GENETIC VARIATION AND HYBRIDIZATION WITH WALLEYE IN MONTANA SAUGER POPULATIONS DETERMINED BY PROTEIN ELECTROPHORESIS AND MICROSATELLITE ANALYSIS

by

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Executive Summary

Saugers (*Sander canadensis*) are native to Montana, but walleyes (*S. vitreus*) have been introduced and stocked extensively. Concern has been expressed on the decline of sauger populations in Montana. Altered river flows and reservoir water levels, construction of dams, and hybridization with introduced walleyes are among the factors cited for this decline. This study surveyed genetic variation in Montana sauger populations by protein electrophoresis and determined the extent of hybridization and introgression between native saugers and introduced walleyes.

Sauger specimens were collected from 21 sites; 16 sites in Montana and four sits in neighboring Wyoming and Alberta, Canada, from drainages that flow into Montana, and one site from North Dakota, downstream from Montana on the Missouri River system. Four (*mMDH-1*, PGM-1*, ALAT** and *IDDH**) diagnostic loci between saugers and walleyes and two (*sMDH-3** and *PROT-3**) informative loci in saugers were used to detect sauger-walleye hybrids. Hybrid and introgressed fish were found at 12 of the 18 sites examined after pooling to address low sample sizes at three sites. Hybridization rates ranged from 0-22% in the Missouri River drainage and 0-4% in the Yellowstone River drainage, although rates of up to 10% were observed in potential Yellowstone River brood fish, and 20.4% in Lake Sakakawea, ND. Several microsatellite loci offer potential for analysis of hybridization between walleye and sauger. Brood stock to be used for supplemental sauger stocking should be genetically screened to prevent the propagation and accidental stocking of hybrids, but a more reliable way of conducting this screening will be required.

Two (*EST** and *SOD-2**) of the 35 loci analyzed were polymorphic in Montana saugers. Montana populations showed moderate structuring **(**F_{ST} = 0.091**)** and were partitioned into two

main genetic groups. These two genetic groups did not coincide with the two main river drainages, the Missouri River and Yellowstone River drainages. One of these groups consisted of fish from the Missouri River below Fort Peck Reservoir dam and the Milk River below the Fresno Reservoir dam, and fish from the Boysen Reservoir in Wyoming. The other main group contained a mixture of fish from both the Missouri River and Yellowstone River drainages. Milk River saugers from above the Fresno Reservoir dam had significantly different allele frequencies from those below the dam. Significant genetic heterogeneity was found among all of the sauger composite populations examined. Several composite populations showed significant deviations from Hardy-Weinberg expectations, all due to heterozygote deficits, likely caused by the Wahlund effect because sampling was not confined to spawning populations, something that should be avoided in future studies of genetic variation in Montana sauger. Due to the population differentiation present in Montana sauger populations, they should be managed individually and stock transfer is not recommended.

Microsatellite DNA analysis revealed a high level of genetic variability in Montana sauger populations, with most of the variation occurring within rather than among populations. Nevertheless, the Bighorn River population from Wyoming was significantly different from all of the other populations examined. Microsatellite DNA analysis offers promise for future studies on Montana sauger populations because non-lethal samples such as scales could be examined for genetic variation once DNA has been extracted from cells attached to them. This would permit a more thorough sampling of spawning populations and a possible analysis of any archived sauger scale samples to search for historic trends in genetic variation in Montana sauger.

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INTRODUCTION

Sauger (*Sander canadensis*, formerly *Stizostedion canadense*) a large predatory fish in the family Percidae, is native to Montana. The congeneric walleye (*Sander vitreus*, formerly *Stizostedion vitreum*) is not native to Montana, but has been introduced and extensively stocked across the state into drainages containing saugers. In a recent review, McMahon and Gardner (2001) expressed concern about the decline in Montana sauger populations since the late 1980s. They attributed this decline to: (1) low river flows and reservoir water levels, (2) habitat loss and migratory barriers, (3) competition and hybridization with walleye, (4) interaction with other species, and (5) over exploitation. A severe drought in the late 1980s was thought to be responsible for the decline, but an apparent lack of rebound in sauger abundance despite improved flow conditions raised major concerns (McMahon and Gardner 2001). The role of entrainment in diversion channels in causing non-angling mortality was recently confirmed by tagging studies (Jaeger 2004), although significant over exploitation was not shown in this study.

The historical distribution of saugers in Montana (Figure 1) included the Missouri River and its major tributaries downstream of the Great Falls, as well as the Yellowstone River and its major tributaries downstream, including the Clark Fork (McMahon and Gardner 2001). In the past, saugers likely occupied about 3,376 km of Montana's riverine habitat. The distribution of saugers presently has declined by 53% from historical levels to an estimated 1570 km. Their decline has been more widespread in tributaries where currently saugers occupy only 479 km from the estimated 1896 km distribution in the past, a 75% reduction (McMahon and Gardner 2001). The current distribution of saugers in the Missouri River is limited to the main-stem Missouri and a few sections of the Marias and Milk rivers. Saugers are considered rare or absent in other major tributaries such as the Teton, Judith, Musselshell, and Poplar rivers. In the

Yellowstone River drainage, the present distribution is confined to the lower main-stem Yellowstone. Saugers are considered rare or absent in major tributaries such as the Bighorn and Powder rivers. However, saugers are present in a small section of the upper Powder River and are found in the Bighorn River system in Wyoming (McMahon and Gardner 2001).

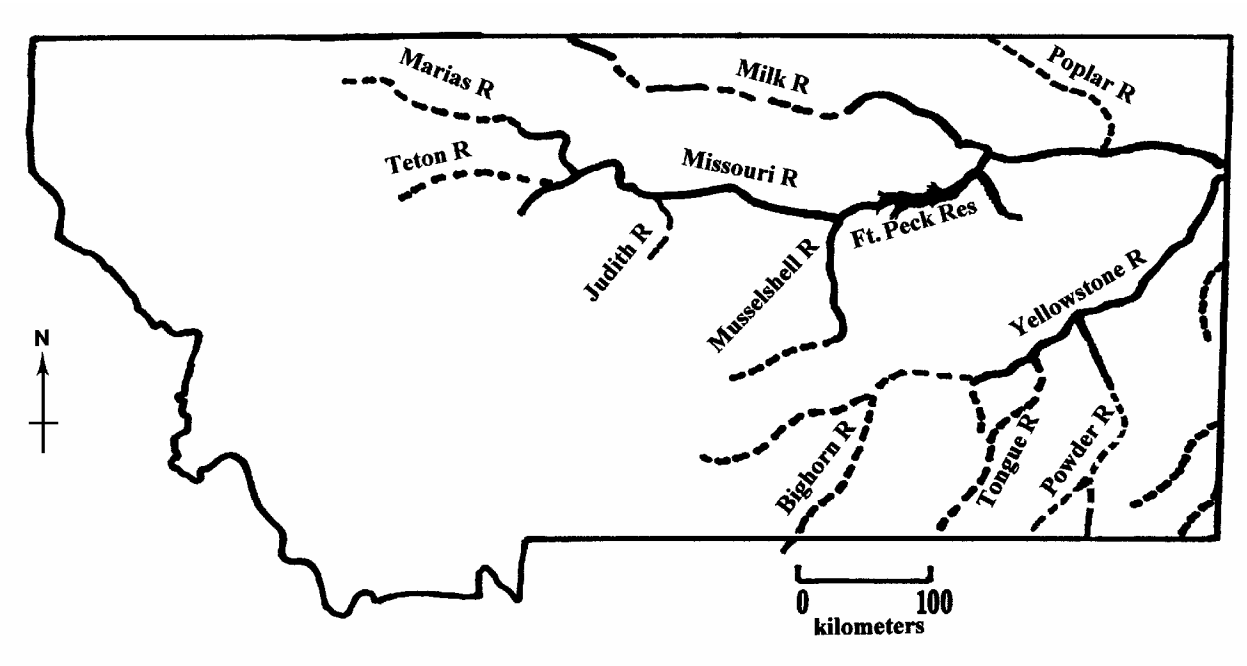

Figure 1. Estimated historical and present distribution of saugers in Montana. Solid lines represent areas where saugers are still present and dashed lines represent areas where saugers were present historically but are now rare or absent (redrawn from McMahon and Gardner 2001).

Literature devoted to describing the distribution of intraspecific genetic variation in fishes has increased dramatically in the recent past (reviewed by Ryman and Utter 1987; Utter 1991; May 2003). Protein electrophoretic analyses have revealed substantial amounts of genetic variation among different populations of a single species (Ward et al. 1989; Todd 1990; Echelle et al. 1999; Mitchell et al. 2002; White et al. 2005). These studies support the hypothesis that geographic variation occurs among populations of the same species.

Genetic variation inherent in a species can be partitioned into two components, intra-population variation (variation within a single population) and inter-population (variation among different populations). Loss of variation among individuals of a population occurs due to natural selection and genetic drift, processes that are intensified when populations become small, while variation among populations occurs due to populations subdivision, local adaptation, and lack of gene flow. Loss of genetic variation can result from mixing of previously isolated populations, stocking, or gene flow, resulting in one homogenous population (Meffe 1986; Philipp and Claussen 1995). In addition to loss of variation due to mixing of populations, out-breeding depression, the loss of fitness in offspring produced by distantly related parents, results in the loss of co-adapted gene complexes suited for local environments (Templeton 1986).

In addition to protein electrophoresis, other molecular genetic markers such as mitochondrial DNA (Ward et al. 1989; Brunner et al. 1998; Billington 2003; White et al. 2005) and microsatellites (Hansen et al. 1999; Lemaire et al. 2000; De Innocentiis et al. 2001; Eldridge et al. 2002; Meldgaard et al. 2003; White et al. 2005) have been used in population structure studies. All these genetic markers yield relatively concordant amounts of genetic differentiation among populations with a few exceptions (Lemaire et al. 2000; De Innocentiis et al. 2001). However, many loci need to be analyzed when estimating genetic differentiation to infer historical levels of gene flow and patterns of genetic exchange among populations (Campton 1987, 1990; Allendorf and Seeb 2000).

Knowledge of the genetic structure of natural populations is essential for effective management in conservation biology (Frankel 1974). Such information is particularly significant in riverine fishes because restriction of gene flow is possible and levels of population subdivision are thus increased (Meffe 1986; Allendorf and Leary 1988; Mitchell et al. 2002; Meldgaard et al.

2003). The limited studies that exist on genetic variation in sauger populations have reported low amounts of allozyme variation (Uthe et al. 1966; Billington et al. 1990, 1996, 1997a; White and Schell 1995; Kreuger et al. 1997). White and Schell (1995) concluded that the little genetic variation that they observed in Ohio River sauger populations show that they exist as a panmictic unit, or the migratory barriers in the river do not significantly affect gene flow among the sauger populations. There are no prior data documenting genetic variation within and among sauger populations in Montana. Insight on the extent of genetic variation in Montana sauger populations and the partitioning of such variation will provide valuable information on sauger population subdivision or stock structure and provide an assessment of the risk of inbreeding.

Interspecific hybridization is a widespread phenomenon in fishes partially due to their employment of external fertilization (Schwartz 1981). However, hybridization is more common among freshwater species than in marine species (Hubbs 1955). Introgression, the incorporation of genes of one species into the gene complex of another species, occurs when viable first generation (F_1) hybrids reproduce with either of the parental species. In extreme cases, introgression will lead to the formation of hybrid swarms where the genes of parent species are distributed randomly in a population with no F_1 hybrids or pure parental taxa (Leary et al. 1995) or even the emergence of a new taxon (DeMarais et al. 1992). Some workers argue, however, that lack of introgression is not benign, it is equivalent to wasted reproductive effort that may increase the ability of one species to eliminate another species, especially if the species attains maturity earlier and has higher fecundity or when the species is more abundant (Leary et al. 1993; Leary et al. 1995; Konishi et al. 2003). Intraspecific hybridization also occurs, but it is not easily detected due to genetic similarities between the species (Leary et al. 1995).

Saugers and walleyes are known to hybridize naturally (Stroud 1948; Flammang and Willis 1993; Van Zee et al. 1996; Billington et al. 1997a, 2004, 2005a; Billington and Koigi 2004; White et al. 2005) and artificially under experimental conditions (Nelson 1968; Hearn 1986). Their hybrids, especially saugeye (an F_1 hybrid resulting from a cross between a female walleye and a male sauger) have been produced and extensively stocked in central United States because they can withstand warm, eutrophic waters with high flushing rates better than walleyes, and they have faster growth rates than either of the parental species (Lynch et al. 1982; Johnson et al. 1988; Leeds 1989; Stahl et al. 1996).

Several external morphological characteristics distinguish saugers from walleyes (Trautman 1981; Page and Burr 1991). Saugers have darker skin pigmentation (dark-yellow to brown), scaled cheeks, three dark saddles that extend all the way down the sides of their bodies, and a series of dark speckles arranged in a number of lines across their first dorsal fin. Walleyes, have lighter skin pigmentation (light-yellow to green), un-scaled cheeks, thirteen short, lightly colored saddles that extend slightly down the sides of their bodies, and a dark blotch on the posterior end of the first dorsal fin. First generation (F_1) hybrids are likely to be intermediate for the characteristics of the parents but features of both parents are often expressed (Trautman 1981). Consequently, it is usually very difficult to distinguish backcrosses of these F_1 hybrids to either of the parental species by morphology because they tend to closely resemble one of the parental species. In addition, Nelson (1968) found that embryo and larval F_1 hybrids tended to closely resemble the female parent.

Hybrids can be easily detected by genetic screening when diagnostic loci between the species involved have been identified (Campton 1987, 1990). Saugers and walleyes show fixed allelic differences at four protein coding loci: *mMDH-1** for malate dehydrogenase (E.C

1.1.1.37) and *PGM-1** for phosphoglucomutase (E.C. 5.4.2.2) in muscle tissue, and *ALAT** for alanine aminotransferase (E.C. 2.6.1.2) and *IDDH** for L-iditol 2-dehydrogenase (E.C. 1.1.1.14) in liver tissue (Clayton et al. 1973; Billington et al. 1990; Todd 1990; Van Zee et al. 1996). Two additional loci, *sMDH-3** (also known as *sMDH-B**, for malate dehydrogenase) (E.C. 1.1.1.37) and *PROT-3** (general muscle protein – which has no E.C. number), are informative in saugers (polymorphic in walleyes, but fixed for one allele in saugers) (Billington et al. 1990), and one additional locus *SOD-2** (superoxide dismutase) (E.C. 1.15.1.1) is informative in walleyes (polymorphic in saugers, but fixed for one allele in walleyes) (Billington and Koigi 2004). Enzyme numbers are those recommended by the International Union of Biochemistry and Molecular Biology, Nomenclature Committee (IUBMBNC 1992), and genetic nomenclature follows that recommended by Shaklee et al. (1990). By using these loci, it is possible to screen *Sander* specimens by protein electrophoresis to confirm species identification, detect F_1 hybrids, and second-generation (F_2) hybrids or backcrossed individuals (often jointly referred to as F_x hybrids). The F_1 hybrids will be heterozygous at all of the four diagnostic loci, while the F_x hybrids will be heterozygous at some loci and homozygous at the others. The direction of the backcrossing can then often be inferred from the homozygous alleles.

Studies have established the natural occurrence of sauger-walleye hybrids, introgression between the two species, and the difficulties of distinguishing saugers, walleyes, and their hybrids using morphological characteristics (Flammang and Willis 1993; Ward and Berry 1995; Van Zee et al. 1996; Billington et al. 1996, 1997a, 2004, 2005a; White et al. 2005). Considering the reported cases of natural hybridization between these two *Sander* species and further complications of extensive stocking of saugeyes, Billington (1997) recommended genetic

screening of both walleyes and saugers prior to their use as brood stock in order to maintain genetic integrity of sauger and walleye populations.

Destruction of habitat is largely responsible for the extinction of native fish species (Echelle et al. 1999). However, introduced species have been reported to significantly contribute to further loss of native species. A state survey on the use of native and non-native species in fishing programs reported that 36% of the states had more non-native sport fishes than they did native ones (Horak 1995). Horak (1995) also reported that 75% of the non-native fish had become well established and did not need further stocking to thrive. Therefore, management agencies need to assess native fish populations before implementing the introduction of non-native species in their recreational programs (Horak 1995; Lassuy 1995).

Hybridization between native saugers and introduced walleyes is among the factors that have been attributed to the decline of Montana saugers (McMahon and Gardner 2001). Previous studies have reported hybridization and introgression between saugers and walleyes in Montana ranging from 0-15% (McMahon and Gardner 2001; Koigi et al. 2004). The Montana Department of Fish, Wildlife and Parks (MDFWP) have used supplemental stocking to maintain sauger populations. They collect brood fish from the wild, spawn them and raise the fry and fingerlings for stocking. By so doing, there is a danger that they may inadvertently propagate sauger-walleye hybrids.

In this study, we surveyed genetic variation by protein electrophoresis and microsatellite DNA analysis in saugers from 21 sites (16 from Montana plus five from adjacent watersheds in Alberta, Wyoming, and North Dakota) to determine how this variation is partitioned. The extent of hybridization and introgression between native Montana saugers and introduced walleyes is also investigated by protein electrophoresis at four diagnostic and two informative loci. The

management implications of using wild saugers as brood stock for supplemental stocking and the use of protein electrophoretic screening to detect hybrids are also examined. In addition, the management implications of stocking walleyes and/or hybrids in Montana are discussed.

METHODS

Sample collection

Montana Department of Fish Wildlife and Parks (MDFWP) personnel collected sauger specimens from 16 sites in Montana (11 sites in the Missouri River drainage and five sites from the Yellowstone River drainage) (Table 1). Most samples could not be obtained during the spring spawning season due to personnel constraints; samples were obtained during the summer and fall. As a consequence, we used major barriers such as dams or geographic distance to separate groups of sample sites in our analysis (W. Gardner – personal communication) rather than these sampling sites as in a previous report (Billington et al. 2005b). All samples were frozen and shipped to Troy University (TROY) via overnight express courier, where they were screened by protein electrophoresis. Five neighboring sites, one from the upper Milk River, Alberta, Canada, which drains into the Missouri River system, three from the Bighorn River system in Wyoming which drains into the Yellowstone River system (the Boysen Reservoir on the Wind River which is a tributary of the Bighorn River, the Bighorn River, and the Bighorn Reservoir), and one population from Lake Sakakawea, North Dakota, downstream of the confluence between the Missouri and Yellowstone rivers were also included in this study. Samples from Alberta were collected by the Alberta Conservation Association and shipped to TROY via the MDFWP. Samples from the Bighorn River and Bighorn Reservoir were collected by the Wyoming Game and Fish Department and shipped to TROY via the MDFWP, while the samples from the Boysen Reservoir, Wyoming, were collected by the Wyoming Game and Fish Department, frozen and shipped directly to TROY, as were samples from Lake Sakakawea, North Dakota, that were collected by the North Dakota Department of Game and Fish (Table 1, and Figures 2 and 3). From now on in this report, all of the populations surveyed will be referred

to as Montana sauger populations for brevity, because all of the drainages from outside of Montana run into or out of the state.

Table 1. Sampling sites and drainages, site codes, their geographical locations (latitude and longitude), and sample sizes of the 16 Montana, one Alberta, three Wyoming, and one North Dakota sauger sampling sites examined in this study.

Sampling sites	Site codes	Latitude	Longitude	Sample size
Missouri River Drainage				
Marias River	MAR	47.940˚N	110.519˚W	21
Judith River	JUD	47.707˚N	109.650˚W	16
Middle Missouri River upper reach	MMU	48.005˚N	110.263˚W	25
Middle Missouri River lower reach	MML	47.624˚N	108.677˚W	30
Fort Peck Reservoir upper	FPU	47.569˚N	107.944˚W	2
Fort Peck Reservoir lower	FPL	47.690˚N	107.390˚W	11
Lower Missouri River upper reach	LMU	48.080˚N	105.521˚W	23
Lower Missouri River lower reach	LML	47.967˚N	103.996˚W	31
Milk River upper reach (Alberta)	MKU	49.151˚N	112.208˚W	6
Milk River Fresno Reservoir	MKF	48.601˚N	109.944˚W	9
Milk River middle reach	MKM	48.579˚N	109.231˚W	32
Milk River lower reach	MKL	48.060˚N	106.290˚W	42
Yellowstone River Drainage				
Yellowstone River upper reach	YSU	46.260˚N	106.690˚W	10
Yellowstone River middle reach	YSM	46.270˚N	106.670˚W	33
Yellowstone River lower reach	YSL	47.160˚N	104.310˚W	52
Powder River	POW	46.718˚N	105.405˚W	10
Tongue River	TON	46.414˚N	105.864˚W	2
Boysen Reservoir, Wind River (Wyoming)	BOY	43.967˚N	108.605˚W	17
Bighorn River (Wyoming)	BHU	44.406˚N	108.313˚W	30
Bighorn Reservoir (Wyoming)	BHRV	45.161˚N	108.013˚W	1
Lake Sakakawea, North Dakota	SAK	47.724˚N	102.189˚W	44

Due to the small sample size (N = 2), the Tongue River sample (TON) was pooled with the adjacent Yellowstone River middle reach sample (YSM) for further analysis, the Fort Peck Reservoir upper sample (FPU) (N=2) was pooled with the adjacent Middle Missouri River lower

(MML) sample, and the Bighorn Reservoir sample (BHRV) (*N*=1) was pooled with the adjacent Bighorn River (BHU) sample. Thus, 18 sites will be referred to in further analysis.

Protein electrophoresis

Upon arrival at TROY the fish remained frozen at –20°C until analysis began. Small pieces (0.5 g) of muscle, liver, and eye tissue were extracted from each specimen and placed into a 1.5 ml microcentrifuge tube with 400-500 μL of distilled water and homogenized with a sonic dismembrator (Fisher Model 100) using three 10-second pulses. Ten microliters of each homogenate were loaded into each of the 12 wells of the applicator base (Helena Laboratories, Beaumont, Texas). Sample aliquots were applied to Titan III cellulose acetate gels and electrophoresed for 20 min at 200 V (Hebert and Beaton 1993). Two buffer systems were used; continuous 1X tris-glycine buffer (TG) pH = 8.5 and CAAPM buffer pH = 7.0 (Billington et al. 1990; Hebert and Beaton, 1993; Van Zee et al. 1996). The CAAPM buffer is more commonly known as tris-citrate buffer (Clayton and Tretiak 1972). Histochemical staining recipes of May (1992), and Hebert and Beaton (1993) were used to examine the products of 35 presumptive loci (Table 2). Gels could be scored after 2-15 minutes of staining for the diagnostic sauger and walleye alleles. Patterns at *ALAT** were visualized with UV light.

Six of the loci examined are useful for the detection of hybridization and introgression between saugers and walleyes. We screened at two loci known to be diagnostic between saugers and walleyes that can be resolved in muscle tissue: *mMDH-1** (walleye **100* allele; sauger **140* allele) and *PGM-1** (walleye **100* allele; sauger **80* allele), and two diagnostic loci that can be resolved in liver tissue *ALAT** (walleye **100* allele; sauger **85* allele) and *IDDH** (walleye **100* allele; sauger **-10* allele) (Billington et al. 1990; Van Zee et al. 1996). In addition, two informative loci in saugers were also screened *sMDH-3** and *PROT-4** from muscle (Billington

et al. 1990; Hebert and Beaton 1993; Van Zee et al. 1996). Walleyes are polymorphic for *sMDH-3** having three alleles (**70*, **100* and **120*), while saugers only have the **120* allele. Walleyes are polymorphic at *PROT-3** having two alleles (**100* and **160*), whereas saugers only exhibit the **160* allele. Therefore, if any fish identified as a sauger contained any of the other walleye alleles it must have received them through hybridization. Tissue extracts from a known walleye, sauger and saugeye were included on each batch of gels as mobility reference standards. The frequency of hybrid and introgressed individuals in each population was then determined.

Genetic nomenclature for protein electrophoretic data follows that recommended by Shaklee et al. (1990) and the enzyme numbers used are those recommended by the International Union of Biochemistry and Molecular Biology, Nomenclature Committee (IUBMNC 1992). Measures of genetic variability including mean number of alleles per locus (*A*), percent polymorphic loci (*P*), and average heterozygosity (*H*) were calculated following Pasteur et al. (1988). Deviations from Hardy-Weinberg Equilibrium (HWE), Rogers' (1972) genetic distances, and F-statistics (Wright 1969) were analyzed with the Genes in Populations 2.2 computer program (May et al. 1995). Gene flow among the populations, expressed as the number of migrants per generation (*Nm*), was estimated with POPGENE 1.31 (Yeh et al. 1999). A dendogram of genetic distances among the populations was constructed by using the un-weighted pair group method with arithmetic averages (UPGMA) in Genes in Populations 2.2 (May et al. 1995). Contingency chi-squared (χ^2) analysis of allele frequencies among populations at each locus and heterogeneity chi-square (χ^2) analyses among populations for both polymorphic loci were also calculated (Clarke 1980).

Table 2. Protein designations, Enzyme Commission numbers (IUBMBNC 1992), tissue sources and buffer systems used, and presumptive loci identified in the survey of genetic variation in Montana sauger.

Proteins examined, with abbreviations		**Enzyme Commission number**	**Tissue**	**Buffer system**	**Number loci**
Acid phosphatase	ACP	3.1.3.2	Liver	CAAPM	2
Adenylate kinase	AK	2.7.3.2	Muscle	TG	1
Alcohol dehydrogenase	ADH	1.1.1.1	Liver/Eye	TG	1
Aldehyde oxidase	AO	1.2.3.1	Liver/Eye	TG	1
Alkaline phosphatase	ALP	3.1.3.1	Liver/Eye	CAAPM	1
Alanine aminotranferase	ALAT	2.6.1.2	Liver/Eye	TG	1
Aspartate aminotransferase	AAT	2.6.1.1	Liver/Muscle	TG	2
Esterase	EST	3.1.1.1	Liver/Eye	CAAPM	1
Fumarase	FUM	4.2.1.2	Muscle	TG	1
Glucose-6-phosphate dehydrogenase	G6PDH	1.1.1.49	Muscle	TG	1
Glucose-6-phosphate isomerase	GPI	5.3.1.9	Muscle	TG	1
Glycerol-3-phosphate dehydrogenase	G3PDH	1.1.1.8	Liver/Muscle	TG	2
L-Iditol-2-dehydrogenase	IDDH	1.1.1.14	Liver	TG	1
Isocitrate dehydrogenase	IDHP	1.1.1.42	Muscle/Liver	TG	2
Lactate dehydrogenase	LDH	1.1.1.27	Liver/Eye	TG	3
Malate dehydrogenase	MDH	1.1.1.37	Muscle	TG	3
Malic enzyme	ME	1.1.1.40	Muscle	TG	1
General muscle protein	PROT	–.–.–.–	Muscle	TG	3
Phosphoglucomutase	PGM	5.4.2.2	Muscle	TG	2
6-phosphogluconate dehydrogenase	6GPDH	1.1.1.44	Liver/Eye	TG	2
Superoxide dismutase	SOD	1.15.1.1	Liver	TG	2
Xanthine dehydrogenase	XDH	1.1.1.20	Liver/Eye	TG	1

Microsatellite analysis.

Individual muscle plugs corresponding to the fish analyzed for protein variation were sub-sampled from the fish used for protein electrophoretic analysis, frozen, and shipped overnight to the Molecular Conservation Genetics Laboratory (MCGL) at the Wisconsin Cooperative Fishery Research Unit of the University of Wisconsin at Stevens Point. Upon arrival, all samples were cataloged into a master tissue database maintained in the MCGL and stored at -20°C until prepared for microsatellite analysis.

DNA was originally extracted from all received samples using QIAgen DNeasy® Tissue Kit[1] (QIAgen, Inc., Valencia, CA). Due to issues related to sample quality, nearly all tissues had to be re-extracted using the Promega Wizard® Genomic DNA Purification kit (Promega, Inc., Madison, WI) to yield quantities of DNA appropriate for subsequent PCR amplification. The manufacturer's suggested protocol for animal tissues was used for both extraction procedures.

Eight microsatellite loci (Table 3) previously developed for walleye (Borer et al. 1998; Eldridge et al. 2002) were amplified as multiplex PCR reactions (Table 4; reaction conditions available upon request from MCGL). Amplicons were size fractionated via polyacrylimide gel electrophoresis on an ABI PRISM® 377 DNA sequencer (Applied Biosystems, Foster City, CA). An internal lane standard (GeneFlo 625, Chimerx, Inc., Milwaukee, WI) was included in all samples to facilitate sizing of bands using GeneScan (Applied Biosystems). All fragment sizes were confirmed and entered manually into a Genetic Analysis in Excel v6 (GenAlEx; Peakall and Smouse 2005; Appendix II) spreadsheet for subsequent data analysis.

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[1] Use of Trade names throughout this report does not imply endorsement by the Federal Government

Table 3. Microsatellite loci used, primer sequence (forward/reverse), and allele size ranges observed for the sauger used in this study and a number of studies based on Wisconsin walleye (Frankowiak 2005). Multiplex combinations are listed as footnotes (reactions conditions available upon request).

Locus	Primer Sequence (5' – 3')	Allele size range for sauger	Allele size range for walleye	Reference
Svi-2[1]	CAACCAGACCCAATCCCTTG GGGCCGAGTATATCAGTTAAC	195-271	189-219	Eldridge et al. 2002
Svi-4[1]	ACAAATGCGGGCTGCTGTTC GATCGCGGCACAGATGTATTG	101-141	105-117	Eldridge et al. 2002
Svi-7[1]	GAAACCTTACAAAAGCCTGG TTATCTGCACTTCTACAGGC	164-226	161-171	Eldridge et al. 2002
Svi-17[2]	GCGCACTCTCGCATAGGCCCTG CGTTAAAGTCCTTGGAAACC	96-116	104-118	Borer et al. 1999
Svi-18[3]	GATCTGTAAACTCCAGCGTG CTTAAGCTGCTCAGCATCCAGG	120-126	Not analyzed	Borer et al. 1999
Svi-20[3]	CAAGTGCGCAATGGTGCATTAC GAATGAAGAAATGCACCCATGC	160-198	149-179	Eldridge et al. 2002
Svi-26[2]	CGAACTACTTATCTTCTGGC GTAAGTGTGAATCAGCCAGAC	151-195	155-191	Eldridge et al. 2002
Svi-33[2]	CAGGACTGCTGTGTATAGACTTG GATATAGCTTTCTGCTGGGGTC	87-145	86-106	Borer et al. 1999

[1] Multiplex A, [2] Multiplex B, [3] Multiplex C

Sample genotypic data was grouped into conglomerates (Table 4) based on major barriers in the river systems such as dams or geographic distance (W. Gardner – personal communication). All conglomerate sample groups were examined for conformance to Hardy-Weinberg expectations (HWE) using a Fisher's exact test as employed by GenAlEx v6 (Peakall

and Smouse 2005). Tests of linkage or gametic disequilibrium were performed to ensure independence of microsatellite loci using Powermarker v3.25 (Liu and Muse 2005). Sequential Bonferroni correction was applied throughout this research when multiple comparisons were conducted. Base genetic diversity measures were assessed using expected heterozygosity (H_e), mean number of alleles per locus adjusted for differing sample sizes based on the rarefaction method of Kalinowski (2004) as implemented in HP-RARE (Kalinowski 2005), and mean number of private alleles per locus based on the same rarefaction methods. Comparisons between population groups were performed using the nonparametric Kruskal-Wallis test as implemented in Minitab® v14 (Minitab, Inc., State College, PA).

The amount of genetic structure in the data was assessed using AMOVA and pairwise comparisons of Φ_{ST}. This approach allows the hierarchical apportionment of molecular variance into within group proportions, among groups within region proportions, and between region proportions. Regions were varied as was inclusion of populations in the regions (see results for more detail) but in general, tests were based on two primary regions, Missouri River mainstem and Yellowstone River. Tests were conducted using the AMOVA option of GenAlEx v6 with 999 permutations of the codominant genetic data.

A final analysis of the data consisted of Neighbor-joining clustering of populations using Nei's (1983) genetic distance. Confidence in the resulting topology was inferred using 1000 bootstrap pseudoreplicates mapped onto an unrooted tree (Figure 5).

Table 4. Sample sites with number of fish genotyped in parentheses used in the microsatellite study. Samples are consistent with the locations outlined in Table 1. Samples are grouped in conglomerates based on major barriers in the river systems such as dams or geographic distance (W. Gardner – personal communication).

Conglomerate Populations & Sample Sites	Population Code	Genetic Analysis Sample size
Above Fort Peck Population	**AFP**	**74**
Fort Peck Reservoir lower		6
Fort Peck Reservoir upper		2
Judith River		15
Marias River		19
Middle Missouri River lower reach		13
Middle Missouri River upper reach		19
Below Fort Peck Population	**BFP**	**115**
Milk River upper reach (Alberta)		6
Milk River Fresno Reservoir		8
Milk River middle reach		38
Milk River lower reach		19
Lower Missouri River lower reach		16
Lower Missouri River upper reach		28
Yellowstone River Population	**YEL**	**94**
Yellowstone River upper reach		10
Yellowstone River middle reach		31
Yellowstone River lower reach		42
Powder River		9
Tongue River		2
Bighorn River Population	**BHR**	**23**
Bighorn River (Wyoming)		22
Bighorn Reservoir (Wyoming)		1
Lake Sakakawea Population	**SAK**	**38**

RESULTS

Hybridization and introgression between saugers and walleyes in Montana

Three (m*MDH-1*, PGM-1** and *IDDH**) of the four diagnostic loci between saugers and walleyes were resolved for all sauger specimens, but only 254 of 447 specimens were resolved for *ALAT** (Appendix I). In the Milk River upper reach (MKU) population only muscle plugs were collected for 12 of the 18 specimens (NT – no tissue for the liver samples in Appendix I). Therefore, these 12 samples were screened only for the muscle loci.

Saugers containing walleye alleles were found at 12 of the 18 sites (after data pooling due to small sample sizes) surveyed (Table 5). Hybridization rates ranged from 0-22% in the Missouri River drainage and from 0-4% in the Yellowstone River drainage (Table 5). In the Missouri River drainage (Appendix I), one fish from the Judith River (JUD 11), one fish from the Marias River (MAR 12), five fishes from the middle Missouri River upper reach (MMU 2, 3, 4, 8, and 9), and five fishes in the lower Missouri River upper reach (LMU 1, 2, 8, 10, and 13), and one fish from the lower Missouri River lower reach (LML 30) were backcrosses to sauger, while one fish from the Fort Peck Reservoir (FPL 3) was an F_1 hybrid. In the Milk River (Appendix I), one fish from the upper Milk River (MKU 1), two fish from the Milk River middle reach (MKM 17 and 19), and two fish from the Milk River lower reach (MKL 11 and 24) were backcrosses to sauger. In the Yellowstone River drainage (Appendix I), one fish from the Yellowstone River middle reach (YSM 18), and two from the Yellowstone River lower reach (YSL 36 and YSL 48) were backcrosses to sauger. All of the hybridization found in the Yellowstone River drainage occurred in Montana, no hybridization and introgression was detected in the sauger populations from Wyoming (BOY, BHU, or BGRV). In Lake Sakakawea, 20.4% of saugers possessed walleye alleles, with eight fishes (SAK 8, 17, 19, 20, 23, 26, 39, and

43) being backcrosses to sauger and one fish (SAK 22) being a multigenerational hybrid (it had two walleye alleles at *ALAT**). Of the hybrids found in this study, only one was an F_1 hybrid (FPL 3), one was a multigenerational hybrid (SAK 22), while the remainder were all backcrossed individuals possessing predominantly sauger alleles.

Table 5. Hybridization rates observed in 16 Montana sauger populations and five neighboring populations from Wyoming and Alberta, Canada.

Populations	**Site codes**	**Number of hybrids/total population**	**Percentage hybrids**
Missouri River Drainage			
Marias River	MAR	2/25	9.5
Judith River	JUD	1/16	6.3
Middle Missouri upper reach	MMU	5/25	20.0
Middle Missouri lower reach	MML	0/30	0.0
Fort Peck Reservoir upper	FPU	0/2	0.0
Fort Peck Reservoir lower	FPL	1/11	9.1
Lower Missouri upper reach	LMU	5/23	21.7
Lower Missouri lower reach	LML	1/31	3.2
Milk River upper reach (AB)	MKU	1/18	5.5
Milk River Fresno Reservoir	MKF	0/9	0.0
Milk River middle reach	MKM	2/32	6.3
Milk River lower reach	MKL	2/42	4.8
Yellowstone River Drainage			
Yellowstone River upper reach	YSU	0/10	0.0
Yellowstone River middle reach	YSM	1/33	3.0
Yellowstone River lower reach	YSL	2/52	3.8
Powder River	POW	0/10	0.0
Tongue River	TON	0/2	0.0
Boysen Reservoir; Wind River (WY)	BOY	0/17	0.0
Bighorn River (WY)	BHU	0/30	0.0
Big Horn Reservoir (WY)	BHRV	0/1	0.0
Lake Sakakawea, ND	SAK	9/44	20.4

In the Missouri River drainage (Table 5), the percentage of hybrids was highest in the middle Missouri upper reach (MMU) and lower Missouri upper reach (LMU) (20% and 21.7%

respectively). Hybrids also occurred at high frequency (20.4%) in Lake Sakakawea. Lower frequencies of hybrid and introgressed fishes were found in the Yellowstone River drainage (Table 5); the percentage of hybrids was highest in the Yellowstone River middle reach and Yellowstone River lower reach populations (3.0 % and 3.8 % respectively).

For subsequent analysis, all individuals that possessed walleye alleles will be removed from analysis, because they were not pure sauger. Only fish that did not possess sauger alleles will be analyzed for protein variation and microsatellite DNA analysis.

Comments on sauger-walleye hybridization based on microsatellite DNA data

Based on the distribution of allele frequencies at the eight microsatellites screened in this study, future analysis of walleye-sauger hybridization may be aided by the use of microsatellite genotyping. Several loci (Svi-2, Svi-7, and Svi-17) show allele frequency distributions in Wisconsin walleye (Frankowiak 2005; B. Sloss and R. Frankowiack – unpublished data) that overlap only at the extremes with the allele distributions observed in the Montana sauger sampled in this study. In particular, Svi-2 shows two alleles in the sauger (205 and 195) that were observed in only one fish each and are 36+ base pairs different in size than the smallest sauger allele (241) observed for several fish. When compared to walleye allele ranges, these two alleles fall within the middle of those observed for Wisconsin walleye (189-219). Several other analyses will be conducted on these data in the future to further examine the utility of these markers in identifying F_x hybrids; a difficult identification based on allozymes alone due to the relatively small number of diagnostic and polymorphic loci.

Protein genetic variation in Montana sauger populations

The protein electrophoretic population data for each individual site were originally reported by Billington et al. (2005b). Unfortunately, due to personnel constraints it was not

possible to for the MDFWP to sample most populations at their spawning sites during the spring. Samples were largely collected during the summer and fall. Thus, these samples likely represent mixtures of distinct sub-populations, as exemplified by the heterozygote deficiencies seen at many of these sites. Therefore, in this report we decided to form conglomerate populations based upon combining sites that were separated by barriers such as dams and major geographic barriers. Foe example, fish from the Upper Milk River above Fresno Reservoir (UMK), had been shown to be genetically discrete from those below the Fresno Reservoir, by Billington et al. (2005). These conglomerate populations are listed in Table 6. While there are no major barriers to sauger traveling from the Missouri River system below Fort Peck Reservoir (BFP) to the Yellowstone River (YEL) and Lake Sakakawea (SAK), a heterogeneity χ^2 test (χ^2 = 72.20, 4 df [degrees of freedom], $p<0.001$) showed that these three composite populations to be genetically discrete, so they were not pooled into a single composite.

Polymorphism was detected at two (*EST** and *SOD-2**) of the 35 loci analyzed. At the *EST** locus three alleles (**60*, **85* and **100*) were resolved (Table 7 and Figure 2); however, the **60* allele was very rare with only a single *60/100 heterozygote found in the Bighorn River (BHU) population. This single **60* allele was pooled with the **85* alleles in this population for subsequent analysis. Two alleles (**100* and **130*) were resolved at the *SOD-2** locus (Table 7 and Figure 3). The genotypes at both loci of every individual screened are presented in Appendix I. However, only data from fish that did not contain walleye alleles will be used for further analysis.

The percentage of polymorphic loci ($P_{0.99}$) in Montana saugers was 5.7%. The mean number of alleles per locus (*A*) was 1.09. Mean heterozygosity (*H*) for all loci was 0.020. Significant deviations from HWE were found in three of the 7 population conglomerates (42.9%)

at the *SOD-2** locus and four of the 7 population conglomerates at the *EST** locus (57.1%) (Table 7). At both loci, the HWE deviations were due to heterozygote deficiencies, likely due to the Wahlund effect, reduced heterozygosity due to the mixing of discrete sub-populations.

Table 6. Conglomerate populations used in protein electrophoretic data analysis on Montana sauger.

Conglomerate population	**Code**	**Sites involved**
Missouri River system above Fort Peck Reservoir	AFP	MAR, JUD, MMU, MML, FPU, FPL
Upper Milk River, above Fresno Reservoir	UMK	MKU, MKF
Missouri River system below Fort Peck Reservoir and Milk River below Fresno Reservoir	BFP	LMU, LML, MKM, MKL
Yellowstone River system in Montana	YEL	YSU, YSM, YSL, POW, TON
Lake Sakakawea, ND	SAK	SAK
Boysen Reservoir, WY	BOY	BOY
Bighorn River system, WY	BHR	BHU, BHRV

Contingency chi-square analysis at the *EST** locus showed highly significant population differentiation among all Montana populations (χ^2 = 111.64, 6 df, p<0.001). Contingency chi-square analysis at the *SOD-2** locus showed significant population differentiation among all populations examined (χ^2 = 18.15, 6 df, p<0.01). Heterogeneity chi-squared analysis based on both polymorphic loci showed highly significant differences among all Montana populations (χ^2 = 129.79, 12 df, p<0.001).

Table 7. Allele frequencies observed at the *EST** and *SOD-2** loci in 7 composite Montana sauger populations, along with their sample sizes (*N*). Populations and loci with significant deviations from Hardy Weinberg expectations (HWE) are indicated by an asterisk; all deviations were heterozygote deficits.

	Composite Population						
Allele	AFP	UMK	BFP	YEL	SAK	BOY	BHR
EST*							
**60*	0.000	0.000	0.000	0.000	0.000	0.000	0.016
**85*	0.094	0.036	0.386	0.058	0.208	0.471	0.097
**100*	0.906	0.964	0.614	0.942	0.792	0.529	0.887
HWE	*		*	*		*	
N	96	14	118	104	36	17	31
SOD-2*							
**100*	0.646	0.786	0. 508	0.591	0.625	0.412	0.548
**130*	0.354	0.214	0. 492	0.409	0.375	0.588	0.452
HWE	*				*		*
N	96	14	118	104	36	17	31

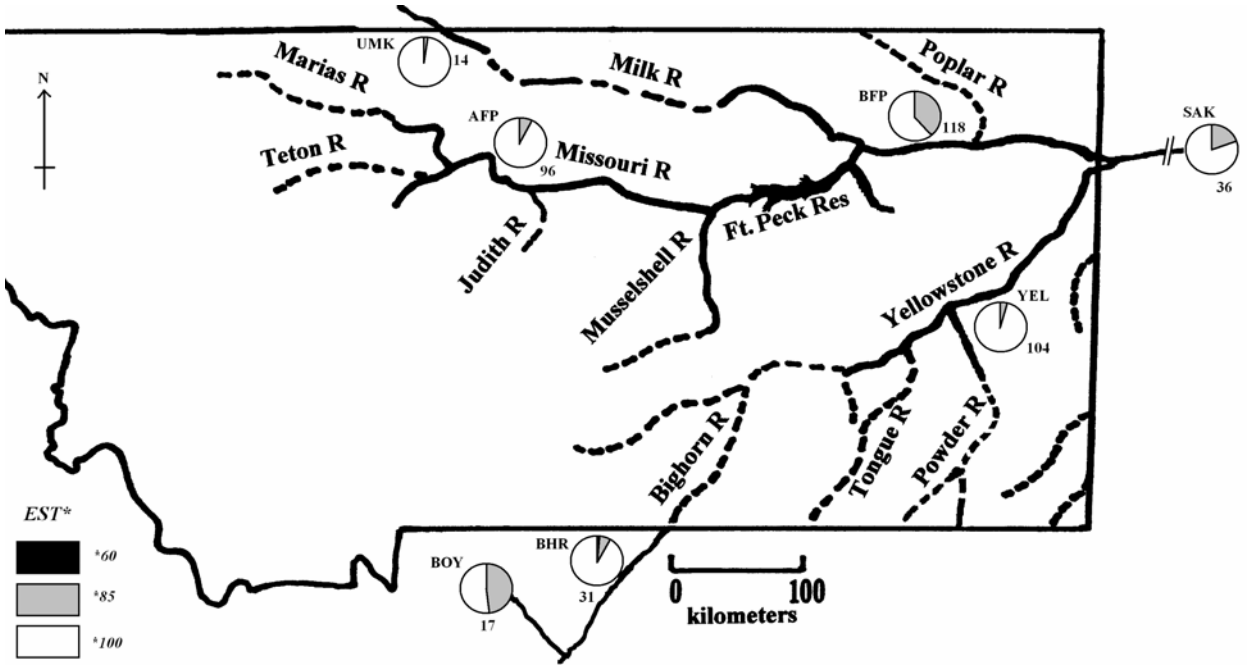

Figure 2. The distribution and relative frequencies observed for *EST** alleles in the Montana sauger conglomerate populations surveyed. Site codes and sample sizes are also included.

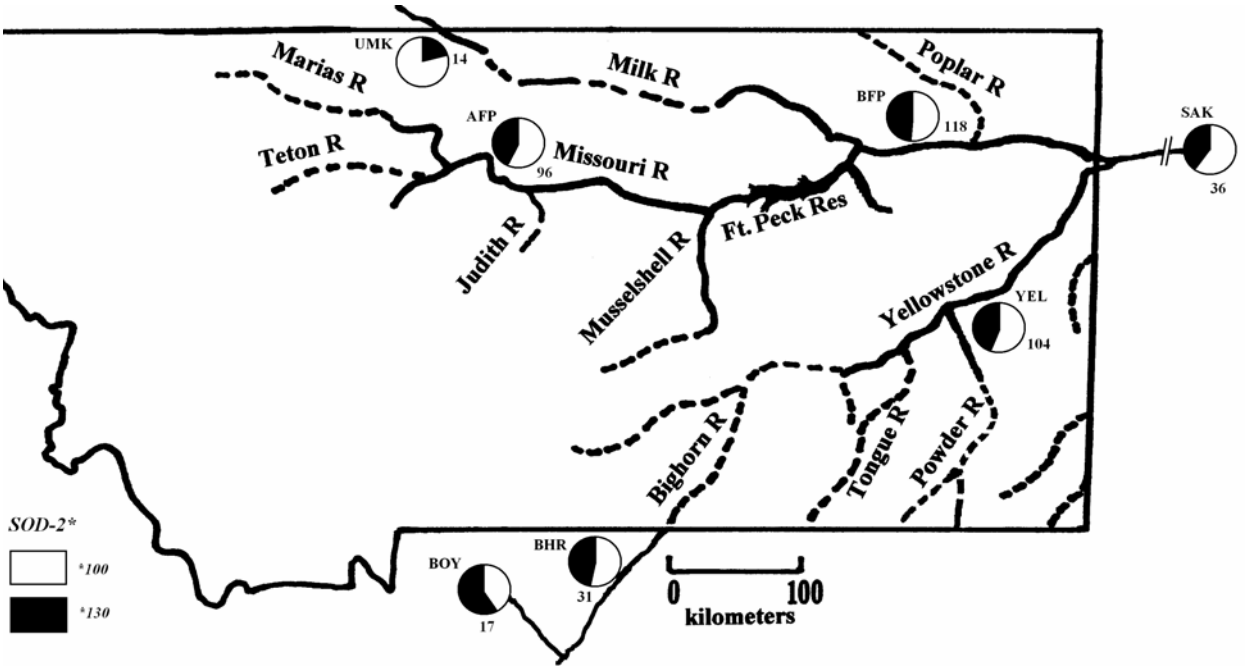

Figure 3. The distribution and relative frequencies observed for *SOD-2** alleles in the Montana sauger conglomerate populations surveyed. Site codes and sample sizes are also included.

Genetic distance values among pairs of conglomerate sauger populations surveyed were low ranging from 0.002-0.005. The UPGMA dendogram derived from Rogers' (1972) genetic distance demonstrated the relationships among conglomerate populations (Fig. 4). The populations were clustered into two main groups. The genetic distance between these two groups was 0.014. One group consisted of populations from the Missouri River below Fort Peck (BFP) and the Boysen Reservoir (BOY) that showed a genetic distance of 0.005 between each other. The second group consisted of all of the other populations in no geographically significant order (Fig. 4). The smallest genetic distance (0.002) was between the Missouri River populations above Fort Peck Reservoir (AFP) and the Yellowstone River populations (YEL). The Bighorn River population clustered next to these two populations at a genetic distance of 0.003, followed by Lake Sakakawea at 0.005. The upper Milk River above the Fresno Reservoir population (UMK) clustered with this group next at a genetic distance of 0.008, but in a separate main group from the populations in the middle and lower Milk River and the Missouri River below Fort Peck Reservoir (BFP). These two populations (UMK and BFP) exhibit significantly different allelic frequencies as shown by a significant heterogeneity χ^2 value ($\chi^2 = 20.98$, 2 df, $p < 0.001$). This confirms the genetic differences in the sauger populations above and below the Fresno Reservoir on the Milk River reported by Billington et al. (2005b).

The F_{ST} values among Montana sauger populations showed moderate genetic subdivision ($F_{ST} = 0.091$). Hartl (1980) described F_{ST} values of 0.05-0.15 as representing moderate population structuring, 0.15-0.25 for high population structuring and, >0.25 for very high population structuring. However, the proportional reduction in heterozygotes due to inbreeding (F_{IS}) was very high ($F_{IS} = 0.318$). The estimated gene flow in the sauger populations surveyed was 2.497 migrants per generation (*Nm*).

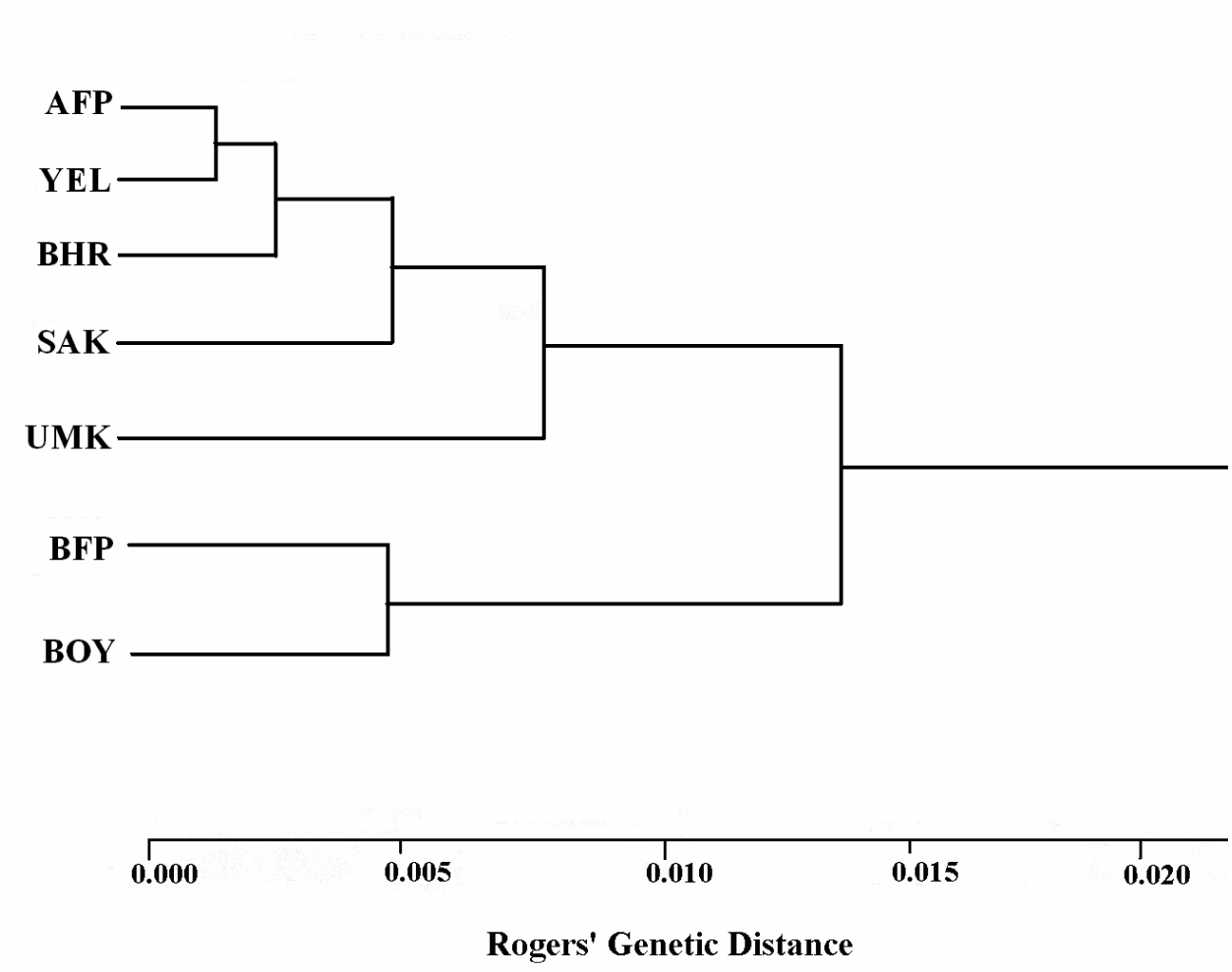

Figure 4. Un-weighted pair group method with arithmetic averages (UPGMA) dendogram showing Rogers' (1972) genetic distances among Montana sauger populations (see Table 6 for composite population site codes).

Microsatellite data

Tissue sample quality. Overall, 421 tissue samples were received at the MCGL from Troy University. The yields (ng/µl) of DNA following the QIAgen extractions were extremely low and the laboratory technician commented on the poor state of samples (a large quantity of water with partially degraded muscle plugs) upon arrival. Although not mandatory, a high quality tissue yields high quantity and quality (molecular weight) of DNA for downstream applications

such as microsatellite genotyping. In an effort to save samples, MCGL conducted an experimental set of 96 extractions comparing our yields from the QIAgen extractions and the Promega Wizard® Genomic DNA purification kit. This kit uses a salt-based precipitation technique (versus the membrane filtration of QIAgen) and we've found it often can be used when low molecular weight DNA is the predominant DNA in a sample. The experiment was conducted across identical tissue samples. The use of the Promega kit improved the yields (mean = 68.8 ng/µl versus QIAgen mean = 13.2 ng/µl)) and was subsequently the method we used to extract (or re-extract) the source DNA we used for all microsatellite genotyping.

Despite the improved yields using the Promega kit, overall issues with tissue quality resulted in a number of individuals failing to amplify or amplifying only a few loci (generally the smaller loci). This resulted in an initial dataset that had a large number of missing loci and/or complete multilocus genotypes missing. We therefore truncated the dataset to include only those individuals which amplified a majority of loci (5 loci or more) in all analyses. All samples identified as hybrids (either F_1 or F_x) were also eliminated from analysis. The resulting dataset consisted of 344 individuals (Appendix II). Note, samples from the Boysen Reservoir were not analyzed for microsatellite DNA.

Sample conglomerates. The suggestion to group samples into larger geographical conglomerates was primarily based on two issues: 1) sample sizes that were too small to conduct standard genetic diversity assessment with a reasonable confidence (i.e., n <10), and 2) the observed deviations from Hardy-Weinberg expectations (HWE) observed in the allozyme data originally analyzed by Billington et al. (2005b). These groupings were logical and necessary based on the fact that the majority of samples were not collected from spawning aggregates of sauger but from post-spawning, potentially mixed origin groups.

Genetic diversity and Hardy-Weinberg expectations. Levels of genetic diversity were high and show the overall utility of this suite of microsatellite markers to quantify the genetic diversity of sauger populations (Table 8). The number of alleles per locus (corrected via rarefaction) ranged from 6.34 (Bighorn River population) to 9.75 (Lake Sakakawea) with no significant differences among populations (Kruskal-Wallis p-value = 0.406). Likewise, the number of unique or private alleles per grouping (corrected for unequal sample sizes via rarefaction) ranged from 3.03 (Bighorn River) to 9.30 (Lake Sakakawea) with the average number of private alleles/locus/population showing no significant differences (Kruskal-Wallis p-value = 0.406). The mean H_e of the populations (0.739) ranged from 0.673 (Bighorn River) to 0.761 (Lake Sakakawea).

Exact tests of HWE resulted in 3/40 locus/population comparisons that were significantly out of HWE based on sequential Bonferroni adjustment ($\alpha_o = 0.05/40 = 0.00125$). A review of the distribution of observed and expected genotypes showed no discernible pattern (i.e., no consistent deficit of heterozygosity indicative of a Wahlund effect) and was confirmed by running an exact test for heterozygote deficiency in GenePop v3.3 (updated version of GenePop 1.2, Raymond and Rousset 1995). Although this proportion (7.5%) is slightly higher than the 5% expected by chance, the lack of consistent violations of HWE for any given locus and/or population is indicative of HWE for the groupings. A multi-locus linkage disequilibrium test employed in PowerMarker (Liu and Muse 2005) showed no locus combinations (up to 3 loci) were significantly linked following sequential Bonferroni correction suggesting all eight loci are segregating independently.

Table 8. Summary of genetic diversity measures for sample groups where N = mean number of individuals genotyped/locus, A_u = uncorrected allele diversity, A_d = allelic diversity corrected for different sample sizes based on rarefaction (Kalinowski 2004), A_e = effective number of alleles, H_o = direct count, observed heterozygosity/loci, and H_e = mean expected heterozygosity. The Private allelic richness is based on the rarefaction method of Kalinowski (2004). The number of private alleles represents the sum total of all observed private alleles in a given conglomerate population.

Sample	**N**	$\mathbf{A_u}$	$\mathbf{A_d}$	$\mathbf{A_e}$	$\mathbf{H_o}$	$\mathbf{H_e}$	**Private Allelic Richness**	**# of Private Alleles**
AFP	70.87	11.75	8.80	5.69	0.734	0.743	0.553	3
BFP	110	13.50	9.37	6.19	0.744	0.757	0.687	6
YEL	91.5	13.25	9.15	6.04	0.749	0.758	0.807	8
SAK	35.87	11.62	9.75	6.05	0.700	0.761	1.163	6
BHR	22.5	6.50	6.35	3.59	0.724	0.674	0.379	0

Genetic Structure. An AMOVA conducted to compare the levels of genetic variance attributable to within populations versus among population proportions showed 99% of the genetic variance was attributable to genetic diversity within populations and only 1% of the variance attributable to among population differences. This is consistent with the suggestions in Leary's review (Leary 2005) of (Billington et al. 2005b). However, when placing populations into distinct regions (Missouri River Drainage initially consisting of AFP, BFP, and SAK versus Yellowstone River Drainage initially consisting of YEL and BHR) and conducting a hierarchical AMOVA, a significant amount of between population/region diversity (1%; p = 0.01) was apparent suggesting the regions as defined were not optimal. We re-organized regions to consist of three

regions, the Missouri River (as defined previously), the Bighorn River (a population that was apparently divergent), and the Yellowstone group. This resulted in an AMOVA result showing no within region variance, but a significant proportion of among region variance (1%, p-value = 0.01).

A pairwise analysis of Φ_{ST} values showed all pairwise comparisons of Bighorn River to be significantly different (Table 9). This is a strong indicator of the relative divergence of the Bighorn River sauger versus the rest of the samples included in this study. More importantly, this result makes sense geographically as the Bighorn River sample sites are located in a headwater area of the Yellowstone River that is isolated from the other sample sites on the Yellowstone (and the other Missouri River sites for that matter) by a significant stretch that is thought to not contain sauger. Therefore, the isolation of this site is apparent geographically, species distribution-wise, and, now, genetic evidence suggests the gene pools have been isolated for a significant period of time to allow a buildup of genetic divergence.

Table 9. Matrix of pairwise population Φ_{ST} values (below diagonal) and p-values (above diagonal) assessing the null hypothesis of $\Phi_{ST} = 0$ (i.e., no significant differences). Significance following sequential Bonferroni adjustment in Italics.

	AFP	**BFP**	**YEL**	**SAK**	**BHR**
AFP	***	0.087	0.054	0.225	*0.001*
BFP	0.002	***	*0.008*	0.430	*0.001*
YEL	0.002	*0.004*	***	0.025	*0.001*
SAK	0.001	0.000	0.005	***	*0.001*
BHR	*0.025*	*0.024*	*0.025*	*0.034*	***

The only other populations showing significant differences were the below Fort Peck (BFP) population and the Yellowstone River populations. A result that at first glance was surprising given the occurrence of the confluence of the Yellowstone River to the Missouri River within the BFP stretch. This result could be due to many factors but we would suggest it is one of two primary issues. First, the confluence of the Yellowstone River is significantly downstream of the majority of BFP samples (SAK is the exception). If there is a downstream gradient or cline to the genetic diversity, this result could be expected. Second, it is a somewhat spurious result not indicative of the regional genetic divergence patterns of sauger; more related to some sampling, ecological or biological issue within this conglomerate.

In order to determine which of these two options were preferred, we conducted a distance analysis (Nei et al. 1983) with NJ clustering. The resulting unrooted NJ tree (Figure 5) shows the highly divergent Bighorn River population but little other relevant structure in the data. Therefore, we would conclude the likely result is Yellowstone River and the BFP population differences are not the result on ancestral patterns of divergence but more likely a bias in sample selection or the construction of conglomerates.

Based on the preponderance of genetic data on the structuring of populations, there appears to be little genetic structure in the mainstem portion of the Missouri River. The primary genetic divergence in this system appears to be the Bighorn River versus the remaining samples. However, one should be careful of interpreting this as no genetic differences throughout the mainstem Missouri River. This is not the case. It does appear there is a large amount of gene flow among most populations but the approach of constructing conglomerates of sample sites is rife with the possibility of missing important genetic units within a given region. For example, it is highly possible that one of the tributaries (e.g., Judith River or Marias River) is in fact

divergent but the level of sampling (i.e., sample sizes) and the distribution of samples is not sufficient at present to address this issue. If this issue is relevant to MDGFP management decisions, we would suggest a focus on obtaining spawning sauger with a minimum n = 50 from each spawning aggregate. Through this approach the potential for genetic divergence within conglomerates can be more confidently assessed.

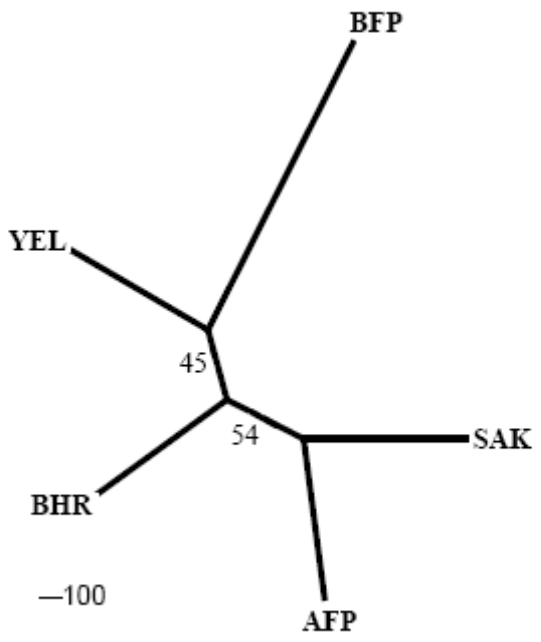

Figure 5. Unrooted neighbor joining tree based on Nei's (1983) genetic distance. Node values represent the percent of 1000 bootstrap pseudoreplicates supporting the topology.

DISCUSSION

Hybridization and introgression between saugers and walleyes in Montana

Hybridization was found between native saugers and introduced walleyes at 11 of the 18 sites surveyed after the samples surveyed with very low sizes (one or two fish) had been pooled with those from adjacent sites (FPU with FPL, TON with YSM, and BHRV with BHU). Hybridization rates ranged from 0-22% in the Missouri River drainage, 0-4% in the Yellowstone River drainage, and were 20.4% in Lake Sakakawea. These rates are comparable with other values reported in other hybridization and introgression studies of Montana sauger populations (McMahon and Gardner 2001), which ranged from 0-10% in the Missouri River system and 0-15% in the Yellowstone River system (Table 10). The value of 20.4% for Lake Sakakawea was about double that reported (10%) by Ward (1982) but he only used two diagnostic loci. Higher values were recorded in two populations from the Missouri River drainage, MMU (20.0%) and LMU (21.7%) populations. These two sampling sites represent the upper most locations in their respective reaches. Sauger densities are much lower and about equal to the walleye densities in the MMU compared to the MML where saugers predominate (W. Gardner; personal communication). Population densities of saugers and walleyes are presently unknown in the LMU and LML areas. Therefore, hybridization is likely heightened by the two species occurring together in relatively large numbers. The low hybridization values recorded in the Yellowstone River drainage likely indicate that sauger-walleye hybrids might have been underestimated in this study due to the small sample sizes for some populations. However, in a survey of potential brood stock from the Yellowstone River middle reach (YSM) 10% (5/50 individuals) of the fish in 2003, 4.1% (3/72) fish in 2004, and 9.4% (5/53) in 2005 contained walleye alleles (Table 11).

Except for the one F_1 hybrid found in the Fort Peck Reservoir lower reach population (FPL) the rest of the hybrids were all backcrosses to sauger and the presence one multigenerational hybrid found in Lake Sakakawea (SAK) showing that introgression of walleye alleles into saugers is occurring. However, MDFWP personnel tried to collect fish that looked like sauger, so this might explain why most of the individuals with walleye alleles were backcrosses and only a single F_1 hybrid was found.

Table 10. The frequency of hybridization between saugers and walleyes reported in Montana and its neighboring states (redrawn and updated from McMahon and Gardner 2001).

Location	**Date**	**Number**	**Percent hybrids**	**References**
Fort Peck Lower	1995	158	9.5	Leary and Allendorf (1997)
Fort Peck Lower	1997	50	6.0	Billington (1998)
Middle Missouri River	1996	14	0.0	Billington et al. (1997b)
Middle Missouri River	1999	109	4.5	Billington and Sloss (1990)
Lower Missouri River	1996	85	4.7	Leary (1998)
Milk River	1999	52	7.7	Billington et al. (2001)
Lower Yellowstone River	1995	48	14.6	Leary and Allendorf (1997)
Lake Sakakawea, ND	1991	279	10.0	Ward (1992)
Lewis and Clark Lake, SD	1995	50	10.0	Van Zee et al. (1996)
Lewis and Clark Lake, SD	2002	224	8.9	Billington and Koigi (2004)
Lake Sharpe, SD	2002	118	3.3	Billington and Koigi (2004)
Lake Francis Case, SD	2002	178	3.3	Billington and Koigi (2004)
Bighorn Lake and River, WY	1995	164	0.0	Kreuger et al. (1997)
Boysen Reservoir, WY	1995	98	0.0	Kreuger et al. (1997)

All fish were screened for *PGM-1**, *mMDH-1**, *sMDH-3**, *PROT-3**, and *IDDH** (Appendix 1). The *ALAT** locus could not be scored for a significant proportion of the fish. However, 254 of the 403 specimens (63%) could be evaluated at *ALAT**. This might have been

due to increased storage time before the samples could be screened (Billington and Koigi 2004) leading to breakdown at this locus. However, a pilot study conducted by Billington et al. (2003) to determine whether reliable data could be obtained by electrophoresis actually revealed that *PGM-1** was the locus most susceptible to thermal break down, yet every sample in the current survey was scored at this locus.

Table 11. Number of potential sauger brood fish from the middle Missouri River lower reach (MML) and the middle reach of the Yellowstone River (YSM) in Montana found to contain walleye alleles during genetic screening, and total number of fish screened in different years.

Population and Year	**Number with walleye alleles/number tested**	**Percentage**	**Reference**
MML 1999	5/109	4.6	Billington and Sloss (1999)
MML 2000	1/22	4.5	Billington et al. (2002)
MML 2001	1/26	3.8	Billington et al. (2002)
MML 2002	2/74	2.7	N. Billington and R. N. Koigi – unpublished data
MML 2003	4/133	3.0	N. Billington and R. N. Koigi – unpublished data
MML 2004	4/106	3.8	Koigi et al. (2004)
MML 2005	11/133	8.3	Koigi et al. (2005)
YSM 2003	5/50	10.0	Koigi et al. (2004)
YSM 2004	3/72	4.2	Koigi et al. (2004)
YSM 2005	5/53	9.4	Koigi et al. (2005)

With only four diagnostic loci, it is important to note that there is a 6.25% chance of missing introgressed alleles. This is because the probability of misidentifying a backcrossed individual as a parental is $(½)^n$, where n is the number of diagnostic loci between the two species under examination (Campton 1990). The addition of three informative loci nonetheless, reduces the possibility of missing introgressed alleles, but not to the extent that resolution would have been improved by having three additional diagnostic loci. In addition, liver samples were not

collected for 12 samples from the Milk River upper reach population (MKU), therefore all the diagnostic loci could not be tested in all fish, further increasing the likelihood that some hybrids could have been missed.

Various studies (Ward and Berry 1995; Van Zee et al. 1996; Billington et al. 1997a, 2004; White et al. 2005) have reported cases of hybridization between saugers and walleyes and have shown that morphological analysis is usually inferior compared to protein electrophoresis for identification of sauger-walleye hybrids. This study provides further evidence that morphology alone is insufficient for identifying saugers, walleyes, and their hybrids. Twenty-three fish that were hybrids or introgressed individuals were identified as saugers in Montana plus an additional nine fish from Lake Sakakawea, yet they possessed walleye alleles.

Hybridization can occur when the spawning periods of the species involved overlap, where there is a shortage of spawning sites, or when one species is more abundant than another so that individuals find it difficult to find conspecific individuals to spawn with (Campton 1987, 1990; Konishi et al. 2003). Saugers and walleyes have not co-existed for long in Montana; they likely have not developed reproductive isolating mechanisms and their spawning periods might be overlapping, leading to hybridization. In addition, saugers in the upper and middle Missouri River are threatened by walleyes migrating downstream from the Canyon Ferry where they were illegally introduced (Yerk 2000). Montana saugers are declining therefore; it is possible that there are fewer conspecific individuals to spawn with, thus forcing saugers to spawn with walleyes. This study does not report any significant increase in hybridization between saugers and walleyes in most Montana populations. However, continued stocking and range expansion of walleyes may lead to the formation of hybrid swarms contributing further to the decline of sauger. If such populations arise, there will likely be no pure saugers remaining presenting

serious problems in conservation efforts of saugers in Montana. The MDFWP should consider increasing the anglers' daily allowable catch of walleyes and reduce that of saugers in an effort to reduce the likelihood of hybridization. In addition, they should consider increasing the supplemental stocking of saugers in Montana.

Hybridization and introgression has caused major problems in many other fish species, especially in salmonids. For instance the westslope cutthroat trout *Oncorhyncus clarki lewisi*, also native to Montana, has greatly declined and now only occupies 2.5% of their historical range (Allendorf and Leary 1988). Their decline has been attributed to introgressive hybridization with the introduced rainbow trout and the Yellowstone cutthroat trout (*Oncorhyncus clarki bouvieri*) resulting in the formation of hybrid swarms. The existence of the remaining westslope cutthroat trout populations continue to be threatened by the migration of rainbow trout both upstream and downstream (Allendorf and Leary 1988; Leary et al. 1995).

Several nonnative species have been introduced into most states to support sport-fishing programs, in some cases leading to the extinction of native species. In an analysis of 40 extinct taxa, Miller et al. (1989) cited introduced species as responsible for 68% (27 cases) of these extinctions. Although some of these extinctions may be due to a combination of factors, Lassuy (1995) suggests that habitat loss is not necessarily a precursor to the severe effects caused by introduced species. Conversely, natural populations and their supporting ecosystems can be made more vulnerable to the impacts of introduced species when their habitat is degraded. Four native fish species in the Colorado River are considered endangered due to a combination of dams and introduced species (Minckley 1991).

In Montana, the construction of dams and water diversion structures on rivers has likely affected sauger populations. Saugers are highly migratory and depend heavily on unimpeded

habitats provided by large rivers (Collette et al. 1977; Carlander 1997; McMahon and Gardner 2001; Jaeger 2004), perhaps making saugers more susceptible to the effects of introduced walleyes. In California, extensive water projects combined with introduced species that can better tolerate the degraded habitats has resulted in the decline of many species, and again introduced species were cited as a primary factor in the status of 49% of species that are now extinct, endangered or require protection (Moyle and Williams 1990).

The MDFWP collect sauger brood stock from the wild to raise fry and fingerling for supplemental stocking, but there is a serious risk of inadvertently including individuals that possess walleye alleles. This would be potentially detrimental to the genetic integrity of the saugers in Montana presenting further risk to sauger populations in the state. Ward and Berry (1995) warned that the potential exists to seriously impact the genetic integrity of recipient natural populations following stocking because a few hybrids or backcrossed individual accidentally included as brood fish can result in the production of many hundreds of thousands of fry and fingerlings containing foreign alleles. Therefore, every effort should be made to prevent the inadvertent culture and stocking of hybrid or introgressed individuals by fisheries management agencies. Data collected between 1999-2005 in surveys of potential sauger brood stock from the lower reach of the middle Missouri River (MML) and Yellowstone River middle reach (YSM), showed the percentage of fish that contained walleye alleles ranged from 2.7-10.0% (Table 11); these fish were either not used as brood fish, or if they were used then the fertilized egg batches were destroyed. No fish collected in this study from the MML contained walleye alleles, but fish from MMU did. Given the highly migratory nature of saugers, fish that spawned in the lower reach of the middle Missouri River could easily migrate to the upper reach of the middle Missouri River. In addition, in 2005 the proportion of hybrids increased in saugers

collected from MML later in the spawning run (Koigi et al. 2005). It is important that saugers to be used as brood fish to produce fry and fingerlings for supplemental stocking continue to be screened by protein electrophoresis or other genetic analysis such as microsatellites once markers have been developed, prior to spawning in order to maintain the genetic integrity of the fish produced. Concern over the potential inclusion of hybrids in sauger brood stock and difficulties in being able to adequately screen them by protein electrophoresis has led MDFWP to suspend their sauger spawning program.

Genetic screening of potential brood fish is an important precaution that can be taken: (1) to ensure correct species identification, and (2) to prevent the inadvertent inclusion of F_1 or F_x hybrids in fish culture operations (Ward and Berry 1995; Billington 1997, 1998; Billington et al. 2002). One concern with screening potential sauger brood fish is that it is normally only possible to collect muscle samples from live fish either by a fin clip technique (Billington et al. 1996) or with a biopsy needle (e.g., McAndrew 1981). With only two diagnostic loci that can be scored in muscle (*mMDH-1** and *PGM-1**) it will be possible to confirm species identification and to eliminate all of the F_1 hybrids, but $(½)^n = (½)^2$ in this case = 25% of the backcrosses would likely be missed (equation from Campton 1990). The use of the two additional diagnostic loci in liver could improve the chances of detecting backcrosses to 6.25%, but MDFWP personnel were concerned about performing surgery on fish that were about to be spawned in the field to collect liver samples such as has been used for largemouth bass by Harvey et al. (1984).

This percentage is reduced somewhat further in saugers because additional information can be gained from the two muscle loci that are informative in saugers (*sMDH-3** and *PROT-3**). An alternative approach would be to sacrifice the adults once they have been spawned, screen all four diagnostic loci (because the two liver loci could now be included) plus all of the

relevant informative loci by electrophoresis and then destroy any batches of fertilized eggs that derive from hybrid or introgressed fishes. However, many fisheries managers are unwilling to sacrifice mature fish that might spawn for many years on the slight chance of them possibly being a hybrid or backcrossed individual.

The use of non-lethal muscle sampling techniques such as biopsy needles (McAndrew 1981) or fin-clips (Billington et al. 1996) and rapid electrophoretic screening by using cellulose acetate electrophoresis facilitates the identification of hybrid and backcrossed individuals in the hatchery or brood fish (Billington et al. 1996, 1997b). However, it is likely to prove impractical to collect liver samples from large numbers of potential brood fish by surgery (for example with the methods of Harvey et al. 1984 or Leitner and Isely 1994) to permit the screening of *ALAT** and *IDDH**. Although a needle biopsy procedure for collecting liver samples from *Micropterus* spp. has been reported (Van Meter 1995), it would need to be refined for use on *Sander* species. Thus, it will be important to search for additional loci that are diagnostic between saugers and walleyes that can be scored in muscle.

Genetic variation in Montana sauger populations

Protein electrophoresis

This is the first study that surveys genetic variation at protein coding loci in Montana sauger populations. This is also the first study that has revealed significant amounts of genetic variation among sauger populations. Other studies on the population genetics of saugers (Uthe et al. 1966; Billington et al. 1990, 1996, 1997a; White and Schell 1995; Kreuger et al. 1997) have reported little or no genetic variation.

Genetic variation was detected at two of the 35 loci (*EST** and *SOD-2**) surveyed in Montana sauger populations. Polymorphism at the *EST** locus in sauger has been reported in

other studies albeit at low levels and additional alleles to the ones found in this study appear to occur in the eastern part of the sauger distribution (Billington et al. 1990; White and Schell 1995; Barr et al. 2006). Polymorphism at the *SOD-2** locus was first reported by Billington and Koigi (2004). The **100* allele has been observed throughout the North American range of saugers, but the **130* allele has only been observed in sauger populations from the Missouri River drainage (Billington and Koigi 2004; Barr et al. 2006). Billington et al. (1996, 1997a) reported polymorphism at the *PGM-1** locus, with saugers from the Peoria Pool of the Illinois River having four alleles; the relative mobility of these alleles were **50, *70, *80* and **90* against a walleye **100* allele reference. The **50, *70,* and **90* alleles all occurred at low frequencies in Peoria Pool. In this study *PGM-1** was not polymorphic, however, one **50/*80* heterozygote was observed in potential brood stock collected from the lower reach of the middle Missouri River (MML) in the spring spawn of 2003, another one in spring 2004, and one more in spring 2005 (N. Billington and R. N. Koigi, Troy University, unpublished data). This rare allele might have been detected in the main survey if the sample size was much larger. This study shows that sauger populations in Montana are moderately structured ($F_{ST} = 0.091$).

Gene flow is the most important determinant of population structure, because it determines to what extent each local population of a species is an independent evolutionary unit (Slatkin 1993). Therefore, if gene flow among neighboring populations is strong, the populations evolve together, while if it is limited, then each population evolves autonomously. The population genetic parameter (*Nm*) measures the number of migrants per generation and provides an indication of the differentiation among populations. An *Nm* value >1 indicates gene flow action against genetic differentiation among populations (Gall 1987). However, Mitchell et al. (2002) state that 2.25 migrants are required to sustain significant gene flow among

populations. In this study, the *Nm* value of 2.497 exceeds both 1.00 and 2.25 migrants per generation, showing that there is reasonable gene flow among Montana sauger populations. The relatively high F_{IS} value (0.318) was likely influenced by the heterozygote deficits caused by the Wahlund effect, because a mixture of sub-populations were sampled in the summer and fall, rather than spawning populations had sampling occurred in the spring. However, a low mean heterozygosity value (0.020) was recorded for Montana saugers and the mean number of alleles (*A*) was only 1.09.

The low genetic distance values show little divergence among Montana sauger populations. The dendogram did not cluster sauger populations based on the two main (Missouri River and Yellowstone River) drainages. However, there were two distinct groups including a group that largely consisted of populations from the middle and the lower Milk River and the lower Missouri River, populations that are below the Fresno Reservoir dam on the Milk River and the Fort Peck dam on the Missouri River (BFP), and the Boysen Reservoir in Wyoming. The rest of the populations from both the Missouri River drainage and the Yellowstone River Drainage (including the Bighorn River, WY) are interspersed showing clustering expected for a migratory species, where no separation of the individuals in subpopulations is observed. However, there were significant differences among the Yellowstone River (YEL), Missouri River system below Fort Peck Reservoir (BFP) and Lake Sakakawea, North Dakota (SAK). In addition, the Milk River population above the Fresno Reservoir was genetically different from the Milk River below the Fresno Reservoir (Billington et al. 2005b), and the UMK population was significantly different from the Missouri River system population below Fort Peck Reservoir (BFP), see below.

Saugers are a highly migratory species (Collette et al. 1977, Carlander 1997; McMahon and Gardner 2001; Jaeger 2004) and they have a tendency to travel long distances, especially during spawning. In a natural environment with very little human influence, their populations will tend to be panmictic. However, the alteration of such habitat by construction of dams and other water diversion structures may cause populations to become discreet (Mitchell et al. 2002; Meldgaard et al. 2003).

In Montana, numerous dams and water diversion structures have been constructed. These structures likely interfere with the migration of saugers across their range by blocking or impeding their movements. On the Milk River for instance, there are seven major water diversions, all of which are considered migratory barriers (McMahon and Gardner 2001), plus the Fresno Reservoir dam, a major barrier to fish movement (W. Gardner, MDFWP, personal communication). In this study, two populations from the upper reaches of the Milk River (MKU, MKF) were divergent from the two lower populations (MKM, MKL) (Billington et al. 2005b), supporting the inference that the Fresno Reservoir dam on the Milk River likely plays a major role in the structuring of the Milk River sauger populations. This was confirmed in this study by the heterogeneity chi-squared test ($\chi^2 = 20.98$, df = 2, $p<0.001$), showing there were significant differences between the above-dam (UMK) and below-dam sauger populations on the Milk River below the Fresno Reservoir dam and the Missouri River below Fort Peck Reservoir (BFP).

The effects of dams and other water diversion structures may lead to severe changes in genetic variation. For instance, if an impassable dam separates two populations, a fraction of the fry will drift over the dam or with water released from the dam to the downstream population, thereby creating gene flow from upstream to downstream, but not vice versa. Once downstream, these fish cannot return upstream causing unidirectional transport of alleles. As a result, the size

of the upstream population is likely to decrease and genetic drift will likely increase leading to population differentiation. Under natural conditions, the drift of fry downstream would be balanced by migration of adults upstream.

Several problems are associated with the study of declining populations. One major concern is the extent to which sampling depletes populations in conservation genetic studies. Secondly, because of small sample sizes, such as is the case in some of the populations in this study, the statistical analysis may be weakened. However, the findings provide a general insight for conservation practices of the declining species.

Montana sauger populations show some level of genetic population structuring; therefore conservation efforts should focus on maintaining this genetic variation. Stock transfer should not be conducted at present as there may already be local adaptations that will be lost if populations are mixed. Perhaps additional sampling is required particularly focusing on spawning populations before firm recommendations can be made on these issues.

Microsatellite DNA variation

This is the first study that surveys genetic variation at microsatellite DNA loci in sauger populations. Eight microsatellite loci that were previously developed for walleye (Borer et al. 1998; Eldridge et al. 2002) were successfully amplified in Montana sauger. Levels of genetic diversity were high with these markers in Montana sauger; numbers of alleles (corrected for rarefaction) ranged from 6.34-9.75, although there were no significant differences among the populations analyzed. Note that for the microsatellite work the BFP population included the upper Milk River samples, there was no significant heterogeneity in these samples for the microsatellites compared to the protein analysis. In addition, no samples from the Boysen

Reservoir (BOY) were analyzed for microsatellite DNA variation. Observed heterozygosity values ranged from 0.700-0.749.

Unlike the protein genetic variation, there were no consistent patterns of deviations from Hardy-Weinberg expectations at the microsatellite loci. The majority (995) of the genetic variance detected was within populations rather than among them (1%). When the data was partitioned into three distinct regions consisting of the Missouri River drainage (AFP, BFP, and SAK), the Yellowstone River (YEL), and the Bighorn River (BHR), a significant proportion of among-region variance was detected; this was primarily due to the divergence of the Bighorn River from all of the other population conglomerates. The only other significant difference between population pairs was the Yellowstone River (YEL) and the Missouri River Population below Fort Peck (BFP). This pair-wise comparison was also significantly different in a heterogeneity χ^2 test for the protein data ($\chi^2 = 69.78$, df =2, $p<0.001$). In contrast, the protein variation analysis also showed differences among YEL, BFP and SAK. However, the unrooted tree that resulted from the distance analysis with NJ clustering (Fig. 5) showed the BHR population to be highly divergent from the other populations but that there was little other structure to the microsatellite data. That there is considerable gene flow among populations may reflect the highly mobility of sauger (Jaeger 2004). However, the approach of constructing conglomerates of sample sites that were sampled in the summer and fall likely meant that genetic units throughout the region were missed. Future work should focus on sampling spawning populations of sauger with minimum population sizes of 50. Non-lethal sampling of scale samples can be used to screen microsatellite DNA (e.g. Nielsen et al. 1997) and the additional loci and alleles that can be scored with this method should hopefully reveal genetic differences among spawning populations. Such an approach has been used for walleye from Escanaba Lake

(Frankoviak 2005). Archived scale samples from Montana sauger samples, should they exist, could be used to examine changes in genetic variation in Montana sauger over time.

MANAGEMENT IMPLICATIONS

The sauger is native to Montana, but the congeneric walleye is not native to Montana, but has been introduced and extensively stocked across the state into drainages containing saugers. Concern has been expressed on the decline of Montana sauger populations. Various reasons have been attributed to this decline including: (1) low river flows and reservoir water levels, (2) habitat loss and migratory barriers, (3) competition and hybridization with walleyes, (4) interaction with other species, and (5) over exploitation. A severe drought in the late 1980s was thought to be responsible for the decline, but an apparent lack of rebound in sauger abundance despite improved flow conditions raised major concerns (McMahon and Gardner 2001).

In this study, genetic variation in sauger from Montana populations was surveyed and how that variation is partitioned was determined. There was evidence of heterozygote deficits for the polymorphic allozyme loci in Montana populations, related to Wahlund effects likely related to sampling sites during the summer and fall. Montana sauger populations showed moderate population structuring. Microsatellite DNA analysis revealed additional genetic variation, most of which was found within rather than among populations. The Bighorn River population was the only one that appeared to be divergent from the other population conglomerates examined. However, it appears that the constraint on sampling the populations during the summer and fall rather than at spring spawning time has reduced the likelihood of detecting genetic variation among Montana sauger populations. Further work could include non-lethal sampling of spawning populations by using microsatellite DNA analysis on scale samples. Archived scale samples could provide further information on historic changes in genetic variation in Montana sauger.

In order to maintain the genetic variation present among the sauger populations in Montana, it is important that populations are managed individually at present. Stock transfers should not be conducted until more information on spawning populations can be conducted. Some populations appear to be divergent, for example the Bighorn River population by the microsatellite analysis and the populations from the Milk River above the Fresno Reservoir dam (UMK) have significantly different allele frequencies from those below the dam (BFP) by the allozyme analysis.

Hybridization and introgression was found between native saugers and introduced walleyes in 11 of the 18 sauger populations surveyed after pooling to address low sample sizes in three populations. The hybridization rates ranged from 0-22% in the Missouri River drainage and 0-4% in the Yellowstone River drainage. Hybridization and introgression rates in the current study are comparable with values reported in previous studies of Montana sauger populations, which ranged from 0-15% in the Missouri River system and 0-10% in the Yellowstone River system. However, higher values (up to 22%) were recorded in two populations (MMU and LMU) in the Missouri River drainage, populations where the proportion of walleyes is much higher. Although hybridization rates have not increased significantly in Montana, stocking of walleyes in Montana, especially into drainages with drastic declines of saugers should be limited as hybridization with walleyes presents a long-term threat to the declining sauger populations as many appear to be hybrid swarms. It is recommended that MDFWP should consider increasing the daily allowable catch of walleyes by anglers and reduce that of saugers, and also increase supplemental stocking of saugers, as methods for reducing hybridization. At present Montana does not stock saugeye in its waters and it is recommended that this practice continue. The presence of saugeye which are able to reproduce with both

walleye and sauger would severely compromise the genetic integrity of both of these species in Montana, and they would be a competitor for food. If sauger stock is to be resumed in Montana, the use of additional diagnostic loci will be required to more reliably eliminated fish containing walleye alleles will be needed. With further work microsatellite DNA analysis could contribute towards this goal also.

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APPENDIX I - Electrophoretic data

Sample	*PGM-1**	*mMDH-1**	*sMDH-3**	*PROT-3**	*ALAT**	*IDDH**	*SOD-2**	*EST**	Genetic Identification
Judith River									
JUD1	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
JUD2	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
JUD3	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
JUD4	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
JUD5	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
JUD6	80/80	140/140	120/120	160/160		-10/-10	100/130	100/100	Sauger
JUD7	80/80	140/140	120/120	160/160		-10/-10	100/100	100/100	Sauger
JUD8									
JUD9	80/80	140/140	120/120	160/160		-10/-10	100/100	100/100	Sauger
JUD10	80/80	140/140	120/120	160/160		-10/-10	100/130	100/100	Sauger
JUD11	**80/100**	140/140	**100/120**	160/160		-10/-10	100/130	100/100	**Backcross to Sauger**
JUD12	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
JUD13	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
JUD14	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
JUD15	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
JUD16	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
JUD17	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
Middle Missouri River upper									
MMU1	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	85/100	Sauger
MMU2	80/80	140/140	120/120	160/160	85/85	**-10/100**	100/130	100/100	**Backcross to Sauger**
MMU3	80/80	140/140	120/120	160/160	**85/100**	-10/-10	100/130	100/100	**Backcross to Sauger**
MMU4	80/80	140/140	120/120	160/160	**85/100**	-10/-10	130/130	100/100	**Backcross to Sauger**
MMU5	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
MMU6	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	85/85	Sauger
MMU7	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
MMU8	80/80	140/140	120/120	160/160	**85/100**	-10/-10	130/130	100/100	**Backcross to Sauger**
MMU9	80/80	140/140	120/120	160/160	**85/100**	-10/-10	100/130	100/100	**Backcross to Sauger**
MMU10	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
MMU11	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
MMU12	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
MMU13	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
MMU14	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
MMU15	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger

Sample	*PGM-1**	*mMDH-1**	*sMDH-3**	*PROT-3**	*ALAT**	*IDDH**	*SOD-2**	*EST**	Genetic Identification
MMU16	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
MMU17	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
MMU18	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
MMU19	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	85/85	Sauger
MMU20	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
MMU21	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
MMU22	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
MMU23	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
MMU24	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
MMU25	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
Marias River									
MAR1	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
MAR2	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
MAR3	80/80	**100/140**	120/120	160/160	85/85	-10/-10	100/130	100/100	**Backcross to Sauger**
MAR4	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
MAR5	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
MAR6	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
MAR7	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
MAR8	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
MAR9	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
MAR10	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
MAR11	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
MAR12	80/80	140/140	**100/120**	160/160	**85/100**	**-10/100**	130/130	100/100	**Backcross to Sauger**
MAR13	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	85/100	Sauger
MAR14	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
MAR15	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
MAR16	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
MAR17	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
MAR18	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
MAR19	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
MAR20	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
MAR21	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
Middle Missouri River lower									
MML1	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	85/100	Sauger
MML2	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
MML3	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
MML4	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger

Sample	*PGM-1**	*mMDH-1**	*sMDH-3**	*PROT-3**	*ALAT**	*IDDH**	*SOD-2**	*EST**	Genetic Identification
MML5	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	85/100	Sauger
MML6	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
MML7	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
MML8	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
MML9	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
MML10	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
MML11	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
MML12	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
MML13	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
MML14	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
MML15	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
MML16	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
MML17	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
MML18	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
MML19	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
MML20	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
MML21	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
MML22	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	85/85	Sauger
MML23	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
MML24	80/80	140/140	120/120	160/160		-10/-10	100/130	85/85	Sauger
MML25	80/80	140/140	120/120	160/160		-10/-10	100/100	100/100	Sauger
MML26	80/80	140/140	120/120	160/160		-10/-10	130/130	85/100	Sauger
MML27	80/80	140/140	120/120	160/160		-10/-10	100/130	100/100	Sauger
MML28	80/80	140/140	120/120	160/160		-10/-10	100/130	100/100	Sauger
MML29	80/80	140/140	120/120	160/160		-10/-10	100/130	85/85	Sauger
MML30	80/80	140/140	120/120	160/160		-10/-10	100/100	85/100	Sauger
Fort Peck Reservoir upper									
FPU 1	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
FPU 2	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
Fort Peck Reservoir lower									
FPL1	80/80	140/140	120/120	160/160		-10/-10	130/130	100/100	Sauger
FPL2	80/80	140/140	120/120	160/160		-10/-10	100/100	100/100	Sauger
FPL3	**80/100**	**100/140**	**100/120**	**100/160**		**-10/100**	100/100	100/100	**F_1 hybrid**
FPL4	80/80	140/140	120/120	160/160		-10/-10	130/130	100/100	Sauger
FPL5	80/80	140/140	120/120	160/160		-10/-10	100/130	100/100	Sauger
FPL6	80/80	140/140	120/120	160/160		-10/-10	100/100	100/100	Sauger

Sample	*PGM-1**	*mMDH-1**	*sMDH-3**	*PROT-3**	*ALAT**	*IDDH**	*SOD-2**	*EST**	Genetic Identification
FPL7	80/80	140/140	120/120	160/160		-10/-10	100/130	100/100	Sauger
FPL8	80/80	140/140	120/120	160/160		-10/-10	100/100	100/100	Sauger
FPL9	80/80	140/140	120/120	160/160		-10/-10	100/130	100/100	Sauger
FPL10	80/80	140/140	120/120	160/160		-10/-10	100/100	100/100	Sauger
FPL11	80/80	140/140	120/120	160/160		-10/-10	100/130	85/85	Sauger
Lower Missouri River upper									
LMU1	80/80	140/140	120/120	**100/160**		-10/-10	100/100	85/100	**Backcross to Sauger**
LMU2	**80/100**	**100/140**	**100/120**	160/160		-10/-10	130/130	85/85	**Backcross to Sauger**
LMU3	80/80	140/140	120/120	160/160		-10/-10	100/130	85/100	Sauger
LMU4	80/80	140/140	120/120	160/160		-10/-10	130/130	85/85	Sauger
LMU5	80/80	140/140	120/120	160/160		-10/-10	100/130	100/100	Sauger
LMU6	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	85/100	Sauger
LMU7	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
LMU8	80/80	140/140	120/120	160/160	**85/100**	-10/-10	130/130	100/100	**Backcross to Sauger**
LMU9	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
LMU10	80/80	140/140	120/120	160/160	**85/100**	-10/-10	100/100	100/100	**Backcross to Sauger**
LMU11	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	85/100	Sauger
LMU12	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	85/100	Sauger
LMU13	80/80	140/140	120/120	160/160	85/85	**-10/100**	130/130	100/100	**Backcross to Sauger**
LMU14	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
LMU15	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
LMU16	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
LMU17	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
LMU18	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
LMU19	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
LMU20	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	85/85	Sauger
LMU21	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	85/100	Sauger
LMU22	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
LMU23	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	85/100	Sauger
Lower Missouri River lower									
LML1	80/80	140/140	120/120	160/160		-10/-10	100/130	85/100	Sauger
LML2	80/80	140/140	120/120	160/160		-10/-10	100/100	85/100	Sauger
LML3	80/80	140/140	120/120	160/160		-10/-10	100/100	85/100	Sauger
LML4	80/80	140/140	120/120	160/160		-10/-10	100/100	100/100	Sauger
LML5	80/80	140/140	120/120	160/160		-10/-10	100/130	85/85	Sauger
LML6	80/80	140/140	120/120	160/160		-10/-10	100/100	100/100	Sauger

Sample	*PGM-1**	*mMDH-1**	*sMDH-3**	*PROT-3**	*ALAT**	*IDDH**	*SOD-2**	*EST**	Genetic Identification
LML7	80/80	140/140	120/120	160/160		-10/-10	100/100	85/100	Sauger
LML8	80/80	140/140	120/120	160/160		-10/-10	100/100	85/85	Sauger
LML9	80/80	140/140	120/120	160/160		-10/-10	100/130	85/85	Sauger
LML10	80/80	140/140	120/120	160/160		-10/-10	100/130	85/85	Sauger
LML11	80/80	140/140	120/120	160/160		-10/-10	100/100	85/85	Sauger
LML12	80/80	140/140	120/120	160/160		-10/-10	100/100	100/100	Sauger
LML13	80/80	140/140	120/120	160/160		-10/-10	100/130	85/100	Sauger
LML14	80/80	140/140	120/120	160/160		-10/-10	130/130	85/100	Sauger
LML15	80/80	140/140	120/120	160/160		-10/-10	100/130	85/100	Sauger
LML16	80/80	140/140	120/120	160/160		-10/-10	100/100	85/85	Sauger
LML17	80/80	140/140	120/120	160/160		-10/-10	100/130	100/100	Sauger
LML18	80/80	140/140	120/120	160/160		-10/-10	130/130	85/85	Sauger
LML19	80/80	140/140	120/120	160/160		-10/-10	100/100	85/100	Sauger
LML20	80/80	140/140	120/120	160/160		-10/-10	100/130	100/100	Sauger
LML21	80/80	140/140	120/120	160/160		-10/-10	130/130	85/100	Sauger
LML22	80/80	140/140	120/120	160/160		-10/-10	100/100	100/100	Sauger
LML23	80/80	140/140	120/120	160/160		-10/-10	130/130	85/85	Sauger
LML24	80/80	140/140	120/120	160/160		-10/-10	130/130	100/100	Sauger
LML25	80/80	140/140	120/120	160/160		-10/-10	100/130	100/100	Sauger
LML26	80/80	140/140	120/120	160/160		-10/-10	130/130	100/100	Sauger
LML27	80/80	140/140	120/120	160/160		-10/-10	130/130	100/100	Sauger
LML28	80/80	140/140	120/120	160/160		-10/-10	130/130	100/100	Sauger
LML29	80/80	140/140	120/120	160/160		-10/-10	130/130	100/100	Sauger
LML30	80/80	140/140	120/120	160/160		**-10/100**	100/130	100/100	**Backcross to Sauger**
LML31	80/80	140/140	120/120	160/160		-10/-10	100/100	100/100	Sauger
Milk River upper, AB									
MKU1	80/80	140/140	120/120	160/160		**-10/100**	100/100	100/100	**Backcross to Sauger**
MKU2	80/80	140/140	120/120	160/160		-10/-10	100/100	100/100	Sauger
MKU3	80/80	140/140	120/120	160/160		-10/-10	100/100	100/100	Sauger
MKU4	80/80	140/140	120/120	160/160		-10/-10	100/100	100/100	Sauger
MKU5	80/80	140/140	120/120	160/160		-10/-10	100/130	100/100	Sauger
MKU6	80/80	140/140	120/120	160/160		-10/-10	100/130	85/100	Sauger
MKU7	80/80	140/140	120/120	160/160	NT	NT	NT	NT	Sauger
MKU8	80/80	140/140	120/120	160/160	NT	NT	NT	NT	Sauger
MKU9	80/80	140/140	120/120	160/160	NT	NT	NT	NT	Sauger
MKU10	80/80	140/140	120/120	160/160	NT	NT	NT	NT	Sauger
MKU11	80/80	140/140	120/120	160/160	NT	NT	NT	NT	Sauger

Sample	*PGM-1**	*mMDH-1**	*sMDH-3**	*PROT-3**	*ALAT**	*IDDH**	*SOD-2**	*EST**	Genetic Identification
MKU12	80/80	140/140	120/120	160/160	NT	NT	NT	NT	Sauger
MKU13	80/80	140/140	120/120	160/160	NT	NT	NT	NT	Sauger
MKU14	80/80	140/140	120/120	160/160	NT	NT	NT	NT	Sauger
MKU15	80/80	140/140	120/120	160/160	NT	NT	NT	NT	Sauger
MKU16	80/80	140/140	120/120	160/160	NT	NT	NT	NT	Sauger
MKU17	80/80	140/140	120/120	160/160	NT	NT	NT	NT	Sauger
MKU18	80/80	140/140	120/120	160/160	NT	NT	NT	NT	Sauger
Milk River Fresno Reservoir									
MKF1	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
MKF2	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
MKF3	80/80	140/140	120/120	160/160		-10/-10	100/130	100/100	Sauger
MKF4	80/80	140/140	120/120	160/160		-10/-10	130/130	100/100	Sauger
MKF5	80/80	140/140	120/120	160/160		-10/-10	100/100	100/100	Sauger
MKF6	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
MKF7	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
MKF8	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
MKF9	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
Milk River middle reach									
MKM1	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	85/85	Sauger
MKM2	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	85/85	Sauger
MKM3	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
MKM4	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	85/100	Sauger
MKM5	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
MKM6	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	85/100	Sauger
MKM7	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	85/85	Sauger
MKM8	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
MKM9	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
MKM10	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	85/85	Sauger
MKM11	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
MKM12	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
MKM13	80/80	140/140	120/120	160/160		-10/-10	100/130	100/100	Sauger
MKM14	80/80	140/140	120/120	160/160		-10/-10	100/130	100/100	Sauger
MKM15	80/80	140/140	120/120	160/160		-10/-10	130/130	100/100	Sauger
MKM16	80/80	140/140	120/120	160/160		-10/-10	100/130	85/85	Sauger
MKM17	**80100**	140140	120120	160160		**-10/100**	100/130	85/85	**Backcross to Sauger**
MKM18	80/80	140/140	120/120	160/160		-10/-10	130/130	85/85	Sauger

Sample	*PGM-1**	*mMDH-1**	*sMDH-3**	*PROT-3**	*ALAT**	*IDDH**	*SOD-2**	*EST**	Genetic Identification
MKM19	80/80	140/140	120/120	160/160		**-10/100**	100/130	85/85	**Backcross to Sauger**
MKM20	80/80	140/140	120/120	160/160		-10/-10	100/130	100/100	Sauger
MKM21	80/80	140/140	120/120	160/160		-10/-10	100/130	85/100	Sauger
MKM22	80/80	140/140	120/120	160/160		-10/-10	130/130	100/100	Sauger
MKM23	80/80	140/140	120/120	160/160		-10/-10	100/130	85/85	Sauger
MKM24	80/80	140/140	120/120	160/160		-10/-10	130/130	100/100	Sauger
MKM25	80/80	140/140	120/120	160/160		-10/-10	130/130	100/100	Sauger
MKM26	80/80	140/140	120/120	160/160		-10/-10	100/130	85/100	Sauger
MKM27	80/80	140/140	120/120	160/160		-10/-10	130/130	85/100	Sauger
MKM28	80/80	140/140	120/120	160/160		-10/-10	100/130	100/100	Sauger
MKM29	80/80	140/140	120/120	160/160		-10/-10	100/130	85/85	Sauger
MKM30	80/80	140/140	120/120	160/160		-10/-10	100/100	100/100	Sauger
MKM31	80/80	140/140	120/120	160/160		-10/-10	100/130	85/85	Sauger
MKM42	80/80	140/140	120/120	160/160		-10/-10	100/130	100/100	Sauger
Milk River lower reach									
MKL1	80/80	140/140	120/120	160/160		-10/-10	130/130	85/100	Sauger
MKL2	80/80	140/140	120/120	160/160		-10/-10	100/130	100/100	Sauger
MKL3	80/80	140/140	120/120	160/160		-10/-10	130/130	85/100	Sauger
MKL4	80/80	140/140	120/120	160/160		-10/-10	100/130	85/100	Sauger
MKL5	80/80	140/140	120/120	160/160		-10/-10	100/100	85/100	Sauger
MKL6									
MKL7	80/80	140/140	120/120	160/160		-10/-10	130/130	100/100	Sauger
MKL8	80/80	140/140	120/120	160/160		-10/-10	100/100	100/100	Sauger
MKL9	80/80	140/140	120/120	160/160		-10/-10	100/100	85/85	Sauger
MKL10	80/80	140/140	120/120	160/160		-10/-10	100/100	100/100	Sauger
MKL11	80/80	140/140	120/120	160/160		**-10/100**	100/100	100/100	**Backcross to Sauger**
MKL12	80/80	140/140	120/120	160/160		-10/-10	100/100	100/100	Sauger
MKL13	80/80	140/140	120/120	160/160		-10/-10	130/130	85/85	Sauger
MKL14	80/80	140/140	120/120	160/160		-10/-10	130/130	100/100	Sauger
MKL15	80/80	140/140	120/120	160/160		-10/-10	100/130	100/100	Sauger
MKL16	80/80	140/140	120/120	160/160		-10/-10	100/100	85/85	Sauger
MKL17	80/80	140/140	120/120	160/160		-10/-10	100/130	85/85	Sauger
MKL18	80/80	140/140	120/120	160/160		-10/-10	100/130	85/85	Sauger
MKL19	80/80	140/140	120/120	160/160		-10/-10	100/130	85/100	Sauger
MKL20	80/80	140/140	120/120	160/160		-10/-10	100/100	100/100	Sauger
MKL21	80/80	140/140	120/120	160/160		-10/-10	130/130	85/85	Sauger
MKL22	80/80	140/140	120/120	160/160		-10/-10	100/130	85/100	Sauger

Sample	*PGM-1**	*mMDH-1**	*sMDH-3**	*PROT-3**	*ALAT**	*IDDH**	*SOD-2**	*EST**	Genetic Identification
MKL23	80/80	140/140	120/120	160/160		-10/-10	100/130	85/85	Sauger
MKL24	80/80	140/140	120/120	160/160		**-10/100**	100/130	85/85	**Backcross to Sauger**
MKL25	80/80	140/140	120/120	160/160		-10/-10	100/130	85/85	Sauger
MKL26	80/80	140/140	120/120	160/160		-10/-10	130/130	100/100	Sauger
MKL27	80/80	140/140	120/120	160/160		-10/-10	100/130	100/100	Sauger
MKL28	80/80	140/140	120/120	160/160		-10/-10	100/130	85/100	Sauger
MKL29	80/80	140/140	120/120	160/160		-10/-10	100/100	100/100	Sauger
MKL30	80/80	140/140	120/120	160/160		-10/-10	130/130	85/85	Sauger
MKL31	80/80	140/140	120/120	160/160		-10/-10	100/100	85/85	Sauger
MKL32	80/80	140/140	120/120	160/160		-10/-10	130/130	100/100	Sauger
MKL33	80/80	140/140	120/120	160/160		-10/-10	100/130	85/100	Sauger
MKL34	80/80	140/140	120/120	160/160		-10/-10	100/100	85/85	Sauger
MKL35	80/80	140/140	120/120	160/160		-10/-10	100/130	100/100	Sauger
MKL36	80/80	140/140	120/120	160/160		-10/-10	100/130	85/85	Sauger
MKL37	80/80	140/140	120/120	160/160		-10/-10	100/130	100/100	Sauger
MKL38	80/80	140/140	120/120	160/160		-10/-10	100/130	100/100	Sauger
MKL39	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
MKL40	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
MKL41	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	85/100	Sauger
MKL42	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
MKL43	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
Yellowstone River upper reach									
YSU 1	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
YSU 2	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
YSU 3	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
YSU 4	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
YSU 5	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
YSU 6	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
YSU 7	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
YSU 8	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
YSU 9	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
YSU 10	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
Yellowstone River middle reach									
YSM1	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
YSM2	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
YSM3	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger

Sample	*PGM-1**	*mMDH-1**	*sMDH-3**	*PROT-3**	*ALAT**	*IDDH**	*SOD-2**	*EST**	Genetic Identification
YSM4	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
YSM5	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
YSM6	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
YSM7	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
YSM8	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
YSM9	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
YSM10	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
YSM11	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
YSM12	80/80	140/140	120/120	160/160		-10/-10	130/130	100/100	Sauger
YSM13	80/80	140/140	120/120	160/160		-10/-10	100/100	100/100	Sauger
YSM14	80/80	140/140	120/120	160/160		-10/-10	100/100	100/100	Sauger
YSM15	80/80	140/140	120/120	160/160		-10/-10	130/130	100/100	Sauger
YSM16	80/80	140/140	120/120	160/160		-10/-10	100/100	100/100	Sauger
YSM17	80/80	140/140	120/120	160/160		-10/-10	100/100	100/100	Sauger
YSM18	**80/100**	140140	120120	**100/160**		-10/-10	100/100	100/100	**Backcross to Sauger**
YSM19	80/80	140/140	120/120	160/160		-10/-10	130/130	100/100	Sauger
YSM20	80/80	140/140	120/120	160/160		-10/-10	100/130	100/100	Sauger
YSM21	80/80	140/140	120/120	160/160		-10/-10	100/130	100/100	Sauger
YSM22	80/80	140/140	120/120	160/160		-10/-10	130/130	100/100	Sauger
YSM23	80/80	140/140	120/120	160/160		-10/-10	100/130	100/100	Sauger
YSM24	80/80	140/140	120/120	160/160		-10/-10	100/130	100/100	Sauger
YSM25	80/80	140/140	120/120	160/160		-10/-10	130/130	100/100	Sauger
YSM26	80/80	140/140	120/120	160/160		-10/-10	100/100	100/100	Sauger
YSM27	80/80	140/140	120/120	160/160		-10/-10	100/130	100/100	Sauger
YSM28	80/80	140/140	120/120	160/160		-10/-10	100/100	100/100	Sauger
YSM29	80/80	140/140	120/120	160/160		-10/-10	100/130	100/100	Sauger
YSM30	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
YSM31	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
YSM32	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	85/100	Sauger
YSM33	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
Yellowstone River lower reach									
YSL1	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	85/85	Sauger
YSL2	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
YSL3	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
YSL4	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
YSL5	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
YSL6	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger

Sample	*PGM-1**	*mMDH-1**	*sMDH-3**	*PROT-3**	*ALAT**	*IDDH**	*SOD-2**	*EST**	Genetic Identification
YSL7	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
YSL8	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
YSL9	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
YSL10	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
YSL11	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	85/100	Sauger
YSL12	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
YSL13	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
YSL14	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
YSL15	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
YSL16	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
YSL17	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
YSL18	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
YSL19	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
YSL20	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
YSL21	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	85/100	Sauger
YSL23	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
YSL24	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
YSL25	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
YSL26	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	85/100	Sauger
YSL27	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
YSL28	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
YSL29	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
YSL30	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
YSL31	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
YSL32	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
YSL33	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
YSL34	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	85/85	Sauger
YSL35	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
YSL36	80/80	140/140	120/120	160/160	**85/100**	-10/-10	100/130	85/85	**Backcross to Sauger**
YSL37	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
YSL38	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
YSL39	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
YSL40	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
YSL41	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
YSL42	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
YSL43	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
YSL44	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger

Sample	*PGM-1**	*mMDH-1**	*sMDH-3**	*PROT-3**	*ALAT**	*IDDH**	*SOD-2**	*EST**	Genetic Identification
YSL45	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
YSL46	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
YSL47	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
YSL48	**80/100**	140140	120120	160160	85/85	-10/-10	100/100	85/85	**Backcross to Sauger**
YSL49	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
YSL50	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
YSL51	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
YSL52	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
YSL53	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
Powder River									
POW 1	80/80	140/140	120/120	160/160	85/85	10/10	100/100	85/100	Sauger
POW 2	80/80	140/140	120/120	160/160	85/85	10/10	100/130	85/100	Sauger
POW 3	80/80	140/140	120/120	160/160	85/85	10/10	130/130	100/100	Sauger
POW 4	80/80	140/140	120/120	160/160	85/85	10/10	100/100	100/100	Sauger
POW 5	80/80	140/140	120/120	160/160	85/85	10/10	100/130	100/100	Sauger
POW 6	80/80	140/140	120/120	160/160	85/85	10/10	100/130	100/100	Sauger
POW 7	80/80	140/140	120/120	160/160	85/85	10/10	100/130	100/100	Sauger
POW 8	80/80	140/140	120/120	160/160	85/85	10/10	130/130	100/100	Sauger
POW 9	80/80	140/140	120/120	160/160	85/85	10/10	100/100	100/100	Sauger
POW 10	80/80	140/140	120/120	160/160	85/85	10/10	100/100	100/100	Sauger
Tongue River									
TON1	80/80	140/140	120/120	160/160	85/85	10/10	130/130	100/100	Sauger
TON2	80/80	140/140	120/120	160/160	85/85	10/10	130/130	85/85	Sauger
Boysen Reservoir, WY									
BOY1	80/80	140/140	120/120	160/160	85/85	10/10	130/130	100/100	Sauger
BOY2	80/80	140/140	120/120	160/160	85/85	10/10	100/130	100/100	Sauger
BOY3	80/80	140/140	120/120	160/160	85/85	10/10	130/130	100/100	Sauger
BOY4	80/80	140/140	120/120	160/160	85/85	10/10	130/130	100/100	Sauger
BOY5	80/80	140/140	120/120	160/160	85/85	10/10	130/130	100/100	Sauger
BOY6	80/80	140/140	120/120	160/160	85/85	10/10	100/130	100/100	Sauger
BOY7	80/80	140/140	120/120	160/160	85/85	10/10	100/100	85/85	Sauger
BOY8	80/80	140/140	120/120	160/160	85/85	10/10	100/130	85/85	Sauger
BOY9	80/80	140/140	120/120	160/160	85/85	10/10	100/130	85/85	Sauger
BOY10	80/80	140/140	120/120	160/160	85/85	10/10	100/130	85/10	Sauger
BOY11	80/80	140/140	120/120	160/160	85/85	10/10	130/130	85/85	Sauger
BOY12	80/80	140/140	120/120	160/160		10/10	100/100	85/85	Sauger
BOY13	80/80	140/140	120/120	160/160		10/10	100/130	85/85	Sauger

Sample	*PGM-1**	*mMDH-1**	*sMDH-3**	*PROT-3**	*ALAT**	*IDDH**	*SOD-2**	*EST**	Genetic Identification
BOY14	80/80	140/140	120/120	160/160		10/10	100/100	100/100	Sauger
BOY15	80/80	140/140	120/120	160/160		10/10	130/130	100/100	Sauger
BOY16	80/80	140/140	120/120	160/160		10/10	100/130	85/85	Sauger
BOY17	80/80	140/140	120/120	160/160		10/10	100/130	85/100	Sauger
Bighorn Reservoir, WY									
BHRV1	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	85/100	Sauger
Bighorn River, WY									
BHU1	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
BHU2	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
BHU3	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	85/100	Sauger
BHU4	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	85/100	Sauger
BHU5	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	85/100	Sauger
BHU6	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
BHU7	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
BHU8	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
BHU9	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
BHU10	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
BHU11	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
BHU12	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
BHU13	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
BHU14	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
BHU15	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
BHU16	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
BHU17	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
BHU18	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
BHU19	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
BHU20	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
BHU21	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
BHU22	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	85/100	Sauger
BHU23	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
BHU24	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
BHU25	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
BHU26	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	85/100	Sauger
BHU27	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
BHU28	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	60/100	Sauger
BHU29	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
BHU30	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger

Sample	*PGM-1**	*mMDH-1**	*sMDH-3**	*PROT-3**	*ALAT**	*IDDH**	*SOD-2**	*EST**	Genetic Identification
Lake Sakakawea, ND									
SAK 1	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
SAK 2	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
SAK 3	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
SAK 4	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	85/100	Sauger
SAK 5	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
SAK 6	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
SAK 7	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	85/85	Sauger
SAK 8	**80/100**	**100/140**	120/120	160/160	**85/100**	-10/-10	130/130	100/100	**BxSar**
SAK 9	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
SAK 10	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	85/100	Sauger
SAK 11	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
SAK 12	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
SAK 13	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
SAK 14	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
SAK 15	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
SAK 16	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	85/100	Sauger
SAK 17	80/80	140/140	120/120	160/160	85/85	**-10/100**	130/130	85/85	**BxSar**
SAK 18	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	85/85	Sauger
SAK 19	80/80	**100/140**	120/120	**100/160**	85/85	**-10/100**	100/130	85/85	**BxSar**
SAK 20	**80/100**	**100/140**	**100/120**	160/160	85/85	**-10/100**	100/100	100/100	**BxSar**
SAK 21	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	85/100	Sauger
SAK 22	80/80	**100/140**	120/120	160/160	**100/100**	-10/-10	130/130	100/100	**BxSar/Fx**
SAK 23	80/80	**100/140**	120/120	160/160	85/85	**-10/100**	100/130	85/85	**BxSar**
SAK 24	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
SAK 25	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
SAK 26	**80/100**	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	**BxSar**
SAK 27	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	85/85	Sauger
SAK 28	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	85/100	Sauger
SAK 29	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	85/100	Sauger
SAK 30	80/80	140/140	120/120	160/160	85/85	-10/-10	100/130	100/100	Sauger
SAK 31	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
SAK 32	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	85/100	Sauger
SAK 33	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
SAK 34	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
SAK 35	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
SAK 36	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger

Sample	***PGM-1****	***mMDH-1****	***sMDH-3****	***PROT-3****	***ALAT****	***IDDH****	***SOD-2****	***EST****	**Genetic Identification**
SAK 37	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
SAK 38	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	85/100	Sauger
SAK 39	80/80	**100/140**	120/120	160/160	**85/100**	-10/-10	100/130	85/100	**BxSar**
SAK 40	80/80	140/140	120/120	160/160	85/85	-10/-10	130/130	100/100	Sauger
SAK 41	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger
SAK 42	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	85/100	Sauger
SAK 43	80/80	140/140	120/120	160/160	**85/100**	-10/-10	100/130	85/100	**BxSar**
SAK 44	80/80	140/140	120/120	160/160	85/85	-10/-10	100/100	100/100	Sauger

APPENDIX II – microsatellite data

Genotypes of individuals assayed

ID#	Pop	Svi2		Svi4		Svi7		Svi17		Svi18		Svi20		Svi26		Svi33	
1	AFP	249	253	117	119	180	192	96	98	120	122	170	186	159	165	121	141
2	AFP	249	263	117	139	184	192	98	98	120	122	172	172	153	155	105	129
3	AFP	253	253	117	139	184	198	96	98	120	122	176	186	165	187	103	107
4	AFP	205	257	113	139	172	180	96	116	120	126	176	176	159	167	87	133
5	AFP	249	251	115	119	180	180	96	98	122	122	164	180	159	159	131	133
6	AFP	245	253	115	117	180	200	98	98	120	120	172	188	159	159	123	133
7	AFP	251	263	119	135	186	196	96	98	120	122	180	194	159	165	129	131
8	AFP	249	257	117	119	180	198	96	96	124	124	180	188	153	159	125	131
9	AFP	251	265	117	117	0	0	96	98	120	122	170	182	0	0	0	0
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11	AFP	253	267	107	139	220	224	96	98	120	122	184	188	159	159	129	131
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25	AFP	253	263	107	117	192	220	96	96	122	122	180	188	159	181	121	129
26	AFP	263	265	107	119	196	198	96	96	122	122	164	168	159	161	103	141
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28	AFP	253	253	117	123	180	190	96	96	120	122	172	172	159	165	103	139
29	AFP	253	265	117	117	194	200	96	96	120	122	164	172	161	161	129	131

30	AFP	249	265	117	117	192	192	96	100	122	122	170	188	153	159	91	103
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32	AFP	251	265	117	137	0	0	96	98	120	122	186	188	0	0	103	109
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35	AFP	253	253	119	139	180	180	96	96	120	122	174	180	165	187	91	123
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39	AFP	249	263	137	137	180	218	96	96	120	122	168	180	159	159	103	135
40	AFP	249	263	117	121	180	198	96	98	120	122	168	182	165	181	103	137
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42	AFP	253	257	117	117	180	194	96	96	120	120	168	186	153	165	107	137
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47	AFP	249	257	117	139	180	198	96	96	120	120	178	180	159	177	103	131
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57	AFP	245	263	123	139	180	180	96	96	120	122	164	176	169	181	107	137
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77	BFP	243	253	117	139	0	0	96	98	120	122	172	178	165	187	131	133
78	BFP	243	253	117	117	180	192	96	96	120	120	184	184	153	159	107	131
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81	BFP	243	261	119	119	222	224	96	96	120	122	172	186	159	187	129	131
82	BFP	249	265	115	117	180	220	96	96	120	120	172	186	187	187	103	103
83	BFP	253	255	117	139	190	226	96	96	122	122	168	186	153	159	103	133
84	BFP	249	249	117	139	192	194	96	96	120	122	180	184	165	189	129	135
85	BFP	245	253	117	123	180	194	96	96	120	122	170	184	153	165	103	107
86	BFP	243	257	117	123	200	208	96	96	120	122	180	186	165	165	103	133
87	BFP	253	253	117	117	198	220	96	96	120	120	186	186	153	187	117	133
88	BFP	253	265	117	117	0	0	96	98	120	120	172	186	165	189	133	137
89	BFP	249	261	107	139	180	200	96	96	122	122	180	180	159	159	121	139
90	BFP	0	0	0	0	0	0	98	98	120	120	172	172	187	189	103	123
91	BFP	263	265	113	117	194	200	96	98	120	122	172	180	189	189	123	129
92	BFP	0	0	117	137	0	0	96	98	122	122	184	188	165	165	107	137
93	BFP	253	263	139	139	192	192	96	96	120	120	172	190	0	0	91	103
94	BFP	253	271	117	117	194	198	98	98	120	122	172	180	159	159	103	139
95	BFP	253	255	119	119	196	196	96	100	120	120	178	180	155	159	137	137
96	BFP	263	267	117	139	194	200	96	96	122	122	168	190	153	159	99	115
97	BFP	243	251	113	121	184	222	96	96	120	122	164	178	153	153	103	107

98	BFP	251	251	123	139	180	184	96	96	120	122	180	184	159	165	105	129
99	BFP	249	253	117	117	180	190	96	98	122	122	168	194	159	189	107	121
100	BFP	249	253	115	117	180	192	96	98	122	122	172	182	159	159	103	137
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102	BFP	253	253	115	139	0	0	96	98	120	120	178	188	159	177	119	129
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105	BFP	253	259	117	119	190	198	98	98	120	120	172	188	165	165	129	131
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107	BFP	255	265	117	137	194	200	96	98	120	122	180	180	165	187	103	141
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122	BFP	253	255	119	139	180	184	96	96	120	124	180	180	153	187	103	107
123	BFP	251	253	117	117	192	194	0	0	120	122	180	180	0	0	0	0
124	BFP	245	255	107	117	194	222	98	98	120	122	176	186	159	161	103	129
125	BFP	251	265	117	119	188	194	98	100	120	122	188	194	0	0	0	0
126	BFP	249	253	115	119	180	180	0	0	120	120	0	0	0	0	0	0
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128	BFP	241	257	117	119	180	192	96	96	120	120	172	182	159	165	107	129
129	BFP	253	255	119	119	180	180	96	116	122	122	188	188	159	181	109	129
130	BFP	257	265	117	117	194	220	96	96	120	120	174	184	159	189	107	129
131	BFP	249	253	107	123	180	184	96	96	120	122	172	170	153	187	103	117

132	BFP	249	253	107	139	184	192	96	96	120	120	164	172	165	165	103	129
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134	BFP	249	253	107	117	180	192	96	96	120	120	172	184	161	161	129	135
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143	BFP	253	265	115	117	192	220	96	96	120	122	168	184	153	165	103	129
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161	BFP	0	0	107	119	0	0	96	96	120	122	178	180	0	0	91	107
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163	BFP	243	255	115	117	200	220	98	98	120	120	172	188	165	187	107	127
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189	BFP	249	257	119	139	186	198	0	0	120	120	186	190	0	0	0	0
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191	YEL	249	259	115	117	190	200	96	98	122	122	182	190	159	187	137	139
192	YEL	253	253	117	117	180	180	96	96	122	122	172	172	165	185	103	107
193	YEL	253	261	115	127	180	190	96	96	122	122	172	190	185	187	105	137
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219	YEL	263	265	139	139	192	194	96	98	120	122	176	184	0	0	103	103
220	YEL	249	267	115	119	0	0	96	98	120	122	170	190	159	165	123	133
221	YEL	253	267	115	137	194	208	96	96	122	122	170	186	153	159	107	131
222	YEL	249	265	117	139	192	202	96	98	122	122	178	182	153	165	103	125
223	YEL	257	265	117	139	180	196	96	98	120	120	172	182	153	165	111	127
224	YEL	253	253	117	137	192	200	96	100	122	122	170	184	159	159	107	123
225	YEL	251	263	115	117	190	192	98	98	122	122	172	186	159	185	129	137
226	YEL	249	253	119	139	194	198	96	96	120	122	170	180	165	189	103	131
227	YEL	0	0	123	137	196	200	96	96	120	122	170	172	0	0	103	105
228	YEL	257	265	117	137	180	192	96	98	120	122	170	170	183	189	129	131
229	YEL	263	265	137	139	180	200	96	96	122	122	180	182	155	187	103	131
230	YEL	251	253	107	139	190	194	96	96	122	122	164	176	159	183	133	139
231	YEL	0	0	117	117	180	184	96	96	122	122	180	182	159	181	121	137
232	YEL	249	253	117	139	180	190	96	96	122	122	172	188	155	161	107	131
233	YEL	253	253	119	123	196	208	96	98	120	122	170	188	165	181	103	119

234	YEL	253	267	117	119	180	196	96	96	120	122	172	180	181	187	103	135
235	YEL	249	253	117	119	184	200	98	100	120	120	168	194	159	165	103	137
236	YEL	249	251	117	119	222	224	96	98	122	124	172	180	153	159	97	105
237	YEL	263	263	117	137	180	200	96	96	122	122	184	186	153	165	103	135
238	YEL	249	251	139	141	180	190	96	96	120	122	164	164	159	159	103	103
239	YEL	249	263	117	117	192	194	96	96	122	122	170	180	159	159	103	135
240	YEL	251	259	117	137	180	190	96	96	120	122	180	180	155	165	121	139
241	YEL	249	261	117	139	180	226	96	98	120	122	180	198	161	163	103	103
242	YEL	253	253	113	117	194	200	96	96	0	0	0	0	155	159	103	129
243	YEL	251	253	137	139	180	180	96	98	120	122	178	180	159	165	105	139
244	YEL	263	265	137	139	180	196	98	98	120	122	178	190	159	165	103	103
245	YEL	259	263	117	117	190	192	96	98	122	122	180	184	153	159	93	139
246	YEL	253	265	117	137	180	180	96	98	120	122	180	188	165	183	103	133
247	YEL	251	253	115	139	198	200	96	98	120	122	186	188	159	165	103	119
248	YEL	249	261	117	119	194	220	96	98	120	122	170	172	159	165	121	131
249	YEL	251	253	117	139	180	180	96	98	120	122	164	190	159	159	127	131
250	YEL	247	263	117	119	186	198	98	98	120	120	172	172	165	173	129	135
251	YEL	253	261	115	117	186	196	96	96	122	122	182	188	153	155	137	137
252	YEL	249	259	117	123	192	200	96	98	122	122	172	172	0	0	127	131
253	YEL	253	253	117	139	196	218	96	96	122	122	172	172	165	165	91	139
254	YEL	249	253	117	139	180	220	96	98	122	122	184	186	0	0	131	133
255	YEL	249	263	137	139	196	196	96	98	120	122	172	184	0	0	121	129
256	YEL	249	253	115	117	180	226	96	98	120	122	172	184	181	189	103	133
257	YEL	249	253	115	117	0	0	98	98	120	120	188	188	159	159	103	137
258	YEL	253	257	119	137	194	198	96	98	120	122	180	184	161	187	103	103
259	YEL	253	253	115	119	180	224	96	98	120	120	172	190	187	189	131	137
260	YEL	249	249	115	117	184	192	96	96	120	122	182	182	187	189	121	133
261	YEL	253	267	117	117	0	0	96	96	120	122	172	182	0	0	103	121
262	YEL	263	265	117	141	192	194	96	98	120	122	164	186	153	181	133	137
263	YEL	253	263	101	137	172	180	96	96	122	122	168	172	0	0	107	131
264	YEL	249	253	117	137	180	192	96	98	122	122	184	192	159	187	129	133
265	YEL	253	253	107	119	192	198	96	96	120	122	172	172	163	165	103	129
266	YEL	253	265	139	139	194	202	96	96	120	122	172	188	187	189	129	135
267	YEL	0	0	107	139	198	200	96	96	122	122	168	172	165	187	105	121

268	YEL	263	263	117	139	184	194	96	96	122	122	180	182	153	153	103	137
269	YEL	259	263	123	137	200	208	98	98	120	122	172	188	159	165	139	145
270	YEL	249	259	107	117	180	222	98	98	120	122	180	184	159	159	137	141
271	YEL	253	255	119	139	180	194	96	96	120	120	170	172	187	187	103	129
272	YEL	253	253	107	139	192	208	96	96	122	122	172	172	159	165	103	107
273	YEL	249	259	137	139	190	200	96	98	120	120	176	180	177	187	121	137
274	YEL	259	263	117	117	184	222	96	96	120	122	170	176	159	159	133	137
275	YEL	253	265	139	139	194	196	98	98	120	122	180	180	159	187	129	133
276	YEL	249	249	119	119	180	196	96	96	120	122	180	180	159	181	129	137
277	YEL	245	253	107	137	192	216	96	96	122	122	172	176	159	165	119	121
278	YEL	249	263	113	119	180	182	96	96	122	122	182	190	159	187	131	141
279	YEL	253	253	115	117	180	194	96	98	122	122	172	176	153	159	131	139
280	YEL	249	265	121	139	180	192	98	98	122	122	164	182	159	195	107	131
281	YEL	253	253	113	119	184	192	96	96	122	122	182	186	165	187	133	141
282	YEL	249	267	107	137	192	222	96	98	122	122	172	182	165	189	129	137
283	YEL	253	263	107	139	190	192	96	98	120	122	164	194	163	165	127	139
284	SAK	249	253	119	137	192	194	0	0	120	120	180	182	0	0	103	137
285	SAK	253	265	119	119	192	196	96	96	120	122	172	188	189	193	107	131
286	SAK	249	265	107	139	180	192	96	98	120	122	168	180	159	159	91	131
287	SAK	253	253	119	139	180	188	0	0	120	120	164	164	0	0	123	139
288	SAK	249	253	117	139	180	200	96	96	122	122	186	186	159	159	119	129
289	SAK	251	251	115	119	184	198	98	98	120	122	180	188	159	183	109	135
290	SAK	249	265	119	119	180	200	0	0	120	120	164	164	0	0	123	135
291	SAK	253	267	119	137	180	192	96	98	120	120	164	168	159	165	103	129
292	SAK	251	267	119	137	180	192	96	98	122	122	176	186	151	151	103	139
293	SAK	249	265	117	119	200	208	96	98	120	122	176	184	161	161	123	131
294	SAK	253	253	115	117	192	214	96	96	120	120	178	184	165	187	129	129
295	SAK	253	267	117	137	184	200	96	96	122	122	170	170	159	159	91	103
296	SAK	253	263	117	119	180	192	96	96	122	122	164	168	159	165	107	109
297	SAK	253	255	115	115	200	200	96	98	120	122	164	180	159	189	91	107
298	SAK	195	249	109	139	174	192	96	96	120	120	178	190	161	171	99	137
299	SAK	249	263	115	137	180	198	0	0	120	120	178	178	159	161	109	139
300	SAK	257	267	119	121	190	194	96	98	122	122	172	180	153	161	129	129
301	SAK	253	265	117	133	200	222	0	0	122	122	180	180	153	153	103	133

302	SAK	241	263	107	119	192	208	96	96	120	122	180	188	161	161	129	129
303	SAK	245	249	117	117	180	194	0	0	120	122	164	188	0	0	103	131
304	SAK	249	263	117	119	190	198	96	98	120	120	172	182	159	159	103	103
305	SAK	253	263	115	117	180	184	96	96	120	122	184	184	165	187	115	133
306	SAK	249	251	107	117	190	200	96	96	120	120	182	182	165	165	103	121
307	SAK	253	261	121	135	180	200	96	98	120	120	174	178	155	161	107	139
308	SAK	249	253	107	115	170	180	0	0	0	0	0	0	0	0	111	133
309	SAK	253	261	117	123	220	224	96	96	120	122	168	180	159	159	117	129
310	SAK	253	263	115	141	180	194	96	96	120	122	172	184	153	159	121	127
311	SAK	257	263	117	119	180	200	98	98	120	122	186	186	159	181	103	129
312	SAK	251	263	117	123	180	216	96	96	122	122	176	176	183	187	123	137
313	SAK	245	253	117	139	180	188	96	96	120	120	180	180	159	165	123	139
314	SAK	257	265	113	117	200	208	96	98	120	122	180	186	165	185	131	133
315	SAK	249	267	119	119	186	192	96	98	122	122	172	180	159	185	103	105
316	SAK	253	253	113	117	190	194	96	98	122	122	172	178	161	161	105	129
317	SAK	253	263	117	119	180	202	98	98	122	122	178	180	165	165	103	107
318	SAK	253	263	117	117	192	198	96	98	122	122	0	0	161	165	103	137
319	SAK	253	265	119	137	180	180	98	98	120	120	172	172	159	165	131	137
320	SAK	263	265	113	117	180	200	0	0	122	122	170	178	0	0	131	137
321	SAK	253	253	117	135	194	198	96	98	120	120	186	190	153	159	123	129
322	BHR	253	253	117	139	192	194	96	98	120	120	172	180	161	189	129	135
323	BHR	0	0	0	0	0	0	96	96	124	126	180	184	187	191	103	129
324	BHR	249	265	119	119	180	180	96	96	120	122	172	182	159	161	103	129
325	BHR	257	265	117	139	192	194	96	96	120	122	180	184	187	189	125	131
326	BHR	253	265	119	139	180	182	96	96	120	120	172	182	159	161	129	131
327	BHR	249	253	115	117	180	200	96	98	122	122	180	190	187	189	103	129
328	BHR	249	253	117	119	192	194	96	96	120	122	170	190	165	187	129	129
329	BHR	0	0	137	139	180	220	96	96	122	122	164	172	187	189	129	129
330	BHR	249	249	119	139	180	192	96	98	120	122	172	178	165	189	131	135
331	BHR	249	265	117	119	192	192	96	96	120	120	182	184	159	165	129	129
332	BHR	249	253	117	139	180	194	96	98	120	122	172	172	159	165	103	103
333	BHR	249	263	115	117	180	210	96	96	122	122	172	172	187	189	131	135
334	BHR	253	263	107	117	192	194	96	98	120	122	172	180	159	159	135	137
335	BHR	249	253	117	139	200	210	96	98	122	122	172	172	187	187	119	129

336	BHR	249	253	139	139	180	184	96	98	120	122	172	188	159	189	129	135
337	BHR	245	265	117	139	192	200	96	96	120	122	172	180	181	191	131	135
338	BHR	249	253	117	119	180	192	96	98	120	122	178	184	155	159	129	135
339	BHR	253	265	117	119	180	180	96	96	120	122	178	180	159	159	103	139
340	BHR	257	265	115	117	192	194	96	96	122	122	172	182	159	187	129	129
341	BHR	249	249	139	139	200	210	96	96	120	122	172	172	165	187	129	131
342	BHR	249	265	139	139	192	192	96	96	122	122	172	184	187	189	129	131
343	BHR	263	265	117	139	194	194	96	98	120	122	180	180	187	187	103	129
344	BHR	249	263	117	139	184	200	96	96	120	122	172	176	159	159	129	131

APPENDIX III

Publications and presentations resulting from this work

This section lists the publications and presentations that have already arisen from this work to date. We plan to submit further manuscripts from this work to Transactions of the American Fisheries Society and to Conservation Biology.

Publications:

(1) Koigi, R.N. 2004. Genetic variation, hybridization and introgression in Montana sauger populations. Master's thesis. Department of Biological and Environmental Sciences, Troy University, Troy, Alabama. 77 pp.

(2) Koigi, R., N. Billington, D. Porter, F. White, W. Gardner, and V. Riggs. 2004. Hybridization between native sauger and introduced walleye in Montana, and in Yellowstone river sauger brood stock. Gene Families and Isozymes Bulletin 37:28.

(3) Koigi, R. N., N. Billington, J. Xiong, J. Gaston, P. T. Ezell, W. Gardner and V. Riggs. 2005. Use of isozyme markers to document genetic variation in Montana sauger and to screen for hybrids with walleye in sauger brood fish. Gene Families and Isozymes Bulletin 38:12.

Published abstracts:

(1) Koigi, R. N., N. Billington, and W. Gardner. 2004. Genetic variation, hybridization and introgression in Montana sauger populations. Southeastern Biologist 51: 200.

(2) Koigi, R. N., N. Billington, and W. Gardner. 2004. Conservation genetics of Montana sauger. Journal of the Alabama Academy of Sciences 75: 59.

(3) Koigi, R. N., N. Billington, W. Gardner. 2005. Conservation genetics of Montana sauger. Southeastern Biology 52: 125-126.

(4) Barr, A, R. N. Koigi, R. E. Creech, J. Gaston, and N. Billington. 2006. Genetic variation in sauger populations determined by protein electrophoresis. Southeastern Biologist 53: 165-166.

(5) Koigi, R. N., J. Xiong, N. Billington, and W. Gardner. 2006. Genetic variation in Montana sauger and hybridization with walleye. The Journal of the Alabama Academy of Science 78 – in press.

(6) Gaston, J., R. N. Koigi, R. E. Creech, P. T. Ezell, and N. Billington. 2006. Hybridization and introgression between sauger and walleye determined by protein electrophoresis. The Journal of the Alabama Academy of Science 78 – in press.

(7) Barr, A. and N. Billington. 2006. Protein electrophoretic distribution of genetic variation in sauger populations. The Journal of the Alabama Academy of Science 78 – in press.

Technical reports:

(1) Billington, N., R. N. Koigi, and J. Xiong. 2005. Genetic variation in Montana sauger populations determined by protein electrophoresis and hybridization with walleye**.** Technical Report of the Department of Biological and Environmental Sciences, Troy University to Montana Department of Fish, Wildlife and Parks. 66 pp. December 6, 2005.

Presentations:

(1) Billington, N., R. N. Koigi, and W. Gardner. 2003. Genetic variation in Montana sauger populations: stock structure, hybridization and conservation genetics. AFS NCD Walleye Technical Committee Summer Meeting, 24th July, Wassau, WI, U.S.A.

(2) Billington, N., R. N. Koigi, and W. Gardner. 2004. Genetic variation, hybridization and introgression in Montana sauger populations. 53rd Annual Meeting of the Great Plains Fishery Workers Association, 3rd Feb., Winnipeg, Manitoba, Canada.

(3) Koigi, R. N., N. Billington, and W. Gardner. 2004. Conservation genetics of Montana sauger. 81st Annual Meeting of the Alabama Academy of Science, 19th March, University of Montevallo, Montevallo, AL., U.S.A.

(4) Koigi, R. N., N. Billington, and W. Gardner. 2004. Genetic variation, hybridization and introgression in Montana sauger populations. Poster paper 65th Annual Meeting at the Association of Southeastern Biologists, 15th April, Memphis, TN, U.S.A.

(5) Billington, N., R. N. Koigi, and W. Gardner. 2004. Conservation genetics of Montana sauger. 134th Annual Meeting of the American Fisheries Society, 24th August, Madison, WI, U.S.A.

(6) Koigi, R. N., N. Billington, W. Gardner. 2005. Conservation genetics of Montana sauger. 66th Annual Meeting of the Association of Southeastern Biologists, 14th April Florence, AL, 2005.

(7) Koigi, R. N., J. Xiong, N. Billington, and W. Gardner. 2006. Genetic variation in Montana sauger and hybridization with walleye. 83rd Annual meeting of the Alabama Academy of Science, 17th March, Troy University, Troy, AL, U.S.A.

(8) Gaston, J., R. N. Koigi, R. E. Creech, P. T. Ezell, and N. Billington. 2006. Hybridization and introgression between sauger and walleye determined by protein electrophoresis. 83rd Annual meeting of the Alabama Academy of Science, 17th March, Troy University, Troy, AL, U.S.A.

(9) Barr, A. and N. Billington. 2006. Protein electrophoretic distribution of genetic variation in sauger populations. 83rd Annual meeting of the Alabama Academy of Science, 17th March, Troy University, Troy, AL, U.S.A. (Ms. Barr received the Alabama Academy of Science – Section I Biological Sciences – Student Research Award for her paper)

(10) Barr, A, R. N. Koigi, R. E. Creech, J. Gaston, and N. Billington. 2006. Genetic variation in sauger populations determined by protein electrophoresis. 67th Annual Meeting of the Association of Southeastern Biologists, 30th March, Gatlinburg, TN.