## **Evaluation of genetic purity of Kansas walleye populations**

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#### Introduction

There are several important considerations regarding walleye *Sander vitreus* management in Kansas. One such consideration is genetic purity of stocks, including potential hybridization with congeners, and back-crossing with saugeye *Sander vitreus* × *Sander canadensis* that are stocked by the Kansas Department of Wildlife, Parks, and Tourism (KDWPT). Introgression of sauger *Sander canadensis* alleles into walleye populations could have implications for broodstock collection and resulting stocking of progeny, unintended downstream genetic contamination of stocks, and genetic fitness of compromised populations. As such, the genetic purity of Kansas walleye populations is periodically assessed.

In 2000, KDWPT obtained tissue samples from walleye in 10 Kansas reservoirs to determine the genetic purity of walleye populations. In that study, malate dehydrogenase (MDH) and phosphoglucomutase (PGM) activities were examined, which were used to discriminate between walleye and sauger alleles. Data indicated sauger alleles were present in what were thought to be pure Kansas walleye populations. Backcrossing was also detected, but the exact nature of the hybridization was undetermined. Since it has been 15 years since this one and only genetic examination of Kansas walleye populations, further investigation of the purity of Kansas walleye populations was warranted. Therefore, the objective of this study was to investigate the genetic purity of ten priority percid waters in Kansas.

### **Methods**

Voucher specimens of walleye, sauger, and saugeye (i.e., 5-10 individuals each) were collected in April 2015. These fish were used to represent a genetic baseline of MDH and PGM alleles to which all subsequent tissue samples were compared. Voucher specimens were

C until processed.

Each tissue biopsy tube was assigned a number to identify the sample and reservoir from which it was obtained. Tissues were thawed and homogenized in a buffer consisting of Tris-DTA to produce a protein extract. Proteins contained in the extract were separated in a Tris-HCl buffer system utilizing starch gel electrophoresis as described by Murphy et al. (1996). Slices of electrophoresed gels were stained to determine MDH and PGM activities (Murphy et al. 1996). Stained electrophoretic patterns obtained for each tissue sample were compared to those established from voucher specimens to determine the genetic purity of the sample.

#### **Results and Discussion**

A total of 783 percids was tested for genetic purity from 10 study reservoirs in 2015 (Table 1). Reservoirs that had no evidence of sauger alleles or backcrossing included Cedar Bluff, Hillsdale, Marion, Webster, Wilson, and Lovewell Reservoirs. Samples from Cheney, Glen Elder, and Kirwin Reservoirs showed low incidences of backcrossing, which was indicated by banding patterns on the gels that did not match walleye, sauger, or saugeye, suggesting a multigenerational backcross. Sauger alleles were detected in two samples at Milford Reservoir.

Reservoirs absent of sauger alleles or backcrosses in 2000 and 2015 were Cedar Bluff, Marion, and Wilson. One sample each from Kirwin and Cheney Reservoirs were identified as a backcrosses in 2015, although all samples were pure walleye from these reservoirs in 2000. Conversely, all samples from Hillsdale and Webster Reservoirs were free of sauger alleles in 2015 samples; however, both reservoirs had low incidences of saugeye and backcrosses in 2000. Milford Reservoir had similar levels of occurrences of sauger alleles between 2000 and 2015, but incidence of sauger alleles decreased at Glen Elder reservoir since 2000. Overall, there were fewer instances of sauger alleles and backcrosses in Kansas reservoirs in 2015 (5 out of 783 = 0.6%) compared to 2000 (15 out of 674 = 2.2%).

Currently, Cedar Bluff and Hillsdale Reservoirs are the main brood populations for Kansas walleye propagation. These results do not indicate genetic integrity of walleye are compromised in these waters. Milford Reservoir is also used as a brood water; however, walleye eggs from this reservoir are mainly used in saugeye production. Walleye production using gametes collected at Milford Reservoir may not be prudent if genetic integrity of stocked walleye is a priority. Additionally, sauger alleles are persisting in Milford Reservoir, albeit at low levels. Whether these alleles are persisting due to natural recruitment of hybrid fish or contamination from on-the-water saugeye production is unknown. Regardless, all attempts to prohibit fertilized saugeye eggs from entering Milford Reservoir should continue during saugeye production.

Stocking progeny produced from genetically-pure walleye broodstock has been a priority in Kansas, although gametes have been collected from waters deemed impure in 2000 (Table 3). Some populations that are dependent on stocking have shown a decrease in sauger allele frequency (e.g., Glen Elder and Hillsdale Reservoirs). However, new genetic contamination, albeit at low frequencies, has appeared in Cheney and Kirwin Reservoirs. While regular

stockings occur at all four of these reservoirs, it is unclear whether changes in sauger allele frequencies are due to stocking genetically contaminated progeny. Regardless, future stockings should only occur from brood waters with pure walleye genetics if genetic purity is deemed a high priority in Kansas reservoirs.

Table 1. Genetic identity of percids from 10 Kansas reservoirs sampled in 2015. Backcrossing is a term used to indicate that the banding pattern observed did not match either walleye, sauger, or saugeye. The exact nature of the cross is not known.

Reservoir	Pure walleye	Sauger alleles detected	Backcross	
Cedar Bluff (N=100)	100		_	
Hillsdale (N=100)	100			
Marion (N=69)	69			
Webster (N=66)	66			
Wilson (N=100)	100			
Lovewell (N=11)	11			
Cheney (N=76)	75		1	
Glen Elder (N=103)	102		1	
Kirwin (N=60)	59		1	
Milford (N=103)	101	2		

Table 2. Genetic identity of percids from eight Kansas reservoirs sampled in 2000 (adapted from Fields and Phillip 2000).

Reservoir	Pure walleye	Saugeye	Backcross
Hillsdale (N=70)	69		1
Marion (N=76)	76		
Webster (N=83)	80	1	2
Wilson (N=70)	70		
Cheney (N=74)	74		
Glen Elder (N=100)	91	8	1
Kirwin (N=100)	100		
Milford (N=97)	95	2	
Jeffrey Energy Center (N=19)	19		

Table 3. Number of walleye eggs (in millions) taken from Kansas Reservoirs and from out of state trades (OS) from 2000-2015. Number of eggs from 2000-2006 are only those received by Milford Hatchery. Number of eggs from 2007-2015 are eggs received by all Kansas hatcheries.

-	GELR	HILR	MARR	KIRR	MILR	CHNR	ELDR	LOVR	WEBR	CDBR	OS
2000	37.3	54.6	11.6		2.0			1.6			
2001	53.6	21.1	2.9	42.4							
2002	66.5	25.3	0.1	58.4		4.2					4.9
2003	26.1	47.8		47.9		15.4			9.8		2.9
2004		53.4		26.0	17.5	28.7			14.2		1.0
2005		47.1	1.3	0.2	30.1				11.2		
2006		45.6			4.9	18.7				45.0	
2007		46.7	1.5							46.7	4.4
2008		54.2			14.2					40.4	
2009		44.7			0.4					40.9	
2010		36.7								59.8	
2011		23.5								66.0	
2012		21.0								39.1	40.2
2013		13.9								59.4	
2014		37.5			1.8					61.5	
2015		31.0								54.7	

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