

# **Stock Identification of Yukon River Chinook and Chum Salmon using Microsatellites**

Report to Yukon River Panel : Project CRE 79-12

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## **Abstract**

Stock identification of chum and Chinook salmon migrating past the Eagle, Alaska sonar site near the Yukon-Alaska border was conducted in 2012 through analysis of microsatellite variation. Variation at 14 microsatellites was surveyed for 642 chum salmon and variation at 15 microsatellites was surveyed for 344 Chinook salmon collected from the sonar site. For chum and Chinook salmon, all fish sampled at the Eagle sonar site were analyzed.

The analysis of chum salmon samples indicated that spawning populations from the White River drainage were estimated to comprise 47% of the fish sampled that migrated past the sonar site, while 53% were estimated to have been from mainstem Yukon River chum salmon spawning populations. The analysis of Chinook salmon migrating past the Eagle sonar site that were sampled indicated that the major regional stocks contributing to the run were the Teslin River (38%), mainstem Yukon River (19%), Carmacks area tributaries (13%), Pelly River (10%), Stewart River (6%), upper Yukon tributaries (6%), lower Yukon tributaries (4%), and White River (4%).

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## Introduction

Chum salmon (*Oncorhynchus keta*) and Chinook salmon (*O. tshawytscha*) are widely distributed throughout the Yukon River drainage, spawning in tributaries ranging from the extreme headwaters (e.g. Teslin River, British Columbia) to near the mouth of the river (e.g. Andreafsky River, Alaska). Management for conservation of biodiversity within the drainage requires knowledge of genetic variation among populations as well as population-specific information from fisheries. Effective management of fisheries in major drainages like the Yukon River generally requires information on the harvest and timing of specific populations, should managers wish to change exploitation rates on specific populations for conservation purposes. For example, the Canada/U.S. Yukon River Salmon Agreement has established escapement targets and harvest sharing provisions for Canadian-origin salmon stocks. It is therefore important to develop a management system that allows managers to assess accurately the status of these stocks in fisheries throughout the drainage during the season so that management decisions can ensure that treaty obligations are achieved. Accurate post-season run reconstructions are essential in evaluating whether management actions were consistent with meeting overall objectives and Treaty obligations. Run reconstructions are also important in monitoring the productivity of stocks and assessing the adequacy of current escapement targets and both pre-season forecasting and in-season run assessment techniques. Without this knowledge, managing to achieve Treaty obligations is difficult and severely limits the assessment of factors influencing stock productivity, which appear to have fluctuated widely in recent years.

Stock identification of chum and Chinook salmon migrating through the mainstem Yukon River is a continuing management concern. Although allozyme-based methods of stock identification have proven useful in the estimation of chum salmon stock composition in mixed-stock fisheries (Shaklee et al. 1999), and differentiation at allozyme loci occurs among Yukon

River chum salmon (Beacham et al. 1988; Wilmot et al. 1992), the level of population discrimination available in the Yukon River was not sufficient for population-specific applications. Variation in microsatellite loci has been applied in other species requiring discrimination among salmonid populations within watersheds (Small et al. 1998; Beacham and Wood 1999; Beacham et al. 2001), and has been shown to be useful in stock discrimination in Chinook salmon (Banks et al. 2000). Variation at microsatellite loci has been particularly useful for population-specific estimates of stock composition of Fraser River Chinook salmon (Beacham et al. 2003) and Yukon River Chinook salmon (Beacham et al. 2008a). Microsatellites have also been useful for chum salmon stock identification on a Pacific-Rim basis (Beacham et al. 2009).

In 2012, we surveyed variation at 14 microsatellite loci for chum salmon and 15 microsatellite loci for Chinook salmon. Samples were obtained from salmon live-captured in the test fishery associated with the sonar program near Eagle, Alaska. We used microsatellite variation to estimate stock composition in the samples collected.

## Materials and Methods

### Collection of DNA Samples and Laboratory Analysis

Tissue samples were collected from adult chum salmon migrating past the Eagle sonar site between August 3, 2012 and September 27, 2012. All chum salmon caught during this period were sampled. Axial processes were taken from sampled fish and DNA was extracted as described by Withler et al. (2000). Samples from all Chinook salmon sampled at the Eagle sonar site from July 14, 2012 to August 19, 2012 were analyzed.

Once chum salmon genomic DNA was available, surveys of variation at the following 14 microsatellite loci were conducted: *Ots3* (Banks et al. 1999), *Oke3* (Buchholz et al. 2001), *Oki2* (Smith et al. 1998), *Oki100* (Beacham et al. 2008b), *Ots103* (Nelson and Beacham 1999), *Omm1070* (Rexroad et al. 2001), *Omy 1011* (Spies et al. 2005), *One101*, *One102*, *One104*, *One111*, and *One114* (Olsen et al. 2000), *Ssa419* (Cairney et al. 2000), and *OtsG68* (Williamson et al. 2002). Microsatellites were size fractionated in an Applied Biosystems (ABI) 3730 capillary DNA sequencer, and genotypes were scored by GeneMapper software 3.0 (Applied Biosystems, Foster City, CA) using an internal lane sizing standard.

For Chinook salmon, the following 15 microsatellite loci were surveyed for genetic variation: *Ots100*, *Ots101*, *Ots104*, *Ots107* (Nelson and Beacham 1999), *Ssa197* (O'Reilly et al. 1996), *Ogo2*, *Ogo4* (Olsen et al., 1998), *Oke4* (Buchholz et al., 2001), *Omy325* (O'Connell et al, 1997), *Oki100* (Beacham et al. 2008b), *Omm1080* (Rexroad et al. 2001), *Ots201b*, *Ots211* (Grieg 2003), and *Ots2*, *Ots9* (Banks et al. 1999). Microsatellites were size fractionated in an ABI 3730 capillary DNA sequencer, and genotypes were scored by GeneMapper software 3.0 (Applied Biosystems, Foster City, CA) using an internal lane sizing standard.

In general, polymerase chain (PCR) reactions were conducted in 10 µl volumes consisting of 0.06 units of Taq polymerase, 1µl of 30ng DNA, 1.5-2.5mM MgCl<sub>2</sub>, 1mM 10x buffer, 0.8mM dNTP's, 0.006-0.065µM of labeled forward primer (depending on the locus),

0.4 $\mu$ M unlabeled forward primer, 0.4 $\mu$ M unlabeled reverse primer, and deionized H<sub>2</sub>O. PCR was completed on an MJResearch™ DNA Engine™ PCT-200 or a DNA Engine Tetrad™ PCT-225. The amplification profile involved one cycle of 2 min @ 92°C, 30 cycles of 15 sec @ 92°C, 15 sec @ 52-60°C (depending on the locus) and 30 sec @ 72°C, and a final extension for 10 min @ 72°C. Specific PCR conditions for a particular locus could vary from this general outline. Further information on laboratory equipment and techniques is available at the Molecular Genetics Laboratory website at <http://www.pac.dfo-mpo.gc.ca/science/facilities-installations/pbs-sbp/mgl-igm>.

### Baseline Populations

The baseline survey consisted of microsatellite analysis of chum salmon from 9 locations and microsatellite analysis of Chinook salmon from 24 locations within the Canadian portion of the drainage (Table 1). All annual samples available for a specific sample location were combined to estimate population allele frequencies, as was recommended by Waples (1990).

### Estimation of Stock Composition

Analysis of fishery samples was conducted with a Bayesian procedure (BAYES) as outlined by Pella and Masuda (2001). Each locus was assumed to be in Hardy-Weinberg equilibrium, and expected genotypic frequencies were determined from the observed allele frequencies and used as model inputs. For BAYES, the initial FORTRAN-based computer program as outlined by Pella and Masuda (2001) required large amounts of computer analytical time when applied to stock identification problems with a baseline as comprehensive as employed in the current study. Given this limitation, a new version of the program was developed by our laboratory as a C-based program which is available from the Molecular Genetics Laboratory website (Neaves et al. 2005). In the analysis, ten 20,000-iteration Monte



Carlo Markov chains of estimated stock compositions were produced, with initial starting values for each chain set at 0.90 for a particular population which was different for each chain. Estimated stock compositions were considered to have converged when the shrink factor was  $< 1.2$  for the 10 chains (Pella and Masuda 2001). The last 1,000 iterations from each of the 10 chains were then combined, and for each fish the probability of originating from each population in the baseline was determined. These individual probabilities were summed over all fish in the sample, and divided by the number of fish sampled to provide the point estimate of stock composition. Standard deviations of estimated stock compositions were determined from the last 1,000 iterations from each of the 10 chains incorporated in the analysis.

## Results and Discussion

### 1.0 Chum Salmon

On a seasonal basis, chum salmon spawning populations from the White River drainage accounted for 47% of the run, whereas mainstem spawning Yukon River populations were estimated to comprise 53% of the chum salmon migrating past the Eagle sonar site (Table 2). Mainstem spawning and White River populations were present throughout the sampling period, but the proportion of White River chum salmon declined as the season progressed. Teslin River chum salmon were most prevalent during the September 27<sup>th</sup> sample, the latest period in the migration past the sonar sampling site.

### 2.0 Chinook Salmon

Based upon sampling at the Eagle sonar site, populations which tended to migrate earlier in the run included those from the White River and lower Yukon tributaries (Chandindu River, Klondike River). The mainstem spawning stock returned noticeably later than the other stocks (Table 3). The Teslin River stock was relatively abundant throughout the run. The analysis of Chinook salmon migrating past the Eagle sonar site indicated that the major regional stocks contributing to the run were the Teslin River (38%), mainstem Yukon River (19%), Carmacks area tributaries (13%), Pelly River (10%), Stewart River (6%), upper Yukon tributaries (6%), lower Yukon tributaries (4%), and the White River (4%). The application of the estimated stock compositions to estimate stock-specific abundance as determined at the sonar site was outlined in the report of the Joint Technical Committee of the Yukon River Panel for 2012.

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**Table 1.** Baseline used to estimate stock compositions of chum and Chinook salmon from the Eagle sonar site 2012.

Region	Populations
Chum salmon	
Mainstem Yukon River	Mainstem Yukon at Pelly River, Tatchun Creek, Big Creek and Minto
White River	Kluane River, Kluane Lake, Donjek River
Teslin River	Teslin River
Yukon early	Chandindu River
Chinook salmon	
Upper Yukon tributaries	Whitehorse hatchery, Takhini River
Teslin River	Jennings River, Morley River, Nisutlin River, Teslin River, Teslin Lake
Carmacks Area tributaries	Little Salmon, Big Salmon, Tatchun Creek
Mid-mainstem/Nordenskiold	Mainstem Yukon River, Nordenskiold River
Pelly River	Little Kalzas River, Blind Creek, Earn River, Glenlyon, Hoole River, Pelly River
Stewart River	Mayo River, Stewart River
North Yukon mainstem tributaries	Chandindu River, Klondike River
White River	Tincup Creek, Nisling River

**Table 2.** Estimated percentage stock composition of chum salmon migrating past the Eagle sonar site, 2012. Stock compositions were estimated using 14 microsatellite loci and the baseline outlined in Table 1. Standard error of the estimated stock composition is in parentheses.

Sample size Populations	August 3 1		August 23- September 9 236		September 10-22 208		September 22-26 207	
	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
Big Creek	0.5	(13.7)	38.8	(12.1)	26.7	(10.9)	7.8	(3.5)
Minto	0.2	(13.3)	0.3	(1.0)	0.4	(0.8)	0.1	(0.4)
Pelly	0.0	(13.1)	4.4	(13.1)	6.6	(13.4)	48.2	(5.1)
Tatchun	0.0	(13.8)	0.0	(0.2)	0.1	(0.5)	0.1	(0.4)
Donjek	0.0	(14.0)	43.3	(21.9)	27.0	(33.0)	38.1	(13.1)
Kluane	0.0	(13.5)	0.0	(0.3)	5.6	(16.7)	0.1	(0.3)
Kluane Lake	0.0	(14.2)	11.3	(22.6)	33.5	(33.3)	4.6	(13.8)
Teslin	0.0	(12.3)	0.0	(0.2)	0.0	(0.3)	0.8	(1.0)
Chandindu	99.3	(28.5)	1.7	(1.6)	0.1	(0.3)	0.1	(0.4)
Regions								
Mainstem	0.7	(24.6)	43.6	(4.5)	33.8	(5.0)	56.2	(4.1)
White	0.0	(21.8)	54.7	(4.2)	66.1	(5.0)	42.8	(4.0)
Teslin	0.0	(12.3)	0.0	(0.2)	0.0	(0.3)	0.8	(1.0)
Yukon summer	99.3	(28.5)	1.7	(1.6)	0.1	(0.3)	0.1	(0.4)



**Table 2.** Continued

Sample size Populations	September 27 41		Seasonal 642	
	Estimate	SE	Estimate	SE
Big Creek	8.6	(14.9)	23.9	(23.2)
Minto	18.4	(16.7)	4.7	(13.9)
Pelly	43.7	(22.1)	14.2	(21.7)
Tatchun	1.3	(3.5)	4.6	(13.6)
Donjek	0.1	(1.0)	42.2	(21.2)
Kluane	24.7	(7.8)	5.0	(14.9)
Kluane Lake	1.0	(3.1)	5.4	(16.2)
Teslin	1.9	(3.5)	0.1	(0.2)
Chandindu	0.1	(1.0)	0.1	(0.2)
Regions				
Mainstem	72.1	(8.5)	47.3	(2.5)
White	25.8	(7.5)	52.6	(2.5)
Teslin	1.9	(3.5)	0.1	(0.2)
Yukon summer	0.1	(1.0)	0.1	(0.2)

**Table 3.** Estimated percentage stock composition of Chinook salmon migrating past the Eagle sonar site in 2012. Stock compositions were estimated using 15 microsatellite loci and the baseline outlined in Table 1. Standard errors of the estimates are in parentheses.

Sample size Populations	July 10-16		July 17-23		July 24-31		August 1-6	
	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
Takhini	1.7	(10.4)	2.2	(2.3)	0.0	(0.2)	4.0	(2.3)
Whitehorse	0.0	(6.3)	2.0	(2.3)	6.2	(2.1)	0.9	(0.9)
Jennings	0.0	(5.0)	0.5	(2.1)	0.4	(1.1)	0.2	(0.9)
Morley	0.1	(5.3)	17.7	(7.7)	5.0	(2.9)	2.3	(3.0)
Nisutlin	0.0	(6.6)	0.0	(0.6)	0.0	(0.3)	2.4	(2.6)
Teslin_	14.4	(19.6)	2.5	(5.2)	28.6	(6.2)	41.3	(8.2)
Teslin Lake	0.5	(7.8)	14.0	(7.6)	2.0	(2.8)	1.8	(2.4)
Big_Salmon	14.2	(20.1)	14.4	(8.7)	1.4	(2.9)	7.3	(6.1)
L._Salmon	0.2	(6.8)	0.4	(1.9)	10.1	(4.0)	5.3	(5.7)
Tatchun_	1.3	(9.6)	0.0	(0.6)	0.0	(0.3)	0.0	(0.3)
Nordenskiold	0.0	(5.8)	0.2	(1.0)	0.1	(0.4)	0.5	(1.3)
Yukon_main	0.0	(6.5)	0.1	(1.0)	0.4	(0.9)	0.0	(0.2)
Blind_Cr_	17.8	(21.2)	8.6	(7.7)	14.2	(5.4)	21.2	(8.0)
Earn_River	0.0	(5.9)	0.4	(1.6)	7.0	(2.7)	1.8	(2.5)
Glenlyon	0.0	(5.7)	1.2	(3.5)	0.2	(0.8)	0.2	(0.6)
Hoole	22.9	(23.5)	0.3	(1.3)	0.8	(1.2)	0.0	(0.3)
Little_Kalzas	0.0	(5.4)	1.9	(2.3)	2.8	(1.7)	2.0	(1.6)
Pelly	27.2	(25.2)	13.8	(7.2)	0.9	(2.0)	3.0	(2.7)
Mayo	0.0	(6.5)	3.4	(5.0)	2.6	(3.1)	2.1	(3.0)
Stewart	0.0	(5.5)	5.8	(5.6)	5.2	(3.7)	1.1	(2.1)
Chandindu	0.0	(5.1)	6.6	(4.6)	4.9	(2.1)	0.5	(1.1)
Klondike	0.0	(5.7)	1.8	(3.7)	0.2	(0.9)	0.6	(1.2)
Nisling	0.0	(6.4)	1.9	(3.5)	5.7	(2.3)	1.4	(1.7)
Tincup	0.0	(6.6)	0.2	(1.1)	1.2	(1.0)	0.0	(0.2)
Regions								
Yukon upper	1.7	(12.0)	4.2	(3.2)	6.2	(2.1)	4.9	(2.5)
Yukon Teslin	15.0	(22.9)	34.8	(9.2)	36.0	(6.5)	48.1	(8.3)
Yukon Carmacks	15.6	(22.8)	15.0	(9.0)	11.6	(4.4)	13.1	(5.6)
Yukon mainstem	17.8	(22.0)	8.7	(7.8)	14.6	(5.4)	21.2	(8.0)
Yukon Pelly	50.0	(25.1)	17.6	(7.5)	11.7	(3.5)	7.0	(3.3)
Yukon Stewart	0.0	(8.5)	9.2	(5.6)	7.8	(3.3)	3.2	(3.2)
Yukon Lower	0.0	(7.6)	8.4	(4.5)	5.2	(2.0)	1.2	(1.4)
Yukon White	0.0	(9.2)	2.1	(3.7)	7.0	(2.5)	1.4	(1.7)

**Table 3. Continued**

Sample size Populations	August 7-13 44		August 14-21 3		Seasonal 344	
	Estimate	SE	Estimate	SE	Estimate	SE
Takhini	8.3	(4.9)	0.2	(5.0)	2.8	(1.1)
Whitehorse	4.6	(3.1)	0.0	(4.0)	3.6	(1.0)
Jennings	0.0	(0.4)	0.0	(4.4)	0.0	(0.3)
Morley	0.6	(1.7)	0.0	(4.2)	5.9	(1.9)
Nisutlin	0.5	(1.8)	0.0	(4.9)	0.1	(0.3)
Teslin_	33.7	(11.3)	0.5	(6.4)	27.8	(4.5)
Teslin Lake	0.7	(1.8)	0.1	(4.3)	4.1	(1.7)
Big_Salmon	0.9	(2.3)	0.2	(4.9)	6.7	(2.8)
L._Salmon	0.5	(2.0)	1.0	(6.8)	4.6	(2.3)
Tatchun_	0.0	(0.5)	0.3	(4.4)	0.0	(0.1)
Nordenskiold	8.7	(5.2)	0.1	(4.4)	1.6	(1.2)
Yukon_main	0.0	(0.5)	0.0	(4.3)	0.0	(0.2)
Blind_Cr_	29.5	(12.0)	97.4	(19.9)	18.8	(4.4)
Earn_River	0.0	(0.4)	0.0	(4.8)	3.4	(1.4)
Glenlyon	0.3	(1.3)	0.0	(4.5)	0.1	(0.4)
Hoole	0.1	(0.8)	0.0	(5.2)	0.3	(0.5)
Little_Kalzas	0.1	(0.7)	0.0	(4.1)	2.1	(0.9)
Pelly	0.0	(0.5)	0.0	(4.0)	3.8	(1.6)
Mayo	0.1	(0.7)	0.1	(5.7)	1.0	(1.4)
Stewart	9.7	(5.5)	0.0	(3.6)	5.4	(1.9)
Chandindu	0.0	(0.5)	0.0	(5.0)	3.5	(1.1)
Klondike	0.0	(0.4)	0.0	(4.2)	0.1	(0.4)
Nisling	0.0	(0.7)	0.0	(3.7)	3.6	(1.2)
Tincup	1.7	(2.2)	0.0	(4.8)	0.7	(0.6)
Regions						
Yukon upper	12.8	(5.6)	0.2	(6.6)	6.4	(1.5)
Yukon Teslin	35.5	(11.4)	0.7	(10.5)	37.8	(4.6)
Yukon Carmacks	10.1	(6.0)	1.6	(10.2)	13.0	(3.1)
Yukon mainstem	29.5	(12.0)	97.4	(19.8)	18.8	(4.4)
Yukon Pelly	0.5	(1.8)	0.0	(10.1)	9.7	(2.0)
Yukon Stewart	9.8	(5.5)	0.1	(6.8)	6.4	(1.9)
Yukon Lower	0.0	(0.7)	0.0	(6.4)	3.6	(1.1)
Yukon White	1.7	(2.2)	0.0	(5.9)	4.3	(1.3)