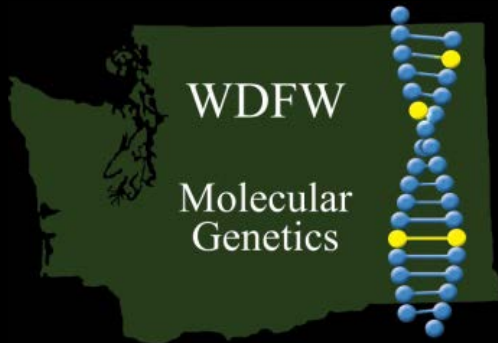


# DNA-Based Pedigree Analysis of Chinook Salmon from the Yakima River

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## Background

- Joint project between WA Department of Fish and Wildlife (WDFW) and Yakama Nation (YN)
- Project objective is to assess the relative reproductive success of Chinook in the upper Yakima River
- Collection of hatchery-origin adult males and females, jacks, and precocious male Chinook occurred at Roza Dam from 2003 – 2006
- Collection of both hatchery- and natural-origin Chinook has occurred from 2007 - present
- Genetic analysis using microsatellite DNA loci is used to determine parentage. Methodology used for the analysis is the same as we have used for the Cle Elum spawning channel

## 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.3.7
- Data was binned using GAPS allele naming

# Cherril setting up DNA extraction



## 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
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- Data was binned using GAPS allele naming

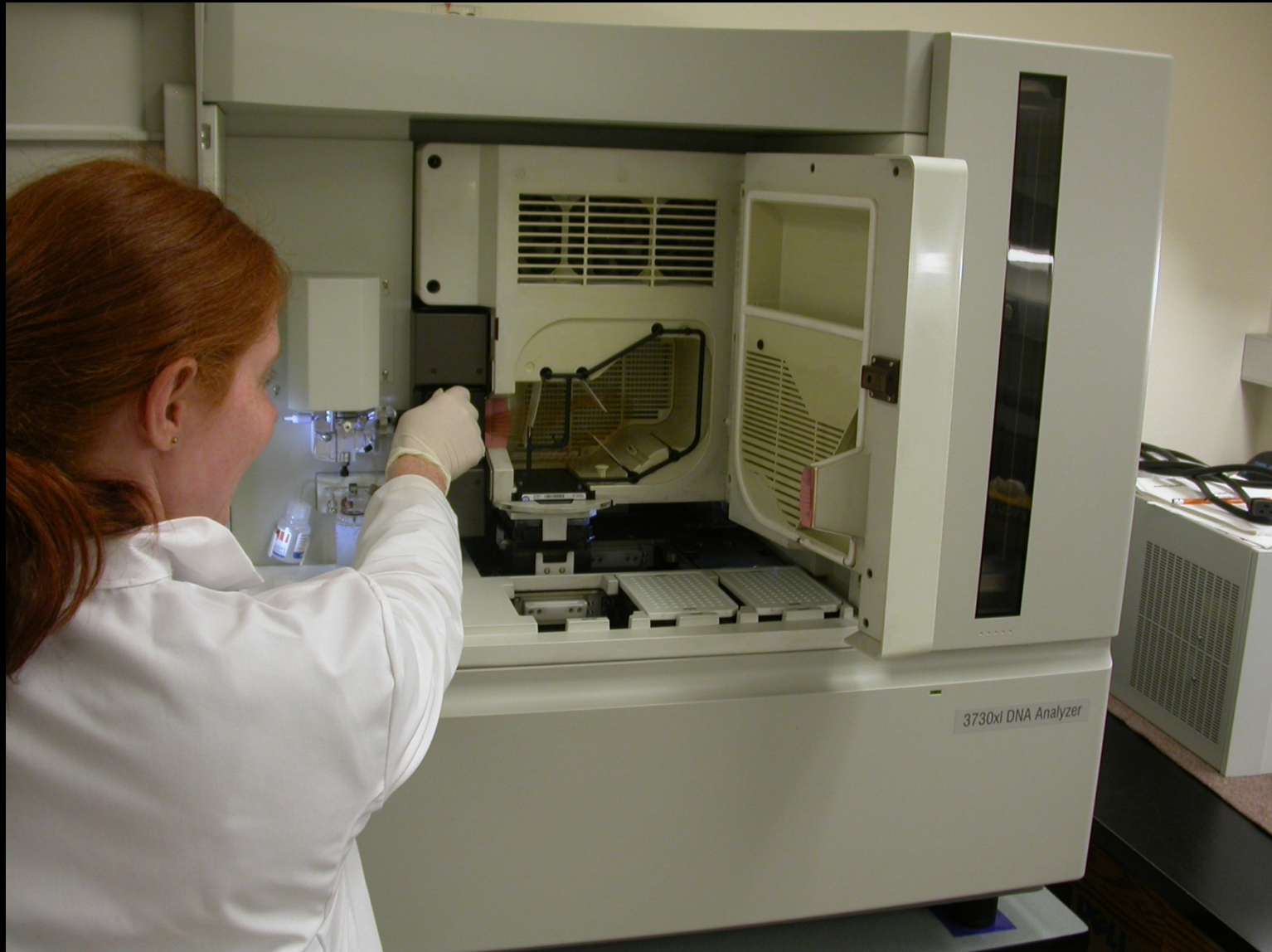
# Cheryl setting up PCR reaction



## 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.3.7
- Data was binned using GAPS allele naming

# Jennifer loading the ABI-3730





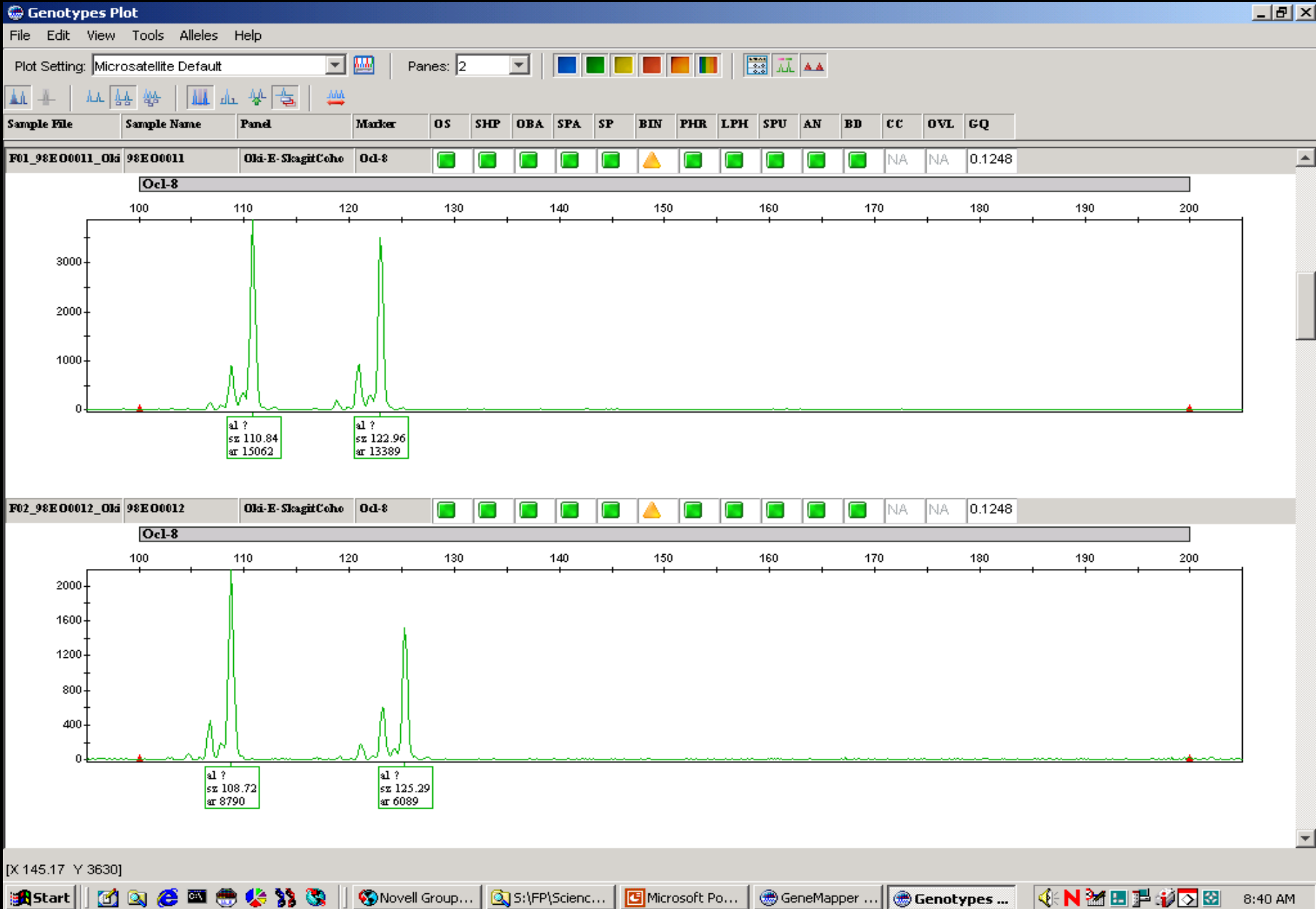
## Laboratory Methods

- 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.3.7 (~40,000 individual electropherograms)
- Data was binned using GAPS allele naming

# Jennifer Scoring an Electropherogram



# Electropherogram – Ocl-8



## 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.3.7
- Data was binned using GAPS allele naming

## Locus Data

Locus	N Alleles	N parents Genotyped	H <sub>o</sub>	H <sub>e</sub>	Excl (1)	Excl (2)
Ogo-2	11	2,186	0.825	0.821	0.475	0.648
Ogo-4	11	2,188	0.801	0.806	0.456	0.632
Oki-100	26	2,117	0.919	0.904	0.682	0.811
Omm-1080	44	2,162	0.937	0.961	0.852	0.920
Ots-201b	29	2,118	0.915	0.904	0.679	0.809
Ots-208b	29	2,115	0.930	0.941	0.787	0.880
Ots-211	28	2,123	0.930	0.931	0.757	0.861
Ots-212	24	2,182	0.887	0.887	0.631	0.774
Ots-213	29	2,185	0.921	0.936	0.769	0.869
Ots-3M	9	2,185	0.652	0.651	0.254	0.435
Ots-9	6	2,186	0.678	0.656	0.237	0.400
Ots-G474	13	2,190	0.362	0.367	0.072	0.211
Ssa-197	25	2,180	0.902	0.906	0.683	0.812
Ssa-408	27	2,160	0.728	0.916	0.709	0.830

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

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

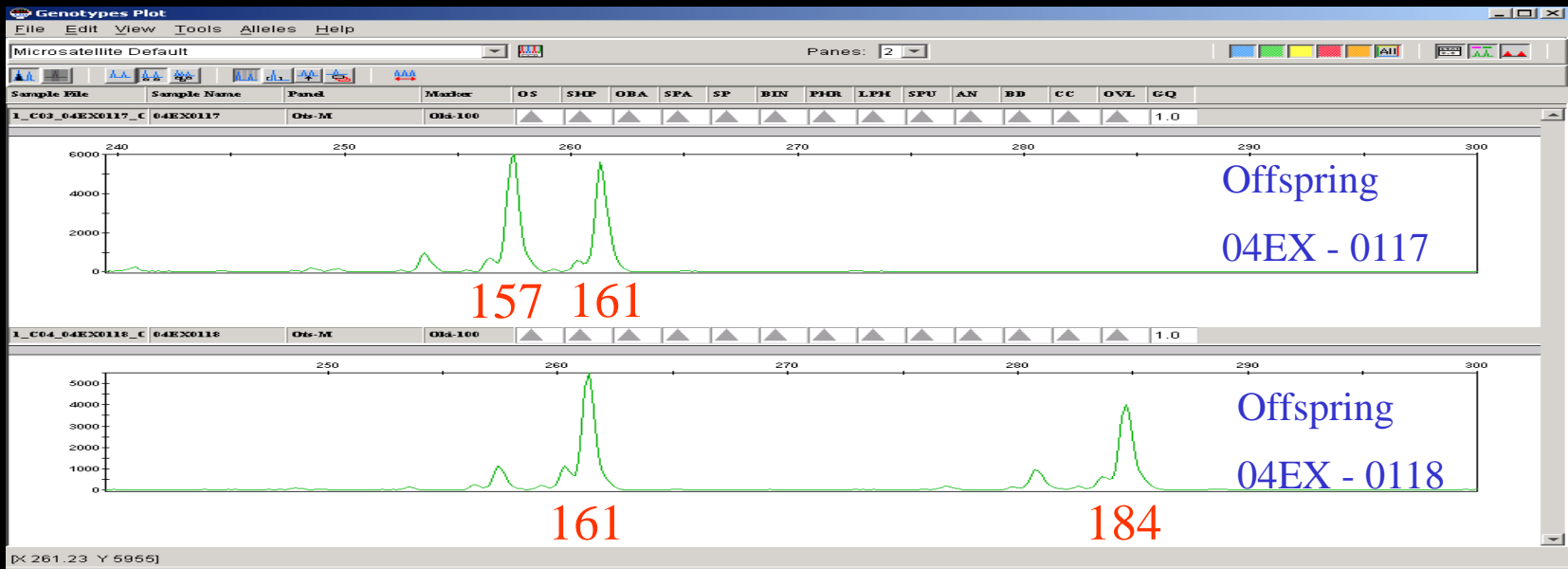
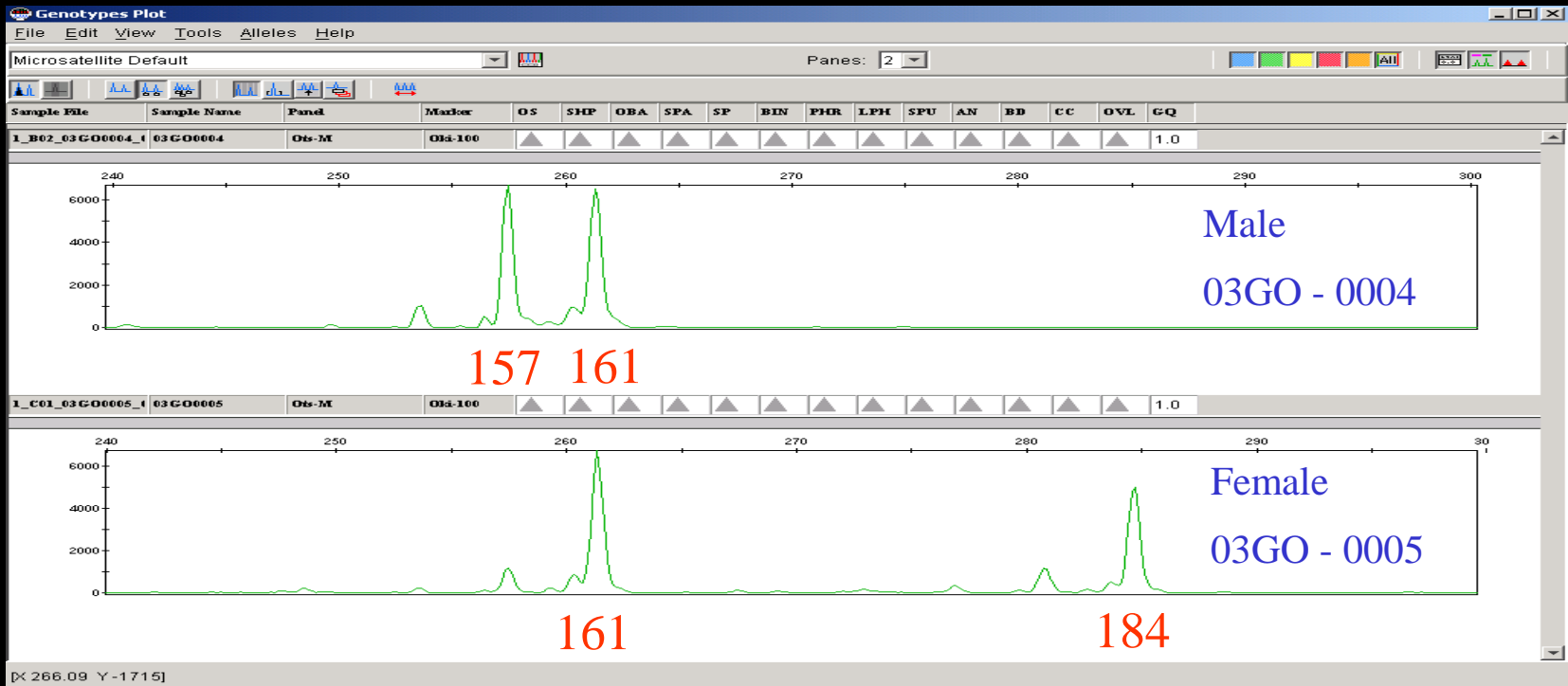
# Evaluation of Parentage Assignments

- Maximum likelihood parentage assignments performed with the program CERVUS 3.0
- Assignments for offspring were calculated for the most likely male and female parent pair. The parent pair assignment with two mismatches or less was accepted
- Individuals that did not assign to a parent pair were then analyzed for a female parent only and male parent only (assignments with zero or one mismatches were accepted)

## 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

# 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

**Expected proportion -  
Hatchery- and Natural-origin Chinook in 2007 return**

- **2,284 – Hatchery-origin Chinook count at Roza Dam**
  - **1,558 / 1,147 – Natural-origin Chinook count at Roza Dam  
(411 – Natural-origin Chinook brood)**
  - **$2,284 / 3,431 = 0.6657 - P$  ;  $1,147 / 3,431 = 0.3343 - Q$**
- 
- **44.3% Hatchery-origin (H X H) –  $P^2$**
  - **44.5% Hatchery & Natural-origin (H X N & N X H) –  $2PQ$**
  - **11.2% Natural-origin (N X N) -  $Q^2$**

**Observed returns -  
Hatchery- and Natural-origin Chinook**

- **229 / 1,153** offspring were assigned parental pair  
Hatchery X Hatchery (**19.9%**)
- **443 / 1,153** offspring were assigned a mother only  
Hatchery X Natural (**38.4%**)
- **163 / 1,153** offspring were assigned a father only  
Natural X Hatchery (**14.1%**)
- **318 / 1,153** offspring did not assign a mother or father  
Natural X Natural (**27.6%**)

## Comparison of Expected and Observed Percentages of Hatchery and Natural-Origin Chinook

	Expected	Observed
<b>H X H</b>	<b>45.0%</b>	<b>19.9%</b>
<b>H X N &amp; N X H</b>	<b>44.0%</b>	<b>52.5%</b>
<b>N X N</b>	<b>11.0%</b>	<b>27.6%</b>

# Conclusions

- Preliminary data –
  - Still need to calculate assignment errors (probability of assigning incorrect parent)
  - Estimate significance of the assignments
    - The number of observed natural-origin Chinook is higher than expected
    - The number of observed hatchery-origin Chinook is lower than expected
    - More hatchery-origin females assigned as a parent than hatchery-origin males

## Future Work

- Statistical analysis of 1999 and 2000 brood to determine an error rate for calculating N X N offspring in the 2007 and 2008 collections
- Analysis of 2004 adults (completed this year)
- Analysis of 2008 offspring (scheduled for this upcoming year)
- Analysis of third generation (2011 and 2012 returns)

## Acknowledgements

- BPA funds for the YKFP supported this work effort
- Mark Johnston and crew from the Yakama Nation at Roza Dam for collecting samples
- Jennifer Von Barga for all laboratory analysis