

Hierarchical patterns of population structure in the endangered Fraser River white sturgeon (*Acipenser transmontanus*) and implications for conservation

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Abstract: The Fraser River system consists of five white sturgeon (*Acipenser transmontanus*) management units, two of which are listed as endangered populations under Canada's Species at Risk Act. The delineation of these management units was based primarily on population genetic analysis with samples parsed by collection location. We used polysomic microsatellite markers to examine population structure in the Fraser River system with samples parsed by collection location and with a genetic clustering algorithm. Strong levels of genetic divergence were revealed above and below Hells Gate, a narrowing of the Fraser canyon further obstructed by a rockslide in 1913. Additional analyses revealed population substructure on the Fraser River above Hells Gate. The Middle Fraser River (SG-3) and Nechako River were found to be distinct populations, while the Upper Fraser River, although currently listed as an endangered population, represented a mixing area for white sturgeon originating from SG-3 and Nechako. Differences between these results and previous genetic investigations may be attributed to the detection of population mixing when genetic clustering is used to infer population structure.

Résumé : Le système du fleuve Fraser consiste en cinq secteurs de protection de l'esturgeon blanc (*Acipenser transmontanus*), dont deux comprenant des populations figurant sur la liste des populations en voie de disparition en vertu de la *Loi sur les espèces en péril* du Canada. La délimitation de ces secteurs de protection a principalement reposé sur l'analyse génétique des populations d'échantillons groupés selon le lieu d'échantillonnage. Nous avons utilisé des marqueurs microsatellites polysomiques pour examiner la structure des populations dans le système du fleuve Fraser sur la base d'échantillons groupés selon le lieu d'échantillonnage et à l'aide d'un algorithme de groupement génétique. Des degrés élevés de divergence génétique ont été relevés en amont et en aval de Hells Gate, une section étroite du canyon du Fraser obstruée de surcroît par un éboulement rocheux survenu en 1913. D'autres analyses ont révélé une sous-structure des populations dans le fleuve Fraser en amont de Hells Gate. Ainsi, le moyen Fraser (SG-3) et la rivière Nechako renferment des populations distinctes, alors que la population du haut Fraser, bien que figurant sur la liste des populations en voie de disparition, représente une zone de mélange d'esturgeons blancs issus du SG-3 et de la Nechako. Les différences entre ces résultats et ceux d'études génétiques antérieures peuvent être attribuées à la détection du mélange de populations rendue possible par l'utilisation du groupement génétique pour établir la structure des populations.

[Traduit par la Rédaction]

Introduction

The white sturgeon (*Acipenser transmontanus*) is the largest freshwater fish in North America, with spawning populations in the Sacramento, Columbia, Snake, and Fraser River systems. White sturgeon are long-lived and late maturing, with reports of individuals >80 years of age and females reaching sexual maturity as late as at 18–34 years in the Fraser River system (Scott and Crossman 1973; COSEWIC 2003). Although white sturgeon in the Sacramento–San Joaquin, Lower Columbia, and Lower Fraser systems may access the ocean

and are known to utilize marine habitat (Chadwick 1955; Galbreath 1985; Veinott et al. 1999), white sturgeon do not require salt water to complete their life cycle, and several land-locked populations (e.g., Kootenai River) exist. In the late spring and early summer, Fraser River adult white sturgeon make upstream migrations to spawning grounds (COSEWIC 2003). White sturgeon are iteroparous broadcast spawners, with males spawning every 2–5 years and females spawning every 3–8 years in the Fraser River (Semakula and Larkin 1968).

Six populations of white sturgeon are recognized in Canada, and four of these are listed as endangered under the federal

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Species at Risk Act: the Upper Columbia, Kootenay, Nechako, and Upper Fraser river populations (COSEWIC 2003; Fisheries and Oceans Canada 2007). Current threats to white sturgeon in Canada include habitat modification due to impoundment, development of riparian and (or) floodplain areas, decreased water quality, bycatch in salmonid fisheries, and poaching (COSEWIC 2003; Fisheries and Oceans Canada 2007). The Upper Columbia and Nechako River populations are characterized by ongoing recruitment failure, which constitutes a major threat to their persistence (Hildebrand et al. 1999; Nechako White Sturgeon Recovery Initiative 2004).

White sturgeon abundance in the Fraser River declined precipitously at the turn of the 20th century because of overharvest for flesh and caviar (Lane 1991; Echols 1995). Bycatch in salmonid fisheries was likely another large source of mortality for Fraser River white sturgeon throughout much of the 20th century (Echols 1995). Recent population estimates suggest the number of adult white sturgeon inhabiting the Fraser River is 11 000, with a majority (75%) of these found in the Lower Fraser River (Fisheries and Oceans Canada 2007). Much research has focused on population assessment and recovery planning in the endangered Nechako and Upper Fraser River populations (e.g., Golder Associates, Ltd. 2006; Lheidli T'enneh First Nations 2009; Triton Environmental Consultants Ltd. 2010). However, success of white sturgeon restoration efforts in the Fraser River depends upon the correct delineation populations.

Smith et al. (2002) designated four populations in the Fraser River, based largely on an examination of population structure using data from the mitochondrial control region and four microsatellites. These are the Lower Fraser River (below Hells Gate), Middle Fraser River (Hells Gate to river kilometre (rkm) 553), Nechako River, and Upper Fraser River (from above the Nechako confluence to McBride). Hells Gate is a narrowing of the Fraser canyon at rkm 211 (COSEWIC 2003) that was further obstructed by a rockslide in 1913. These authors distinguished populations based primarily on the mtDNA control region data, as data from the four disomic microsatellites provided little resolution of population structure because of low levels of polymorphism (Smith et al. 2002). Currently the Lower Fraser River is managed as two separate units, SG-1 (rkm 0 to 153) and SG-2 (rkm 154 to 211) based on an earlier analysis of the Smith et al. (2002) data set and demographic data (Nelson et al. 1999; McKenzie 2000). However, no study has examined population structure on the Fraser River using highly polymorphic polysomic microsatellite markers recently developed for white sturgeon (Börk et al. 2008).

The use of a microsatellite data set allows for the inference of population structure without relying on sample location information to a priori parse samples for analysis. Traditional analyses used to infer population structure, such as F_{ST} or analysis of molecular variation (AMOVA), are sensitive to the manner in which samples are defined for analysis. Collections of samples often are defined for analysis by the geographic region in which they were collected. This approach can be problematic in long-lived species with high dispersal capabilities such as sturgeon, where individuals from multiple populations may inhabit the same geographic region during nonreproductive times (Dugo et al. 2004; Bott et al. 2009; Israel et al. 2009). Spurious signals of population structure

might be generated if samples from multiple spawning populations are contained in a single collection for analysis. Genetic clustering programs such as STRUCTURE (Pritchard et al. 2000) allow for the inference of population structure from genetic data alone, reducing the risk of erroneously identifying population mixtures as distinct populations.

Here, we use 13 polysomic microsatellite loci to examine fine-scale population structure in the Fraser River system. We reveal strong levels of genetic divergence above and below Hells Gate. A hierarchical examination of population structure below and above Hells Gate suggests that only three distinct populations inhabit the Fraser River system, which has implications for management and recovery efforts.

Materials and methods

DNA extraction and genotyping

Fin clips were collected from subadult and adult white sturgeon sampled throughout the Fraser River system (Fig. 1; Table 1). DNA was extracted from tissue using the Qiagen PureGene DNA extraction kit and quantified on a FLA 5100 fluorimeter (Fujifilm). Polymerase chain reaction (PCR) was performed in Life Technologies (LT) GeneAmp 9700 thermal cyclers using fluorescently labeled primers for 13 microsatellite loci: AciG 2, AciG 35, AciG 52, AciG 53, AciG 110, AciG 140, As 015, Atr 105, Atr 107, Atr 109, Atr 117, Atr 1101, Atr 1173 (Rodzen and May 2002; Zhu et al. 2005; Börk et al. 2008). PCR conditions and thermal profiles for amplification of these loci have been published previously (Drauch Schreier et al. 2012). All samples were genotyped on an ABI 3730 Genetic Analyzer (LT). A total of 1.0 μ L of diluted PCR product was added to 8.85 μ L of highly deionized formamide (The Gel Company) and 0.15 μ L of Rox 400 HD size standard (LT). GeneMapper v4.0 software (LT) was used for allele scoring.

Belonging to sturgeon ploidy group B, white sturgeon are ancient octoploids, possessing ~250 chromosomes (Birstein et al. 1993; van Eenennaam et al. 1998; Drauch Schreier et al. 2011). The highly duplicated nature of the white sturgeon nuclear genome precluded the scoring of allele dosages. Therefore, we treated each microsatellite allele as a present or absent dominant locus, which created a binary allelic phenotype of ones and zeros for each individual (Rodzen and May 2002; Drauch Schreier et al. 2011; Pfeiffer et al. 2011).

Data analysis

Genetic diversity

Previous work with large genotype data sets has shown that in heavily sampled populations of long-lived organisms such as sturgeon, multiple tissue samples may be collected from the same individual over time (A. Drauch Schreier, unpublished data). Therefore, we used the program GenoType (Meirmans and van Tienderen 2004) to look for duplicate samples in the Fraser River white sturgeon data set. The rate of allelic dropout at the 13 microsatellite loci was estimated by re-genotyping (in triplicate) 95 samples of multiple ages and tissue types collected from several locations across the species' range on the ABI 3730xl (LT). This is similar to the "multiple tube approach" recommended by Taberlet and Luikart (1999) to quantify allelic dropout in genotyping non-invasively collected DNA samples. The replicate genotyping

Fig. 1. Map of Fraser River system and regions from which white sturgeon samples were collected. SG-1, Lower Fraser management unit located rkm 0–153; SG-2, Lower Fraser management unit located rkm 154–211; SG-3, Middle Fraser River management unit; UFR, Upper Fraser River; NKO, Nechako River; SL, Stuart Lake; FL, Fraser Lake.

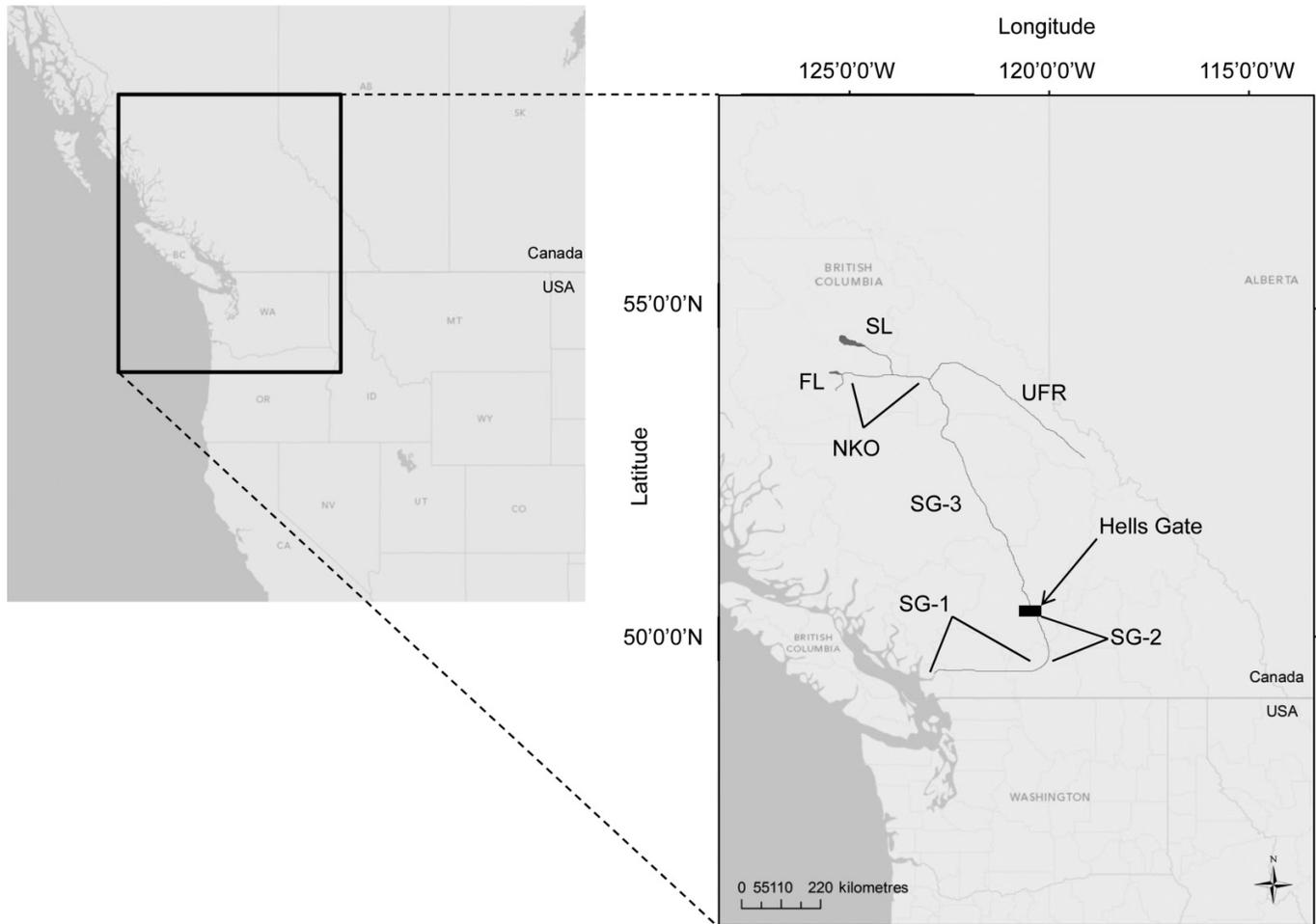


Table 1. Total number of alleles (A_T), private alleles (A_P), and Shannon's index (H') calculated across 13 microsatellites for each of seven regions sampled in the Fraser River system.

Region	N	A_T	A_P	H'
SG-1	38	182	19	18.37
SG-2	38	177	14	17.34
SG-3	40	104	0	10.68
NKO	50	122	2	13.15
SL	23	119	0	12.91
FL	13	104	0	12.11
UFR	47	110	0	11.83

Note: N indicates sample size for each region; SG-1, Lower Fraser management unit located rkm 0–153; SG-2, Lower Fraser management unit located rkm 154–211; SG-3, Middle Fraser River management unit; NKO, Nechako River; SL, Stuart Lake; FL, Fraser Lake; UFR, Upper Fraser River.

we conducted revealed an allelic dropout rate of 1.2%, and therefore up to two mismatches were allowed in identifying duplicate samples (204 total alleles \times 1.2%). We used GenAlEx version 6.3 (Peakall and Smouse 2006) to calculate the total

number of alleles and number of private alleles, or alleles unique to a particular region, in each collection as defined by geographic sampling location. As unequal sample sizes may bias estimates of genetic diversity, we also calculated Shannon's diversity index (H') in the program FAMD (Schlüter and Harris 2006) to examine genetic diversity levels in each collection. Monomorphic loci were removed, and the calculation was conducted with log base 10 and band presences divided by N .

Population structure analysis

We wished to compare patterns of population structure revealed when samples were parsed by geographic criteria or by genetic criteria in a genetic clustering algorithm. We conducted an analysis of molecular variation (AMOVA) in GenAlEx version 6.3 to examine the proportion of genetic diversity partitioned among collections defined by geographic criteria (Table 1). Random permutations were conducted (9999) to assess the significance of Phi-PT (Peakall et al. 1995), an analogue of F_{ST} more appropriate for dominant data that can provide a measure of pairwise genetic divergence. 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 sampling locations. A PCO is an ordination method that utilizes a distance matrix (in this case a genetic distance matrix) to illustrate similarities between cases.

We used the genetic clustering program STRUCTURE version 2.3.3 (Pritchard et al. 2000) to explore the number of possible populations (K) existing within the Fraser River system. Initial exploratory analyses of the full data set used a relatively short burn-in (50 000) and a small number of iterations (100 000) to test the likelihood of $K = 1$ to $K = 20$. We conducted longer analyses (burn-in 500 000; 1 000 000 iterations) for the most likely K values revealed by the exploratory analyses (Pritchard et al. 2010). For the full Fraser River data set, we examined $K = 1$ to $K = 10$, and for analyses of substructure both above and below Hells Gate, we examined $K = 1$ to $K = 4$. The admixture model was used and correlated allele frequencies were assumed for each STRUCTURE analysis. Six replicates were conducted for each K . The LOCPRIOR model (Hubisz et al. 2009) was employed when sampling location information was found to be informative by the program to improve the STRUCTURE's ability to identify the true K in the presence of weak genetic differentiation. This approach allows geographic information to be incorporated into the analysis only if it is found to be correlated to genetic clustering results (Hubisz et al. 2009).

We first examined the mean likelihood value ($\ln \Pr(X|K)$) for each possible number of populations in the program STRUCTURE HARVESTER (Earl and vonHoldt 2011). We selected the value of K with the highest likelihood as the best estimate of the number of populations in the Fraser River. As this method has a tendency to overestimate K when analyzing dominant data (Pritchard et al. 2010), we also examined the STRUCTURE output using the metric of ΔK (Evanno et al. 2005) in STRUCTURE HARVESTER. Individual Q values were examined to inform selection of the most likely K when multiple K values seemed equally likely from examination of the likelihood function and ΔK . In this analysis, a Q value referred to an individual's proportional ancestry in each genetic cluster. The program CLUMPP (Jakobsson and Rosenberg 2007) was used to compile individual assignments across all six replicates for the most likely K . Individual Q values were plotted for visualization in DISTRUCT 1.1 (Rosenberg 2004). The partitioning of genetic diversity was then re-examined in GenAlEx version 6.3 and FAMM as described above using population delineations based on genetic clustering results. A Bonferroni correction was conducted to account for multiple pairwise comparisons in the pairwise Phi-PT analysis.

We conducted additional STRUCTURE analyses to evaluate the possibility of cryptic population substructure above and below Hells Gate. We used exploratory analyses to test the likelihood of $K = 1$ to $K = 4$ and $K = 1$ to $K = 3$, respectively. Full STRUCTURE analyses were then conducted for the most likely K values ($K = 1$ to $K = 4$) in the manner described above for analysis of the full data set. The program NTSys-PC 2.2 (Rohlf 2009) was used to calculate a Jaccard similarity matrix from samples collected above Hells Gate and to conduct a PCO to visualize those data.

Results

Genetic diversity

No duplicate samples were identified in the Fraser River data set. Across 13 microsatellite loci, a total of 204 alleles was detected in the Fraser River. The greatest numbers of alleles and private alleles were found in regions below Hells Gate (Table 1). The lowest level of genetic diversity was detected in Fraser Lake, which was also the region with the smallest sample size (Table 1). Shannon's index was highest below Hells Gate (in SG-1 and SG-2) and lowest in the Middle Fraser (SG-3) (Table 1).

Population structure analysis

The global Phi-PT value when samples were parsed by geographic sampling location was 0.051 ($P = 0.0001$), which indicated that 5% of genetic diversity was partitioned among regions while 95% was partitioned within regions. Pairwise Phi-PT analyses revealed significant genetic divergence among all regions with the exceptions of the Nechako River, Stuart Lake, and Fraser Lake as well as the comparison between Fraser Lake and the Upper Fraser River (Table 2). The PCO revealed that 54.48% and 37.04% of variance was explained by the first two axes (Fig. 2). The PCO also illustrated a close relationship among the Nechako River, Stuart Lake, and Fraser Lake samples (Fig. 2).

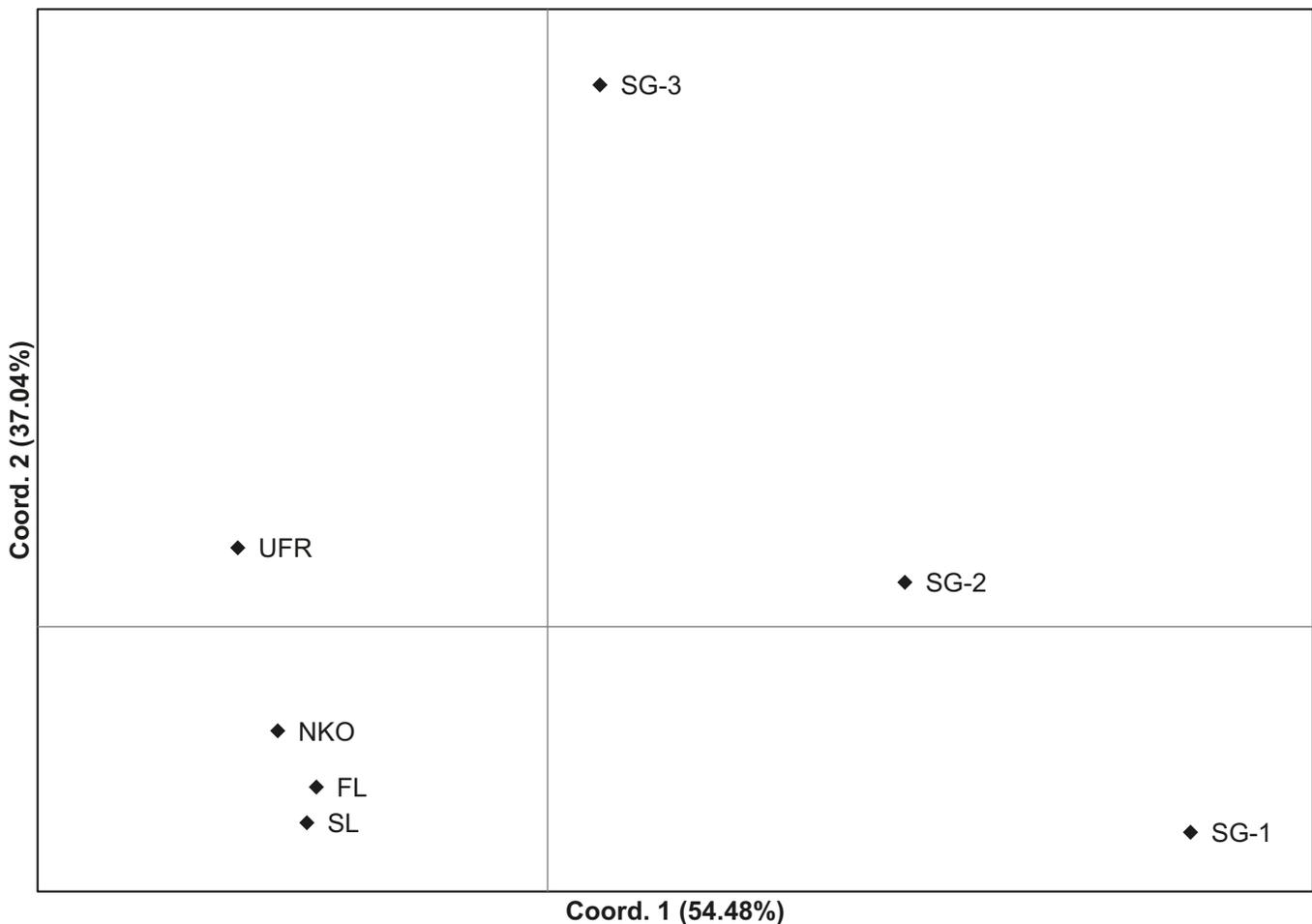
Geographic sampling labels were found to be informative in elucidating population structure in the Fraser River data set, and therefore the LOCPRIOR model was applied by STRUCTURE. Initial examinations of $\ln \Pr(X|K)$ (Fig. 3) and ΔK suggested that the most likely number of genetic clusters in the Fraser River system was three. However, Q values indicated that this was likely an overestimation of population structure. When two clusters were assumed ($K = 2$), STRUCTURE clearly identified two distinct groups where all individuals inherited a high proportion of their genome from one lineage or the other (mean Q values = 0.99 and 0.93, respectively). When three putative clusters were tested, individuals primarily showing ancestry in the cluster 1 lineage now were evenly divided between clusters 1 and 3 (blue and yellow lineages in Fig. 4b). For example, when $K = 2$ was assumed, individual X showed a high proportion of ancestry in cluster 1 with a membership coefficient of 0.95, but when $K = 3$ was assumed, individual X 's ancestry was evenly divided between clusters 1 and 3, each with a membership coefficient of ~ 0.50 (Fig. 4b). No individual showed a high proportion of ancestry in the cluster 3 lineage.

When $K = 2$ was assumed, the two genetic clusters 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 (Fig. 4a). The Fraser River above Hells Gate cluster included individuals sampled in the Middle Fraser (SG-3), Upper Fraser, and Nechako Rivers, as well as Stuart Lake and Fraser Lake. Some individuals from the Lower Fraser sampled just below Hells Gate (SG-2) showed a high proportion of ancestry in the Fraser River above Hells Gate, although no individuals sampled above Hells Gate showed ancestry in the Lower Fraser cluster (Fig. 4a). 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 popula-

Table 2. Pairwise genetic divergence among regions sampled in the Fraser River system.

	SG-1	SG-2	SG-3	NKO	SL	FL	UFR
SG-1		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
SG-2	0.020*		0.0001	0.0001	0.0001	0.0010	0.0001
SG-3	0.105*	0.043*		0.0001	0.0001	0.0001	0.0001
NKO	0.081*	0.040*	0.062*		0.4730	0.4730	0.0018
SL	0.077*	0.044*	0.076*	0.000		0.4790	0.0004
FL	0.073*	0.040*	0.070*	0.000	0.000		0.0480
UFR	0.100*	0.051*	0.051*	0.013*	0.024*	0.016	

Note: Phi-PT values are below the diagonal, and *P* values are above the diagonal. Significant values are indicated with an asterisk. SG-1, Lower Fraser management unit located rkm 0–153; SG-2, Lower Fraser management unit located rkm 154–211; SG-3, Middle Fraser River management unit; NKO, Nechako River; SL, Stuart Lake; FL, Fraser Lake; UFR, Upper Fraser River.

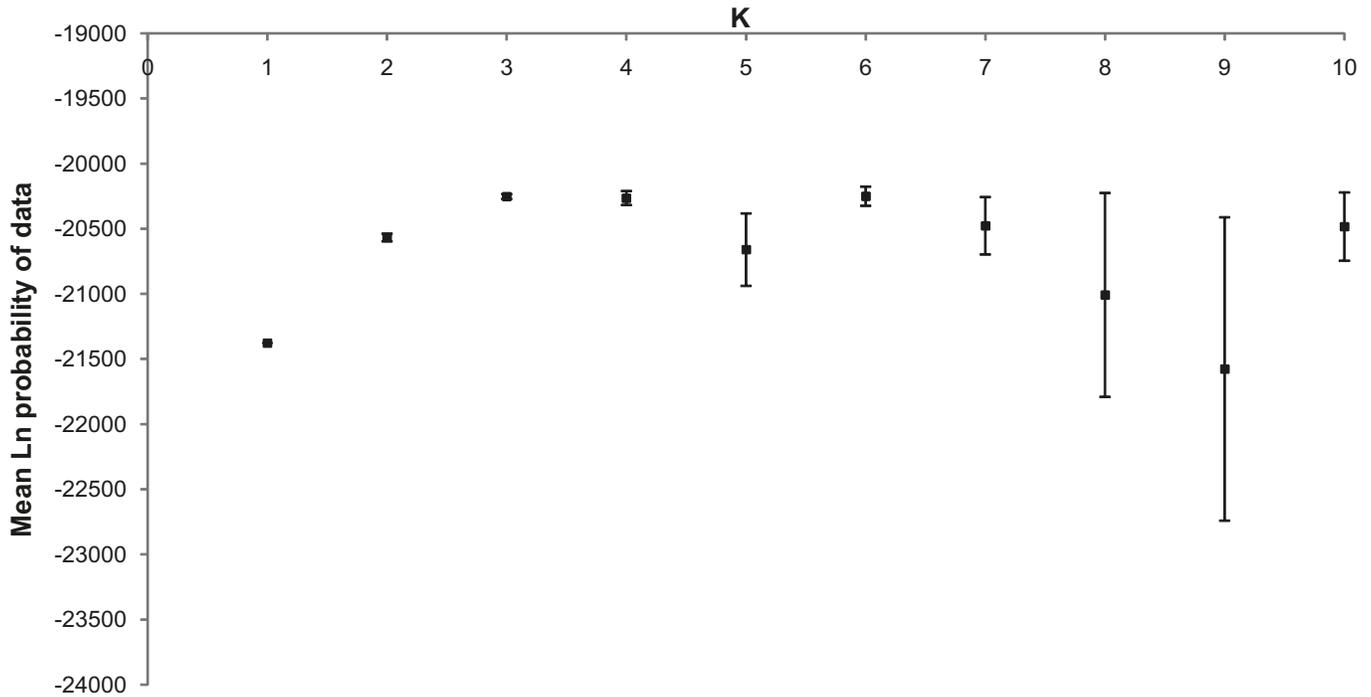
Fig. 2. Principal coordinates analysis illustrating genetic relationships among white sturgeon inhabiting different regions of the Fraser River.

tion substructure in the reaches below and above Hells Gate, respectively. Individuals sampled in SG-2 that showed ancestry in the Fraser River above Hells Gate were added to that collection for substructure analyses. 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 (Fig. 5). In both scenarios, individuals sampled in SG-2 that showed ancestry in the Fraser River above Hells Gate cluster appeared to originate specifically in SG-3, the Middle Fraser River (Figs. 6a, 6b).

If it was assumed there were two clusters above Hells Gate ($K = 2$), SG-3 was a genetically distinct population (mean $Q = 0.99$), and the majority of individuals sampled in the

Fig. 3. Mean Ln Pr($X|K$) values for STRUCTURE analysis of population structure within the entire Fraser River system. Vertical lines denote standard deviation.



Nechako River, Stuart Lake, and Fraser Lake showed ancestry in a second population (mean Q values = 0.94, 0.98, 0.93, respectively; Fig. 6a). Two individuals sampled in the Nechako River and two sampled in Fraser Lake showed greater ancestry in the SG-3 population (mean Q values = 0.72, 0.79, respectively; Fig. 6a), which may be indicative of migration or historical admixture. The Upper Fraser River consisted of a mixture of individuals with ancestry in the SG-3 and Nechako–Stuart Lake–Fraser Lake populations (mean Q values = 0.86, 0.88, respectively). Several individuals in the Upper Fraser River had intermediate levels of ancestry in the SG-3 and Nechako–Stuart Lake–Fraser Lake populations, which suggested that some admixture may be occurring between these populations (Fig. 6a).

When $K = 3$ is assumed above Hells Gate, the SG-3 collection remained a genetically distinct population (mean $Q = 0.97$), and most individuals from the Nechako River, Stuart Lake, and Fraser Lake still showed ancestry primarily in a second distinct population (mean Q values = 0.91, 0.94, 0.74, respectively; Fig. 6b). The Upper Fraser River still consisted of a mixture of individuals showing ancestry primarily in one of three different clusters. The third cluster (represented in green in Fig. 6b) contained only 15 individuals, but Q values were high (mean $Q = 0.89$; Fig. 6b). One individual from the Nechako River showed ancestry in nearly equal proportions to the SG-3 and this third cluster, while an individual from the Upper Fraser River showed nearly equal ancestry to the SG-3 and Nechako–Stuart Lake–Fraser Lake population, suggesting the possibility of some admixture (Fig. 6b). The PCO analysis showed distinction among all three genetic clusters, with the third cluster falling out as most divergent (Fig. 7). The two putative admixed individuals sam-

pled in the Nechako and Upper Fraser were located intermediately between the two populations to which they showed nearly equal proportions of ancestry (Fig. 7).

A recalculation of genetic diversity metrics for the populations inferred from genetic clustering analysis (Lower Fraser, SG-3, Nechako–Stuart Lake–Fraser Lake, third Upper Fraser population) revealed high levels of genetic diversity and a very high number of private alleles in the Lower Fraser below Hells Gate (Table 3). When Phi-PT was recalculated using the STRUCTURE groupings, the amount of genetic variance partitioned among populations increased to 9% ($P = 0.0001$). Pairwise Phi-PT values showed similar relationships among populations as the Phi-PT analysis based on regional groupings (Table 4). Significant levels of genetic divergence were found among all populations identified by STRUCTURE (Bonferroni-adjusted $\alpha = 0.008$; Table 4).

Discussion

Our analysis of genetic diversity and population structure in Fraser River white sturgeon revealed strong levels of genetic divergence above and below Hells Gate. Additional population substructure was revealed in the Fraser River above Hells Gate, although no substructure was identified in the Lower Fraser below Hells Gate when genetic clustering was used. These results differ from previous investigations of white sturgeon population structure and may have implications for management and recovery planning.

High levels of genetic divergence above and below Hells Gate on the Fraser River are supported by three types of analyses. First, the great disparity in genetic diversity between populations located above and below Hells Gate and the high

Fig. 4. Bar histogram from STRUCTURE depicting individual assignments in the entire Fraser River system. 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. (a) $K = 2$, (b) $K = 3$. The location of Hells Gate on the Fraser River is indicated. SG-1, Lower Fraser management unit located rkm 0–153; SG-2, Lower Fraser management unit located rkm 154–211; SG-3, Middle Fraser River management unit; NKO, Nechako River; SL, Stuart Lake; FL, Fraser Lake; UFR, Upper Fraser River.

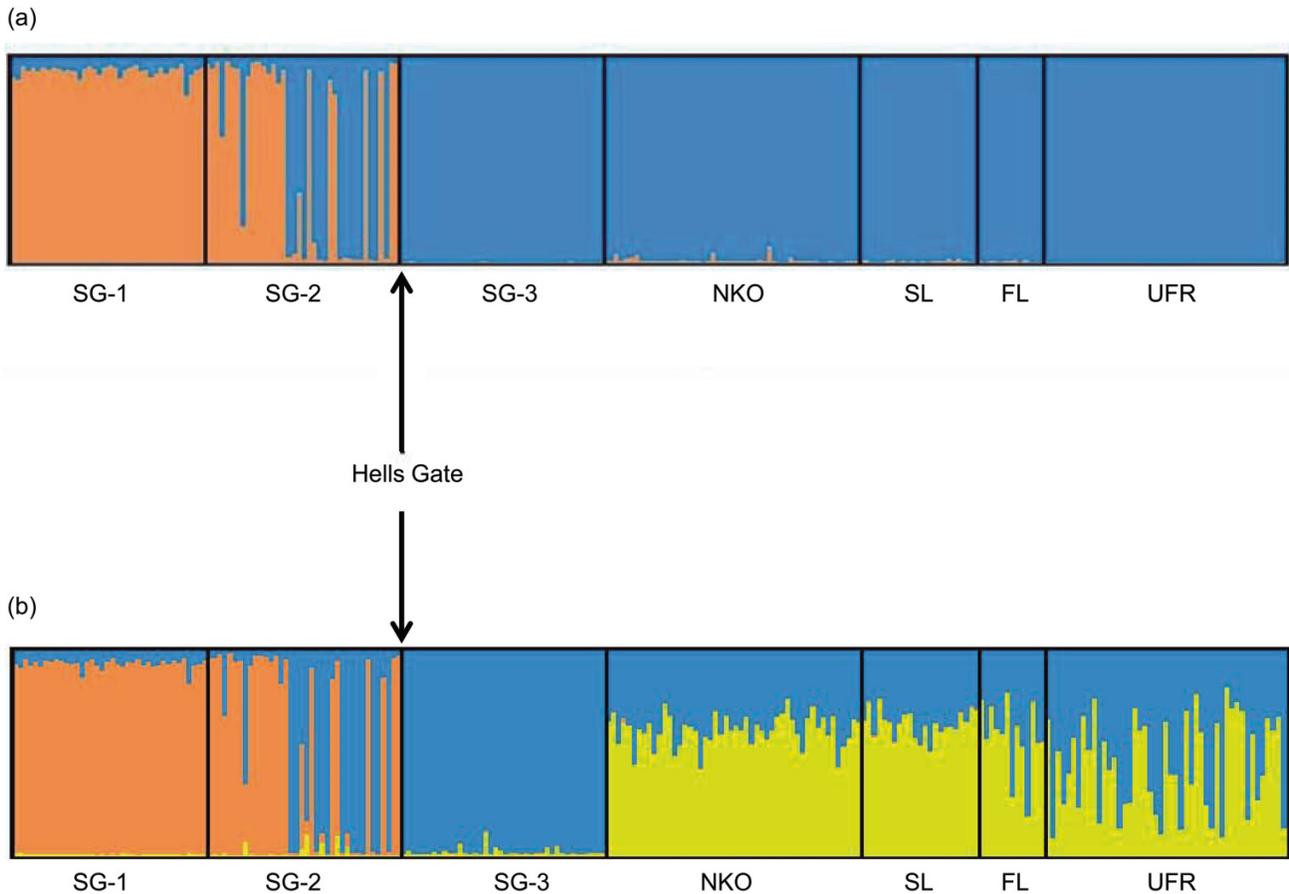


Fig. 5. Mean $\ln \Pr(X|K)$ values for STRUCTURE analysis of population structure within the Fraser River above Hells Gate. Vertical lines denote standard deviation.

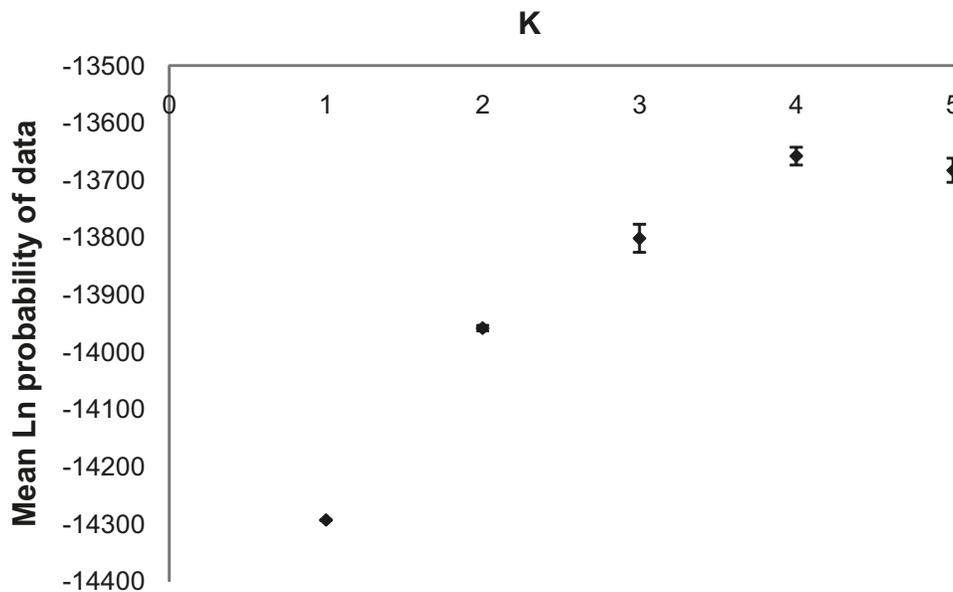
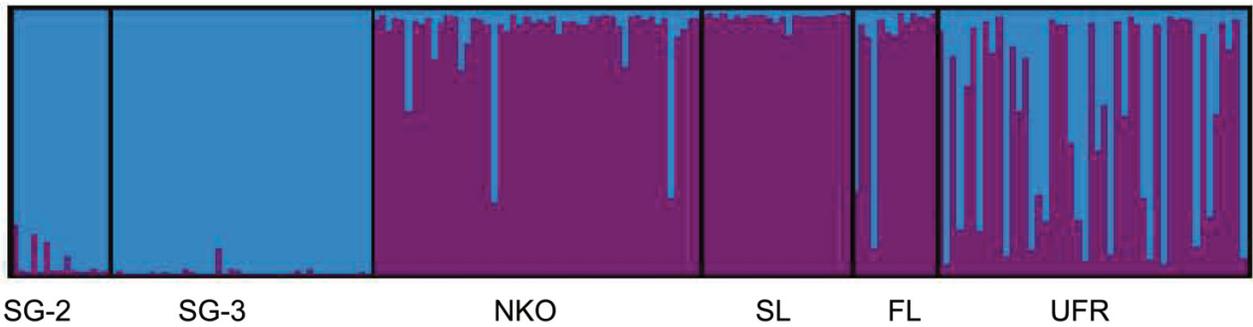


Fig. 6. Bar histogram outputs from STRUCTURE depicting individual assignments in the Fraser River above Hells Gate. Individuals sampled in SG-2 but had ancestry in the Fraser River above Hells Gate were included in this analysis. Each bar represents one individual genome, each color represents a population 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) $K = 3$. SG-2, individuals sampled in Lower Fraser management unit located rkm 154–211; SG-3, Middle Fraser River management unit; NKO, Nechako River; SL, Stuart Lake; FL, Fraser Lake; UFR, Upper Fraser River.

(a)



(b)

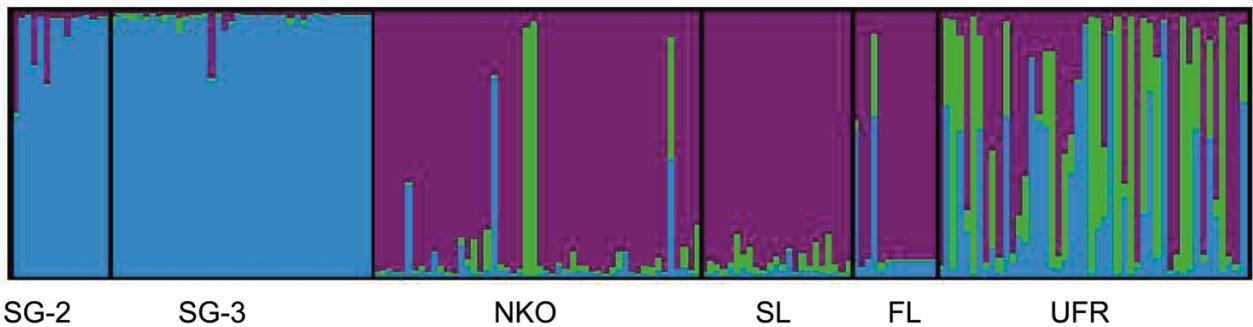
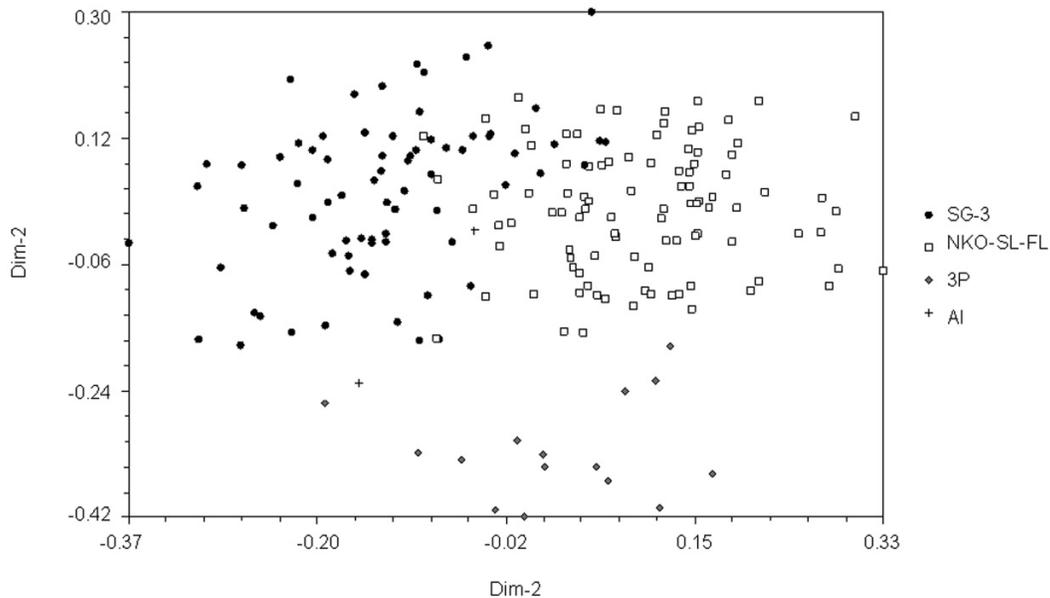


Fig. 7. Principal coordinate analysis of a Jaccard similarity index calculated for the Upper Fraser River above Hells Gate. SG-3, cluster containing Middle Fraser River and some Nechako and Upper Fraser River samples; NKO–SL–FL, cluster containing Nechako River, Stuart Lake, and Fraser Lake, and some Upper Fraser River samples; and UFC3, third cluster detected above Hells Gate containing 15 individuals sampled in the Nechako River, Fraser Lake, and Upper Fraser River; AI, putative admixed individuals.



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Table 3. Total number of alleles (A_T) and private alleles (A_P) detected at 13 microsatellites for populations detected by genetic clustering analysis of the Fraser River system.

Population	N	A_T	A_P	H'
LFR	60	198	72	19.05
SG-3	74	110	2	11.24
NKO-SL-FL	99	126	4	13.08
UFC3	15	81	0	7.996

Note: N indicates sample size for each cluster; LFR, Lower Fraser River (SG-1 and SG-2); SG-3, cluster containing Middle Fraser River and some Nechako River and Upper Fraser River samples; NKO-SL-FL, cluster containing Nechako River, Stuart Lake, and Fraser Lake, and some Upper Fraser River samples; and UFC3, third cluster detected above Hells Gate containing 15 individuals sampled in the Nechako River, Fraser Lake, and Upper Fraser River.

Table 4. Genetic divergence between Fraser River populations as identified by genetic clustering analysis.

	LFR	SG-3	NKO-SL-FL	UFC3
LFR		0.0001	0.0001	0.0001
SG-3	0.099		0.0001	0.0001
NKO-SL-FL	0.081	0.061		0.0001
UFC3	0.165	0.165	0.124	

Note: Phi-PT values are below the diagonal, and P values are above the diagonal. LFR, Lower Fraser River (SG-1 and SG-2); SG-3, cluster containing Middle Fraser River and some Nechako River and Upper Fraser River samples; NKO-SL-FL, cluster containing Nechako River, Stuart Lake, and Fraser Lake, and some Upper Fraser River samples; and UFC3, third cluster detected above Hells Gate containing 15 individuals sampled in the Nechako River, Fraser Lake, and Upper Fraser River. All values were significant.

number of private alleles (72) in the Lower Fraser River support genetic isolation between the two regions. Second, pairwise Phi-PT analysis of sampling locations revealed higher levels of genetic divergence in comparisons between SG-1 and the Fraser River above Hells Gate and between SG-2 and the Fraser River above Hells Gate than between SG-1 and SG-2. The low but significant level of genetic differentiation between SG-1 and SG-2 may be due to the presence of individuals originating from the Middle Fraser River in the SG-2 collection. Finally, when examining the entire Fraser River system, STRUCTURE identified two distinct genetic clusters corresponding to the regions below and above Hells Gate, with little evidence of admixture between them.

Similar patterns of strong genetic differentiation among fish populations above and below Hells Gate have been observed in several salmonid species (coho salmon (*Oncorhynchus kisutch*), Small et al. 1998; Chinook salmon (*Oncorhynchus tshawytscha*), Teel et al. 2000; sockeye salmon (*Oncorhynchus nerka*), Withler et al. 2000). Withler et al. (2000) found that the magnitude of genetic divergence in pairwise comparisons between sockeye salmon populations above and below Hells Gate was independent of the geographic distance between them, although isolation by distance was observed among populations located either above or below the barrier. Small et al. (1998) proposed that an ice dam remaining in the Fraser canyon after glacial recession may have extended genetic isolation between Lower and Upper

Fraser fish populations even after postglacial 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 the Lower Fraser River and the Fraser River above Hells Gate is that there was a second unknown postglacial recolonization source for the Lower Fraser (McCusker et al. 2000; Teel et al. 2000; Withler et al. 2000).

Low levels of genetic diversity in the Fraser River above Hells Gate relative to the Lower Fraser River and the high number of private alleles in the Lower Fraser also may be evidence of different recolonization routes for above and below the Fraser canyon. The Fraser River above Hells Gate 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 River was likely recolonized via migration of individuals along the continental shelf, from the Lower Columbia (Brown et al. 1992) and possibly one other source population. The second possible source population for recolonization of the Lower Fraser is unknown, although some authors have proposed a Beringian refugium (Teel et al. 2000) and other coastal refugia (Vancouver Island, Queen Charlotte Islands; McCusker et al. 2000) for Lower Fraser salmonid populations. Given that white sturgeon have been captured in marine waters along the Alaskan coast and long-distance marine dispersal has been documented for this species (Galbreath 1985; Welch et al. 2006; Ruiz-Campos et al. 2011), use of Beringian or other coastal refugia seems plausible. A preliminary investigation of mtDNA control region haplotype frequencies across the species' range has shown that the Lower Fraser River population possesses high haplotype diversity as well as a nucleotide diversity level an order of magnitude greater than that of any other white sturgeon population (B. Mahardja, unpublished data). High nucleotide diversity in a recently recolonized population suggests that it is composed of multiple haplotype lineages (Provan and Bennett 2008), providing further support for the hypothesis that two recolonization sources contributed to the Lower Fraser River population.

Higher levels of genetic diversity in the Lower Fraser River relative to the Fraser River above Hells Gate may be due in part to net downstream gene flow over Hells Gate and (or) contemporary gene flow from other white sturgeon populations with access to marine habitat, such as those in the Lower Columbia River and the Sacramento-San Joaquin River system. Differences in genetic diversity levels above and below Hells Gate may also be exacerbated by small population size 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 Fraser River above Hells Gate (McKenzie 2000; Yarmish and Toth 2002). An accelerated rate of genetic drift in the smaller populations inhabiting the Fraser River above Hells Gate relative to the larger Lower Fraser River population may result in lower levels of genetic diversity in the former.

The occurrence of individuals sampled in the Lower Fraser River originating from above Hells Gate corroborates field data documenting the movement of white sturgeon over this barrier (McKenzie 2000; E. Stoddard, Ministry of Forests, Lands, and Natural Resource Operations, #200 – 10428 153rd Street, Surrey, BC V5W 1K4, Canada, personal communica-

tion, 2012). Downstream movement of other species over Hells Gate has been observed (McPhail and Lindsay 1986). Two scenarios can be proposed to explain the absence of individuals sampled above Hells Gate that exhibit ancestry from the Lower Fraser population. The first is that Hells Gate is a contemporary upstream barrier that is impassible by white sturgeon. An alternative explanation is that Hells Gate is passable by sturgeon, and individuals from the SG-3 population may move upstream and downstream over Hells Gate on feeding migrations (E. Stoddard, Ministry of Forests, Lands, and Natural Resource Operations, #200 –10428 153rd Street, Surrey, BC V5W 1K4, Canada, personal communication, 2012). The few individuals sampled below Hells Gate exhibiting mixed ancestry, resulting from mating between Lower Fraser River individuals and migrants originating from above Hells Gate, further supports the second scenario. If Hells Gate is no longer a barrier to both upstream and downstream white sturgeon migration, the little evidence of admixture between the Lower Fraser and SG-3 populations may be due to fairly strong spawning site fidelity at the scale of river reach in the Fraser River. Regional spawning site fidelity, different recolonization sources, and historical isolation (e.g., proposed ice dam in the Fraser canyon; Small et al. 1998) all may have acted to create the signal of strong genetic divergence we observe above and below Hells Gate.

Our analysis of population substructure in the Fraser River below the Hells Gate barrier concurs with that of Smith et al. (2002), who suggested that the Lower Fraser River below Hells Gate be considered a single biogeographic unit. Although low but significant levels of genetic divergence were revealed between the SG-1 and SG-2 samples with pairwise Phi-PT analysis, no population substructure was detected in the region when migrants from the SG-3 population, revealed by genetic clustering analysis, were removed from the SG-2 collection.

The analysis of population substructure in the Fraser River above Hells Gate suggests that within that genetic cluster there are at least two genetically distinct spawning populations: one corresponding to SG-3 (Middle Fraser River; Hells Gate to rkm 553) and another corresponding to the Nechako River system. No genetic differentiation was detected between the Nechako River and Stuart Lake or Fraser Lake, suggesting that individuals sampled in the Stuart River – Stuart Lake and Fraser Lake either (i) use the same spawning site as Nechako River white sturgeon and (or) (ii) use different spawning sites but a high level of gene flow occurs between them. Only a single spawning site has been characterized on the Nechako River thus far, at Vanderhoof, British Columbia (Triton Environmental Consultants Ltd. 2009, 2010). A small conservation aquaculture program did operate in the Nechako River from 2005 to 2009 to mitigate for recruitment failure, but this program used wild captured broodstock from the Vanderhoof spawning location and is unlikely to have had any effects on patterns of population structure above Hells Gate.

The Upper Fraser River appears to be a mixing area for individuals originating from the SG-3 or Nechako River populations. The mixing of nonreproductive subadult and adult sturgeon from different spawning populations has been documented in many different species (Gulf sturgeon (*Acipenser oxyrinchus desotoi*), Dugo et al. 2004; lake sturgeon (*Acipenser fulvescens*), Bott et al. 2009; green sturgeon (*Acipenser medirostris*), Israel et al. 2009). Yarmish and Toth

(2002) have suggested that the 80 km reach of the Middle Fraser River south of the Nechako–Fraser confluence consists of poor white sturgeon habitat (shallow, poorly defied thalweg, few eddies) and may present a dispersal barrier. These authors also suggested a 60 km region of the Lower Nechako River contained similarly unsuitable habitat that may prevent movement of Nechako River white sturgeon into the Fraser River. Efforts to capture sturgeon in these areas have been largely unsuccessful (McKenzie 2000; Yarmish and Toth 2002), which suggests that sturgeon may not continually inhabit these regions. However, the presence of several individuals showing a high proportion of ancestry in SG-3 in the Nechako River and Fraser Lake and the detection of Nechako and SG-3 individuals in the Upper Fraser River suggests that some exchange of individuals is occurring or has occurred historically across these putative barriers. In addition, field data has also identified seasonal movements of white sturgeon through these suboptimal habitats (C. Williamson, Facility Development Manager, Nechako White Sturgeon Conservation Centre, 4051 – 18th Avenue, Prince George, BC, V2N 1B3, Canada, personal communication, 2012). This is further confirmed by recent radio-tracking studies that have revealed some movement of white sturgeon between SG-3 and the Upper Fraser River and the Nechako River (Golder Associates, Ltd. 2006; Lheidli T'enneh First Nations 2008, 2009); future research is required to better characterize habitat use and spawning behavior in the Fraser River above Hells Gate.

It is uncertain whether two or three populations exist in the Fraser River above Hells Gate. STRUCTURE analyses revealed two scenarios with similar likelihoods, indicating that two or three populations in the Fraser River above Hells Gate are possible. The smallest cluster revealed in the $K = 3$ STRUCTURE scenario may be a distinct spawning population or a family group. All but two individuals showing ancestry in this small cluster possessed allele 237 at locus Atr 117, an allele found in few other individuals in the Fraser River above Hells Gate, which supports the hypothesis that this small cluster is a family group. However, PCO of the similarity index matrix calculated for the individuals in the Fraser River above Hells Gate did not show the small cluster to be a tightly clustered group, which is what would be expected for closely related individuals. Additionally, the PCO remains unchanged if data from allele 237 is removed. The detection of individuals that were tagged in the Upper Fraser River in the Nechako River the following winter that exited the Nechako during the spawning season may be evidence of an Upper Fraser River spawning population (C. Williamson, Facility Development Manager, Nechako White Sturgeon Conservation Centre, 4051 – 18th Avenue, Prince George, BC V2N 1B3, Canada, personal communication, 2012). Future study of spawning behavior and spawning site selection in the Fraser River above Hells Gate will help us to better infer the number of populations inhabiting this region.

Our results illustrate the importance of examining population structure with a variety of methods, including those that analyze samples without regard to sampling location. Nelson et al. (1999) concluded that the Lower Fraser River contained two populations and that the Upper Fraser River was a distinct population based on deviations from Hardy Weinberg equilibrium in samples that were parsed by collection location. Similarly, Smith et al. (2002) upheld the designation of the Upper

Fraser River as a distinct population with samples parsed by collection location alone. We also found low but significant levels of population structure between SG-1 and SG-2 on the Lower Fraser River and between the Upper Fraser River and all other regions when analysis was conducted using sampling location to parse samples. However, the inference of population structure using only genetic data revealed that the region designated SG-2 (rkm 154–211) and the Upper Fraser River are actually mixing areas for two or more distinct populations. Population mixtures would result in deviations from Hardy Weinberg equilibrium, and spurious signals of population structure may be inferred without a further examination of the data with a method that uses only genetic data for inference. STRUCTURE (Pritchard et al. 2000) is only one genetic clustering program that infers population structure from genetic data. Other genetic clustering programs such as BAPS2 (Corander et al. 2004) and GeneClass2 (Piry et al. 2004), as well as individual-based genetic distance measures such as similarity indices, will infer genetic relationships among individuals without regard to the location in which they were sampled.

Conservation implications

These genetic data support the delineation of three populations for conservation management in the Fraser River system: the Lower Fraser River below Hells Gate, SG-3 (Middle Fraser River), and the Nechako River. SG-1 and SG-2 do not appear to be genetically distinct populations, although SG-2 appears to contain individuals that have migrated downstream from the SG-3 population. Little sign of admixture between SG-3 and Lower Fraser River fish was detected with this data set. However, the likely detection of downstream migration over Hells Gate in another data set (Nelson et al. 1999) suggests that downstream movement may be common.

Although listed as an endangered population under the Species at Risk Act (Fisheries and Oceans Canada 2007), the Upper Fraser River appears to be a mixing area for the listed Nechako River population and unlisted SG-3 population. It is possible that the small population detected by genetic clustering analysis represents an “Upper Fraser” spawning group, but additional field research is required to corroborate this conclusion. One spawning site has been identified on the Nechako River (Triton Environmental Consultants Ltd. 2009, 2010), but to our knowledge spawning sites have not been characterized on the Middle or Upper Fraser River. The detection of many individuals showing ancestry in the Nechako River population in the Upper Fraser River suggests that the Upper Fraser may contain important habitat for the endangered Nechako population. Reports by the Lheidli T’enneh First Nations (2008, 2009) identify the Grand Canyon area and confluence of the McGregor River in the Upper Fraser as holding areas, and these may represent important overwintering areas for Nechako River white sturgeon. Movement into the Upper Fraser River may also represent a feeding migration by the SG-3 and Nechako River populations following salmonid spawning runs, as salmonids likely represent an important component of the adult white sturgeon diet in this region (McKenzie 2000). Although the Upper Fraser River may not contain a distinct population that warrants listing and protection, the importance of the Upper Fraser River habitat to the listed Nechako River population should be further evaluated.

Low levels of genetic diversity in the Fraser River above Hells Gate may be a reflection of the genetic diversity levels of the postglacial recolonization source or possibly genetic diversity loss due to genetic drift, particularly in the recruitment-limited Nechako River population. Preservation of remaining genetic diversity should be a conservation goal for the Fraser River above Hells Gate, particularly for the recruitment-limited Nechako River population. Continuation of a conservation aquaculture program may represent an important tool for conservation of the Nechako River population to prevent further genetic diversity loss due to mortality while biologists work to restore habitat so that natural recruitment can resume (Anders 1998).

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References

- Anders, P.J. 1998. Conservation aquaculture and endangered species: can objective science prevail over risk anxiety? *Fisheries* (Bethesda, Md.), **23**: 28–31.
- Birstein, V.J., Poletae, A., and Goncharov, B.F. 1993. DNA content in Eurasian sturgeon species determined by flow cytometry. *Cytometry*, **14**(4): 377–383. doi:10.1002/cyto.990140406. PMID:8513694.
- Börk, K.B., Drauch, A.M., Israel, J.A., Pedroia, J.K., Rodzen, J., and May, B.P. 2008. Development of new microsatellite DNA primers for green and white sturgeon. *Conserv. Genet.* **9**(4): 973–979. doi:10.1007/s10592-007-9417-9.
- Bott, K., Kornely, G.W., Donofrio, M.C., Elliott, R.F., and Scribner, K.T. 2009. Mixed-stock analysis of lake sturgeon in the Menominee River sport harvest and adjoining waters of Lake Michigan. *N. Am. J. Fish. Manage.* **29**(6): 1636–1643. doi:10.1577/M08-211.1.
- Brown, J.R., Beckenbach, A.T., and Smith, M.J. 1992. Influence of Pleistocene glaciations and human intervention upon mitochondrial DNA diversity in white sturgeon (*Acipenser transmontanus*) populations. *Can. J. Fish. Aquat. Sci.* **49**(2): 358–367. doi:10.1139/f92-041.
- Chadwick, H.K. 1955. California sturgeon tagging studies. *Calif. Fish Game*, **45**(4): 297–301.
- Corander, J., Waldmann, P., Marttinen, P., and Sillanpää, M.J. 2004. BAPS 2: enhanced possibilities for the analysis of genetic population structure. *Bioinformatics*, **20**(15): 2363–2369. doi:10.1093/bioinformatics/bth250. PMID:15073024.
- COSEWIC. 2003. COSEWIC assessment and update status report on the white sturgeon *Acipenser transmontanus* in Canada [online]. Report by the Committee on the Status of Endangered Wildlife in Canada, Ottawa, Canada. Available from <http://publications.gc.ca/collections/Collection/CW69-14-353-2004E.pdf> [accessed 12 July 2012].

- Fisheries and Oceans Canada. 2007. Recovery potential assessment for white sturgeon [online]. DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2007/014. Available from http://publications.gc.ca/collections/collection_2011/mpo-dfo/Fs70-6-2007-014-eng.pdf [accessed 12 July 2012].
- Drauch Schreier, A., Gille, D., Mahardja, B., and May, B. 2011. Neutral markers confirm the octoploid origin and reveal spontaneous autopolyploidy in white sturgeon, *Acipenser transmontanus*. *J. Appl. Ichthyology*, **27**(Suppl. S2): 24–33. doi:10.1111/j.1439-0426.2011.01873.x.
- Drauch Schreier, A., Rodzen, J., Ireland, S., and May, B. 2012. Genetic techniques inform conservation aquaculture of the endangered Kootenai River white sturgeon, *Acipenser transmontanus*. *Endanger. Species Res.* **16**(1): 65–75. doi:10.3354/esr00387.
- Dugo, M.A., Kreiser, B.R., Ross, S.T., Slack, W.T., Heise, R.J., and Bowen, B.W. 2004. Conservation and management implications of fine-scale genetic structure of Gulf sturgeon in the Pascagoula River, Mississippi. *J. Appl. Ichthyol.* **20**(4): 243–251. doi:10.1111/j.1439-0426.2004.00572.x.
- Earl, D.A., and vonHoldt, B.M. 2011. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv. Genet. Resour.* **4**(2): 359–361.
- Echols, J.C. 1995. Fraser River white sturgeon long-term management objectives, strategies, and uncertainties. M.Sc. thesis, The University of British Columbia, Vancouver, B.C. Lib. Arch. Can. AMICUS No. 23560925.
- Evanno, G., Regnaut, S., and Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol.* **14**(8): 2611–2620. doi:10.1111/j.1365-294X.2005.02553.x. PMID:15969739.
- Galbreath, J.L. 1985. Status, life history, and management of Columbia River white sturgeon, *Acipenser transmontanus*. In *North American sturgeons*. Edited by F.P. Binkowski and S.I. Doroshov. Dr. W. Junk Publishers, Dordrecht, the Netherlands. pp. 119–125.
- Golder Associates, Ltd. 2006. Nechako River white sturgeon tagging project — 2005 data report [online]. Report to the British Columbia Ministry of Environment, Prince George, British Columbia. Available from http://nechakowhitesturgeon.org/uploads/files/Nechako_WST_Tagging_Project_2005_Final_Report_.pdf [accessed 12 July 2012].
- Hildebrand, L., McLeod, C., and McKenzie, S. 1999. Status and management of white sturgeon in the Columbia River in British Columbia, Canada: an overview. *J. Appl. Ichthyol.* **15** (1): 164–172.
- Hubisz, M.J., Falush, D., Stephens, M., and Pritchard, J.K. 2009. Inferring weak population structure with the assistance of sample group information. *Mol. Ecol. Resour.* **9**(5): 1322–1332. doi:10.1111/j.1755-0998.2009.02591.x. PMID:21564903.
- Israel, J.A., Bando, J.K., Anderson, E.C., and May, B. 2009. Polyploid microsatellite data reveal stock complexity among estuarine North American green sturgeon (*Acipenser medirostris*). *Can. J. Fish. Aquat. Sci.* **66**(9): 1491–1504. doi:10.1139/F09-091.
- Jakobsson, M., and Rosenberg, N.A. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, **23**(14): 1801–1806. doi:10.1093/bioinformatics/btm233. PMID:17485429.
- Lane, E.D. 1991. Status of the white sturgeon, *Acipenser transmontanus*, in Canada. *Can. Field Nat.* **105**(2): 161–168.
- Lheidli T'enneh First Nations. 2008. 2007 Assessment of Upper Fraser River white sturgeon; critical habitat identification, population assessment and capacity development [online]. Report prepared for the Aboriginal Funds for Species at Risk Program, Report No. 2007AFSAR1270. Available from http://nechakowhitesturgeon.org/uploads/files/2007_CH_Assessment_CSTC.pdf [accessed 12 July 2012].
- Lheidli T'enneh First Nations. 2009. 2008 Assessment of Upper Fraser River white sturgeon; critical habitat identification, population assessment and capacity development [online]. Report prepared for the Aboriginal Funds for Species at Risk Program, Report No. 2008AFSAR1090. Available from <http://nechakowhitesturgeon.org/uploads/files/2008AFSAR1090.pdf> [accessed 12 July 2012].
- McCusker, M.R., Parkinson, E., and Taylor, E.B. 2000. Mitochondrial DNA variation in rainbow trout (*Oncorhynchus mykiss*) across its native range: testing biogeographical hypotheses and their relevance to conservation. *Mol. Ecol.* **9**(12): 2089–2108. doi:10.1046/j.1365-294X.2000.01121.x. PMID:11123621.
- McKenzie, S. 2000. Fraser River white sturgeon monitoring program — comprehensive report (1995–1999) [online]. Report to the British Columbia Ministry of Water, Land, and Air Protection. RL&L Report No. 815F. Available from http://www.env.gov.bc.ca/wld/fishhabitats/pdf/White_sturgeon_Fraser.pdf [accessed 12 July 2012].
- McPhail, J.D., and Lindsay, C.C. 1986. Zoogeography of the freshwater fishes of Cascadia (the Columbia system and rivers north to the Stikine). In *Zoogeography of North American freshwater fish*. Edited by C.E. Hocutt and E.O. Wiley. John Wiley & Sons, Inc., Hoboken, New Jersey. pp. 615–637.
- Meirmans, P.G., and van Tienderen, P.H. 2004. GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. *Mol. Ecol. Notes*, **4**(4): 792–794. doi:10.1111/j.1471-8286.2004.00770.x.
- Nechako White Sturgeon Recovery Initiative. 2004. Recovery plan for Nechako River white sturgeon [online]. Report prepared by Golder Associates, Ltd. for the Nechako White Sturgeon Recovery Initiative. Available from <http://nechakowhitesturgeon.org/uploads/files/Recovery%20Plan%20July07%202004.pdf> [accessed 12 July 2012].
- Nelson, J., Smith, C., Rubidge, E., and Koop, B. 1999. Genetic analysis of the D-loop region of the mitochondrial DNA and microsatellite loci of the nuclear DNA for white sturgeon (*Acipenser transmontanus*) from British Columbia. Report to BC Fisheries, Victoria, British Columbia.
- Peakall, R., and Smouse, P.E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes*, **6**(1): 288–295. doi:10.1111/j.1471-8286.2005.01155.x.
- Peakall, R., Smouse, P.E., and Huff, D.R. 1995. Evolutionary implications of allozyme and RAPD variation in diploid populations of dioecious buffalograss *Buchloë dactyloides*. *Mol. Ecol.* **4**(2): 135–148. doi:10.1111/j.1365-294X.1995.tb00203.x.
- Pfeiffer, T., Roschanski, A.M., Pannell, J.R., Korbecka, G., and Schnittler, M. 2011. Characterization of microsatellite loci and reliable genotyping in a polyploid plant, *Mercurialis perennis* (Euphorbiaceae). *J. Hered.* **102**(4): 479–488. doi:10.1093/jhered/esr024. PMID:21576288.
- Piry, S., Alapetite, A., Cornuet, J.-M., Paetkau, D., Baudouin, L., and Estoup, A. 2004. GeneClass2: a software for genetic assignment and first-generation migrant detection. *J. Hered.* **95**(6): 536–539. doi:10.1093/jhered/esh074. PMID:15475402.
- Pritchard, J.K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics*, **155**(2): 945–959. PMID:10835412.

- Pritchard, J.K., Wen, X., and Falush, D. 2010. Documentation for *structure*. Version 2.3.3 [online]. Available from http://pritch.bsd.uchicago.edu/structure_software/release_versions/v2.3.3/structure_doc.pdf [accessed 12 July 2012].
- Provan, J., and Bennett, K.D. 2008. Phylogeographic insights into cryptic glacial refugia. *Trends Ecol. Evol.* **23**(10): 564–571. doi:10.1016/j.tree.2008.06.010. PMID:18722689.
- Rice, W.R. 1989. Analyzing tables of statistical tests. *Evolution*, **43**(1): 223–225. doi:10.2307/2409177.
- Rodzen, J.A., and May, B. 2002. Inheritance of microsatellite loci in white sturgeon (*Acipenser transmontanus*). *Genome*, **45**(6): 1064–1076. doi:10.1139/g02-083. PMID:12502251.
- Rohlf, F.J. 2009. NTSYSpc: numerical taxonomy system. Ver. 2.21c. Exeter Software, Setauket, New York.
- Rosenberg, N.A. 2004. DISTRUCT: A program for the graphical display of population structure. *Mol. Ecol. Notes*, **4**(1): 137–138. doi:10.1046/j.1471-8286.2003.00566.x.
- Ruiz-Campos, G., Castro-Aguirre, J.L., and Garcia DeLeon, F.J. 2011. First specimen of the white sturgeon (*Acipenser transmontanus* Richardson, 1836) in coastal waters of Mexico with data on its genetic identity. *Calif. Fish Game*, **97**(1): 36–42.
- Schlüter, P.M., and Harris, S.A. 2006. Analysis of multilocus fingerprinting data sets containing missing data. *Mol. Ecol. Notes*, **6**(2): 569–572. doi:10.1111/j.1471-8286.2006.01225.x.
- Scott, W.B., and Crossman, E.J. 1973. White sturgeon. In *Freshwater fishes of Canada*. Edited by W.B. Scott and E.J. Crossman. Fisheries Research Board of Canada, Ottawa, Ontario, Canada. pp. 96–100.
- Semakula, S.N., and Larkin, P.A. 1968. Age, growth, food, and yield of the white sturgeon (*Acipenser transmontanus*) of the Fraser River, British Columbia. *J. Fish. Res. Board Can.* **25**(12): 2589–2602. doi:10.1139/f68-229.
- Small, M.P., Beacham, T.D., Withler, R.E., and Nelson, R.J. 1998. Discriminating coho salmon (*Oncorhynchus kisutch*) populations within the Fraser River, British Columbia, using microsatellite DNA markers. *Mol. Ecol.* **7**(2): 141–155. doi:10.1046/j.1365-294x.1998.00324.x.
- Smith, C.T., Nelson, R.J., Pollard, S., Rubidge, E., McKay, S.J., Rodzen, J., May, B., and Koop, B. 2002. Population genetic analysis of white sturgeon (*Acipenser transmontanus*) in the Fraser River. *J. Appl. Ichthyol.* **18**(4–6): 307–312. doi:10.1046/j.1439-0426.2002.00418.x.
- Taberlet, P., and Luikart, G. 1999. Noninvasive genetic sampling and individual identification. *Biol. J. Linn. Soc.* **68**(1–2): 41–55. doi:10.1111/j.1095-8312.1999.tb01157.x.
- Teel, D.J., Milner, G.B., Winans, G.A., and Grant, W.S. 2000. Genetic population structure and origin of life history types in chinook salmon in British Columbia, Canada. *Trans. Am. Fish. Soc.* **129**(1): 194–209. doi:10.1577/1548-8659(2000)129<0194:GPSA00>2.0.CO;2.
- Triton Environmental Consultants Ltd. 2009. Adult white sturgeon monitoring — Nechako River 2008 [online]. Report prepared for Rio Tinto Alcan, Report No. 3972. Available from http://nechakowhitesturgeon.org/uploads/files/2008_Sturgeon_Report_FINAL-opt.pdf [accessed 12 July 2012].
- Triton Environmental Consultants Ltd. 2010. Adult white sturgeon monitoring — Nechako River 2009 [online]. Report prepared for the British Columbia Ministry of the Environment, Report No. 4170. Available from http://nechakowhitesturgeon.org/uploads/files/2009_Spawn_Monitoring_lowres.pdf [accessed 12 July 2012].
- van Eenennaam, A.L., Murray, J.D., and Medrano, J.F. 1998. Synaptonemal complex analysis in spermatocytes of white sturgeon, *Acipenser transmontanus* Richardson (Pisces, Acipenseridae), a fish with a very high chromosome number. *Genome*, **41**(1): 51–61.
- Veinott, G., Northcote, T., Rosenau, M., and Evans, R.D. 1999. Concentrations of strontium in the pectoral fin rays of the white sturgeon (*Acipenser transmontanus*) by laser ablation sampling — inductively coupled plasma — mass spectrometry as an indicator of marine migrations. *Can. J. Fish. Aquat. Sci.* **56**(11): 1981–1990. doi:10.1139/f99-120.
- Welch, D.W., Turo, S., and Batten, S.D. 2006. Large-scale marine and freshwater movements of white sturgeon. *Trans. Am. Fish. Soc.* **135**(2): 386–389. doi:10.1577/T05-197.1.
- Withler, R.E., Le, K.D., Nelson, R.J., Miller, K.M., and Beacham, T.D. 2000. Intact genetic structure and high levels of genetic diversity in bottlenecked sockeye salmon (*Oncorhynchus nerka*) populations of the Fraser River, British Columbia, Canada. *Can. J. Fish. Aquat. Sci.* **57**(10): 1985–1998. doi:10.1139/f00-149.
- Yarmish, J.A., and Toth, B.M. 2002. 2001/2002 Assessment of upper Fraser River white sturgeon [online]. Report produced for the Upper Fraser River Nechako Fisheries Council and Fisheries Renewal British Columbia, Prince George, British Columbia. Available from <http://a100.gov.bc.ca/pub/acat/public/viewReport.do?reportId=15939> [accessed 12 July 2012].
- Zhu, B., Liao, X., Shao, Z., Rosenthal, H., and Chang, J. 2005. Isolation and characterization of microsatellites in Chinese sturgeon, *Acipenser sinensis*. *Mol. Ecol. Notes*, **5**(4): 888–892. doi:10.1111/j.1471-8286.2005.01100.x.