DNA Based Parentage Assignments of Chinook Salmon from the Cle Elum Spawning Channel in 2004

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Background

- Joint project between WDFW (WA Dept. of Fish and Wildlife) and YN (Yakama Nation) to assess reproductive success in a closed access spawning channel at the Cle Elum Hatchery

- Difficulty in assessing reproductive success has been in controlling entry to natural spawning areas and collecting representative samples of recently hatched fry

- Microsatellite DNA analysis provide 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 in 2001, 2002, 2003, and 2004

- 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 - 2004

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

- Fry and precocious males (not initially sampled) were recovered 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 (the loci changed from 2001 – 2004)
- 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)

Jennifer setting up DNA extraction

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Sewall setting up PCRs

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Judy loading the ABI-3730

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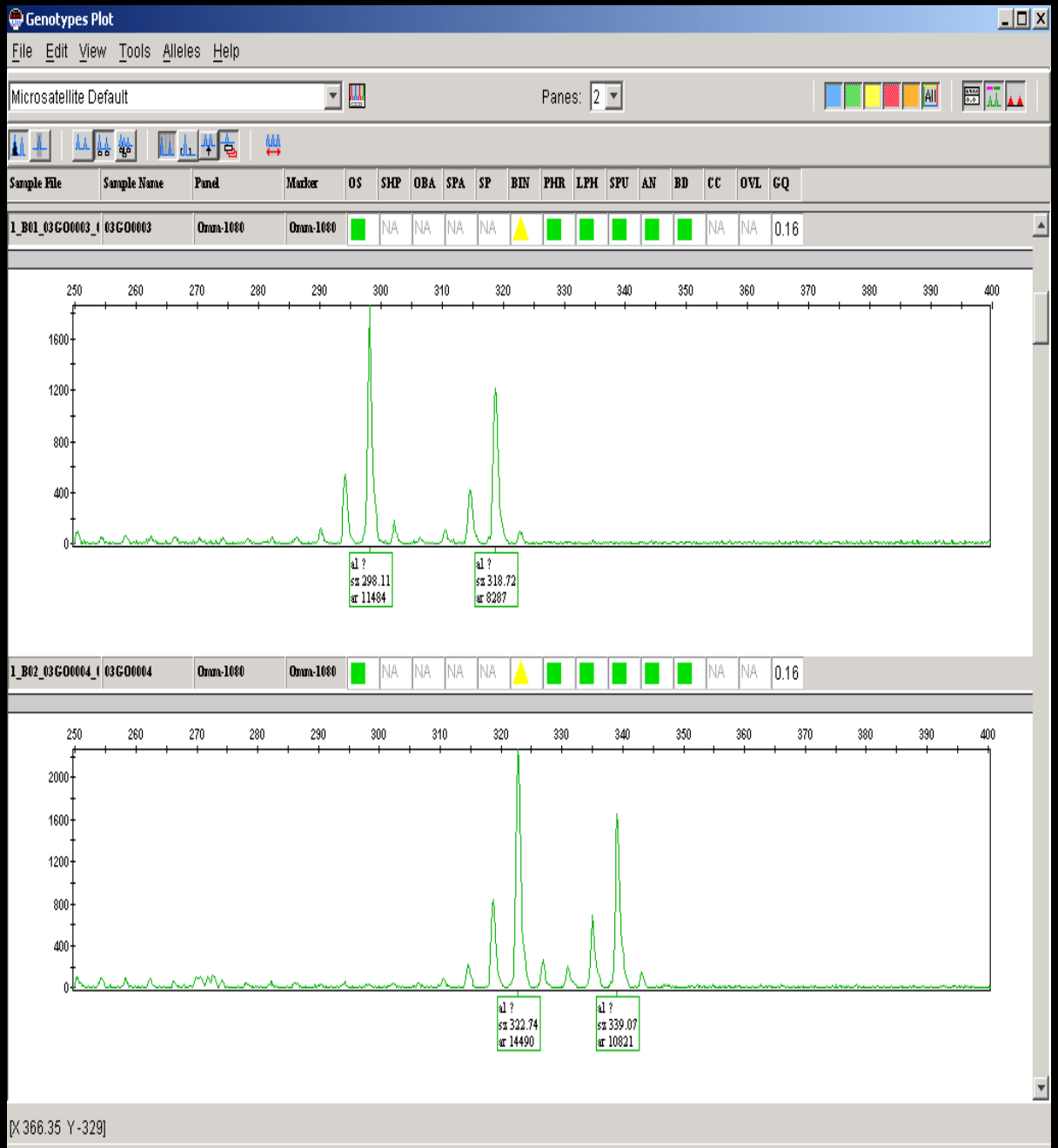
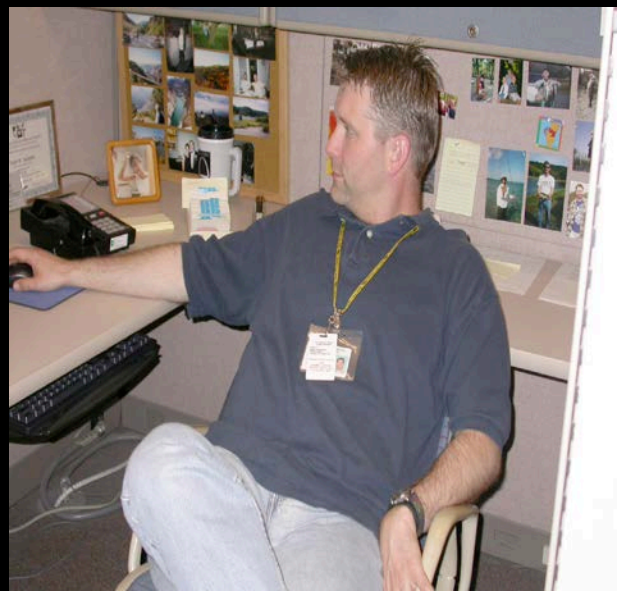
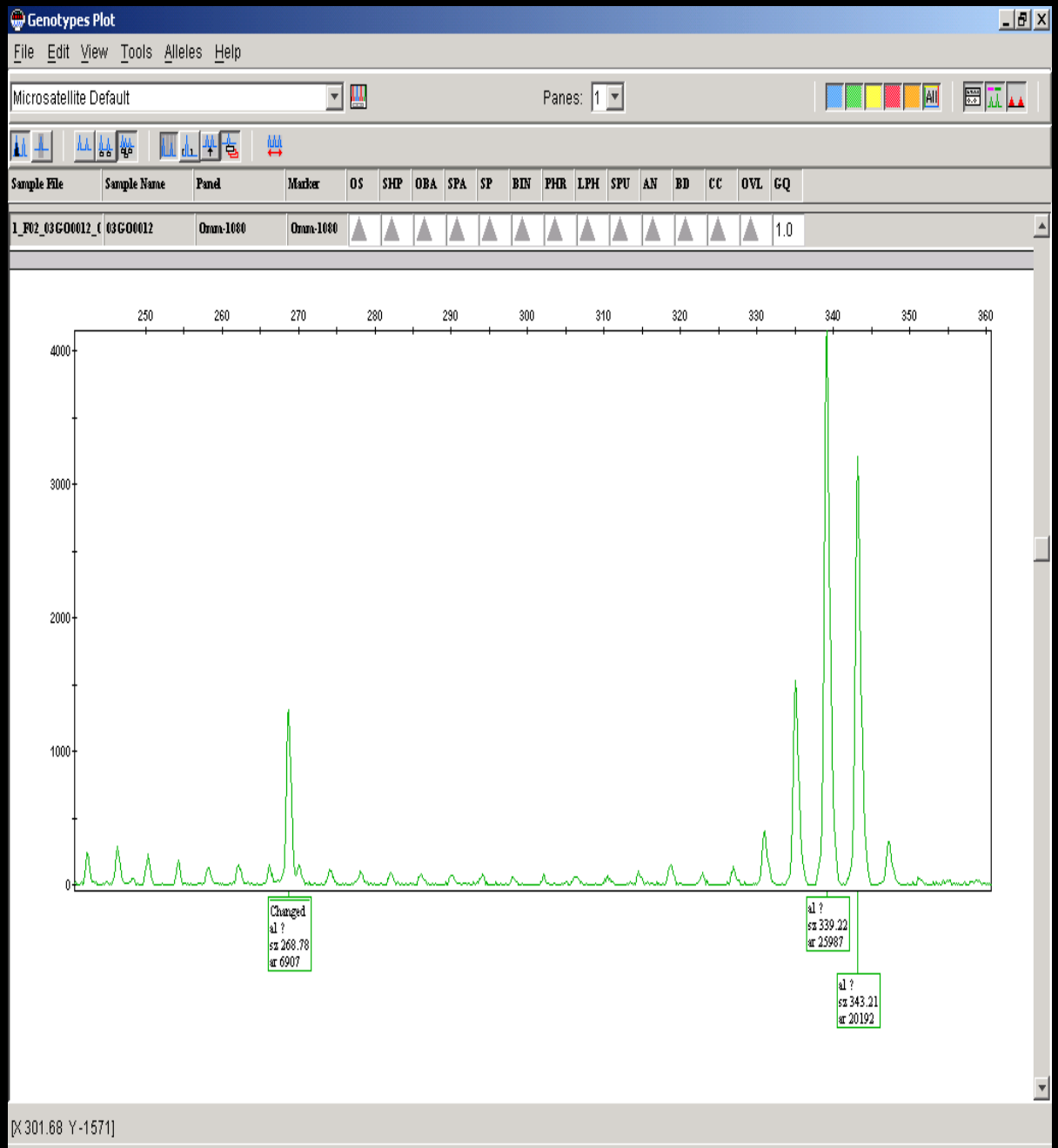
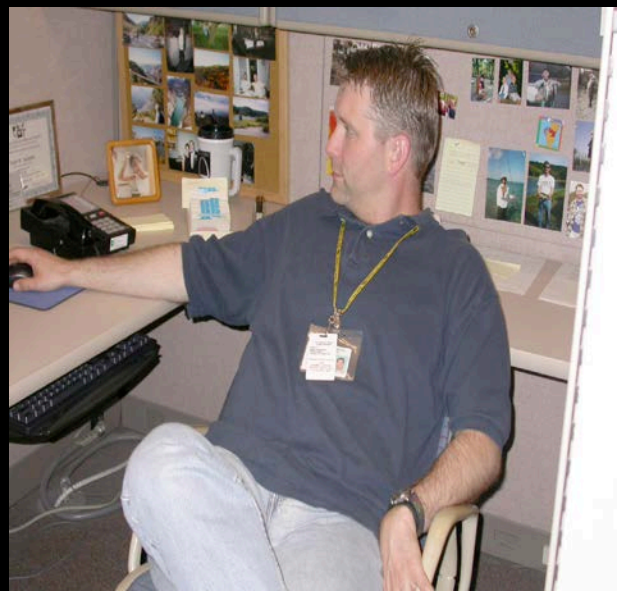
Electropherogram – Oki-100





Todd Scoring an Electropherogram





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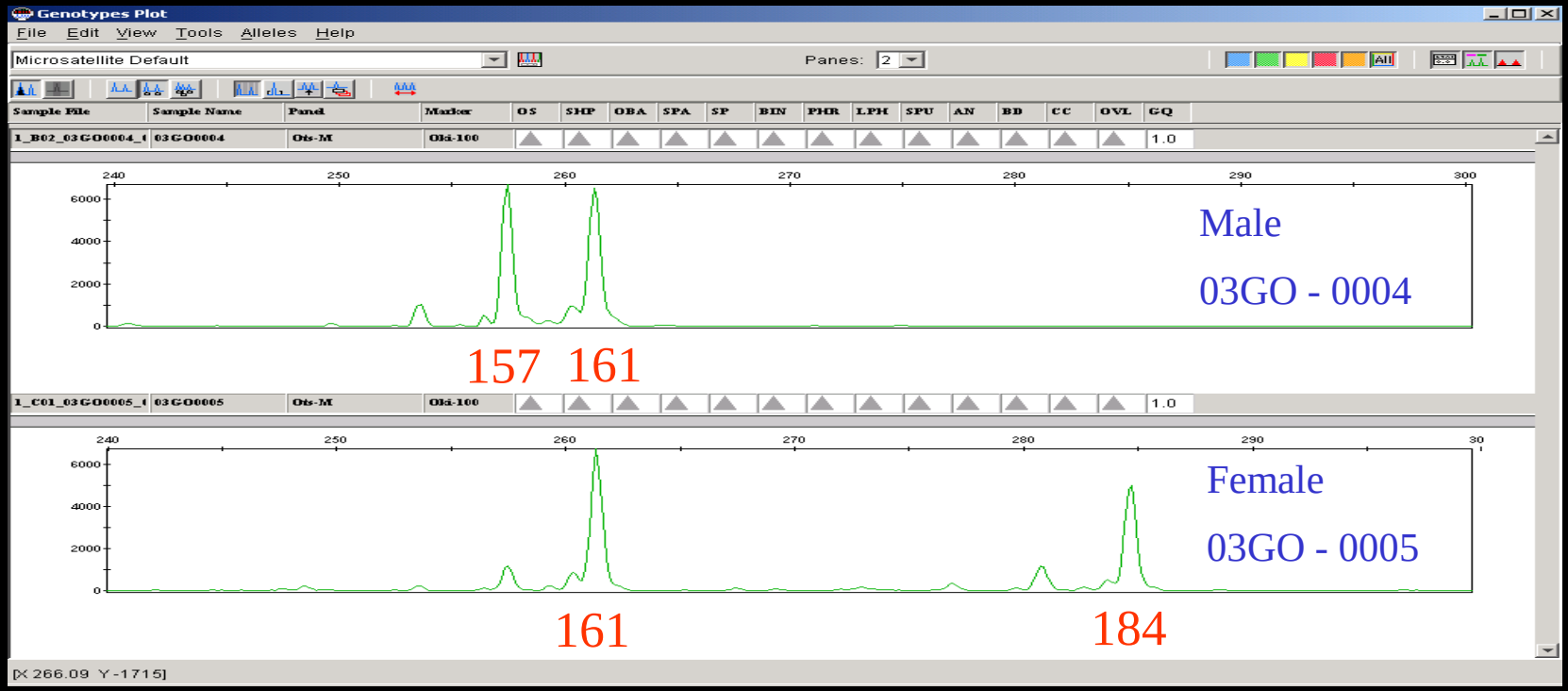
Statistical Methods

- Maximum likelihood parentage assignments performed with the program Cervus 2.0 for a sample of 3,012 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
- Final analysis yielded a total of 2,750 assignments

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

- 2,750 / 3,012 offspring analyzed were assigned parents

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Cumulative Results

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

Results from DNA based parentage analysis in 2004

2004

Females		% of total	Ave. # per Female	Males		% of total	Ave. # per Male
A - H	1,615	58.7%	44.7	A - H	924	33.6%	30.2
A - N	1,135	41.3%	31.4	A - N	1,465	53.3%	37.7
				J - H	65	2.4%	4.7
				J - N	86	3.1%	31.0
				P - H	67	2.4%	3.5
				P - N	137	5.0%	3.8
				P - ?	6	0.2%	0.1
Total	2,750	100.0%		Total	2,750	100.0%	

Cumulative Results from DNA based parentage analysis

2001

Females		% of total	Ave. # per Female	Males		% of total	Ave. # per Male
A - N	774	100.0%	73.2	A - N	754	97.4%	49.0
				J - N	0	0.0%	0.0
				P - N	17	2.2%	17.7
				? - N	3	0.4%	3.1
Total	774	100.0%		Total	774	100.0%	

2002

Females		% of total	Ave. # per Female	Males		% of total	Ave. # per Male
A - H	712	40.1%	19.0	A - H	654	36.9%	21.5
A - N	1,063	59.9%	29.8	A - N	945	53.3%	23.0
				J - H	39	2.2%	5.5
				J - N	5	0.3%	1.4
				P - H	127	7.2%	10.1
				P - N	1	0.0%	0.1
				P - ?	2	0.1%	0.2
Total	1,775	100.0%		Total	1,773	100.0%	

2003

Females		% of total	Ave. # per Female	Males		% of total	Ave. # per Male
A - H	1,317	46.5%	21.0	A - H	829	29.3%	16.1
A - N	1,513	53.5%	24.1	A - N	1,632	57.7%	28.6
				J - H	13	0.5%	2.3
				J - N	75	2.6%	13.1
				P - H	224	7.9%	6.5
				P - N	48	1.7%	3.4
				P - ?	9	0.3%	1.6
Total	2,830	100.0%		Total	2,830	100.0%	

2004

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A - H	1,615	58.7%	44.7	A - H	924	33.6%	30.2
A - N	1,135	41.3%	31.4	A - N	1,465	53.3%	37.7
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				P - N	137	5.0%	3.8
				P - ?	6	0.2%	0.1
Total	2,750	100.0%		Total	2,750	100.0%	

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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