Use of Polysomic Genetic Markers to Address Critical Uncertainties in White Sturgeon Biology and Management

By

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This work is dedicated to my husband, Brian Schreier, and our unconventional family. Their love and companionship has carried me through good times and bad.

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ABSTRACT

The application of genetic markers to investigate evolutionary and ecological questions about white sturgeon, *Acipenser transmontanus*, has been limited due to the species' highly duplicated nuclear genome. Here, polysomic microsatellite markers were used to 1) examine the ancestral level of genome duplication in white sturgeon, 2) examine genetic diversity and patterns of population structure within and among drainages across the species' range, and 3) provide genetic monitoring for a conservation aquaculture program sustaining an endangered white sturgeon population. In the first chapter, we followed the inheritance of eight microsatellite markers in 15 families of white sturgeon from a commercial caviar farm to determine whether white sturgeon (~250 chromosomes) should be classified as tetraploid or octoploid. The eight microsatellite loci were detected predominantly in four or eight copies, with one locus observed in >8 copies. Numbers of alleles per locus, patterns of allele transmission, and inference of gene copy number in parents suggested that white sturgeon should be considered ancient octoploids. The discovery of dodecaploid parents and their decaploid offspring in the farm population, confirmed by flow cytometry analysis, indicated that some aspect of sturgeon aquaculture was inducing spontaneous autopolyploidy in white sturgeon.

Next, microsatellite markers were applied to examine white sturgeon population structure across the species' range. Population assignment testing was used to determine the origin of white sturgeon sampled in non-natal estuaries, or those not containing a spawning population, to evaluate marine dispersal behavior. The Sacramento-San Joaquin River system was found to contain a single white sturgeon population while the

Fraser River exhibited a hierarchical pattern of population structure. Strong levels of genetic divergence were detected above and below a natural barrier, Hells Gate, and fine-scale population substructure was identified above Hells Gate. Population structure in the Columbia River drainage (including the mainstem Columbia and Snake Rivers) was complex and suggested a pattern of isolation by distance. Net downstream gene flow also may have contributed to this pattern, with individuals migrating downstream through impoundments and over barriers with little upstream movement possible. There was no support for the current practice of managing each impounded reach on the Columbia or Snake Rivers as a separate population. Lack of population structure within historically continuous river habitat found across the species' range suggested spawning site fidelity in white sturgeon may occur on a regional scale, with local gene flow among geographically proximate spawning sites. Population assignment of samples collected from non-natal estuaries indicated that all populations with ocean access make marine migrations, and individuals did not necessarily originate from the nearest spawning population.

Finally, microsatellites were used to conduct genetic monitoring of the Kootenai Tribe of Idaho's conservation aquaculture program (CAP) for the endangered Kootenai River white sturgeon population. Continuous recruitment failure in this population has left it entirely dependent on the CAP for reproduction. A genetic profile database of wild broodstock used in the CAP was created to monitor hatchery-induced genetic changes in the Kootenai River population. Broodstock genotypes also were used to evaluate the accuracy of parentage assignment in the Kootenai River population, as hatchery managers soon will depend on this analysis to prevent inbreeding when most sexually

mature adults available for captive breeding will be derived from hatchery production. Numbers of alleles and numbers of alleles per individual per locus were calculated to monitor the amount of wild type genetic diversity captured in broodstock utilized by the CAP. Parentage analysis with 18 microsatellite markers was validated in known hatchery families from the 2010 year class. Genetic diversity in the Kootenai River population was very low relative to other populations examined, likely due to founder effects and genetic drift after isolation from the mainstem Columbia c. 10,000 YBP. In less than one sturgeon generation, 96% of Kootenai River genetic diversity has been captured in broodstock that contributed offspring that survived to release in the Kootenai River and further propagation will likely preserve additional genetic variation. The 18 microsatellite panel improved parentage assignment accuracy and allowed a greater number of assignments relative to the previous panel used for parentage analysis, suggesting that this technique may become a useful tool in the management of this vulnerable population.

CHAPTER 1

Neutral markers confirm the octoploid origin and reveal spontaneous autopolyploidy in white sturgeon, *Acipenser transmontanus*

Andrea Drauch Schreier, Daphne Gille, Brian Mahardja, and Bernie May

ABSTRACT

The role of polyploidy in vertebrate genome evolution remains a fertile area of research and sturgeons (order Acipenseriformes) provide a unique model of genome duplication, with species possessing ~120, ~250 or ~360 chromosomes. Cytogenetic and molecular data have been used to support different hypotheses about the number of genome duplications in this polyploid series; however, few studies have examined inheritance in sturgeons, although evaluation of polysomic segregation ratios is crucial to inferring ancestral genome duplication level in a polyploid species. Here we examine the inheritance of eight microsatellite loci in fifteen white sturgeon (*Acipenser transmontanus*) families of known parentage to infer the level of genome duplication. Microsatellites were detected as four or eight copy loci. Numbers of alleles per locus, transmission frequencies of informative alleles, and gene copy numbers in parents reveal an ancient octoploid origin for white sturgeon. Comparison to the lake sturgeon genome suggests the ~250 chromosome state in sturgeon was achieved by multiple independent polyploid events. The discovery of spontaneous autopolyploids via microsatellite analysis and flow cytometry provides additional evidence of the plasticity of highly duplicated sturgeon genomes.

INTRODUCTION

The ancient sturgeons and paddlefishes (order Acipenseriformes) provide a unique model of genome evolution in vertebrates, as their radiation contains multiple polyploidization events (Birstein et al. 1997). Acipenseriformes evolved from a common ancestor with a diploid chromosome number of 60 (Dingerkus and Howell 1976; Birstein and Vasil'ev 1987). Early investigations of sturgeon genome size and structure by karyotype or flow cytometry assorted extant species into three groups: those possessing ~120 (group A), ~250 (group B), or ~500 chromosomes (group C; Birstein and Vasil'ev 1987; Birstein et al. 1993; Blacklidge and Bidwell 1993). The arrangement of the ~120 chromosome American paddlefish, *Polyodon spathula*, karyotype into groups of tetrads (Dingerkus and Howell 1976) led researchers to infer that all Acipenseriformes with ~120 chromosomes were similarly tetraploid derived, those possessing ~250 chromosomes were octoploid derived, and the sole species with ~500 chromosomes, *Acipenser mikadoi*, was hexadecaploid (16n) derived. Recent revisions to these classifications include the discovery of ~360 chromosomes in the shortnose sturgeon, *Acipenser brevirostrum*, (Kim et al. 2005) and only ~250 chromosomes in *Acipenser mikadoi* (Vishnyakova et al. 2008). Group C has been revised to include only the shortnose sturgeon with 360 chromosomes.

Some have proposed reconsideration of ploidy classifications for sturgeon. Fontana (1994) examined the nucleolar organizing regions (NORs) of four sturgeon species and concluded that the group A species had fully diploidized; therefore the group B species should be considered tetraploid, and *A. brevirostrum* hexaploid. Several subsequent papers have upheld these designations (Tagliavini et al. 1999; Fontana 2002;

Fontana et al. 2008). Ludwig et al. (2001) used the numbers of alleles per locus at six microsatellite loci in 20 sturgeon species to support the new categorizations. Others suggest these revisions are inappropriate. Birstein (2005) indicates that the high number of NORs in group A relative to teleost fishes does indeed reflect ancient tetraploidy. Also, Vasil'ev (2009) argues that the presence of duplicated genes and the karyotypic structure of the genome support an ancient tetraploid origin for the group A species, with subsequent diploidization and gene silencing. He further proposes two different scales for considering relationships between ploidy groups within the Acipenseriformes: the evolutionary scale of diploid (2n ≈60), tetraploid (2n ≈120), octoploid (2n ≈250), and dodecaploid (2n ≈360) as well as the recent scale of diploid (2n ≈120), tetraploid (2n ≈250) and hexaploid (2n ≈360), reflecting the procession to diploidization in each lineage (Vasil'ev 2009).

Regardless, designating group B and group C species as tetraploid and hexaploid based on extant levels of diploidization of the group A ploidy class may oversimplify and misrepresent the evolutionary history of Acipenseriformes. It is possible that group B and C species diverged from group A species before 120 chromosome genomes were fully diploidized and if this were the case, higher levels of polysomy might be expected in species with 250 and 360 chromosomes. No studies either inferring ploidy level or mechanisms of genome duplication cited above include gene segregation data in their analyses. However, these data are essential as only examination of segregation ratios in known families can provide evidence of polysomic inheritance and therefore the ancestral level of genome duplication in a species (Wright et al. 1983; Allendorf and Thorgaard 1984; Marsden et al. 1987; David et al. 2003).

The white sturgeon (*Acipenser transmontanus*), a species with ~250 chromosomes (Fontana 1994; Hedrick et al. 1991; Van Eenennaam et al. 1998a), is the largest North American freshwater fish, native to large river systems and estuaries along the west coast of the continent. Analysis of synaptonemal complex formation in white sturgeon spermatocytes suggested that although highly duplicated, the male white sturgeon genome may be fully diploidized as only bivalent pairing was observed (Van Eenennaam et al. 1998b). However, a previous study examining inheritance of microsatellite loci in white sturgeon found evidence of polysomy and genome duplication higher than tetraploidy (Rodzen and May 2002). Here, we follow the inheritance of eight additional microsatellite loci from known families of white sturgeon to examine levels of genome duplication. We use numbers of alleles per locus, transmission frequencies of informative alleles, and gene copy numbers in parents to reveal an ancient octoploid origin for white sturgeon. Comparison to the lake sturgeon genome suggests the ~250 chromosome state in sturgeon was achieved by multiple independent polyploid events. As genotype data suggest the presence of alternative ploidy states among the individuals examined, we employ flow cytometry to provide additional evidence of spontaneous autopolyploidy in a captive population of white sturgeon. The documentation of viable spontaneous autopolyploids in captive white sturgeon further suggests the plasticity of highly duplicated sturgeon genomes.

METHODS

Sample Collection

Fifteen full- and half-sibling white sturgeon families were created at Sterling Caviar LLC (Elverta, California) from crosses of five female (Y243, 065, Y3, Y192, 062) and three male (7219, 1178, 3d09) broodstock. Fin clips were collected from the eight broodstock and 48 one day post-hatch offspring from each cross were sacrificed for DNA extraction (N = 720). DNA was obtained from all samples using a standard DNA extraction kit (Promega).

Blood samples were provided by farm personnel from 3 yr old male (N = 3) and 7 yr old female (N = 3) white sturgeon to examine alternative ploidy levels within the farm population with flow cytometry. Approximately 2.0 mL of blood was drawn from the caudal vein and mixed with 2.0 mL of heparin to prevent clotting. A single chicken blood sample was provided by UC Davis Hopkins Avian Facility personnel (Protocol # 15053) for use as a diploid control in flow cytometric ploidy analysis. All blood samples were stored on ice on their way to the lab for processing.

Microsatellite Genotyping

PCR was conducted with labeled primers for eight microsatellite loci developed for white, green (*A. medirostris),* or Chinese (*A. sinensis)* sturgeon (Table 1.1). Amplification reactions consisted of 1.0 µl of 10X reaction buffer, 0.2 mM of each dNTP (Promega), 1.5 – 3.0 mM $MgCl_2$, 5.0 µM each of forward and reverse primers, 0.375 U Taq polymerase (Promega GoTaq) and dH_20 to a final volume of 10 µl. PCR was performed in either GeneAmp® 9700 PCR systems (Life Technologies; LT) or MJ Dyad™ DNA Engines (BioRad; Table 1.1). PCR product was diluted with dH_20 and 1.0 µl of diluted product then was added to 9.0 µl of highly deionized formamide (The Gel

Company) and 0.1 µl of LT Rox 400 HD size standard. Samples were denatured for 3 min at 95^{o}C before loading on an LT ABI 3130xl Genetic Analyzer for fragment analysis. Size-calling of alleles was conducted in GeneMapper v4.0 software. Stutter was present at several loci (AciG 43, 52, 53, 110, As015) and the height ratios of adjacent peaks were used to distinguish between stutter peaks and true alleles.

Flow Cytometry

Chicken blood collected for ploidy analysis by flow cytometry was mixed with equal parts of 2% dextran sulfate in a polystyrene tube and incubated at 37°C for 45 min. The resulting leukocyte layer was transferred to a new container and centrifuged at 1200 rpm for 5 min. The supernatant was aspirated and the chicken cell pellet was further processed in the same manner as white sturgeon samples. Approximately 1.0 – 2.0 mL of heparinized sturgeon blood was mixed with equal parts of 5.0 mM EDTA in phosphate buffered saline (PBS) and centrifuged at 1200 rpm for 5 min. The supernatant was aspirated and ACK buffer (1.55 M NH_4Cl and 0.1 M $KHCO_3$) was applied to the white sturgeon blood cells and chicken leukocytes for 4 min to lyse any remaining erythrocytes. The lysis reaction was stopped by the addition of 3.0 – 4.0 mL of staining media (SM: PBS with 2.0 mM EDTA). Leukocytes were centrifuged at 1200 rpm for 5 min and pellets were treated with ACK buffer a second time. Leukocyte cell pellets were re-suspended in 500 µl of SM and counted by hemacytometer using Trypan blue and an inverted lens microscope. Aliquots of 1.0 – 2.0 x 10^6 cells were transferred to polystyrene tubes containing 1.0 – 2.0 mL of PBS and centrifuged at 1200 rpm for 5 min. Leukocytes were fixed in 200 µl of 70% ethanol for 10 min on ice and washed twice with 3.0 – 4.0 mL of SM. Fixed leukocytes were centrifuged at 2000 rpm for 5 min, re-

suspended in 250 μl of RNase (100 μg/mL in PBS), and incubated at room temperature for 30 min. The leukocytes were washed with SM and centrifuged at 2000 rpm for 5 min. The supernatant was aspirated and leukocytes re-suspended in 250 – 500 μL of propidium iodide (PI; 40 μg/mL in PBS) for flow cytometry analysis.

Microsatellite Data Analysis

We evaluated the level of genome duplication in white sturgeon in two ways. First, we examined the number of alleles per individual found at each locus in both the parents and progeny. We made comparisons between the average number of alleles per locus observed in families of dams exhibiting a higher ploidy level (Y192 and 065) and the nine remaining families with two sample t-tests, using the harmonic mean to account for differences in sample size (Sokal and Rohlf, 1981). Second, we examined the dosage of informative alleles in each parent. Due to 1) the high numbers of alleles detected per individual, 2) size specific amplification bias in loci with allele size ranges spanning >100 base pairs, and 3) our inability to detect null alleles in a duplicated genome, inferring allele dosage from electrophoretic data was impossible. Therefore, we used the frequency that an informative allele (allele unique to one parent) was transmitted to progeny in half-sibling families to determine parental allele dosage. We calculated 95% confidence intervals (CIs) around the frequency of allelic transmission and applying the rules of Mendelian inheritance, alleles with transmission frequency CIs overlapping 0.5 (50% of offspring inherited an allele) were considered to be present in a single copy in the parent, whereas CIs overlapping 0.83 suggested two copies of an allele. CIs with a lower bound > 0.83 indicated more than two copies of an allele were present in the parent (Rodzen and May 2002). In ambiguous cases, where the lower bounds of transmission

frequency CIs were greater than 0.5 but upper limits were less than 0.83, we conservatively classified the allele as present in a single dose. Applying a Mendelian model of inheritance was deemed to be appropriate as previous research indicated that microsatellite alleles were transmitted 1:1 from parent to offspring in white sturgeon (Rodzen and May 2002).

We also evaluated white sturgeon genome structure by examining patterns of allelic transmission. We calculated the number of informative, single copy alleles that were transmitted by one parent to individual offspring. If a microsatellite system was present as a single tetrasomic locus, a parent could transmit a maximum of two alleles to an offspring. At a microsatellite system consisting of two tetrasomic loci or one octosomic locus, a single offspring could inherit up to four informative alleles from one parent. In this analysis, allelic transmission was examined on a per family basis as different allele combinations were informative in different crosses.

Finally, we looked for evidence of disomy in the white sturgeon genome by examining segregation frequencies of informative, single copy allelic pairs transmitted by parents. In these analyses, we assumed that double reduction was negligible in white sturgeon, a premise supported by the absence of multivalent pairing observed in male white sturgeon (Van Eenennaam et al. 1998b), although pairing is unknown in female white sturgeon. In the case where a primer pair is amplifying two or more disomic loci, certain alleles should never be co-transmitted to the same offspring, as alleles at the same disomic locus should segregate during meiosis. In contrast, two alleles originating from a single tetrasomic locus, two tetrasomic loci, or a single octosomic locus have a non-zero probability of co-transmission (1/6, 1/4, and 15/70, respectively). We used these

Mendelian ratios to construct simple chi square tests to examine conformance of each locus to disomic, tetrasomic, and octosomic models of inheritance. Only pairs of informative, single copy alleles were included in this analysis. The presence of many microsatellite systems consisting of disomically inherited loci would suggest that white sturgeon are well progressed along the evolutionary path to diploidization.

Flow Cytometry Data Analysis

Nuclear DNA content of the chicken and white sturgeon leukocytes was estimated by analyzing PI fluorescence with a BD FACScan flow cytometer (Becton Dickinson) with a single argon laser with a 488 nm excitation beam and BD CellQuest analysis software (Becton Dickinson). We measured the fluorescence of 5,000 – 30,000 stained leukocyte nuclei per sample. DNA content was estimated by comparing the fluorescence and genome size (2.5 pg/nucleus) of the diploid chicken standard to the resulting white sturgeon fluorescence by the equation DNA (pg) = 2.5 x (S/C), where S and C correspond to the mean fluorescence values of the white sturgeon and chicken leukocytes respectively (Rasch et al. 1970).

RESULTS

Microsatellite Data

The numbers of alleles per individual per locus ranged from 1 - 11 (mean 2.06 – 7.65) across eight loci and 15 white sturgeon families (Table 1.2). Dams 065 and Y192 appeared to exhibit a higher level of genome duplication than other parents. At presumed

eight copy loci (see below), dam Y192 possessed from 6 - 10 alleles per locus, while other white sturgeon parents possessed from 4 – 7 alleles per locus (Table 1.2). Offspring in the families of Y192 and 065 had a significantly greater average number of alleles per locus at 8 of 8 and 5 of 8 loci, respectively (Table 1.2).

Segregation of informative alleles in offspring revealed that gene copy number ranged from 4 - 13 in parents, with the highest levels of duplication found in 065 and Y192, although dam 062 possessed at least 10 gene copies at AciG 35 (Table 1.3). Most other parents possessed eight or fewer gene copies at each locus, although sire 3d09 had nine copies at AciG 52 (Table 1.3). All loci were present in at least four copies (AciG 53, 140), with some loci in eight or more copies (AciG 2, 35, 43, 52, 110, As015; Table 1.3). At AciG 53 and 140, offspring exhibited 1 - 4 alleles per individual and offspring segregation data revealed that parents possessed 2 - 5 gene copies per locus. Only dams 065 and Y192 possessed greater than 4 copies at these loci. The remaining loci were inferred to be in ≥8 gene copies, with individuals possessing from 2 - 9 alleles per locus, excluding 065 and Y192 and their families (Table 1.2). AciG 2 only exhibited 2 - 5 alleles per locus, but the presence of up to five alleles in many individuals suggests it too is duplicated in > 4 copies. We infer AciG 35 to be present in more than eight copies, as three parents (Y192, 065, 062) possessed > 8 copies of locus AciG 35, including one parent (065) with >12 copies (Table 1.3). Three of the progeny of 062 possessed nine alleles at AciG 52, although we can only infer that 062 has a minimum of 7 or 8 gene copies at this locus, as two of her alleles are uninformative (Tables 1.2, 1.3).

The number of single copy informative alleles transmitted to individual offspring ranged from 0 - 4 in 065, 0 - 6 in Y192, and 0 - 4 in the remaining parents. Informative

alleles present in >1 copy were not considered so these intervals represent minimum estimates. This analysis was not possible in AciG 2 or AciG 53 due to a paucity of informative alleles, but at four copy locus AciG 140, parents transmitted up to two single copy alleles to individual offspring. At As015, a locus also limited by few informative alleles, this analysis was only possible with families of Y192, and she transmitted 0-6 alleles to individual offspring.

The frequency with which informative single copy alleles were inherited together within families provided some evidence of disomic inheritance in two loci. In AciG 43, alleles *305* and *347* from dam Y243 were inherited independently in family Y243x1178, although allele *305* was found at a relatively low frequency in this family (20% of offspring). In family Y192x7219, alleles *313* and *317* were never inherited together but allele *317* was present in only 10% of offspring. Several AciG 43 alleles in multiple families were observed at lower frequencies than expected, suggestive of meiotic drive or some other non-Mendelian process (Table 1.3). Alleles *254* and *262* at AciG 35 from sire 7219 were not inherited together in family Y3x7219 and these alleles were found in 50% and 48% of offspring, respectively. This allelic pair was informative only in one other family Y3x3d09, where *254* and *262* were inherited together in nine offspring. For the majority of allele pairs across the eight loci, patterns of inheritance conformed to models consistent with a single tetrasomic locus (7.1%), two tetrasomic loci (11%), multiple polysomic models (60%), or no model (21%) of inheritance.

Flow Cytometry Data

We estimated the DNA content of three female and three male white sturgeon relative to a diploid chicken control using flow cytometry. Figure 1 is a representative plot that combines flow cytometry histogram data from two females (Fe 1 and Fe 2) with markedly different PI fluorescences as well as the chicken control. Genome size ranged from 8.51 pg/cell to 11.39 pg/cell in white sturgeon, indicating that the presence of multiple ploidy classes (Table 1.4).

DISCUSSION

These results support previous work with microsatellite markers suggesting that the white sturgeon genome is highly duplicated (Rodzen and May 2002). None of the markers examined here appear to follow the model of Mendelian disomic inheritance. This refutes the claims of others that white sturgeon are fully diploidized (Fontana 2002; Van Eenennaam et al. 1998b) and is consistent with Rodzen and May (2002), who found only one sex-specific disomic microsatellite marker in white sturgeon. At this locus, Atr 113, two males exhibited disomic inheritance while two females appeared tetrasomic. Ludwig et al. (2001) report one microsatellite locus presumed to be disomic in white sturgeon, but this conclusion was based only on the number of alleles per locus and no inheritance data were included. Also the samples examined in Ludwig et al. (2001) originated from an endangered white sturgeon population found by others to have very low genetic diversity (see Chapters 2, 3). Polysomic inheritance in white sturgeon characterized here and in Rodzen and May (2002) suggests that although bivalent pairing of chromosomes is occurring at least in males, not enough genetic differences have

accumulated between chromosome copies to result in consistent preferential pairing of homologs (Stift et al. 2008). Lacking any information on meiotic chromosome behavior in female white sturgeon, we assume for the species a model of random bivalent pairing among homologous chromosomes (Rodzen and May 2002; Stift et al. 2008).

Pooling data from this study and Rodzen and May (2002), we find ten loci in > 4 copies in the white sturgeon genome, suggesting that the species is octoploid derived and the classification of white sturgeon as tetraploid is invalid. One possible explanation for the presence of both four copy and eight copy loci in white sturgeon is the duplication of individual gene loci (Ludwig et al. 2001). AciG 35 appears to be an example of individual locus duplication, as it is found in a minimum of ten copies in an 8n individual (062) and in at least 13 copies in an individual with a more highly duplicated genome (065). However, if white sturgeon were evolutionary tetraploids that had experienced some tandem gene duplications, one would expect to observe an abundance of four copy loci with few loci exhibiting higher levels of duplication. Examining the 17 microsatellites characterized thus far in white sturgeon, we find there are actually more loci in > 4 copies (10) than there are four copy loci (7), suggesting that localized gene duplication is an unlikely explanation for the presence of the eight copy loci (Rodzen and May 2002; this study).

We might expect to observe four copy and eight copy loci in a genome shaped by an allopolyploid event. In an 8n allopolyploid, one might expect to find microsatellites systems consisting of two tetrasomic loci. One of two tetrasomic loci in a pair might become fixed for a null allele, or lost altogether from the genome, and therefore be undetectable by PCR, producing microsatellites detected in only four copies such as

AciG 140 and AciG 53. Given that modern sturgeon species are known not only to hybridize both in the wild and in culture (Burtsev 1972; Pirogovskiĭ et al. 1986; Sokolov and Vasil'ev 1986a; Birstein et al. 1997; Ludwig et al. 2009) but also produce viable offspring (Burtsev 1972; Sokolov and Vasil'ev 1986b,c; Arefjev 1989), allopolyploid steps in the radiation of the Acipenseriformes seem plausible. Vasil'ev (1999) and Fontana et al. (2008) provide models of Acipenseriform evolution containing allopolyploid steps.

The alternative scenario of genome duplication in white sturgeon is autopolyploidy. In an ancient 8n autopolyploid, microsatellites originally present in eight copies as octosomic loci would gradually decay into pairs of disomic loci if bivalent pairing becomes nonrandom. A more recently derived 8n autopolyploid would possess loci predominantly inherited in octosomic ratios. Alleles inherited together at four copy locus AciG 140 exhibit behavior consistent with transmission from a single tetrasomic locus while allelic pairs at loci with ≥8 copies conform to patterns expected for alleles originating from either a single tetrasomic locus, two tetrasomic loci, or conform to multiple models of inheritance. Conformance to multiple models may be due to the similarity in segregation ratios expected for various inheritance models (e. g. 0.25 for two tetrasomic loci vs. 0.21 for a single octosomic locus) or to an inheritance pattern intermediate to tetrasomy and octosomy. The sample sizes used here do not give us the statistical power to differentiate between such close ratios. Additional examination of the white sturgeon genome with more markers and larger sample sizes is required to determine the mode of polyploidization in *A. transmontanus*.

Another group B species for which microsatellite inheritance data are available is the lake sturgeon. Although lake sturgeon and white sturgeon exhibit nearly identical genome sizes, lake sturgeon microsatellites were shown previously to be inherited as single tetrasomic loci, pairs of disomic loci, or single disomic loci (Pyatskowit et al. 2001; McQuown et al. 2002; Welsh and May 2006). It is uncertain if the two locus types in lake sturgeon reflect segmental allopolyploidy (Stebbins 1947; Johnson et al. 1987; Wolfe 2001) or simply greater progression towards diploidization in another ancient octoploid. Differences in genome structure suggest that lake sturgeon and white sturgeon may be the result of different polyploidization events, which is supported by the most recent sturgeon phylogenies (Peng et al. 2007; Krieger et al. 2008). This reinforces the idea that it is impossible to make generalizations about the evolution of an entire ploidy class based only on data from a single species in that group.

The dams Y192 and 065 provide another example of the plasticity of sturgeon genomes, as these individuals possess a greater number of gene copies than other parents examined in this study. At loci present in eight copies (AciG 2, 43, 52, As015), Y192 exhibits a minimum of 10 - 11 copies per locus and 065 possesses a minimum of 6 – 11 copies per locus. All of the alleles possessed by Y192 and 065 are found in other parents or in wild individuals from the Sacramento-San Joaquin population, the source for most broodstock at Sterling, suggesting high allele numbers in these individuals aren't due to PCR artifacts. Only one other parent exhibited >8 copies at one of the eight copy loci. Although sire 3d09 possesses a minimum of nine copies at AciG 52, he exhibits ≤ 8 or ≤ 4 copies at other eight and four copy loci, respectively, suggesting these data reflects either a novel individual duplication or an overestimation of copy number. Only two of 134

offspring from crosses of 3d09 with 8n females possess nine alleles, which is fewer than would be expected if 3d09 regularly were transmitting more than four alleles to his progeny.

The offspring of Y192 and 065 have significantly more alleles per locus than offspring from other crosses, confirming that higher copy number in these dams is not due to sample contamination or PCR artifacts. We detect fewer numbers of alleles per locus and gene copies in 065 and her offspring than in Y192 and her offspring, as 065 possesses a greater number of copies of just a few common alleles, many of which are uninformative.

The cause of the unusual gene copy number in these females is uncertain, although one possibility is that they descended from the fusion of an unreduced gamete with a normal gamete. Pyatskowit et al. (2001) and McQuown et al. (2002) discovered hatchery-reared lake sturgeon that were the products of unreduced gametes donated by one parent in artificial crosses, although the cause of abnormal meiosis was unclear. Three wild adult white sturgeon sampled in the Columbia and Sacramento Rivers exhibited unusually high numbers of alleles per locus across loci, suggesting that the phenomenon of unusual gene copy number is not exclusive to captive rearing (ADS, unpublished data). Karyotypic and cytological studies have revealed differences in chromosome number and DNA content between wild and captive bred white sturgeon as well as wild sturgeon originating from different populations (Hedrick et al. 1991; Blacklidge and Bidwell 1993; Fontana 1994; Van Eenennaam et al. 1998b; Zhou et al. 2011). Blacklidge and Bidwell (1993) report individual lake sturgeon and Gulf sturgeon (*Acipenser oxyrinchus desotoi*) with genome sizes that are 3n to the 2n genome size

measured in the species. It was suggested that interspecific hybridization may have caused the observed increase in genome size (Blacklidge and Bidwell 1993), but as Y192 and 065 are the progeny of controlled crosses in captivity, hybridization can be ruled out. Zhou et al. (2011) conducted flow cytometry analysis on nine sturgeon species and discovered several examples of intraspecific variation in genome size, particularly amongst captive bred individuals. One of twelve adult white sturgeon examined by these authors possessed a genome size 1.5 times larger than the typical white sturgeon genome (Zhou et al. 2011).

Assuming white sturgeon are octoploid derived and Y192 and 065 are the products of unreduced gametes, they would be dodecaploid (12n). The transmission of six (AciG 43, AciG 52, AciG 110) and seven (As015) informative alleles to each offspring by Y192 and up to five informative alleles (AciG 52) by 065 is consistent with dodecaploidy. If Y192 and 065 are dodecaploids, their crosses with 8n males would produce decaploid (10n) offspring. Indeed, the progeny of Y192 and 065 exhibited up to ten alleles at eight copy loci and 11 alleles at AciG 35. Although we were unable to perform flow cytometric analysis on the offspring of Y192 and 065, we discovered two individuals (Fe 2 and Fe 3) in a sample of six white sturgeon from the caviar farm that possessed unusually large genome sizes (Table 1.4). Previous investigation of genome size in sturgeon showed the putative 12n shortnose sturgeon (*Acipenser brevirostrum*) had an average DNA content of 13.075 ± 0.051 pg/cell while the 8n white sturgeon had a mean genome size of 9.463 ± 0.043 (Blacklidge and Bidwell 1993). Genome sizes of 10.44 pg/cell (Fe 3) and 11.39 pg/cell (Fe 2) seem to represent an intermediate 10n genome size between the previously observed 8n and 12n genome sizes in the

Acipenseriformes. The parentage of Fe 2 and Fe 3 is unknown, but they originate from the same year class and may be sisters or half-sisters. The other female, Fe 1, originates from the same year class as Fe 2 and Fe 3 but may not share common parents. The males sampled (M1 – M3) represent a different year class from the females and similarly may have different parentage. Unfortunately, we were unable to obtain blood samples Y192 and 065 to confirm their dodecaploidy. Farm records indicate thatY192 is still living and she will be sampled for flow cytometric analysis at the next available opportunity.

Alternative ploidy states within extant species have been documented in the wild for other fish species and several amphibians (Bogart 1980; Legatt and Iwama 2003). Diploid and octoploid populations of the frog *Ceratophyrs ornata* have been discovered and both diploid and tetraploid populations of *Odontophrynus spp*. exist (Bogart 1980). Legatt and Iwama (2003) suggest that spontaneous polyploidy may be relatively common in certain fish lineages. One species exhibiting spontaneous polyploidy is the dojo loach, *Misgurnus anguillicaudatus,* which can be found in diploid, triploid, and rare tetraploid forms in the wild (Arai et al. 1993). Spontaneous polyploids often are morphologically indistinguishable from individuals of "normal" ploidy (e.g. this study) and the prevalence of this phenomenon in fishes may be underappreciated (Legatt and Iwama 2003). Autopolyploids may be intentionally produced in aquaculture to improve growth rate and carcass quality of food fishes and invertebrates (Piferrer et al. 2009). However, the induction of autopolyploidy would be unintended in the farmed population we describe.

The fate of spontaneous autopolyploids may be determined by a number of factors, including their fertility, the presence pre- or post-zygotic isolating mechanisms between spontaneous autopolyploids and "normal" individuals, and their competitive

advantage or disadvantage to the "normal" population. We can only partially address the first two factors. The presence of 10n individuals in the farm population suggests that 12n females are fertile and can produce viable offspring when crossed with normal 8n males. This is not unexpected in a species where bivalent chromosome pairing at meiosis may be the norm. The 10n form is clearly viable, although nothing is known about its fertility relative to 8n individuals. Backcrosses of a decaploid to either an octoploid or dodecaploid would produce aneuploid 9n or 11n offspring, respectively. Even if these individuals were viable, their unbalanced chromosome number (9n or 11n) may lead to unusual pairings at meiosis and the production of aneuploid gametes. Certain triploid salamanders, however, have been observed to bypass the constraints of aneuploidy by producing unreduced triploid gametes through pre-meiotic endomitotic replication (Bogart 1980). Further research is required to determine the viability and fertility of 9n and 11n aneuploid forms.

The discovery of spontaneous autopolyploidy in white sturgeon here and in Zhou et al. (2011) calls into question the notion that genome duplication in lower vertebrates must necessarily occur through hybridization. Future research examining meiotic processes in sturgeon of both sexes may help us to better understand the potential for autopolyploidy in this lineage. Likewise, studies examining the reproductive potential of naturally produced hybrids, particularly the fertility of progeny from interploid crosses, will greatly increase our understanding of the role of hybridization in sturgeon speciation.

Our findings have implications for sturgeon conservation as well. Several conservation aquaculture programs are operating to preserve endangered sturgeon species such as the pallid sturgeon (*Scaphirhynchus albus;* Oldenburg et al. 2004), Kootenai

River white sturgeon, an endangered distinct population segment (Ireland et al. 2002), and Adriatic sturgeon (*Acipenser naccarii*; Congiu et al. 2011). As similar artificial spawning techniques are utilized across sturgeon aquaculture programs, it is possible that the unintentional production of spontaneous autopolyploids is occurring in multiple facilities. Screening of additional sturgeon production facilities is necessary to ascertain the prevalence of spontaneous autopolyploidy in captive bred sturgeon. This need is most pressing in conservation focused programs, as some alternative ploidy states may exhibit lowered fertility. Introducing individuals with low fertility into wild populations would be counterproductive to conservation aquaculture goals. Experiments to identify environmental and genetic factors that may lead to spontaneous autopolyploidy in captive bred sturgeon are required so artificial spawning techniques can be modified, if necessary, to prevent its occurrence.

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Table 1.1. Conditions for microsatellite PCR in white sturgeon families.

Locus	$MgCl_2$ (mM)	Ta (°C)	Annealing Time (s)	Reference
AciG 2	1.5	60	30	Börk et al. (2008)
AciG 53	2.0	56	105	Börk et al. (2008)
AciG 140	2.5	56	30	Börk et al. (2008)
AciG 35	3.0	56	30	Börk et al. (2008)
AciG 43	2.0	56	30	Genbank: HM459582[†]
AciG 52	2.0	56	105	Börk et al. (2008)
AciG 110	2.0	56	30	Börk et al. (2008)
As015	2.5	56	30	Zhu et al. (2005)

[†]Forward primer sequence: TAATACAGCGGGGATGGAA

Reverse primer sequence: GCACAGTGAAAGCACGGTAA

Table 1.2. Numbers of microsatellite alleles observed in parents and offspring at each locus. Four copy loci: AciG 53, 140. Eight copy loci: AciG 2, 35, 43, 52, 110, As015. Asterisks indicate significantly greater mean number of alleles per individual relative to 9 families from dams Y243, Y3, and 062 ($P<0.001$).

Locus	Alleles															
	Y243	F_1	065	F_1	Y3	F_1	Y192	F_1	062	F_1	7219	F_1	1178	F_1	3d09	F_1
		($\overline{X}$)		($\overline{X}$)		($\overline{X}$)		($\overline{X}$)		($\overline{X}$)		($\overline{X}$)		($\overline{X}$)		($\overline{X}$)
AciG 2	3	1-3	3	1-3	2	1-3	2	1-3	3	1-4	3	1-4	3	1-4	2	1-3
		(2.49)		(3.10*)		(2.32)		(2.59*)		(2.56)		(2.64)		(2.61)		(2.55)
AciG 53	3	1-3	2	1-2	2	1-2	4	2-4	2	1-2	2	1-4	2	1-4	2	1-4
		(2.41)		(1.96)		(1.96)		(3.24*)		(1.92)		(2.25)		(2.32)		(2.32)
AciG 140	3	1-4	3	1-4	2	1-4	3	2-5	3	1-4	2	1-4	4	2-5[b]	3	2-5[b]
		(2.67)		(3.28*)		(2.43)		(3.14*)		(2.62)		(2.54)		(3.04)		(2.90)
AciG 35	6	4-8	6	4-10	6	3-8	6	5-9	5	5-8	7	4-9	6	3-10	7	5-10
		(6.14)		(7.65*)		(6.08)		(7.37*)		(6.82)		(6.69)		(6.70)		(7.04)
AciG 43	6	2-7	5	2-9	6	2-7	9	4-9	4	3-7	5	2-8	5	2-9[b]	3	2-8
		(4.31)		(5.67*)		(4.37)		(6.22*)		(4.69)		(5.17)		(5.36)		(4.62)
AciG 52	6	4-8 (5.58)	7	4-10	5	3-8	10	5-11	7	4-9[a]	6	4-10[b]	4	4-9[b]	5	4-11[b]
				(6.98*)		(5.60)		(7.41*)		(6.02)		(6.68)		(5.79)		(6.48)
AciG 110	6	3-7 (5.35)	4	3-6	5	3-7	8	4-9	4	3-6	6	2-8	5	3-9[b]	4	3-9[b]
				(4.75)		(4.78)		(6.51*)		(4.87)		(5.22)		(5.43)		(4.75)
As015	4	2-5 (3.84)	5	2-7	4	2-6	9	4-10	4	3-6	4	3-9[b]	5	3-9[b]	4	2-10[b]
				(4.94*)		(4.57)		(7.12*)		(4.67)		(4.97)		(5.18)		(4.92)

[a]One offspring from 062x7219 and 2 offspring from 062x3d09 had 9 alleles at AciG 52. [b]Offspring with unusually high allele numbers are from families with 065 and Y192 as dams

Table 1.3. Minimum estimates of gene copy number at microsatellite loci in white sturgeon parents. Only informative alleles (alleles unique to one parent in a cross; A_I), are used in this analysis. N is the number of offspring evaluated. Dams are Y243, 065, Y3, Y192, 062; sires are 1178, 7219, 3d09.

Locus	Parent	Genotype	Min Copy No.	A_I	Allele count / N	Transmission frequency ± 95% CI	Est. dosage
AciG 2	Y243	*283/287/295*	3	*295*	27/48	0.56±0.14	1
	065	*271/283/287*	4	*271*	117/143	0.82±0.06	2
	Y3	*283/295*	2	*295*	25/48	0.52±0.14	1
	Y192	*283/295*	4	*295*	48/48	1.00	>2
	062	*283/287/299*	3	*299*	67/144	0.47±0.08	1
	1178	*283/287/295*	3	*287*	38/95	0.40±0.10	1
				295	39/95	0.41±0.10	1
	7219	*283/287/295*	3	*287*	59/96	0.61±0.10	1
				295	38/96	0.40±0.10	1
	3d09	*283/287*	3	*287*	84/96	0.88±0.07	2
AciG 53	Y243	*214/218/222*	3	*222*	72/144	0.50±0.08	1
	Y192	*214/218/222/242*	5	*222*	119/144	0.83±0.06	2
				242	74/144	0.51±0.08	1
AciG 140	Y243	*164/172/180*	3	*164*	57/96	0.59±0.10	1

Table 1.3 continued

Locus	Parent	Genotype	Min Copy No.	A_I	Allele count / N	Transmission frequency ± 95% CI	Est. dosage
	065	*158/164/180*	5	*158*	30/84	0.63±0.14	1
				164	91/96	0.95±0.04	>2
	Y3	*164/172*	2	*164*	46/96	0.48±0.10	1
	Y192	*164/168/172*	4	*164*	79/96	0.82±0.08	2
				168	66/144	0.46±0.08	1
	062	*164/172/180*	3	*164*	56/96	0.58±0.10	1
	1178	*158/164/172/180*	4	*158*	91/191	0.48±0.07	1
				172	29/47	0.62±0.14	1
				180	48/95	0.51±0.10	1
	7219	*172/180*	4	*172*	47/48	0.98±0.04	>2
				180	52/96	0.54±0.10	1
	3d09	*158/172/180*	4	*158*	92/192	0.48±0.07	1
				172	37/48	0.77±0.12	2
				180	48/96	0.50±0.10	1
AciG 35	Y243	*250/254/258/266/274/286*	8	*250*	96/96		>2

Table 1.3 continued

Locus	Parent	Genotype	Min Copy No.	A_I	Allele count / N	Transmission frequency ± 95% CI	Est. dosage
				254	17/48	0.35±.0.13	1
				266	70/96	0.73±0.09	1
				274	45/96	0.47±0.10	1
	065	*238/242/250/254/282/286*	13	*238*	127/142	0.89±0.05	>2
				242	119/142	0.84±0.06	2
				250	47/47	1.00	>2
				254	40/47	0.85±0.10	2
				282	75/95	0.79±0.08	2
	Y3	*238/250/258/274/286/306*	7	*238*	121/143	0.85±0.06	2
				250	30/48	0.63±0.14	1
				274	67/96	0.70±0.09	1
	Y192	*238/250/254/266/270/282*	11	*238*	71/144	0.49±0.08	1
				250	48/48	1.00	>2
				254	24/48	0.50±0.14	1
				266	81/96	0.84±0.07	2

Table 1.3 continued

Locus	Parent	Genotype	Min Copy No.	A_I	Allele count / N	Transmission frequency ± 95% CI	Est. dosage
				270	96/96	1.00	>2
				282	48/96	0.50±0.10	1
	062	*238/250/254/274/278*	10	*238*	125/144	0.87±0.05	2
				250	48/48	1.00	>2
				254	48/48	1.00	>2
				274	47/96	0.49±0.10	1
				278	31/96	0.65±0.13	1
	1178	*258/266/274/282/286/306*	7	*258*	126/143	0.88±0.05	2
				266	63/142	0.44±0.08	1
				274	47/95	0.49±0.10	1
				282	61/143	0.43±0.08	1
				286	53/96	0.55±0.10	1
				306	101/191	0.53±0.07	1
	7219	*250/254/258/262/278/286/306*	8	*254*	24/48	0.50±0.14	1
				258	72/144	0.50±0.08	1

Table 1.3 continued

Locus	Parent	Genotype	Min Copy No.	A_I	Allele count / N	Transmission frequency ± 95% CI	Est. dosage
				262	112/240	0.47±0.06	1
				278	89/192	0.46±0.07	1
				286	83/96	0.86±0.07	2
				306	119/192	0.62±0.07	1
	3d09	*254/258/262/270/278/286/306*	7	*254*	32/48	0.67±0.13	1
				258	63/143	0.44±0.08	1
				262	138/239	0.58±0.06	1
				270	102/191	0.53±0.07	1
				278	98/191	0.51±0.07	1
				306	93/191	0.49±0.07	1
				286	46/96	0.48±0.10	1
AciG 43	Y243	*301/305/309/347/351/363*	7	*301*	28/95	0.29±0.09[a]	1
				305	21/96	0.22±0.08[a]	1
				347	21/48	0.44±0.14	1
				351	73/95	0.77±0.08	2

Table 1.3 continued

Locus	Parent	Genotype	Min Copy No.	A_I	Allele count / N	Transmission frequency ± 95% CI	Est. dosage
				363	55/96	0.57±0.10	1
	065	*293/297/305/347/351*	10	*293*	117/143	0.82±0.06	2
				297	72/95	0.76±0.09	2
				305	82/95	0.86±0.07	2
				347	44/47	0.94±0.07	>2
				351	66/96	0.69±0.09	1
	Y3	*297/301/309/313/351/354*	6	*297*	26/95	0.27±0.09[a]	1
				301	32/96	0.33±0.09[a]	1
				313	26/48	0.54±0.14	1
				351	53/96	0.55±0.10	1
				354	78/143	0.55±0.08	1
	Y192	*289/293/301/305/309/313/317/351/363*	11	*289*	80/144	0.56±0.08	1
				293	77/144	0.53±0.08	1
				301	41/96	0.43±0.10	1
				305	88/96	0.92±0.05	>2

Table 1.3 continued

Locus	Parent	Genotype	Min Copy No.	A_I	Allele count / N	Transmission frequency ± 95% CI	Est. dosage
				313	21/48	0.48±0.14	1
				317	40/144	0.28±0.07[a]	1
				351	42/96	0.44±0.10	1
				363	52/96	0.54±0.10	1
	062	*293/297/305/347*	8	*293*	133/144	0.92±0.04	>2
				297	28/48	0.58±0.14	1
				305	67/96	0.70±0.09	1
				347	38/48	0.79±0.11	2
	1178	*301/309/313/351/359*	6	*301*	53/95	0.56±0.10	1
				309	72/95	0.76±0.09	2
				313	56/143	0.39±0.08[a]	1
				351	18/48	0.38±0.14[a]	1
				359	107/238	0.45±0.06	1
	7219	*297/305/309/347/363*	5	*297*	79/143	0.55±0.08	1
				305	25/48	0.52±0.14	1

Table 1.3 continued

Locus	Parent	Genotype	Min Copy No.	A_I	Allele count / N	Transmission frequency ± 95% CI	Est. dosage
				309	70/96	0.73±0.09	1
				347	49/96	0.51±0.10	1
				363	73/144	0.51±0.08	1
	3d09	*309/313/347*	4	*309*	82/96	0.85±0.07	2
				313	49/144	0.34±0.08[a]	1
				347	48/96	0.50±0.10	1
AciG 52	Y243	*178/182/186/194/198/228*	8	*178*	37/48	0.77±0.12	2
				186	46/94	0.49±0.10	1
				198	114/142	0.80±0.06	2
				228	51/94	0.54±0.10	1
	065	*178/182/184/190/192/194/198*	11	*178*	34/47	0.72±0.13	2
				184	92/143	0.64±0.08	1
				190	138/143	0.97±0.03	>2
				192	87/143	0.61±0.08	1
				198	114/143	0.80±0.06	2

Table 1.3 continued

Locus	Parent	Genotype	Min Copy No.	A_I	Allele count / N	Transmission frequency ± 95% CI	Est. dosage
	Y3	*172/182/186/198/210*	6	*172*	63/143	0.44±0.08	1
				186	74/96	0.77±0.08	2
				198	72/143	0.50±0.08	1
	Y192	*178/182/190/194/198/202/206/210/224/242*	11	*178*	34/48	0.71±0.13	2
				190	99/143	0.69±0.07	1
				198	61/143	0.43±0.08	1
				202	79/143	0.55±0.08	1
				206	75/143	0.52±0.08	1
				224	82/143	0.57±0.08	1
				242	72/143	0.50±0.08	1
	062	*178/182/184/186/190/192/194*	7	*178*	22/37	0.59±0.16	1
				184	64/123	0.52±0.09	1
				186	60/86	0.70±0.10	1
				190	62/123	0.50±0.09	1
				192	66/123	0.54±0.09	1

Table 1.3 continued

Locus	Parent	Genotype	Min Copy No.	A_I	Allele count / N	Transmission frequency ± 95% CI	Est. dosage
	1178	*182/186/194/210*	6	*186*	59/95	0.62±0.10	1
				194	44/48	0.92±0.08	>2
				210	59/132	0.45±0.08	1
	7219	*178/182/188/194/210/228*	8	*178*	21/48	0.44±0.14	1
				188	187/240	0.78±0.05	2
				194	41/48	0.85±0.10	2
				210	77/144	0.53±0.08	1
				228	91/192	0.47±0.07	1
	3d09	*178/182/188/194/210*	9	*178*	44/48	0.92±0.08	>2
				188	183/227	0.81±0.05	2
				194	41/48	0.85±0.10	2
				210	59/132	0.45±0.08	1
AciG 110	Y243	*262/291/299/303/307/327*	6	*291*	71/144	0.49±0.08	1
				299	43/96	0.45±0.10	1
				307	53/96	0.55±0.10	1

Table 1.3 continued

Locus	Parent	Genotype	Min Copy No.	A_I	Allele count / N	Transmission frequency ± 95% CI	Est. dosage
				327	70/144	0.49±0.08	1
	065	*262/292/299/303*	6	*299*	138/143	0.97±0.03	>2
				303	29/48	0.60±0.14	1
	Y3	*262/292/303/323/335*	6	*303*	43/48	0.90±0.09	2
				323	40/95	0.42±0.10	1
				335	69/143	0.48±0.08	1
	Y192	*262/266/291/292/299/301/303/347*	11	*266*	42/48	0.88±0.09	2
				291	77/144	0.53±0.08	1
				299	90/96	0.94±0.05	>2
				301	75/144	0.52±0.08	1
				303	23/48	0.48±0.14	1
				347	70/144	0.49±0.08	1
	062	*262/295/296/299*	5	*295*	61/96	0.64±0.10	1
				296	63/144	0.44±0.08	1
				299	74/96	0.77±0.08	2

Table 1.3 continued

Locus	Parent	Genotype	Min Copy No.	A_I	Allele count / N	Transmission frequency ± 95% CI	Est. dosage
	1178	*262/266/292/295/303*	7	*266*	155/191	0.81±0.05	2
				292	75/96	0.78±0.08	2
				295	93/191	0.49±0.07	1
				303	27/48	0.56±0.14	1
	7219	*262/266/292/299/303/323*	6	*266*	88/192	0.46±0.07	1
				292	52/96	0.54±0.10	1
				299	24/48	0.50±0.14	1
				303	25/48	0.52±0.14	1
				323	96/192	0.50±0.07	1
	3d09	*262/292/307/319*	5	*292*	82/96	0.85±0.07	2
				307	97/192	0.51±0.07	1
				319	114/240	0.48±0.06	1
As015	Y243	*189/193/197/209*	4	*209*	44/96	0.46±0.10	1
	065	*189/193/197/213/221*	6	*213*	112/143	0.78±0.07	2
				221	94/143	0.66±0.08	1

Table 1.3 continued

Locus	Parent	Genotype	Min Copy No.	A_I	Allele count / N	Transmission frequency ± 95% CI	Est. dosage
	Y3	*191/193/197/209*	5	*191*	64/143	0.45±0.08	1
				209	79/96	0.82±0.08	2
	Y192	*185/191/193/197/209/213/221/225/233*	11	*185*	52/95	0.55±0.10	1
				191	60/143	0.42±0.08	1
				209	68/95	0.72±0.09	1
				213	127/143	0.89±0.05	>2
				221	62/143	0.43±0.08	1
				225	69/143	0.48±0.08	1
				233	72/143	0.50±0.08	1
	062	*189/193/209/213*	5	*209*	51/96	0.53±0.10	1
				213	113/144	0.78±0.07	2
	1178	*185/189/193/197/209*	7	*185*	103/190	0.54±0.07	1
				189	75/95	0.79±0.08	2
				197	40/48	0.83±0.10	2
				209	23/47	0.49±0.14	1

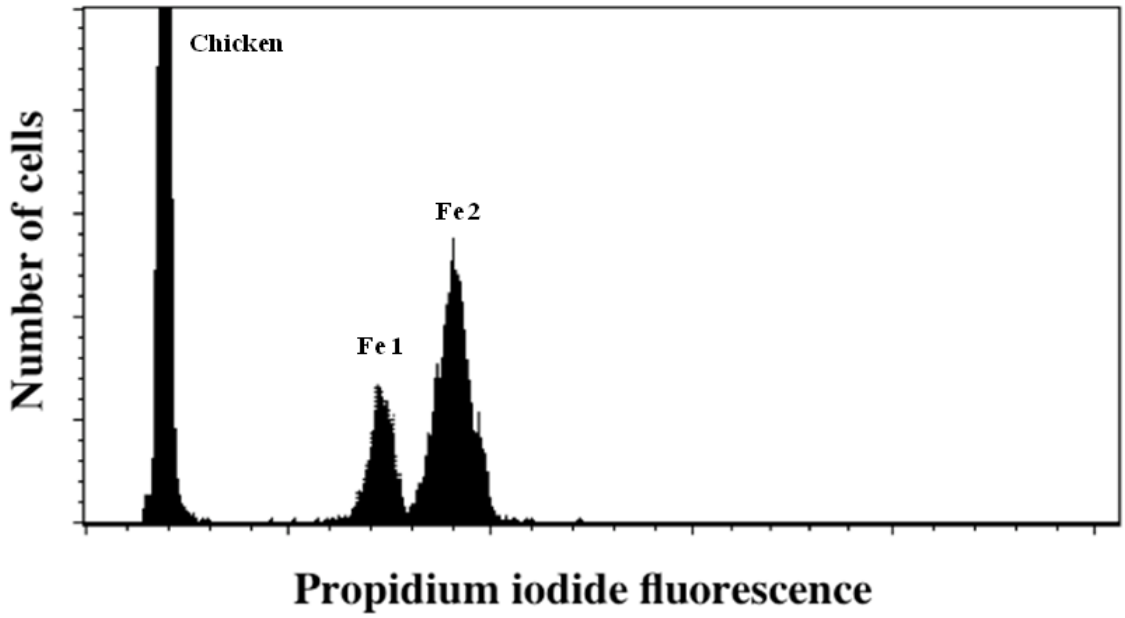
Table 1.3 continued

Locus	Parent	Genotype	Min Copy No.	A_I	Allele count / N	Transmission frequency ± 95% CI	Est. dosage
	7219	*189/193/197/217*	7	*189*	75/95	0.79±0.08	2
				197	48/48	1.00	>2
				217	135/240	0.56±0.06	1
	3d09	*189/193/197/217*	6	*189*	73/96	0.76±0.08	2
				197	38/48	0.79±0.11	2
				217	121/239	0.51±0.06	1

Table 1.4. Mean propidium iodide (PI) fluorescence and estimated DNA content relative to the chicken control.

Sample	Mean PI fluorescence	Estimated DNA content (pg)	Putative Ploidy
Chicken	79.84	2.50	2n
Female 1 (Fe 1)	293.9	9.20	8n
Female 2 (Fe 2)	363.9	11.4	10n
Female 3 (Fe 3)	333.4	10.4	10n
Male 1 (M1)	279.8	8.76	8n
Male 2 (M2)	271.9	8.51	8n
Male 3 (M3)	282.7	8.85	8n

Figure 1.1. Representative flow cytometry histogram of two female white sturgeon (Fe 1 and Fe 2) and chicken control. Fe 1 (8n) and Fe 2 (10n) represent two of three ploidy states we detected in white sturgeon with neutral markers. Using the chicken genome as a diploid standard, we calculate that Fe 2 has a genome size intermediate to that previously described for 8n white sturgeon and 12n shortnose sturgeon (Blacklidge and Bidwell 1993). Fe 2 appears to be decaploid (10n), the same ploidy state detected in the offspring of dodecaploid (12n) dams Y192 and 065.



CHAPTER 2

Population structure in the ancient octoploid white sturgeon, *Acipenser transmontanus*, within and among drainages revealed with polysomic microsatellite markers

Andrea Drauch Schreier, Brian Mahardja, and Bernie May

ABSTRACT

The white sturgeon *Acipenser transmontanus* is the largest fish inhabiting North American freshwater. Examination of population structure in white sturgeon has been limited by the highly duplicated nature of the species' genome. Here, we report results from an examination of white sturgeon population structure within and among drainages using 13 polysomic microsatellite loci. Genetic diversity levels varied widely among regions, with the lowest levels observed in the endangered Kootenai River DPS and the highest levels detected in regions with access to estuarine and marine habitat (Sacramento-San Joaquin, Lower Columbia, Lower Fraser rivers). The highest levels of genetic divergence were detected between the Kootenai River and all other regions. Low levels of divergence were revealed among regions within the Columbia River and between regions with ocean access. Patterns of population structure varied among drainages, with no population structure detected in the Sacramento-San Joaquin, hierarchical genetic structure detected in the Fraser, and a complicated pattern of isolation by distance revealed in the Columbia/Snake. Population assignment techniques were used to identify the origin of individuals captured in three non-natal estuaries along the West Coast. Migrants from the Sacramento-San Joaquin, Lower Columbia, and

Lower Fraser rivers were identified in these collections. Individuals captured in non-natal estuaries did not necessarily originate from the nearest source population. Our results provided little support for the current practice of managing each impounded reach of the Columbia and Snake rivers as a distinct population. Hierarchical patterns of population structure observed in the Fraser River suggest a second post-glacial recolonization source for Lower Fraser white sturgeon. The lack of population structure in the Sacramento-San Joaquin and the complex pattern of population structure observed in the Columbia suggested that the scale of spawning site fidelity in white sturgeon be reconsidered. Because all white sturgeon populations with ocean access exhibit marine dispersal behavior, the magnitude of marine movements should be determined to support interjurisdictional management.

INTRODUCTION

The white sturgeon *Acipenser transmontanus* is the largest fish inhabiting the freshwaters of North America, ranging from Ensenada, Mexico to the Gulf of Alaska (Moyle 2002; Figure 1). Spawning populations occur in the Sacramento-San Joaquin, Columbia, and Fraser rivers. White sturgeon are long-lived, late-maturing fishes that may attain 80+ years of age, with age of sexual maturity in females increasing with latitude (12-34 years; Scott and Crossman 1973; Moyle 2002) . Like most North American sturgeons, white sturgeon experienced severe harvest pressure at the turn of the twentieth century due to high demand for caviar and flesh. Significant declines in harvest rates due to near collapse of white sturgeon populations led to fishery restrictions and

closures across the species range (Craig and Hacker 1940; Rieman and Beamesderfer 1990; Moyle 2002).

The current status of white sturgeon varies widely across the species range. They are highly abundant in the lower Columbia River where a small commercial and large recreational fishery exist (McCabe and Tracy 1994). Recreational fisheries for white sturgeon may be found in the other regions of the Columbia, as well as the Sacramento-San Joaquin, Snake, and Fraser rivers. However, several impounded regions in the mainstem Columbia and Snake rivers contain collections of white sturgeon unable to sustain harvest. The Lower Fraser white sturgeon fishery is catch-and-release only and no fishing is permitted in the Middle Fraser, Upper Fraser, or Nechako rivers (BC MFLNR 2011). Additionally, white sturgeon are listed under the Species at Risk Act in Canada and the Kootenai River distinct population segment (DPS) is listed under the Endangered Species Act in the United States (USFWS 1994; COSEWIC 2003). Factors currently limiting white sturgeon populations include habitat degradation, habitat fragmentation and modification of seasonal flow regimes by impoundment, pollution, non-native species, and overharvest. In some areas (Upper Columbia, Kootenai, Nechako rivers), recruitment limitations for decades or more have shifted the age structure of white sturgeon populations and continue to threaten their persistence (Hildebrand et al. 1999; Anders et al. 2002; McAdam et al. 2005). Conservation aquaculture programs currently sustain white sturgeon reproduction in the Upper Columbia and Kootenai rivers (Drauch Schreier et al. 2011a; Drauch Schreier et al. 2011b).

Previously, population genetic analysis of white sturgeon was hindered by the highly duplicated nature of the species' genome. White sturgeon are ancient octoploids, possessing ~240 chromosomes (Birstein and Vasil'ev 1987), and nuclear loci such as microsatellites may be found in four, eight, or 12 copies (Rodzen and May 2002; Drauch Schreier et al. 2011c). Initial examinations of population structure relied upon allozymes or mitochondrial DNA to avoid complications with the polyploid nuclear genome (Bartley et al. 1985; Setter and Brannon 1992; Brown et al. 1992 a, b). Although these studies provided preliminary evidence of genetic structure on a regional scale, the markers used did not provide enough resolution to examine fine-scale patterns of population structure. More recent examinations of population structure (Smith et al. 2002; Rodzen et al. 2004) have used limited numbers of microsatellites to provide finer resolution. Smith et al. (2002) used a small number of disomically inherited microsatellites in conjunction with mitochondrial control region sequence data to identify four populations in the Fraser River drainage. Using eight polysomic microsatellites, Rodzen et al. (2004) reported a significant global F_{ST} of 0.11 in an examination of population structure including samples from several regions in the Columbia, Fraser, and Sacramento rivers. However, no study to date has provided a detailed examination of genetic structure with exhaustive sample coverage including all regions of the species' range.

Here we use data from thirteen polysomic microsatellite loci to examine white sturgeon population structure across the species' range. Geographic sample coverage is increased in this study compared to previous studies. Study goals were to 1) characterize genetic diversity within and among regions inhabited by white sturgeon, 2) examine

population structure both within and among drainages, and 3) use population assignment testing to assign individuals sampled in non-natal estuaries (estuaries without a spawning population) to their population of origin.

MATERIALS AND METHODS

Sample Collection and DNA Extraction

Tissue samples were collected throughout three major river systems containing spawning populations of white sturgeon: the Sacramento-San Joaquin, Columbia, and Fraser river drainages (Figure 2.1; Table 2.1). Within heavily impounded rivers such as the Columbia and Snake rivers, care was taken to obtain samples from nearly all impounded reaches. The majority of samples were collected from subadult and adult fish during routine population monitoring by state, federal, and tribal management agencies or public utility companies. Samples from individuals incidentally captured in coastal fisheries (Winchester Bay, Tillamook Bay) and involved in a stranding event in Port Susan Bay were included in this study to evaluate marine dispersal behavior and the potential for mixing of different spawning populations in non-natal estuaries.

DNA was extracted from tissue using the Qiagen PureGene DNA extraction kit and the Qiagen DNeasy blood and tissue kit was used to extract DNA from blood samples. DNA was quantified on a Fujifilm FLA 5100 fluorimager and diluted to 10 ng. PCR was performed in Life Technologies (LT) GeneAmp 9700 thermal cyclers using fluorescently labeled primers for thirteen microsatellite loci as described in Drauch Schreier et al. (2011a; Table 2.2). A total of 1.0 µL of diluted PCR product was added to

8.85 µL of highly deionized formamide (The Gel Company) and 0.15 µL of Rox 400 HD size standard (LT). Genotyping was conducted on either an LT ABI 3130xl or 3730 Genetic Analyzer using GeneMapper v4.0 software. Positive controls were genotyped on each platform to ensure conformity of allele binning between instruments. Due to the highly duplicated nature of the white sturgeon genome the scoring of gene dosage was impossible; therefore, microsatellites could not be genotyped as codominant loci. Instead, each microsatellite allele was treated as a present/absent dominant locus, producing a binary allelic phenotype of 1's and 0's for each individual (Rodzen and May 2002; Israel et al. 2009; Pfeiffer et al. 2011).

Analysis of Duplicates

The program GenoType (Meirmans and Tienderen 2004) was used to identify duplicate samples in the white sturgeon dataset. We estimated the rate of allelic dropout through quality control tests re-genotyping 96 unique samples of multiple ages and tissue types collected from several sample locations on the 3730xl. This experiment revealed an allelic dropout rate of 1.2% and therefore up to two mismatches were allowed in identifying duplicate samples. One individual from each pair of duplicate samples was removed from the dataset before further analyses were conducted.

Genetic Diversity

The number of shared and private alleles was calculated in GenAlEx version 6.3 (Peakall and Smouse 2006) to characterize levels of genetic diversity in each region sampled. We used a random number generator in Excel 2007 to create a subsample of each region equal to the smallest sample size in the dataset (N=60; Lower Fraser) in order

to compare levels of genetic diversity among regions. Numbers of alleles and private alleles were then recalculated.

Without the ability to score gene dosage, it was not possible to estimate heterozygosity levels in white sturgeon populations, at least in the traditional sense. However, we calculated the average number of alleles per individual per locus for each population as a proxy for heterozygosity. Populations characterized by high levels of heterozygosity will possess a high mean number of alleles per individual, while populations characterized by low levels of heterozygosity will possess a low mean number of alleles per individual.

An analysis of molecular variation (AMOVA) was conducted in GenAlEx version 6.3 (Peakall and Smouse 2006) to examine the proportion of genetic diversity partitioned within and among regions. Random permutations were conducted (9999) to assess the significance of Phi-PT (Peakall et al. 1995), an analogue of F_{ST} most appropriate for dominant data that provides a measure of pairwise genetic divergence among populations. A sequential Bonferroni correction (Rice 1989) was conducted to account for multiple pairwise comparisons using $\alpha = 0.05$. Principle coordinates analysis (PCO) also was conducted in the program GenAlEx version 6.3 to visualize genetic relationships among regions across the species' range. Two PCO analyses were conducted, one including all regions and one excluding the genetically divergent Kootenai River DPS.

Population Structure Analyses

We parsed our population structure analyses into within river and range-wide examinations. We wanted to examine population structure on the finest scale possible

(within drainage analysis) and also examine relationships among populations from different drainages (among drainage analysis). When analyzing the full dataset, some distinct populations, as identified by the within river analysis, were subsampled to reduce the computational time of the analysis.

Within Drainage Population Structure

We first explored population structure within river drainages. The program Structure version 2.3.3 (Pritchard et al. 2000) was used to explore the number of possible populations (K) existing within each drainage system (Sacramento-San Joaquin, Columbia/Snake, Fraser). Additional analyses were conducted to look for cryptic population structure within the Fraser River above Hells Gate, a narrowing of Fraser Canyon that was obstructed by a rock slide in 1913-1914. Exploratory analyses were first performed using a relatively short burn-in (50,000) and small number of iterations (100,000) to test the likelihood of K = 1 to K = 20 for each river system and ascertain the general shape of the likelihood curve. Examination of cryptic substructure in the Fraser River above and below Hells Gate used exploratory analyses to test the likelihood of K = 1 to K = 4 and K = 1 to K = 3, respectively. Longer analyses (burn-in 500,000; 1,000,000 iterations) were conducted only for the most likely range of K values found in the exploratory analyses (Table 2.3; Pritchard et al. 2010). Each Structure analysis utilized the admixture model and assumed correlated allele frequencies among populations. Six replicates were conducted for each K. The LOCPRIOR model (Hubisz et al. 2009), which incorporates sampling location information as a prior when found to be informative by Structure, was utilized to increase the program's ability to identify the true K in the presence of weak differentiation among populations. Structure results were

interpreted in two ways. First we examined the mean likelihood value (Ln Pr(X|K)) for each possible number of populations in the program Structure Harvester (Earl 2009). The K with the highest likelihood value was interpreted as the best estimate of the number of populations in each river system. However, this method has a tendency to overestimate K, particularly when analyzing dominant data (Pritchard et al. 2010). Therefore, we also used the ΔK metric of Evanno et al. (2005) to interpret Structure results for each system. The Evanno et al. (2005) method is based on the principle that at the true K, the likelihood function Ln Pr(X|K) begins to asymptote or increase slightly. Delta K (ΔK), or the second order rate of change of Ln Pr(X|K), identifies the break in the slope of the Ln Pr(X|K) function which Evanno et al. (2005) assert occurs at the true K. When multiple K values seemed equally likely via examination of the likelihood function and ΔK, individual Q values were examined to select the most likely K. We used the program CLUMPP (Jakobsson and Rosenberg 2007) to compile individual assignments across all replicates for the most likely K and individual Q values were plotted in the program Distruct 1.1 (Rosenberg 2003) for visual examination.

Range-Wide Population Structure

Structure 2.3.3 was then used to examine population structure among white sturgeon populations across the species' range. The computational time of analyzing all 2,056 samples in Structure was prohibitive; therefore, we randomly sub-sampled populations identified by the within-drainage analysis using a random number generator in Excel 2007 to reduce the total number of samples analyzed to 855. Exploratory analyses (burn-in 50,000 with 100,000 iterations) examined the likelihood of K = 1 to K = 20, while full analyses (burn-in 500,000 with 1,000,000 iterations) explored K = 1 to K

= 10 (Table 2.3). The admixture model was used and correlated allele frequencies among populations assumed. Six replicates were conducted for each K. As with the within-drainage analyses, the LOCPRIOR model was implemented when sampling location was informative and Structure results were interpreted by examining the Ln Pr(X|K) function, the ΔK metric, and individual Q values. The program CLUMPP compiled individual assignments across replicates for the most likely K and individual Q values were plotted in Distruct 1.1 for visual examination.

We conducted a second analysis of molecular variation (AMOVA) in GenAlEx version 6.3 to examine how genetic diversity was partitioned among populations identified by Structure. Random permutations were conducted (9999) to assess the significance of Phi-PT and a Bonferroni correction was conducted to account for multiple pairwise comparisons. The corrected P value denoting significance at $\alpha = 0.05$ was 0.002. Principle coordinates analysis (PCO) was conducted in GenAlEx version 6.3 to visualize genetic relationships among populations. Two PCO analyses were conducted, one with and one without the genetically divergent Kootenai River DPS.

Origin of Non-Natal Estuary Samples

We examined the origin of white sturgeon individuals captured in the non-natal estuaries in two ways. First, we used the prior population information (USEPOPINFO=1) model in the program Structure version 2.3.3 to assign these samples to populations identified by Structure in the within-drainage population structure analyses. We used the admixture model and assumed correlated allele frequencies among populations. The analysis consisted of a burn-in of 500,000 with 1,000,000

iterations. We also used the "allocation" procedure in AFLPOP (Duchesne and Bernatchez 2002) to assign non-natal estuary samples to source populations previously identified by within-drainage Structure analyses. AFLPOP is similar to Structure in that it assigns individuals to genetic clusters; however, AFLPOP uses dominant allele frequencies in pre-defined source populations to determine the likelihood that a particular genotype originates from a population. It uses a threshold minimum log difference (MLD) value to assign individuals to a source population. With an MLD of 2, an individual has to be 10^2 times more likely to belong to the most likely population than any other population to be assigned. We first used the "Simulation: many iterations" procedure to selection the appropriate MLD value to maximize the number of correct allocations to source populations while minimizing the rate of non-allocations. Ten simulations were conducted and 1000 genotypes were randomly generated for each source population. The MLD value that maximized the number of correct allocations and minimized the number of non-allocations was MLD = 0.8. The "allocation" procedure in AFLPOP assigned individuals of unknown origin to the source population from which it most likely originated based on similarity of allele frequencies. We used a zero frequency replacement value of 1/(N+1). Only samples without missing data could be included in this analysis. Migrants from the Upper Fraser River population detected in the Lower Fraser River were excluded from the Lower Fraser River source population for AFLPOP analyses.

RESULTS

A total of 19 sample pairs were classified as duplicates in the white sturgeon genotype database, two pairs from the Sacramento-San Joaquin and 17 pairs from the Columbia/Snake. Nine of these duplicate pairs originated from the Transboundary Reach. Samples from this region were originally collected for a site fidelity study and several individuals were recaptured in different locations and tissue collected multiple times. Remaining duplicate pairs originated from the Lower Columbia (below Bonneville), the Kootenai River, and Hells Canyon Complex on the Snake River. No duplicates were detected in the Fraser River or among non-natal estuary samples. The majority of these pairs exhibited no or one mismatch while two pairs possessed two mismatches.

Genetic Diversity

Across 13 microsatellite loci, a total of 275 alleles were detected. The total number of alleles found within regions ranged from 97 (Kootenai River) to 236 (Sacramento-San Joaquin; Table 2.4). No private alleles were detected in the Kootenai River or Middle Snake, while the Sacramento-San Joaquin had the highest number of private alleles (18; Table 2.4). When the total number of alleles and private alleles were recalculated for the subsampled regions (N=60), the highest level of genetic diversity was detected in the Lower Fraser, with 198 alleles and 13 private alleles (Table 2.5). Similarly high levels of genetic diversity were detected in the other two regions with access to marine habitat, the Sacramento-San Joaquin and Lower Columbia (Table 2.5).

The number of alleles per microsatellite locus ranged from seven (AciG 2) to 35 (Atr 107; Table 6). AciG 35 had the greatest number of alleles per individual per locus

while AciG 53 had the fewest. The Columbia River regions tended to have greater numbers of alleles per individual per locus while the Kootenai River had the lowest (Table 2.6).

A Phi-PT value of 0.09 (P = 0.0001) indicated significant levels of genetic differentiation among regions. A total of 9% of genetic diversity was partitioned among regions while 91% of variation was found within regions. An examination of pairwise Phi-PT values revealed significant levels of genetic differentiation among nearly all comparisons with the highest levels between the Kootenai River and all other regions (Table 2.7). The comparison between the Middle Columbia and Lower Snake was not significant. The lowest levels of genetic differentiation were found among the Lower Columbia, Middle Columbia, Transboundary Reach, and Lower Snake river regions. Similarly low levels of divergence were found for one out-of-drainage comparison between the Lower Columbia and Lower Fraser rivers (Table 2.7). The Middle Snake showed low differentiation from the Lower Snake but higher levels of divergence from the Columbia River regions. Interestingly, the Lower Fraser showed higher levels of genetic divergence from the Upper Fraser than from the Sacramento-San Joaquin and Lower Columbia (Table 2.7). The PCO including all regions revealed that 40.8% and 30.7% of variance was explained by the first two axes (Figure 2.2). The Kootenai River was separated from all others along Axis 1, while other regions were primarily distinguished along Axis 2 (Figure 2.2). The second PCO excluding the Kootenai River (50.4% and 26.9% variance explained) exhibited similar results, although less similarity between the Lower Columbia and both Lower Fraser and Upper Fraser was evident (Figure 2.3). The second PCO also showed greater similarity among the Middle

Columbia, Transboundary Reach, and Lower Snake. Note that all regions with access to marine habitat were found in a single quadrant in both Figures 2.2 and 2.3.

Within-Drainage Population Structure

Sacramento-San Joaquin River System

Exploratory analyses suggested that the most likely number of populations (K) in the Sacramento-San Joaquin sample was between one and six, so more extensive exploration of the data was conducted for those values of K. Examination of likelihood values, Ln Pr(X|K), from the longer analysis indicated that the Sacramento – San Joaquin river system was most likely a single population (Figure 2.4). The Evanno method suggested that K=4 was the most likely number of populations in the Sacramento-San Joaquin. However when Structure assumed four populations, each individual was assigned evenly to each population (mean Qs = 0.23, 0.25, 0.24, and0.26), suggesting this was an overestimate of the true population number. Here, a Q value represents an individual's estimated membership coefficient in each identified population. It is important to note that ΔK cannot evaluate the likelihood of K=1 so a comparison between the likelihood of K=1 and K=4 by this method is not possible.

Columbia/Snake River

In the Columbia/Snake River drainage, sampling location labels were found to be informative ($r \leq 1$) by Structure and the LOCPRIOR model was employed. Examination of Ln Pr(X|K) and ΔK both indicated the most likely number of populations in the data was three (Figure 2.5). In the mainstem Columbia River, from the Lower Columbia to the Transboundary Reach and including the Lower Snake River (Ice Harbor Pool to Hells

Canyon), individuals assigned to two populations, with membership to the second population increasing on an upstream cline (Figure 2.6). Individuals in the Middle Snake River (Brownlee Pool to Upper Salmon Falls Pool) assigned strongly to the second population with high Q values (mean Q = 0.99). The Kootenai River was identified as a distinct population with all individuals from the Kootenai River assigned to a single population with high Q values (mean Q = 0.99). In subsampling for the range-wide population structure analysis, we distinguished between the Lower Columbia below Bonneville Dam (downstream population), the Columbia/Lower Snake (Dalles Pool to Transboundary Reach on the Columbia, Ice Harbor Pool to Hells Canyon Complex on the Snake), and Middle Snake (upstream population).

Fraser River

As in the Columbia River, geographic sampling labels were informative in elucidating population structure in the Fraser River dataset and therefore the LOCPRIOR model was applied by Structure. Initial examinations of Ln Pr(X|K) (Figure 2.7) and ΔK suggested that the most likely number of populations was three. However, Q values indicated that this may have been an overestimation of population structure. When two populations were assumed (K=2), Structure clearly identified two distinct populations with all individuals assigning strongly to one or the other (mean Qs = 0.99 and 0.93, respectively). When three putative populations were tested, individuals that had assigned strongly to population 1 now assigned evenly to populations 1 and 3. For example, when K=2 was assumed, individual X assigned to population 1 with a membership coefficient of 0.95, but when K=3 was assumed, individual X assigned to both populations 1 and 3, each with a membership coefficient of ~0.50. No individuals assigned strongly to

population 3. Therefore, we concluded that the most likely number of subpopulations in the Fraser River system was two.

The two populations detected in the Fraser River system corresponded to the Lower Fraser River below Hells Gate (SG-1 and SG-2) and the remaining Fraser River system located above Hells Gate, hereafter referred to as the Upper Fraser cluster (Figure 2.8). The Upper Fraser cluster included individuals sampled in the Middle Fraser (SG-3), Upper Fraser, and Nechako Rivers. A few individuals from the Lower Fraser sampled just below Hells Gate (SG-2) assigned to the Upper Fraser cluster (Figure 2.8). Because of the ambiguity in K and previous work suggesting higher levels of substructure in the Fraser River (Smith et al. 2002), we conducted additional Structure analyses to examine the possibility of substructure in the reaches below and above Hells Gate , respectively. Geographic sampling labels were found to be informative and the LOCPRIOR model was applied in Structure. No additional population structure was detected below Hells Gate. Both Ln Pr(X|K) and ΔK suggested up to three populations (K=3) existed in the Fraser River above Hells Gate, although the likelihood for K=2 was similar (Figure 2.9). If K=2 was assumed, the Middle Fraser River (SG-3) consisted of a genetically distinct population (mean Q = 0.99) and the majority of individuals sampled in the Nechako/Stuart Lake/Fraser Lake group assigned to a second population (mean Q = 0.93; Figure 2.10a). The Upper Fraser River consisted of a mixture of individuals originating from the Middle Fraser and Nechako populations. One individual in Nechako/Stuart Lake/Fraser Lake and several in the Upper Fraser River had intermediate levels of membership in the Middle Fraser and Nechako populations, which suggested that some admixture may be occurring between these populations. When K=3 is assumed, the SG-3

collection remained a genetically distinct population (mean Q = 0.98) and most individuals from the Nechako River/Stuart Lake/Fraser Lake collection still assigned primarily to a second distinct population (mean Q = 0.88; Figure 2.10b). The Upper Fraser River still consisted of a mixture of individuals assigning strongly to three different populations. Population 3 (represented in green in Figure 2.10b) contained only 15 individuals but those that assigned to it did so with high Q values (mean Q = 0.88; Figure 2.10b). All but two individuals assigned to population 3 possessed allele *237* at locus Atr 117, an allele found in few other individuals in the Fraser River above Hells Gate. Similar signatures of admixture were detected in the Upper Fraser River when three populations were assumed.

Range-wide Population Structure

Sampling information was found to be informative in the range-wide Structure analysis and the LOCPRIOR model was implemented by Structure. The Ln Pr(X|K) plot suggested that the most likely number of populations was between four and seven (Figure 2.11), while ΔK suggested two was the true number of populations. The slope of the curve between K=1 and K=2 indeed exhibited the greatest rate of change, but the likelihood plot clearly indicated that K=2 was not the most likely number of populations. We used our knowledge of species life history and the results of the within-drainage Structure analyses and regional Phi-PT analyses to conclude that K=6 was the most likely number of populations in the dataset.

When K=6 was assumed, the Sacramento-San Joaquin (mean Q = 0.97), Middle Snake (mean Q = 0.94), Kootenai (mean Q = 0.99), Lower Fraser (mean Q = 0.77), and

Upper Fraser cluster (mean Q = 0.96) were five distinct populations (Figure 2.12). Individuals sampled downstream of McNary Dam on the Columbia tended to assign to one population (mean Q = 0.51) while individuals sampled from the McNary Reservoir to the Transboundary Reach tended to assign to the Middle Snake population (mean Q = 0.60), although an isolation by distance pattern was still evident at this level (Figure 2.12). Individuals from the Lower Snake tended to assign to the Middle Snake population (mean Q = 0.68). Downstream migrants from the Upper Fraser cluster into the Lower Fraser were still detected at this level (Figure 2.12).

When individuals were parsed into the six populations identified by Structure, the Phi-PT value increased to 0.10 (P = 0.0001), meaning the proportion of genetic diversity partitioned among populations was 10%. Partitioning the Fraser River above Hells Gate into two populations (for a total of eight populations) did not change the partitioning of genetic diversity within and among populations. An examination of pairwise Phi-PT values revealed similar patterns to the Phi-PT analysis based on sampling regions, with the highest levels between the Kootenai River and all other populations (Table 2.8). All pairwise comparisons were significant. The lowest levels of genetic differentiation were found between the Lower Columbia and the Lower Fraser. Relatively low levels of genetic differentiation were detected between the Lower Columbia and Middle Columbia/Middle Snake (Table 2.8). The Sacramento-San Joaquin population showed lower levels of divergence from the Lower Columbia and Lower Fraser, two other populations with ocean access, compared to the Middle Columbia/Middle Snake, Kootenai, and Upper Fraser populations. As found in the regional analysis, the Lower Fraser showed higher levels of genetic divergence from the Fraser River above Hells

Gate than from the Sacramento-San Joaquin, Lower Columbia, and Columbia/Lower Snake populations (Table 2.8). The PCO including all populations identified by Structure revealed that 52.6% and 26.7% of variance was explained by the first two axes (Figure 2.13). The Kootenai River population was separated from all others along Axis 1, while other populations were primarily distinguished along Axis 2 (Figure 2.13). Similar to the regional analysis, all populations with access to marine habitat were found in the lower left quadrant of Figure 2.13. In the second PCO excluding the Kootenai River population, 56.2% and 29.6% of variance was explained by the first two axes. When the Kootenai population was removed, less similarity was evident between the Middle Columbia/Middle Snake population and Upper Fraser River cluster (Figure 2.14). Similar to the PCO analyses based on regional groupings, a close genetic relationship between the Lower Columbia and Lower Fraser populations was shown although the similarity was less pronounced when the Kootenai population was removed (Figures 2.13, 2.14).

Origin Non-Natal Estuary Samples

Neither Structure nor AFLPOP was able to assign several samples collected in non-natal estuaries to their population of origin. The analysis using prior population information in Structure assigned 6/39 individuals to a source population with Q values ranging from 0.504 to 0.600 (mean Q = 0.549; Table 2.9). Q values in this analysis refer to the posterior probability of belonging to a particular population. Four of these individuals were included in AFLPOP analyses (WB1198, WB198, WB1598, PS0905) but the remaining two could not be included due missing data at one or more loci. In the initial AFLPOP allocation analysis, 14/37 individuals were allocated to a source population. The difference in the total number of non-natal estuary samples analyzed by

Structure and AFLPOP is due to the inability of AFLPOP to accommodate missing data. There was consensus between both analysis methods; the four individuals analyzed by both programs were assigned to the same source population by each (Table 2.9). In some cases, individuals were allocated to the nearest source population. One Tillamook Bay (AFLPOP) and six of Winchester Bay samples (Structure and AFLPOP) allocated to Columbia River populations (Tables 2.9, 2.10). On the other hand, only two Port Susan samples could be allocated and neither was allocated to the Lower Fraser or Lower Columbia (Structure and AFLPOP; Tables 2.9, 2.10). To see if allocation rates could be improved in AFLPOP, we combined individuals from the Lower Columbia, Middle Columbia, Transboundary Reach, and Lower Snake into a single source population, as both Structure and AMOVA analyses suggested low levels of genetic differentiation between individuals sampled in these regions (Table 2.11). This did reduce the number of non-allocations and the number of individuals allocated to the Columbia River increased. There was also an increase in the number of individuals assigned to the Sacramento-San Joaquin and Lower Fraser populations when the Lower Columbia was not included as a separate source population. One individual from Port Susan Bay assigned to the geographically proximate Lower Fraser when the combined Columbia River/Lower Snake source was used (Table 2.11). Interestingly, in both the Structure and AFLPOP analyses, a total of nine individuals from the three non-natal estuary collections assigned to the geographically distant Sacramento-San Joaquin population.

The prior population model in Structure can also be used to detect migrants in predefined populations. When assigning the non-natal estuary samples Structure also detected a single individual in the Lower Columbia River (below Bonneville Dam) that

assigned to the Sacramento-San Joaquin River population (Q = 0.55). Several individuals from the Dalles Pool and one individual from the John Day Pool assigned to the Lower Columbia population. One individual from the Dalles Pool (Q = 0.50) and one from the John Day Pool (Q = 0.80) assigned to the Lower Fraser. All individuals from the Lower Fraser River identified by previous Structure analyses as migrants from above Hells Gate were assigned to the Upper Fraser cluster in this analysis as well.

DISCUSSION

Here we report genetic diversity and reveal significant levels of population structure among white sturgeon collections within and among drainages across the species' range. Populations suggested by Structure generally corresponded to regional designations (Table 2.1), with the exception of the Columbia/Snake drainage. Both within-drainage and range-wide population structure analyses recognize distinct populations from the Sacramento-San Joaquin, Kootenai, Lower Fraser, and Upper Fraser Rivers, with substructure identified in the Upper Fraser. Within-drainage and range-wide analyses for the Columbia River system indicated that Columbia fish were distinct, but the pattern of population structure within the river was complex. At the upstream extent of the white sturgeon range in the Columbia system, the Middle Snake represents a distinct population, while the Middle Columbia, Transboundary Reach, and Lower Snake samples show varying levels of admixture between the Middle Snake population and a less distinct Lower Columbia River group. This pattern is consistent with net downstream gene flow and isolation by distance. Below we discuss the implications of

these results by drainage and in a range-wide context and describe how these data can be used to improve the management of white sturgeon.

Genetic Diversity

The Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) and shortnose sturgeon (*Acipenser brevirostrum*),distributed along the East Coast of North America, have been shown to exhibit high levels of microsatellite genetic diversity in the middle of their ranges while regions at the northern and southern extremes of their ranges possess less genetic diversity (King et al. 2001; Grunwald et al. 2002). This pattern is not replicated in white sturgeon. Genetic diversity levels in the regions inhabited by white sturgeon were highest in regions with access to ocean habitat (Sacramento-San Joaquin, Lower Columbia, Lower Fraser) and lowest in upriver regions (Middle Snake, Upper Fraser above Hells Gate, Kootenai). Very low levels of genetic diversity in the Kootenai River were expected based on findings of other studies (Rodzen et al. 2004; Drauch Schreier et al. 2011a). White sturgeon inhabiting the Kootenai River were isolated from the rest of the Columbia River by Bonnington Falls in 10,000 – 12,000 years ago (Northcote 1973). Very low levels of genetic diversity in the Kootenai River suggest that the Kootenai River population was founded by few individuals. A lack of gene flow and decades-long recruitment failure also have likely contributed to genetic diversity loss in the Kootenai River. In the Columbia River and Fraser River systems, there are several possible explanations for higher levels of genetic diversity in downstream regions with access to marine habitat than in upriver regions. First, estuarine and marine habitat is highly productive and populations with access to these resources may have had larger historical population sizes. Genetic drift may be more efficient at removing genetic diversity in

smaller upriver populations. Second, many upriver reaches (Transboundary Reach, Kootenai River, Nechako River) have suffered from recruitment limitations for decades and genetic diversity may be lost as adults die without successfully contributing offspring to the next generation. Finally, net downstream movement of individuals has been documented in the Columbia River (Parsley et al. 2007), Snake River (Lepla and Chandler 1995, 1997), and Fraser River (this study), and this process may introduce "upstream" alleles into the downstream regions, increasing their level of genetic diversity. This may result in genetic diversity loss for upriver regions over time as alleles move downstream and are not replaced by upstream gene flow.

With the exception of the Kootenai River, white sturgeon possess high levels of genetic diversity despite great reductions in abundance due to severe overharvest at the turn of the century. From the late 1800s to the early 1900s, harvest of white sturgeon in all three drainage systems declined precipitously as populations collapsed (Craig and Hacker 1940; Moyle 2002; Echols 1995). Bottleneck tests in lake sturgeon (*Acipenser fulvescens*), a species that underwent similar levels of exploitation, revealed no signature of genetic bottleneck in the face of severe demographic declines (DeHaan et al. 2006). Welsh et al. (2008) describe similarly high levels of genetic diversity in lake sturgeon populations across the species' range despite large spatial variability in population size and viability. Zhang et al. (2003) report a high N_{ef}/N ratio for Chinese sturgeon (*Acipenser sinensis*) suggestive of a bottleneck but see no evidence of genetic diversity decrease in that species. Quattro et al. (2002) describe high levels of genetic diversity in endangered shortnose sturgeon populations recovering from exploitation. Retention of genetic diversity in spite of population declines has been observed in other long-lived

organisms with long generation times (Kuo and Janzen 2004; Lawrence et al. 2008; Pittman et al. 2011). In white sturgeon, this so-called "storage effect," where long-lived individuals retain genetic diversity over long periods (Quattro et al. 2002) may be facilitated by polyploidy, where up to eight copies of each allele may be maintained in a single individual.

Within-Drainage Population Structure

Sacramento-San Joaquin River System

The Sacramento-San Joaquin collection contains a single population despite that fact that historically, this system contained two rivers with available spawning habitat. Although flows on the San Joaquin River have been significantly reduced by agricultural diversion of water for ~60 years (Pelley 2009), capture of adult white sturgeon and white sturgeon larvae in the San Joaquin River (Kohlhorst 1976; Dubois et al. 2009; Dubois et al. 2010) and on the San Joaquin side of the delta (Stevens and Miller 1970) suggests that spawning may have occurred in the San Joaquin historically. Additionally, in the spring of 2011, a year where average daily flows in the San Joaquin were two to three times higher than the average daily flows over the previous 20 years, 23 white sturgeon eggs were sampled in the San Joaquin River near rkm 142 (Zac Jackson, US Fish and Wildlife Service, pers comm.). The lack of population structure in the Sacramento-San Joaquin system in spite of likely historical spawning in both rivers suggests that 1) there is a high rate of gene flow between them, enough to prevent the accumulation of significant genetic differences or 2) a genetically distinct San Joaquin population existed but is now extinct. A high rate of gene flow between the two rivers is plausible as adults spending a

majority of their time feeding in the rivers' shared estuary (San Francisco Bay system) have equal access to both rivers when flows are adequate. The degradation of the San Joaquin River in recent decades has likely reduced opportunities for successful spawning in the river and the current collection of adults sampled from the Sacramento-San Joaquin may originate from spawning in the Sacramento River. Examination of tissue samples collected from adults in the Sacramento-San Joaquin system before modification of the San Joaquin River would allow us to distinguish between these two scenarios.

Columbia/Snake River

Patterns of population structure within the Columbia River system were more complex than those in the Sacramento-San Joaquin or Fraser River systems. The within-river analysis of population structure suggests that three populations exist in the Columbia River drainage. One is the Kootenai River population, which is highly divergent from all other white sturgeon populations. The distinctiveness of the Kootenai River population is likely due to its history of isolation and subsequent genetic drift. Structure analyses identified two other clusters in the Columbia River system, one associated with Lower Columbia and a Middle Snake cluster at the upstream extent, with individuals in the Middle Columbia, Transboundary Reach, and Lower Snake exhibiting signs of admixture between the two. It is likely that this pattern is due to isolation by distance along the length of the Columbia River and possibly net downstream gene flow.

The patterns of population structure revealed here suggest that historically there was some degree of spawning site fidelity in Columbia/Snake white sturgeon, with one population preferring to spawn in areas further upstream in the Columbia and Snake and

another population preferentially spawning lower in the Columbia River system. As contemporary spawning sites are known to be distributed throughout the Columbia even in the presence of impoundments (Parsley and Kappenman 2000), it is likely that multiple spawning sites were located throughout the system historically. Before the Columbia and Snake rivers were impounded, white sturgeon had the ability to migrate unimpeded from the Columbia River estuary upstream to Columbia Lake on the Columbia River and Shoshone Falls on the Snake River. It is unknown whether the two populations detected here mixed in the productive Columbia River estuary during non-reproductive times or if a non-migratory "resident" white sturgeon population that never or rarely used estuary habitat inhabited the upper reaches of the system. In either case, gene flow between spawning sites in the uppermost and lowermost regions of the system would have been minimal due to prohibitive distances between them. Gene flow between geographically proximate sites along the length of the Columbia River drainage would have been greater, which would lead to the observed cline in population membership from the downstream to upstream reaches of the system. The genetic signature of the upstream population found in individuals sampled in the lower reaches of the Columbia also may be explained by net downstream export of genes via downstream dispersal of individuals through one or more impoundments on the Columbia River. Evidence suggests that net downstream gene flow over natural barriers in the Columbia may occur as well. Several individuals in the Transboundary Reach, directly downstream of Bonnington Falls, showed some ancestry in the Kootenai River population but no individuals in the Kootenai River showed ancestry in the Columbia (Figure 2.6). While white sturgeon movement downstream through certain Columbia River dams and natural barriers may occur,

upstream movement remains relatively rare (Parsley et al. 2007) and is likely impossible at Bonnington Falls. Therefore genes from the upstream population are exported to the downstream population but reciprocal gene flow is often not possible.

It is important to note that impoundment is a relatively recent disturbance when considering the long generation time of sturgeons. Only ~3-4 white sturgeon generations have passed since the first impoundment was constructed on the Columbia River. It is likely that not enough time has passed since the beginning of habitat fragmentation for it to have significantly altered patterns of population structure. For example, the first dam constructed on the Columbia River, Grand Coulee Dam, isolates the Transboundary Reach from the rest of the river. However, the within-drainage Structure analysis reveals very little difference in proportional population membership among collections ranging from the McNary reservoir (seven impoundments down) to the Transboundary Reach. Over time, however, genetic divergence among white sturgeon collections isolated in impounded regions is expected to increase, depending on the rate of downstream migration which likely varies by impoundment. Uppermost regions of the Columbia/Snake system are expected to differentiate more quickly due to isolation from gene flow from downstream reaches.

Fraser River

Our initial Structure analysis of the Fraser River system revealed a scale of population structure that differed from that presented by Smith et al. (2002). Using four disomic microsatellite markers and mtDNA control region sequence, Smith et al. (2002) classified collections of white sturgeon in the Fraser River system into four evolutionary

significant units, the Lower Fraser River below Hells Gate, the Mid-Fraser River above Hells Gate to rkm 553, the Upper Fraser River (above the confluence of the Nechako) and the Nechako River. However, our initial analysis only detected two populations, the Lower Fraser River and the Upper Fraser cluster consisting of the Middle Fraser, Nechako, and Upper Fraser rivers. An additional analysis of samples collected on the Fraser River above Hells Gate identified additional substructure, providing a picture of fine scale population structure more similar to the results of Smith et al. (2002). We first discuss the results of the initial examination of structure on the entire Fraser River dataset and then discuss the implications of the second analysis of the Fraser River above Hells Gate.

The initial examination of population structure utilizing all samples collected in the Fraser River revealed a strong signal of genetic divergence between the Lower Fraser and the Fraser River above Hells Gate. Similar patterns of high levels of genetic differentiation among fish populations above and below Hells Gate have been observed in several salmonid species (coho salmon, *Oncorhynchus kistuch*, Small et al. 1998; chinook salmon, *Oncorhynchus tshawytscha*, Teel et al. 2000; sockeye salmon, *Oncorhynchus nerka*, Withler et al. 2000). Small et al. (1998) proposed that an ice dam remaining in Fraser Canyon after glacial recession may have extended genetic isolation between Lower and Upper Fraser fish populations even after post-glacial recolonization of both regions, which may explain the high levels of genetic divergence between them. Another hypothesis for the magnitude of genetic divergence among fish populations inhabiting Lower Fraser and the Fraser River above Hells Gate is that there was a second

unknown post-glacial recolonization source for the Lower Fraser (Small et al. 1998; Teel et al. 2000; Withler et al. 2000).

Additional evidence suggests the possibility of a second post-glacial recolonization source for Lower Fraser white sturgeon. Brown et al. (1992a) detected higher levels of haplotypic diversity in Fraser River white sturgeon relative to Columbia River white sturgeon and a more recent examination of white sturgeon phylogeography has replicated these findings (BM, unpublished data). These results are contrary to expectations given that the Columbia River was considered the most likely source of post-glacial recolonization for the Fraser River. Microsatellite data also reveal very high levels of genetic diversity in the Lower Fraser River when sample size is accounted for in diversity calculations (Table 2.5). A recolonized population is expected to have less genetic diversity than its source population due to founder effects (only a small number of recolonizers relative to source population size) and subsequent genetic drift (Hewitt 1996). Brown et al. (1992) hypothesized that a higher level of anthropogenic disturbance (historic overharvest and impoundment) in the Columbia River relative to the Fraser could have resulted in greater genetic diversity loss in the former. However, Lower Fraser white sturgeon also were subjected in to an intense fishery for caviar and flesh at the turn of the 20th century (Lane 1991) and impoundment in the Columbia is a relatively recent phenomenon (~3-4 sturgeon generations), likely not long enough to affect patterns of genetic diversity on the Columbia. Also, the Lower Fraser possesses the highest number of private alleles of any population examined once unequal sample size is accounted for (Table 2.5). One would not expect the Lower Fraser to possess many

alleles not found in the Lower Columbia if the Lower Columbia were its only recolonization source.

Lower levels of genetic diversity in the Fraser River above Hells Gate relative to the Lower Fraser may be evidence of different recolonization routes for above and below Fraser Canyon. The Upper Fraser was likely recolonized by the Columbia River via connections between Mid- and Upper Fraser glacial lakes and the Columbia River (McPhail and Lindsay 1986). The Lower Fraser was likely recolonized via migration of individuals along the continental shelf, from the Lower Columbia and possibly one other source population. Low levels of genetic divergence revealed in AMOVA and PCO analyses support a close genetic relationship between the Lower Fraser and the Lower Columbia. The second possible source population for recolonization of the Lower Fraser is unknown, although some authors have proposed a Beringian refuge for Lower Fraser salmonid populations (Teel et al. 2000). Given that white sturgeon have been captured in marine waters along the Alaskan coast and long-distance dispersal has been documented for this species (Welch et al. 2006), a Beringian refuge seems plausible. However, it is also possible that higher levels of genetic diversity in the Lower Fraser relative to the Upper Fraser cluster are due to contemporary gene flow from other populations with access to marine habitat and exacerbated by small population size (and subsequent genetic drift) in the Fraser River above Hells Gate. The abundance estimate for Lower Fraser white sturgeon is an order of magnitude greater than that for the Upper Fraser River above Hells Gate (RL&L 2000; Yarmish and Toth 2002), which lends some credence to the idea that differences in genetic diversity may be at least partially due to genetic drift.

The occurrence of individuals sampled in the Lower Fraser originating from above Hells Gate corroborates field data documenting the movement of white sturgeon over this barrier (E. Stoddard, BC Forests, Lands, and Natural Resources, pers. comm.). Downstream movement of other species over Hells Gate has been observed (McPhail and Lindsay 1986). The absence of individuals sampled above Hells Gate exhibiting ancestry from the Lower Fraser population suggests that Hells Gate provided a velocity barrier to gene flow even before the physical barrier provided by the rockslide of 1913-1914. Only approximately ~4-5 white sturgeon generations have passed between the rockslide and the time of sample collection, and one would not expect to observe such strong levels of genetic divergence among populations isolated for so few generations. Pairwise Phi-PT analyses indicate higher levels of genetic differentiation between the Lower and Upper Fraser populations than between the Lower Fraser and all other populations with the exception of the Kootenai River.

The analysis of population substructure in the Fraser River above Hells Gate suggests that there are at least two genetically distinct spawning populations in that region, one corresponding to the Middle Fraser River and the other corresponding to the Nechako River system. The Upper Fraser River appears to be a mixing area for individuals originating from the Middle Fraser or Nechako River populations. The mixing of non-reproductive subadult and adult sturgeon from different spawning populations has been documented in many different species (Atlantic sturgeon, Waldman et al. 2002; Gulf sturgeon, *Acipenser oxyrinchus desotoi*, Dugo et al. 2004; shortnose sturgeon, Walsh et al., 2001; lake sturgeon, Bott et al. 2009; green sturgeon, *Acipenser medirostris*, Israel et al. 2009). Mixing was somewhat unexpected in the Upper Fraser

given that tagging studies have revealed no movement between the Upper Fraser and Middle Fraser or Nechako (Yarmish and Toth 2001). Some have suggested that the 80 km reach of the Middle Fraser south of the Nechako/Fraser confluence consists of poor white sturgeon habitat and may present a dispersal barrier (Yarmish and Toth 2001). These authors also suggested a 60 km region of the Lower Nechako presented similarly unsuitable habitat which may prevent movement of Nechako River white sturgeon into the Fraser. However, genetic data indicating that mixing of Nechako River and Middle Fraser River origin individuals occurs in the Upper Fraser River suggests that movement across both putative barriers is possible.

Range-wide Population Structure

Examining population structure both on a within-drainage and range-wide scale with Structure allowed us to detect fine-scale population structure within rivers, which may not have been possible in a single analysis of the entire dataset, as well as evaluate genetic relationships among white sturgeon populations inhabiting different drainages. When examining white sturgeon across the species range in Structure, K=4 through K=7 had similar likelihoods and variances. It this scenario, the Structure manual suggests selecting the lowest K value if biologically reasonable (Pritchard et al. 2010). However, when K=4 was assumed (data not shown), individuals from the Sacramento-San Joaquin, Lower Columbia, and Lower Fraser essentially assigned to a single cluster. When K = 5 is assumed (data not shown), the Sacramento-San Joaquin population was found to be distinct, but the Lower Columbia and Lower Fraser collections were nearly identical, showing membership to a Sacramento-San Joaquin group and a Columbia River group. These two outcomes are contrary to our understanding of species biology, as most

sturgeon species exhibit some degree of natal philopatry, often on the scale of drainages (King et al. 2001; Israel et al. 2004; Welsh et al. 2008; but see Smith et al. 2002). Although recaptures of tagged individuals suggest that white sturgeon do migrate to non-natal estuaries, we have no evidence to indicate that these migrations are for spawning. When K=7 (data not shown), no individuals assigned to the seventh cluster suggesting this was an overestimate of the number of populations in the dataset. We used our knowledge of species life history and the results of the within-drainage Structure analysis to conclude that K=6 was the most likely number of populations in the dataset. This is supported by the authors of Structure, who suggest that program users strongly consider the biological feasibility of their results when determining the number of populations in their dataset (Pritchard et al. 2010).

On the range-wide scale, Structure revealed the Sacramento-San Joaquin, Middle Snake, Kootenai, Lower Fraser, and Upper Fraser (above Hells Gate) to be genetically distinct populations. Previous analysis using allozymes also concluded that the Sacramento-San Joaquin population was genetically distinct from other white sturgeon collections based on the presence and high frequency of a private allele (Bartley et al. 1985). One the range-wide scale, the distinctiveness of the Lower Columbia River (below Bonneville Dam) population is called into question, although the Structure analysis focused within the Columbia River drainage suggested it was somewhat differentiated from the other reaches of the Columbia and Snake rivers. When the Lower Columbia population is expanded to include all individuals sampled below McNary Dam, as suggested by range-wide analysis in Structure, the pairwise Phi-PT analysis supports the distinctiveness of this collection from the rest of the Columbia and Snake (Table

2.12). It is possible that a low level of gene flow from other populations with ocean access, such as the Sacramento-San Joaquin and Lower Fraser could be contributing to the distinctiveness of Lower Columbia population detected in the within-drainage analysis for the Columbia River. This is supported by the detection of a migrant from the Sacramento-San Joaquin population in the Lower Columbia River below Bonneville Dam, although it is unknown if white sturgeon detected in non-natal estuaries represent straying events or foraging migrations. The PCO analysis using populations defined by Structure also corroborates the hypothesis of some gene flow (historical or contemporary) between the three populations with access to marine habitat (Sacramento-San Joaquin, Lower Columbia, Lower Fraser), as all three populations cluster in the same quadrant of the PCO plot that includes the Kootenai River (Figure 2.13). When the Kootenai population is removed, the populations with ocean access still appear more closely related to each other than to the Middle Columbia/Middle Snake or Upper Fraser populations (Figure 2.14). It is uncertain if the very close relationship identified for the Lower Columbia and Lower Fraser River by the PCO analysis is due to contemporary gene flow because the Columbia River is likely one source for post-glacial recolonization of the Fraser River.

Patterns of population structure revealed within and among drainages have implications for our understanding of spawning site fidelity in sturgeons. Many anadromous species such as salmonids exhibit strict spawning site fidelity, where reproductively mature individuals exclusively migrate to the spawning site from which they originated. This behavior leads to patterns of significant genetic divergence among populations using different spawning sites. However, the scale of population structure in

continuous river habitat, as described here, suggests that there is gene flow between geographically proximate white sturgeon spawning sites within a river system. There is no evidence of population structure within the collection of individuals sampled from the Lower Columbia River (below Bonneville Dam) despite the fact that it is characterized by spawning sites on two rivers, one 12 km downstream of Bonneville Dam on the Columbia and another on the Lower Willamette River (Chapman and Jones 2010). In the Lower Fraser, six spawning sites are distributed over 60 rkm (Perrin et al. 2003), and no substructure was detected there. Previous examinations of white sturgeon population structure within the Transboundary Reach, a 300 km area known to contain at least four spawning sites (L. Hildebrand, Golder & Associates, pers comm.), provided little evidence of population structure (Drauch Schreier et al. 2011d). In this study, we observe little genetic difference between the Middle Columbia, Transboundary Reach, and Lower Snake despite the fact that these regions are distributed in two major river systems. The pattern of isolation by distance revealed here in the Columbia and Snake Rivers suggests that there is gene flow between geographically proximate white sturgeon populations (in the absence of natural barriers) as opposed to many genetically discrete spawning populations.

Spawning site fidelity has been examined most extensively in lake sturgeon and several authors report use of multiple spawning sites within and among years by some individuals (Lyons and Kempinger 1992, Rusack and Mosindy 1997). Applying the theory of Wright (1931), the use of multiple spawning sites by a few individuals would be enough to reduce genetic differentiation among spawning populations. Similarly, Welsh and McLeod (2010) found no genetic differentiation among adult lake sturgeon

sampled at five suspected spawning sites distributed over 30.5 rkm on the Namakan River, Ontario, which also suggests gene flow among spawning sites at that geographic scale. An investigation of population structure of shovelnose sturgeon (*Scaphyrinchus platyorinchus*) in continuous river habitat (Middle Mississippi River and Lower Missouri River) found little genetic differentiation between the Middle Mississippi and Lower Missouri groups (Schrey et al. 2009), although it was uncertain whether this result was due to mixing of individuals from different reproductive groups during non-reproductive times. Straying between river drainages has been documented in shovelnose and lake sturgeon (Schrey et al. 2009; Homola et al. 2010). Quattro et al. (2002) showed that hatchery reared shortnose sturgeon stocked into the Savannah River recolonized the Edisto River via dispersal through marine habitat, although movement by hatchery reared individuals that may not be properly imprinted on natal habitat does not provide sufficient evidence of straying. Dugo et al. (2004) documented three adult Gulf sturgeon from the Pearl River spawning population at the known spawning site in the adjacent Pascagoula River, but it is unknown whether they reproduced. Acoustic tagging studies are required to further characterize spawning site fidelity in sturgeons. Acoustic tagging arrays may be constructed near known spawning sites and reproductively mature individuals can be tagged with long lived acoustic tags for remote detection as they utilize one or more spawning sites in subsequent reproductive cycles. This might provide information about sex specific differences in spawning site fidelity, which may explain differences in patterns of population structure revealed by mtDNA and nuclear genetic markers in several studies of sturgeon population structure (King et al. 2001; Ludwig et al. 2003; Drauch Schreier et al. 2011d).

Non-Natal Estuary Samples

The assignment of samples collected from Winchester Bay, Tillamook Bay, and Port Susan Bay to all three populations with coastal access (Sacramento-San Joaquin, Lower Columbia below Bonneville Dam, Lower Fraser) provides further evidence of the ability of white sturgeon to engage in long distance dispersal. Contrary to expectation, individuals sampled in non-natal estuaries did not necessarily assign to the most geographically proximate population. In Winchester Bay, most individuals that could be assigned originated from the Columbia River, although two assigned to the Sacramento-San Joaquin population. In Tillamook Bay, the three populations with access to marine habitat were represented in similar proportions. In Port Susan Bay, however, the majority of individuals that could be assigned were found to originate from the Sacramento-San Joaquin population. This represents a dispersal distance similar to that reported by Welch et al. (2006), who described a recapture in the Lower Fraser River of an individual first encountered in the Klamath River in California. The preponderance of migrants from the Sacramento-San Joaquin system despite its great geographic distance from the non-natal estuaries sampled could be due to differences in marine habitat use among populations. In shortnose sturgeon, populations at the northernmost and southernmost extremes of the species range tend to utilize marine habitat more frequently than populations in the mid-Atlantic portion of the range (Kynard 1997; Grunwald et al. 2002). Atlantic sturgeon populations at the northern portion of the range tend to make more marine migrations than populations at the southern end of the range (Grunwald et al. 2008; Peterson et al. 2008).

Several migrants from the Sacramento-San Joaquin and Lower Fraser were detected in the Columbia River collection, one each in the unimpounded Lower Columbia, Dalles Pool, and John Day Pool. Presumably the migrants discovered in the impounded reaches are individuals that were trapped above Bonneville Dam when its construction was completed in 1938. Interestingly, no migrants were detected in the Sacramento-San Joaquin or Lower Fraser River collections. No detection of migrants in the Lower Fraser could be due to a small sample size relative to the Columbia and Sacramento-San Joaquin collections; if some migrants from other spawning populations are present, sampling may not have been extensive enough to detect them. White sturgeon tagged in the Columbia River have been recaptured in the Lower Fraser River (T. Nelson, Fraser River Sturgeon Conservation Society, pers comm.), although it is unclear whether these fish are individuals born in the Columbia visiting the Fraser or individuals originating from the Fraser that were tagged while feeding in the Columbia River estuary. The lack of migrants in the Sacramento-San Joaquin system could due to its location at the southernmost edge of the species range. Israel et al. (2009) and Lindley et al. (2011) found that green sturgeon on the West Coast make annual northward migrations to feeding grounds in various estuaries and coastal regions. It is possible that northward migration occurs more frequently than southward migration in white sturgeon as well. However, capture of both white sturgeon and green sturgeon off the coast of Mexico (Rosales-Casián and Almeda-Jáuregui 2009; Ruiz-Campos et al. 2011) suggests that some southward movement occurs for both species. Further sampling of white sturgeon along the continental shelf and in non-natal estuaries is required to further clarify patterns of marine movement in this species.

MANAGEMENT IMPLICATIONS

Management and conservation of white sturgeon can be greatly enhanced by an improved understanding of genetic relationships among white sturgeon populations across the species range. In the Columbia and Snake rivers, collections of individuals trapped between impoundments are often treated as "populations" for the purpose of management and conservation (Parsley et al. 2007). Our findings do not provide support for the management of impounded reaches as distinct populations. Evidence of gene flow between geographically proximate regions, either due to historical movement between regions that are now impounded or due to unidirectional downstream movement over dams suggests that the genetic units be defined on a larger scale. It would be difficult to divide the Columbia system into distinct genetic units for management as strong genetic differentiation only exists between white sturgeon inhabiting system's extremes (Lower Columbia and Middle Snake) and little genetic divergence is observed among white sturgeon collections throughout most of the system (Middle Columbia, Transboundary Reach, Lower Snake). Hatchery supplementation is being considered for various impounded reaches of the Columbia to mitigate for hydroelectric projects and to improve white sturgeon fisheries. We recommend selecting broodstock from the reach where stocking is to occur or from geographically proximate impounded reaches. We advise against selecting broodstock from the Lower Columbia River and geographically proximate reaches (Bonneville Pool, Dalles Dam Pool, John Day Pool) for stocking into the Transboundary Reach or Middle Snake and vice versa, to preserve any unique genetic

differences that might exist at the upstream and downstream extremes of the species range.

In the Fraser River, patterns of population structure are similar to those described by Smith et al. (2002) although our results do not support a treating the Upper Fraser River as a distinct population. We recommend that the Lower Fraser (below Hells Gate), the Middle Fraser (between Hells Gate and rkm 553), and the Nechako River system be classified as distinct populations. Our results suggest that the Upper Fraser is a mixing zone for Middle Fraser and Nechako River white sturgeon. Additional characterization of spawning habitat and reproductive behavior in white sturgeon above Hells Gate would increase our understanding of how the Upper Fraser River is used by different spawning populations of white sturgeon.

Management of chronic recruitment failure in white sturgeon may be improved by a greater understanding of different aspects of spawning behavior, including spawning site fidelity. As factors responsible for recruitment failure likely vary between locations, knowledge of how white sturgeon select available spawning habitat (spatially and temporally) will improve managers' ability to pinpoint the causes and possibly provide mitigation. We reveal that the scale of spawning site fidelity in sturgeon may differ from the salmonid model, with gene flow between geographically proximate spawning sites. This suggests that recruitment failure might best be managed on a regional scale rather than on a "per spawning site" basis, at least in populations that utilize multiple spawning sites.

Illegal harvest continues to be a significant problem for white sturgeon managers as the black market price for white sturgeon caviar provides a great incentive for exceeding harvest limits or harvesting large, old females in protected size classes. Anti-poaching efforts may be enhanced if the areas where poaching is occurring can be identified. The identification of genetically distinct populations across the white sturgeon range suggests that wildlife forensic techniques might be applied to determine the origin of confiscated sturgeon products. In particular, individuals originating from the Sacramento-San Joaquin, Kootenai, Lower Fraser, and Upper Fraser (above Hells Gate) rivers should be assignable to their source populations. It may be difficult, however, to pinpoint the region of the Columbia River or Snake River from which a white sturgeon product originated. We recommend combining samples from the Lower and Middle Columbia and Transboundary Reach with samples from the Lower Snake to create a general Columbia River baseline for use in white sturgeon forensic investigations. As the Middle Snake was revealed to be genetically distinctive in the within-drainage and range-wide population structure analyses, it may provide a useful baseline. It may be possible to determine the origin of a sturgeon product originating from the Fraser River above Hells Gate on a finer scale, although we have not yet investigated this possibility using the USEPOPINFO model in Structure or likelihood based analyses in AFLPOP. A sturgeon product that assigned to the Upper Fraser (above Hells Gate) baseline might be tested further with a Middle Fraser and Nechako River baseline to determine the origin of that sample on a finer geographic scale. Unfortunately, the rate of non-assignment in both Structure and AFLPOP for non-natal estuary samples suggests that in some cases, a sample may not be assignable to its population of origin. The development of

codominant SNP markers for white sturgeon would likely increase our ability to determine the origin of illegal white sturgeon products.

Another critical unknown hindering management of white sturgeon is their propensity for marine dispersal. The sympatric green sturgeon makes seasonal marine migrations (Lindley et al. 2008) and individuals from both the Southern and Northern Distinct Population Segments (DPS) often congregate in non-natal estuaries (Israel et al. 2009; Lindley et al. 2011). On the east coast, shortnose sturgeon, Atlantic sturgeon, and gulf sturgeon are known to disperse through marine habitat to access non-natal estuaries at various life stages (Dovel and Beggren 1983, cited in Grunwald et al. 2008; Waldman et al. 2002; Dugo et al. 2004). Tagging data indicates that white sturgeon also engage in marine dispersal behavior, although the degree to which they do this is unknown (Chadwick 1955; Galbreath 1985; Brennan and Cailliet 1991; DeVore et al. 1999; Welch et al. 2006). Although population assignment testing of individuals sampled in non-natal estuaries cannot approach the question of what proportion of individuals embark on marine migrations, this analysis reveals that long distance movements are made. Characterization of movement rates and non-natal estuary use is important for a species that ranges over several interjurisdictional boundaries and where populations are characterized by widely varying levels of viability. For example, protective harvest regulations in the Sacramento-San Joaquin white sturgeon population are more stringent that those in the Lower Columbia River, which contains the most productive sturgeon fishery in North America (DeVore et al. 1995). Likewise, white sturgeon are listed under the Species at Risk Act in Canada and harvest of Fraser River white sturgeon is illegal. Stable isotope studies conducted by Veinott et al. (1999) suggest that up to 10% of the

Lower Fraser population enters marine habitat where that proportion may be subject to harvest in a fishery outside of Canadian jurisdiction. Additional research to quantify the rate of non-natal habitat use by the Sacramento-San Joaquin, Lower Columbia, and Lower Fraser River populations is recommended. Of particular interest is non-natal estuary use by the endangered Lower Fraser River population. Lindley et al. (2011) provide an example of how tagging studies may be conducted using long-lived acoustic tags and an existing acoustic array already present along the West Coast of North America to characterize marine habitat use by sturgeons over a large geographic scale.

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Table 2.1. Samples used for examination of population structure in white sturgeon.

Drainage/Region	River Reach/Estuary	N	Tissue type
Sacramento-San Joaquin River (S-SJ)	San Pablo Bay	135	Fin clip
	Suisun Bay	480	Fin clip
	Sacramento River	42	Fin clip
	Napa River	3	Fin clip
Lower Columbia	Lower Columbia (LC)[a]	97	Pectoral fin ray
	Dalles Pool (DD)	59	Fin clip
	John Day Pool (JD)	59	Fin clip
Middle Columbia	McNary Pool (MCN)	28	Fin clip
	Priest Rapids Pool (PR)	4	Fin clip
	Wanapum Pool (WN)	30	Fin clip
	Rock Island Pool (RI)	4	Fin clip
	Rocky Reach Pool (RR)	9	Fin clip
	Wells Pool (WP)	11	Fin clip
	Chief Joseph Pool (CJ)	5	Fin clip
Upper Columbia	Transboundary Reach (TR)	330	Fin clip
	Kootenai (KT)	98	Fin clip
Lower Snake	Ice Harbor Pool (IH)	48	Fin clip
	Lower Monumental Pool (LM)	49	Fin clip
	Hells Canyon Complex (HC)	97	Fin clip
Middle Snake	Brownlee Pool (BL)	28	Blood
	Swan Falls Pool (SF)	47	Fin clip
	CJ Strike Pool (CJS)	41	Fin clip
	Lower Salmon Falls Pool (LSF)	19	Fin clip
	Upper Salmon Falls Pool (USF)	50	Fin clip
Lower Fraser	SG-1	38	Fin clip
	SG-2	38	Fin clip
Upper Fraser	Middle Fraser River (SG-3)	40	Fin clip
	Nechako River/Stuart Lake/Fraser Lake (NK/SL/FL)	86	Fin clip
	Upper Fraser (UF)	47	Fin clip
Non-natal estuaries	Winchester Bay (WB)	22	Fin clip
	Tillamook Bay (TB)	6	Fin clip
	Port Susan (PS)[b]	11	Fin clip

[a]Lower Columbia refers to the reach from the mouth of the Columbia River estuary to Bonneville Dam.

[b]Port Susan Bay is located in the Whidbey Island Basin of Puget Sound

Table 2.2. Microsatellite loci used to genotype white sturgeon samples. TD indicates a touchdown profile was used for amplification.

Microsatellite	**T_A (^oC)**	**Reference**
AciG 2	60	Börk et al. (2008)
AciG 35	56	Börk et al. (2008)
AciG 52	56	Börk et al. (2008)
AciG 53	56	Börk et al. (2008)
AciG 110	56	Börk et al. (2008)
AciG 140	56	Börk et al. (2008)
As015	56	Zhu et al. (2005)
Atr 105	TD	Rodzen and May (2002)
Atr 107	TD	Rodzen and May (2002)
Atr 109	TD	Rodzen and May (2002)
Atr 117	TD	Rodzen and May (2002)
Atr 1101	57	Rodzen and May (2002)
Atr 1173	TD	Rodzen and May (2002)

Table 2.3. Structure analyses conducted for white sturgeon population delineation.

River system	Exploratory Analysis	Full Analysis
Sacramento-San Joaquin	K = 1 to K = 20	K = 1 to K = 6
Columbia	K = 1 to K = 20	K = 1 to K = 10
Fraser	K = 1 to K = 20	K = 1 to K = 10
Fraser (below Hells Gate)	K = 1 to K = 4	K = 1 to K = 4
Fraser (above Hells Gate)	K = 1 to K = 3	K = 1 to K = 4
Full dataset	K = 1 to K = 20	K = 1 to K = 10

Table 2.4. Total number of alleles and private alleles detected in white sturgeon within regions. N refers to the sample size while A_T is the total number of alleles and A_P the total number of private alleles detected across 13 microsatellite loci.

Population	**N**	**A_T**	**A_P**
Sacramento-San Joaquin	660	236	18
Lower Columbia	214	217	3
Middle Columbia	91	178	1
Transboundary Reach	328	187	1
Kootenai	376[a]	97	0
Lower Snake	194	192	4
Middle Snake	184	123	0
Lower Fraser	60	198	10
Upper Fraser	112	133	1

[a]This value calculated from genetic monitoring dataset for Kootenai River population (see Chapter 3)

Table 2.5. Total number of alleles and private alleles detected in white sturgeon within regions after subsampling to smallest population sample size (N=60).

Population	**N**	**A_T**	**A_P**
Sacramento-San Joaquin	60	196	8
Lower Columbia	60	189	2
Middle Columbia	60	172	3
Transboundary Reach	60	160	2
Kootenai	60	77	0
Lower Snake	60	164	2
Middle Snake	60	107	1
Lower Fraser	60	198	13
Upper Fraser	60	124	1

Table 2.6. Average number of alleles per individual per locus detected in white sturgeon within subsampled regions (N=60). A_T is the total number of alleles detected at each locus. S-SJ = Sacramento-San Joaquin, LC = Lower Columbia, MC = Middle Columbia, TR = Transboundary Reach, KT = Kootenai, LS = Lower Snake, MS = Middle Snake, LF = Lower Fraser, UF = Upper Fraser.

		Population								
Locus	**A_T**	**S-SJ**	**LC**	**MC**	**TR**	**KT**	**LS**	**MS**	**LF**	**UF**
AciG 2	7	2.25	2.37	2.59	2.33	2.56	2.43	2.40	2.47	2.27
AciG 35	21	5.87	5.97	5.85	5.82	5.02	5.80	5.53	5.70	5.18
AciG 52	26	5.03	5.27	5.27	5.30	4.40	5.25	4.76	5.33	4.75
AciG 53	11	2.27	2.28	2.13	2.00	1.73	2.03	2.02	2.22	1.95
AciG 110	28	4.77	4.62	4.60	4.65	3.63	4.33	3.97	4.52	4.37
AciG 140	10	2.18	2.40	2.30	2.45	1.78	2.20	1.98	2.42	2.12
As015	24	4.77	4.81	4.85	4.25	3.00	4.58	4.08	4.90	4.53
Atr 105	12	2.47	2.54	2.74	2.90	2.38	2.74	2.76	2.52	2.83
Atr 107	35	4.42	4.53	4.92	4.48	2.98	4.76	4.81	4.68	4.37
Atr 109	31	4.20	4.10	3.66	3.42	2.68	3.50	2.85	4.00	2.93
Atr 117	33	3.90	4.10	3.91	3.78	2.88	4.03	3.90	3.73	3.53
Atr 1101	10	2.82	2.59	2.30	2.23	2.10	2.07	1.75	2.32	2.32
Atr 1173	24	3.48	3.42	3.14	3.32	2.57	3.30	3.09	3.20	3.13

Table 2.7. Pairwise genetic divergence among regions inhabited by white sturgeon. Phi-PT values are below the diagonal and P-values are above the diagonal. S-SJ = Sacramento-San Joaquin, LC = Lower Columbia, MC = Middle Columbia, TR = Transboundary Reach, KT = Kootenai, LS = Lower Snake, MS = Middle Snake, LF = Lower Fraser, and UF = Upper Fraser. Significant values are indicated with an asterisk.

Population	S-SJ	LC	MC	TR	KT	LS	MS	LF	UF
S-SJ		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
LC	0.043*		0.0005	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
MC	0.084*	0.023*		0.0001	0.0001	0.0050	0.0001	0.0001	0.0001
UC	0.093*	0.029*	0.026*		0.0001	0.0004	0.0001	0.0001	0.0001
KT	0.222*	0.171*	0.169*	0.156*		0.0001	0.0001	0.0001	0.0001
LS	0.094*	0.025*	0.016	0.010*	0.186*		0.0001	0.0001	0.0001
MS	0.171*	0.083*	0.050*	0.050*	0.240*	0.022*		0.0001	0.0001
LF	0.040*	0.022*	0.055*	0.057*	0.193*	0.056*	0.118*		0.0001
UF	0.122*	0.065*	0.082*	0.079*	0.209*	0.075*	0.136*	0.082*	

Table 2.8. Pairwise Phi-PT values for the six populations of white sturgeon identified by range-wide Structure analysis. S-SJ = Sacramento-San Joaquin, LC = Lower Columbia (from river mouth to McNary Dam), MC/MS (McNary to Transboundary Reach, Lower Snake, Middle Snake), KT = Kootenai, LF = Lower Fraser, and UF = Upper Fraser. All values were significant ($P < 0.0001$).

Population	S-SJ	LC	MC/MS	KT	LF	UF
S-SJ						
LC	0.040					
MC/MS	0.120	0.038				
KT	0.225	0.164	0.178			
LF	0.041	0.018	0.069	0.209		
UF	0.128	0.061	0.078	0.215	0.083	

Table 2.9. Structure assignments of six samples from non-natal estuaries. Q values, representing the posterior probability that an individual originated from the population to which it was assigned, are found in parentheses. NA indicates that missing data precluded a sample from being included in AFLPOP analysis. S-SJ = Sacramento-San Joaquin; LC = Lower Columbia; C/LS = Middle Columbia, Transboundary Reach, Lower Snake; MS = Middle Snake; LF = Lower Fraser; UF = Upper Fraser.

Individual	Sampling location	Source Population	AFLPOP Assignment
WB1198	Winchester Bay	S-SJ (0.504)	S-SJ
WB1298	Winchester Bay	LC (0.509)	LC
WB1598	Winchester Bay	C/LS (0.580)	C/LS
WB1998	Winchester Bay	C/LS (0.539)	NA
WB2398	Winchester Bay	S-SJ (0.600)	NA
PS0905	Port Susan Bay	S-SJ (0.560)	S-SJ[a]

[a]Assignment possible when LC individuals combined with Middle Columbia, Upper Columbia, and Lower Snake for Columbia River source

Table 2.10. Allocation of non-natal estuary samples to source populations identified by Structure using AFLPOP with an MLD of 0.8. N is the sample size. WB = Winchester Bay, TB = Tillamook Bay, PS = Port Susan. S-SJ = Sacramento-San Joaquin; LC = Lower Columbia; C/LS = Middle Columbia, Transboundary Reach, Lower Snake; MS = Middle Snake; LF = Lower Fraser; UF = Upper Fraser.

	N	**S-SJ**	**LC**	**C/LS**	**MS**	**KT**	**LF**	**UF**	**None**
WB	20	1	6	3	0	0	0	0	10
TB	6	0	1	0	0	0	1	0	4
PS	11	1	0	1	0	0	0	0	9

Table 2.11. Allocation of non-natal estuary samples to source populations identified by Structure using AFLPOP (MLD = 0.8) with individuals from the Lower Columbia, Middle Columbia, Transboundary Reach, and Lower Snake combined into a single source. N is the sample size. WB = Winchester Bay, TB = Tillamook Bay, PS = Port Susan. S-SJ = Sacramento-San Joaquin; LC/C/LS = Lower Columbia, Middle Columbia, Transboundary Reach, Lower Snake; MS = Middle Snake; LF = Lower Fraser; UF = Upper Fraser.

	N	**S-SJ**	**LC/C/LS**	**MS**	**KT**	**LF**	**UF**	**None**
WB	20	2	12	0	0	0	0	6
TB	6	1	2	0	0	2	0	1
PS	11	4	3	0	0	1	0	3

Figure 2.1. Distribution map for white sturgeon, *Acipenser transmontanus*. Blue rectangles indicate natural dispersal barriers while red dashes indicate approximate locations of major impoundments. 1 Sacramento River, 2 San Joaquin River, 3 San Pablo/Suisun Bays, 4 Napa River, 5 Lower Columbia River, 6 Middle Columbia River, 7 Transboundary Reach, 8 Lower Snake River, 9, Middle Snake River, 10 Kootenai River, 11 Lower Fraser River, 12 Middle Fraser River, 13 Upper Fraser River, 14 Nechako River, 15 Stuart Lake, 16 Fraser Lake, 17 Winchester Bay, 18 Tillamook Bay, 19 Port Susan Bay, 20 Hells Gate, 21 Bonnington Falls, 22 Shoshone Falls.

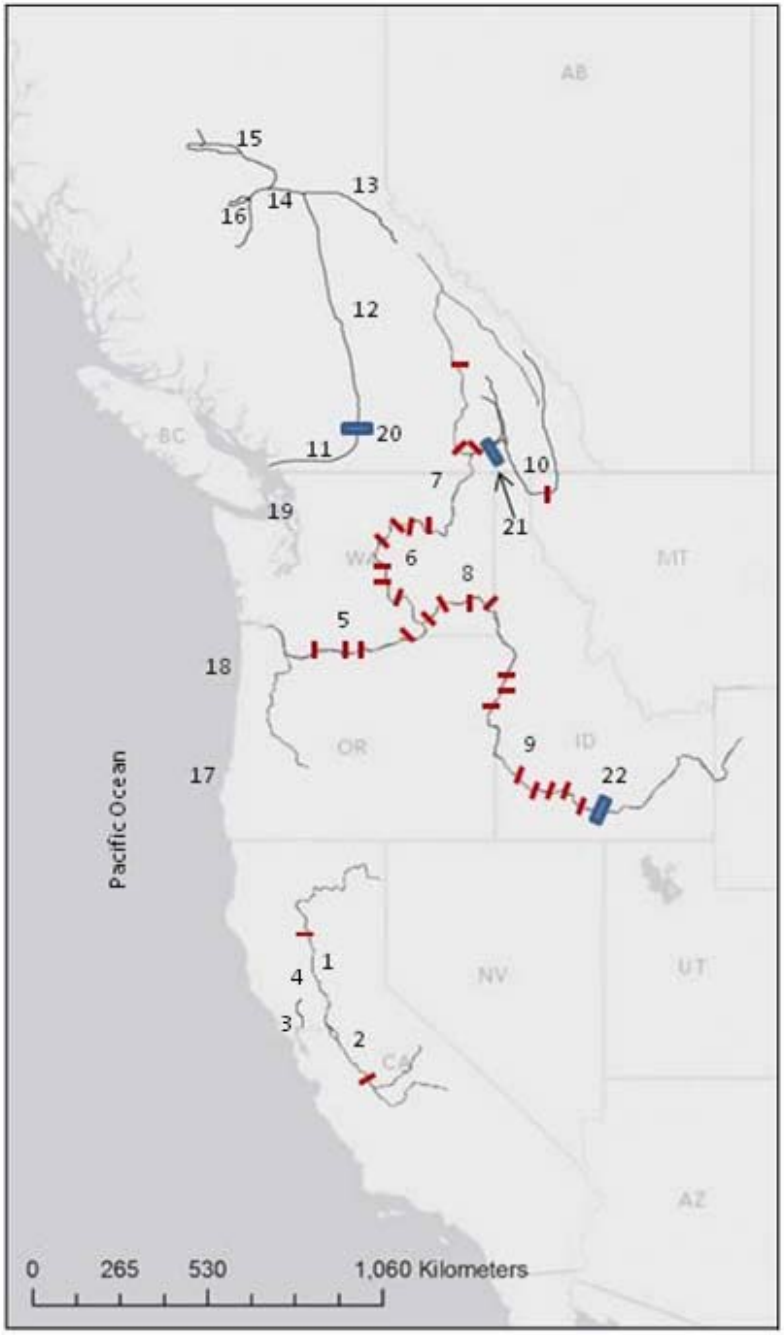


Figure 2.2. Principle coordinates analysis illustrating genetic relationships among white sturgeon inhabiting different regions. S-SJ = Sacramento-San Joaquin, LC = Lower Columbia, MC = Middle Columbia, TR = Transboundary Reach, KT = Kootenai, LS = Lower Snake, MS = Middle Snake, LF = Lower Fraser, and UF = Upper Fraser.

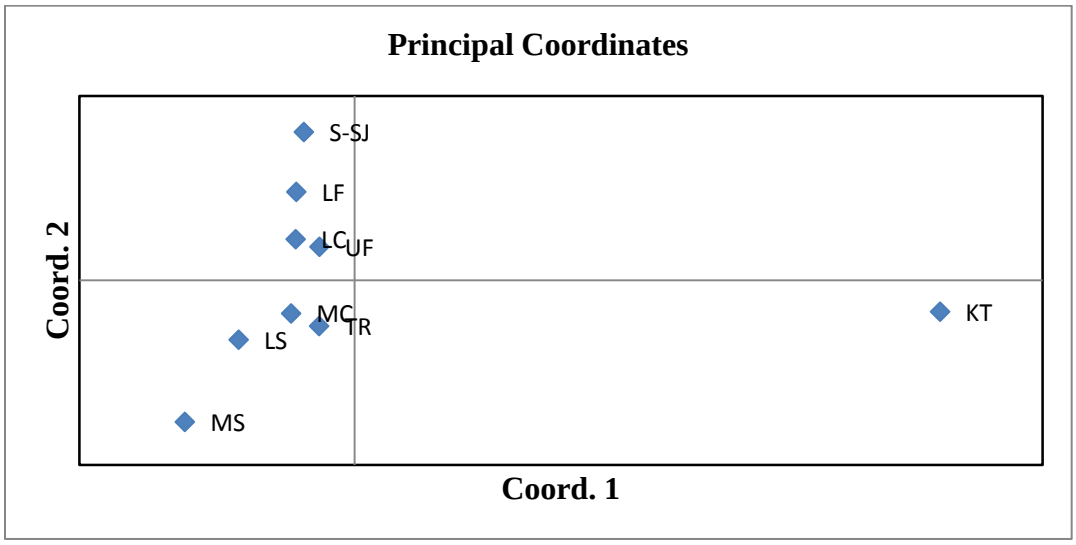


Figure 2.3. Principle coordinates analysis illustrating genetic relationships among white sturgeon inhabiting different regions, excluding the Kootenai River. S-SJ = Sacramento-San Joaquin, LC = Lower Columbia, MC = Middle Columbia, TR = Transboundary Reach, LS = Lower Snake, MS = Middle Snake, LF = Lower Fraser, and UF = Upper Fraser.

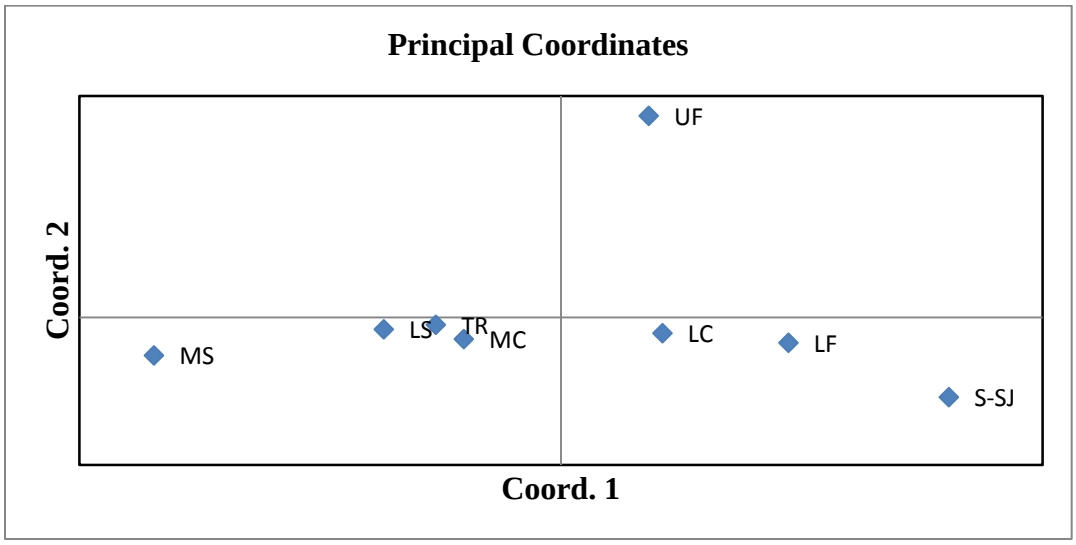


Figure 2.4. Mean Ln Pr(X|K) values for Structure examination of population structure within the Sacramento-San Joaquin system. Vertical lines denote standard deviation.

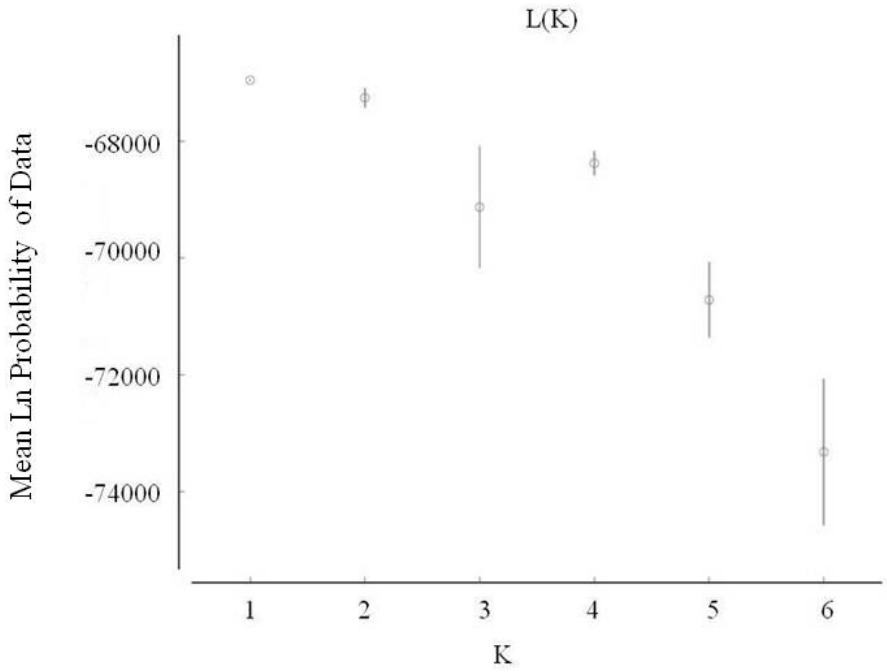


Figure 2.5. Mean Ln Pr(X|K) values for Structure analysis of population structure within the Columbia/Snake drainage. Vertical lines denote standard deviation.

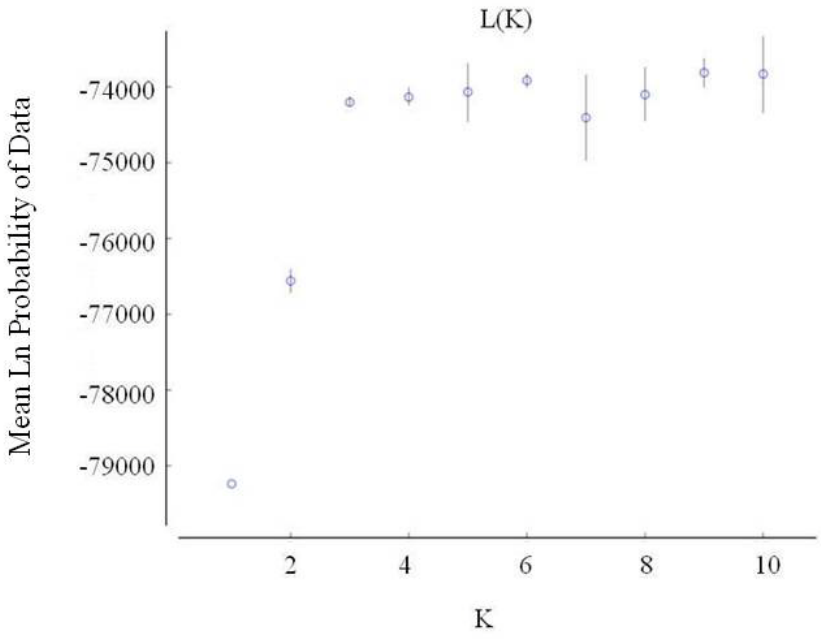


Figure 2.6. Bar histogram output from Structure depicting individual assignments in the Columbia/Snake drainage. Each bar represents one individual genome, each color represents a population identified by Structure, and the proportion of each color in each bar represents the proportional assignment of each individual to each population. See Table 2.1 for population abbreviations.

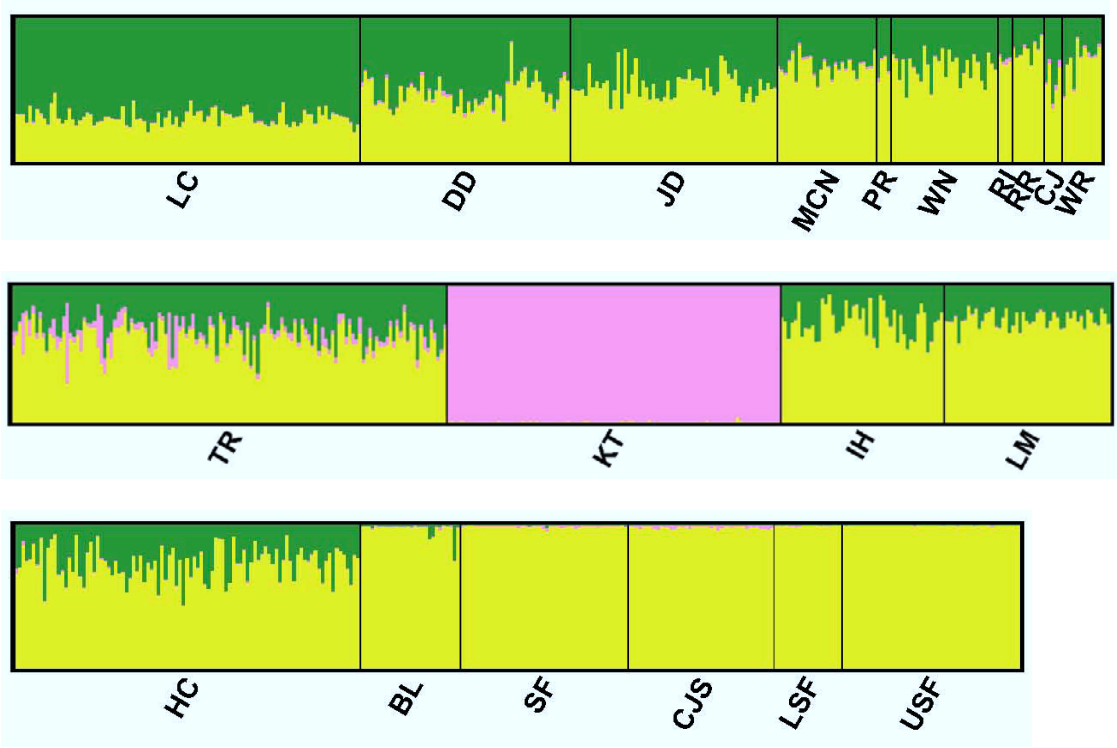


Figure 2.7. Mean Ln Pr(X|K) values for Structure analysis of population structure within the entire Fraser River system. Vertical lines denote standard deviation.

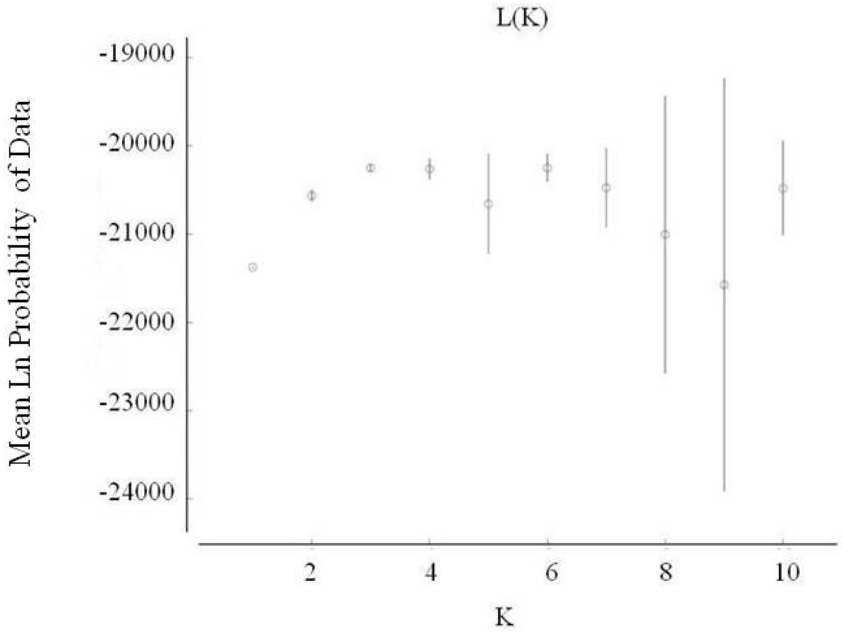


Figure 2.8. Bar histogram from Structure depicting individual assignments in the Fraser River. Each bar represents one individual genome, each color represents a population identified by Structure, and the proportion of each color in each bar represents the proportional assignment of each individual to each of two populations. The location of Hells Gate on the Fraser River is indicated. See Table 2.1 for population abbreviations.

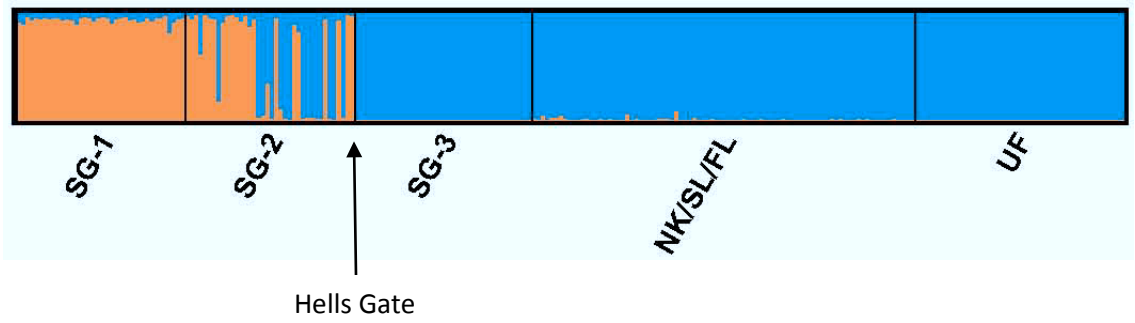


Figure 2.9. Mean Ln Pr(X|K) values for Structure analysis of population structure within the Fraser River above Hells Gate. Vertical lines denote standard deviation.

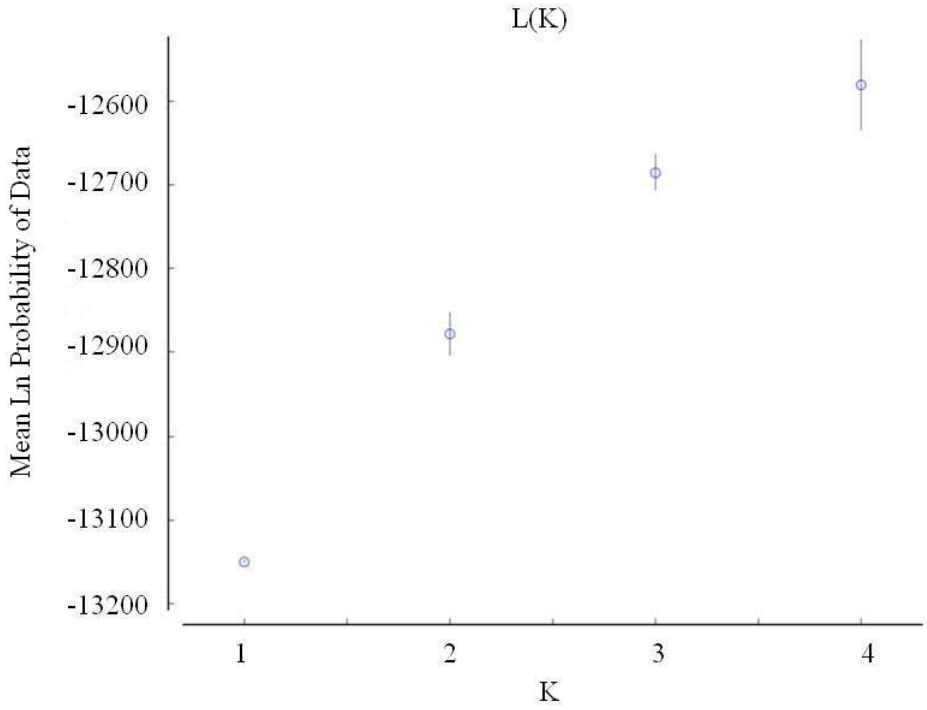
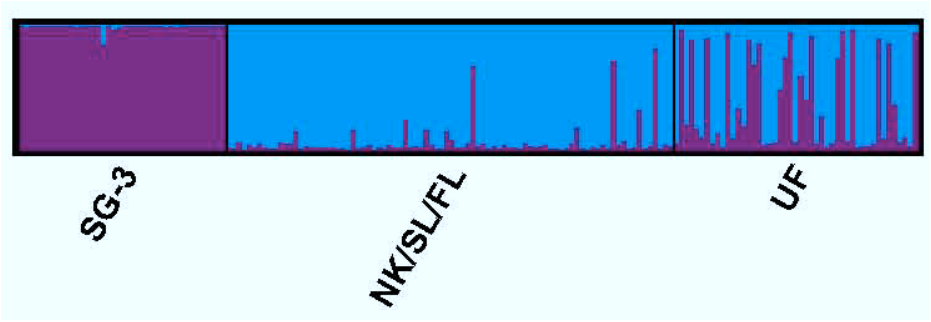


Figure 2.10. Bar histogram outputs from Structure depicting individual assignments in the Fraser River above Hells Gate. Each bar represents one individual genome, each color represents a population identified by Structure, and the proportion of each color in each bar represents the proportional assignment of each individual to each population. A) K = 2, B) K = 3. See Table 2.1 for population abbreviations.

A.



B.

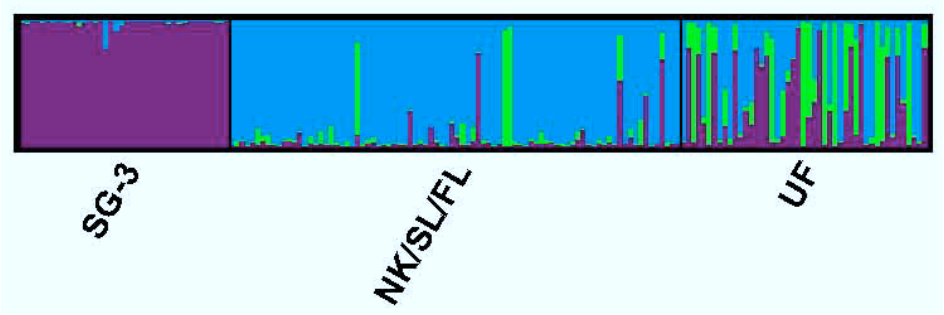


Figure 2.11. Mean Ln Pr(X|K) values for Structure analysis of population structure across the species' range. Vertical lines denote standard deviation.

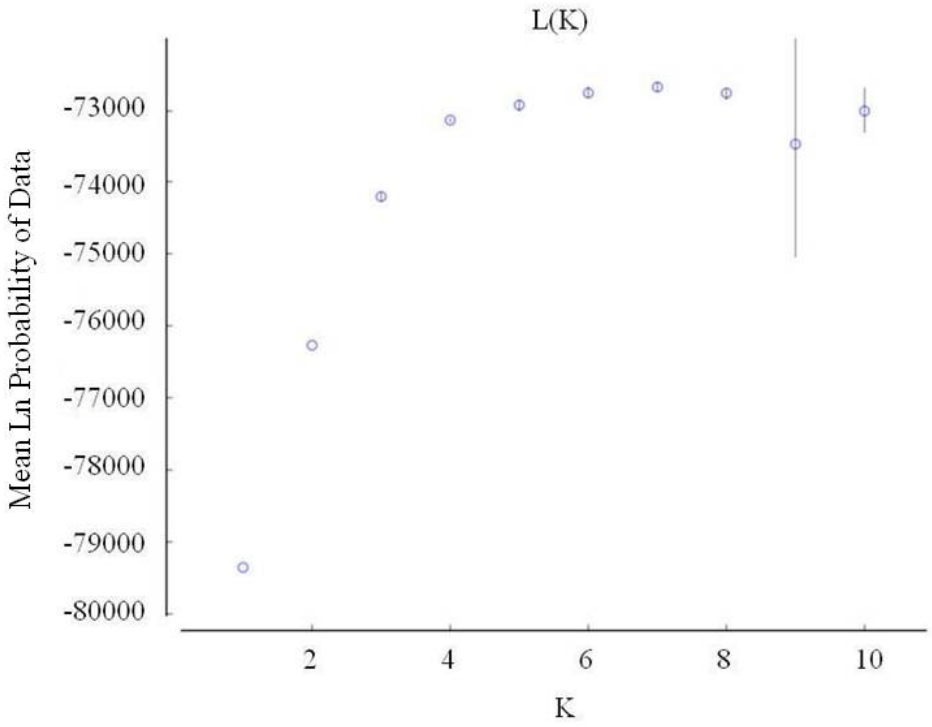


Figure 2.12. Bar histogram outputs from Structure depicting individual assignments in the range-wide dataset. Each bar represents one individual genome, each color represents a population identified by Structure, and the proportion of each color in each bar represents the proportional assignment of each individual to each population. See Table 2.1 for population abbreviations. ColR = Columbia River (DD to TR).

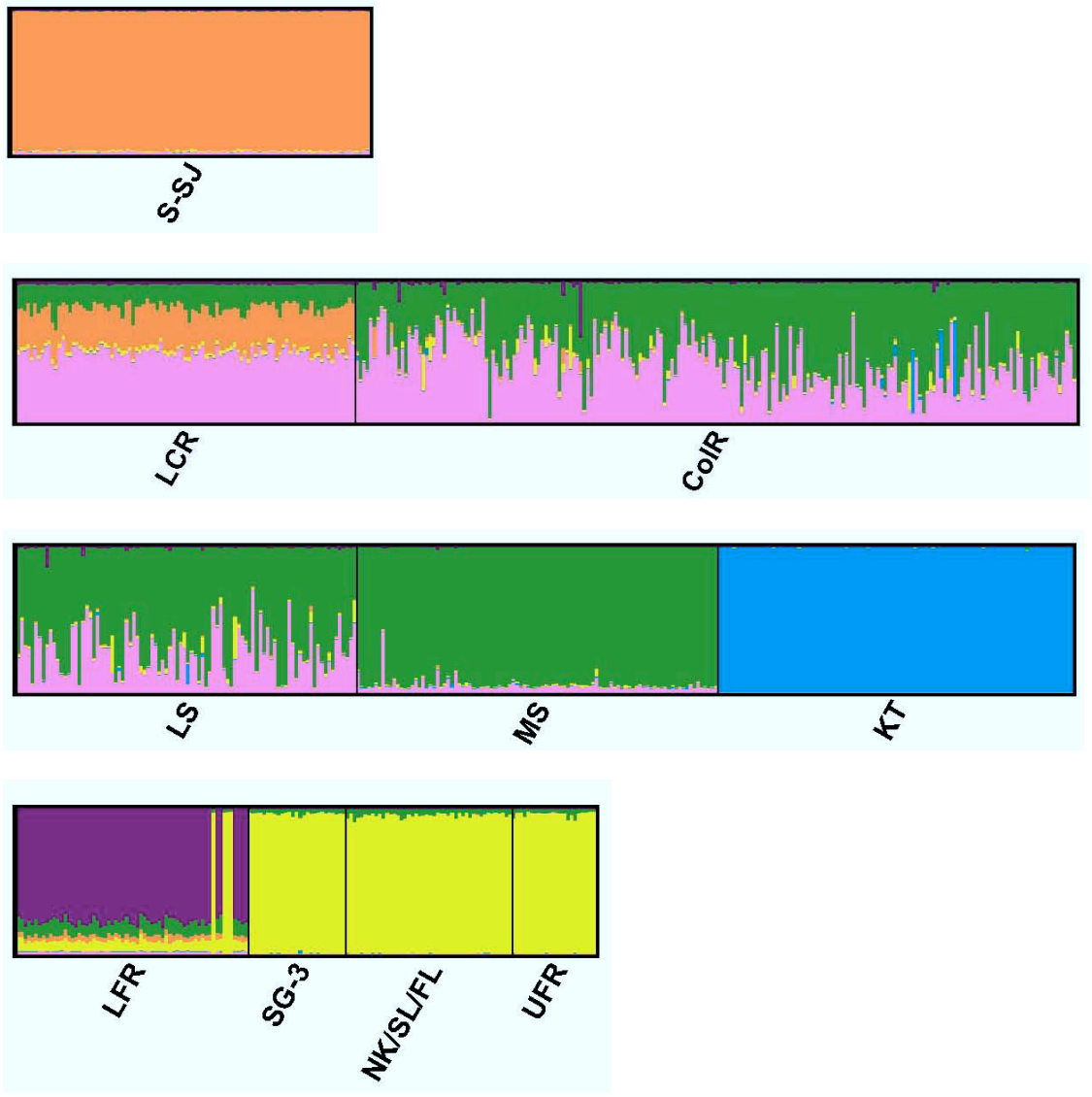


Figure 2.13. Principle coordinates analysis of six white sturgeon populations identified by Structure in range-wide analysis. S-SJ = Sacramento-San Joaquin, LC = Lower Columbia (from river mouth to McNary Dam), MC/MS (McNary to Transboundary Reach, Lower Snake, Middle Snake), KT = Kootenai, LF = Lower Fraser, and UF = Upper Fraser.

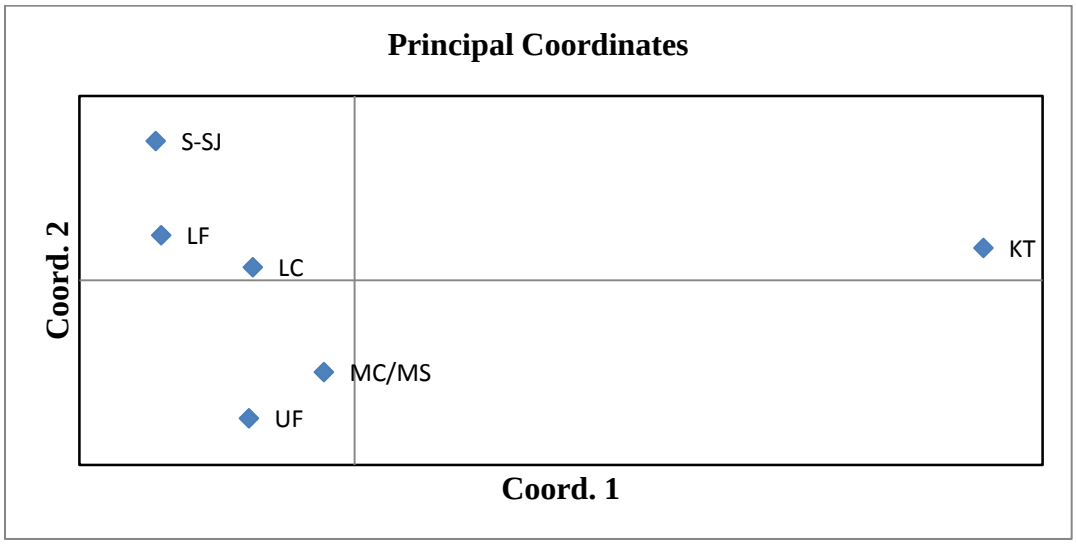
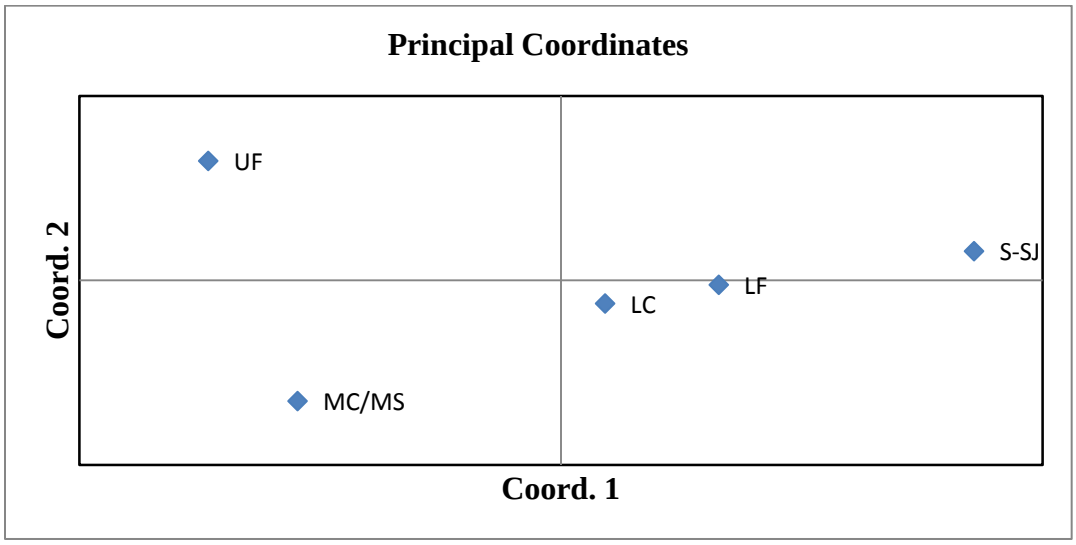


Figure 2.14. Principle coordinates analysis of six white sturgeon populations identified by Structure in range-wide analysis, excluding the Kootenai River population. S-SJ = Sacramento-San Joaquin, LC = Lower Columbia (from river mouth to McNary Dam), MC/MS (McNary to Transboundary Reach, Lower Snake, Middle Snake), LF = Lower Fraser, and UF = Upper Fraser.



CHAPTER 3

Genetic techniques inform conservation aquaculture of the endangered Kootenai River white sturgeon, *Acipenser transmontanus*

Andrea Drauch Schreier, Jeff Rodzen, Sue Ireland, and Bernie May

ABSTRACT

Large river-resident and diadromous fishes are globally threatened by environmental degradation, overharvest, and a rapidly changing climate. Conservation aquaculture is a tool that, used in concert with ecological restoration and harvest regulation, can protect the unique genetic, morphological, and behavioral characteristics of imperiled populations. Although conservation aquaculture programs are designed to minimize genetic impacts to wild populations, founder effects, domestication, and inbreeding may occur. Genetic monitoring may be used in the context of adaptive management to reduce deleterious genetic impacts of captive breeding in wild populations. Here we use the conservation aquaculture program for the endangered Kootenai River white sturgeon (*Acipenser transmontanus*) as a case study to illustrate how genetic tools might improve captive breeding programs for large river fishes. We use microsatellite markers to reveal very low levels of genetic diversity in the Kootenai River white sturgeon relative to other populations across the species range. We show that by using high numbers of broodstock, the conservation aquaculture program has captured 96% of the population's microsatellite diversity in hatchery-released progeny in only 10 years. We validate the power of parentage analysis to identify family relationships

between individual white sturgeon using a panel of 18 microsatellite loci. Parentage analysis will become crucial for inbreeding avoidance in the Kootenai River white sturgeon aquaculture program in ~2020-2030, when the majority of broodstock available for captive breeding will originate from the hatchery.

INTRODUCTION

River fishes worldwide are threatened by habitat fragmentation and loss, modification of natural flow regimes, pollution, loss of habitat connectivity due to impoundment, invasive species, and overharvest (Jelks et al. 2008). Although restoration of habitat and reduction or elimination of harvest are the best means to recover vulnerable fish populations, lack of available personnel, funding, and political will and enforcement present significant challenges to these efforts. In addition, the current imperiled status of many fish populations often is the result of many interrelated environmental and demographic changes that have occurred over long periods of time. It may be difficult to pinpoint the most limiting factor(s) on which to focus management action for population restoration. One conservation tool that might be used in concert with the identification of limiting factors and ecological restoration is conservation aquaculture.

Conservation aquaculture is the use of captive propagation to sustain imperiled species or populations and preserve local characteristics in the face of severe declines. Although similar to supplementation programs in that hatchery releases occur into pre-existing natural populations, the goals of conservation and traditional supplementation hatcheries are quite different. Traditional supplementation hatcheries primarily seek to

increase the abundance of target populations, often to enhance fisheries or attain related fisheries management goals, while in conservation aquaculture programs the preservation of unique genetic diversity, phenotypes, and behaviors is of ultimate concern (Kincaid 1993; Anders 1998). Conservation aquaculture programs might be seen as a "stopgap" measure to slow population decline while restoration activities to alleviate conditions limiting natural recovery are underway (Ireland et al. 2002a, b). Traditional supplementation hatcheries, however, are often viewed as mitigation for the effects of environmental decline in and of themselves. Special techniques to minimize genetic changes in the hatchery environment are particularly emphasized in conservation aquaculture programs to minimize changes to the natural population as well as to maximize survival of captive-bred progeny.

It is well established that hatchery-induced genetic changes may include domestication, founder effects, and inbreeding. Domestication occurs through intentional or unintentional human-mediated selection or the relaxation of the natural selective regime in the hatchery environment (Waples 1999). Decreasing egg sizes in a chinook salmon (*Oncorhynchus tshawytscha*) hatchery program (Heath et al. 2003) and decreasing reproductive success of steelhead (*Oncorhynchus mykiss*) with increasing numbers of generations in captivity (Araki et al. 2007a) provide examples of how modified selection regimes may reduce fitness in the wild. A founder effect is a reduction in genetic diversity in the natural population that can occur if only small numbers of broodstock are used in captive breeding. Low levels of genetic diversity may reduce a population's ability to adapt to environmental challenges (Lacy 1997). Inbreeding depression may occur in broodstock populations of limited size. Inbreeding

depression reduces the fitness of inbred individuals, limiting their ability to contribute surviving offspring to future generations. The acquisition and handling of a sufficiently large number of broodstock is often operationally difficult or impossible.

Although it is inevitable that captive breeding will induce some genetic changes in natural populations (Waples 1999), genetic tools can be used in the adaptive management of conservation aquaculture programs to minimize these changes. Genetic monitoring allows managers to characterize baseline levels of genetic diversity in a target population which can be used to evaluate genetic diversity loss as a result of the conservation aquaculture program. Broodstock numbers, mating schemes, and release strategies then can be adaptively modified to minimize genetic diversity loss. Multilocus genotypes can be used as "genetic tags" to track broodstock used in the hatchery and returned to the wild population. Relatedness analyses (Kozfkay et al. 2008) or parentage assignment (this study) can be used to prevent the mating of close relatives when pedigree information is limited, thus reducing the likelihood of inbreeding depression.

From a genetic perspective, large fishes are particularly suited for conservation aquaculture due to their long generation time and iteroparity. Species with lengthy generation times can retain genetic diversity for long periods of time, even in the face of severe demographic declines (Quattro et al. 2002; Lippé et al. 2006; Lawrence et al. 2008; Moyer et al. 2009). Imperiled populations of large river-resident or diadromous fishes may still possess moderate to high levels of genetic diversity which can be preserved by conservation aquaculture programs utilizing sufficient numbers of broodstock. Iteroparity ensures that an individual receives multiple chances to pass genetic material to the next generation, slowing genetic diversity loss. Multi-year

stocking programs using different wild-caught adults as broodstock each year have been shown to reduce founder effects traditionally associated with hatchery programs (Heggenes et al. 2006; Drauch and Rhodes 2007; Rourke et al. 2009, 2010).

Here we describe how genetic tools have been used to guide a conservation aquaculture program for an endangered population of the white sturgeon, *Acipenser transmontanus*. The white sturgeon is considered the largest freshwater fish species in North America, currently inhabiting major river systems along the West Coast from the Sacramento River, California to the Fraser River, British Columbia. Although land-locked populations confirm that white sturgeon can complete their life cycle in freshwater, white sturgeon inhabiting the lower portions of river systems regularly use estuary and coastal habitat, suggesting they are more accurately classified as diadromous (Parsley et al. 2008). Like all North American sturgeon species, white sturgeon were subject to severe harvest pressure at the turn of the 20th century for the caviar fishery, which lead to significant population declines across the species range. White sturgeon are currently listed as endangered under the Species at Risk Act in Canada and a distinct population segment of white sturgeon in the Kootenai River has been listed as endangered by the US Fish and Wildlife Service (USFWS).

A tributary of the Columbia River, the Kootenai River runs through British Columbia, Canada, and the states of Montana and Idaho, USA (Figure 3.1). Kootenai River white sturgeon inhabit the river reach between Kootenai Falls and Bonnington Falls (Duke et al. 1999). Bonnington Falls has acted as a natural barrier isolating Kootenai River white sturgeon from other Columbia River populations for 10,000-12,000 years (Alden 1953; Northcote 1973). The Kootenai River white sturgeon population has

exhibited declines in abundance since the 1960s and almost no recruitment since 1974 (Duke et al. 1999; Paragamian et al. 2005), although natural spawning events have been observed (Ireland et al. 2002b). The population currently consists of ~1,000 individuals, primarily aging adults (Beamesderfer 2009). Numerous limiting factors have contributed to the decline of Kootenai River white sturgeon including Libby Dam, which was completed in 1975 (Duke et al. 1999). Located 42 rkm upstream of Kootenai Falls, Libby Dam has reduced downstream productivity in the Kootenai River system as well as decreased spring flows by 50% and increased winter flows by 300% (Duke et al. 1999). Other factors contributing to white sturgeon decline are diking and channelization of the Kootenai River between Bonners Ferry and Kootenay Lake, agricultural development of the river valley, and decreases in water quality from mining and industry (Duke et al. 1999; Paragamian et al. 2005).

On September 6, 1994, the Kootenai River white sturgeon population was listed as endangered by the USFWS. One component of the recovery plan for this species was to implement conservation aquaculture to sustain the population until habitat restoration could re-establish natural recruitment. Before white sturgeon were formally listed, the Kootenai Tribe of Idaho examined the feasibility of a conservation aquaculture program and made experimental releases of a small number of hatchery-reared juveniles in 1990 (Ireland et al. 2002b). Success of their initial efforts lead to the establishment of the Kootenai Tribe of Idaho (Kootenai Tribe or Tribe) white sturgeon conservation aquaculture program (CAP), which began large-scale releases in 1999. Each year, fisheries managers from the Tribe collect male and female broodstock from the Kootenai River, spawn them in the Kootenai Tribe of Idaho Tribal Hatchery, and return them to the

river. The target number of females spawned each year is 12 and often up to 20 male broodstock are captured and spawned annually (C. Lewandowski, Kootenai Tribal Hatchery, pers. comm.). While most females are mated with multiple males, each male is mated with only a single female. This creates a full-sib and maternal half-sib families. There is no mixing of milt from multiple males, and the full sibling families are reared in separate circular tanks. Some families are reared at the Kootenai Tribe of Idaho hatchery facility while others are transported to the Kootenay Trout Hatchery in British Columbia, Canada, as a failsafe measure against disease or equipment failure. Variance in reproductive success among females is common and family sizes are variable at release. Over the duration of the Tribe's CAP, captive born white sturgeon have been released from the hatcheries anywhere from the fertilized egg stage to 16 - 18 months of age, with recent releases at older ages due to the discovery of density-dependent competition among age-0 individuals post-release (Justice et al. 2009).

Goals of the Kootenai Tribe's CAP include restoration of a natural age and size structure to the Kootenai River population and maintenance of genetic diversity until habitat restoration allows natural recruitment to resume (Ireland et al. 2002b). A genetic management plan developed for the CAP called for genetic monitoring of the program to prevent genetic diversity loss and avoid inbreeding depression (Kincaid 1993). In the early 2000s, microsatellite loci were developed for genetic monitoring of Kootenai River white sturgeon (Rodzen and May 2002). Although these loci have no known adaptive significance, they can be used as monitoring tools to detect negative genetic changes induced by the CAP such as founder effects and genetic drift which tend to reduce the effective population size, N_e, of the wild population. Components of genetic monitoring

included 1) characterizing levels of genetic diversity in the Kootenai River population (Rodzen et al. 2004a; this paper), 2) documenting the amount of genetic diversity represented by Kootenai River broodstock, and 3) using parentage analysis to design mating schemes when most broodstock available to the hatchery will be of captive origin (projected to occur in 2020-2030; Paragamian et al. 2005; Beamesderfer 2009). In this paper, we use genetic monitoring of the Kootenai Tribe's CAP as a case study of how genetic tools might be applied to conservation aquaculture of other vulnerable river-resident and diadromous fish populations. We present a characterization of neutral genetic diversity in this endangered population and an assessment of how this genetic diversity is being preserved by the Tribe's CAP. We also describe the validation of microsatellite markers for parentage analysis in hatchery families of known parentage, as parentage analysis to describe family relationships between putative broodstock will necessary to prevent inbreeding in later years of the program.

METHODS

Sampling and DNA Extraction

For the characterization of genetic diversity in the Kootenai River population and Kootenai Tribe CAP broodstock, DNA was extracted from randomly sampled adults (N = 201) and those used as broodstock in the CAP since 2002 (N = 175) using a PureGene (Qiagen) extraction kit. Only those individuals spawned in the hatchery that produced surviving offspring are classified as broodstock for genetic monitoring purposes. To validate parentage assignment accuracy, DNA was extracted from 23 to 24 young of year

from each of eleven families (N = 261) belonging to the 2010 year class produced by the CAP.

Microsatellite Genotyping

Adult samples for genetic monitoring were genotyped at 14 microsatellite loci: AciG 2, AciG 35, AciG 43, AciG 52, AciG 53, AciG 110, AciG 140, As015, Atr 105, Atr 107, Atr 109, Atr 117, Atr 1101, and Atr 1173 (Table 3.1). Broodstock and YOY from the 2010 year class were genotyped at these 14 loci and four additional microsatellite loci specifically optimized to increase the power of parentage analysis (AciG 46, AciG 51, AciG 61, AciG 203; Drauch Schreier et al. 2011a). PCR conditions for Atr loci are previously described in Rodzen and May (2002). As015, AciG 2, AciG 35, AciG 43, AciG 52, AciG 53, AciG 110, and AciG 140 were amplified in reactions containing ~10 ng DNA, 1.0 µl of 10X reaction buffer, 0.2 mM of each dNTP (Promega), 1.5 – 3.0 mM $MgCl_2$, 5.0 µM each of forward (labeled) and reverse (unlabeled) primers, 0.375 U Taq polymerase (Promega GoTaq) and dH_20 to a volume of 10 µl. PCR reactions for AciG 46, 51, 61, and 203 were performed in a 10 µl total volume containing ~10 ng DNA, 1.0 µl of 1X PCR reaction buffer w/ $MgCl_2$ mix (Roche), 0.2 mM of each dNTP, 0.2 µM of each forward and reverse primer, 0.04 µM of M13 universal tailed primer, and 0.75 unit of FastStart Taq DNA polymerase (Roche).

PCR product (1.0 µl) was added to 9.0 µl of highly deionized formamide and 0.2 µl of either Life Technologies (LT) Liz 600 (AciG 46, 51, 61, and 203) or Rox 400HD (all other loci) size standards. Fragment analysis was conducted on an LT ABI 3730xl Genetic Analyzer and analyzed using GeneMapper v4.0 software. White sturgeon are an

octoploid derived species with microsatellite loci detected in four or eight copies (Drauch Schreier et al., 2011b). Since it was impossible to determine the number of copies of each microsatellite allele in this complex genome, we were unable to obtain codominant genotypes. Instead, each microsatellite allele was scored as a present/absent dominant locus, creating a binary phenotype of 1's and 0's for each individual (Rodzen and May 2002; Israel et al. 2009; Pfeiffer et al. 2011; Drauch Schreier et al., 2011b).

Data Analysis

Genetic Monitoring

Before any genetic analyses were conducted, the program GenoType (Meirmans and Tienderen 2004) was used to identify duplicate samples in the large (N = 376) genetic profile database we maintain for Kootenai River sturgeon. Preliminary quality control tests with 95 white sturgeon samples collected throughout the species range genotyped at 14 loci revealed a rate of allelic dropout (non-amplification of an allele in replicate PCR reactions) of 1.2%. Therefore two mismatches were allowed between putative duplicate samples. Fourteen pairs of samples were found to be identical and one multilocus genotype from each pair was removed to avoid any bias in genetic diversity analyses. The first task of genetic monitoring was to characterize levels of genetic diversity in the Kootenai River population as a whole. The total number of microsatellite alleles across 14 loci in the Kootenai River adult samples was calculated in the program GenAlEx version 6.3 (Peakall and Smouse 2006).

We also compared genetic diversity in the endangered Kootenai River population with other white sturgeon populations across the species range. We subsampled 100 adults from the Kootenai River dataset and compared genetic diversity levels in the

Kootenai River to those in populations from the lower Columbia River below Bonneville Dam (LCR; N = 98), the Transboundary Reach (TR; N = 101), and the Sacramento River (N = 101). GenAlEx was used to calculate the total number of alleles and number of private alleles in each population. Although a traditional measure of heterozygosity cannot be estimated in the polyploid species, we used the average number of alleles per individual per locus as a proxy for heterozygosity. We compared the average number of alleles per individual per locus at each locus among populations using a Kruskal Wallis test (VassarStats; http://faculty.vassar.edu/lowry/VassarStats.html). For loci where significant differences were detected, we used the Dunn test (VassarStats; Dunn 1964; Zar 1999) to make pairwise comparisons between the Kootenai River and each other population.

The second task of genetic monitoring was to determine whether the Kootenai Tribe's CAP was adequately representing Kootenai River genetic diversity in each year class. We initially examined the pool of broodstock used in each year (2002, 2004 - 2010) separately. Tissue samples from 2003 broodstock were unavailable for analysis. Treating each annual broodstock pool as a different "population," we used GenAlEx to calculate the total number of alleles detected in each pool and compared these values to the number of alleles detected in the total population. To determine the cumulative amount of genetic diversity captured by the Tribe's CAP, we calculated the number of alleles detected across all broodstock (2002, 2004 - 2010) and compared this value to the total number of alleles detected in the Kootenai River population.

Parentage Analysis

The log-likelihood (LOD) method of Gerber et al. (2000) was implemented in the program Parent.exe (Rodzen et al. 2004b) to assign parentage to offspring of the eleven full-sib families of known parentage containing 23 to 24 individuals each. All 18 microsatellite loci were used for parentage analysis and only 2010 male and female broodstock were included as possible parents in the analysis. To calculate LOD scores, we took the log of the likelihood that individual X is the parent of offspring A and divided it by the likelihood that individual X and offspring A are unrelated (Gerber et al. 2000). LOD scores were then summed across all loci. An individual was first assigned to the most likely sire and then to the most likely dam. To evaluate the significance of such assignments, we calculated a statistic, delta (δ), to quantify how much more likely the "best" parent was to be the true parent than was the "second best" parent identified (Marshall et al. 1998). Delta is simply the difference of the LOD scores between the most likely and second most likely parent of a given offspring. For example, a δ of 3 for a certain possible parent means that particular animal was 10^3 (= 1000) times more likely to be the true parent than the second most likely possible parent. Parentage analysis accuracy was assessed for both sires and dams with and without implementing the δ criterion.

RESULTS

Genetic Monitoring

A total of 97 alleles at 14 microsatellite loci were detected in the Kootenai River white sturgeon population. Comparison of genetic diversity among white sturgeon populations revealed over twice as much genetic diversity in the lower Columbia River,

Transboundary Reach, and Sacramento River populations compared to an equal sized subsample from the Kootenai River population (Figure 3.2). No private alleles were detected in the Kootenai River population, although 16, 9, and 26 private alleles were observed in the lower Columbia River, Transboundary Reach, and Sacramento River, respectively (Figure 3.2).

A Kruskal Wallis test to compare the mean number of alleles per locus per individual, a proxy for heterozygosity in this high order polyploid, revealed significant differences among populations at all but one locus (AciG 2; Table 3.2). A Dunn's test using the Kootenai River as a "control" to which all other populations were compared found that the Kootenai River population had significantly fewer alleles per locus per individual than the other populations at 11 of 14 loci (Table 3.2). At loci Atr 105 and Atr 1101, the Kootenai River population had significantly fewer alleles per locus per individual than one and two other populations, respectively (Table 3.2).

When single Kootenai Tribe CAP brood years are considered, between 75 – 83 out of 97 alleles are detected each year (Figure 3.4). When the genetic diversity represented in CAP broodstock was considered cumulatively across brood years 2002 and 2004-2010, 93 alleles detected in the population were represented in the broodstock. Assuming each broodstock cross produces offspring that survive after release, 96% of genetic diversity remaining in the Kootenai River population has been propagated in the Tribe's CAP.

Parentage Analysis

Parentage assignment in the 2010 year class achieved high levels of success, with 70.8 – 100% of individuals assigning to the correct sire and dam across eleven families (overall 92.8% to correct sire and 91.1% to correct dam) without using the δ criterion (Table 3.3). Assignments in families KT-D128 and KT-6FB7 were complicated by a missing genetic sample from dam KT-38E5 and the sire for family KT-523F was unknown. Tabulation of the numbers of successful assignments did not consider assignments to dams in families KTD-128 or KT-6FB7 or to sire in KT-38E5. Assignment success tended to be higher in families where genetic samples from both parents were available, as previously reported in Rodzen et al. (2004b).

A distribution of δ values for correct and incorrect assignments was plotted (Figure 3.3) and a threshold δ value of 2.5 was selected for parentage analysis. We selected this arbitrary value to minimize the number of incorrect assignments while maximizing the number of correct assignments possible. An assignment made with δ of ≥ 2.5 indicates that the "best" parent is at least 316 times ($10^{2.5}$) more likely to be the true parent than the second "best" parent. When the δ criterion of confidence was utilized as a threshold for making assignments, overall accuracy increased with 28 incorrect assignments being eliminated. Only six incorrect assignments were made with the δ threshold of 2.5, compared to 34 without implementing the δ criterion. However, 29 assignments to the correct sire and 25 to the correct dam not meet the δ criterion of confidence and thus, those assignments were not made (Table 3.3).

DISCUSSION

Very low levels of microsatellite genetic diversity in Kootenai River white sturgeon reported here corroborates previous work using other genetic marker types. Initial examinations of genetic diversity in white sturgeon with allozyme markers discovered few polymorphic loci and lower levels of heterozygosity in Kootenai River white sturgeon relative to other populations examined (Bartley et al. 1985; Setter and Brannon 1992). Examination of mitochondrial DNA control region sequence found only two haplotypes in the Kootenai River while other populations possessed four to 11 haplotypes (Anders 2002). A more recent phylogeographic study detected only one haplotype in the Kootenai River while other populations possessed four to 15 haplotypes (B. Mahardja, University of California Davis, pers. comm.).

There are several possible explanations for such low levels of genetic diversity in the Kootenai River population, none of which is mutually exclusive. First, the Kootenai River Valley was glaciated during the Pleistocene and the Kootenai River was recolonized by what was likely a small number of white sturgeon founders. Significantly lower numbers of alleles per locus per individual, a proxy for heterozygosity, in the Kootenai River population relative to other white sturgeon populations further supports a founder effect and subsequent inbreeding. Furthermore, Bonnington Falls has acted as a downstream barrier to fish movement in the Kootenai River for 10,000 – 12,000 years (Alden 1953; Northcote 1973), isolating Kootenai River white sturgeon from gene flow from conspecific populations in the Columbia and Snake Rivers. Finally, more recent demographic declines due to harvest and ecological disturbances coupled with several decades of recruitment failure have likely contributed to genetic diversity loss via genetic drift (Anders et al. 2002).

Nonetheless, genetic monitoring indicates that the Kootenai Tribe's CAP is adequately preserving the neutral genetic diversity that remains in the Kootenai River population. Since microsatellites are generally selectively neutral markers (Jarne and Lagoda 1996), we use them here as indicators of hatchery-induced genetic and demographic changes that may have deleterious effects on the wild population. Loss of neutral genetic diversity from founder effects and genetic drift may indicate a decrease in N_e and a loss of alleles of adaptive significance, which may reduce a population's ability to adapt to environmental changes over time. Representing as many adults as possible in the CAP maximizes the N_e and increases the possibility of preserving all adaptive genetic variants extant in the Kootenai River population. When broodstock genotypes are examined cumulatively, they contain nearly all alleles detected in adult white sturgeon sampled from the Kootenai River population. This study did not consider the contributions of 51 broodstock used in early experimental releases by the Tribe's CAP in 1990 – 1998 (Ireland et al. 2002a) from which genetic samples were not archived. Therefore, the proportion of genetic diversity represented by the hatchery program reported here should be considered a minimum estimate.

To date, genetic data have been collected from 362 unique Kootenai River adults, which represents ~30% of the population at large by recent estimates (Beamesderfer 2009). Although it is possible that we have not yet detected some rare alleles in the population, continued capture of novel broodstock increases the likelihood that unsampled genetic diversity will be represented in future years of the program. As the Kootenai Tribe's CAP has only been conducting large-scale releases for about a decade (1/2 sturgeon generation), it is likely that a large majority of extant Kootenai River white

sturgeon genetic diversity will be propagated by the program within a single generation (~20 years to sexual maturity in females).

Despite low levels of genetic diversity, we show that accurate parentage analysis is possible in the Kootenai River population when using 18 microsatellite loci. Previous validation experiments using fewer loci examined the feasibility of accurate parentage assignment in the Kootenai River population. Rodzen et al. (2004a) had difficulty resolving correct parentage relationships using only eight microsatellite loci. Drauch et al. (2006) found higher levels of assignment accuracy with 14 loci but few assignments were possible when the δ criterion was applied. Increasing the parentage panel to 18 microsatellite loci has provided high levels of assignment accuracy similar to those achieved in Drauch et al. (2006) as well as an increase in the number of assignments possible using the δ criterion. With the 14 marker panel, hatchery managers may have had to weigh the costs and benefits of accepting assignments not meeting the δ criterion of confidence in years when few broodstock were available. The most risk-averse mating scheme would exclude individuals for which parentage could not be resolved with confidence to reduce the chance of accidentally mating close relatives. However, additional power from increasing the marker panel to 18 loci has reduced this concern by increasing the proportion of assignments that meet the δ criterion. Implementing the δ criterion in the 18 marker panel has also alleviated concerns about the proportion of misassignments, as misidentifying close relatives as unrelated individuals could lead to inbreeding in the CAP. Only six of 452 assignments were made to an incorrect parent when implementing the δ criterion with the 18 marker panel.

Parentage analysis will become a crucial component of genetic management in the Kootenai Tribe's CAP in the early 2020's, when it is predicted that the majority of adults at large in the Kootenai River will have been born in captivity (Beamesderfer 2009). There also exists the potential for parent-offspring mating in a long-lived species such as white sturgeon. As the Tribe has archived tissue from nearly all hatchery broodstock since the initiation of large-scale releases, we will be able to use parentage analysis and the broodstock genotype archive to determine the familial relationships among captive born adults brought into the hatchery. Knowledge of familial relationships will allow hatchery personnel to avoid crosses between parent-offspring, full-siblings, and half-siblings, which is essential in a population that already exhibits a low level of genetic diversity.

Genetic markers might also be used to genetically "tag" all wild adults handled by hatchery personnel. Tag loss has been reported in shortnose sturgeon (*Acipenser brevirostrum*) marking programs and an ~6% rate of tag non-detection (either due to tag shedding or the failure of scanners to detect tags) has been observed in adult Kootenai River white sturgeon by Tribal fisheries personnel and state fisheries managers (Smith et al. 2002; D. Wakkinen, Idaho Department of Fish and Game, pers. comm.). Genetic tags provide the most accurate way to track the use of broodstock in the hatchery and prevent multiple spawning of the same individual. The maintenance of a broodstock genetic archive can allow for "family tagging" of hatchery-reared progeny too small for insertion of individual identification tags. Parentage assignment can identify the family from which an individual originated, which may be used in future studies examining family specific variance in survival of stocked individuals (Rourke et al. 2009). Field

assessments have shown that average survival in the first year after stocking is 27%, and increases to an average of 84% and near 100% at ages two and three, respectively (Justice et al. 2009). Evaluating how much genetic diversity survives the age-1 post-stocking bottleneck will provide us the most accurate assessment of genetic diversity loss in the Kootenai River population, as we are unable to calculate effective population size, N_e, using the dominant marker approach to the microsatellite dataset.

In addition to the genetic monitoring described above, genetic data can be implemented in other ways to inform conservation aquaculture programs for large river-resident and diadromous fishes. If genetic samples are available from the wild population before the initiation of stocking, a comparison of genetic diversity levels and partitioning of genetic variation in the historical and contemporary populations can allow one to evaluate genetic changes due to stocking events (e.g. Rourke et al. 2010). Genetic data also might be used to estimate the number of broodstock required to represent a target percentage of genetic diversity in hatchery-reared progeny. Rodzen et al. (2004a) used a re-sampling analysis to show that 80-90% of Kootenai River genetic diversity at eight microsatellite loci could be represented by 30-40 broodstock. This information may be used to predict how long a conservation aquaculture program may need to operate to achieve program goals. For example, if it is estimated that 200 broodstock are required to adequately represent a population's genetic diversity, the necessary duration of a conservation aquaculture program can be estimated by the predicted availability of broodstock in each year.

This manuscript has focused on the use of neutral genetic markers to inform hatchery programs, as less is known about changes to adaptive genetic diversity in

captivity which might occur through domestication. Chebanov et al. (2002) describes how selection of early spawning females for a hatchery program for Azov Sea stellate (*Acipenser stellatus*) and Russian sturgeon (*Acipenser gueldenstaedtii*) has reduced the temporal extant of spawning in the wild populations in the course of just 15 years. However, various hatchery practices might be adopted to reduce the risk of domestication, such as the random selection of different wild born adults as broodstock each year and equalizing family sizes to reduce overrepresentation of individuals better adapted to captive conditions (Araki et al. 2007b; Frankham 2008). The KTOI-CAP reduces domestication risks by randomly sampling wild broodstock from many locations in the Kootenai River throughout several months leading up to the spawning season. The program also avoids representing the same individuals in multiple brood years; only a single female broodstock has been spawned more than once in the twelve years the large scale conservation aquaculture program has been in operation.

The identity and magnitude of selective pressures driving adaptation to captivity are difficult to quantify and likely will vary in different captive breeding programs (Allendorf et al. 2010). Similarly, adaptation to similar selective pressures may differ among populations of the same species due to differences in genomic architecture (Frankham 2008). Advances in the burgeoning field of conservation genomics may provide the capability to monitor conservation aquaculture programs for changes in allele frequency at multiple adaptive genes throughout the genome (Allendorf et al. 2010). The complex nature of the polyploid white sturgeon genome will likely present serious technical challenges to such genomic studies, as has been seen with previous genomic studies of sturgeons.

Although there are uncertainties surrounding the long-term effects of conservation aquaculture that should be addressed by future research, this technique remains an important tool for the conservation of large river-resident and diadromous fishes. When deciding whether to implement a conservation aquaculture program for an imperiled population, it is important to consider the alternative to reproductive intervention. In a critically endangered population such as the Kootenai River white sturgeon with no natural recruitment, choosing not to implement a conservation aquaculture program will result in continued decline in population size. Not implementing such a program has consequences such as irreversible genetic diversity loss, lowered reproduction and survival due to Allee effects, and an increased likelihood that a stochastic event(s) may lead to extinction (Anders 1998; Ireland et al. 2002a). By preserving local genetic diversity and preventing further population size declines, the benefits of conservation aquaculture may outweigh the risks, particularly when negative side effects are minimized through careful planning and genetic monitoring.

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Table 3.1. Microsatellites used for genetic monitoring and parentage assignment in the Kootenai Tribe's CAP. TD1 and TD2 refer to touchdown thermal profiles. The first step of TD1 contains a T_A decrease (T_A = 65 ^oC to T_A = 58 ^oC, -0.5^oC/cycle), and the second step has a constant T_A (54^oC). Both steps of TD2 contain T_A decreases: 1) T_A = 68^oC to T_A = 58 ^oC, -2^oC/cycle, and 2) T_A = 58^oC to T_A = 50^oC, -2^oC/cycle.

Microsatellite	**$MgCl_2$ (mM)**	**T_A (^oC)**	**Annealing Time (s)**	**Reference**
Atr 105	1.6	TD1	60, 60	Rodzen and May (2002)
Atr 107	1.6	TD1	60, 60	Rodzen and May (2002)
Atr 109	2.1	TD1	60, 60	Rodzen and May (2002)
Atr 117	3.1	TD1	60, 60	Rodzen and May (2002)
Atr 1101	1.8	57	30	Rodzen and May (2002)
Atr 1173	1.3	TD1	60, 60	Rodzen and May (2002)
As015	2.5	56	30	Zhu et al (2005)
AciG 2	1.5	60	30	Börk et al. (2008)
AciG 35	3.0	56	30	Börk et al. (2008)
AciG 43	2.0	56	30	Genbank: HM459582[a]
AciG 52	2.0	56	105	Börk et al. (2008)
AciG 53	2.0	56	105	Börk et al. (2008)
AciG 110	2.0	56	30	Börk et al. (2008)
AciG 140	2.5	56	30	Börk et al. (2008)
AciG 46	2.0	TD2	300, 120	Börk et al. (2008)
AciG 51	2.0	TD2	300, 120	Börk et al. (2008)
AciG 61	2.0	TD2	300, 120	Börk et al. (2008)
AciG 203	2.0	TD2	300, 120	Börk et al. (2008)

[a]Forward primer sequence: TAATACAGCGGGGATGGAA
Reverse primer sequence: GCACAGTGAAAGCACGGTAA

Table 3.2. Average number of alleles per individual per locus in the Kootenai River (KT), Lower Columbia River (LCR), Transboundary Reach (TR), and Sacramento River (SAC). P values are from a Kruskal Wallis test indicating significant differences among groups. Asterisks indicate where the number of alleles per individual per locus is significantly lower than LCR, TR, and SAC using Dunn's test (P = 0.05).

	Population				
Locus	KT (N=100)	LCR (N=98)	TR (N=100)	SAC (N=101)	P
AciG 2	2.46	2.29	2.46	2.28	0.1223
AciG 35	4.85*	5.81	5.78	5.74	< 0.0001
AciG 43	3.96*	5.46	4.75	5.25	< 0.0001
AciG 52	4.58*	5.52	5.51	5.15	< 0.0001
AciG 53	1.80*	2.32	2.18	2.29	< 0.0001
AciG 110	3.80*	4.71	4.51	4.95	< 0.0001
AciG 140	1.73*	2.49	2.36	2.11	< 0.0001
As015	3.02*	5.33	4.60	4.96	< 0.0001
Atr 105	1.42[a]	2.63	2.85	2.35	< 0.0001
Atr 107	3.25*	4.56	4.32	4.25	< 0.0001
Atr 109	2.64*	3.99	3.58	4.05	< 0.0001
Atr 117	2.75*	4.05	3.70	4.16	< 0.0001
Atr 1101	2.03[†]	2.69	2.18	2.71	< 0.0001
Atr 1173	2.49*	3.41	3.45	3.33	< 0.0001

[a]Sig diff from TR only

[†]Sig diff from SAC and LCR

Table 3.3. Parentage assignment accuracy using 24 possible parents of the 2010 year class and 18 microsatellite loci (additional loci AciG 46, AciG 51, AciG 61, and AciG 201). The proportion of individuals assigned correctly to each parent with and without the δ criterion is shown. Numbers in parentheses are the numbers of offspring which assigned to the correct parent but with a δ value below the threshold.

	Assignments without δ		**Assignments with δ**	
Family	**Correct Sire**	**Correct Dam**	**Correct Sire**	**Correct Dam**
KT-D128	24/24	NA[a]	24/24 (0)	NA[a]
KT-6FB7	19/24	NA[a]	12/24 (7)	NA[a]
KT-OC72	24/24	24/24	23/24 (1)	24/24 (0)
KT-6CD2	22/23	22/23	19/23 (3)	18/23 (4)
KT-3F5C	22/24	23/24	18/24 (4)	19/24 (4)
KT-6F60	22/23	22/23	20/23 (3)	23/23 (0)
KT-54D0	21/24	24/24	17/24 (4)	21/24 (3)
KT-E401	23/23	17/23	19/23 (4)	14/23 (3)
KT-1193	24/24	22/24	24/24 (0)	21/22 (1)
KT-523F	NA[b]	17/24	NA[b]	9/17 (8)
KT-8749	20/24	24/24	16/24 (4)	22/24 (2)

[a]No genetic sample from dam KT-38E5 is available.
[b]Sire is unknown.

Figure 3.1. Range of white sturgeon, *Acipenser transmontanus*, in the Kootenai River Basin. Map courtesy of Ray Beamesderfer.

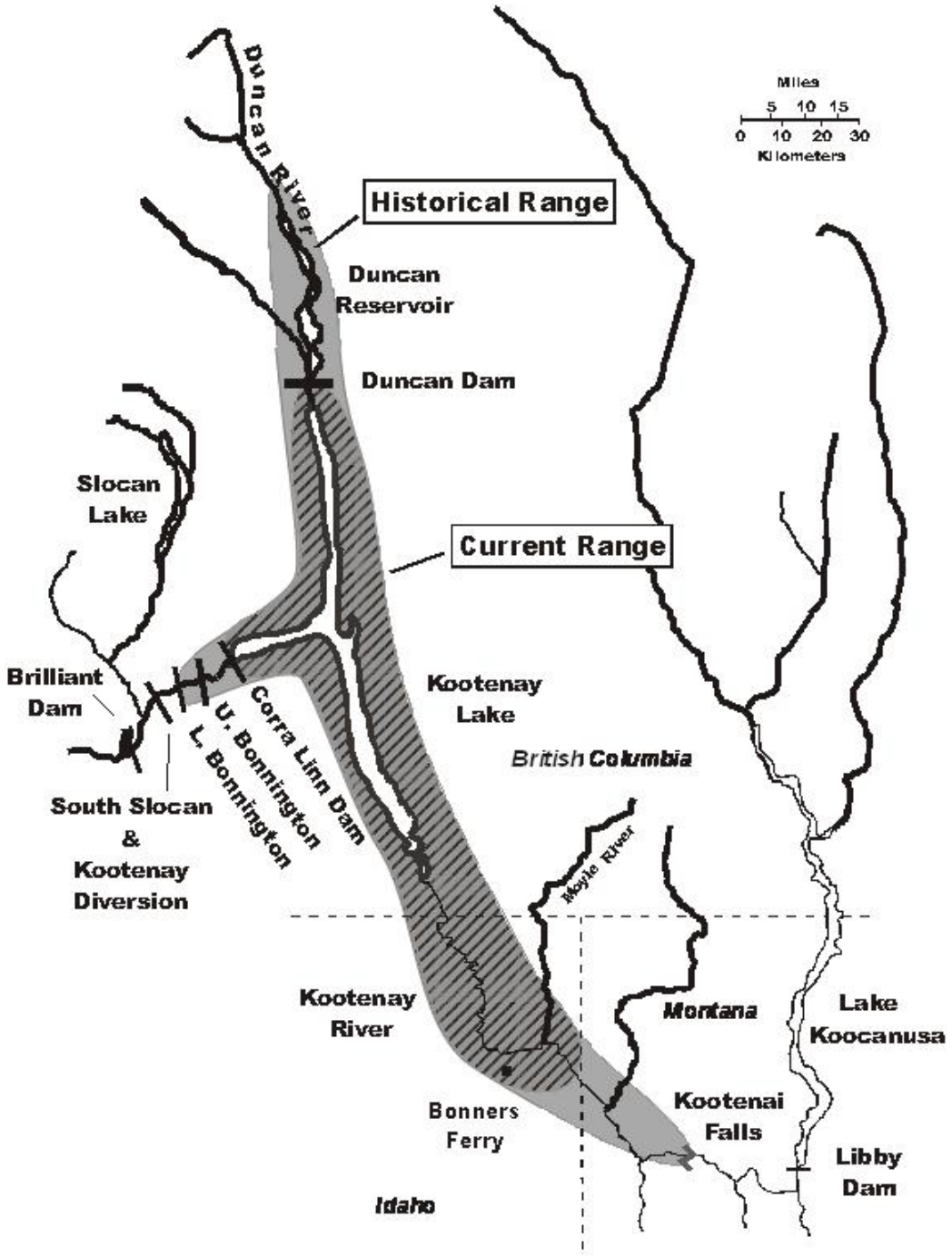

Figure 3.2. Total number of alleles and private alleles detected in a subsample of the Kootenai River dataset (KT; N = 100) and samples collected from the Lower Columbia River (LCR; N = 98), Transboundary Reach (TR; N = 100), and Sacramento River (SAC; N = 101).

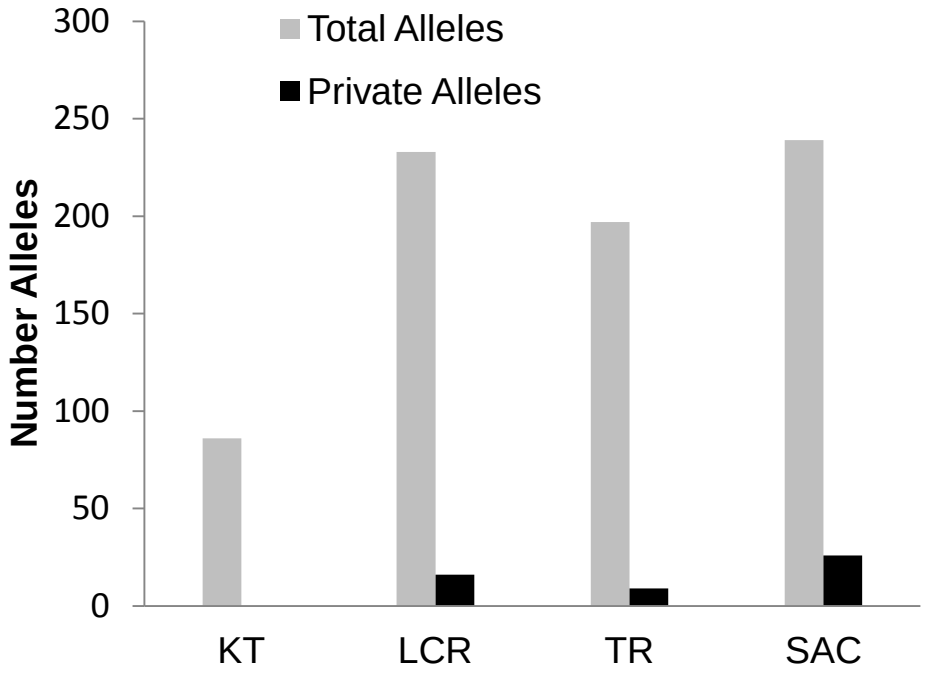

Figure 3.3. Distribution of δ values for correct and incorrect assignments in the Kootenai Tribe's CAP 2010 year class. A vertical dotted line indicates the selected δ value of 2.5.

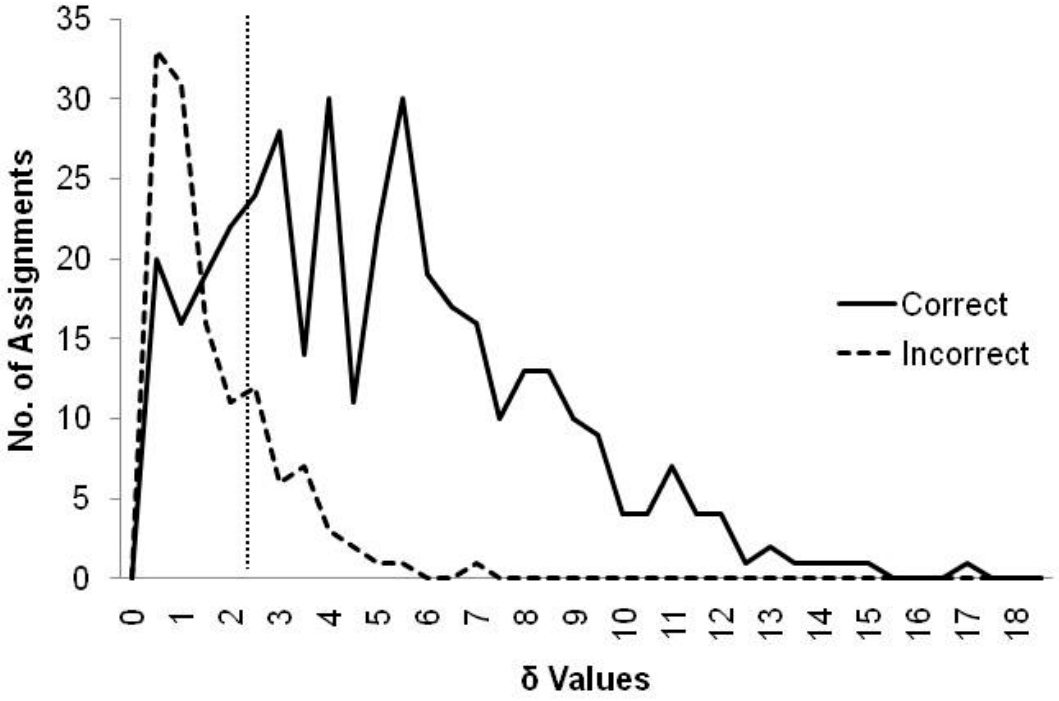

Figure 3.4. Proportion of Kootenai River population microsatellite alleles represented by the Kootenai Tribe's CAP broodstock from 2002, 2004-2010. 'Total' refers to the proportion of population alleles that were captured by broodstock over all years of the program for which genetic monitoring was conducted.