INTROGRESSION BETWEEN INTRODUCED WALLEYE AND NATIVE SAUGER IN FORT PECK RESERVOIR AND THE YELLOWSTONE RIVER, MONTANA

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Abstract. - *Stizostedion* were collected from Fort Peck Reservoir and the Yellowstone River, Montana. Allozyme electrophoresis of liver and muscle tissue indicated introgression of introduced walleye, *S. vitreum,* genes into the native sauger, *S. canadense,* and of sauger genes into the walleye in both locations. We believe this introgression is largely due to misidentification of sauger and fish of hybrid origin as walleye during walleye spawning operations. This introgression seriously threatens the genetic integrity of the native sauger populations.

INTRODUCTION

Hybridization and introgression between native and introduced fish species is common (reviewed by Campton 1987; Leary et al. 1995). Sauger, *Stizostedion canadense*, are native to the upper Missouri and Yellowstone river drainages of Montana (Brown 1971; Scott and Crossman 1973). Walleye, *S. vitreum*, are not native to this area (Brown 1971; Scott and Crossman 1973). It is unknown when walleye were introduced into Montana waters, but they now exist extensively within the range of sauger (Brown 1971).

Walleye support a popular sport fishery in Fort Peck Reservoir in the Missouri River drainage and in the Yellowstone River drainage below Miles City, Montana. With the exception of fishing regulations, Montana Fish, Wildlife, and Parks (MFWP) does not manage the walleye population in the Yellowstone River drainage. Natural reproduction in the river is considered insufficient to support the sport fishery and most fish are believed to be migrants from Lake Sakakawea, North Dakota (Vic Riggs, MFWP, personal communication).

In contrast, MFWP actively manages the walleye fishery in Fort Peck Reservoir (Bill Wiedenheft, MFWP, personal communication). Natural reproduction is considered insufficient to support the fishery. Thus, during the spring walleye are trapped in the vicinity of Big Dry Creek and spawned. The eggs are hatched in a hatchery and the resulting fish released into the reservoir. During spawning operations fish considered to be sauger or possibly of hybrid origin are released unspawned.

This study had two objectives. The first was to determine using allozyme electrophoresis how accurately MFWP personnel could distinguish sauger, walleye, and fish of hybrid origin in the field. The second was to use allozyme electrophoresis to estimate the extent of hybridization

between walleye and sauger in Fort Peck Reservoir and the Yellowstone River drainage.

MATERIALS AND METHODS

Samples

During July and August 1995, 158 percids were collected from 19 areas of Fort Peck Reservoir using gill nets. Fish were identified as walleye, sauger, or hybrids in the field and assigned an identification number. A labeled sample of muscle and liver from each fish was frozen for subsequent allozyme electrophoresis. All fish captured were sacrificed so this can be considered a random sample of percids from the reservoir.

A similar procedure was used to collect 48 percids from seven locations in the Yellowstone River below Miles City during September and October 1995. The only difference between this and the Fort Peck Reservoir sample is that one fish caught by an angler thought to be of hybrid origin was purposely included in the Yellowstone River sample.

Electrophoresis

We used horizontal starch gel electrophoresis to screen the products of 42 loci (genes) coding for enzymes expressed in eye, heart, liver, or muscle tissue (Table 1) obtained from two morphologically identified walleye and two morphologically identified sauger collected from the Yellowstone River, Montana for evidence of genetic variation. The purpose of this was to search for loci that did not appear to share alleles (form of a gene) in common between the walleye and sauger. Such loci are commonly termed diagnostic loci because subsequently the alleles detected at them can be used to determine whether tissue samples came from a walleye, sauger, or a fish of hybrid origin. Samples from a walleye would have alleles characteristic of only walleye

(homozygous for walleye alleles) at all diagnostic loci. Likewise, samples from a sauger would be homozygous for sauger alleles at all diagnostic loci. Samples from a first generation hybrid would have alleles characteristic of both species (heterozygous) at all diagnostic loci. Samples from later generation hybrids would be heterozygous at some diagnostic loci and homozygous at others.

Only the diagnostic loci were analyzed from the tissues collected for this study. Each gel contained a known walleye sample as a reference standard. Electrophoresis followed the procedures of Leary and Booke (1990). Stains used to reveal the position of particular enzymes in the gels after electrophoresis followed the recipes of Harris and Hopkinson (1974) and Allendorf et al. (1977). Nomenclature of loci and alleles followed the recommendations of Shaklee et al. (1990).

RESULTS

Diagnostic loci

Three loci (*ALAT**, *IDDH**, and *PGM**) were found to be diagnostic between walleye and sauger. In all cases, the product of the allele characteristic of walleye had a faster electrophoretic mobility than the product of the allele characteristic of sauger. Billington et al. (1990) also found these loci to be diagnostic between the species. Todd (1990) did not analyze *ALAT** or *IDDH**, but did find *PGM** to be diagnostic. The only other locus we are aware of reported to be diagnostic between the species is mitochondrial malate dehydrogenase (Clayton et at. 1973), but we were not capable of consistently resolving the product of this locus.

Field versus laboratory identification

In the Fort Peck Reservoir sample, 109 fish were homozygous for the allele characteristic of walleye at all diagnostic loci. These fish, therefore, appear to have been walleye. All of these fish were identified as walleye in the field. The sample also contained 34 fish that were homozygous for alleles characteristic of sauger at all diagnostic loci. All but one of these fish were identified as sauger in the field. The remaining fish (ID number 33) was identified as a walleye in the field. The sample also contained 15 fish that appeared to be of hybrid origin as they were heterozygous at at least one diagnostic locus (Table 2). None of these fish were identified as hybrids in the field; six were identified as walleye, nine as sauger. (Table 2).

The Yellowstone River sample contained 11 fish that were homozygous for alleles characteristic of walleye at all diagnostic loci. All but one of these were identified as walleye in the field. The remaining fish (ID L) was identified as a sauger in the field. This sample also contained 30 fish that were homozygous for alleles characteristic of sauger at all diagnostic loci. All but two of these fish were identified as sauger in the field. The remaining fish (ID numbers 24 and 25) were identified as being hybrids in the field. This sample also contained seven fish that appeared to be of hybrid origin (Table 2). Of these, two were identified as hybrids, two as walleye, and three as sauger in the field (Table 2).

Hybridization and introgression

A hybrid index score was calculated for each fish in both samples. The allele characteristic of walleye at each diagnostic locus was given a value of zero and the allele characteristic of sauger a value of one. The hybrid index is the sum of these allele values over all

three diagnostic loci. Thus, a pure walleye would have a hybrid index of zero, a pure sauger an index of six, and fish definitely of hybrid origin intermediate values.

The hybrid index scores divide the fish in both samples into two groups. In the Fort Peck Reservoir sample, the first group of fish have scores of zero or one (Fig. 1) indicating these fish to be either pure walleye or at least back crosses between walleye and first generation hybrids. Thus, there is conclusive evidence of introgression of native sauger genes into the introduced walleye population in the reservoir.

The second group of fish in this sample have scores ranging from four to six (Fig. 1) indicating they are either pure sauger or at least back crosses between sauger and first generation hybrids. Thus, there is also conclusive evidence of introgression of introduced walleye genes into the native sauger population in the reservoir.

The extent of introgression is noticeably greater from walleye to sauger than it is from sauger to walleye. In the introgressed walleye, 99.4% of the alleles among the three diagnostic loci originated from walleye and only 0.6% from sauger. In the introgressed sauger, 95.9% of the alleles among the diagnostic loci originated from sauger and 4. 1 % from walleye.

Similar results were obtained from the Yellowstone River sample (Fig. 1). There is one group of fish indicating introgression of native sauger genes into introduced walleye and another group indicating introgression of introduced walleye genes into native sauger. The extent of introgression is also greater from walleye to sauger than from sauger to walleye. In the introgressed walleye, 98.6% of the alleles originated from walleye and in the introgressed sauger, 95.7% of the alleles originated from sauger (Note the latter value is calculated excluding the fish purposely included in the sample because it was believed to be a hybrid).

The next question we addressed was whether the alleles characteristic of walleye were randomly distributed among the fish in the introgressed sauger in both samples. If so, this would indicate that introgression had been occurring for a number of generations and had attained equilibrium so that essentially there are now no pure sauger in one or both populations.

We used the procedure of Hill (1974) to examine whether there was a positive association between alleles characteristic of walleye between diagnostic loci (gametic phase disequilibrium) in both samples. In the Fort Peck Reservoir sample, there was a significant positive association between walleye alleles at *ALAT** and *IDDH** (Table 3). In the Yellowstone River sample, there was a significant positive association between walleye alleles for all possible pairs of loci (Table 3). Thus, in both populations the walleye alleles were not randomly distributed among individuals indicating that introgression had not reached equilibrium and that pure sauger still exist in both populations.

We could not meaningfully test for the presence of gametic phase disequilibrium in the introgressed walleye samples. The extent of introgression in both samples was so low that no fish was heterozygous at more than one diagnostic locus (Fig. 1).

DISCUSSION

Hybridization and introgression between naturally sympatric walleye and sauger populations appears to be rare (Clayton et al. 1973; Billington et al. 1988; Ward et al. 1989; Todd 1990). In contrast, it appears to be common where walleye or first generation hybrids have been introduced (Ward 1992; White and Schell 1995).

Given the limited natural reproduction of walleye, we believe that artificial propagation is

mainly responsible for the introgression of sauger alleles into the walleye populations in Fort Peck Reservoir and the Yellowstone River. The data clearly indicate the field identification is not completely accurate. A sauger from Fort Peck Reservoir was misidentified in the field as a walleye. Such mistakes during spawning operations would result in the production of first generation hybrids. Furthermore, 6 of the 15 fish of hybrid origin in the Fort Peck Reservoir sample were misidentified as walleye. During spawning operations these mistakes would result in introgression of sauger alleles into the walleye population. Similar mistakes are also probably made during walleye spawning operations in Lake Sakakawea (Ward 1992) from which the Yellowstone River fish are believed to originate.

The hybrids occasionally created during artificial propagation are also probably at least partially responsible for the introgression of walleye alleles into the native sauger populations. Of course, we cannot rule out the possibility that sauger and walleye may occasionally hybridize in the wild also contributing to introgression. If this is also the case, then artificial propagation would serve to increase the extent of introgression.

The difference in the extent of introgression from sauger to walleye compared to walleye to sauger may also partially be a consequence of artificial propagation. During spawning operations, there is some selection against hybrid reproduction which may occur to a lesser extent or not at all in the wild.

Introgression from walleye to sauger has not yet resulted in the formation of random hybrid swarms (i.e. populations in which no individual is a pure sauger). The presence of introgression, however, does pose a serious threat to the genetic integrity of the native sauger populations. Given the high proportion of fish of hybrid origin in the Fort Peck Reservoir (24%)

and Yellowstone River (14%) sauger samples it may be too late to prevent the formation of random hybrid swarms. Possibly the best that can be done now is to prevent increasing the extent of introgression. One means of accomplishing this would be to cease walleye artificial propagation. An alternative would be to perform pair matings during spawning operations and subsequently use biochemical techniques to identify the parents. Eggs or the progeny from undesirable matings could then be discarded.

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TABLE I - Enzymes and loci screened for the existence of diagnostic loci between walleye and sauger. Tissues: E = eye, H = heart, L = liver, M = muscle. Buffer is the buffer system used to analyze a particular enzyme.

Enzyme	Loci	Tissue	Buffer
Acid phosphatase	*ACP-1*, ACP-2**	L	AC^+
Adenylate kinase	*AK**	M	AC
Alanine aminotransferase	*ALAT**	L	MF
Alcohol dehydrogenase	*ADH**	L	RW
Aspartate aminotransferase	*sAAT-1**	L	AC, RW
	*sAAT-2**	E	AC, RW
	*sAAT-3**	H, M	AC, RW
Creatine kinase	*CK-A**	M	RW
	*CK-B**	H	RW
Dipeptidase	*PEPA**	E, H, L, M	RW, SR
Fumarate hydratase	*FH-1*, FH-2**	E, H, L, M	AC
Glucose-6-phosphate isomerase	*GPI-B1*, GPI-B2**	M	RW

Enzyme	Loci	Tissue	Buffer
Glyceraldehyde-3-phosphate dehydrogenase	*GAPDH**	E,H	AC^+
Glycerol-3-phosphate dehydrogenase	*G3PDH-1**	M	AC
	*G3PDH-2**		AC
Iditol dehydrogenase	*IDDH**	L	RW
Isocitrate dehydrogenase	*IDHP-1**	L	AC
	*IDHP-2**	M	AC
Lactate dehydrogenase	*LDH-A**, *LDH-C**	E, H	RW, SR
	*LDH-B**	E, H, L, M	RW, SR
Malate dehydrogenase	*MDH-A**	E, H, L, M	AC
	*MDH-B**	M	AC
Malic enzyme	*MEP**	H, L, M	AC
Mannose-6-phosphate isomerase	*MPI-1**	E, H, L, M	MF
	*MPI-2**	E	MF
Phosphoglucomutase	*PGM**	M	AC

Enzyme	Loci	Tissue	Buffer
Phosphogluconate dehydrogenase	*PGDH**	E, H, L, M	AC
Phosphoglycerate kinase	*PGK-1**	H	AC^+
	*PGK-2**	E, H, L, M	AC^+
Phosphoglycerate mutase	*PGAM-1**	H	AC^+
	*PGAM-2**	E, H, L, M	AC^+
Pyruvate kinase	*PK-1**	H, M	MF
	*PK-2**	H	MF
Superoxide dismutase	*sSOD**	L	RW
Triose-phosphate isomerase	*TPI-1**	E, H, L, M	RW, SR
	*TPI-2**	E, H	RW, SR
Tripeptidase	*PEPB**	E, H, L, M	SR
Xanthine dehydrogenase	*XDH**	L	RW

AC = N- (3-aminopropyl) - morpholine and citric acid buffer of Clayton and Tretiak (1972). pH 6.5 for liver and muscle.

AC^+ = same as AC except 2 drops beta-mercapto ethanol and 15mg beta-nicotinamide adenine dinucleotide are added for every 225 ml of gel buffer. pH 6.9 for eye and muscle, 6.3 for liver.

MF = Tris-boric acid-EDTA buffer of Markert and Faulhaber (1965).

RW = Tris-citric acid buffer of Ridgway et al. (1970).

SR = Tris-citric acid buffer of Gall and Bentley (1981).

TABLE 2. - Genotypes of individuals that appear to be of hybrid origin at the diagnostic loci between walleye and sauger. S = homozygous for alleles characteristic of sauger. W = homozygous for alleles characteristic of walleye. W/S heterozygous for alleles characteristic of both walleye and sauger.

Field ID number	**Field ID species**	**Locus and genotype** *ALAT**	*IDDH**	*PGM**
Fort Peck Reservoir				
41	Walleye	S	W/S	S
43	Walleye	W	W/S	W
45	Walleye	W	W/S	W
53	Walleye	W/S	W	W
78	Sauger	W/S	W/S	S
86	Sauger	S	W/S	S
88	Sauger	W/S	S	S
97	Walleye	W/S	W	W
107	Sauger	W/S	S	S
123	Sauger	W/S	S	S
124	Sauger	W/S	S	S
128	Sauger	W/S	W/S	S
156	Walleye	W/S	W/S	S
157	Sauger	S	S	W/S
158	Sauger	S	S	W/S

Field ID number	Field ID species	Locus and genotype *ALAT**	*IDDH**	*PGM**
Yellowstone River				
5	Walleye	W/S	W	W
8	Sauger	W/S	S	S
23	Hybrid	W/S	W/S	S
C	Sauger	S	W/S	S
E	Sauger	W/S	S	W/S
T	Walleye	W/S	W/S	W/S
U	Hybrid	W/S	W/S	W/S

TABLE 3. - Gametic phase disequilibrium (see text for explanation) relative to maximum possible value between pairs of diagnostic loci in sauger populations introgressed with walleye in Fort Peck Reservoir (above diagnol) and the Yellowstone River (below diagnol). * = $P < 0.05$, ** = $P < 0.01$, *** $P < 0.001$.

	*ALAT**	*IDDH**	*PGM**
*ALAT**	-	0.543**	-1.000
*IDDH**	0.635**	-	-1.000
*PGM**	1.00***	0.461*	-

Figure 1. - Hybrid index scores for percids collected from Fort Peck Reservoir an the Yellowstone River, Montana.

Hybrid Index Scores