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Population genetic analysis of white sturgeon (*Acipenser transmontanus*) in the Fraser River

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Summary

White sturgeon (*Acipenser transmontanus*) in the Fraser River are listed as imperiled (the second highest possible rating) by the British Columbia Conservation Data Centre. A difficulty in trying to protect this species in the Fraser River and elsewhere is the lack of knowledge regarding their population biology. Variation in the mitochondrial DNA control region and at four microsatellite loci was examined in order to characterize white sturgeon samples from throughout the Fraser River mainstem and from a major tributary, the Nechako River. Samples from the adjacent Columbia River were analyzed for comparison. In contrast to previous work, present data indicate that white sturgeon population structure in this region reflects post-glacial dispersal more than it does recent anthropogenic effects. The data divided the Fraser into four biogeographic regions: (i) the lower Fraser, below Hell's Gate; (ii) the middle Fraser, between Hell's Gate and river km 553; (iii) the upper Fraser, above the Nechako confluence; and (iv) the Nechako River. These four groups are concordant with those suggested by tag and recapture and catch per unit effort data, and are separated by what have been identified as barriers to white sturgeon migration. Based on concordance between these different types of data, it is argued that the four groups identified here merit evolutionarily significant unit (ESU) status.

Introduction

White sturgeon (*Acipenser transmontanus*) are large (individuals over 6 m) members of the family Acipenseridae inhabiting rivers along the Pacific coast of North America. While this species has been described as anadromous (Scott and Crossman, 1973), purely freshwater populations do exist and the role of marine migrations is poorly understood (although see Choudhury and Dick, 1998). Longevity of individuals (over 100 years in some instances) and their preference for deep turbid habitat have made the study of white sturgeon population biology difficult.

A commercial fishery for Fraser River white sturgeon was established in the late 1800s. By 1905, over-fishing had reduced annual harvests to $< 4\%$ of what they had been 8 years previous (fishery and management history summarized in Echols, 1995). Present-day management of the sport fishery, and decisions regarding habitat restoration are confounded by a lack of knowledge of this species' population biology. Specifically, knowledge of whether or not barriers to gene flow exist within the Fraser, or between the Fraser and the Columbia rivers is prerequisite for the conservation management of this species.

In assessing the utility of molecular genetic markers for characterizing white sturgeon populations, it is important to consider the relative complexity of this species' nuclear genome. Evolution of the family Acipenseridae has been characterized by several polyploidization events (summarized in Birstein et al., 1997). Genetic markers in individuals may exhibit either tetrasomic or disomic inheritance, depending on the locus examined (May et al., 1997). The white sturgeon genome is thought to be octoploid, but in an advanced stage of diploidization (Van Eenennaam et al., 1998a). Further, different individuals are known to have different chromosome numbers (Van Eenennaam et al., 1998b). Assumptions regarding the inheritance of individual loci need to be tested before inferences based on those loci are made.

The goal of the present study was to characterize population structure in Fraser River white sturgeon.

Materials and Methods

White sturgeon were sampled along the length of the Fraser River mainstem and in the Nechako River, as well as in a few regions in the Columbia system (Fig. 1). Pectoral fin clips were collected from each individual and stored in 95% EtOH. Genomic DNA was extracted from each sample after Nelson et al. (1998).

Microsatellites

Microsatellite primers examined were *LS19, LS34, LS57, LS68* (May et al., 1997), *Aox23, Aox27* (Lubinski et al., 1998), *Atr1* (forward-GTACTTTCCTTCTTTTTAAGGTCC TGG, reverse-CAACTAATGTCACAGTACGTCCC), *Atr2* (forward-TTCTATGTAAAACAACTTGTATTACTAG, reverse-TAGATAAACTATGCCGCTCATCC), *Atr3* (forward-TTTTCATCATTAAAAGACCAGAGCC, reverse-TT ATTCTATAAGCAACATTTCAATTTCC).

One microlitre of DNA extract served as template for each polymerase chain reaction (PCR). PCR was performed in 25 μl volume with 100 pmol each primer, 80 μM each nucleotide, 20 mM tris-pH 8.8, 2 mM $MgSO_4$, 10 mM KCl, 0.1% Triton X-100, 10 mM $(NH_4)SO_4$, 0.1 mg ml^{-1} bovine serum albumin and 1 U of *Taq* DNA polymerase. PCRs were carried out in a PTC200 thermal cycler (MJ Research). Initial denaturation of 3 min at 94°C was followed by 30 cycles of 94°C for 30 s, annealing temperature for 30 s, and 72°C for 30 s. PCR products were size-fractionated on 10% 19 : 1 acrylamide to bis-acrylamide gels, in 2X TAE buffer and stained with ethidium bromide. Photographs of the gels were taken

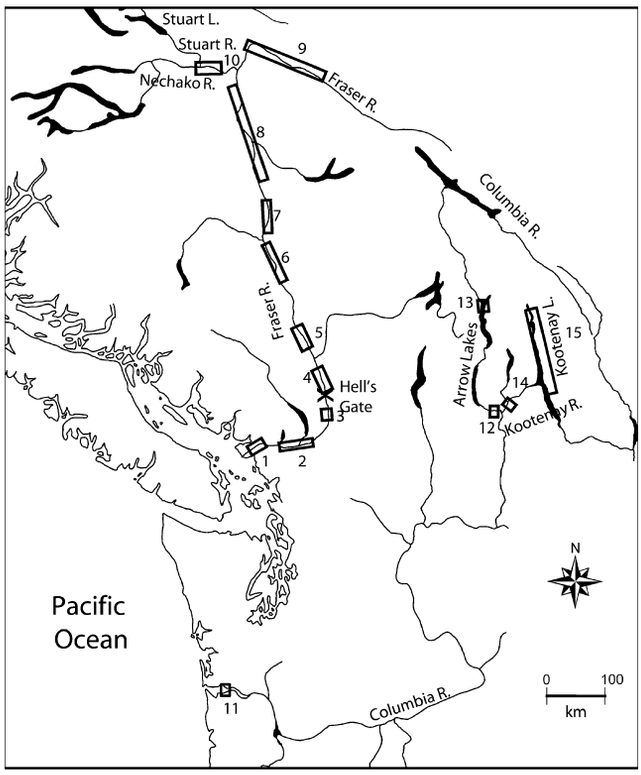

Fig. 1. Sample collection sites: 1) Fraser 1, 2) Fraser 2, 3) Fraser 3, 4) Fraser 4, 5) Fraser 5, 6) Fraser 6, 7) Fraser 6, 7) Fraser 7, 8) Fraser 8, 9) Fraser 9, 10) Nechako R., 11) lower Columbia R., 12) upper Columbia R., 13) Arrow L., 14) Kootenay R., 15) Kootenay L. Numbers of individuals examined for microsatellite and mtDNA variation are listed in Table 1 and Table 3, respectively

digitally, and transferred to Bio Image Intelligent Quantifier (B. I. Systems Corp.). Products not clearly scoreable by this method (all except *Atr* primers) were subsequently run through 5% Long Ranger Gel (FMC Bioproducts) on an ABI377 DNA sequencer and analyzed using Genescan (ABI) software.

Pedigree analysis was performed by examining each locus in eight sets of known parents with 22–48 offspring each. Banding patterns were compared with both disomic and tetrasomic Mendelian expectations using a χ^2 test. Loci that were unscoreable, monomorphic, or showed multiple departures from Mendelian expectations were discarded. The remaining loci were *Atr1, Atr2, Atr3,* and *Aox27*. *Aox27* exhibited 1–4 bands per individual and ratios that matched tetrasomic Mendelian expectations. The 3 *Atr* loci exhibited asymmetric banding (May et al., 1997), with ratios which matched disomic Mendelian expectations. These loci were treated as disomic in the following analysis.

Mitochondria

A subset of the samples assayed for microsatellite variation were also examined for mitochondrial DNA (mtDNA) control region (d-loop) restriction fragment length polymorphism (RFLP). The PCR primers L185 and H740 (Brown et al., 1993) were used to amplify 598 bp of the mtDNA d-loop. PCR was performed in 50 μl volume in a cocktail identical to that described above. Reactions were cycled as follows: an initial denaturation of 3 min at 94°C, followed by 30 cycles of 94°C for 30 s, 45°C for 30 s and 72°C for 1 min. PCR products were purified using QIAquick Purification Kit 96 (QIAGEN, Valencia, CA, USA), and digested with *Mse* I, *Sfc* I (New England Biolabs, Beverly, MA, USA) and *Hsp*92II (Promega, Madison, WI, USA), as per the manufacturer's instructions. Digests were electrophoresed through either 7% (*Sfc* I) or 10% (*Mse* I and *Hsp*92II) 19 : 1 acrylamide : bis-acrylamide gels and viewed as described above.

Mitochondrial variation within sites was quantified as haplotype diversity (h) and nucleotide diversity (π) (Nei, 1987, pp. 179 and 256) using Arlequin (Schneider et al., 1999).

Relationships among samples

Because of the relatively small size of the Fraser 8 sample (Fig.1), it was not included in analyses of population structure. PHYLIP (Felsenstein, 1995) was used to calculate pairwise genetic distance (D_{CSE}) (Cavalli-Sforza and Edwards, 1967) between all sites based on both microsatellite and on mtDNA data. The complete data set and one containing only Fraser River samples were subject to unweighted pair-group method with arithmetic mean (UPGMA) analysis. Concordance among microsatellite loci was examined using 10^3 bootstrap replicates. Correlation between microsatellites and mtDNA was calculated and tested using a permutation procedure (Mantel, 1967) in Arlequin.

Statistical differences between sites were tested using nucleotide divergence (Nei, 1987, p. 276) for mtDNA data and F_{ST} (Weir and Cockerham, 1984) for microsatellite data, each with a null distribution based on 10^5 replicates. The proportion of genetic variance explained by UPGMA structures (among group variance), as well as the potential of the present data to detect finer structure (among site within group variance) were assessed with analysis of molecular variance (AMOVA) (Excoffier et al., 1992).

Results

Microsatellites

Microsatellite allele frequencies are listed in Table 1. UPGMA of microsatellite data divided the Fraser River samples among three groups with ≥50% bootstrap support (Fig. 2). Pairwise F_{ST} estimates between sites within these groups were not significant ($P > 0.05$), while those between sites among groups were significant ($P < 0.05$). An exception is that the Nechako and uppermost Fraser samples were not significantly different ($P = 0.189$). The same three Fraser River groups appeared when the Columbia samples were included in the analysis. The lower Fraser group joined the Columbia samples (58% bootstrap), whereas the other two Fraser groups were distinct from the Columbia samples.

Mitochondria

The mtDNA RFLP assay revealed nine compound haplotypes (Table 2). UPGMA of mtDNA data divided Fraser River samples among three major branches (Fig. 3). Nucleotide divergence indicated that although the Nechako and uppermost Fraser were on the same UPGMA branch, the difference between them was highly significant ($P < 0.000$). Variation among sites on the other two branches was not significant ($P \leq 0.05$). The same four Fraser River groups appeared when the Columbia samples were included in the analysis.

Haplotype diversity ranged tenfold from $h = 0.07$ in Kootenay Lake to $h = 0.79$ in the upper Columbia (Table 3). Relative diversity of the Columbia and Fraser rivers contrasted with predictions based on earlier work. Haplotype diversity

Table 1
Observed allele frequencies at 4 microsatellite loci in white sturgeon from the Fraser and Columbia rivers. Site numbers correspond to Fig. 1. Names of alleles correspond to the size of the PCR product (base pairs)

		Atr1		*Atr2*		*Atr3*		*Aox27*					
Site	n	130	134	184	193	128	132	126	130	134	138	142	146
1	56	0.581	0.419	0.580	0.420	0.955	0.045	0.012	0.054	0.381	0.345	0.161	0.048
2	55	0.372	0.628	0.548	0.452	0.877	0.123	0.014	0.027	0.373	0.386	0.136	0.064
3	46	0.349	0.651	0.535	0.465	0.977	0.023	0.033	0.006	0.250	0.328	0.322	0.061
4	50	0.245	0.755	0.480	0.520	1.000	–	0.021	–	0.201	0.325	0.433	0.021
5	50	0.510	0.490	0.449	0.551	1.000	–	0.050	–	0.160	0.340	0.435	0.015
6	50	0.418	0.582	0.552	0.448	1.000	–	0.015	–	0.165	0.410	0.405	0.005
7	56	0.381	0.619	0.510	0.490	1.000	–	0.022	–	0.179	0.304	0.446	0.049
8	10	0.400	0.600	0.500	0.500	1.000	–	–	–	0.313	0.188	0.500	–
9	43	0.465	0.535	0.593	0.407	1.000	–	0.032	–	0.129	0.427	0.411	–
10	50	0.420	0.580	0.615	0.385	1.000	–	–	–	0.214	0.541	0.245	–
11	50	0.333	0.667	0.542	0.458	0.949	0.051	0.006	0.017	0.344	0.450	0.156	0.028
12	50	0.296	0.704	0.460	0.540	0.970	0.030	0.027	0.016	0.282	0.564	0.064	0.048
13	20	0.400	0.600	0.531	0.469	0.975	0.025	–	0.038	0.200	0.675	0.050	0.038
14	50	0.367	0.633	0.418	0.582	1.000	–	–	0.011	0.223	0.670	0.069	0.027
15	50	0.270	0.730	0.071	0.929	0.930	0.070	–	–	0.203	0.622	0.174	–

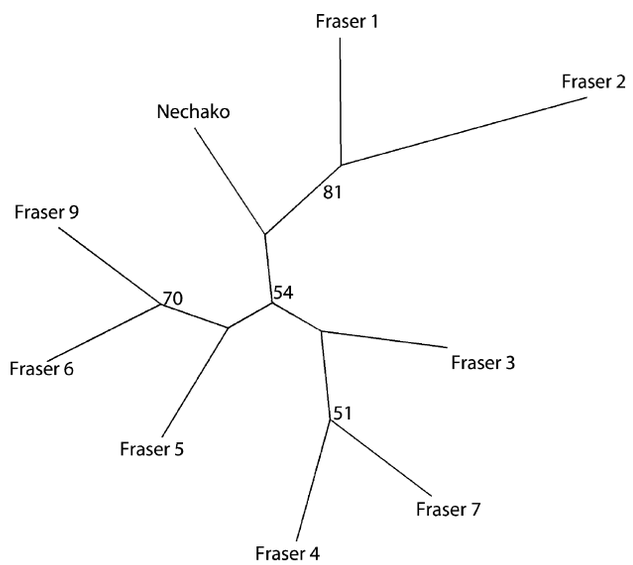

Fig. 2. UPGMA dendrogram based on microsatellite D^{CSE} for Fraser R. white sturgeon. Numbers on branches indicate the percentage of 1000 bootstrap replicates that support grouping the sites distal to that branch

Table 2
Nomenclature for composite m + DNA haplotypes. The defining fragment used to identify each single locus haplotype is given in parentheses (in base pairs) the first time that haplotype is listed. Gel images showing these bands are available at http://web.uvic.ca/~bioweb/people/koop/sturgeon.html

Single restriction enzyme haplotype			
*Hsp*92II	*Mse*I	*Sfc*I	Composite haplotype
a (88 bp)	a (233 bp)	a	1
b	a	a	2
b	b	a	3
a	b	a	4
b	b	b (136 bp)	5
c (259)	b	b	6
b	a	b	7
d (64)	b	b	8
d	b	a	9

within the Fraser ($h = 0.71$) was similar to that based on RFLP of the entire molecule ($h = 0.70$) (Brown et al., 1992), but lower than that based on d-loop sequence ($h = 0.97$) (Brown et al., 1993). Haplotype diversity in the Columbia ($h = 0.63$) was also lower than that based on d-loop sequence ($h = 0.93$) (Brown et al., 1993), but much higher than that based on RFLP of the entire molecule ($h = 0.08$) (Brown et al., 1992).

A positive correlation was observed between D_{CSE} based on microsatellite data and that based on mtDNA data ($r^2 = 0.34$, $P < 0.000$).

AMOVA

Analysis of molecular variance revealed that 78.3% ($P = 0.002$) of mtDNA variation and 95.5% ($P = 0.004$) of microsatellite variation observed in the Fraser River was found among individuals within the sample sites. Therefore, 21.7% of the mtDNA data and 4.5% of the microsatellite data were useful for investigating relationships among sites. AMOVA was used to partition this variation between the categories 'among groups' and 'among sites within groups'. Division of the Fraser into three groups (Fig. 2) best explained microsatellite variation, partitioning 5.0% ($P < 0.001$) 'among groups' and −0.5% ($P = 0.933$) 'among sites within groups'. Division of the Fraser into four groups (Fig. 3) best-explained mtDNA variation, partitioning 19.9% ($P < 0.001$) 'among groups' and 1.8% ($P = 0.161$) 'among sites within groups'. Small and nonsignificant 'among sites within groups' variance for both markers indicated that population structure on a finer scale would not be resolved with this data.

Discussion

Both microsatellite and mtDNA RFLP data appear useful for describing population structure in white sturgeon. Pedigree analysis was successful in identifying limitations of the markers considered. As several loci were discarded based on our inability to interpret them, the variation reported here is likely only partially representative of that present in the white sturgeon genome. Mitochondrial data in the present study provided greater resolution than the microsatellite data, and provided greater inferential power because of an unambiguous inheritance pattern. With all that is unknown regarding the

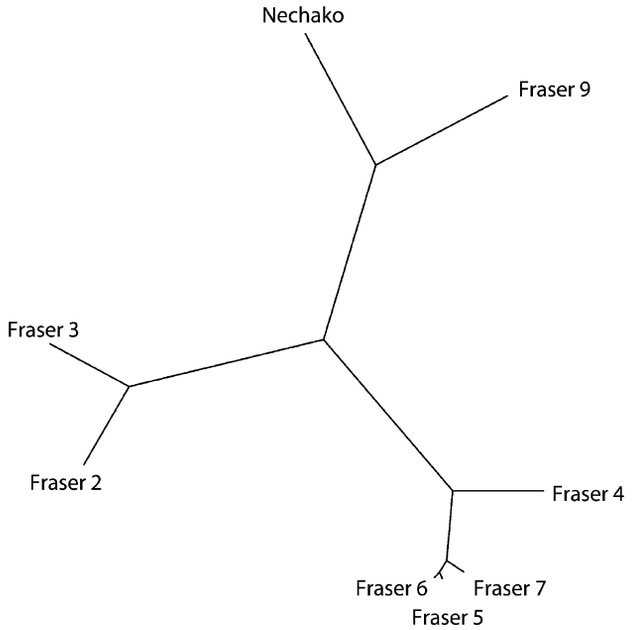

Fig. 3. UPGMA dendrogram based on mitochondrial D_{CSE} for Fraser River white sturgeon

basic biology of white sturgeon, extrapolation of structure at any genetic marker to species population structure must be viewed as speculative. Correlation and overall concordance between the two marker types lent some confidence in this case to making such an assumption.

Based on previous discussions (Brown et al., 1992, 1993), relatively little genetic diversity was expected to be seen in the upper Columbia. During the last glacial retreat, Kootenay Lake was isolated from the remaining Columbia River sites by falls shortly after it was freed from ice (summarized by Northcote, 1973). This allowed only a short founding period, and Kootenay Lake is known to be correspondingly less diverse than lower regions of the Columbia system (Bartley et al., 1985; Setter and Brannon, 1992). When Kootenay Lake was removed from the present analysis, haplotype diversity was higher in the Columbia basin than in the Fraser. More mtDNA haplotypes and microsatellite alleles were seen in the upper Columbia than the upper Fraser. Bernatchez and Wilson (1998) summarized data for several anadromous fish species which, despite being subject to recent anthropogenic bottlenecks, still exhibit phylogeographic patterns reflecting post-glacial dispersal. The observed distribution of genetic variation can be explained without the invocation of an anthropogenic bottleneck over-riding the signal of post-glacial dispersal.

Within the Fraser, the present data support that of Brown et al. (1992) in identifying the lower region as the most diverse. Because the Fraser and Columbia enter the Pacific Ocean only 500 km distant from each other, the potential contribution of inter-river migration to this diversity is of interest. Evidence for potential migration includes the presence of white sturgeon on (mostly the west coast of) Vancouver Island (D. Lane, pers. comm.), sturgeon tagged in the Columbia River and subsequently recaptured off the coast of northern Washington State (Galbreath, 1985), and evidence that some Fraser River white sturgeon make marine migrations (Veinott et al., 1999). Several aspects of the present data reveal that white sturgeon in the lower Fraser are similar to those in the Columbia. It cannot, however, be determined based on these data whether the similarity is because of migration, or a relatively short coalescence time. The upper limit to the lower Fraser region is the velocity barrier at Hell's Gate rapids. White sturgeon are not believed to be able to migrate upstream of this barrier, but downstream movement has been recorded (McKenzie, 2000). Divergence of this region from the rest of the Fraser, with uncertainty regarding exactly where the border is, suggests that the Fraser River below Hell's Gate is a distinct biogeographic region, but that it is not a single or closed breeding unit.

Sites between Hell's Gate and Fraser 7 are grouped together, with neither marker type showing significant differences between them. The site upon which the two markers contrast is Fraser 9. Despite significant effort over 2 years of sampling, very few sturgeon were observed in the 80 km region south of the Nechako–Fraser confluence (McKenzie, 2000). Yarmish and Toth (2001) noted that this region was broad and shallow, lacking deep pools and channel structure. These authors suggested that this region, as well as a similar region in the lower 60 km of the Nechako River, acts as migration barriers for white sturgeon. Tag and recapture data frequently revealed large movements (>32 km) within the upper Fraser, but no

Table 3
Observed frequencies of compound m + DNA haplotypes at several sites in the Fraser and Columbia rivers. Site numbers correspond to Fig. 1. Haplotype diversity (h) measures variation based on identity/non-identity in state, while nucleotide diversity (π) takes into account the distance between states

		Composite haplotype										
Site	n	1	2	3	4	5	6	7	8	9	h	π
2	31	–	0.13	0.39	0.35	0.06	0.03	0.03	–	–	0.725	0.369
3	27	–	0.11	0.44	0.19	0.04	–	0.22	–	–	0.732	0.372
4	26	–	–	0.54	–	0.12	–	0.35	–	–	0.600	0.247
5	30	–	0.13	0.30	–	0.13	–	0.43	–	–	0.710	0.254
6	30	–	0.07	0.30	–	0.13	–	0.50	–	–	0.660	0.247
7	28	–	0.11	0.32	–	0.04	–	0.54	–	–	0.619	0.246
8	5	–	0.20	0.60	–	0.20	–	–	–	–	0.700	0.200
9	30	–	0.31	0.69	–	–	–	–	–	–	0.435	0.109
10	29	–	0.38	0.41	–	–	–	0.21	–	–	0.665	0.211
12	30	0.13	0.37	0.27	0.03	0.10	0.03	–	0.03	0.03	0.793	0.376
13	18	–	0.33	0.50	0.06	–	–	–	0.06	0.06	0.667	0.247
14	28	0.11	0.56	0.15	0.15	–	–	–	–	0.04	0.643	0.316
15	27	–	0.96	0.04	–	–	–	–	–	–	0.074	0.019

evidence of movement between the upper Fraser and the middle Fraser or the Nechako (Toth et al., 2000; Yarmish and Toth, 2001). The authors of those studies proposed that the relatively large movements within the upper Fraser might be an adaptive response to the unique environment faced by these fish (i.e. lower temperature, seasonal ice and more sporadic anadromous food sources). Although microsatellite data did not reveal divergence here, mtDNA data are supported by tag and recapture data in identifying divergence between the upper and middle Fraser.

Nechako River white sturgeon are of special interest for conservation as the stock consists mostly of older fish and exhibits low spawning success and recruitment. This is likely due to altered thermal and hydrographic regimes, and leaves sturgeon in this region susceptible to extirpation (McKenzie, 2000). Microsatellite and mtDNA data concurred that the Nechako was distinct from the middle Fraser. While microsatellite data were unclear regarding distinction between the upper Fraser and the Nechako (majority bootstrap, but no significance), mtDNA data (specifically haplotype 7) provided much more conclusive evidence of this divergence. All Nechako sturgeon examined were sampled ≥72 km from the Fraser confluence, as sampling below this area failed to produce a single fish (McKenzie, 2000). This observation supports the suggestion that the lower Nechako acts as a migration barrier to white sturgeon.

Data presented here suggest the division of Fraser River white sturgeon into four biogeographic groups: (i) the lower Fraser, below Hell's Gate; (ii) the middle Fraser, between Hell's Gate and river km 553 (km 553 is the northern limit of Fraser 7); (iii) the upper Fraser, above the Nechako confluence; and (iv) the Nechako River. These four groups are concordant with those suggested by tag and recapture and catch-per-unit-effort data, and are separated by what have been identified as barriers to white sturgeon migration. The lack of significant 'among site within region' variance indicates that structure on a finer scale than the one described is not detectable with the current data.

McPhail and Carveth (1993) suggested adoption of the evolutionarily significant unit (ESU) as the basic conservation element for this region's ichthyofauna. An ESU may be defined as a population which is substantially reproductively isolated from conspecific populations, and which represents an important component of the evolutionary legacy of the species (Waples, 1991). The present data revealed statistically significant differences which, given the limitations of what is known about this species' population biology, is interpreted as 'substantial reproductive isolation'. To evaluate the second condition it is noted that the environments, and thus selective regimes, faced by these groups are diverse. White sturgeon in the upper Fraser and Nechako grow more slowly, have to move greater distances to feed, and likely spend more time at a low metabolic rate under winter ice (McKenzie, 2000; Yarmish and Toth, 2001). As Hell's Gate prevents upstream movement, sturgeon living above it are unlikely to have made marine migrations, while sturgeon living below it may have (Veinott et al., 1999). Based on the combination of our genetic data with sturgeon habitat and ecologic data, we suggest that the four groups identified here merit designation as ESUs.

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References

Bartley, D. M.; Gall, G. A.; Bentley, B., 1985: Preliminary description of the genetic structure of white sturgeon, *Acipenser transmontanus,* in the Pacific Northwest. In: North American sturgeons: biology and aquaculture potential. F. P. Binkowski and S. I. Doroshov (Eds). Dr W. Junk Publishers, Dordrecht, The Netherlands, pp. 105–109.

Bernatchez, L.; Wilson, C. C., 1998: Comparative phylogeography of nearctic and palearctic fishes. Mol. Ecol. **7,** 431–452.

Birstein, V. J.; Hanner, R.; DeSalle, R., 1997: Phylogeny of the Acipenseriformes: Cytogenetic and molecular approaches. Env. Biol. Fish. **48,** 127–156.

Brown, J. R.; Beckenbach, A. T.; 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,** 358–367.

Brown, J. R.; Beckenbach, A. T.; Smith, M. J., 1993: Intraspecific DNA sequence variation of the mitochondrial control region of white sturgeon (*Acipenser transmontanus*). Mol. Biol. Evol. **10,** 326–341.

Cavalli-Sforza, L. L.; Edwards, A. W., 1967: Phylogenetic analysis. Models and estimation procedures. Am. J. Hum. Genet. **19,** 233–257.

Choudhury, A.; Dick, T. A., 1998: The historical biogeography of sturgeons (Osteichthyes: Acipenseridae): a synthesis of phylogenetics, palaeontology and palaeogeography. J. Biogeog. **25,** 623–640.

Echols, J. C., 1995: Review of Fraser River white sturgeon, *Acipenser transmontanus*. Canada Department of Fisheries & Oceans, Vancouver, B.C.

Excoffier, L.; Smouse, P. E.; Quattro, J. M., 1992: Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics **131,** 479–491.

Felsenstein, J., 1995: PHYLIP, Version 3.5c. University of Washington, Seattle.

Galbreath, J. L., 1985: Status, life history and management of Columbia River white sturgeon, *Acipenser transmontanus*. In: North American sturgeons: biology and aquaculture potential. F. P. Binkowski and S. I. Doroshov (Eds). Dr W. Junk, Dordrecht, The Netherlands. pp. 119–125.

Lubinski, B. A.; King, T. L.; Dorschner, M. O., 1998: *Acipenser oxyrhnchus oxyrhynchus* microsatellite *Aox27* repeat region. Genbank accession no. AF067812.

Mantel, N., 1967: The detection of disease clustering and a generalized regression approach. Cancer Res. **27,** 209–20.

May, B.; Krueger, C. C.; Kincaid, H. L., 1997: Genetic variation at microsatellite loci in sturgeon: primer sequence homology in *Acipenser* and *Scaphirhynchus*. Can. J. Fish. Aquat. Sci. **54,** 1542–1547.

McKenzie, S., 2000: Fraser River white sturgeon monitoring program – comprehensive report (1995–1999). British Columbia Ministry of Water, Land and Air Protection. RL&L Report No. 815F. Victoria, B.C. 92 pp. + App. pp.

McPhail, J. D.; Carveth, R., 1993: A foundation for conservation: the nature and origin of the freshwater fish fauna of British Columbia. BC Environment, 39pp.

Nei, M., 1987: Molecular evolutionary genetics. Columbia University Press, New York, 512pp.

Nelson, R. J.; Beacham, T. D.; Small, M. P., 1998: Microsatellite analysis of the population structure of a Vancouver Island sockeye salmon (*Oncorhynchus nerka*) stock complex using nondenaturing gel electrophoresis. Mol. Mar. Biol. Biotech. **7,** 312–319.

Northcote, T., 1973: Some impacts of man on Kootenay Lake and its salmonids. Great Lakes Fisheries Commission, Ann Arbor, Michigan. Tech. Report No 2.

Schneider, S.; Roessli, D.; Excoffier, L., 1999: Arlequin: a software for population genetic data analysis version 2.001. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Scott, W. B.; Crossman, E. J., 1973: Freshwater fishes of Canada. Fish. Res. Board Can. Bull. **184,** 96–100.

Setter, A.; Brannon, E., 1992: A summary of stock identification research on white sturgeon of the Columbia River (1985–1990). Final Report January 1985–July 1991. Report to Bonneville Power Administration, Bonneville, Contract No. 1989BP97298, Project No. 198904400. BPA Report DOE/BP-97298-1, 105 pp.

Toth, B. M.; Yarmish, J. A.; Smith, R. G., 2000: 1999/2000 Assessment of upper Fraser River white sturgeon. The Lheidli T'enneh Band, Prince George, B.C. Upper Fraser Nechako Fisheries Council file: 230499-025, 31 pp.

Van Eenennaam, A. L.; Murray, J. D.; Medrano, J. F., 1998a: Synaptonemal complex analysis in spermatocytes of white sturgeon, *Acipenser transmontanus* Richardson (Pisces, Acipenseridae), a fish with a very high chromosome number. Genome **41,** 51–61.

Van Eenennaam, A. L.; Murray, J. D.; Medrano, J. F., 1998b: Mitotic analysis of the North American white sturgeon, *Acipenser transmontanus* Richardson (Pisces, Acipenseridae), a fish with a very high chromosome number. Genome **41,** 266–271.

Veinott, G.; Northcote, T.; Rosenau, M.; 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,** 1981–1990.

Waples, R. S., 1991: Definition of "species" under the Endangered Species Act: application to pacific salmon. US Dept. Comm., NOAA Tech. Memo. NMFS F/NWC-194, 29 pp.

Weir, B. S.; Cockerham, C. C., 1984: Estimating F-statistics for the analysis of population structure. Evolution **38,** 1358–1370.

Yarmish, J. A.; Toth, B. M., 2001: 2000/2001 Assessment of upper Fraser River white sturgeon. The Lheidli T'enneh Band, Prince George, B.C. Upper Fraser Nechako Fisheries Council file: 0400-060, 42 pp.

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