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Sex ratios of juvenile and adult Chinook salmon in the Kuskokwim and Yukon rivers

Final Report for Study 02-097

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FINAL REPORT SUMMARY PAGE

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Study Number: 02-097

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Geographic Area: Kuskokwim River/Yukon River

Information Type: Subsistence Fishery Harvest Monitoring

Issue(s) Addressed: A contributing factor to low returns of Chinook salmon in the Yukon and Kuskokwim rivers may be low proportions of returning females. Skewed sex-ratios have been noted at weirs in several tributaries with low proportions of females returning to spawn (Gisasa R. 1994-2000 average 30%, range 17-42%; Tuluksak R. 1991-1994 average 20%, range 14-29%; Kwethluk R. 1998 & 2000, average 23%). It is unclear whether the sex ratio is skewed due to environmentally-influenced sex-inversion during embryo development, differential survival, or other, as yet unknown reasons.

Study Cost: $25,011

Study Duration: June 2002 to September 2003

Abstract: Some populations of Chinook salmon (*Oncorhynchus tshawytscha*) from western Alaska display persistent and often extreme adult sex ratio bias. Sex ratio bias in Pacific salmon is known to occur during early juvenile development and in adults during ocean migration. In this study we used a combination of phenotypic sex and a genetic sex marker, the growth hormone pseudogene (*GHp*), to distinguish between these two possibilities in Chinook salmon from the Kwethluk, Tuluksak, and Gisasa Rivers in western Alaska. The primary objectives were to; 1) compare the genetic and phenotypic gender of adult Chinook salmon, and 2) estimate the genetic sex ratio of age-2. Chinook salmon juveniles. Three results support a tentative conclusion that sex ratio distortion in these populations is due to gender-biased marine survival rates related to gender differences in life history strategies. These results are; 1) adult genetic and phenotypic sex ratios are generally similar and are male-biased, 2) juvenile genetic sex ratios are not male-biased, and 3) the average age-at-maturation for males is significantly less than for

females. Our conclusion is tentative because some results allow for alternative interpretation. Six recommendations for further study are provided to verify the conclusion.

Key Words: Chinook salmon, sex-ratio bias, genetic sex marker, Yukon River, Kuskokwim River, *Oncorhynchus tshawytscha*.

Project Data: Description – Data for this study consist of biological collections (fin tissue and DNA samples) and information (date, location, age, and length of adult samples, phenotypic sex of adult and some juvenile samples, and genetic sex of all adult and juvenile samples. Format – Fin tissue samples stored in 90% ethanol. Sampling and genetic data are stored in a Microsoft Excel spreadsheet. Custodian(s) – U.S. Fish and Wildlife Service, Conservation Genetics Laboratory, 1011 East Tudor Road, Anchorage, Alaska 99503. Availability – Access to biological samples and data is available upon request to the custodian(s).

Report Availability: Please contact either the author(s) to obtain a copy of this report.

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INTRODUCTION

Some populations of Pacific salmon (*Oncorhynchus* spp.) display persistent and often extreme sex ratio bias. Distorted sex ratios have been reported in populations of adult coho salmon (*O. kisutch*, Holtby and Healy 1990; Spidle et al. 1998), adult Chinook salmon (*O. tshawytscha*, Halupka et al. 2000) and juvenile sockeye salmon (*O. nerka*, Craig et al. 1996). Gender imbalance may adversely impact the genetic health of a population if the overall abundance is small and rapidly declining (Frankham et al. 2002). Such a scenario may exist in some Chinook salmon populations on the Yukon and Kuskokwim Rivers in western Alaska (e.g. Gates and Harper 2003). The development of an effective conservation and management plan for these and other salmon populations requires knowledge of when (at what life stage) the sex ratio becomes unbalanced and the likely factor(s) influencing gender abundance. Sex ratio distortion is known to occur during early juvenile development and in adults during ocean migration (Holtby and Healy 1990; Craig et al. 1996). Genetic sex markers (Devlin et al. 1991; Du et al. 1993) can be used to distinguish between these two possibilities (e.g. Spidle at al. 1998) and help focus conservation efforts on the life-stage factors that influence sex ratio.

Distorted sex ratios in Pacific salmon (*Oncorhynchus* spp.) have been associated with various environmental factors during early juvenile development in freshwater. For example, water temperature appears to be an important variable influencing sex determination in embryos of some salmon species. Craig et al. (1996) attributed female biased sex ratios (62% to 84% phenotypic female) in juvenile sockeye salmon and kokanee (*O. nerka*) to temperature manipulation during incubation in a hatchery. While it is not clear how the developmental mechanism controlling sex is influenced by temperature, Craig et al. (1996) suggest there is an important pre-hatch window in which sex determination (phenotypic gender) in embryonic salmon is sensitive to temperature. Other factors that may cause phenotypic sex reversal (contrasting phenotypic and genetic gender) in developing salmon include pH, exogenous sex steroids, and pollutants (Nagler et al. 2001 and references therein). For example, Hunter et al. (1986) describe a post-hatch window in which sex determination is sensitive to the level of exogenous estrogen. During this period, male Chinook salmon can be sex-reversed by exposure to high concentrations of estrogen. Some artificial compounds such as detergents and pesticides may act as estrogens. These "environmental estrogens" may cause sex reversal if present in the high enough concentrations. Nagler et al. (2001) suggest that environmental estrogens as well as temperature fluctuations during incubation may explain evidence of sex reversal in wild Chinook salmon from the Hanford Reach on the Columbia River, Washington.

Distorted sex ratios have also been associated with gender specific foraging behavior and age-at-maturation in saltwater. For example, the male-biased sex ratios observed in some populations of adult coho salmon (*O. kisutch*) are thought to be the outcome of sex-specific tolerance for risk while foraging (Holtby and Healy 1990; Spidle et al. 1998). Holtby and Healy (1990) posit there are two general types of coho salmon populations that reflect the opposing outcomes of natural and sexual selection. In populations with a balanced sex ratio, the males tolerate a relatively high degree of risk (mortality similar to females) during foraging because the breeding

environment dictates they achieve a relatively large size for successful reproduction. In populations with a male-biased sex ratio, the males tolerate a relatively low degree of risk (mortality less than females) during foraging because the breeding environment does not strongly favor large males. Gender-specific age-at-maturation may also result in biased sex ratios. In some populations of adult Chinook salmon the males generally return at an earlier age and in greater abundance than females. Halupka et al. (2000) found that the ratio of adult male to female Chinook salmon (*O. tshawytscha*) averaged 4:1 in four of 30 southeast Alaska populations sampled intermittently over 20 years. The males in these populations were on average approximately one year younger than the females. Presumably, the life history trade-offs differ between the sexes. For males, the cost of spending less time in the ocean (small size) is offset by a higher probability of survival and reproductive success.

Another factor that may also contribute to distorted sex ratios in some salmon populations is sex-biased harvest. Some studies have shown a correlation between harvest method (e.g. drift gillnet mesh size) and sex ratio in the catch (Molyneaux and DuBois 1999). Unfortunately, it is difficult to correlate these results to a single population because most salmon fisheries occur in areas where many populations mingle. For example, males are generally more abundant than females in some tributaries of the Kuskokwim River drainage in Alaska despite evidence that males tend to be harvested at the same rate (or higher) than females in the lower river fishery targeting these populations (Molyneaux and DuBois 1999).

In this study a genetic sex marker specific for the Y chromosome in Chinook salmon (Du et al. 1993) was used to estimate the genetic sex ratio in juveniles and adults from populations in four western Alaska rivers (Kwethluk, Tuluksak, and Gisasa rivers, and Big Creek). The former three populations have exhibited declining abundance during the period 1990-2003 and persistent male-biased sex ratios. Weirs operated intermittently between 1990 and 2003 indicate the percentage of adult male Chinook salmon averages 78% in the Kwethluk and Tuluksak rivers and 70% in the Gisasa River (Roettiger et al. 2003; Gates and Harper 2003; Wiswar 2001). The Big Creek Chinook salmon generally displays a balanced sex ratio and this sample served as a control. The primary objectives were to; 1) compare the genetic and phenotypic gender of adult Chinook salmon returning to the Tuluksak, Kwethluk and Gisasa Rivers and Big Creek, 2) estimate the genetic sex ratio of age-2. Chinook salmon juveniles from those same drainages, and 3) test for intersex development (individuals with male and female gonads) in juvenile Chinook salmon. Our study results indicate that adult genetic and phenotypic sex ratios are similar and heavily favor males whereas the juvenile genetic sex ratios are not male-biased. These results support a tentative conclusion that the factors influencing sex ratio in these populations are gender-based differences in life history strategies such as age at return and marine foraging behavior which result in gender-biased marine survival rates.

METHODS

Sample Collection, DNA Preparation, and Genetic Sexing

Fin tissue samples were collected from juvenile and adult Chinook salmon in the Kwethluk and Tuluksak Rivers and Big Creek (Figure 1, Table 1). Some juvenile samples included whole fish (~ 75 mm in length) so that the gonads could be removed and examined histologically for intersex development. Only samples of adult Chinook salmon were collected from the Gisasa River because logistical constraints prevented the collection of juveniles. Adult Chinook salmon were collected over a two to four week period at weirs operated by the U.S. Fish and Wildlife Service (USFWS) to enumerate annual escapement. All adult samples were assigned sex based on external appearance (phenotypic sex), measured for length (mid eye to fork of tail), and a scale was taken to estimate age at maturation. Juvenile Chinook salmon were collected in baited minnow traps from multiple locations within each river.

Each fin tissue sample was placed in a 2 ml vial with 90% ethanol and stored for processing at the University of Idaho. Genomic DNA was isolated from the fin tissue using the protocol of Nagler et al. (2001). Genetic sex was determined by testing each individual for the presence of the growth hormone pseudogene (*GHp*), a male-specific genetic marker in Chinook salmon (Du et al. 1993), using real-time quantitative polymerase chain reaction (PCR) as described by Nagler et al. (2004). Whole fish samples were placed in 10 ml conical tubes containing 30% formalin for storage. The gonads were removed by dissection, processed for routine histological examination (stained with hematoxylin), and examined using a compound microscope. Intersex development was determined by comparing the histological and genetic sex of the juvenile samples.

Data Analysis and Interpretation

A log-likelihood ratio test for contingency tables was used to determine if adult phenotypic and genetic sex ratios were significantly different. A log-likelihood ratio goodness of fit test (*G*-test) was used to determine if juvenile and adult genetic sex ratios deviated from unity (1:1). Each test had one degree of freedom so a correction for continuity was applied by adjusting the observed frequencies by 0.5 to minimize *G* (Sokal and Rohlf 1995). A two-sample t-test was used to determine if the average age at maturation differed between male and female Chinook salmon in each population.

Data interpretation and inference regarding the source(s) of sex ratio distortion in Chinook salmon from the Kwethluk, Tuluksak, and Gisasa rivers was based on the four possible outcomes of the data analysis. 1) the adult genetic and phenotypic sex ratios do not differ significantly and the juvenile genetic sex ratios are 1:1. Under this scenario, the sex ratio distortion is likely related to factors that differentially influence marine survival of the two sexes such as gender-specific foraging behavior and age-at-maturation. 2) the adult genetic and phenotypic sex ratios

do not differ significantly and the juvenile genetic sex ratios are not 1:1. Under this scenario, the sex ratio distortion could have a genetic basis and be related to factors influencing segregation of sex genes during reproduction or to gender-biased mortality in juveniles. 3) the adult genetic and phenotypic sex ratios do differ significantly and the juvenile genetic sex ratio are 1:1. Under this scenario, the sex ratio distortion is likely related to phenotypic sex reversal caused by one or more environmental factors during early juvenile development. 4) the adult genetic and phenotypic sex ratios do differ significantly and the juvenile genetic sex ratios are not 1:1. Under this scenario, the sex ratio distortion could be related to phenotypic sex reversal during early juvenile development but may also have a genetic basis.

RESULTS

The genetic sex ratios of adult Chinook salmon ranged from 16.7% (Kwethluk River, 2002) to 60.7% female (Big Creek, 2000). Four of the six adult samples differed significantly from 1:1 (Gisasa River, 2001; Kwethluk River, 2002; Tuluksak River, 2002; Big Creek, 2001). The former three samples were dominated by males whereas the Big Creek 2001 sample was dominated by females.

Genetic and phenotypic sex differed for some adult Chinook salmon samples, ranging from 3 (Big Creek, 2000) to 60 individuals (Gisasa River, 2001, Table 2). The fraction of individuals in the two categories of disagreement (phenotypic female/genetic male, phenotypic male/genetic female) varied across populations (Table 2). For example, the percentage of phenotypic males identified as genetic females averaged 58.9% over all populations, but ranged from 15.4% (Kwethluk River, 2002) to 70.0% (Gisasa River, 2001). The genetic and phenotypic sex ratios differed in all but the Tuluksak River 2002 sample (Table 3). The differences were statistically significant ($P < 0.05$) in the Gisasa River 2001.

The genetic sex ratios for juvenile Chinook salmon ranged from 49.5% (Big Creek, 2001) to 67.2% female (Tuluksak River, 2003, Table 1, Figure 2). The genetic sex ratio of the Tuluksak River juvenile sample differed significantly from 1:1. No evidence of intersex was found in gonad samples from 68 juvenile Chinook salmon from the Kwethluk and Tuluksak rivers and Big Creek (Table 1).

Adult Chinook salmon males were, on average, younger than females in the three test populations and in Big Creek (Table 4). The mean age differences were greatest (> 1.0 year, $P < 0.001$) for the Kwethluk and Tuluksak samples. The mean age differences were also statistically significant for the samples from the Gisasa River ($P < 0.001$) and Big Creek 2000 ($P < 0.004$), but not Big Creek 2001 ($P = 0.238$).

DISCUSSION

Three results support a tentative conclusion that sex ratio distortion in Chinook salmon from the Kwethluk, Tuluksak and Gisasa rivers is due to gender-biased marine survival rates related to gender differences in life history strategies (outcome number 1 above) and not due to phenotypic sex reversal. These results are 1) adult genetic and phenotypic sex ratios are generally similar and are male-biased , 2) juvenile genetic sex ratios are not male-biased, and 3) the average age at maturation for males is significantly less than for females. The conclusion is tentative and must be viewed with caution because some results allow for alternative interpretation and because juvenile samples are not available for the Gisasa River population. The results supporting the conclusion, as well as the caveats and recommendations for further study, are discussed in detail below.

Adult Genetic and Phenotypic Sex Ratios

The adult Chinook salmon genetic sex ratios were, with the exception of the Gisasa River sample, not statistically different from the phenotypic sex ratios and indicate a male-biased adult population in the Kwethluk and Tuluksak rivers. If the sex ratio distortion were due to phenotypic sex reversal occurring during early juvenile development then the adult genetic and phenotypic sex ratios would differ significantly. This is not the case for the Kwethluk or Tuluksak population samples (Table 3), suggesting something other than sex-reversal, such as gender-biased marine survival, is causing the sex ratio distortion. Support for gender-biased marine survival also comes from the two inter-annual sample replicates from the Tuluksak River. While the adult sex ratio estimates for the sequential years vary substantially, the genetic and phenotypic sex ratios are not significantly different within each year.

Sex reversal, however, cannot be entirely excluded. Evidence for phenotypic sex reversal is strongest for the Gisasa River Chinook salmon because the genetic and phenotypic sex ratios are statistically different ($P < 0.05$). There are also differences in genetic and phenotypic sex at the individual level in all adult samples, including Big Creek (Table 2). These individuals represent a relatively small fraction (4.5% to 11%) of the samples from the Kwethluk and Tuluksak Rivers and Big Creek (2000) but comprise over 20% of the individuals from Gisasa River and Big Creek (2001). It is interesting that both categories of disagreement (i.e. female phenotype/genetic male, male phenotype/genetic female) were observed in each adult population sample. Sex reversal typically occurs in one direction, depending upon the environmental factor influencing sex determination (e.g. Hunter et al. 1986; Craig et al. 1996; Nagler et al. 2001). So, while phenotypic sex reversal cannot be excluded, previous studies suggest it is unlikely because the direction of reversal should be one way. A more likely explanation for this finding is error in either the genetic or phenotypic sexing or both. Recently, Chowen and Nagler (2005) documented evidence of sexing error using the *GHp* marker to determine genetic sex in four Columbia River Chinook salmon populations. The magnitude of error averaged 19% in males and 23% in females but varied greatly across populations. The accuracy of phenotypic sexing based on external characteristics of adults captured in river is unknown (Hyer and Schleusner

2005). In order to identify and quantify the sexing error associated with both methods of sex determination used in this study, additional sampling and analysis is needed.

Juvenile Genetic Sex Ratios

The juvenile genetic sex ratios for the Tuluksak and Kwethluk River Chinook salmon were not male-biased (Figure 1). If the adult sex ratio distortion favoring males has a genetic basis, then the genetic sex ratios for adults and juveniles should be similar. This is not the case. In fact, the genetic sex ratio for the juvenile Chinook salmon from the Kwethluk River does not differ significantly from 1:1.

Interestingly, the sample of juvenile Chinook salmon from the Tuluksak River exhibits a female-biased sex ratio (Table 1). The female percentage (67.2%) is significantly different from 50% ($P < 0.001$). It is possible that this sex ratio bias has a genetic explanation (e.g. female chromosome bias in gametes or during fertilization). If this were the case, then this extreme female bias should persist in the adults. Instead, the sex-ratio bias favors males in the adults and the bias is equally, if not more, extreme (Figure 1). These contrasting results could again be due to error in genetic sexing using the *GHp* marker. In a sample of 18 juveniles from Big Creek 2002, 7 individuals had contrasting genetic and phenotypic sex (as opposed to intersex individuals which have both male and female gonads). As with the adult samples, both categories of disagreement (i.e. female phenotype/genetic male, male phenotype/genetic female) were observed. Additional analysis of juvenile samples from the Tuluksak and Kwethluk rivers is underway to test for congruent genetic and phenotypic sex.

Average Age at Maturation

Although the data was limited to only one or two return years, male Chinook salmon were, on average, younger than females in the Tuluksak, Kwethluk and Gisasa rivers. This trend was most extreme in the Kwethluk and Tuluksak Rivers where the difference exceeded one year. Gender differences in age at maturation have been noted in other Chinook salmon populations (e.g. Halupka et al. 2000, 2003) and have been correlated with gender differences in abundance. The fact that males in these populations return to spawn at a younger age suggests they may experience a lower rate of marine mortality and hence survive at a greater rate than the females. The trade off for males is that they sacrifice size (correlated with age) at reproduction. Holtby and Healy (1990) suggested that such a trade off (greater marine survival but smaller size at maturity) is more likely for males where selection for size may not be as strong as for females. It is also possible that females are more willing to take risk when foraging at sea in order to attain a larger size. Such a dichotomy has been suggested for coho salmon (Hotlby and Healy 1990). These two possible explanations (age at maturation versus marine foraging behavior) for gender-biased marine survival are not mutually exclusive, however, they are only conjecture and require a complete analysis of cohort survival by sex for multiple cohorts to evaluate.

Sex-biased harvest

It is also possible that the average age at maturation and the distorted sex ratios in these populations is the result of sex-biased harvest. Molyneaux et al. (2004) found that the female percentage in the subsistence gillnet fishery was higher than the average female percentage at escapement projects along the Kuskokwim River. Alternatively, Molyneaux & DuBois (1999) found that male chinook salmon tended to be harvested in higher numbers than females in the commercial gillnet fishery that targets a mixed stock containing some Tuluksak and Kwethluk River fish. The two studies indicate that the harvest may be sex-biased but that the direction of the bias depends on the fishery. It is not clear what the cumulative impact of subsistence and commercial harvest is on the sex ratio of adult spawners. Moreover, it is difficult to correlate these results to a single population because both fisheries occur in the Kuskokwim River (downstream of the Tuluksak and Kwethluk Rivers) where the populations mingle.

Recommendations

Further study is needed to verify the primary conclusion that the sex ratio distortion in these Chinook salmon populations is due to gender-biased marine survival rates in adults and not due to phenotypic sex reversal in juveniles. The following analyses are recommended:

1) Use the genetic sex marker *Oty*1 (Devlin et al. 1991) to verify genetic sex of each individual for which genetic and phenotypic sex disagree (n = 124). Like *GHp*, *Oty*1 is an established genetic sex marker in Chinook salmon. Recent tests comparing the two markers indicate *Oty*1 is more accurate and not subject to intermittent error like *GHp* (Chowen and Nagler 2005). If the *GHp* genetic sex is confirmed using Oty1, then the sexing disagreements in these populations may in fact be due to phenotypic sex reversal. Consider doing recommendation 3.

2) Verify the method of phenotypic sexing of adults by using post-spawning Chinook salmon that can be sacrificed and assigned sex by gonad examination. Alternatively, methods such as ultra-sound may be used to identify male and female gonads in live adults at weirs. Use the genetic marker *Oty*1 to characterize genetic sex.

3) Sample juvenile Chinook salmon from the Gisasa River for genetic sexing.

4) Use histological analysis (J. Nagler, pers. comm.) to determine phenotypic sex of whole juvenile samples of Chinook salmon (underway).

5) Conduct a cohort analysis to verify the age-at-maturation results and to test for differences in marine survival rates between sexes. In lieu of juvenile abundance data, relative marine survival could be estimated by computing total return for each sex for a given cohort and assuming a 1:1 sex ratio at the time juveniles enter saltwater.

6) Conduct a detailed examination of harvest rate on male and female Chinook salmon in the fishery.

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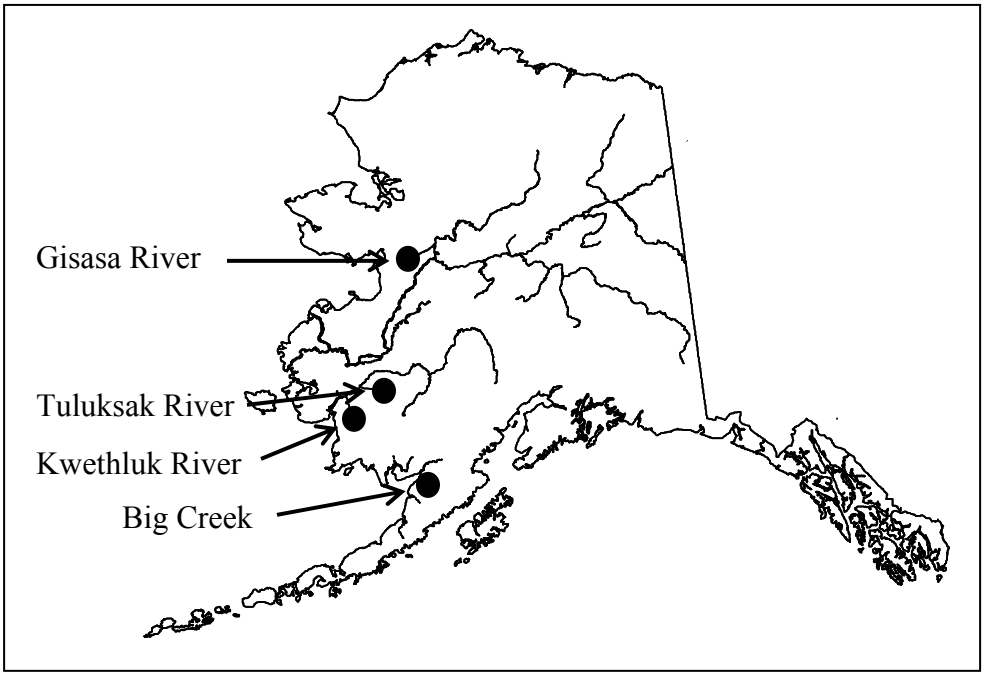

Figure 1. Map showing the locations of the Kwethluk, Tuluksak, and Gisasa rivers, and Big Creek

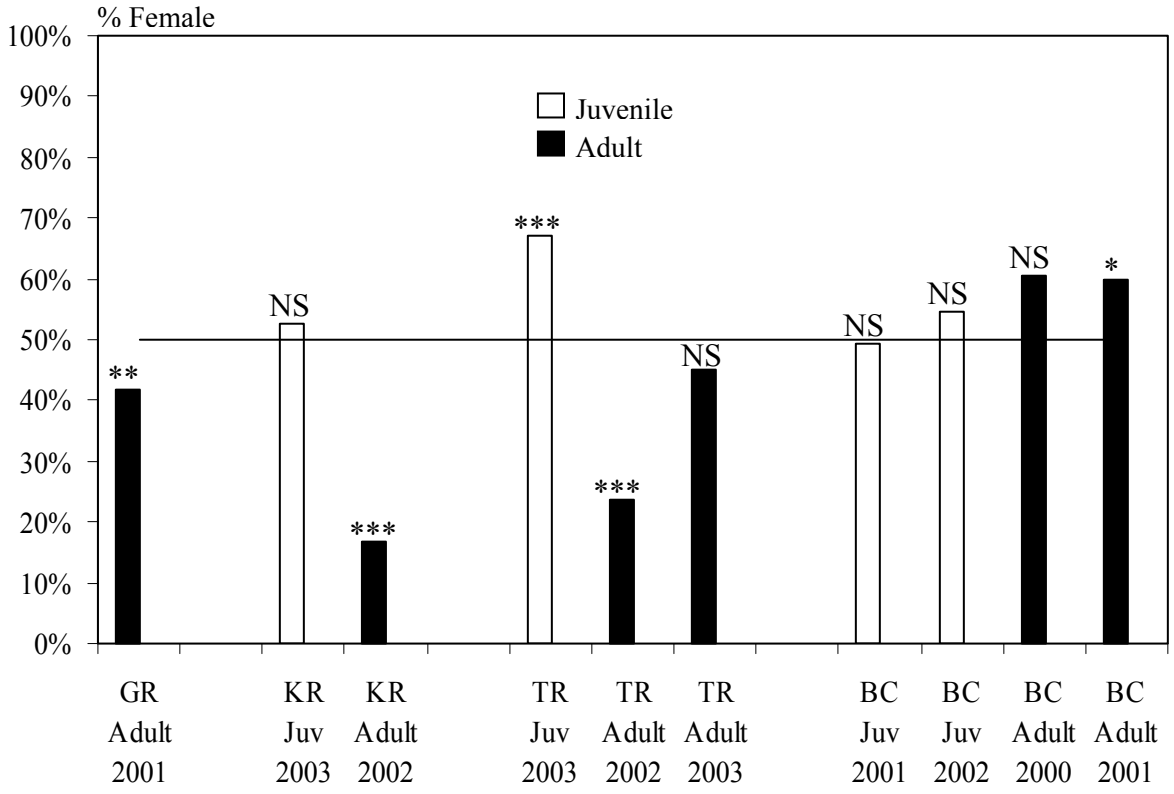

Figure 2. Histogram showing the female percentage of juvenile and adult Chinook salmon samples from the Gisasa (GR), Kwethluk (KR), and Tuluksak (TR) rivers, and Big Creek (BC). Sex was determined using the sex-linked genetic marker *GHp*. Samples that deviate significantly from a 1:1 sex ratio are denoted with an asterisk (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, NS = not significant).

Table 1. Summary of sample data and genetic sex for four Chinook salmon populations from three river drainages. Sample sizes (n) are shown for adult (A) and juvenile (J) life stages (LS). The number of males (M) and females (F) were determined using the sex-linked genetic marker GHp. Genetic sex ratios were tested for deviation from unity (1:1) using the G-test statistic. Significant test results are indicated with an asterisk (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, NS = not significant). The number of juveniles exhibiting intersex (IS) relative to the number of whole juveniles (WJ) is shown.

Drainage Population	LS	year	n	M	F	%F	*G*		IS/WJ
Yukon R.									
Gisasa R.	A	2001	295	172	123	41.7%	7.85	**	
Kuskokwim R.									
Kwethluk R.	J	2003	144	68	76	52.8%	0.34	NS	0/25
	A	2002	288	240	48	16.7%	138.13	***	
Tuluksak R.	J	2003	192	63	129	67.2%	22.45	***	0/25
	A	2002	187	143	44	23.5%	54.01	***	
	A	2003	93	51	42	45.2%	0.69	NS	
Naknek R.									
Big Creek	J	2001	192	97	95	49.5%	0.01	NS	
	J	2002	95	43	52	54.7%	0.67	NS	0/18
	A	2000	61	24	37	60.7%	2.38	NS	
	A	2001	130	52	78	60.0%	4.84	*	

Table 2. The number of adults (n_d) in each Chinook salmon population sample assigned different genetic and phenotypic sex. The percent of disagreements in each sample (% n) relative to the sample size (n) is shown. The disagreements are categorized as female phenotype/genetic male (F_PM_G) and male phenotype/genetic female (M_PF_G).

Drainage Population	Year	n	n_d	% n	F_P M_G	$\%F_P$ M_G	M_P F_G	$\%M_P$ F_G
Yukon River								
Gisasa R.	2001	295	60	20.3%	18	30.0%	42	70.0%
Kuskokwim R.								
Kwethluk R.	2002	288	13	4.5%	11	84.6%	2	15.4%
Tuluksak R.	2002	187	12	6.4%	6	50.0%	6	50.0%
	2003	93	10	10.8%	7	70.0%	3	30.0%
Naknek R.								
Big Creek	2000	61	3	4.9%	1	33.3%	2	66.7%
	2001	130	26	20.0%	8	30.8%	18	69.2%
total		568	124	21.8%	51	41.1%	73	58.9%

Table 3. The number of male (M) and female (F) Chinook salmon in adult samples (n) from four populations in three river drainages. Sex was determined using the sex-linked genetic marker *GHp* and external appearance (pheno). The genetic and phenotypic sex ratios were tested for similarity using the *G*-test statistic. Phenotypic and genetic sex ratios that differed significantly are indicated with an asterisk (* = $P < 0.05$, NS = not significant).

Drainage Population	year	sex ID	n	M	F	%F	*G*	
Yukon R.								
Gisasa R.	2001	*GHp*	295	172	123	41.7%	4.17	*
		pheno	295	196	99	33.6%		
Kuskokwim R.								
Kwethluk R.	2002	*GHp*	288	240	48	16.7%	0.94	NS
		pheno	288	231	57	19.8%		
Tuluksak R.	2002	*GHp*	187	143	44	23.5%	0.00	NS
		pheno	187	143	44	23.5%		
	2003	*GHp*	93	51	42	45.2%	0.35	NS
		pheno	93	47	46	49.5%		
Naknek R.								
Big creek	2000	*GHp*	61	24	37	60.7%	0.03	NS
		pheno	61	25	36	59.0%		
	2001	*GHp*	130	52	78	60.0%	1.56	NS
		pheno	130	62	68	52.3%		

Table 4. Average age of male (A_M) and female (A_F) Chinook salmon sampled for this study. The difference in age between sexes was tested using a two-sample t-test (*P*-value shown).

		male		female			
Population	year	n	A_M	n	A_F	A_F-A_M	*P*
Yukon River							
Gisasa R.	2001	158	5.06	111	5.75	0.69	<<0.001
Kuskokwim River							
Kwethluk R.	2002	209	4.59	41	5.88	1.29	<<0.001
Tuluksak R.	2002	123	4.69	42	5.86	1.17	<<0.001
	2003	47	4.87	40	5.98	1.11	<<0.001
Naknek River							
Big Creek	2000	18	4.11	35	4.77	0.66	<0.004
	2001	40	5.33	58	5.57	0.24	0.238

NON-DISCRIMINATION STATEMENT

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