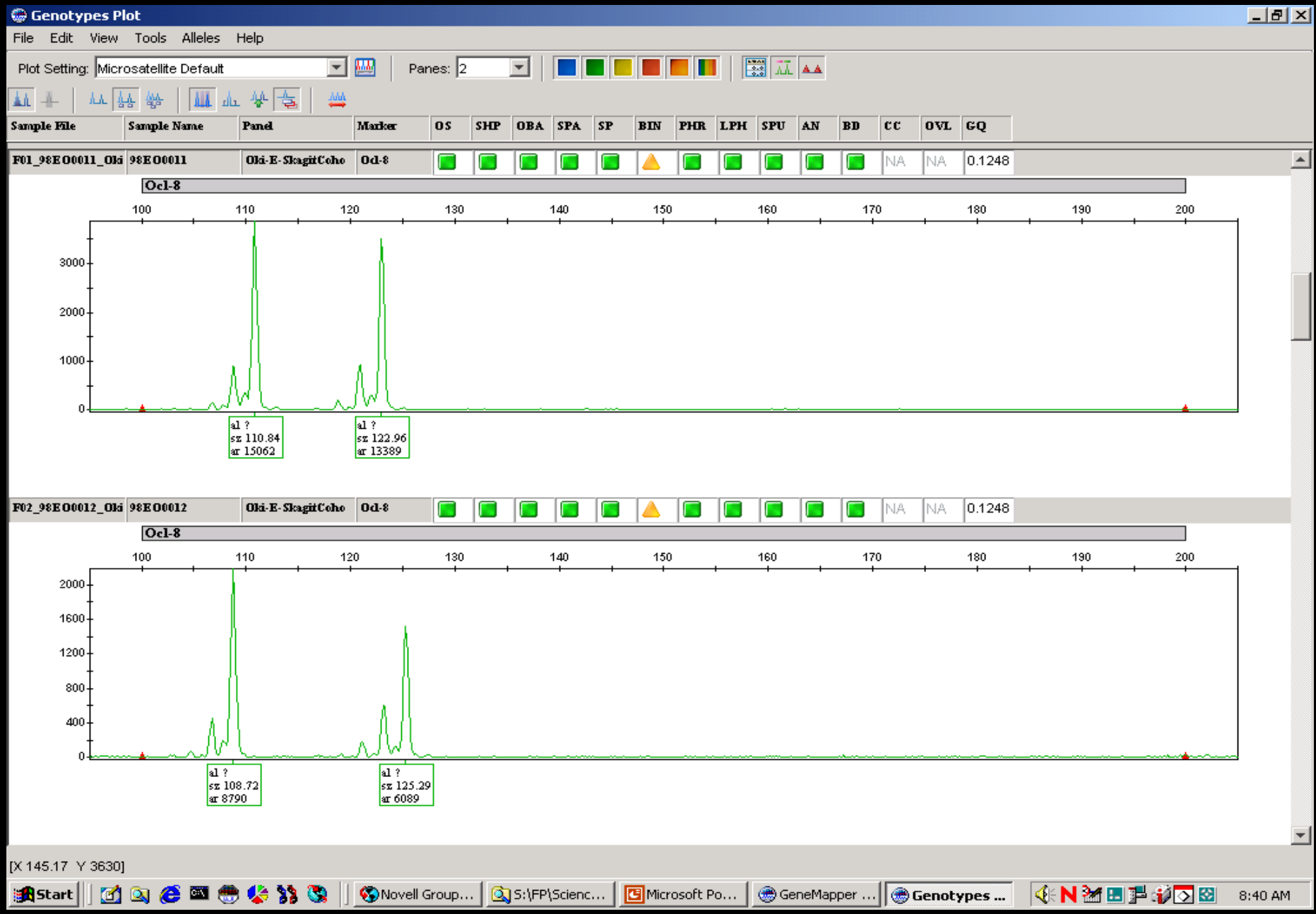
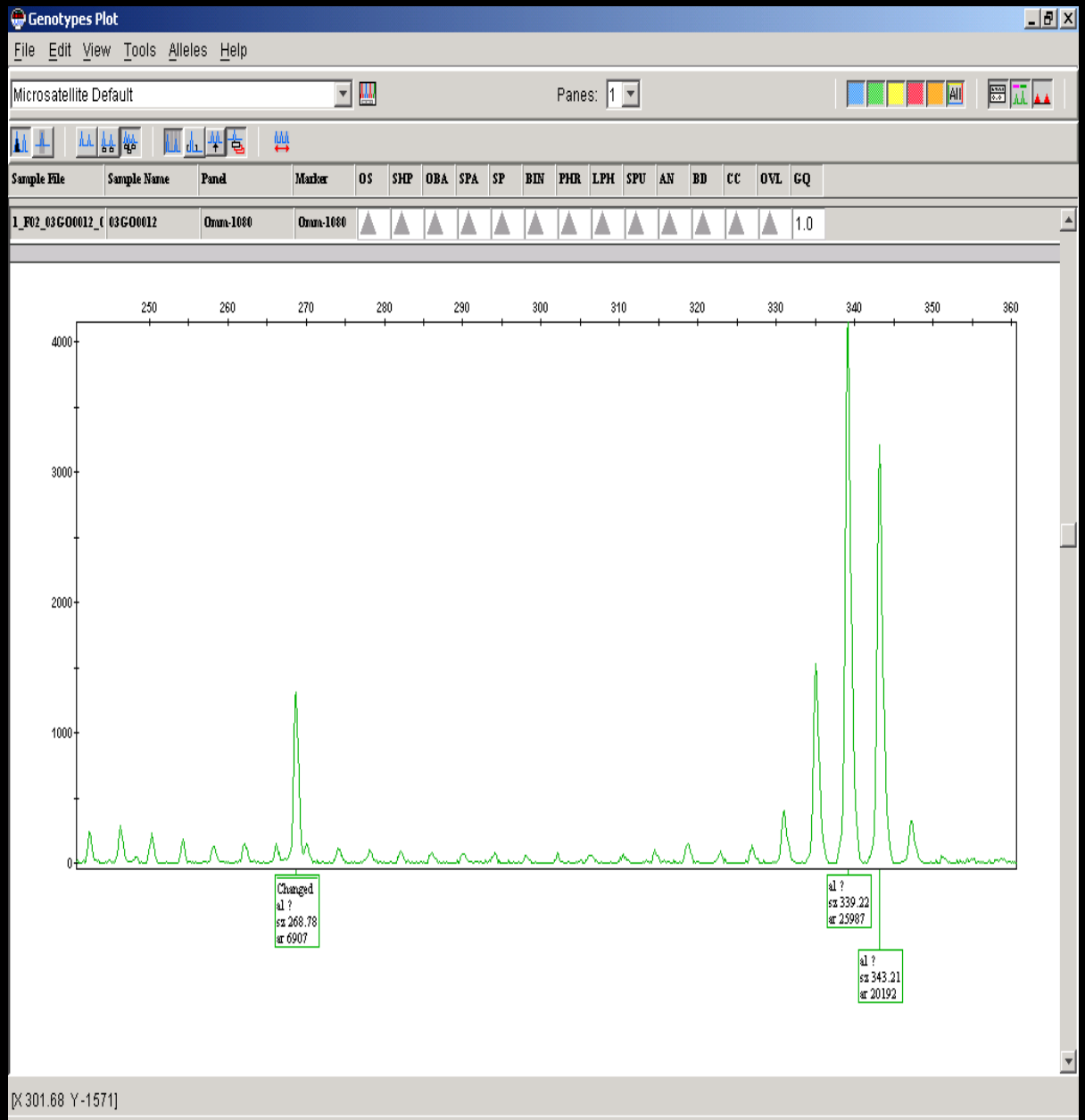
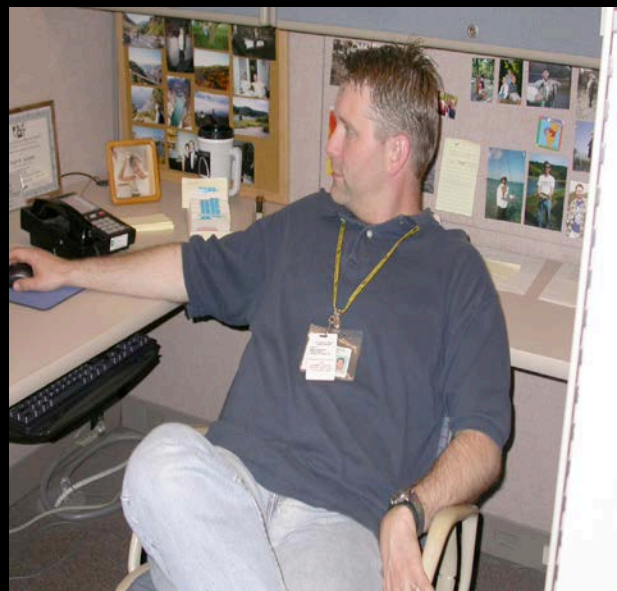
- PCR amplification was performed using microsatellite loci
- Amplified products were run through an ABI-3730 Genetic Analyzer
- Electropherograms were scored using Genemapper software v.3.0 (~31,940 individual electropherograms)
- Data was exported and binned using Microsatellite Binner v.1.h (available from S.F. Young, WDFW)

Electropherogram – Ocl-8



Todd Scoring an Electropherogram





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Locus Data

Locus	N Alleles	N parents Genotyped	H_o	H_e	Excl (1)	Excl (2)
Ogo-4	11	97	0.732	0.787	0.423	0.603
Oki-100	21	97	0.928	0.906	0.672	0.804
Omm-1080	35	97	0.959	0.958	**0.828**	**0.906**
Ots-201b	21	97	0.928	0.914	0.691	0.817
Ots-208b	25	97	0.918	0.945	0.784	0.879
Ots-211	23	97	0.897	0.929	0.735	0.847
Ots-212	18	97	0.825	0.876	0.591	0.744
Ots-213	23	97	0.918	0.937	0.759	0.863
Ots-G474	10	97	0.351	0.425	**0.097**	**0.248**
Ssa-408	18	97	0.763	0.923	0.714	0.833
Total					0.999	1.000

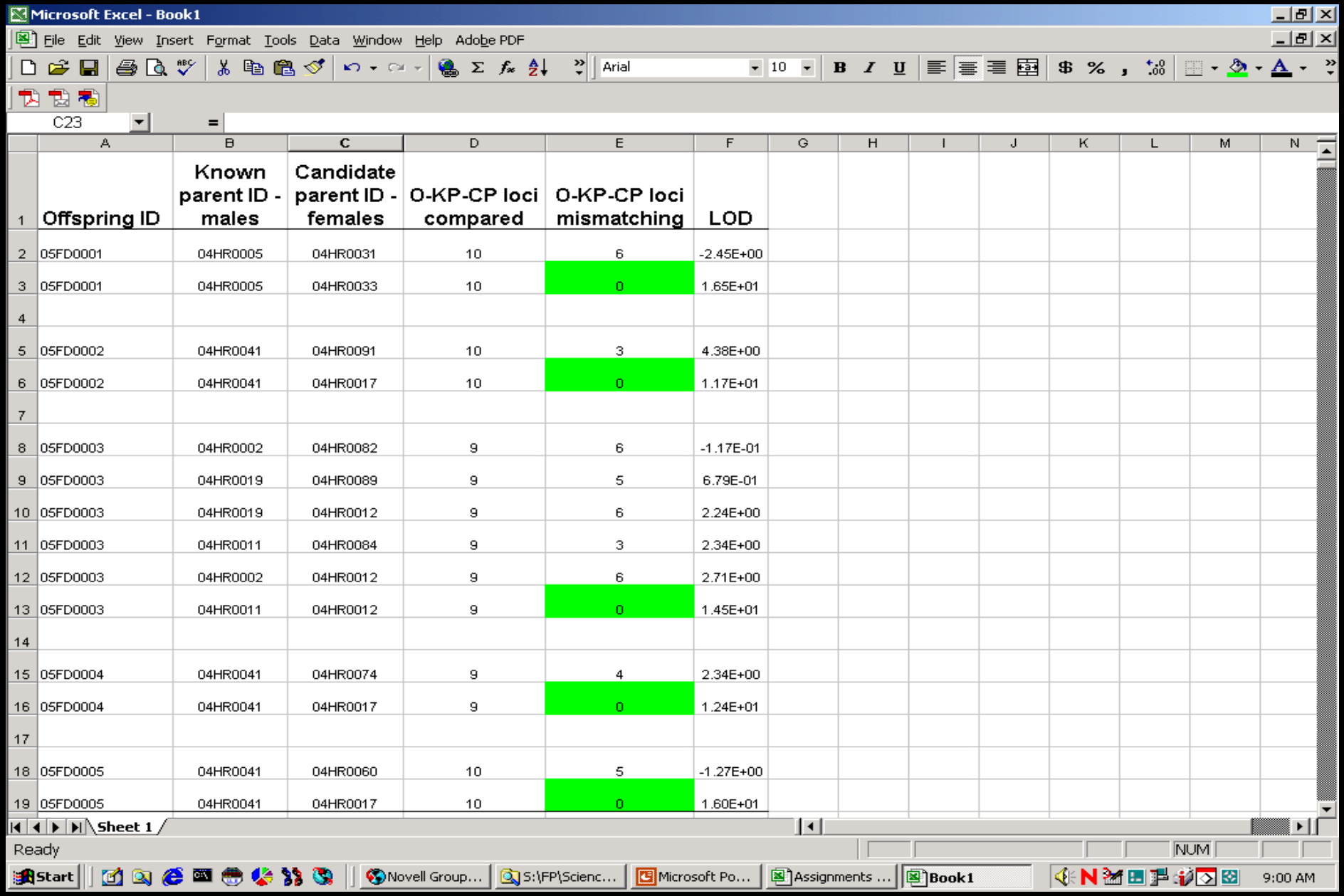
Excl (1) = Exclusionary ability of the locus when neither parent is known

Excl (2) = Exclusionary ability of the locus when one parent is known

Evaluation of Parentage Assignments

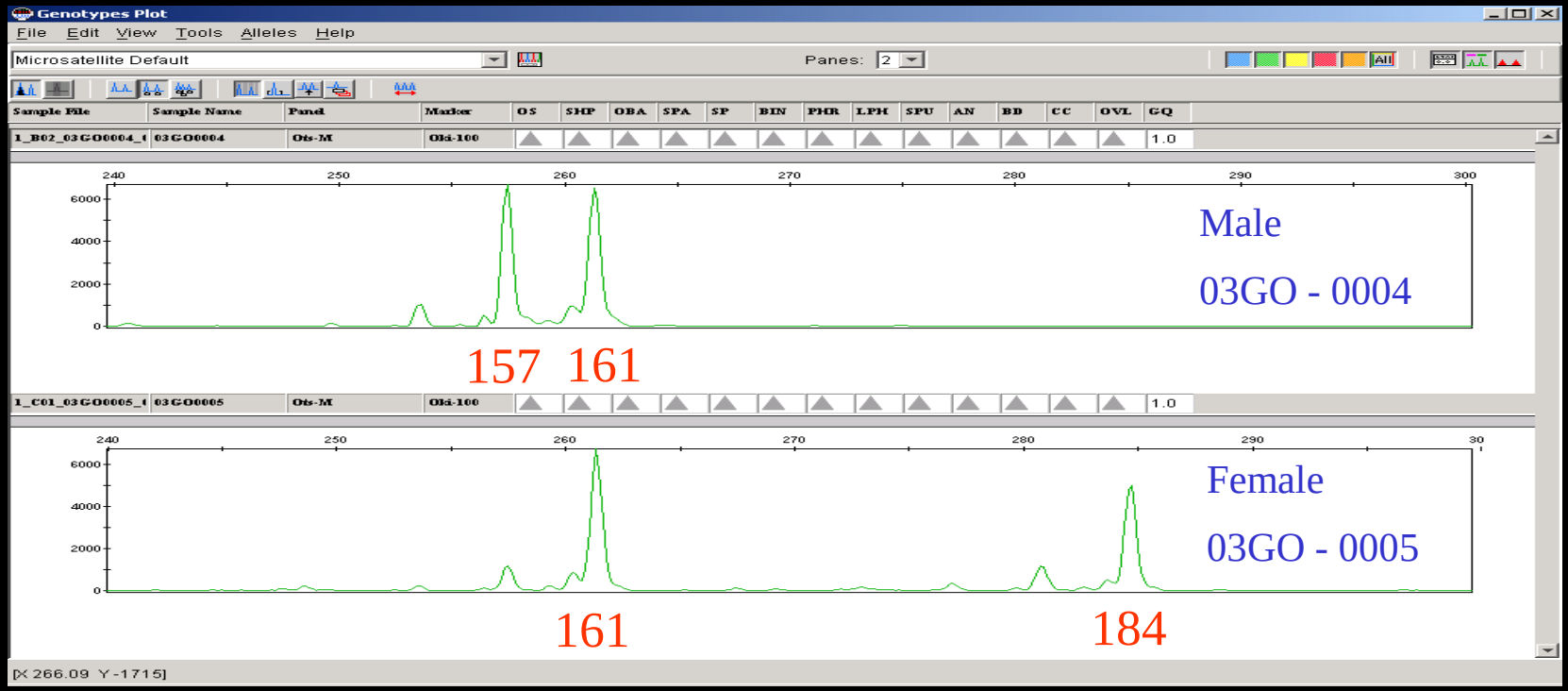
- Maximum likelihood parentage assignments performed with the program Cervus 2.0 for a sample of 3,000 offspring
- Assignments for offspring were calculated for all females that had a positive log of odds (LOD) score (multiple females will assign to an offspring at this stage)
- Assignments were then calculated for the two most likely male parents using the females as a known parent (again multiple parents will assign to an offspring at this stage)
- All assignments with negative LOD scores or greater than two mismatches were excluded as too unlikely
- Assignments for two different sets of parents with equal numbers of mismatches were excluded because a confident assignment could not be determined

Cervus output file



	A	B	C	D	E	F
1	Offspring ID	Known parent ID - males	Candidate parent ID - females	O-KP-CP loci compared	O-KP-CP loci mismatching	LOD
2	05FD0001	04HR0005	04HR0031	10	6	-2.45E+00
3	05FD0001	04HR0005	04HR0033	10	0	1.65E+01
4						
5	05FD0002	04HR0041	04HR0091	10	3	4.38E+00
6	05FD0002	04HR0041	04HR0017	10	0	1.17E+01
7						
8	05FD0003	04HR0002	04HR0082	9	6	-1.17E-01
9	05FD0003	04HR0019	04HR0089	9	5	6.79E-01
10	05FD0003	04HR0019	04HR0012	9	6	2.24E+00
11	05FD0003	04HR0011	04HR0084	9	3	2.34E+00
12	05FD0003	04HR0002	04HR0012	9	6	2.71E+00
13	05FD0003	04HR0011	04HR0012	9	0	1.45E+01
14						
15	05FD0004	04HR0041	04HR0074	9	4	2.34E+00
16	05FD0004	04HR0041	04HR0017	9	0	1.24E+01
17						
18	05FD0005	04HR0041	04HR0060	10	5	-1.27E+00
19	05FD0005	04HR0041	04HR0017	10	0	1.60E+01

Electropherogram – Oki-100



Mismatching

	Oki-100	Ots-3M	Ots-213
Female – 1	100/100	100/100	100/100
Female – 2	200/200	200/200	200/200
Male –1	120/120	120/120	120/120
Male – 2	240/240	240/240	240/240
Offspring – 1	100/120	100/120	100/120
Offspring – 2	200/240	200/240	200/240
Offspring – 3	100/120	100/120	100/240

Causes of Mismatching

- Germ-line mutation – a parent passes a changed allele to their offspring (sequence or allele changes during replication)

- PCR error (or process error) – error introduced by poor amplification from lower quality DNA extracts

- Genotyping error – inadvertent human error and computer software error in scoring due to multiple peaks being selected

Results

- 2,892 / 3,000 offspring analyzed were assigned parents

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Cumulative Results 2001 - 2005

2001

Males	Females	# fry assigned parents/Total Analyzed
18*	11*	774 / 961 = 80.5%

*Only includes natural-origin fish

2002

Males	Females	# fry assigned parents/Total Analyzed
65	41	1,775 / 2,000 = 88.8%

2003

Males	Females	# fry assigned parents/Total Analyzed
61	44	2,830 / 3,000 = 94.3%

2004

Males	Females	# fry assigned parents/Total Analyzed
51[a]	26	2,750 / 3,012 = 91.3%

[a] = 22 additional precocious males were recovered in the spring

2005

Males	Females	# fry assigned parents/Total Analyzed
47[a]	23	2,892 / 3,000 = 96.4%

[a] = 27 additional precocious males were recovered in the spring

Acknowledgements

- BPA funds for the YKFP supported this work effort
- Steve Schroder (WDFW) and Curt Knudsen (Oncor Consulting) designed the experimental spawning channel
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