Lake Sturgeon Population Genetics in the Saskatchewan and Winnipeg Rivers

Final Report

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INTRODUCTION

The lake sturgeon (*Acipenser fulvescens*) is the largest freshwater fish in North America and is native to the Great Lakes, Hudson Bay, and the Mississippi River drainages (Auer 1996). Lake sturgeon populations have been severely reduced from historical levels of abundance, primarily through overfishing in the late 1800's (Auer 1999) and habitat degradation (Noakes et al. 1999). The population dynamics (Bruch 1999), movements (Rusak and Mosindy 1997), and population genetics (Guenette et al. 1993; Ferguson and Duckworth 1997) have been investigated in most of the drainages inhabited by lake sturgeon. Previous studies of lake sturgeon mitochondrial DNA variation were successful at determining drainage-level post glacial history but were unable to detect the fine scale genetic variation needed to identify management units in each drainage (Ferguson and Duckworth, 1997). Microsatellite DNA variation should prove to be useful in discriminating between management units for species such as lake sturgeon, which have traditionally exhibited low levels of genetic variation with traditional markers such as mtDNA haplotypes and allozymes (Carvalho and Hauser, 1994; O'Connell and Wright, 1997).

The present project addresses lake sturgeon management concerns expressed by regional and provincial agencies in Saskatchewan and Manitoba. The main objective of this study was to determine if lake sturgeon populations in Saskatchewan, Manitoba, and Ontario are genetically differentiated using microsatellite DNA markers (May et al. 1997; McQuown et al. 2000; Pyatskowit et al., 2001).

POPULATION SAMPLES

Lake sturgeon tissue samples were collected in the Spring, Summer, and Fall of 2000 from 3 riverine systems in Saskatchewan, Manitoba, and Ontario (Table 1). All these river systems are located within the Hudson/James Bay drainage basin. In Ontario, lake sturgeon tissues were collected from spawning grounds in the Rainy River (Manitou and Long Sault Rapids) and from Lake of the Woods, near the mouth of Rainy River. In Manitoba, tissue samples were collected from three separate rivers; the Nelson River, the Winnipeg River, and the Saskatchewan River. The Saskatchewan River flows through both Saskatchewan and Manitoba. In Manitoba, the Saskatchewan River collections wee made at a point close to the Saskatchewan /Manitoba border known as The Pas. In Saskatchewan, all tissue samples were taken from various points along the Saskatchewan River. Samples were collected from Bigstone Rapids (≈ 40 km from SK/MB border), Centre Angling (≈50-100 km from SK/MB border), Torch River (≈100 km from SK/MB border), and the Forks (≈300 km from SK/MB border). There is a large hydropower station (EB Campbell dam, Saskatchewan Power) downstream from the Forks which prevents fish movement between this area and the other sampling localities.

METHODOLOGY

Genomic DNA was isolated from fin tissues using a standard phenol/chloroform extraction procedure. DNA samples were extracted from a total of 330 individual lake sturgeon. Three microsatellite loci (Table 2) were amplified via the polymerase chain reaction (PCR) for each individual. PCR products were separated electrophoretically on polyacrylamide gels and visualized using a Hitachi FMBIO II Systems scanner. A fourth locus (Aox27), known to be polymorphic in lake sturgeon from the Great Lakes

(McQuown, unpub. data), was also screened but was found to be monomorphic (i.e. fixed alleles with no variation) in fish from the Hudson/James Bay drainage (Robinson, unpub. data).

Deviations from Hardy-Weinberg (HW) proportions and linkage disequilibrium were tested using Genepop 3.2a software (Raymond and Rousset 1995). Population samples were compared to one another and tested for significant differences using three different types of pairwise tests appropriate for the type of data and number of individuals collected. Pairwise tests based on allele frequencies (genic differentiation) and genotype frequencies (genotypic differentiation) were executed using the population differentiation option of Genepop 3.2a (Raymond and Rousset 1995). Pairwise F_{ST} tests (based on heterozygote defecit among populations) were conducted using the population differentiation submenu of FSTAT 2.9.1 (Goudet 1995).

RESULTS

The 3 microsatellite loci showed relatively high levels of polymorphism. The numbers of alleles per locus ranged from 7 (Afu 68) to 10 (Afu 68b). Observed mean heterozygosities (Table 3) ranged from 0.5714 (Torch River SK, n=14) to 0.7000 (Winnipeg River MB, n=50). Based on observed and expected heterozygsities (Table 3), levels of genetic diversity were typically higher for samples collected in Manitoba and Saskatchewan than those taken from the Rainy River system in Ontario. All loci conformed to HW proportions in samples collected in Manitoba and Saskatchewan. Locus Afu68 did not meet HW expectations in samples collected from the Rainy River ($p<0.005$) and Lake of the Woods ($p\approx0.005$). No evidence for linkage disequilibrium (non random association of genes) was detected among any of the three loci tested. It

should be mentioned that small sample sizes from Torch River SK (n=14), Bigstone Rapids SK (n=26), and Lake of the Woods (n=25) negate the possibility of drawing statistically sound conclusions from pairwise population comparisons involving these samples.

Pairwise population differentiation tests based on genic and genotypic frequencies detected significant differences between, but not within, the population samples from the river systems tested (Table 4). Allelic and genotypic frequencies measured in the Nelson and Winnipeg River samples were significantly different from each other and from all other population samples tested. Similarly, allelic and genotypic frequencies from samples collected in Ontario (Rainy River system) were significantly different from samples collected in the Saskatchewan and Winnipeg River drainages. No significant genic or genotypic differences were detected within the Saskatchewan River system, and all population samples collected in this system were significantly different from the Winnipeg, Nelson, and Rainy River systems.

Pairwise F_{ST} tests (Table 5) also detected strongly significant differences between, but not within individual river systems without exception. The Nelson and Winnipeg River samples were significantly different from each other and from all other population samples tested. No significant differences were detected between samples collected in the Saskatchewan River, and all Saskatchewan River samples were consistently different from samples collected in the Winnipeg, Nelson, and Rainy Rivers. Although the number of loci used in this study is below the number usually accepted as a minimum for such studies, increasing the number of loci would not likely have a large impact on the results as the differences detected between the tested systems are highly significant.

INTERPRETATION OF RESULTS

The significant genetic differences detected between lake sturgeon populations in the Rainy, Saskatchewan, Winnipeg, and Nelson river systems are indicative of unique genetic stocks in each of these river systems. Policies and conservation efforts aimed at improving natural lake sturgeon populations through wise management should not involve the translocation of fish or transfer of gametes between these river systems. Any artificial reproduction programs aimed at supplementing the natural stocks should use parent broodstock from the river system where the stocking will occur. In light of this data, fisheries managers should adopt a precautionary stock-based approach towards lake sturgeon management, and develop strategies and improvement plans on a river by river basis.

CONCLUSIONS

- Based on an analysis of 3 polymorphic microsatellite loci, lake sturgeon populations in the Winnipeg, Nelson, Saskatchewan, and Rainy Rivers are genetically different from one another and as such should be managed as discrete stocks.
- Enhancement efforts for Canadian lake sturgeon populations should make provisions for the preservation of these unique stocks.
- Such provisions to enhancement strategies for these populations should include measures to prevent translocations of fish (eggs, larvae, young-of-the-year, juveniles, or adults) and gametes between river systems).

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Table 1: Summary of lake sturgeon (*Acipenser fulvescens*) tissue collections from spring and summer 2000 field seasons in Ontario, Manitoba, and Saskatchewan.

Collection Location	N	Collection Date
ONTARIO		
Rainy River - River Mouth	25	Aug./Sept 2000
Rainy River Hatchery – Broodstock	42	April 2000
MANITOBA		
Saskatchewan River, The Pas	33	August 2000
Winnipeg River, Sturgeon Rapids	50	May 2000
Nelson River, Landing River Stretch	50	May 2000
SASKATCHEWAN		
Saskatchewan River - Bigstone Rapids	26	May-Aug 2000
Saskatchewan River - Centre Angling	50	June 2000
Saskatchewan River - Torch River	14	Summer 2000
Saskatchewan River - The Forks	40	May-Sept 2000

Table 2: Lake sturgeon microsatellite loci.

Locus Name	Primer Sequence (5′ to 3′)	GenBank Acc. Number
Spl 120	F: ATTCCATGAGCAACACCACA R: TGATGGTCTGATGAGATCGG	AF276189
Afu 68b	F: AACAATATGCAACTCAGCATAA R: AGCCCAACACAGACAATATC	U72739*
Afu 68	F: TTATTGCATGGTGTAGCTAAAC R: AGCCCAACACAGACAATATC	U72739

*Afu 68b was designed from the clone for Afu 68

Table 3: Genetic characteristics of microsatellite loci analyzed in adult lake sturgeon from Canadian Rivers. Populations are listed by province, all locations listed for SK are located in the Saskatchewan River system. N_{FISH} refers to the number of fish analyzed per locus per population. H_{OBS} and H_{EXP} refer to the observed and expected frequencies of heterozygotes.

Locus & Characteristic	**Populations Sampled**									**Totals**
	Ontario			**Manitoba**		**Saskatchewan**				
	L. Woods	Rainy R.	Nelson R.	Winnipeg R.	Sask. R. Pas	Torch River	Bigstone Rap.	C. Angling	The Forks	
AFU 68										
N_{FISH}	25	40	50	50	33	14	24	46	40	322
$N_{ALLELES}$	4	6	5	5	4	4	6	5	5	
Size Range (bp)	115-135	115-135	115-139	111-135	115-135	115-135	111-135	111-135	111-135	111-139
H_{OBS}	0.1600	0.1429	0.3800	0.5000	0.6061	0.4286	0.6538	0.5200	0.5750	
H_{EXP}	0.3224	0.3176	0.5012	0.4800	0.6979	0.7064	0.6677	0.5800	0.7423	
AFU 68b										
N_{FISH}	25	42	50	50	33	14	26	50	40	330
$N_{ALLELES}$	6	7	7	8	7	5	7	8	6	
Size Range (bp)	168-196	168-196	160-202	160-202	160-202	160-188	160-192	160-202	160-192	160-202
H_{OBS}	0.7600	0.6667	0.8000	0.8000	0.6061	0.5000	0.5769	0.6600	0.5500	
H_{EXP}	0.7168	0.7443	0.7470	0.7762	0.7048	0.5293	0.5104	0.6074	0.5538	
SPL 120										
N_{FISH}	25	42	50	50	33	14	26	50	40	330
$N_{ALLELES}$	7	6	8	6	5	5	5	5	5	
Size Range (bp)	252-288	252-284	248-288	252-284	252-284	252-284	252-284	252-284	252-284	248-288
H_{OBS}	0.8800	0.6667	0.8000	0.8000	0.8788	0.7857	0.8077	0.8200	0.6250	
H_{EXP}	0.7884	0.7236	0.7946	0.7412	0.7897	0.7007	0.7700	0.7900	0.7355	
MEAN H_{OBS}	0.6000	0.4921	0.6600	0.7000	0.6970	0.5714	0.6795	0.6667	0.5833	
MEAN H_{EXP}	0.6092	0.5952	0.6809	0.6658	0.7308	0.6455	0.6494	0.6591	0.6787	

Table 4: Genic and genotypic pairwise tests showing samples compared, chi-square values and associated and p-values for each test. Population samples are coded as follows: NR= Nelson River, MB; WR= Winnipeg River, MB; SC= Saskatchewan River Centre Angling, SK; SF= Saskatchewan River Forks, SK; SP= Saskatchewan River Pas, MB; BR= Saskatchewan River Bigstone Rapids, SK; LW= Lake of the Woods, ON; RR= Rainy River, ON.

Population Comparison			Genotypic Differentiation		Genic Differentiation	
			χ^2	P-Value	χ^2	P-Value
NR	&	WR	23.464	0.0006*	23.355	0.0007*
NR	&	SC	37.632	<0.0001*	36.691	<0.0001*
NR	&	SF	46.06	<0.0001*	∞	<0.0001*
NR	&	SP	30.102	<0.0001*	28.197	0.0001*
NR	&	ST	24.353	0.0004*	22.301	0.0012*
NR	&	BR	41.692	<0.0001*	41.133	<0.0001*
NR	&	LW	∞	<0.0001*	∞	<0.0001*
NR	&	RR	27.194	0.0001*	30.983	<0.0001*
WR	&	SC	∞	<0.0001*	∞	<0.0001*
WR	&	SF	∞	<0.0001*	∞	<0.0001*
WR	&	SP	41.923	<0.0001*	∞	<0.0001*
WR	&	TR	30.417	<0.0001*	34.825	<0.0001*
WR	&	BR	∞	<0.0001*	∞	<0.0001*
WR	&	LW	∞	<0.0001*	∞	<0.0001*
WR	&	RR	∞	<0.0001*	∞	<0.0001*
SC	&	SF	10.882	0.0921	12.608	0.0497
SC	&	SP	7.207	0.3021	8.02	0.2366
SC	&	TR	14.227	0.0272	14.183	0.0277
SC	&	BR	6.23	0.3979	7.351	0.2896
SC	&	LW	∞	<0.0001*	∞	<0.0001*
SC	&	RR	∞	<0.0001*	∞	<0.0001*
SF	&	SP	14.377	0.0257	15.448	0.0170
SF	&	TR	12.957	0.0437	13.622	0.0342
SF	&	BR	7.239	0.2993	8.389	0.2109
SF	&	LW	∞	<0.0001*	∞	<0.0001*
SF	&	RR	∞	<0.0001*	∞	<0.0001*
SP	&	TR	12.013	0.0616	11.076	0.0861
SP	&	BR	10.201	0.1164	9.509	0.1469
SP	&	LW	∞	<0.0001*	∞	<0.0001*
SP	&	RR	44.575	<0.0001*	∞	<0.0001*
TR	&	BR	8.975	0.1750	8.811	0.1845
TR	&	LW	∞	<0.0001*	∞	<0.0001*
TR	&	RR	32.495	<0.0001*	43.826	<0.0001*
BR	&	LW	∞	<0.0001*	∞	<0.0001*
BR	&	RR	∞	<0.0001*	∞	<0.0001*
LW	&	RR	5.216	0.5165	7.575	0.2709

* = Significant at Bonferonni (Rice, 1989) adjusted $p < 0.0014$.

∞ =Very large χ^2 values.

Table 5: Pairwise F_{ST} comparisons between sturgeon population samples. Population samples are coded as follows: NR= Nelson River, MB; WR= Winnipeg River, MB; SC= Saskatchewan River Centre Angling, SK; SF= Saskatchewan River Forks, SK; SP= Saskatchewan River Pas, MB; BR= Saskatchewan River Bigstone Rapids, SK; LW= Lake of the Woods, ON; RR= Rainy River, ON.

	WR	**SC**	**SF**	**SP**	**BR**	**LW**	**RR**
NR	0.0163*	0.0200*	0.0572*	0.0285*	0.0591*	0.0423*	0.0171*
WR	-	0.0461*	0.0974*	0.0499*	0.0958*	0.0624*	0.0397*
SC	-	-	0.0146	0.0030	0.0081	0.1096*	0.0660*
SF	-	-	-	0.0097	0.0004	0.1630*	0.1176*
SP	-	-	-	-	0.0064	0.1066*	0.0821*
BR	-	-	-	-	-	0.1780*	0.1353*
LW	-	-	-	-	-	-	0.0062

*=Significant at Bonferonni (Rice, 1989) adjusted $p < 0.001786$.