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Frequency of Natural Hybridization between Saugers and Walleyes in the Peoria Pool of the Illinois River, as Determined by Morphological and Electrophoretic Criteria

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Abstract.—External morphological characteristics and protein electrophoresis at two diagnostic loci were used to determine the proportion of 704 *Stizostedion* samples collected from the Peoria Pool of the Illinois River during March 1995 that were saugers *S. canadense*, walleyes *S. vitreum*, or their hybrids. Morphological analyses indicated that 616 (87.5%) fish were saugers, 58 (8.2%) were walleyes, and 30 (4.3%) were hybrids; electrophoretic analyses indicated that 625 (88.8%) fish were saugers, 50 (7.1%) were walleyes, and 29 (4.1%) were hybrids. Clear discrepancies between the morphological and electrophoretic analyses affected at least 43 (6.1%) fish. Only 2% of saugers were hybrids, but at least 14% of walleyes possessed sauger alleles. Polymorphism at the *PGM-1** locus in Peoria Pool saugers was also identified. We recommend electrophoretic screening for hybrids if saugers or walleyes are collected for use as broodstock from waters where they co-occur.

Saugers *Stizostedion canadense* and walleyes *S. vitreum*, two extensively managed North American percids, are known to hybridize naturally (Stroud 1948; Clayton et al. 1973; Nelson and Walburg 1977; Billington et al. 1988; Todd 1990; Van Zee et al. 1996). Several external morphological characteristics allow separation of saugers from walleyes (Trautman 1981). Typically saugers have darker skin pigmentation (dark yellow to brown) than walleyes (light yellow to green), scaled cheeks (walleyes have unscaled cheeks), three dark saddles that reach all the way down the sides of their bodies (walleyes have up to 13 short, lightly colored saddles that reach less than one-fifth of the way down the side of the body), and a series of dark speckles arranged in a number of lines across their first dorsal fin (walleyes have just one large dark blotch at the posterior end of the first dorsal fin). First-generation (F_1) hybrids tend to be intermediate for these characteristics but often express features of both parental species (Trautman 1981). However, it is often difficult to identify hybrids from parental species by morphological characteristics (Flammang and Willis 1993; Ward and Berry 1995; Van Zee et al. 1996), especially if hybrids have backcrossed with the parental species.

Genetic screening can detect hybrid fishes if diagnostic loci have been identified between the species involved (Campton 1990). Walleye and sauger show fixed allelic differences at four protein coding loci: *mMDH-1** for malate dehydrogenase (1.1.1.37) and *PGM-1** for phosphoglucomutase (5.4.22) from muscle; and *ALAT** for alanine aminotransferase (2.6.1.2, formerly glutamic-pyruvate transaminase) and *IDDH** for L-iditol 2-dehydrogenase (1.1.1.14, formerly sorbitol dehydrogenase) from liver (Clayton et al. 1973; Billington et al. 1990; Todd 1990; White and Schell 1995; Van Zee et al. 1996). Enzyme numbers were 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 electrophoretically screen North American *Stizostedion* samples to confirm specific identification, detect F_1 hybrids (which will be heterozygous at all of the diagnostic loci), or second generation (F_2 or backcrossed) hybrid individuals. Backcrossed individuals will be heterozygous at a portion of the diagnostic loci, with the remaining loci being homozygous; the direction of backcrossing can be determined by the alleles that are homozygous. Electrophoretic analyses have already proven useful in the examination of walleyes, saugers, and their hybrids (Clayton et al. 1973; Todd 1990; Flammang and Willis 1993; Ward and Berry 1995; White and Schell 1995; Van Zee et al. 1996).

Saugers and walleyes are indigenous to the Peoria Pool of the Illinois River (Forbes 1920); saugers are the predominant *Stizostedion* species, but both species reproduce there naturally. Sauger and walleye spawning areas and periods overlap in the Peoria Pool, and turbidity in the pool during spring ranges up to 140 Jackson turbidity units (Mills et al. 1966), impeding visual identification of conspecific mates. Thus, conditions for natural hybridization appear ideal. In fact, fish suspected of being sauger–walleye hybrids, based on morphological examination, have been collected from the

Peoria Pool, but the extent of hybridization has not been studied previously.

The Illinois Department of Natural Resources (IDNR) uses saugers collected from the Peoria Pool for broodstock to produce fingerlings to restock the Illinois River. Thus, it is important to determine the extent of sauger–walleye hybridization in the Peoria Pool. Because a number of studies have shown that morphological identification of sauger–walleye hybrids may be unreliable, it was important to compare visual and genetic identification methods.

Methods

Stizostedion samples (N = 1,020) from the Peoria Pool of the Illinois River were collected by angling during the 1995 Master's Walleye Circuit Tournament (25–26 March) held in Spring Valley, Illinois. Fish were identified by IDNR personnel as saugers, walleyes, or hybrids based on external morphology, and 388 fish (372 saugers, 14 walleyes and 2 hybrids) were separated for broodstock analysis as detailed by Billington et al. (1996). For this study, we examined 704 fish, which included all of the remaining Peoria Pool fish and 72 fish from the broodstock survey that were screened at both diagnostic loci (see below). Fish identifications were rechecked by the first two authors and the total length (TL) of each fish measured to the nearest millimeter. A small piece of muscle tissue (approximately 0.25 g) was removed with surgical scissors from the posterior portion of the base of the left pectoral fin of each fish and placed into a numbered 1.5-mL microcentrifuge tube which was then frozen (at −20°C) until analyzed electrophoretically (Billington et al. 1996). After sampling, fish were returned to the Peoria Pool and released.

We used two loci, *PGM-1** and *mMDH-1**, that were known to be diagnostic between saugers and walleyes, and that can be screened in muscle tissue. Tissue homogenization and electrophoretic procedures are described by Billington et al. (1996). Gels were scored for the diagnostic alleles (*PGM-1*, walleye **100* allele, sauger **80* allele; *mMDH-1*, walleye **100* allele, sauger **140* allele).

Results

Based on morphological examination, 616 (87.5%) of the fish were identified as saugers, 58 (8.2%) as walleyes, and 30 (4.3%) as hybrids (Table 1). Based on the electrophoretic analyses, 625 (88.8%) fish were identified as saugers, 50 (7.1%) as walleyes and 29 (4.1%) as hybrids. Discrepancies between the results obtained from morphological and electrophoretic analysis affected 43 (6.1%) fish; total lengths for these 43 fish ranged from 305 to 537 mm. Fourteen (2.3%) fish identified as sauger by morphology were identified as hybrids (including backcrosses) by electrophoresis, and 21 fish identified as hybrids by morphology had sauger alleles at both loci. Eight (13.8%) fish identified as walleyes by morphology were misidentified, based on the electrophoretic data, including two fish that had sauger alleles at both loci examined.

TABLE 1.—Comparison of morphological and electrophoretic (based on two diagnostic muscle loci, *PGM-1** and *mMDH-1**) methods of identifying *Stizostedion* species collected from the Peoria Pool of the Illinois River on 25–26 March 1995.

Species identification				
Morphology	Electrophoresis	N	Percentage	Total length (mm)
Sauger	Sauger	602	97.7	277–547
Sauger	F_1 hybrid	8	1.3	328–534
Sauger	Backcross[a]	6	1.0	337–471
Walleye	Walleye	50	86.2	305–412
Walleye	F_1 hybrid	3	5.2	329–409
Walleye	Backcross[b]	3	5.2	341–524
Walleye	Sauger	2	3.4	305–355
Hybrid	Sauger	21	70.0	310–537
Hybrid	F_1 hybrid	1	3.3	366
Hybrid	Backcross[c]	8	26.7	300–520

[a] Five backcrosses were to sauger and one to walleye.
[b] Two backcrosses were to walleye and one to sauger.
[c] Seven backcrosses were to sauger and one to walleye.

Polymorphism was also detected in Illinois River saugers at *PGM-1** during this survey, and three additional alleles (**90*, **70* and **50*, relative to the walleye **100* allele) were observed. All three alleles were rare and occurred as heterozygotes with the common sauger **80* allele; 15 **80/90* heterozygotes, 12 **70/80* heterozygotes, and 5 **50/80* heterozygotes were detected. No significant departures from Hardy–Weinberg expectations were observed at the *PGM-1** locus for Peoria Pool sauger allele frequencies. The relative mobilities of these alleles in saugers would be **113*, **88* and **63*, respectively.

Discussion

There were clear discrepancies between the morphological and electrophoretic methods for identifying *Stizostedion* species and their hybrids in the Peoria Pool. For example, 22 fish identified by morphology as saugers (N = 14) or walleyes (N = 8) were shown to be hybrids by electrophoresis. (In this discussion, backcrosses will also be

referred to as hybrids because they possess alleles of the other species.) Similarly, 21 fish that were suspected of being hybrids were shown to be saugers at the two diagnostic loci screened. However, it is possible that these fish were backcrosses because with only two diagnostic loci, there is a 25% chance of misidentifying a backcross as a parental (Campton 1990). Note that two fish identified by morphology as walleyes had sauger alleles at both diagnostic muscle loci. Given the relatively high rate of hybridization in Peoria Pool walleyes (at least 10.4%), these two fish might be backcrosses that happened to possess sauger alleles at the two loci that we were able to score from muscle. The other two loci that are known to be diagnostic between these two species can only be scored in liver tissue and, thus, cannot be easily screened by nonlethal sampling techniques. We are searching for additional diagnostic loci between saugers and walleyes that can be screened in muscle tissue to reduce the proportion of backcrosses that are misidentified as parental species.

It is possible that *Stizostedion* species in the Peoria Pool are not fixed for species-diagnostic alleles at *PGM-1** and *mMDH-1**. We have examined more than 500 walleyes and more than 1,200 saugers electrophoretically to date and have found that in allopatric populations the two markers we used are fixed between the species. In sympatric populations, however, some individuals of one species have been found with alleles typical of the other species, most usually as heterozygotes (e.g., Billington et al. 1990; Van Zee et al. 1996; this study). While low levels of polymorphism might exist in both saugers and walleyes at these two loci, the fact that both loci are species-diagnostic in allopatric populations suggests that the low levels of gene flow occurring between them in sympatry is due to natural hybridization and introgression. Several other workers have suggested that these two loci, in particular, are good diagnostic loci between the North American *Stizostedion* species (Clayton et al. 1973; Todd 1990; Flammang and Willis 1993; Ward and Berry 1995; White and Schell 1995). Thus, we are reasonably confident about the utility of our diagnostic genetic markers.

Several workers have attempted to determine if there is a critical size at which saugers, walleyes, and their hybrids can be reliably discriminated by morphology. Nelson (1968) suggested that sauger–walleye F_1 hybrids 100 mm TL and longer could be separated from their parental species by morphology. However, Flammang and Willis (1993) found that morphological characters were unreliable for separating juvenile walleyes from purposefully produced saugeyes (walleye female × sauger male hybrids) and recommended electrophoretic verification if fish were less than 200 mm TL. Ward and Berry (1995) observed that the use of skin pigmentation was inadequate to separate saugers and natural sauger–walleye hybrids that ranged in TL from 190 to 564 mm. *Stizostedion* samples from the Peoria Pool that were incorrectly identified by morphology ranged from 305 to 537 mm TL (Table 1). These fish had been visually examined twice by personnel with a great deal of experience in working with saugers, walleyes, and their hybrids. Clearly, morphological analyses cannot reliably discriminate *Stizostedion* species from their hybrids.

Sauger fingerlings have been stocked into the Peoria Pool of the Illinois River annually since 1990 to supplement natural recruitment. Sauger broodfish are collected at the Master's Walleye Circuit Tournament and transported to the LaSalle Fish Hatchery where they are spawned and the offspring are raised to fingerlings. Sauger fry and fingerlings from the LaSalle hatchery are also supplied to other state natural resource agencies for stocking. Hatchery personnel screen sauger broodfish from walleyes and hybrids by examining morphological characteristics, but potential broodfish cannot always be reliably identified to species. Moreover, there is a chance of inadvertently including fish that have walleye alleles (either F_1 hybrids or backcrosses) as broodstock. Thus, the potential exists to seriously impact the genetic integrity of recipient populations because a few hybrids or backcrosses inadvertently included as broodfish can result in the production of many hundreds of thousands of fry and fingerlings containing foreign alleles (Ward and Berry 1995). Even though the percentage of hybrid individuals was low in the Peoria Pool sauger samples (approximately 2%), in some years stocked fish can comprise up to 76% of a year-class (R. Brooks and R. Heidinger, unpublished data). If a substantial proportion of these stocked fish contained walleye alleles, this could seriously compromise the genetic integrity of the sauger population and possibly further contribute to the breakdown of reproductive isolation between the two species in the Peoria Pool. The problems would be more severe if Peoria Pool walleyes were to be used as broodfish for walleye production because at least 14% of these fish contain sauger alleles.

Management Implications

Numerous studies have now reported the occurrence of natural sauger–walleye hybrids, introgression between the two species, and difficulties in using morphological characters to discriminate saugers, walleyes, and their hybrids (Clayton et al. 1973; Billington et al. 1988; Todd 1990; Flammang and Willis 1993; Ward and Berry 1995; Van Zee et al. 1996; this study). In addition, White and Schell (1995) reported the presence of recombinant genotypes between saugers and walleyes in three pools in the Ohio River. These recombinant genotypes were only found in Ohio River pools that were in drainages where saugeyes had been previously stocked. Thus, White and Schell (1995) warned of the potential genetic impact on saugers and walleyes from reproduction between stocked saugeyes and the parental species. Saugeyes have also been extensively stocked into impoundments and other water bodies in the midwestern United States (Lynch et al. 1982; Humphreys et al. 1987; Leeds 1989; Flammang and Willis 1993; Summers et al. 1994; White and Schell 1995); it is often impossible to prevent saugeyes from moving over spillways into downstream waterways. Therefore, given the reported cases of natural hybridization between saugers and walleyes and the further complication of extensive stocking of saugeyes, we recommend genetic screening of both *Stizostedion* species before their use as broodstock in order to maintain the genetic integrity of sauger and walleye gene pools. Genetic screening may also be useful for revealing polymorphism in broodfish, such as occurred at *PGM-1** in Peoria Pool saugers. Because of concerns about hybridization in Peoria Pool saugers, the IDNR initiated genetic screening of its sauger broodstock in 1995 to minimize the number of broodfish containing walleye alleles (Billington et al. 1996).

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