

Genetic Sex of Chinook Salmon in the Columbia River Basin

Final Report
2001 - 2004



This Document should be cited as follows:

Nagler, James, Gary Thorgaard, "Genetic Sex of Chinook Salmon in the Columbia River Basin", 2001-2004 Final Report, Project No. 200100800, 9 electronic pages, (BPA Report DOE/BP-00004752-1)

Bonneville Power Administration
P.O. Box 3621
Portland, OR 97208

This report was funded by the Bonneville Power Administration (BPA), U.S. Department of Energy, as part of BPA's program to protect, mitigate, and enhance fish and wildlife affected by the development and operation of hydroelectric facilities on the Columbia River and its tributaries. The views in this report are the author's and do not necessarily represent the views of BPA.

Genetic Sex of Chinook Salmon in the Columbia River Basin

Project number: 2001-008-00
Contract Number: 00004752

Reporting period: May 2001- March 2003

James J. Nagler,
Department of Biological Sciences,
University of Idaho,
Moscow, ID 83844-3051

Gary Thorgaard,
School of Biological Sciences,
Washington State University,
Pullman, WA 99164-4236

Dennis Dauble
Natural Resources Department,
Environmental Technology Division,
Battelle,
Richland, WA 99352

August 2004

(A) Abstract

It has previously been documented that naturally spawning, female fall-run chinook salmon (*Oncorhynchus tshawytscha*) from the Hanford Reach on the Columbia River carried a male-linked genetic marker, *OtY1*. The primary objective of this study was to investigate the spatial and temporal nature of this observation by sampling adults from the Hanford Reach and two other new sites, the lower Yakima River and at Ives Island below Bonneville Dam, in sequential years (2001 and 2002). A hatchery population on the Columbia River (Priest Rapids Hatchery) was sampled too. A total of 364 male and 359 female fall-run chinook salmon were sampled over the two spawning seasons from all sites. Detection of the male-linked genetic marker *OtY1* in male fish was found to be 100% consistent with the phenotypic male sex in all populations sampled. A variable proportion of females from all sites tested positive for *OtY1*. This ranged from 67% at Ives Island in 2001 to 0% at the Priest Rapids Hatchery in 2002. A significant reduction of *OtY1* incidence in females sampled in 2002 compared to 2001 emerged at all sites. A second objective sought evidence that a YY genotype might exist in the populations sampled. A real-time PCR method based on the growth hormone pseudogene (*GHP*), another male-linked chinook salmon genetic marker, was developed and tested to determine whether a YY genotype might be detected. The experimental variability between different XY individuals precluded its use to discriminate an YY from an XY genotype. During associated work on new chinook salmon sex linked genetic markers a new Y chromosome linked genetic marker, *OtY2*, was discovered.

(B) Introduction

In 1999 we used a molecular test, based on a DNA marker linked to the Y-chromosome (*OtY1*), to investigate whether naturally-spawning male and female fall-run chinook salmon (*Oncorhynchus tshawytscha*) from the Columbia River were faithfully expressing their genetic sex (Nagler et al. 2001). We tested if fall-run chinook salmon from the Hanford Reach of the Columbia River with a male phenotype possessed this marker, and conversely, if phenotypic females did not. Our results showed that a high proportion (84%) of phenotypic female salmon were positive for the male-linked DNA marker. This finding was significant because females positive for *OtY1* had not been previously reported before in any naturally spawning population of Pacific salmon. The biological significance of female chinook salmon carrying a male-linked genetic marker is unknown. The intent of this project was to investigate how widespread the occurrence of this observation was and by doing so provide information to help determine the impact on salmon populations.

The first objective was to determine the temporal and spatial incidence of male-specific DNA markers in female fall-run chinook salmon in the Columbia River Basin. The hypothesis was that we would find evidence for male-linked DNA markers in female-appearing fish throughout the basin. To test this three sites were selected, the Hanford Reach, lower Yakima River, and at Ives Island below Bonneville dam. Each site supports an accessible, naturally spawning population of fall-run chinook salmon that were geographically separated. These sites were sampled in 2001 and 2002 to provide

data to address how widespread this observation might be and whether it was consistently observed in different year classes from each site.

The second objective was to test for the presence of an abnormal YY genotype in male fall-run chinook salmon from the Columbia River Basin. This genotype could potentially occur in offspring of females carrying the male-linked marker (if they were truly XY) upon mating with a normal XY male. If fish with a YY genotype were detected this would support the idea that the “female” parent must have had a XY genotype. Sex reversal of genotypic males (XY) to phenotypic females due to some unknown environmental agent(s) has been raised as a possibility to explain these observations. The hypothesis was that male salmon with a YY genotype could be detected returning to spawn in the populations sampled. Evidence of the scope of the problem we have identified, and presence and number of YY males, might be used to predict effects upon the spawning populations sampled. A second component of this objective was an effort to develop X chromosome linked markers for chinook salmon, that could be used to show whether a YY genotype was present. It was expected due to the nature of this work that additional Y chromosome markers might result also.

(C) Description of project area

The three study sites are all part of the Columbia River watershed. The most up-river site was the Hanford Reach (90 km long) beginning below Priest Rapids Dam (639 km upstream) and extending down to the upper end of McNary Reservoir. This part of the mainstem primarily flows through the Hanford Nuclear Reservation. The Yakima River is a major tributary of the Columbia River that enters the mainstem within the McNary Reservoir. The Yakima River flows through a region predominated by agricultural activities. The Ives Island site is situated approximately 1 km below Bonneville Dam (235 km upstream), the lowermost dam on the mainstem Columbia River. This region is characterized by a collection of small islands (the number depending on water flow and depth).

(D) Methods and Materials

Post-spawned male and female fall-run chinook salmon carcasses were collected in 2001 and 2002 from three different locations within the Columbia River watershed: the Hanford Reach, Yakima River, and vicinity of Ives Island. In addition, fish that had returned to the Priest Rapids Hatchery on the Hanford Reach were also sampled after they were killed for gamete sampling. The phenotypic sex of each fish was confirmed by gross morphological examination of the gonads. A piece of pectoral fin tissue or gonad was removed and preserved in 95% ethanol using scissors and forceps that had been cleaned with either 95% ethanol or a 10% bleach solution. DNA was isolated from each tissue sample using a Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN) and a protocol for fixed or solid tissue. The concentration and quality of each DNA sample was calculated with UV spectrophotometry and the ratio of optical absorbance at A260nm/A280nm, respectively.

For the first objective qualitative polymerase chain reaction (PCR) for the male-linked genetic marker, *OtY1*, was conducted using a *Taq* DNA Polymerase kit (Gibco

BRL, Rockville, MD) with primers Y1 and Y2 as per Devlin et al. (1994). The PCR conditions are described in Nagler et al. (2001).

For the second objective a fluorescence in situ-hybridization technique (Stein et al. 2001) was initially examined. This method was based on obtaining blood samples collected from juveniles and isolating white blood cells (WBCs) that could be probed with a *OtY1* DNA sequence labeled with a fluorescent marker. The intent was to visualize the number of chromosomes marked with the fluorescent probe, where one chromosome marked would indicate an XY while two chromosomes marked would represent a YY individual. During initial testing of the method we were unable to isolate sufficient numbers of high quality WBCs to perform the hybridization. This approach was discontinued in favor of a real-time PCR method to test whether the chinook salmon growth hormone pseudogene (*GHP*) could be used to discriminate between XY and YY male chinook salmon. The *GHP* is another genetic marker, present as a single gene copy, reported to be male-linked in chinook salmon (Du et al. 1993). A real-time PCR approach was used because it is quantitative, with the intent of being able to quantify the amount of *GHP* in XY and YY fish. The YY fish were expected to show twice the amount of *GHP* signal compared to XY fish. The method and conditions for the *GHP* real-time PCR assay are described in Nagler et al. (2004).

(E) Results and Discussion

A total of 364 male and 359 female fall-run chinook salmon were sampled over the two spawning seasons (2001 and 2002) from all sites. Detection of the male-linked genetic marker *OtY1* in male fish was found to be 100% consistent with the phenotypic male sex in all populations sampled from the Columbia River (Table 1). These data provide strong evidence that the *OtY1* genetic marker is male-linked in fall-run chinook salmon from the four sites sampled in this study.

Table 1: Incidence of the male-linked genetic marker *OtY1* in adult male and female fall-run chinook salmon sampled from various sites on the Columbia River in 2001 and 2002.

Site	Sex	2001		2002	
		<i>OtY1</i> positive	<i>OtY1</i> negative	<i>OtY1</i> positive	<i>OtY1</i> negative
Hanford Reach	M	45	0	50	0
	F*	19	27	4	46
Ives Island	M	45	0	20	0
	F*	11	5	2	34
Yakima River	M	38	0	23	0
	F*	8	8	3	47
Priest Rapids Hat.	M	98	0	45	0
	F*	41	54	0	50

* significant difference ($p < 0.05$; Fisher's Exact Test) in the number of positive/negative fish between years

With the females, variable proportions at all sites except the Priest Rapids Hatchery in 2002 tested positive for *OtY1* (Table 1). Therefore, similar to our first report on female fall-run chinook salmon from the Hanford Reach in 1999 (Nagler et al. 2001) a proportion of females from the Hanford Reach site in subsequent years continue to test positive for *OtY1*. Also significant were female fall-run chinook salmon from other naturally spawning populations (i.e., Yakima River and Ives Island) on the Columbia River consistently containing individuals that carry *OtY1* too. Therefore, the occurrence of *OtY1* in female chinook salmon is not exclusive to the Hanford Reach, but found in populations further downstream also.

There was a statistically significant reduction in incidence of females testing positive for *OtY1* between 2001 and 2002 at all sites (Table 1). Among the naturally spawning populations, the greatest decrease was observed at Ives Island where incidence levels went from 67% positive in 2001 to 6% in 2002. At other sites, the decrease was intermediate compared to Ives Island. The Hanford Reach population has been sampled the longest and a profile extending back four years to 1999 can be generated (Table 2). This naturally spawning population shows a steady decrease from a high in 1999 of 84% to 8% in 2002. The possibility that differences between year classes, based on scale aging, might be part of this decrease was shown not to be significant in these populations sampled in 2000 and 2001 (Chowen and Nagler, 2004).

The basis for the pattern of gradual decrease of *OtY1* in females is not known, but a couple of interpretations can be made. If an environmental agent is causing, for example, sex reversal in these fish then the severity of this agent has been steadily diminishing. Fewer and fewer positive fish are being detected in the naturally spawning populations that have been sampled. At the current rate of decline on the Hanford Reach in two more years no phenotypic females will be detectable that carry *OtY1*. An alternative is that the occurrence of *OtY1* in some females is the result of an unusual chromosomal rearrangement in males that resulted in movement of *OtY1* from the Y-chromosome to the X chromosome and/or a non-sex chromosome(s) (i.e., an autosome). Because *OtY1* is tandemly repeated approximately 300 times on the Y chromosome (Devlin et al. 1998) some or all could be translocated to other chromosomes. Upon fertilization the chromosome(s) carrying *OtY1* from the male parent could enter the genome of an otherwise normal female. This would produce a phenotypic female with a “normal” XX genotype, but carrying *OtY1* within the genome. The gradual decline in incidence of *OtY1* positive females would suggest that for some reason these fish are not returning to breed. If this is the case further questions are raised, why are these females being selected against and what would cause male sex chromosomal rearrangements in the first place?

Table 2 Incidence of the male-linked genetic marker *OtY1* in adult female fall-run chinook salmon sampled from the Hanford Reach during 1999-2002.

Year	% <i>OtY1</i> positive (n)	% <i>OtY1</i> negative (n)
1999	84 (42)	16 (8)
2000	72 (34)	28 (13)
2001	41 (19)	59 (27)
2002	8 (4)	92 (46)

A real-time quantitative PCR method utilizing the chinook salmon *GHP* was developed (Nagler et al. 2004) with the purpose being to discriminate between XY and YY male chinook salmon. The intent of this objective being to detect whether a YY genotype existed in any of the populations in the Columbia River sampled in this study. This method turned out to discriminate well between chinook salmon with XY and XX genotypes, that is the presence or absence of a Y chromosome, but could not be used to differentiate between XY and YY. Unfortunately, enough experimental variability exists between different XY individuals that this method is not able to consistently resolve an XY genotype from a YY genotype. Therefore, at this time the presence of YY chinook salmon individuals in the Columbia River is not known.

During work to discover chinook salmon sex chromosome linked genetic markers a new Y-chromosome linked genetic marker was found. This new marker, called *OtY2*, has been accepted for publication (Brunelli and Thorgaard, 2004).

(F) References

- Brunelli, J.P. and Thorgaard, G.H. (2004) A new Y chromosome-specific marker for Pacific salmon. *Trans. Am. Fish. Soc.* (in press)
- Chowen, T.R. and Nagler, J.J. (2004) Temporal and spatial occurrence of female chinook salmon carrying a male-specific genetic marker in the Columbia River watershed. *Environ. Biol. Fish.* 69: 427-432.
- Devlin, R.H., McNeil, B.K., Solar, I.I., and Donaldson, E.M. (1994) A rapid PCR-based test for Y-chromosomal DNA allows simple production of all-female strains of chinook salmon. *Aquaculture* 128: 211-220.
- Devlin, R.H., Stone, G.W., and Smailus, D. E. (1998) Extensive direct-tandem organization of a long repeat DNA sequence on the Y chromosome of chinook salmon (*Oncorhynchus tshawytscha*). *J. Mol. Evol.* 46:277-287.
- Du, S.J., Devlin, R.H., and Hew, C.L. (1993) Genomic structure of growth hormone genes in chinook salmon (*Oncorhynchus tshawytscha*): presence of two functional genes, GH-I and GH-II, and a male-specific pseudogene, GH-?. *DNA and Cell Biology* 12:739-751.
- Nagler, J.J., Bouma, J., Thorgaard, G.H., and Dauble, D.D. (2001) High incidence of a male-specific genetic marker in phenotypic female chinook salmon from the Columbia River. *Environ. Health. Perspect.* 109: 67-69.
- Nagler, J.J., Cavileer, T., Steinhorst, K., and Devlin, R.H. (2004) Determination of genetic sex in chinook salmon (*Oncorhynchus tshawytscha*) using the male-linked growth hormone pseudogene by real-time PCR. *Mar. Biotechnol.* 6:186-191.

Stein, J., Phillips, R.B., and Devlin, R.H. (2001) Identification of the Y chromosome in chinook salmon (*Oncorhynchus tshawytscha*) Cytogen. Cell Genet. 92:108-110.