Validation of DNA Gender Test for Yukon and Kuskokwim River Chinook Salmon

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Introduction

The low percentages of female Chinook salmon commonly observed at escapement monitoring stations (e.g., Table 1) have been of concern for several years. Biologists have speculated whether the low percentage of females is caused by a natural factor, such as gender-specific differences in life history strategies or survival rates (Olsen et al. 2006), or by selective removal of females in downstream fisheries. More recently, biologists have started to question whether the low percentages might be caused by differences in sex-specific capture probabilities or other sampling bias at escapement monitoring sites. The sex composition of salmon escapements is typically estimated using stratified random sampling techniques (Cochran 1977; Roettiger et al. 2005). If sampling is unbiased within a stratum, which has been assumed without verification, such estimates are unbiased (Cochran 1977).

Table 1. Percentage of the Chinook salmon escapement comprised of females observed at select U. S. Fish and Wildlife Service weirs during the 10-year period from 1996 to 2005.

	Kuskokwim River Drainage[a]		Yukon River Drainage[b]	
Year	Tuluksak R.	Kwethluk R.	Andreafsky R.	Gisasa R.
1996	-	-	41.9	19.5
1997	-	-	36.8	26.0
1998	-	-	29.0	16.2
1999	-	-	28.6	26.4
2000	-	21.7	54.3	34.3
2001	-	-	-	49.2
2002	37.8	20.8	21.1	20.7
2003	30.5	18.3	45.3	38.1
2004	35.6	16.7	37.3	30.1
2005	40.8	-	50.2	34.0

[a] Data source: Molyneaux et al. 2006
[b] Data source: JTC 2006

During the 14-16 March, 2006 Technical Review Committee (TRC) meeting, a committee member voiced concerns regarding sampling bias and suggested that the TRC should fund investigations of the potential existence of sampling bias. A subcommittee of the TRC was charged with further discussing the issue. The subcommittee met on 29 March, 2006 and recommended that the TRC request proposals to investigate sampling bias at escapement monitoring stations.

In response to the request of the TRC, U. S. Fish and Wildlife Service (USFWS) staff met to discuss potential methods of investigating sampling bias. The USFWS staff was initially considering use of video, with which they have had success classifying the gender of salmonids (Daum 2005; Anderson et al. 2006; Gates and Palmer 2006).

Assuming that gender can be accurately determined by examining video images of individual fish, the gender composition of the entire escapement would have been compared to the estimate generated via the usual stratified sampling to detect the presence of any sampling bias.

While the applicability of video was being considered, staff became aware of a recent report (Olsen et al. 2004) containing data that suggested the determination of gender by examination of external morphology may be inaccurate. This report contains a comparison of gender classification of Chinook salmon by examination of external morphology (phenotypic gender) and a DNA test for the presence of the growth hormone pseudogene (GHp), a male-specific marker in Chinook salmon (Du et al. 1993). Olsen et al. (2004) found that the disagreement between the two methods of gender classification varied from 4.5% to 20.3% among six different data sets. An additional examination of those data revealed that the difference between the methods appeared to be related to fish length (Jeff Bromaghin, U. S. Fish and Wildlife Service, unpublished). Using the results of the genetic test as the standard, phenotypic classification errors tended to occur for small females and large males. The existence of this pattern suggests that, of the two methods, phenotypic classification is likely to have the greatest error rate, because no link between length and the genetic marker is known to exist.

These observations suggest that a genetic gender test might provide a more accurate standard of comparison to evaluate other techniques. However, the recent literature suggests that genetic gender tests do not perform well for all Chinook salmon populations, performance appears to vary among populations and geographic regions (Chowan and Nagler 2004; Chowan and Nagler 2005; Metcalf and Gemmell 2006). We summarize an investigation to test the utility of two published DNA markers for determining the gender of Chinook salmon specifically from the Yukon and Kuskokwim rivers. The sex determination from genetic analysis was contrasted with the sex determined in the field by visual examination of gonads.

Results

The original group of test samples contained 500 Chinook from the 2005 Pilot Station test fishery that were identified by sex based on examination of the gonads in the field. Using these samples, we tested two Y-Chromosome markers, OtY-1 (Devlin et al. 1991; modified as recommended by Jim Nagler, University of Idaho, personal communication) and OtY-2 (Brunelli and Thorgaard 2004).

Our initial results were problematic. Based on the genetic assays, a significant number of field-called males appeared as females, and vice versa for both markers. In order to rule out any laboratory variability, we then analyzed 100 (50 males and 50 females) Chinook samples from Priest Rapids hatchery on the Columbia River in Washington with OtY-2. The same samples had been previously analyzed with OtY-2 and reported on by Brunelli and Thorgaard (2004). For these samples, our results were unambiguous and matched precisely with the published results, indicating that the genetic sex-identification "errors"

we observed in the Pilot Station samples were not a result of laboratory error. There were likely caused by regional variation in the genetic makeup of the samples, or by errors in the field identification data.

In order to eliminate possible errors associated with the field sex determination of the 2005 Pilot Station samples, we collected 93 new samples of males and females identified by sex in the field by visual examination of gonads from the 2007 Kuskokwim River subsistence fishery. The results were similar to those obtained with the Pilot Station samples; a significant number of field-called males appeared genetically as females, and vice versa, indicating that the discrepancies between the genetic and field sex identification were a result of genetic variation.

As a final test, a blind subset of the 2005 Pilot Station and the 2007 Kuskokwim samples were sent to Gary Thorgaard's laboratory for analysis. Their results were similar to ours, revealing discrepancies between the field sex identification and that obtained with OtY-2. Soon after these results were obtained, we learned that Thorgaard's laboratory is no longer using the OtY-2 marker because of such difficulties; they are currently preparing a manuscript for publication detailing a new, and reportedly improved, sex marker (Joe Brunelli, personal communication).

At this time, we cannot recommend use of either OtY-1 or OtY-2 for use with Chinook salmon from either Yukon or Kuskokwim rivers. When the manuscript detailing the OtY-3 marker is published, we may revisit the issue. Testing of new markers will be faster and cheaper now that we have isolated the DNA from multiple groups of samples that had gonads positively identified in the field. In the meantime, the most reliable method for verifying field-called sex determination appears to be visual inspection of the gonad tissues.

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