Mitochondrial-DNA Confirmation of Southern Walleye in the Mobile Basin, Alabama

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Abstract: The existence of a distinct walleye (*Stizostedion vitreum*) population in south-flowing drainages of the southeastern United States has been suspected for some time. Recently, a mitochondrial-DNA (mtDNA) marker was identified that permitted discrimination of these southern walleyes from northern forms. In order to determine the type and distribution of walleyes in Alabama, mtDNA analysis was conducted on 35 individuals collected from 3 river systems within the state. Thirty-one fish collected in the Mobile Basin were the southern form of walleye, which previously had been identified only in northeastern Mississippi, while 4 fish from the Tennessee River were of the northern form. There was no evidence for the successful establishment of any female walleyes from Ohio that were stocked into 2 impoundments in the Mobile Basin 10–20 years ago. Additional surveys and a careful monitoring program using genetic markers should be implemented to detect any infusion of northern walleyes into the Mobile Basin through the Tennessee-Tombigbee Waterway.

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The occurrence of a unique strain of walleye in the southeastern United States has been suspected for some time. Hackney and Holbrook (1978) described what they called a "Gulf Coast" race of walleye distributed in south-flowing drainages of northwest Georgia, Alabama, and the western panhandle of Florida, and extending westward to northeastern Mississippi and the Pearl River, bordering Louisiana and Mississippi. Indirect evidence that these southern walleyes might be genetically distinct from Mississippi River basin and other northern walleye populations was provided by 2 protein electrophoretic

studies. Significant differences in blood serum protein bands between walleyes from the Tombigbee River drainage in northeastern Mississippi and samples from Iowa, New York, and Pennsylvania were reported by Wingo (1982), while Murphy (1990) observed that 4 protein loci which are highly polymorphic in walleye populations across North America were all monomorphic in Tombigbee River fish. Using mtDNA analysis, Billington et al. (1992) noted walleyes from the Tombigbee River-Luxapalila Creek system, Mississippi, possessed a unique mtDNA haplotype (haplotype 34) that was not present in 67 other North American walleye populations, including fish from Ohio and Pymatuming Lake, Pennsylvania. Recently, this southern haplotype was shown to be highly divergent (an average of 15 restriction site changes; 2.3% sequence divergence) from northern walleye mtDNA haplotypes (Billington and Strange 1995).

Brown (1962) noted that although they were rare, walleyes were recorded in the 2 main southern drainages of Alabama, the Coosa-Alabama River system and the Tombigbee-Black Warrior River system. However, it is not known whether walleyes from these waters are of the southern type, or if they represent northern walleye that were stocked into impoundments on these river systems. Between 1975 and 1985, 74,263 walleye fingerlings (2.5–5.0 cm long) from the Carbon Hill Federal Hatchery in Alabama were stocked into Tuscaloosa Reservoir. These fish originated from the Senecaville Federal Hatchery in Ohio and represent a mixture of 2 walleye populations, 1 from Seneca Lake, Ohio, next to the hatchery, and the other from Pymatuming Lake, Pennsylvania. Likewise, 202,100 walleye fingerlings from the same hatchery and source population were stocked into Lake Mitchell on the Coosa River between 1973 and 1983.

Mitochondrial-DNA analysis can be useful for determining whether fish are of native or stocked origin, if suitable mtDNA markers are identified that allow their discrimination (Billington and Hebert 1991). A mtDNA marker (haplotype 34) characteristic of southern walleyes from the Upper Tombigbee River system and which is quite distinct from northern walleye mtDNA haplotypes was previously identified (Billington et al. 1992, Billington and Strange 1995). Therefore, mtDNA analysis should allow discrimination of northern and southern walleye haplotypes within Alabama, where potential mixtures could occur. This paper describes the results of a mtDNA restriction analysis survey of 35 walleyes collected from 3 river systems in Alabama during 1994 and 1995.

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Methods

Thirty-five walleyes were collected from 3 river systems in Alabama (Fig. 1) using gill nets and electrofishing. Length, weight, and sex data were recorded and fish were aged by examination of otoliths. Livers were removed and placed

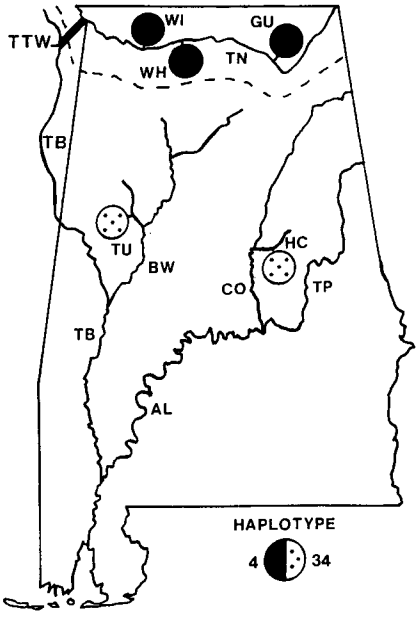

Figure 1. Map showing walleye mtDNA haplotypes and collection locations (Guntersville Tailwater-GU, Hatchet Creek-HC, Tuscaloosa Tailwater-TU, Wheeler Tailwater-WH, Wilson Tailwater-WI). The division between the Tennessee River and the Mobile Bay basins (----), the Tennessee-Tombigbee Waterway (TTW) and major rivers (Alabama River-AL, Black Warrior River-BW, Coosa River-CO, Tallapoosa River-TP, Tennessee River-TN, Tombigbee River-TB) are also shown.

in buffer solution (0.25 M sucrose; 10 mM NaCl; 10 mM Tris-HCl, pH 7.5; 1 mM EDTA, pH 8.0) until processing. Mitochondrial-DNA was extracted from the liver tissue and purified by ultracentrifugation within 72 hours of collection following the procedures described by Billington and Hebert (1988). Pure mtDNA was digested with 10 restriction endonucleases (*Ava* I, *Bcl* I, *Bst*E II, *Cla* I, *Dra* I, *Nci* I, *Nco* I, *Sca* I, *Stu* I, *Taq* I) that reveal diagnostic restriction fragment profiles among walleye populations (Billington et al. 1992). The resulting fragments were end-labeled with ^{32}P, electrophoresed in 1.0% or 1.2% agarose and 4% acrylamide gels and visualized by autoradiography (Billington and Hebert 1988). Restriction fragment patterns were compared with those already identified for walleye haplotypes (Billington and Hebert 1988, Billington et al. 1992, Billington and Strange 1995).

Results and Discussion

Twenty fish were collected in 1994, 13 from Hatchet Creek, 5 from the Tuscaloosa Tailwater, and 2 from the Wilson Tailwater on the Tennessee River. One of the 2 Tennessee River fish was suspected to be a walleye x sauger (*S. canadense*) hybrid by visual identification and this was confirmed by protein electrophoresis of the diagnostic *MDH-1* locus. Nevertheless, as it possessed walleye mtDNA, we included this information in our data set. Four year classes were represented in the 13 fish from Hatchet Creek, 1 from 1988, 1 from 1990, 3 from 1991, and 8 from 1993. Two year classes were represented in fish collected at the Tuscaloosa Tailwater, 1 from 1985 and 4 from 1990. Fifteen fish were

collected in 1995, 12 from Hatchet Creek (all 1993 year class), 1 from the Tuscaloosa Tailwater (1990 year class), and 2 fish from the Tennessee River. Of the 2 Tennessee River fish, 1 was from the Guntersville Tailwater (1994 year class) and 1 from the Wheeler Tailwater (1993 year class). Brown (1962) reported that walleyes were rare in the southern drainages of Alabama. We concur, as only 31 walleyes were collected from Mobile Basin drainages in our 2-year survey. Yet, despite the low numbers of fish collected, a number of year classes were present at each site suggesting successful reproduction in multiple years.

All of the 31 walleyes collected from sites within the Mobile Basin expressed mtDNA restriction fragment patterns typical of haplotype 34 (Fig. 1), whereas the 4 fish collected from the Tennessee River exhibited mtDNA haplotype 4 typical of northern walleyes (Billington et al. 1992, Billington and Strange 1995). Thus, all fish collected in the Mobile Basin of Alabama had the same mtDNA haplotype that was found in walleyes from the Tombigbee River-Luxapalila Creek system of northeastern Mississippi. Our sampling site at Hatchet Creek was above Lake Mitchell where northern walleyes had been previously stocked. We also sampled the tailwater of the Tuscaloosa Dam, which had also been stocked with northern walleyes. The absence of northern walleye mtDNA haplotypes in the Mobile Basin would suggest that stockings of northern walleyes were unsuccessful in these southern drainages, but the examination of more samples and additional sites would be required to confirm this assertion. It should be noted, however, that we would be unable to detect potential hybridization between stocked males of the northern form with indigenous females of the southern form; progeny of such matings would all have the southern form of mtDNA because this molecule is maternally inherited. Moreover, we are not sure if walleyes stocked into Tuscaloosa Reservoir were able to successfully migrate over the dam into the downstream river system.

Given that these southern walleyes represent a unique strain that appears to have evolved in isolation for over a million years (Billington and Strange 1995), all efforts should be made to preserve their genetic integrity. Possible threats include the stocking of northern walleyes into reservoirs in the Mobile Basin and the migration of northern walleye strains into the Mobile Basin from the Tennessee River, through the Tennessee-Tombigbee Waterway (TTW) and down the Tombigbee River. Therefore, we recommend that walleye stocking in the Mobile Drainage Basin should only be undertaken with southern walleyes when there is the risk that stocked fish will escape into river systems. However, the fact that we were unable to detect any northern walleye haplotypes in the Mobile Basin, despite nearly 300,000 northern walleyes being stocked there, suggests that previous stockings with these fish,at least the females, were unsuccessful. A monitoring program could be instituted to determine the degree of invasion of northern walleyes, or walleye x sauger hybrids, into the Mobile Basin through the TTW, or to further screen fish from sites that had previously been stocked with Ohio walleyes for northern haplotypes. As mtDNA genetic markers are available to discriminate between the northern and southern walleye strains, it should be possible to use a non-lethal sampling method, such as tak-

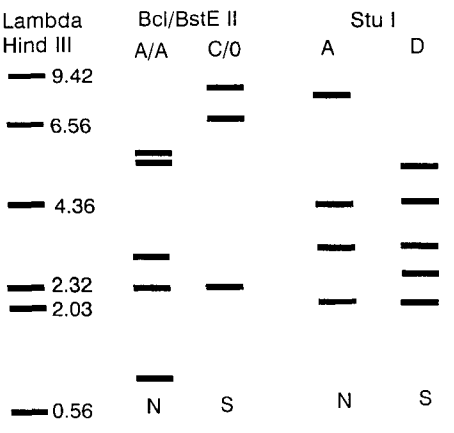

Figure 2. Diagram of a gel showing restriction fragment pattern differences between northern (N) and southern (S) walleye mtDNA for 2 diagnostic restriction enzyme systems (a *Bcl* I/*Bst*E II double digest and a *Stu* I digest) that could be used in a rapid non-lethal screening of fish collected from the Mobile Basin. A lambda Hind III size standard is also shown with fragment sizes in kilobase pairs. Letters at the top of each fragment pattern refer to reference fragment patterns for these enzymes (Billington et al. 1992, Billington and Strange 1995).

ing blood (Wingo and Muncy 1984) or using a fin-muscle sample, to obtain DNA that can be screened for mtDNA restriction fragment profiles (Billington and Hebert 1990). In this way, large numbers of individuals could be sampled relatively efficiently as only 1 or 2 diagnostic endonucleases would be required (e.g., Fig. 2), without the need to sacrifice fish. If the possible invasion of walleye x sauger hybrids was to be detected, additional screening of a fin-muscle sample for the diagnostic *MDH-1* alleles would also be required. Moreover, mtDNA analysis would appear to be the method of choice for screening any walleyes collected in other south-flowing drainages that were previously thought to contain the southern strain of walleye.

Literature Cited

Billington, N. and P. D. N. Hebert. 1988. Mitochondrial DNA variation in Great Lakes walleye (*Stizostedion vitreum*) populations. Can. J. Fish. and Aquat. Sci. 45:643–654.

——— and ———. 1990. Technique for determining mitochondrial DNA markers in blood samples from walleyes. Am. Fish. Soc. Symp. 7:492–498.

——— and ———. 1991. Mitochondrial DNA diversity in fishes and its implications for introductions. Can. J. Fish. and Aquat. Sci. 48 (Suppl. 1):80–94.

——— and R. M. Strange. 1995. Mitochondrial DNA analysis confirms the existence of a genetically divergent walleye population in northeastern Mississippi. Trans. Am. Fish. Soc. 124:770–776.

———, R. J. Barrette, and P. D. N. Hebert. 1992. Management implications of mitochondrial DNA variation in walleye stocks. North Am. J. Fish. Manage. 12:276–284.

Brown, B. E. 1962. Occurrence of the walleye, *Stizostedion vitreum,* in Alabama south of the Tennessee Valley. Copeia 1962:469–471.

Hackney, P. A. and J. A. Holbrook II. 1978. Sauger, walleye, and yellow perch in the

southeastern United States. Pages 74–81 *in* R. L. Kendall, ed. Selected coolwater fishes of North America. Spec. Publ. 11, Am. Fish. Soc., Bethesda, Md.

Murphy, B. R. 1990. Evidence for a genetically unique walleye population in the upper Tombigbee River system of northeastern Mississippi. Southeast Fish. Counc. Proc. 22:14–16.

Wingo, W. M. 1982. Characteristics of walleye in the Tombigbee River and tributaries. M.S. Thesis, Miss. State Univ., Mississippi State. 24pp.

——— and R. J. Muncy. 1984. Sampling walleye blood. Prog. Fish-Cult. 46:53–55.