

Initial microsatellite analysis of wild Kootenai River white sturgeon and subset brood stock groups used in a conservation aquaculture program.

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Executive Summary

This study addressed four issues involving white sturgeon genetics. Microsatellite variation in the Kootenai/y population observed in this study was quantified, and was approximately one third that of other surveyed white sturgeon populations in the Columbia, Fraser, and Sacramento river basins. This finding was consistent with all past allozyme, mtDNA and nuclear DNA analyses that included Kootenai River and Kootenay Lake fish. A somewhat less variable population would be expected in the Kootenai drainage after founding effects associated with post-glacial recolonization, loss of rare alleles in an isolated population due to random genetic drift, and recent demographic and possible genetic bottlenecks. The degree to which lower genetic variability in the Kootenai population might affect population fitness remains unclear.

Third, the level of genetic variability in the Kootenai Hatchery broodstock group was quantified and evaluated. The Kootenai Hatchery broodstock samples represented the genetic variability of the wild population better than expected. We observed that 80 to 90% of the genetic variability identified in the wild Kootenai River population was represented by 30 to 40 broodstock sampled from the same population. Furthermore, high frequency alleles in the wild Kootenai population were generally found at high levels in the broodstock, and alleles that were relatively rare in the wild population were generally found in low frequencies in the broodstock groups. This finding is supported by earlier work that compared mtDNA length variant frequencies in the wild and hatchery broodstock sample groups. Thus, it appears that no changes in broodstock collection techniques or protocols are required in the interest of genetic representation for the Kootenai Hatchery program.

Finally, this study tested the utility of eight microsatellite loci for parentage analysis of Kootenai Hatchery progeny. Because success of parentage assignment is highest when the number of alleles is high and the number of possible parents is low, and because the Kootenai/y population had lower numbers of alleles and more possible parents, more loci will be needed to improve parentage assignment accuracy of Kootenai Hatchery progeny from its current success rates. Producing a white sturgeon microsatellite library and additional loci is recommended to improve the accuracy of future parentage assignment for the Kootenai Hatchery program.

Introduction

The Kootenai River white sturgeon (*Acipenser transmontanus*) population was listed as endangered under the U.S. Endangered Species Act during 1994 due to its small size, failing natural recruitment, right-skewed age class structure, and geographic isolation (Duke et al. 1999; USFWS 1994, 1999). This population also exhibited reduced genetic diversity and variability relative to white sturgeon populations in the Columbia, Snake, Fraser, and Sacramento river drainages according to allozyme and mitochondrial DNA (mtDNA) length variant and sequencing analyses (Bartley et al. 1985; Setter and Brannon 1992; Anders and Powell 2002a, 2002b). Kootenai represents the U.S spelling whereas Kootenay River and Kootenay Lake represent the Canadian spellings of the same river and lake system.

The Kootenai River White Sturgeon Recovery Plan includes efforts to restore natural recruitment and associated habitat conditions, and a conservation aquaculture program to preserve the population's remaining genetic diversity and protect the population from extinction (USFWS 1999). Proportional representation of wild population genetics in the hatchery brood stock and progeny groups is a central issue of the conservation aquaculture program (Anders 1988; Ireland et al. 2002). A recent Chi-square analysis revealed no significant differences when comparing haplotype frequency distributions between the wild population (n=112) and the first 54 brood stock spawned in the Kootenai Hatchery ($p \leq 0.05$; Anders et al. 2000). These results suggested that brood stock selection was sufficiently random across haplotypes such that statistical differences in haplotype frequencies of wild population and brood stock sample groups were non-significant. However, limited discriminatory power is associated with mtDNA length variants.

Due to their highly polymorphic and informative nature, microsatellites have become the markers of choice for many applied conservation genetics research projects in fisheries and wildlife management. Consistent with this trend, a suite of nuclear genetic (microsatellite) markers was recently developed for white sturgeon (Rodzen and May 2002). This study represents the first application of microsatellite techniques to the Kootenai River white sturgeon

population and its individuals used as brood stock in the Kootenai River white sturgeon conservation aquaculture program.

The following report summarizes the results of contracted genetics research conducted in the Genomic Variation Laboratory at the University of California, Davis for the Kootenai Tribe of Idaho. The purpose of the research detailed herein was to: 1) quantify the level of genetic variation within the endangered Kootenai River white sturgeon population, 2) compare it to other populations of white sturgeon, 3) evaluate the level of genetic variability in the animals chosen as broodstock for the KTOI hatchery program, and 4) test the utility of the microsatellite loci for parentage analysis for Kootenai Hatchery progeny.

Methods and materials

Samples

A total of 925 white sturgeon samples were extracted, genotyped, and analyzed (Table 1). These included: 670 samples from wild fish from the Kootenai/y system and other wild populations, a total of 63 broodstock collected from the Kootenai River during 2001, 2003, and 2004, and 24 additional samples from each of eight full-sib families (n=192), for a total of 925 samples (Table 1). The eight full-sib families were produced during 2004 at the Kootenai Hatchery to test the accuracy of parentage analysis. Samples were provided by the Kootenai Tribe of Idaho and from the University of Idaho's Aquaculture Research Institute.

Table 1. Source, collection year, and number of white sturgeon samples extracted, genotyped, and analyzed.

Population or Sample Source	<i>n</i>
1. Wild populations	670
Nechako 1995	49
Lower Fraser 1997	47
CJ Strike 1996	54
McNary 1995	50
Columbia River estuary 1998	38
Sacramento River	38
Kootenai River 1994	13
"KT" 1994	50
Kootenay Lake 1995	49
Kootenai River 1996	88
Kootenay Lake 1996	23
Kootenai River 1997	83
Lake Roosevelt 1998	8
Lake Roosevelt	48
2. Kootenai River broodstock	63
2004 broodstock	30
2003 broodstock	17
2001 broodstock	16
3. Full-sib families	192
FS 261A	24
FS 2972	24
FS 270D	24
FS 3672	24
FS 6453	24
FS 672C	24
FS 193C	24
FS 6532	24
Total	925

All samples were amplified and genotyped for eight microsatellite loci (Atr-100, Atr-105, Atr-107, Atr-109, Atr-113, Atr-114, Atr-117, and Atr-1173) previously described by Rodzen and May (2002). The loci were labeled with fluorescent primers (6Fam, Hex, or Tet), and the PCR products were separated and visualized on an MJ Research Basestation and sized using the Applied Biosystems Genescan 400HD-Rox internal lane standard.

Allele scoring

The white sturgeon is presumptively an octoploid derivative species and does not exhibit simplistic inheritance patterns seen for diploid or even interpretable patterns seen for tetraploids. A previous inheritance study (Rodzen and May 2002) has shown that dosage reading of individual bands in the complex banding patterns is impossible but that most bands are inherited in a 1:1 fashion of presence to absence among the progeny. For this reason they proposed that alleles be treated essentially as loci and scored as being "present" or "absent", thus yielding an array of dominant marker data. This procedure is shown in Appendix II and is detailed in Rodzen et al. (2004). Allele frequencies are then calculated as for a standard dominant, disomic locus where the frequency of the allele $p = 1 - q$, and where q^2 = frequency of individuals in which the allele is absent. Allele frequencies for each population are shown in Appendix I.

Genetic variation within and among populations

Population genetic data from all sample groups were analyzed using a variety of methods. Values of F_{ST} and Nei's standard genetic distances were calculated using POPGENE (Version 1.32) where the individual alleles were treated as independent dominant markers, as suggested by Rodzen and May (2002). The matrices of Nei's distances were then analyzed using PHYLIP version 3.5c (Felsenstein 1993) where the Neighbor-joining method was used to construct unrooted trees and dendrograms.

Initially, all of the collected samples were analyzed at the finest scale of resolution as the populations listed in Table 1. The Kootenai samples were then pooled into either "Kootenay Lake" or "Kootenai River" bins and re-analyzed using POPGENE and NEIGHBOR to compare the Kootenai/y samples as a whole to the other populations. The full-sib groups were included

for some of the analyses to examine the effect of large full-sib groups on the genetic distance / phylogenetics methods.

Parentage testing

To test the utility of the markers for parentage identification, a series of controlled crosses were performed at the Kootenai Hatchery during 2004. In total, eight full-sib families were made and 24 fry from each family were genotyped and analyzed. The families were made from single-pair matings involving eight different Kootenai River males and five females (three females were used twice).

The accuracy of parentage assignment was tested using the algorithms and procedures described in Rodzen et al. (2004). To summarize the methods, a probability that each male and female could be a parent of each offspring is calculated and then the male and female with the highest probability is selected as the most probable parent. A note of interest is that parentage works mostly by process of elimination, meaning that when progeny have an allele that a possible parent does not, that parent is eliminated as a possibility. Thus, as the number of alleles increases, the ability to exclude possible parents also increases.

First, parentage was tested using only the 5 females and 8 males from the 2004 broodstock that were used to make the crosses to see if the correct parents could be identified. Second, all of the broodstock from 2004 were included as possible parents, and the success of parentage assignment was re-tested.

Results

Genetic variation within and among populations

The number of alleles varied greatly across populations, and numbers of observed alleles were positively correlated with sample size across populations (Figure 1, Table 2). Populations with the most observed alleles did not have the largest sample sizes, but were associated with unimpounded lower river systems, near the Pacific Ocean. For example, the Lower Fraser River had 47 samples and 129 alleles, Columbia River estuary had 38 samples and 125 alleles, and the Sacramento River had 38 samples representing 118 alleles (Figure 1, Table 2). Alternatively, Kootenai/y samples had the lowest number of observed alleles. Even when Kootenai/y sample sizes ranged from 49 to 79, observed alleles ranged from only 35 to 37 (Table 2).

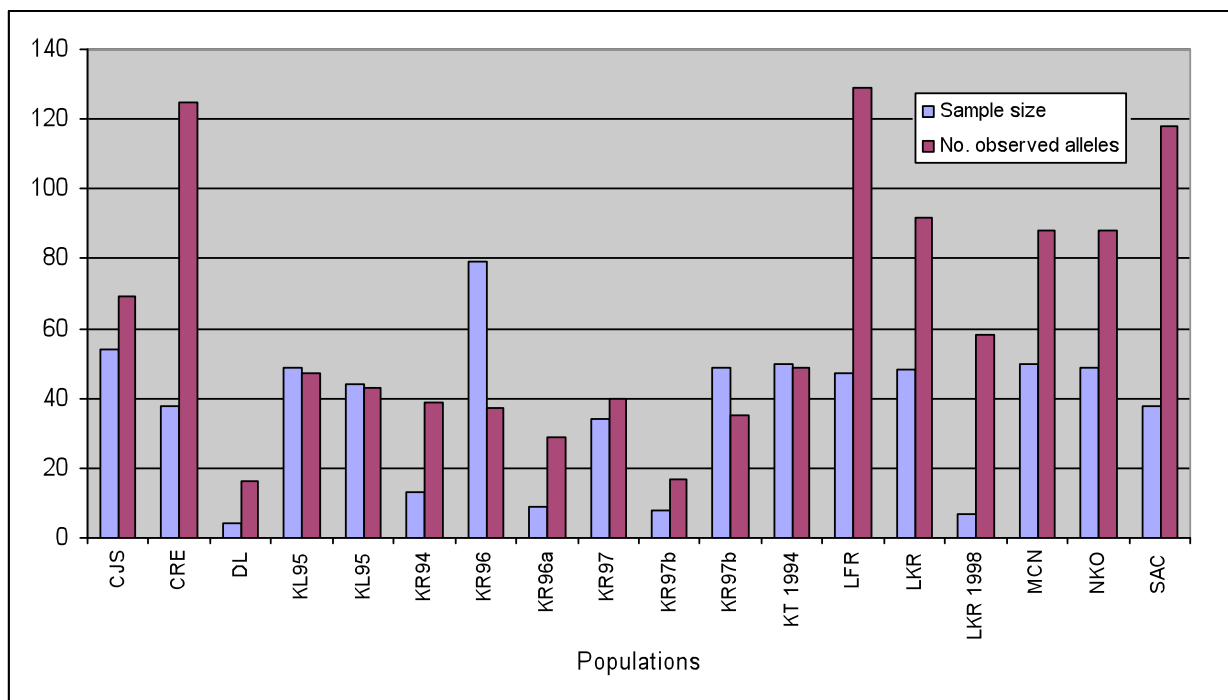


Figure 1. Sample size and number of observed alleles from 18 population or sample groups of white sturgeon analyzed in this study. Sample sizes for each population or sample group are presented in Table 1. Population codes: KL=Kootenay Lake, KR=Kootenai River, KT=Kootenai River, CJS=C. J. Strike Reservoir, CRE=Columbia River estuary, LFR=Lower Fraser River, MCN=McNary Pool, NKO=Nechako River, SAC=Sacramento River.

Table 2. Population or sample source, sample size, number of observed alleles, and number of alleles per sample when sample sizes exceeded 10.

Population or Sample source	<i>n</i>	Number of observed alleles
1. Wild populations	670	
Kootenai River 1997	34	40
Kootenay Lake 1995	49	47
LKR 1998	7	58
Nechako 1995	49	88
Lower Fraser 1997	47	129
Lake Roosevelt	48	92
CJ Strike 1996	54	69
McNary 1995	50	88
Columbia R. estuary 1998	38	125
Kootenai River 1997a	8	17
Kootenai River 1996	79	37
Kootenai River 1997b	49	35
Kootenai River 1994	13	39
KT 1994	50	49
Kootenai River 1996a	9	29
Kootenay Lake 1995	44	43
Duncan Lake	4	16
Sacramento	38	118
2. Full-sib test families	192	
<i>FS 261A</i>	24	40
<i>FS 2972</i>	24	27
<i>FS 270D</i>	24	30
<i>FS 3672</i>	24	21
<i>FS 6453</i>	24	24
<i>FS 627C</i>	24	18
<i>FS 193C</i>	24	24
<i>FS 6532</i>	24	25
3. Kootenai Hatchery Broodstock	63	
2001 broodstock	16	42
2003 broodstock	17	38
2004 broodstock	30	44

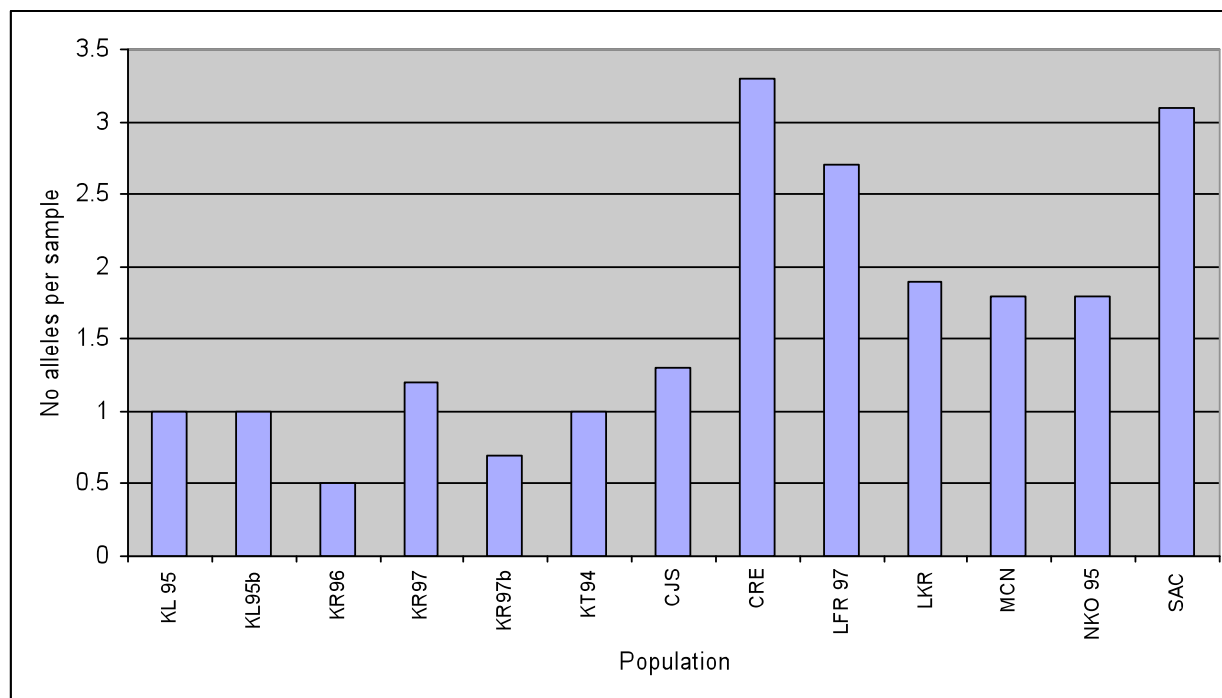


Figure 2. Number of alleles per sample by population or sample group where $n > 10$, comparing genetic variability among Kootenai River, Kootenay Lake, and other white sturgeon populations from throughout the species range. Sample sizes for each population or sample group are presented in Table 1. Population codes: KL=Kootenay Lake, KR=Kootenai River, KT=Kootenai River, CJS=C. J. Strike Reservoir, CRE=Columbia River estuary, LFR=Lower Fraser River, MCN=McNary Pool, NKO=Nechako River, SAC=Sacramento River.

Based on our analyses, a random sample of 30 to 40 fish appeared sufficient to recover approximately 80 to 90% of the genetic variation present in the Kootenai/y population (52 total alleles) (Figure 3, Table 3). Allele frequency distributions for each locus and population or sample group are presented in Appendix II.

The Kootenai/y samples were examined with respect to the total number of alleles detected in the Kootenai system (52 alleles) versus the number of alleles recovered as a function of sample size. This was done to evaluate sample size requirements to recover a certain percentage of the total genetic variation detected. In all cases with sample sizes > 10 (n ranged from 13 to 79), 71 to 94% of the 52 total alleles in the wild population were sampled (Table 3).

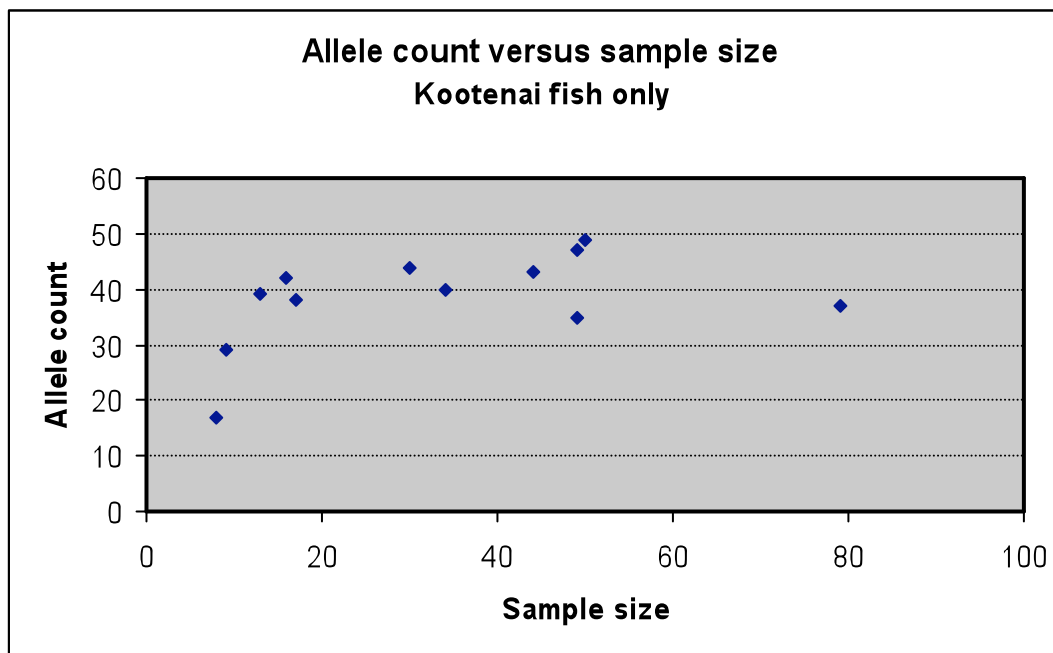


Figure 3. Plot illustrating allele count versus sample size of Kootenai/y samples only.

Table 3. Sample source, number, and numbers of alleles observed and percent of alleles sampled Percent of allelic variation (% of 52 total alleles) recovered in the Kootenai/y samples as a function of sample size.

Sample source	N	No. alleles	Percent of 52 alleles sampled
Kootenai River 1997	34	40	77%
Kootenay Lake 1995	49	47	90%
Kootenai River 1997a	8	17	33%
Kootenai River 1996	79	37	71%
Kootenai River 1997b	49	35	67%
Kootenai River 1994	13	39	75%
KT 1994	50	49	94%
Kootenai River 1996a	9	29	56%
Kootenay Lake 1995	44	43	83%
2001 broodstock	16	42	81%
2003 broodstock	17	38	73%
2004 broodstock	30	44	85%
All broodstock	63	49	94%

The genotype data from the wild Kootenai/y fish were then pooled and randomly resampled to test how many alleles would be recovered by random sampling. Twenty fish were randomly selected and the number of total alleles recovered was tallied. This process was then repeated ten times. Across the ten trials, the number of alleles recovered varied from 42 to 49 alleles, with an average of 45.4 alleles or 87% of the 52 total alleles observed in the wild Kootenai/y population. This finding indicates how a relatively small number of fish (20 in this example) can yield a representative sample of most of the genetic variability in the population.

All sampled populations were then analyzed using POPGENE and PHYLIP to estimate F_{ST} values and Nei's standard genetic distances. When all the populations were analyzed, including the eight full-sib groups and the broodstock, an F_{ST} of 0.22 was observed, indicating significant levels of genetic differentiation between the populations. A dendrogram (Figure 4) and an unrooted Neighbor-joining tree (Figure 5) were constructed from Nei's distances. (Due to their size, Figures 4 through 9 are found at the end of this report, after the references section) Following the analysis of each Kootenai/y sample group, all of the Kootenai/y samples were pooled into either "Kootenay Lake" or "Kootenai River" bins and re-analyzed using POPGENE and PHYLIP. An overall F_{ST} of 0.19 resulted from the pooled analysis, and an unrooted Neighbor-joining tree was created (Figure 6). Figures 4 and 5 are two different visualizations of the same Nei's distances. The overall pattern of the two figures indicates that the Kootenai/y fish appeared to be quite different from the other non-Kootenai/y populations. Also of interest is the amount to which the known full-sib groups diverged from the wild Kootenai/y fish. This divergence resulted from each full-sib group consisting of highly related individuals representing a small percentage of the total genetic diversity found in the wild population. The broodstock also clustered very closely with the wild Kootenai/y fish, again showing that the broodstock are an accurate representation of the genetic diversity found in the whole population. Figure 6 illustrated that the overall pattern of genetic distance was not an artifact of multiple, small samples of the Kootenai/y population.

Since the full-sib families were creating such large outgroups, they were omitted from analysis and new Nei's distances were created in order to more clearly reveal relationships between the broodstock and Kootenay Lake / Kootenai River fish. In their absence the F_{ST} dropped to 0.11,

indicating significant population differentiation but to a much lesser extent. An unrooted Neighbor-joining tree was created and shown in Figure 7. This figure also suggests that the broodstock are very closely related to the wild Kootenai/y population and represent their source population accurately.

Finally, all Kootenai/y samples were analyzed without the influence of non-Kootenai/y fish. These data were analyzed by sequentially including and excluding the known full-sib groups. An F_{ST} of 0.17 was observed when the full-sib groups were included in the analyses. When the full-sib groups were omitted, the F_{ST} dropped to 0.041, indicating only slight differences between the groups of Kootenai fish. These groups were the Kootenay Lake, Kootenai River, “KT”, and the three broodstock classes (2001, 2003, and 2004). An unrooted Neighbor-joining tree for all the Kootenai/y samples is shown in Figure 8, while the tree without the full-sib groups is shown in Figure 9. Figure 8 shows how the full-sib groups branch away from the main population due to the close genetic relationship of full-sibs. Of interest as well is the close clustering of the three broodstock groups with samples randomly drawn from the wild population. In Figure 9, it must be noted that the scale of resolution is about 10 times that of all the other unrooted trees, thus branch lengths appear 10 times the length of previous figures. The main point of Figure 9 is to demonstrate that no particular broodstock year class diverged substantially more than any of the others, indicating that the broodstock represented a random sample of the wild population.

Parentage assignment

Parentage assignment using the multilocus microsatellite genotypes was moderately successful. First, only the 8 sires (males) and 6 dams (females) used to make the test crosses were included as possible parents. Progeny were assigned to the correct sires from 16% to 100% of the time (60% mean success rate across families), and assigned to the correct dams 41% to 96% of the time (66% mean success rate of across families; Table 4).

All broodstock used in the 2004 hatchery crosses were then used as possible parents. This resulted in 14 possible sires and five dams being used. The results of parentage assignment were similar; progeny were correctly assigned to sires 0% to 88% of the time (mean 43% correct

assignment), and correctly assigned to dams 41% to 96% of the time (mean 66% correct assignment; Table 4).

Table 4. Percent correct sire and dam assignments for groups of 24 white sturgeon progeny from test fish and all 2004 broodstock at the Kootenai Hatchery. Note: these sib groups are labeled with “FS” for “Full Sib” in the tables and figures, instead of “KT”.

Family	Test cross fish only		All 2004 Broodstock	
	% Correct sire assignment	% Correct dam correct	% Correct sire assignment	% Correct dam assignment
KT261A	38%	42%	17%	42%
KT2972	17%	42%	0%	42%
KT270D	42%	50%	33%	50%
KT3672	88%	96%	88%	96%
KT6453	92%	42%	46%	42%
KT627C	100%	75%	83%	79%
KT193C	79%	92%	63%	92%
KT6532	21%	92%	17%	83%
Mean	60%	66%	43%	66%

Discussion

White sturgeon inhabiting the Kootenai River and Kootenay Lake have substantially reduced levels of genetic variation compared with white sturgeon populations elsewhere in the Columbia, Fraser, and Sacramento river basins. Using allele counts in this study as a measure of variability, Kootenai/y sturgeon contained approximately one-third the amount of variation found in these other populations. This study, along with all previous white sturgeon genetic studies involving Kootenai River or Kootenay Lake fish failed to find any allozyme markers or private alleles in the mtDNA, or nuclear DNA genomes that did not exist in downstream white sturgeon populations (Bartley et al. 1985; Setter and Brannon 1992; Anders and Powell 2002a, 2002b). Thus, it appears imperative that efforts must continue to be made to preserve what is left of the genetic variation inherent to the Kootenai/y population. However, caution is advised regarding any inferences between this level of variation and overall population fitness. Reduced within-population diversity and variation would be expected in a headwater population like Kootenai River population that has undergone post-glacial recolonization and re-founding, and recent demographic and possibly genetic bottlenecks (Anders and Powell 2002b; Paragamian et al. *In Press*).

In this study, population genetic structure of the Kootenai/y population was also compared to several outgroups, such as the Sacramento, Columbia, and Fraser river populations. It was apparent from the Neighbor-joining trees and allele frequency tables that the Kootenai/y population is unique, although the genetic “uniqueness” stems from its lack of genetic variation rather than any unique or private alleles.

Of particular interest was how well the 2001, 2002, and 2004 broodstock represented the genetic variation of the larger wild Kootenai/y population. From the perspective of both allele frequencies and phylogenetic analyses, the wild population appeared well represented by the broodstock sampled. The high frequency alleles in the Kootenai population were generally found at high levels in the broodstock, and alleles that were low in the wild population were generally found in low frequencies in the broodstock groups. When considering the broodstock

from these three years as one unit, 94% of the observed genetic variation of the wild population was represented in these individuals.

Given that overall levels of genetic variability are low, it was not surprising that 80% of the alleles could be sampled by randomly drawing 30 to 40 broodstock. Given the long generation times and overlapping generations of white sturgeon, it appears that the current protocol of using approximately 20 breeders per year is adequate but should be regarded as the minimum size used. In a simulation test where 20 fish from the Kootenai population were randomly drawn 10 different times, on average 87% of the alleles in the population were recovered in 20 fish. The conservation aquaculture program currently uses a breeding scheme to maximize the number of full sib families produced in a given year, i.e. maximizing the number of contributing males and females, with within-year hatchery broodstock availability and possibly facility space being limiting factors (KTOI 2004).

Since the mathematical models for numbers of individuals to use to maintain a population over time assume discrete generations and diploid genomes, it is difficult to make a mathematically concrete prediction of how many fish must be used per year. This is due to the highly polyploid nature of the white sturgeon genome and the complex and dynamic system of overlapping generations, unequal ages of sexual maturity, and sex-specific differences in spawning periodicity. However, it is our opinion that the Kootenai Hatchery breeding plan recommendations should still be followed, and every attempt should be made to maximize the number of males and females used for each year class, except when that practice would involve the mating of naturally produced siblings in the hatchery. An individual-based empirical demographic and genetic simulation model is currently being developed to evaluate demographic and genetic effects of various Kootenai Hatchery operations on the recipient wild Kootenai River population (Anders and Beamesderfer 2004).

An additional aspect of this study was to evaluate the use of multilocus microsatellite genotypes for the use in parentage determination of recaptured hatchery fish. The loci used in this study were also used in the Rodzen et al. (2004) study where near 100% success rates of parentage assignments were observed. Success in parentage assignment in the current study was

substantially lower and varied greatly between families, ranging from zero to 100%, with mean success rates of parentage assignments across families ranging from 45 to 66% (Table 4).

In the Rodzen et al. (2004) study, these same loci yielded over 120 alleles, while the Kootenai population contained less than 50 alleles. Since parentage assignment works via the process of elimination, the success of parentage assignment depends on the number of alleles, their frequencies, and the actual number of possible parents. Assignment success is highest when the number of alleles is high and the number of possible parents is low. To improve accuracy in the Kootenai population, more loci will need to be employed.

In conclusion, this study accomplished its four purposes. Microsatellite variation in the Kootenai/y population was quantified and reported in this study. However, it exhibited approximately one third that of other surveyed white sturgeon populations in the Columbia, Fraser, and Sacramento river basins. The amount of genetic variability in the Kootenai Hatchery broodstock group was also quantified and evaluated. Thirty to 40 randomly sampled broodstock represented 80 to 90% of the genetic variation found in the wild population. All 67 broodstock from three years sampled represented 94% of the 52 alleles found in the wild population. Thus, it appeared that no changes in broodstock collection techniques or protocols are required in the interest of genetic representation among broodstock used in the Kootenai Hatchery program. Finally, use of the 8 hypervariable microsatellite loci for parentage assignment for Kootenai hatchery progeny was only moderately successful. Parentage assignment accuracy ranged widely due to low levels of variability in the Kootenai/y fish. Creation of a white sturgeon gene library as a tool to develop additional microsatellite loci and primers is recommended to improve the accuracy of parentage assignments with Kootenai/y fish.

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Figure 4. Dendrogram of all populations using Neighbor-Joining of Nei's unbiased genetic distances.

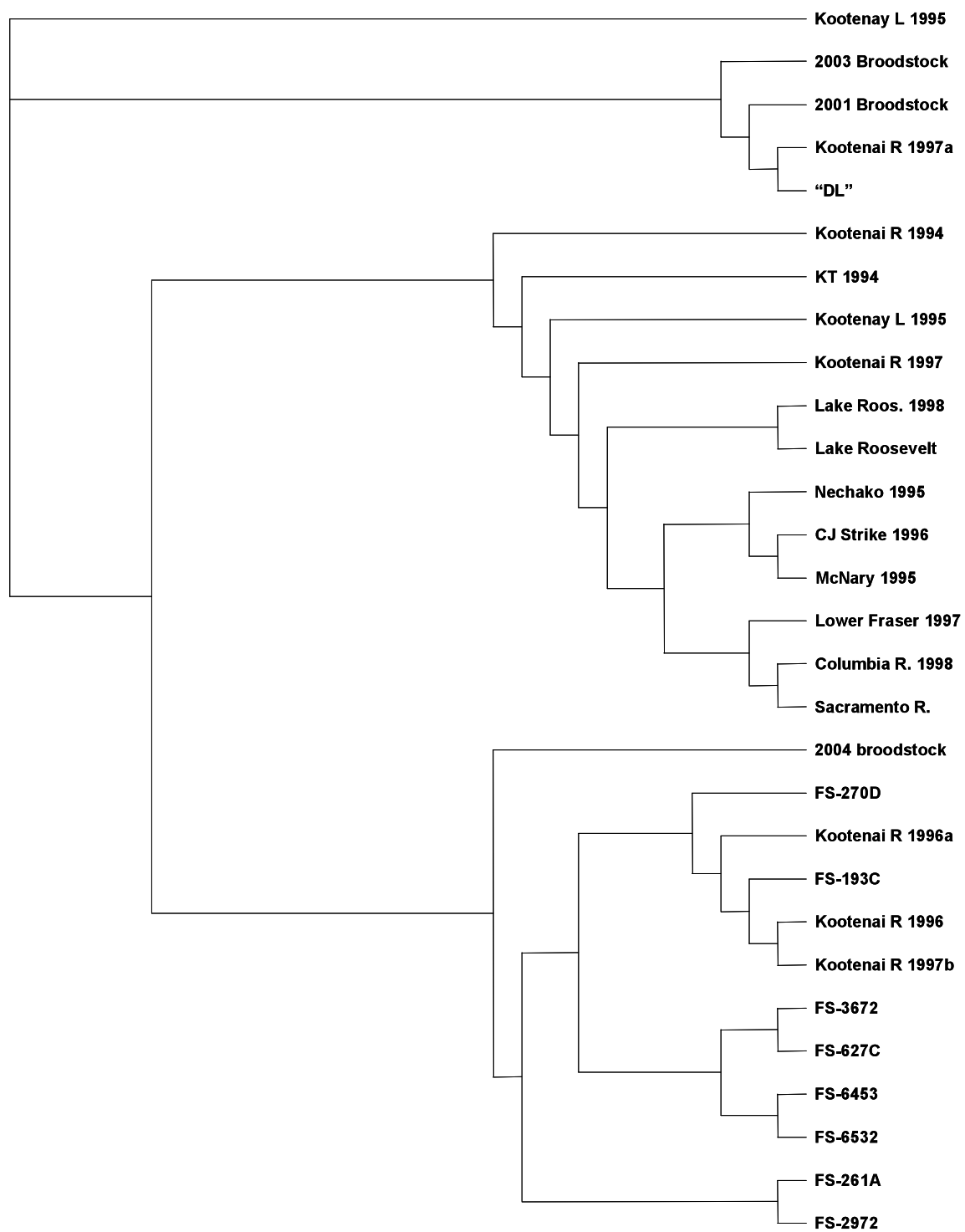
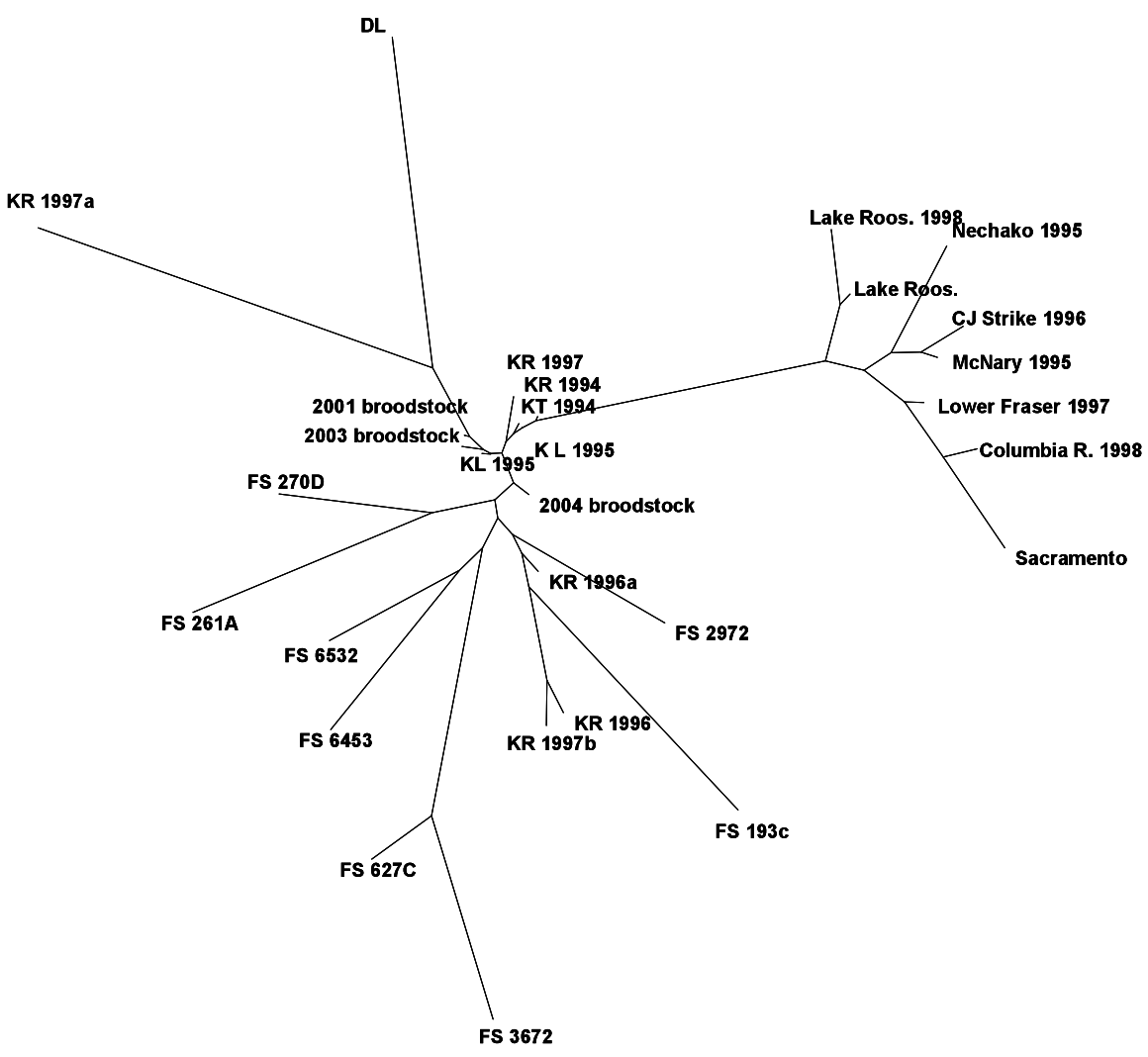


Figure 5. Unrooted tree using Neighbor-Joining of Nei's unbiased genetic distances.

Note: DL and KR 1997a only have 5 or less samples!
KR = Kootenai River; KL = Kootenay Lake



0.01

Figure 6. Unrooted tree using Neighbor-Joining of Nei's unbiased genetic distances and pooled wild Kootenai fish.

Kootenai River includes all "KR" samples (n=184)
Kootenay Lake includes all "KL" samples (n=72)

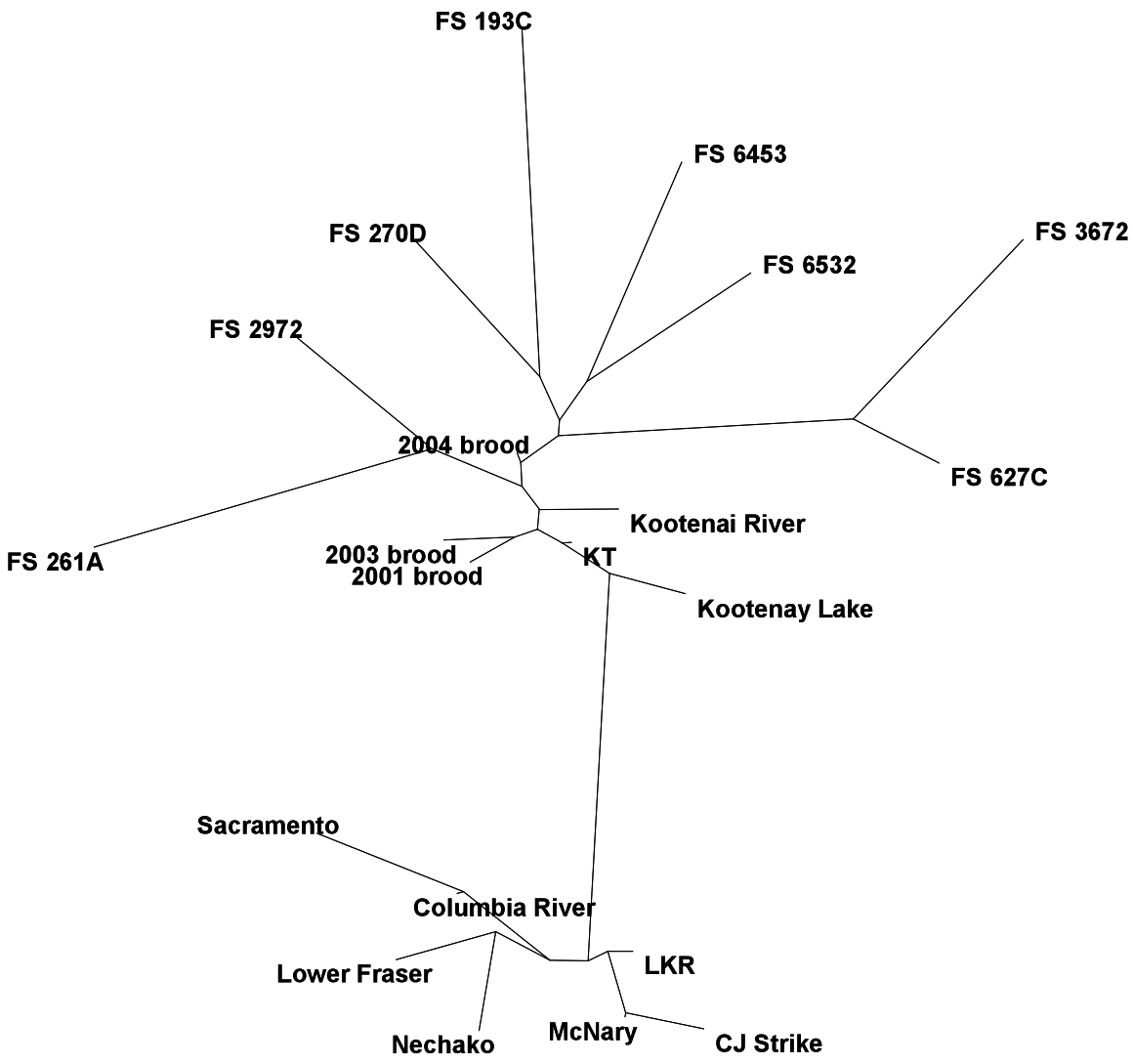
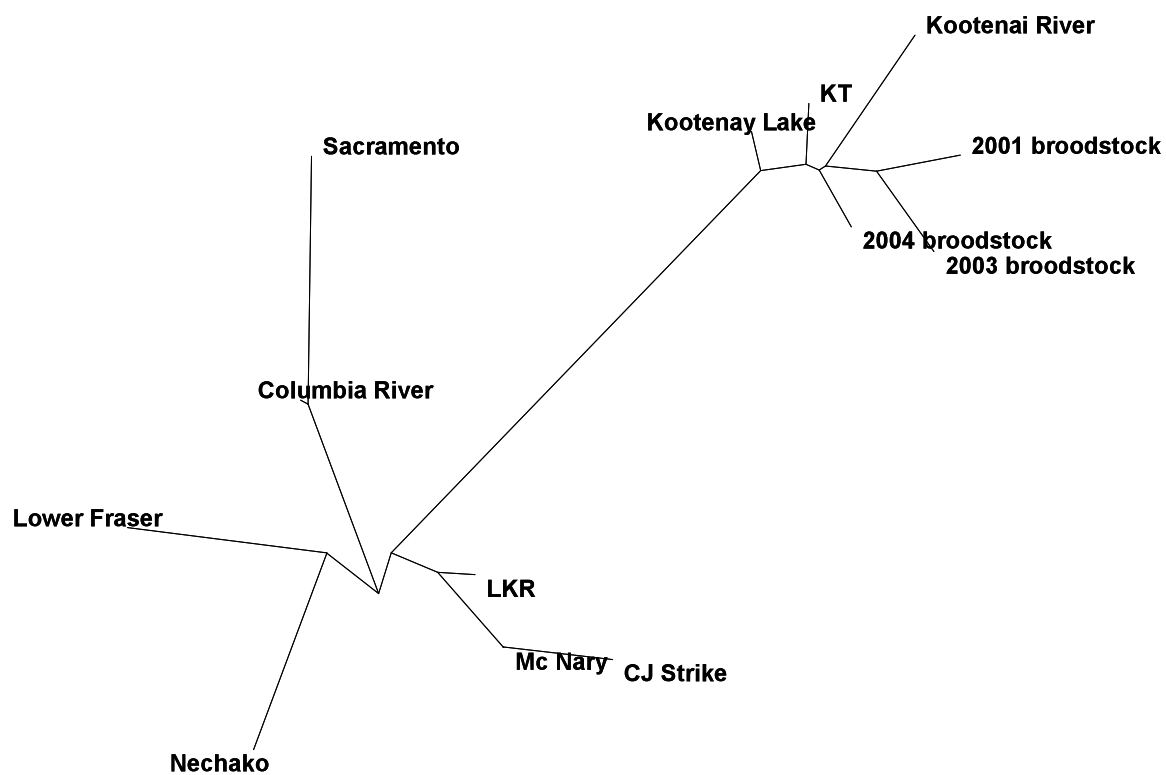


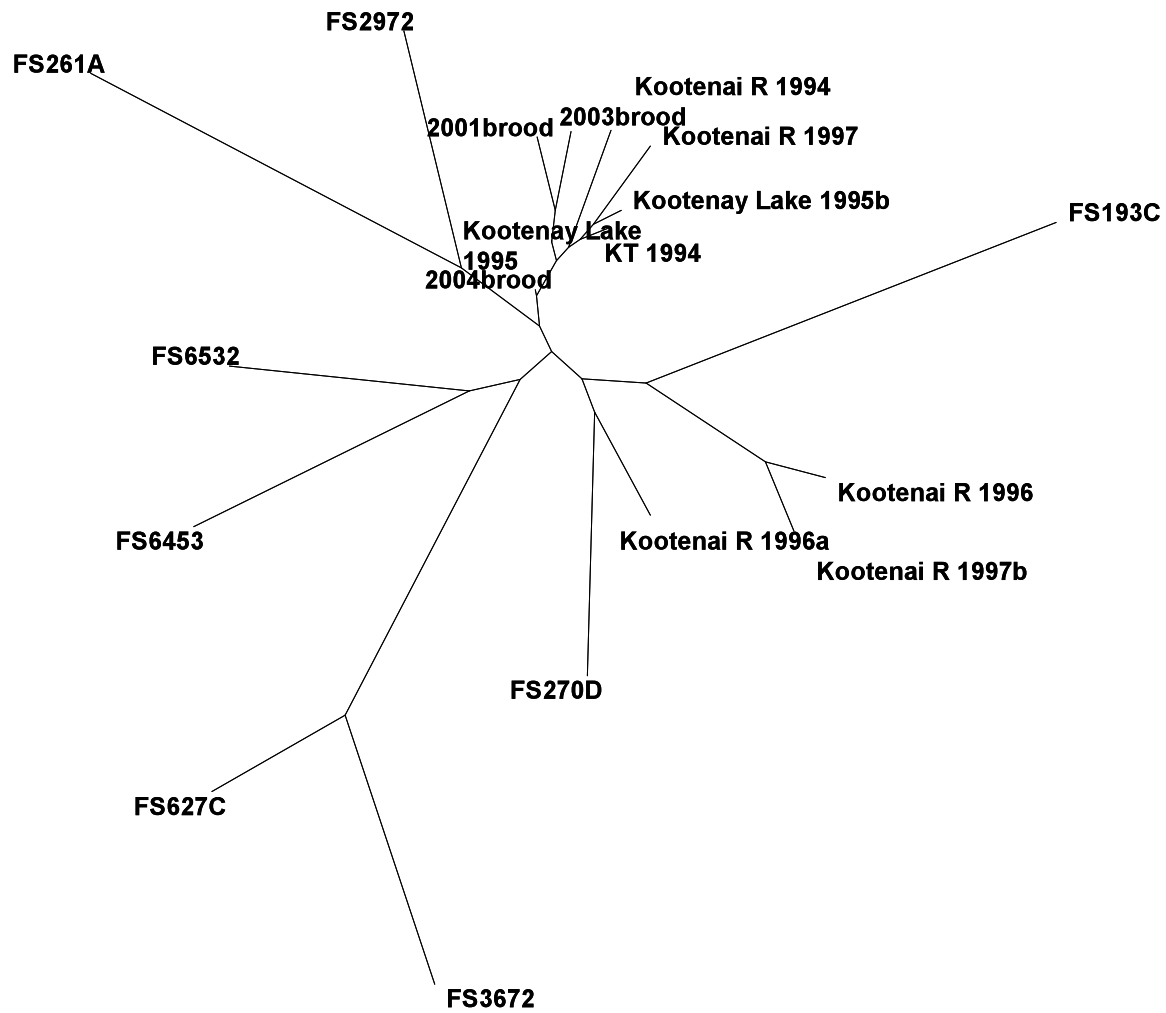
Figure 7. Unrooted tree using Neighbor-Joining of Nei's unbiased genetic distances without the full-sib groups

Overall mean $F_{ST} = 0.11$



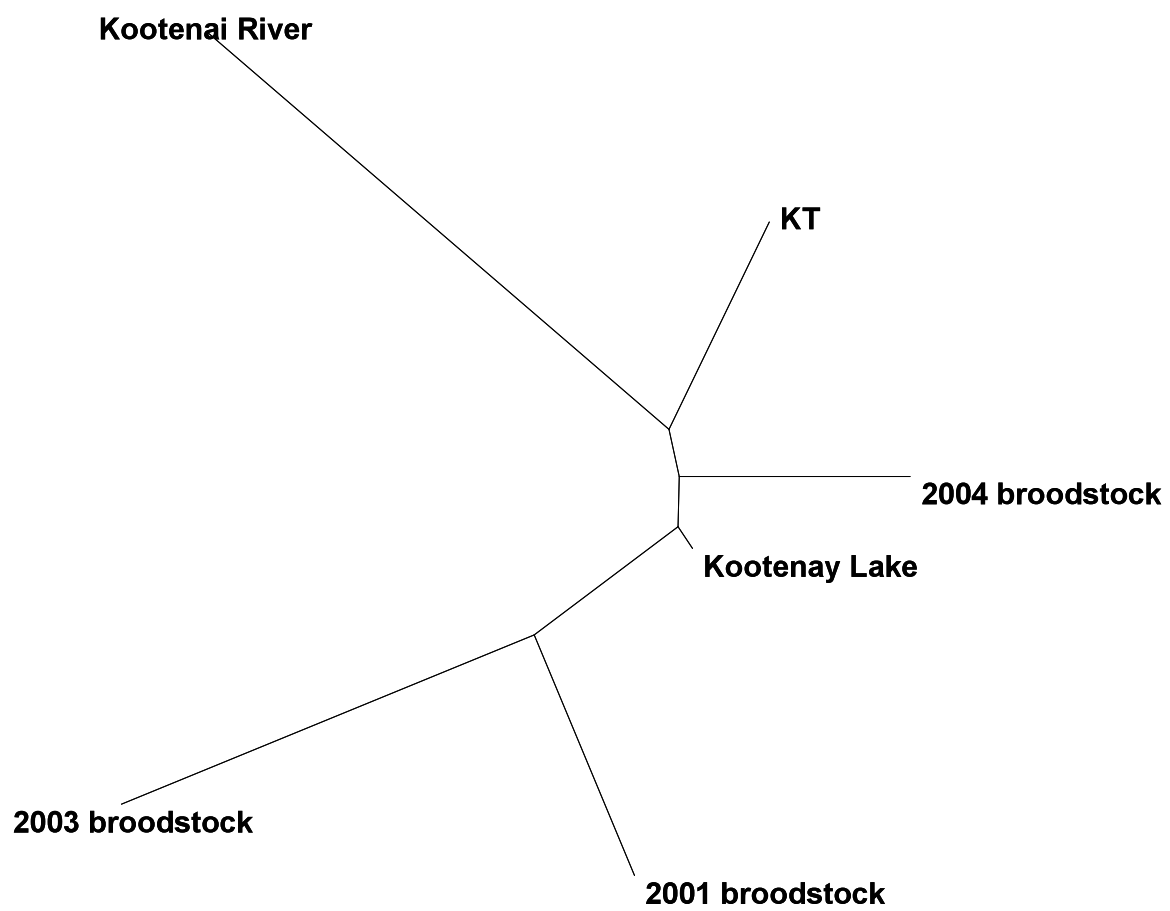
0.01

Figure 8. Unrooted tree using Neighbor-Joining of Nei's unbiased genetic distances of all Kootenai samples.
Overall mean $F_{ST} = 0.17$



0.01

Figure 9. Unrooted tree using Neighbor-Joining of Nei's unbiased genetic distances of wild Kootenai fish and the broodstock.



Appendix I.

Conversion of microsatellite bands to dominant marker loci.

Phenotypic data were converted to dominant loci using the following method. Consider a microsatellite locus with six bands that amplify in a given population. Each band is assigned an arbitrary band ladder number which corresponds to its size in base pairs. There are six bands in this hypothetical population, represented as follows:

Allelic ladder

number (i)	band size (bp)
1	178
2	174
3	170
4	166
5	162
6	158

Now consider an animal with a phenotype of bands 174, 170, and 162bp. This animal has a phenotype of 174,170,162, or using the bands numbered in the allelic ladder, 2,3,5.

Each animal's phenotype is converted into a $1 \times n$ vector where n is the number of bands at the locus. For each band i in the system, the i^{th} element in the vector is assigned a value of 1 if that band is present or a value of 0 if that band is absent in that animal's phenotype. Thus, the 2,3,5 phenotype is converted to a $1 \times n$ vector of [0 1 1 0 1 0], yielding six dominant markers.

For data with more than one microsatellite locus, the process is repeated for each locus j . This produces a $1 \times n_j$ matrix for each locus, where n_j is the number of bands at locus j . These matrices are then horizontally concatenated, resulting in a single $1 \times n_T$ matrix where n_T is the total number of bands across all loci.

Finally, data for all k individuals in the data set are concatenated in a $k \times n_T$ matrix. This matrix now contains dominant marker data for all individuals and all microsatellite loci.

Appendix II. Allele frequencies per locus and per population. *N* denotes sample size, and allele size in bp is noted along the top of the table.

Locus *Atr-100*

Location	<i>N</i>	93	97	101	105	109	122	126	130	134	138	143	147	151
Nechako 1995	49	0.01	0.6	-	-	-	0.15	0.01	0.12	0.07	0.07	0.02	0.12	0.24
Lower Fraser 1997	47	-	0.43	0.06	0.01	-	0.02	0.1	0.1	0.26	0.03	0.08	0.19	0.09
CJ Strike 1996	54	-	0.48	-	-	-	0.05	-	-	0.05	0.13	0.17	0.01	0.03
McNary 1995	50	-	0.49	-	-	-	0.06	-	-	0.05	0.15	0.18	0.01	0.04
Columbia River estuary 1998	38	-	0.41	0.04	0.04	-	0.01	0.14	-	0.19	0.03	0.15	0.11	0.06
Sacramento River	38	0.03	0.41	-	0.05	-	-	0.05	0.03	0.26	0.05	0.16	0.29	0.08
Lake Roosevelt 1998	8	-	0.37	-	-	-	0.11	-	-	0.37	-	0.37	-	-
Lake Roosevelt	48	0.03	0.35	-	-	-	0.07	0.05	-	0.08	0.04	0.27	0.04	0.11
Kootenai River 1994	13	0.05	1	-	-	-	0.15	-	-	-	-	-	-	-
"KT" 1994	50	0.06	0.83	-	-	-	0.08	-	-	-	-	-	-	-
Kootenay Lake 1995	49	0.1	0.64	-	-	0.01	0.24	-	-	-	-	-	-	-
Kootenai River 1996	88	0.21	1	-	-	-	0.18	-	-	-	-	-	-	-
Kootenay Lake 1996	23	0.11	0.62	-	-	-	0.15	-	-	-	-	-	-	-
Kootenai River 1997	83	0.16	0.72	-	-	-	0.29	-	-	-	-	-	-	-
2004 broodstock	30	0.07	0.68	-	-	-	0.29	-	-	-	-	-	-	-
2003 broodstock	17	0.04	0.54	-	-	-	0.62	-	-	-	-	-	-	-
2001 broodstock	16	0.15	0.62	-	-	-	0.35	-	-	-	-	-	-	-
Kootenai R. (pooled)	184	0.17	0.82	-	-	-	0.22	-	-	-	-	-	-	-
Kootenay Lake (pooled)	72	0.1	0.63	-	-	0.01	0.22	-	-	-	-	-	-	-
Kootenai all (pooled)	256	0.15	0.76	-	-	-	0.22	-	-	-	-	-	-	-

Location	<i>N</i>	155	159	168
Nechako 1995	49	-	-	-
Lower Fraser 1997	47	-	0.01	0.01
CJ Strike 1996	54	0.01	-	-
McNary 1995	50	0.01	-	-
Columbia River estuary 1998	38	0.03	-	-
Sacramento River	38	0.03	-	-
Lake Roosevelt 1998	8	0.11	-	-
Lake Roosevelt	48	0.03	-	-
Kootenai River 1994	13	-	-	-
"KT" 1994	50	-	-	-
Kootenay Lake 1995	49	-	-	-
Kootenai River 1996	88	-	-	-
Kootenay Lake 1996	23	-	-	-
Kootenai River 1997	83	-	-	-
2004 broodstock	30	-	-	-
2003 broodstock	17	-	-	-
2001 broodstock	16	-	-	-
Kootenai R. (pooled)	184	-	-	-
Kootenay Lake (pooled)	72	-	-	-
Kootenai all (pooled)	256	-	-	-

Locus Atr-109

Location	N	197	205	212	215	219	223	227	231	235	240	243	247	252
Nechako 1995	49	0.01	-	-	0.2	0.06	-	-	0.02	0.03	-	0.15	0.14	0.04
Lower Fraser 1997	47	0.04	-	0.02	0.03	0.06	0.06	0.04	0.08	0.02	-	0.19	0.23	0.09
CJ Strike 1996	54	-	-	-	0.13	0.04	-	-	0.26	-	0.02	0.02	0.14	0.14
McNary 1995	50	-	-	-	0.07	-	-	0.04	0.1	-	0.01	0.1	0.07	0.15
Columbia River estuary 1998	38	-	-	-	0.02	0.06	0.04	-	-	-	-	0.18	0.18	0.26
Sacramento River	38	0.03	-	-	0.06	0.15	0.03	0.01	0.07	0.03	0.03	0.08	0.11	0.18
Lake Roosevelt 1998	8	-	-	-	0.29	-	-	-	-	-	-	-	0.18	0.42
Lake Roosevelt	48	-	-	-	0.21	0.04	-	0.07	0.11	-	-	0.02	0.06	0.12
Kootenai River 1994	13	-	-	-	-	-	-	-	-	-	-	0.04	0.52	0.04
"KT" 1994	50	-	0.29	-	-	-	-	-	-	-	0.01	0.01	0.27	0.01
Kootenay Lake 1995	49	-	-	-	-	-	-	-	-	-	-	0.01	0.29	0.03
Kootenai River 1996	88	-	-	-	-	-	-	-	-	-	-	-	0.56	-
Kootenay Lake 1996	23	-	-	-	-	-	-	-	-	-	-	-	0.29	-
Kootenai River 1997	83	-	-	-	-	-	-	-	-	-	-	0.01	0.49	-
2004 broodstock	30	-	-	-	-	-	-	-	-	-	-	-	0.25	0.03
2003 broodstock	17	-	-	-	-	-	-	-	-	-	-	-	0.5	-
2001 broodstock	16	-	-	-	-	-	-	-	-	-	-	-	0.4	0.04
Kootenai R. (pooled)	184	-	-	-	-	-	-	-	-	-	-	0.01	0.52	-
Kootenay Lake (pooled)	72	-	-	-	-	-	-	-	-	-	-	0.01	0.29	0.02
Kootenai all (pooled)	256	-	-	-	-	-	-	-	-	-	-	0.01	0.45	0.01

Location	N	256	260	264	268	272	276	280	284	288	292	306
Nechako 1995	49	0.11	0.11	0.23	0.04	0.04	0.19	0.07	0.03	0.03	-	-
Lower Fraser 1997	47	0.19	0.06	0.29	0.22	0.22	0.15	0.19	0.01	0.01	0.02	0.02
CJ Strike 1996	54	0.34	0.01	0.25	0.08	0.04	0.02	0.1	0.03	-	-	-
McNary 1995	50	0.41	0.04	0.34	0.15	0.11	0.04	0.06	0.06	0.06	0.01	-
Columbia River estuary 1998	38	0.26	0.18	0.29	0.21	0.24	0.09	0.06	0.09	0.04	0.06	-
Sacramento River	38	0.15	0.21	0.19	0.26	0.32	0.28	0.03	0.03	-	0.01	-
Lake Roosevelt 1998	8	0.29	0.09	0.29	0.09	0.09	-	0.09	-	-	-	-
Lake Roosevelt	48	0.45	0.07	0.25	0.19	0.14	0.02	0.06	-	0.04	-	-
Kootenai River 1994	13	0.12	0.12	0.22	0.08	0.22	0.08	-	-	-	-	-
"KT" 1994	50	0.16	0.08	0.33	0.23	0.3	0.02	-	-	-	-	-
Kootenay Lake 1995	49	0.16	0.12	0.24	0.24	0.32	0.05	-	-	-	-	-
Kootenai River 1996	88	0.01	0.02	0.31	0.3	0.53	-	-	-	-	-	-
Kootenay Lake 1996	23	0.18	0.06	0.42	0.25	0.25	0.03	-	-	-	-	-
Kootenai River 1997	83	0.07	0.01	0.32	0.38	0.43	-	-	-	-	-	-
2004 broodstock	30	0.11	0.03	0.27	0.34	0.27	0.05	-	-	-	-	-
2003 broodstock	17	0.13	0.06	0.25	0.13	0.34	0.1	-	-	-	-	-
2001 broodstock	16	0.2	0.11	0.35	0.11	0.35	0.04	-	-	-	-	-
Kootenai R. (pooled)	184	0.04	0.03	0.31	0.31	0.46	0.01	-	-	-	-	-
Kootenay Lake (pooled)	72	0.17	0.1	0.28	0.24	0.3	0.05	-	-	-	-	-
Kootenai all (pooled)	256	0.08	0.05	0.3	0.29	0.41	0.02	-	-	-	-	-

Locus Atr-105

Location	<i>N</i>	130	133	138	142	146	150	154
Nechako 1995	49	-	0.83	-	0.41	0.62	0.01	-
Lower Fraser 1997	47	-	1	0.06	0.15	0.63	0.09	0.02
CJ Strike 1996	54	-	1	-	0.31	0.56	0.09	0.01
McNary 1995	50	-	1	-	0.17	0.59	0.07	0.02
Columbia River estuary 1998	38	-	0.7	0.03	0.16	0.76	0.09	0.01
Sacramento River	38	0.03	0.83	0.04	0.15	1	0.17	-
Lake Roosevelt 1998	8	-	1	0.09	-	0.59	0.09	0.09
Lake Roosevelt	48	-	0.85	0.04	0.22	0.51	0.07	0.1
Kootenai River 1994	13	-	1	-	-	0.35	0.04	0.42
"KT" 1994	50	-	1	-	-	0.5	0.01	0.38
Kootenay Lake 1995	49	-	1	-	-	0.45	0.01	0.5
Kootenai River 1996	88	-	1	-	-	0.53	0.01	0.45
Kootenay Lake 1996	23	-	1	-	-	0.37	0.03	0.48
Kootenai River 1997	83	-	1	-	-	0.5	-	0.47
2004 broodstock	30	-	1	-	-	0.34	-	0.59
2003 broodstock	17	-	1	-	-	0.44	-	0.44
2001 broodstock	16	-	1	-	-	0.42	0.03	0.42
Kootenai R. (pooled)	184	-	1	-	-	0.5	0.01	0.46
Kootenay Lake (pooled)	72	-	1	-	-	0.43	0.02	0.5
Kootenai all (pooled)	256	-	1	-	-	0.48	0.01	0.46

Locus Atr-107

Location	N	180	184	188	192	196	200	204	208	213	215	217	219	221
Nechako 1995	49	-	0.03	0.44	0.17	0.04	-	0.29	0.31	-	-	0.02	-	0.13
Lower Fraser 1997	47	0.01	0.37	0.37	0.2	0.16	0.02	0.2	0.23	0.02	-	0.03	0.06	0.06
CJ Strike 1996	54	-	0.1	0.39	0.43	-	-	0.36	0.26	-	-	-	-	0.45
McNary 1995	50	-	0.15	0.34	0.34	0.07	0.01	0.26	0.19	0.03	0.03	0.03	-	0.16
Columbia River estuary 1998	38	-	0.31	0.36	0.27	0.16	0.03	0.16	0.14	0.05	-	0.06	0.01	0.14
Sacramento River	38	-	0.45	0.33	0.41	0.23	-	0.13	0.08	0.03	-	-	-	0.11
Lake Roosevelt 1998	8	-	0.07	0.35	0.35	0.07	-	0.24	0.24	-	-	0.07	-	0.47
Lake Roosevelt	48	-	0.1	0.28	0.35	0.12	0.01	0.3	0.22	0.01	-	0.01	-	0.27
Kootenai River 1994	13	-	0.18	0.09	0.09	-	-	1	0.09	-	-	-	-	0.35
"KT" 1994	50	-	0.07	0.03	0.09	-	-	0.76	0.21	-	-	-	-	0.32
Kootenay Lake 1995	49	-	0.29	0.07	0.08	-	-	0.67	0.18	-	-	-	-	0.44
Kootenai River 1996	88	-	0.01	0.15	0.11	-	0.01	1	0.13	-	-	-	-	0.31
Kootenay Lake 1996	23	-	0.12	0.08	0.12	-	-	0.72	0.22	-	-	-	-	0.45
Kootenai River 1997	83	-	0.04	0.3	0.02	-	-	1	0.11	-	-	-	-	0.42
2004 broodstock	30	-	0.15	0.07	0.02	-	-	0.74	0.19	-	-	-	-	0.51
2003 broodstock	17	-	0.35	0.04	-	-	-	0.54	0.29	-	-	-	-	0.47
2001 broodstock	16	-	0.22	0.04	0.08	-	-	0.61	0.45	-	-	-	-	0.45
Kootenai R. (pooled)	184	-	0.03	0.2	0.07	-	0.01	1	0.12	-	-	-	-	0.35
Kootenay Lake (pooled)	72	-	0.25	0.07	0.09	-	-	0.68	0.19	-	-	-	-	0.44
Kootenai all (pooled)	256	-	0.09	0.16	0.07	-	-	0.83	0.14	-	-	-	-	0.38

Location	N	225	229	232	236	240	244	248	252	256	264
Nechako 1995	49	0.44	-	-	0.15	0.05	0.28	-	0.13	-	-
Lower Fraser 1997	47	0.17	-	0.02	0.14	0.12	0.13	0.05	0.02	0.02	-
CJ Strike 1996	54	0.15	-	-	0.13	0.15	0.29	0.01	-	-	-
McNary 1995	50	0.13	-	-	0.15	0.03	0.19	0.06	-	-	-
Columbia River estuary 1998	38	0.09	0.08	0.01	0.08	0.11	0.18	0.09	-	0.06	0.01
Sacramento River	38	0.11	0.08	0.03	0.05	-	0.13	0.03	-	0.03	-
Lake Roosevelt 1998	8	0.24	-	-	0.07	-	0.07	-	-	-	-
Lake Roosevelt	48	0.24	-	-	0.05	0.09	0.16	0.02	-	-	-
Kootenai River 1994	13	0.29	-	-	-	0.04	0.09	-	-	-	-
"KT" 1994	50	0.17	-	-	-	0.01	0.07	-	-	-	-
Kootenay Lake 1995	49	0.18	-	-	-	-	0.18	0.02	-	-	-
Kootenai River 1996	88	0.02	-	-	-	-	0.39	0.01	-	-	-
Kootenay Lake 1996	23	0.08	-	-	-	-	0.08	-	-	-	-
Kootenai River 1997	83	0.09	-	-	-	-	0.34	-	-	-	-
2004 broodstock	30	0.21	-	-	-	-	0.07	-	-	-	-
2003 broodstock	17	0.2	-	-	-	-	0.15	-	-	-	-
2001 broodstock	16	0.08	-	-	-	0.04	0.22	-	-	-	-
Kootenai R. (pooled)	184	0.07	-	-	-	-	0.35	-	-	-	-
Kootenay Lake (pooled)	72	0.16	-	-	-	-	0.16	0.02	-	-	-
Kootenai all (pooled)	256	0.09	-	-	-	-	0.29	0.01	-	-	-

Locus Atr-114

Location	N	181	185	190	194	198	202	204	208	210	214	218	222	224
Nechako 1995	49	-	-	1	0.16	0.1	0.04	0.05	0.6	0.32	0.39	0.02	0.09	0.01
Lower Fraser 1997	47	-	0.01	0.78	0.28	0.23	0.13	0.15	0.35	0.11	0.33	0.11	0.13	0.02
CJ Strike 1996	54	-	-	0.85	0.21	0.07	0.05	0.04	0.52	0.18	0.3	0.01	0.14	-
McNary 1995	50	-	-	0.84	0.22	0.12	0.06	0.06	0.51	0.32	0.22	0.08	0.14	-
Columbia River estuary 1998	38	0.02	0.02	0.82	0.25	0.17	0.12	0.15	0.69	0.21	0.29	0.05	0.12	0.02
Sacramento River	38	-	-	0.81	0.41	0.13	0.13	0.04	0.55	0.05	0.19	0.05	0.31	-
Lake Roosevelt 1998	8	-	-	0.42	0.29	0.59	0.09	0.18	0.42	0.18	0.29	0.09	0.09	-
Lake Roosevelt	48	-	-	0.73	0.27	0.25	0.04	0.1	0.4	0.19	0.34	0.04	0.05	-
Kootenai River 1994	13	-	-	1	0.55	0.23	-	0.23	0.37	-	-	-	-	-
"KT" 1994	50	-	-	0.74	0.67	0.35	-	0.29	0.3	-	0.2	-	-	-
Kootenay Lake 1995	49	-	-	0.84	0.73	0.3	-	0.34	0.32	0.1	0.2	-	-	-
Kootenai River 1996	88	-	-	0.85	1	0.21	-	0.85	0.18	0.15	0.12	-	-	-
Kootenay Lake 1996	23	-	-	0.59	0.59	0.42	-	0.29	0.18	-	0.18	-	-	-
Kootenai River 1997	83	-	-	0.62	0.78	0.23	-	0.52	0.18	0.08	0.08	-	-	-
2004 broodstock	30	-	-	0.66	0.61	0.41	-	0.22	0.27	0.15	0.17	-	-	-
2003 broodstock	17	-	-	1	0.61	0.45	-	0.38	0.38	0.22	0.22	-	-	-
2001 broodstock	16	-	-	0.74	0.74	0.48	-	0.74	0.18	0.14	0.14	-	-	-
Kootenai R. (pooled)	184	-	-	0.74	0.82	0.22	-	0.63	0.19	0.11	0.1	-	-	-
Kootenay Lake (pooled)	72	-	-	0.79	0.71	0.32	-	0.33	0.3	0.09	0.2	-	-	-
Kootenai all (pooled)	256	-	-	0.75	0.79	0.24	-	0.54	0.21	0.11	0.12	-	-	-

Location	N	230
Nechako 1995	49	-
Lower Fraser 1997	47	0.01
CJ Strike 1996	54	-
McNary 1995	50	0.01
Columbia River estuary 1998	38	-
Sacramento River	38	-
Lake Roosevelt 1998	8	-
Lake Roosevelt	48	-
Kootenai River 1994	13	-
"KT" 1994	50	-
Kootenay Lake 1995	49	-
Kootenai River 1996	88	-
Kootenay Lake 1996	23	-
Kootenai River 1997	83	-
2004 broodstock	30	-
2003 broodstock	17	-
2001 broodstock	16	-
Kootenai R. (pooled)	184	-
Kootenay Lake (pooled)	72	-
Kootenai all (pooled)	256	-

Locus Atr-113 (con't)

Location	<i>N</i>	283	287	291	295	298	303
Nechako 1995	49	0.09	0.02	0.09	0.06	0.02	0.02
Lower Fraser 1997	47	-	0.01	-	-	-	-
CJ Strike 1996	54	-	-	-	-	-	-
McNary 1995	50	-	-	-	-	-	-
Columbia River estuary 1998	38	-	-	-	0.01	-	-
Sacramento River	38	0.03	0.03	0.01	-	-	-
Lake Roosevelt 1998	8	-	-	-	-	-	-
Lake Roosevelt	48	-	-	-	-	-	-
Kootenai River 1994	13	-	-	-	-	-	-
"KT" 1994	50	-	-	-	-	-	-
Kootenay Lake 1995	49	-	-	-	-	-	-
Kootenai River 1996	88	-	-	-	-	-	-
Kootenay Lake 1996	23	-	-	-	-	-	-
Kootenai River 1997	83	-	-	-	-	-	-
2004 broodstock	30	-	-	-	-	-	-
2003 broodstock	17	-	-	-	-	-	-
2001 broodstock	16	-	-	-	-	-	-
Kootenai R. (pooled)	184	-	-	-	-	-	-
Kootenay Lake (pooled)	72	-	-	-	-	-	-
Kootenai all (pooled)	256	-	-	-	-	-	-