Population Structure of Lake Sturgeon within the Namakan River, Ontario

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Introduction

The lake sturgeon, *Acipenser fulvescens*, has a wide range throughout North America, including the Laurentian Great Lakes, Hudson Bay-James Bay, and the Mississippi River. Many populations throughout their range are reduced in size relative to historic population numbers due mainly to overfishing and habitat modifications. Although fishing has been restricted in many jurisdictions, habitat changes, such as the construction and operation of dams, continue to have an effect on some lake sturgeon populations. On the Ottawa River, greater abundance and faster growth of lake sturgeon were observed on reaches that were not impounded (Haxton and Findlay 2008). On the Mattagami River, hydroelectric operations appeared to have an effect on reproductive development (McKinley et al. 1998). Flow regimes on the Sturgeon River had an impact on sturgeon spawning activity (Auer 1996*a*). In the Red River of the North basin, lake sturgeon have been extirpated due to dams blocking access to historic spawning grounds (Aadland et al. 2005).

Studies on lake sturgeon movements can provide additional information on the potential impacts of artificial barriers. Current studies offer conflicting information, with some reporting large migration distances and others reporting minimal movement. In the Great Lakes, a range of adult migration distances from 32-225 km have been reported (reviewed in Auer 1996*b*). Lake sturgeon have also been reported to move between lakes within the Great Lakes basin (Gunderman et al. 2004). Movements of juveniles appear to be shorter (Holtgren and Auer 2004, Smith and King 2005). In the upper Mississippi River, lake sturgeon movements ranged from 3-198 km, with fish moving both upstream and downstream past dams (Knights et al. 2002). In the Ottawa River, movements of four radio-tracked lake sturgeon were limited (Haxton 2003). The sturgeon remained in their respective basins and the maximum distance traveled was 10 km. These studies, however, are often limited by small sample sizes and do not provide any information about whether individuals reproduce at the site to which they migrate.

Genetic studies can add to information elucidated through field techniques by detecting population structure and determining whether there is sufficient migration with subsequent reproduction for groups to be considered a single population. An early study using mitochondrial DNA examined genetic variability in the Hudson Bay/James Bay system and the Great Lakes (Ferguson and Duckworth 1997). Two haplotypes (or genetic variants) predominated in the Hudson Bay/James Bay while only a single haplotype was found in the

Great Lakes. Subsequent studies using microsatellites, a highly variable genetic marker, found substantial population structure within the Great Lakes (McQuown et al. 2003, DeHaan et al. 2006, Welsh et al. 2008). Genetic differences were also observed at finer scales such as in inland lakes (Welsh 2006). Populations with limited gene flow will likely have significant genetic differences. Therefore, genetic data can help identify areas of low reproductive connectivity.

The Namakan River in Ontario contains several rapids that may act as potential barriers to complete migration along the river. The river connects Lac La Croix to Namakan Lake and ultimately flows into the Rainy River system and Hudson Bay. Three hydroelectric facilities are being proposed for construction. Currently, nine natural rapids exist along the river and the objective of this study was to determine if some of these rapids significantly limit gene flow between lake sturgeon in the different stretches of the river. The null hypothesis is that lake sturgeon along the Namakan River constitute a single population with relatively equal amounts of downstream and upstream movement. Insignificant genetic differentiation and insignificant differences in genetic diversity between groups on either side of the rapids would then be expected. Alternatively, sturgeon may be able to move downstream but the rapids may prevent upstream movements. Groups would still be genetically indistinguishable but higher levels of genetic diversity would be expected at downstream locations due to the higher level of immigration (Jager et al. 2001). Finally, the rapids may prevent movement in either direction. Groups on either side of the rapids would then be expected to have significant genetic differences due to limited genetic exchange.

Methods

Fin clips from the tip of the pectoral fin were collected during spring 2007 from lake sturgeon at five spawning sites along the Namakan River: A) below Lady Rapids (n=31), B) below Hay Rapids (n=30), C) below Back Channel (Little Eva Lake) (n=31), D) below Quetico Rapids (Bill Lake) (n=14), and E) below Ivy Falls (n=23). Groups consisted of a mixture of mature and immature fish so not all the samples came from actively spawning adults. However, sampling was conducted during the active spawning season and water temperatures were within the range conducive for spawning. Sampling for mature and ripe individuals can be difficult at a specific site due to spawning periodicity, migration patterns, and fish behavior.

Tissue samples were preserved in 95% ethanol and stored in plastic centrifuge vials. DNA was extracted using either the Promega Wizard SV 96 Genomic DNA Purification System or the Gentra Puregene Tissue Kit, according to manufacturers' protocols. Extracts were then quantified either using a microplate reader or a fluorometer. Twelve microsatellite loci were then used (AfuGs 9, 56, 63, 74, 112, 160, 195, 204; Afu 68, 68b; Spl 120; Aox 27) (described in Welsh and May 2006) in the polymerase chain reaction (PCR). PCR reagents included: 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 µM fluorescently-labeled forward primer, 0.2 µM unlabeled reverse primer, 0.25 U GoTaq polymerase (Promega), and 20 ng of DNA. A BioRad iCycler was used and thermal cycling conditions for all loci except AfuGs 9 and 56 were as follows: 95˚C for two minutes; 40 cycles of 95˚C for 30 seconds, 52˚C for 30 seconds, and 72˚C for 45 seconds; 72˚C for seven minutes, ending with a 4˚C hold.. Thermal cycling conditions for AfuGs 9 and 56 were: 94˚C for one minute; 20 cycles of 92˚C for 30 seconds and 70˚C for 40 seconds with a 0.5˚C decrease in the second step each cycle; 20 cycles of 92˚C for 30 seconds and 60˚C for 40 seconds with a one-second increase in the second step each cycle; ending with a 4˚C hold. PCR products were then pooled into three groups and visualized on a Beckman Coulter CEQ 8000 Genetic Analysis System. Sample proportions in the three groups were: Group 1 (AfuGs 9, 56, 74, 204) – 2:3:2:3; Group 2 (AfuG 195, Afu 68, 68b, Spl 120) – 3:2:5:3; Group 3 (AfuGs 112, 160, Aox 27, AfuG 63) – 2:2:2:3.

Genetic differentiation between groups along the Namakan River was measured using the Weir and Cockerham (1984) estimator of F_{ST} (estimated using the software Arlequin (Schneider et al. 2000)) and pairwise contingency tests of allele frequency heterogeneity (Raymond and Rousset 1995), estimated using the software TFPGA (Miller 1997). F_{ST} values can range from zero to one, with zero signifying no genetic differentiation and one indicating complete differentiation at all loci. The significance of the pairwise F_{ST} comparisons was based on 1023 permutations. For the pairwise contingency tests, 10 batches of 2000 permutations each were run, with 1000 dememorization steps. Significance of both the pairwise F_{ST} and contingency tests was assessed after a sequential Bonferroni correction (Rice 1989). Genetic diversity of each group along the Namakan River was also measured using heterozygosity and allelic richness (number of alleles corrected for differences in sample size; El Mousadik and Petit 1996). TFPGA was used to calculate heterozygosity and the software FSTAT (Goudet 2001)

was used to measure allelic richness. Significant differences in genetic diversity were tested using t-tests.

The statistical tests described above were also used to compare the Namakan River to other sampled spawning populations in the Hudson Bay/James Bay system and in Lake Superior, the Great Lake closest to the Namakan River. If no significant differences had been detected between the five Namakan River groups, they were grouped into a single population for the purpose of this analysis. Additionally, a visual analysis of the genetic relationships among spawning populations were conducted through the construction of a neighbor-joining tree, based on Cavalli-Sforza and Edwards' (1967) chord distance, using the software package Phylip (Felsenstein 2004). Bootstrap values demonstrating statistical support for the groupings in the tree were calculated based on 1000 replicates. Populations used in this analysis include Hudson Bay/James Bay populations (Mattagami River (n=40), Rainy River/Lake of the Woods (n=27)) and Lake Superior populations (Bad River (n=136), White River (n=43), Kaministiquia River (n=85), Black Sturgeon River (n=57), Pic River (n=33), and Goulais River (n=43)). Genetic data for these populations were originally reported in Welsh et al. (2008).

Results

There were no significant genetic differences between the five spawning locations along the Namakan River (Table 1). Pairwise F_{ST} values were low, ranging from 0.00-0.03. All F_{ST} values and pairwise contingency values were not significant. Heterozygosity and allelic richness values were similar among the five different groups (Figure 1). Therefore, there were no significant differences in genetic diversity between any of the five groups.

When comparing the Namakan River to the other Hudson Bay/James Bay locations and Lake Superior locations, all populations had significant genetic differences, with the exception of the Bad and White Rivers of Lake Superior (Table 2). The Namakan River was most similar to the Rainy River/Lake of the Woods and populations from the Hudson Bay/James Bay system clearly separated from the Lake Superior populations (Figure 2). The Namakan River population is highly differentiated from the Lake Superior populations (average F_{ST} = 0.25). The genetic diversity of the Namakan River was lower than the diversity measured in the other populations (Figure 3). Heterozygosity observed in the Namakan River was significantly lower than heterozygosity observed in Lake Superior populations. Allelic richness in the Namakan River

was significantly lower than the Goulais and Pic Rivers of Lake Superior. The Namakan River had an observed heterozygosity of 0.34 and an allelic richness of 2.78.

Discussion

The genetic data suggest that the putative spawning groups along the Namakan River represent a single population. If the rapids along the river presented a barrier to migration, genetic differences would have likely accumulated between the groups. Instead, no significant genetic differences were observed between the groups. Additionally, there were no significant differences in genetic diversity between the five groups, indicating that migration is likely occurring in both directions along the river. The possibility exists that an insufficient amount of time since isolation has passed for the accumulation of genetic differences. However, because the rapids are natural barriers, they have likely been a potential barrier for a relatively long time.

The genetic diversity observed in the Namakan River is lower than the diversity observed in other Hudson Bay/James Bay populations and significantly lower than Great Lakes populations. The diversity is also lower than that observed for most freshwater fishes (average heterozygosity = 0.46; DeWoody and Avise 2000). Possible reasons for low genetic diversity include reduced population size, inbreeding, or isolation from source populations.

The rapids along the Namakan River do not represent reproductive barriers to lake sturgeon and future management actions should preserve the integrity of this population. Fragmentation along rivers resulting from artificial barriers can lead to substantial genetic differentiation evolving within a few generations (e.g., Hanfling and Weetman 2006, Heggenes and Roed 2006). Continual upstream and downstream migration can maintain the genetic diversity along all the segments of the river (Jager et al. 2001, Reid et al. 2008) and prevent further erosion of the remaining genetic diversity in lake sturgeon along the Namakan River.

Literature Cited

Aadland, L.P., T.M. Koel, W.G. Franzin, K.W. Stewart, and P. Nelson. 2005. Changes in fish assemblage structure of the Red River of the North. Pages 293-321 *in* J.N. Rinne, R.M. Hughes, and B. Calamusso, editors. Symposium on changes in fish community structures in large USA rivers. American Fisheries Society, Symposium 45, Bethesda, Maryland.

Auer, N.A. 1996*a*. Response of spawning lake sturgeons to change in hydroelectric facility operation. Transactions of the American Fisheries Society 125: 66-77.

Auer, N.A. 1996*b*. Importance of habitat and migration to sturgeons with emphasis on lake sturgeon. Canadian Journal of Fisheries and Aquatic Sciences 53(Suppl1): 152-160.

Cavalli-Sforza, L.L., and A.W.F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. American Journal of Human Genetics 19:233-257.

DeHaan, P.W., Libants, S.T., Elliott, R.F., and Scribner, K.T. 2006. Genetic population structure of remnant lake sturgeon populations in the upper Great Lakes basin. Transactions of the American Fisheries Society 135:1478-1492.

DeWoody, J., and J. Avise. 2000. Microsatellite variation in marine, freshwater and anadramous fishes compared with other animals. Journal of Fish Biology 56:461-473.

El Mousadik, A., and R.J. Petit. 1996. High level of genetic differentiation for allelic richness among populations of the argan tree [Argania spinosa (L.) Skeels] endemic to Morocco. Theoretical and Applied Genetics 92:832-839.

Felsenstein, J. 2004. PHYLIP (Phylogeny Inference Package) version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.

Ferguson, M.M. and Duckworth, G.A. 1997. The status and distribution of lake sturgeon, *Acipenser fulvescens*, in the Canadian provinces of Manitoba, Ontario and Quebec: a genetic perspective. Environmental Biology of Fishes 48: 299-309.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available: http://www.unil.ch/izea/softwares/fstat.html. (November 2005).

Gunderman, B., Elliott, R., Lang, R., Lychwick, T., Meronek, T., and Kornely, G. 2004. Assessment of remnant lake sturgeon populations in the Green Bay basin, 2002-2003. Report to the Great Lakes Fishery Trust, Project Number 2001.113.

Hanfling B, Weetman D (2006) Concordant genetic estimators of migration reveal anthropogenically enhanced source-sink population structure in the river sculpin, *Cottus gobio*. Genetics 173:1487–1501

Haxton, T.J. 2003. Movement of lake sturgeon, *Acipenser fulvescens*, in a natural reach of the Ottawa River. Canadian Field-Naturalist 117: 541-545.

Haxton, T.J. and Findlay, C.S. 2008. Variation in lake sturgeon (*Acipenser fulvescens*) abundance and growth among river reaches in a large regulated river. Canadian Journal of Fisheries and Aquatic Sciences 65: 645-657.

Heggenes, J. and Roed, K.H. 2006. Do dams increase genetic diversity in brown trout (*Salmo trutta*)? Microgeographic differentiation in a fragmented river. Ecology of Freshwater Fish 15: 366-375.

Holtgren, J.M. and Auer, N.A. 2004. Movement and habitat of juvenile lake sturgeon (*Acipenser fulvescens*) in the Sturgeon River/Portage Lake system, Michigan. Journal of Freshwater Ecology 19: 419-432.

Jager, H.I., Chandler, J.A., Lepla, K.B., and Van Winkle, W. 2001. A theoretical study of river fragmentation by dams and its effects on white sturgeon populations. Environmental Biology of Fishes 60: 347-361.

Knights, B.C., Vallazza, J.M., Zigler, S.J. and Dewey, M.R. 2002. Habitat and movement of lake sturgeon in the upper Mississippi River system, USA. Transactions of the American Fisheries Society 131: 507-522.

McKinley, S., Van der Kraak, G., and Power, G. 1998. Seasonal migrations and reproductive patterns in the lake sturgeon, *Acipenser fulvescens*, in the vicinity of hydroelectric stations in northern Ontario. Environmental Biology of Fishes 51: 245-256.

McQuown, E.C, C.C. Krueger, H.L. Kincaid, G.A.E. Gall, and B. May. 2003. Genetic comparison of lake sturgeon populations: differentiation based on allelic frequencies at seven microsatellite loci. Journal of Great Lakes Research 29:3-13.

Miller, M.P. 1997. Tools for population genetic analyses (TFPGA) 1.3: a Windows program for the analysis of allozyme and molecular population genetic data. Computer software distributed by author.

Raymond, M.L., and F. Rousset. 1995. An exact test for population differentiation. Evolution 49:1280-1283.

Reid, S.M., Wilson, C.C., Mandrak, N.E., and Carl, L.M. 2008. Population structure and genetic diversity of black redhorse (*Moxostoma duquesnei*) in a highly fragmented watershed. Conservation Genetics 9: 531-546.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution 43:223-225.

Schneider, S., D. Roessli, and L. Excoffier. 2000. Arlequin ver 2.000: a software for population genetics data analysis. Available: http://anthro.unige.ch/arlequin. (November 2005).

Smith, K.M. and King, D.K. 2005. Movement and habitat use of yearling and juvenile lake sturgeon in Black Lake, Michigan. Transactions of the American Fisheries Society 134: 1159-1172.

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

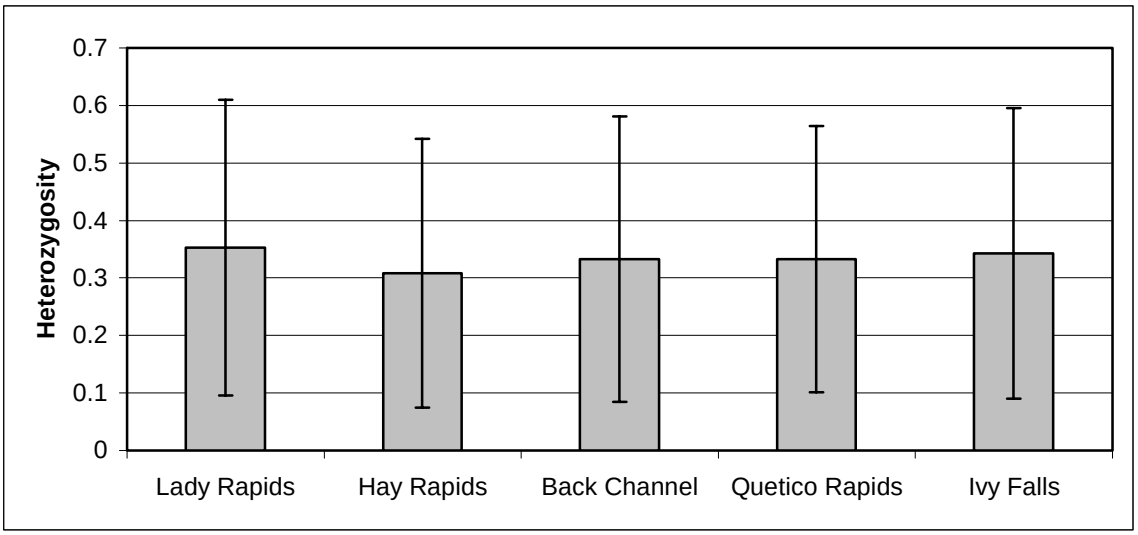
Welsh, A. 2006. Lake sturgeon conservation in the Great Lakes: scaling it up from genetics to policy. Ph.D. dissertation, Department of Animal Science, University of California – Davis.

Welsh, A., and B. May. 2006. Development and standardization of disomic microsatellite markers for lake sturgeon genetic studies. Journal of Applied Ichthyology 22:337-344.

Welsh, A., Hill, T., Quinlan, H., Robinson, C., and May, B. 2008. Genetic assessment of lake sturgeon population structure in the Laurentian Great Lakes. North American Journal of Fisheries Management 28: 572-591.

Figure 1. Genetic diversity of the five putative spawning groups along the Namakan River. Standard deviations are also displayed. A) Observed heterozygosity averaged across all 12 loci. B) Allelic richness averaged across all 12 loci.

A)



B)

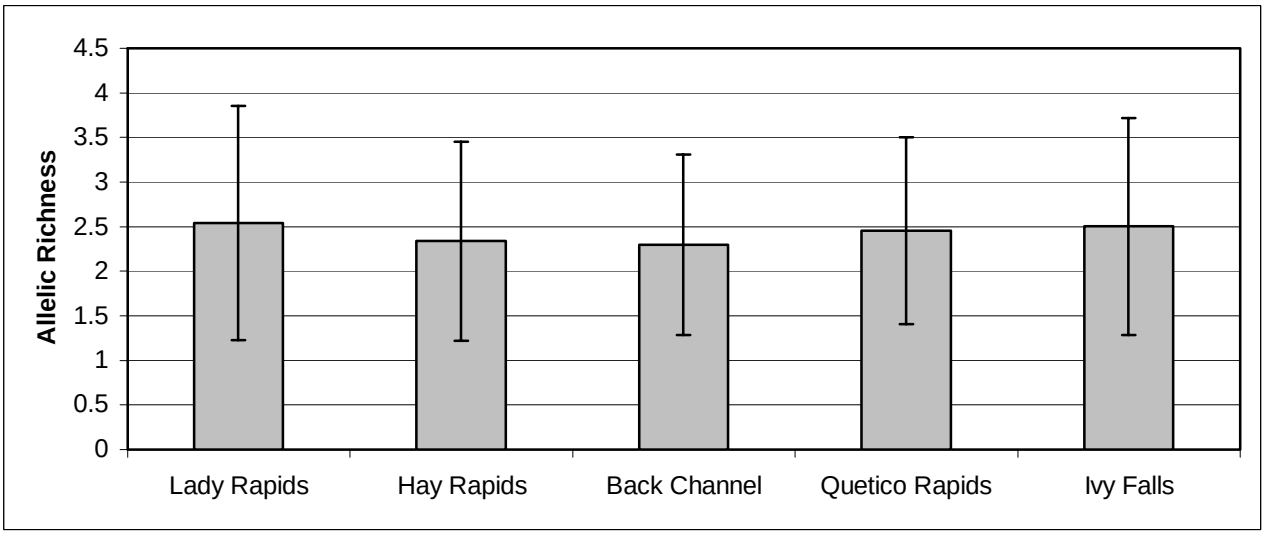


Figure 2. Neighbor-joining tree based on Cavalli-Sforza and Edwards' chord distance. Bootstrap values, or percentage of 1000 replicates supporting the group, that exceed 50 percent are displayed. Genetic distance scale is included.

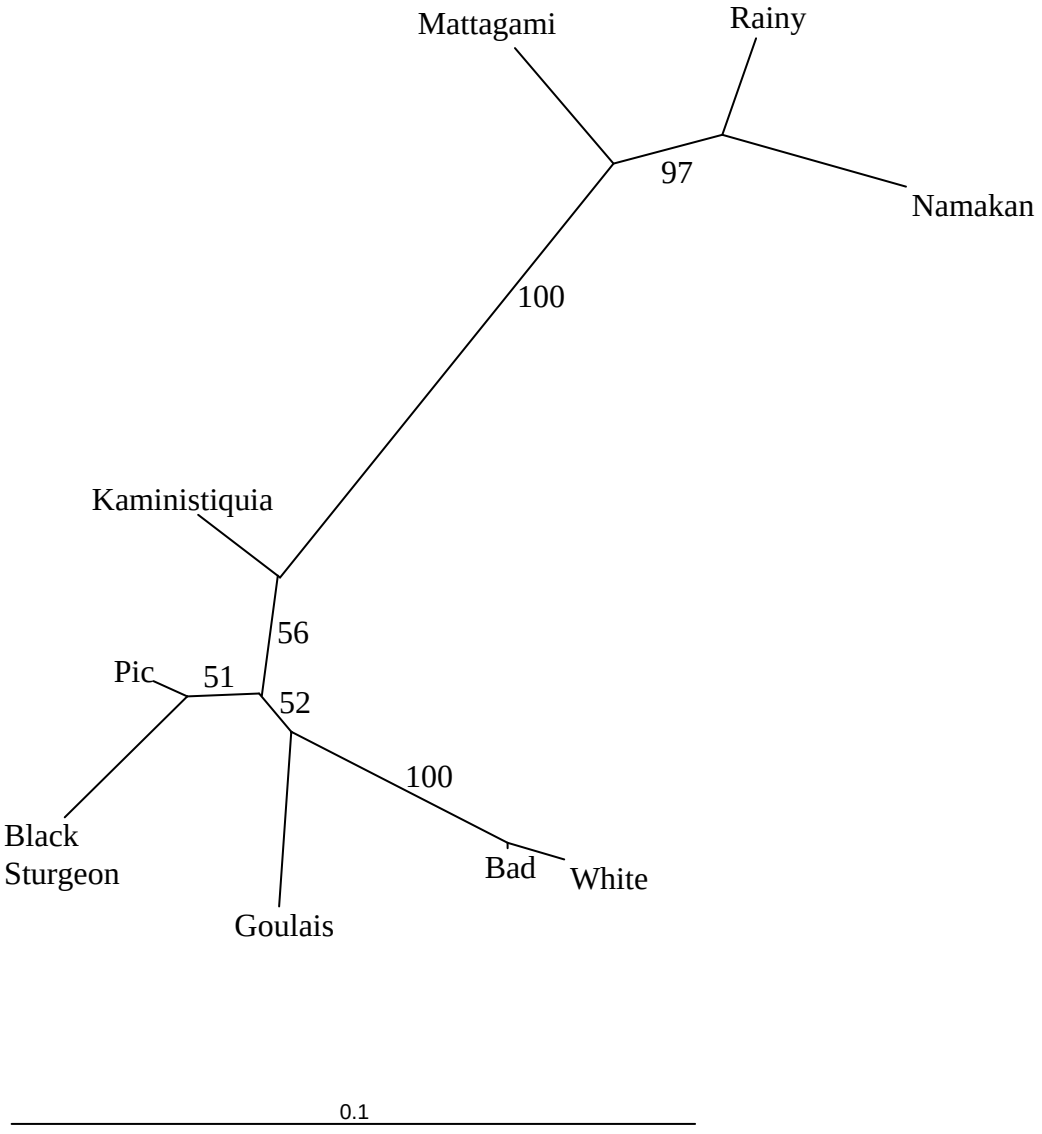
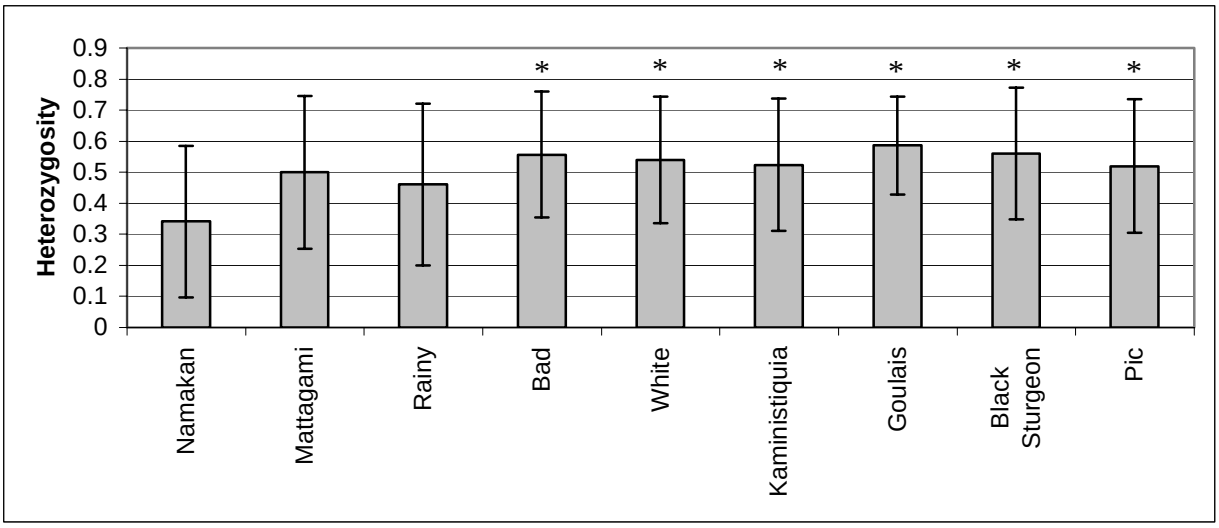


Figure 3. Genetic diversity of the five putative spawning groups along the Namakan River. Standard deviations are also displayed. Asterisks denote populations that have genetic diversity that is significantly different from the Namakan River ($p<0.05$). A) Observed heterozygosity averaged across all 12 loci. B) Allelic richness averaged across all 12 loci.

A)



B)

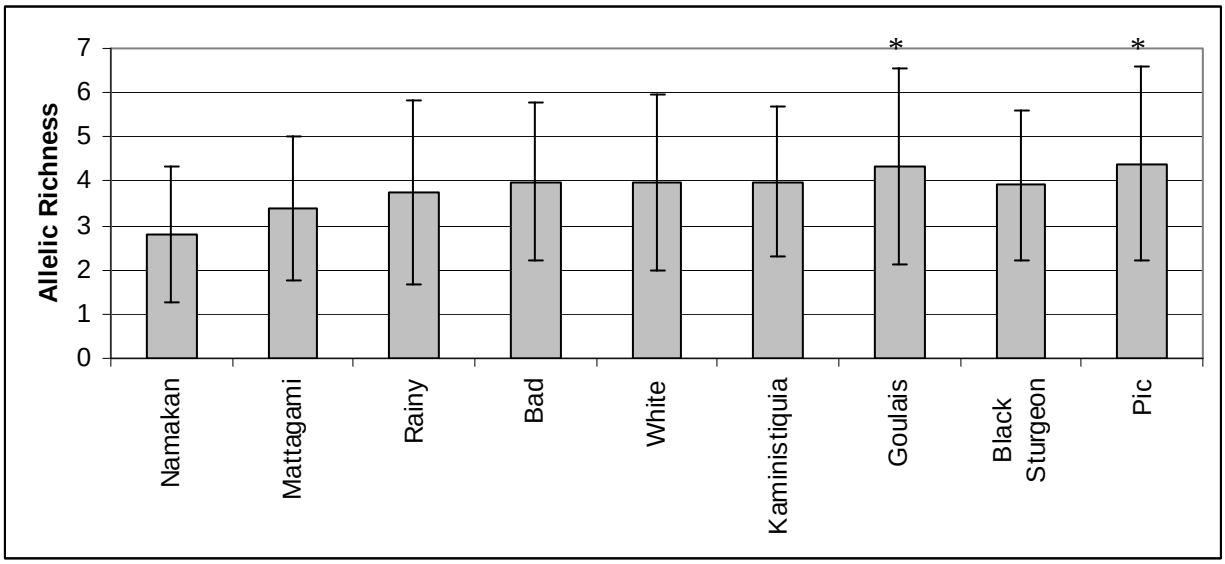


Table 1. Chi-square values from pairwise contingency tests (above diagonal) and pairwise F_{ST} values (below diagonal). No comparisons were significant.

	A	B	C	D	E
A		24.07	24.05	24.60	20.53
B	0.01		25.19	31.53	23.00
C	0.00	0.01		21.96	31.25
D	0.00	0.03	0.00		29.38
E	0.01	0.01	0.01	0.02	

Table 2. Chi-square values from pairwise contingency tests (above diagonal) and pairwise F_{ST} values (below diagonal). Comparisons in bold are **NOT** significant after sequential Bonferroni correction ($p>0.05$ for F_{ST} comparisons).

	Namakan	Mattagami	Rainy	Bad	White	Kaministiquia	Goulais	Black Sturgeon	Pic
Namakan		139.92	155.99	204.89	191.39	213.76	198.24	220.20	218.16
Mattagami	0.16		114.60	198.67	200.45	191.88	206.67	206.10	211.52
Rainy	0.08	0.09		186.66	187.32	184.29	222.69	169.44	173.83
Bad	0.26	0.16	0.18		45.60	202.61	193.65	171.94	175.95
White	0.30	0.18	0.21	**0.00**		188.32	168.98	164.17	166.76
Kaministiquia	0.25	0.15	0.17	0.12	0.13		167.81	195.01	144.55
Goulais	0.26	0.15	0.18	0.08	0.09	0.07		181.77	125.19
Black Sturgeon	0.22	0.15	0.13	0.11	0.13	0.10	0.07		115.70
Pic	0.23	0.16	0.15	0.10	0.12	0.07	0.05	0.03	