THE DISTRIBUTION AND STATUS OF NATIVE WALLEYE (*SANDER VITREUS*) STOCKS IN WEST VIRGINIA

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by

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Abstract

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THE DISTRIBUTION AND STATUS OF NATIVE WALLEYE (*SANDER VITREUS) STOCKS IN WEST VIRGINIA* (45 pp.)

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Walleye (*Sander vitreus*) is a heavily managed fishery, the genetic integrity of which has been affected by introductions of nonnative stocks via hatchery supplementation. DNA analysis on walleye has revealed highly divergent populations of walleye in the Ohio and New rivers. The focus of this project is to identify the distribution and assess the introgression of native walleye populations in West Virginia. PCR-RFLP analysis of mtDNA reveals native walleye are distributed throughout West Virginia, with higher frequencies in the Kanawha/New River system. Analysis of microsatellite DNA markers suggests native walleye stocks within the Ohio and Monongahela Rivers have introgressed with introduced walleye populations. Native walleye stocks in the Kanawha and New Rivers are relatively uninfluenced. The stocks in the Kanawha/New River system should be used for future hatchery supplementation and restoration of a native population of walleye in West Virginia.

Approved

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Introduction

The conservation and management of distinct populations within a managed sport fishery requires an understanding of the population structure of the species from morphological, physiological and genetic standpoints. Knowledge of this structure of a species provides the information needed for understanding of the dynamics of individual populations and the designing of appropriate management practices (Begg and Waldman 1999). The practice of stocking hatchery-reared fish, whether it is for the supplementation of an existing or declining population, the conservation of unique populations, or to introduce new species to an area is a common practice in fisheries management. Agencies also commonly practice "fish exchange programs" in which cohorts of hatchery-reared species are imported (and exported) to other states depending on their individual needs. These practices have lead to the introduction of non-native individuals into pre-existing native populations, which can compromise the genetic integrity of these populations and negatively affect the native species (Ryman and Utter 1987). It has been shown that supplementation by nonnative and/or hatchery-reared individuals can have negative impacts on native fish populations via introgression, hybridization, and outbreeding depression (Leary et al. 1985; Phillip et al. 2002; Gilk et al. 2004).

Walleye (*Sander vitreus,* formerly known as *Stizostedion vitreum*; Figure 1) is one such economically important and intensively managed species both commercially and for sport fishing in the Great Lakes and Mississippi River Basin. It is considered a coolwater species occupying a broad range of habitats from medium to large rivers to large lakes and impoundments (Jenkins and Burkhead 1994). Walleye to the south tend to grow faster, mature quicker, and have a shorter lifespans than those in more northern regions

(Jenkins and Burkhead 1994). It is widely distributed throughout much of North America (Figure 2), the native range extending from the Mackenzie River drainage in northern Canada and the southern half of the Hudson Bay south through the Great Lakes-St. Lawrence basin and most of the Mississippi basin, (Colby et al. 1979; Jenkins and Burkhead 1994), the southern limits of which were considered uncertain until the identification of native haplotypes in the Mobile Basin indicated walleye are native to the Gulf Coast area as well (Billington and Strange 1995). Walleye have been extensively introduced beyond its indigenous range, from the Atlantic Slope (Jenkins and Burkhead, 1994) to Gulf Coat drainages (Billington et al. 1992) and the Pacific Slope (Etnier and Starnes 1993) in an effort to establish new sport fisheries in these areas (Figure 3). Declining population numbers in the last 30-40 years has led to a significant level of hatchery-based enhancement of walleye populations within their native range as well (Colby et al. 1979). For example, the Ohio River has been intensively stocked by various state agencies, in an effort to reestablish a sport fishery in this river. Broodstock, primarily derived from Lake Erie, has been used and has created mixtures of native and introduced stocks (White and Schell 1995). These practices have likely lead to a compromise in the genetic integrity and population/stock structure of many native walleye populations.

Allozyme, mitochondrial DNA (mtDNA) restriction fragment length polymorphisms (RFLPs) and sequence analyses has revealed substantial genetic population differentiation and stock structuring of walleye in the Great Lakes, as well as the Mississipppi River and Ohio River basins (Billington and Herbert 1988; Ward et al. 1989; Billington et al. 1992; Stepien and Faber 1998; McParland et al. 1999; Gatt et al.

2000; Gatt et al. 2003). Glaciation during the Pleistocene played a primary role in shaping this genetic structure. The four glacial advances of the Pleistocene, beginning about one million years ago (Hocutt et al., 1986) altered much of the northern Mississippi basin, impounding and rerouting many tributaries, and forcing walleye inhabiting these areas into refugia to the south and east where the subsequently diverged due to genetic drift and bottlenecks (Billington 1996). After the glaciers receded, a mixing of the walleye populations from the refugia occurred within the Mississippi Basin. The receding glaciers also created the Great Lakes, which were also populated with these walleye populations.

Analysis of intraspecific mtDNA RFLP haplotypes has shown walleye from the Great Lakes to group into four distinct populations consistent with three Pleistocene refugia (Mississippi River, Missouri River, and Atlantic) with a fourth divergent southern population unaffected by the glacial advances (Figure 4) (Billington et al. 1992; Billington 1996). A fifth group of walleye was identified from the Ohio, Kentucky and Tennessee River Basins possessing various haplotypes consistent with the prevalent stocking of these areas with fish from the Great Lakes. This group also included a few individuals possessing divergent haplotypes thought to be remnants of the original walleye population in this region prior to stocking and possibly native to their respective locations (Billington 1996).

Additional studies on the stock structure of walleye have revealed similar divergent haplotypes. In a DNA sequence analysis of walleyes throughout their range, Stepien and Faber (1998) found most haplotypes were consistent with Great Lakes haplotpyes, but also observed one particular haplotype, found in the Ohio River that was

highly divergent from Great Lakes haplotypes. They suggested a time since separation of 1.5 million years from the Great Lakes haplotypes. A survey of walleye in the Ohio River using RFLPs, microsatellites, and allozymes (White et al. 2005) also found a divergent haplotype in the Upper Ohio River. It is likely that these haplotypes are similar to those observed by Billington (1996) and are possibly members of the same native population.

Mitochondrial DNA RFLP analysis of walleye in the New River and Claytor Lake, Virginia (Palmer et al. 2001) revealed unique haplotypes divergent from Great Lakes haplotypes, and suggested a unique New River stock. This haplotype could represent a relic strain of walleye that is native to this river system. White et al. (2005) found identical digest patterns exhibited by both the Ohio River and the New River walleye suggesting a close genetic relationship.

Stepien and Faber hypothesized a Teays River origin for the divergent walleye haplotype found in the Ohio River. The Teays River was a pre-Pleistocene river, dating to the Tertiary (Ver Steeg 1946), that originated in Virginia and flowed north across Ohio and Indiana and connected to the Mississippi River in Illinois (Figure 5, Ver Steeg 1936; Fidlar 1943, Hocutt et al. 1986). The lower two-thirds of the river was impounded by the Nebraskan glacial advance, leaving the upper third (present day Kanawha, New, Big Sandy, Gauley, and Little Kanawha Rivers) isolated (Hocutt et al. 1986). This region is a potential additional walleye refugium.

The river systems of West Virginia offer a unique opportunity to study these distinct populations of walleye given that the hydrologic connection between the native Ohio River walleye population and the native New River walleye population in Virginia

lies with the New and Kanawha rivers. Tributaries of these rivers may also harbor populations possessing similar divergent haplotypes. Aside from the remnants of the Teays River, West Virginia also harbors remnants of another pre-Pleistocene river system, the Old Lower Allegheny River system, which was comprised of what is the present-day Upper Ohio River (above New Martinsville), Monongahela, and Youghiogheny Rivers (Hocutt et al. 1986). This river system flowed north and east to the Atlantic Ocean via the Erigan River (Figure 5, Hocutt et al. 1986) and was not connected with the Teays River. The Erigan River was also buried under glacial advances. Walleye are considered native to all these areas, except for the Potomac River where it is considered introduced (Stauffer et al. 1995, Figure 6).

Historically, walleye have been extensively stocked by the West Virginia Division of Natural Resources (WVDNR) in the Potomac, Monongahela, Kanawha, Ohio, Cheat, and Elk River systems, primarily in impoundments (Chris O'Bara pers. comm). Over 350,000 walleye fingerlings have been stocked into these river systems in the last four years. It is likely that the genetic composition of walleye within the Ohio River and West Virginia have been affected by the stocking of Great Lakes-derived walleye. Understanding the genetic population structure of walleye, in West Virginia is part of a management plan of the WVDNR designed to restore native walleye to the Kanawha and Ohio rivers.

The objective of this study was to identify native walleye populations within the major river systems of West Virginia and assess genetic integrity of these populations. PCR-RFLP analysis of mtDNA was used to identify between native and introduced haplotypes. Microsatellite DNA variation was used to assess the degree of introgression

between native and non-native stocks. This information will aid the WVDNR in the appropriate management of walleye in the state.

Materials and Methods

Collection and DNA extraction

Walleye were collected primarily via boat-mounted pDC electrofishing techniques, in cooperation with the WVDNR. Collections were made from 15 locations from six rivers and four lakes in West Virginia during 2003-2005 (Figure 7). In addition, fish were collected from the Monongahela River at two locations in Pennsylvania (Gray's Landing Lock and Dam and Lock #2). Additional samples, to be used as reference samples, were collected from hatchery-spawned individuals imported into West Virginia from New York (Oneida Lake) and Pennsylvania (Pymatuning Lake) hatcheries. These broodstocks are known to be derived from Lake Erie walleye. WVDNR imports walleyes from both of these hatcheries for use in their walleye introduction program. New River samples, identified as possessing the divergent haplotype, were also obtained from Palmer et al. (2001) to be used as comparison. Tissue samples (fin clips) were taken from each individual and preserved in 95% EtOH in the field and stored at -20°C. Total genomic DNA was extracted using a DNeasy Kit (Qiagen Inc.) following the manufacturer's recommendations. The presence of DNA was determined using 0.8% agarose gel electrophoresis.

mtDNA RFLP analysis

A fragment of the mtDNA genome was amplified using PCR. The universal primers L15926, 5′-TCA AAG CTT ACA CCA GTC TTG TAA ACC-3′ (Kocher et al. 1989) and H16498 5′-CCT GAA GTA GGA ACC AGA TG-3′ (Meyer et al. 1990) were used to amplify an approximately 800bp fragment consisting of the left domain, of the control region from the proline tRNA gene to the central conserved section (Faber and

Stepien 1998, Figure 8). Amplifications were performed in a MJ Research PTC-100-25 thermocycler under the following conditions: 30 cycles of 1 min at 94°C, 25s at 50°C for 30 s, and 45s at 72°C. The presence of amplification products was confirmed with agarose gel electrophoresis.

The PCR amplification products were digested with the restriction endonucleases: *Ase*I and *Bsu*36I (White et al. 2005). Walleye derived from Lake Erie populations ("introduced") contain a diagnostic *Bsu*36I restriction site (CCTNAGG) that produces two fragments of approximately 550 and 250bp (White et al. 2005). The genetically divergent Ohio River walleye haplotype ("native") contain a diagnostic *Ase*I restriction site (ATTAAT) that produces two fragments of approximately 600 and 200bp. Digestion was confirmed using agarose gel electrophoresis. Each individual was identified as possessing the Lake Erie haplotype or Ohio River haplotype based on the digest pattern.

Microsatellite analysis

Microsatellite DNA variation was used to assess the degree of introgression of the native walleye populations. All individuals identified as possessing the native haplotype, as well as a sample of individuals possessing the Lake Erie haplotype, from locations where the native haplotype was observed, were assayed for microsatellite variation. A sample of New River walleyes possessing the native haplotype (Palmer et al. 2001) was also examined.

Microsatellite loci were amplified following the methods outlined in Borer et al. (1999) and amplification products were verified with agarose gel electrophoresis. Two polymorphic loci, *Svi*33 and *Svi*17, that possess diagnostic alleles (78 at *Svi*33 and homozygous 99/99 at *Svi*17; Palmer et al. 2001) were surveyed. Amplified fragments

were separated using 8% polyacrylamide gel electrophoresis. Fragment sizes were determined with Quantity One v.4.1.1 software (BioRad). Allele frequencies were estimated using GenAlEx 6 (Peakall and Smouse 2005). Allele frequencies in native populations were compared to those in the Lake Erie haplotype populations and to New River walleye.

Results

mtDNA RFLP analysis

Three hundred and forty-two walleye were analyzed from the 21 survey localities (Table 1). Some variation in the size of the amplified fragment was observed due to the presence of tandem repeats (Stepien and Faber 1998). Of the 342 walleye sampled, 63 were identified as possessing the Ohio River haplotype (sensu White and Schell 1995). The remainder possessed the Lake Erie haplotype. The Ohio River haplotype was not observed in the Lake Erie-derived New York or Pennsylvania hatchery samples. The New River samples produced a digest pattern similar to that of the Ohio River haplotype.

The Ohio River haplotype was detected in 15 of the survey localities (Figure 9). This haplotype was rare or absent among walleye assayed from the Monongahela and Tygart rivers and the four reservoirs. The average frequency of the Ohio River haplotype in the Ohio River localities was 0.21. This frequency increased in the Kanawha/New River and Elk River (0.52 and 0.75 respectively). The highest number of individuals possessing the Ohio River haplotype was observed from the London and Marmet locks and dam tailwaters on the Kanawha River (N= 16 and 26 respectively). All individuals (N = 4) from Kanawha Falls (Kanawha River – London Pool) possessed the Ohio River haplotype.

The frequency of native haplotypes from the London (sample site 3) and Marmet (sample site 4) sampling locations, however, is likely influenced by recent introductions of New River walleye at both of these localities (Chris O'Bara pers. comm.). Genetic analysis of these walleye (provided by Virginia Polytechnic Institute and State University) identified the broodstock as native New River walleye. Aging analysis of the

walleye collected from both the London and Marmet locales indicated many (N = 15 and 19, respectively) of these walleye could have been among those stocked. These individuals were omitted from the microsatellite analysis.

Microsatellite analysis

Individuals were pooled into a total of seven populations. Five were comprised of the native walleye observed from each river drainage: the Elk, Monongahela, New, Ohio, and Kanawha River populations. A sixth population included the native walleye observed from two of the reservoirs. A seventh population comprised of a sampling of Lake Erie haplotypes observed from each sampling locality from which a native haplotype was also observed. Populations from individual sample sites were not used due to a very small sample size of native haplotypes (N= 1 or 2) observed at most sites. An Analysis of Molecular Variance (AMOVA) revealed significant differentiation among these groups ($p = 0.001$).

Pairwise Wright's F_{ST} values indicated significant differentiation among all comparisons except Lake Erie and Ohio River (Table 2).

Microsatellite allele frequencies based on the 79 assayed individuals are shown in Table 3. Fifteen alleles were observed at the *Svi*33 locus. All seven populations were polymorphic at the *Svi*33 locus. Microsatellite variation in the Elk, Kanawha, Monongahela, New and Reservoir populations was reduced, with only two to four of the fifteen alleles observed. The Ohio River and Lake Erie populations were more variable. The alleles observed in the Ohio River population were similar to those found in the Lake Erie haplotype sample. The diagnostic New River allele (78) was most frequent in the Elk (0.333), Kanawha (0.385), and New River (0.833) populations.

Ten alleles were observed at the *Svi*17 locus. The Monongahela, New, and Kanawha River populations were monomorphic for allele 99, the diagnostic New River allele. The Elk River and Reservoir populations were polymorphic at this locus, with a high frequency at the 99 allele. The Ohio River and Lake Erie populations were both highly polymorphic at this locus.

Discussion

Evidence from both mitochondrial RFLP and microsatellite DNA analyses suggests native walleye populations within the Ohio River have been influenced, via introgression, by introduced Lake Erie-derived walleye. Analysis also suggests relatively little influence, via introgression, on the genetic composition of the Kanawha/New River native walleye populations suggesting limited gene flow with introduced Lake Erie-derived walleye.

mtDNA RFLP analysis

The Lake Erie (introduced) haplotype was detected in all sampling localities except for Kanawha Falls (sample site 2: Kanawha River – London Pool). This is likely due to dispersal from long term introductions at these locations of walleye derived from Lake Erie broodstock. The lack of the introduced haplotype at the Kanawha Falls location may be due to a small sample size at this location (N = 4). The Ohio River (native) haplotype was identified in 15 of the 21 locations, indicating a wide distribution throughout the rivers of West Virginia. The native haplotype was most common in the Kanawha/New River and less common in the Ohio and Monongahela rivers.

Despite the elevated frequencies of Lake Erie haplotypes from recent introductions, the native haplotype was still common in the Kanawha/New River system. This is consistent with one of two explanations. These native populations may be remnants of a historically more widespread population found within the Ohio River sub-basin. Industrialization of the area, with the addition of the lock and dam systems, led to habitat deterioration and to the decline of walleye populations and the reduction in its

range They were restricted to areas less affected by industrialization such as the Upper Kanawha and New rivers and the Upper Ohio River (White et al. 2005).

The higher frequency of the native haplotype in the Kanawha/New River system could also be explained by a Teays River origin/refugium of a native walleye population. These individuals may be descendents of a pre-Pleistocene population within the Teays River basin that was subsequently isolated in the upper portions of the river system. This is further supported by the low frequency of the native haplotype observed from the Monongahela River. The native walleye population may not have extended into the Old Allegheny River system and was isolated to the Teays River system.

A Teays River origin hypothesis was suggested by Stepien and Faber (1998) based on the level of DNA sequence divergence observed between Lake Erie and Ohio River populations (1.5 mya), which is consistent with the time of the Teays River. They suggested the Upper Ohio River population remained in this portion of the Teays River system because of the more pristine water quality and habitat available due to cool mountain runoff and less industrialization.

The New River has also been proposed as a glacial refugium for the northern hogsucker (*Hypentelium nigricans)* (Berendzen et al. 2003). Phylogeographic analysis of the hogsucker recognized a Teays River clade that was subsequently fragmented into western (Interior Highlands and Upper Mississippi River) and eastern (New and Roanoke Rivers) refugia.

Microsatellite analysis

The Ohio River native population has higher genetic diversity than the other native haplotype populations. The lack of differentiation between the Ohio River and

Lake Erie populations provided evidence that the native haplotype populations in the Ohio River have been influenced via introgression with Lake Erie fish previously stocked.

Lower variation in the Reservoir and Monongahela, Kanawha, New and Elk River populations suggest minimal influence from introduced walleye. This could reflect a native walleye stock not influenced by introduced individuals. The sample sizes of native walleye in the Reservoir population was small (N= 2) suggesting sampling bias in this population and will not be considered as an accurate reflection of the native walleye population in these areas. The similarity of the Elk, New and Kanawha River populations at both loci suggests they are likely sampled from a single population. The alleles with the highest frequency of these three populations at both loci were consistent with the alleles considered diagnostic of New River walleye (*Svi*33^{78} and *Svi*17^{99}; Palmer et al. 2001). The Svi33^{78} allele, while not unique to the native haplotype in this study, was found in high frequencies within the Elk, Kanawha and New Rivers. The homozygous *Svi*17^{99}allele, also not unique to the native haplotype, was detected in all Kanawha and New River individuals suggesting they are likely the same Kanawha River/New River population. Two thirds of the individuals collected from the Elk River, a tributary of the Kanawha River, expressed *Svi*17^{99} allele further suggesting that individuals from the Elk River may be from the same Kanawha River/New River population. The homozygous *Svi*17^{99} allele was also detected in all three individuals within the Monongahela River population, indicating a possible similarity to the Kanawha/New River population, but is not considered among the same population due to the difference in alleles expressed at the *Svi*33 locus as well as substantial geographic separation.

The similarity of microsatellite allele frequencies between the Ohio River and the Lake Erie populations suggest evidence of introgression between native and introduced walleye. Introgression, or the movement of alleles from one population to another through hybridization, is a common result of stocking and supportive breeding practices. Hybridization between more distant populations, such as the native walleye and Lake Erie walleye, can have negative impacts on the fitness of native populations as well as the resultant progeny, known as outbreeding depression. Outbreeding depression operates via two mechanisms: the swamping of locally-adapted genes of the native populations resulting in loss of local adaptations of the hybrids and/or the breakdown of locally coadapted gene complexes of native fish (Lynch 1997). Reduced fitness of hybrids, via outbreeding depression has been observed in intensively managed fish species such as trout (Miller et al. 2004), salmon (Gilk et al. 2004) and largemouth bass (Phillip et al. 2002). Hybrids between geographically and/or genetically distant populations may also be more susceptible to infectious diseases (Goldberg et al. 2005).

The introgression observed within the Ohio River likely may have had a negative effect on the genetic integrity of any native walleye populations in these areas. Introgression between introduced lake-derived, and native river-adapted walleye would have a negative effect on the native walleye.

If we consider the Kanawha/New River walleye to be native to this river system then they likely have experienced a high level of historical separation from other walleye populations (Stepien and Faber 1997). It is also likely that during this period of separation they became adapted to the river environment, which may be important to the success of walleye in this region (Billington et al 1992). This population has persisted

despite many introductions of Great Lakes-derived walleye. These local adaptations could provide certain advantages in stocking progeny from Kanawha/New River broodstock into the rivers of West Virginia. Native, naturally-reproducing populations of largemouth bass have been shown to outperform any introduced non-local stock (Phillip et al. 2002). A certain survival advantage, via increases in abundance over non-native walleye, has also been observed in walleye in Minnesota lakes (Eldridge et al. 2002). It is possible that the New River walleye broodstock would have better survival rates than any Lake Erie or other non-native walleye in this region.

There is evidence that walleye exhibit homing behavior to natal spawning sites and spawning philopatry (Ward et al. 1989; Jennings et al. 1996; Stepien and Faber 1998). While many consider this a learned behavior, Jennings et al. (1996) suggests there is a heritable genetic response to environmental cues in returning to natal spawning areas, which could provide further evidence of local adaptation. They observed differences in spawning habitat preferences between river-derived and lake-derived walleye. The river-derived walleye chose more lotic river habitats, while the lake-derived walleye chose more lake-like shallow habitats. Any introduced lake-derived walleye, would be at a disadvantage during spawning in a more lotic river environment such as the Kanawha/New River. Introduced walleye may need a lake-based environmental cue they would not otherwise receive in a riverine environment. There have also been anecdotal reports of larger walleye found in areas containing native walleye (Palmer et al. 2000, White et al. 2005, Hackney and Holbrook 1979) further indicating a possible adaptive advantage of native walleye.

Management Implications

The microsatellite analysis from this study provides evidence that the populations of native walleye in the Upper Kanawha River and New River are likely a single, panmictic population. This population has likely remained a relatively pure native population despite long-term introductions of non-native walleye populations and deserves management as a unique population. Walleye that evolved in this river system should have greater fitness that introduced fishes. Future introductions into the river systems of West Virginia should utilize this native walleye population as broodstock.

The WVDNR has recognized the existence of a native walleye population within West Virginia. A walleye management plan has recently been proposed that includes enhancing walleye populations within the Kanawha River, the Ohio River upstream from, and including the Belleville Pool, and the Little Kanawha River by introductions of native walleye. Based on our results demonstrating limited introgression in the Kanawha/New River. Restoring native walleye into the Kanawha River may be a reasonable goal. There is still suitable walleye habitat less affected by industrialization in the Upper Kanawha/New River system. Alternatively, the native walleye in the Ohio River show evidence of considerable introgression with Great Lakes walleye. The habitat of the Ohio River, primarily below Pike Island Locks and Dam, has been heavily affected by industrialization. This has reduced riffle habitat and increased siltation, reducing shallow gravel habitats preferable for a native river-adapted walleye to spawn. Tagging studies have shown that the locks and dams also impede the movement of walleye along the length of these rivers (Schell et al. 2004). Therefore, it would likely be more difficult to achieve the goal of restoring a genetically pure native population to this area.

Alternatively, introducing additional native walleye into the Ohio River may aid in enhancement of the fitness of the existing native walleye populations.

Evidence from the RFLP survey in this study shows a very low frequency of native walleye haplotypes in the reservoirs and impoundments of West Virginia. Many of the reservoirs and impoundments were stocked with walleye derived from Lake Erie origin to enhance existing walleye populations or establish walleye populations in these areas. Since populations of Lake Erie- derived walleye have been established in these impoundments it may be acceptable to continue stocking of the reservoirs with Lake Erie-derived walleye provided that the risk of dispersal from the reservoir into the rivers is minimal.

Restoring a native population of walleye into the Upper Kanawha and New River will require hatchery supplementation. Walleye from within the same watershed should be used when the goal is augmentation of the local populations. Consequently, the practice of importing and introducing Lake Erie derived walleye into the river systems of West Virginia should be curtailed. It would be expected that any future introgression between introduced lake-derived populations of walleye would have a negative effect on the river-adapted native walleye in the Kanawha River.

Currently the WVDNR does not maintain a hatchery-raised strain for sauger (a close relative of the walleye) broodstock. Wild individuals are collected annually and used for broodstock. These individuals are then tagged and released but are not used subsequent years. This approach should be incorporated for native walleye stocking. Hatchery strains tend to be less fit, have lower performance, and lower genetic variation than wild stocks (Leary et al. 1985; McLean et al. 2004; Reisenbichler and Rubin 1999).

Hatchery-spawned eggs derived from wild New River broodstock are noticeably larger and they resultant fry are more aggressive than those of the established strains of Lake Erie origin (Rodney Null WVDNR, pers. comm).

Walleye intended for use as New River broodstock should be taken from the Upper Kanawha River or New River area. Based on the RFLP analysis of this study, no pure native walleye populations exist in West Virginia. All individuals collected for use as broodstock will have to be screened to ensure the haplotype identity and the presence of the diagnostic microsatellite alleles, *Svi*17^{99} and *Svi*33^{78} (Palmer et al. 2001). This process will have to be carried out every year if wild individuals are to be used for broodstock.

An alternative hatchery plan would be to establish a multi-year class "wild" population of native walleye. This would be established in a reservoir or lake previously uninhabited by walleye, so there will be no possibility of influence from other walleye populations. Walleye desired for broodstock will not have to be screened every year and there would be ample genetic variation between year classes to minimize the negative implications of establishing a hatchery strain. Since walleye exhibit wide fluctuations in year-class strength, aging analysis of this population may be needed in the future to estimate the success of each year-class to determine if additional supplementation would be needed.

Conclusions

This study has identified native walleye stocks throughout West Virginia. Although common within the Ohio River, data suggests native walleye stocks have introgressed with other walleye populations, thus compromising the genetic integrity of these stocks and making them unsuitable for future use as native walleye hatchery broodstock. Native walleye from the Kanawha/New River system exhibited limited introgression. This population should be maintained and conserved as a unique genetic stock. Native walleye from this area should be used for hatchery brood stock in maintaining and restoring native walleye to these rivers. We recommend additional sampling of walleye in West Virginia to clarify the distribution of the New River walleye, primarily within the Kanawha/New River system. I recommend further analysis of walleye from Kanawha Falls on the Kanawha River as all individuals collected in this study possessed the native haplotype. Additional sampling should occur within the Elk and Tygart rivers. It is recommended that walleye should be sampled upstream of any reservoirs, since stocking of Lake Erie walleye in these lakes has likely impacted the stock structure downstream of the dams. Any other large tributaries of the Kanawha River and New River, with appropriate habitat, warrant sampling as well (i.e. Bluestone, Gauley, and Greenbrier Rivers). Additional sampling should also be carried out within the Big Sandy and Tug Fork Rivers as they are both tributaries of the Ohio River.

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TABLE 1.—Water bodies, sampling sites, sample sizes, and frequency of the Ohio River and Lake Erie haplotypes of *Sander vitreus* in West Virginia. Site number corresponds to the sample localities in Figure 7. Abbreviations are as follows: N, sample size; OR, number of individuals possessing the Ohio River haplotype; LE, number of individuals possessing the Lake Erie haplotype; Freq. OR, the frequency of Ohio River haplotypes in the sample. Reference samples are not included within sample set.

Waterbody	Location	Site #	N	OR	LE	Freq. OR
Elk River	Clendenin	1	4	3	1	0.75
Kanawha River	Kanawha Falls	2	4	4	0	1.00
	London L&D	3	53	16	37	0.30
	Marmet L&D	4	45	26	19	0.58
	Winfield L&D	5	5	1	4	0.20
Monongahela River	Gray's Landing L&D	6	14	0	14	0.00
	Lock and Dam #2	7	10	2	8	0.10
	Hildebrand L&D	8	3	0	3	0.00
	Morgantown L&D	9	12	1	11	0.08
	Opekiska L&D	10	12	0	12	0.00
New River	Sandstone Falls	11	5	1	4	0.20
Ohio River	Belleville L&D	12	8	2	6	0.25
	Hannibal L&D	13	7	1	6	0.14
	Racine L&D	14	6	1	5	0.17
	Robert C. Byrd L&D	15	4	1	3	0.25
	Willow Island L&D	16	8	2	6	0.25
Tygart River	Tygart Dam	17	22	0	22	0.00
Cheat Lake		18	11	0	11	0.00
Stonewall Jackson Lake		19	16	1	15	0.06
Summersville Lake		20	83	1	82	0.01
Sutton Lake		21	10	0	10	0.00

TABLE 2.— Pair-wise F_{ST} values of native and introduced walleye populations. The Lake Erie population is comprised of a sample of Lake Erie haplotypes identified from all sampling localities in which a native haplotype was also observed. F_{ST} values are below the diagonal. All values are different from 0 indicating differentiation between populations. F_{ST} values and allele frequencies were determined using GenAlEx 6 (Peakall and Smouse 2005).

	Elk	Monongahela	New	Ohio	Kanawha	Reservoir	Lake Erie
Elk	0.000						
Monongahela	0.151	0.000					
New	0.171	0.391	0.000				
Ohio	0.130	0.294	0.279	0.000			
Kanawha	0.129	0.202	0.188	0.230	0.000		
Reservoir	0.149	0.200	0.395	0.228	0.313	0.000	
Lake Erie	0.113	0.236	0.280	0.088	0.216	0.176	0.000

TABLE 3.—Microsatellite allele frequencies for native walleye (*Sander vitreus*) populations in West Virginia. Native individuals were pooled into populations based on their respective sampling drainage. Populations from individual sampling sites were not used due to small sample size of native walleye (N= 1 or 2) at most sites. The Lake Erie population is comprised of a sample of Lake Erie haplotypes identified from all sampling localities in which a native haplotype was observed.

		Population						
Locus	**Allele**	**Elk**	**Monongahela**	**New**	**Ohio**	**Kanawha**	**Reservoir**	**Lake Erie**
		N = 3	N = 3	N = 3	N = 7	N = 13	N = 2	N = 12
Svi33	**76**	*	*	*	0.214	*	*	*
	78	0.333	*	0.833	0.286	0.385	*	0.042
	82	*	*	*	0.143	*	*	*
	84	*	*	*	0.071	*	*	0.083
	86	0.333	0.667	0.167	*	0.115	0.750	0.042
	88	0.167	0.167	*	0.143	0.462	*	0.042
	90	*	*	*	0.071	*	*	*
	92	*	*	*	*	*	*	0.042
	94	*	*	*	*	0.038	*	0.125
	96	0.167	*	*	*	*	0.250	0.125
	98	*	*	*	0.071	*	*	0.250
	100	*	0.167	*	*	*	*	0.083
	102	*	*	*	*	*	*	0.083
	104	*	*	*	*	*	*	0.042
	108	*	*	*	*	*	*	0.042
Svi17	**95**	*	*	*	*	*	0.500	*
	97	*	*	*	0.500	*	*	*
	99	0.667	1.000	1.000	0.214	1.000	0.500	0.250
	101	0.333	*	*	0.071	*	*	0.042
	103	*	*	*	*	*	*	0.125
	105	*	*	*	0.071	*	*	0.250
	107	*	*	*	0.071	*	*	*
	109	*	*	*	*	*	*	0.250
	111	*	*	*	*	*	*	0.083
	115	*	*	*	0.071	*	*	*

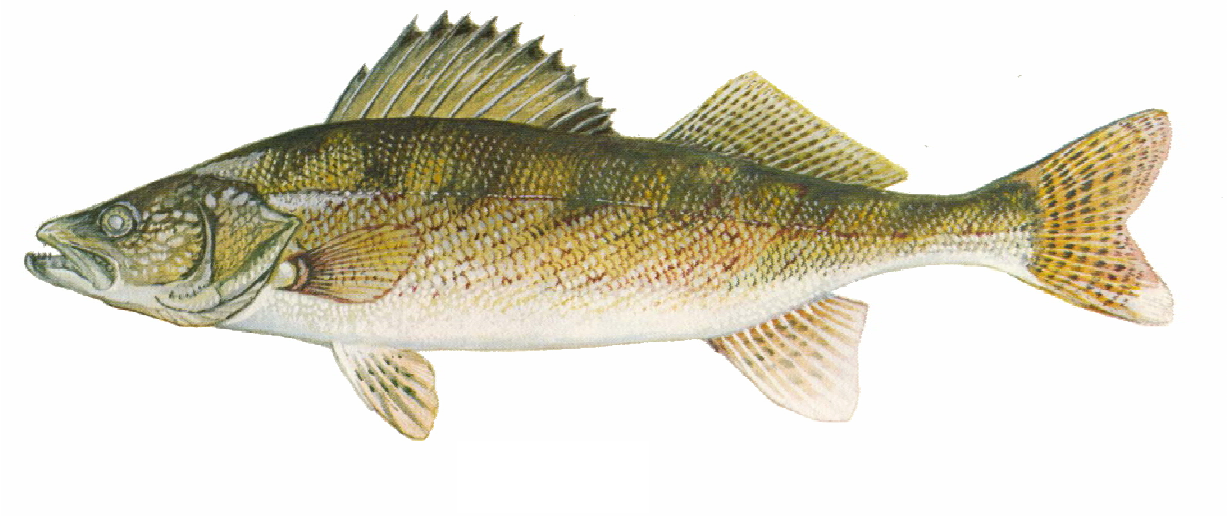

FIGURE 1.—The walleye, *Sander vitreus* (picture from WVDNR)

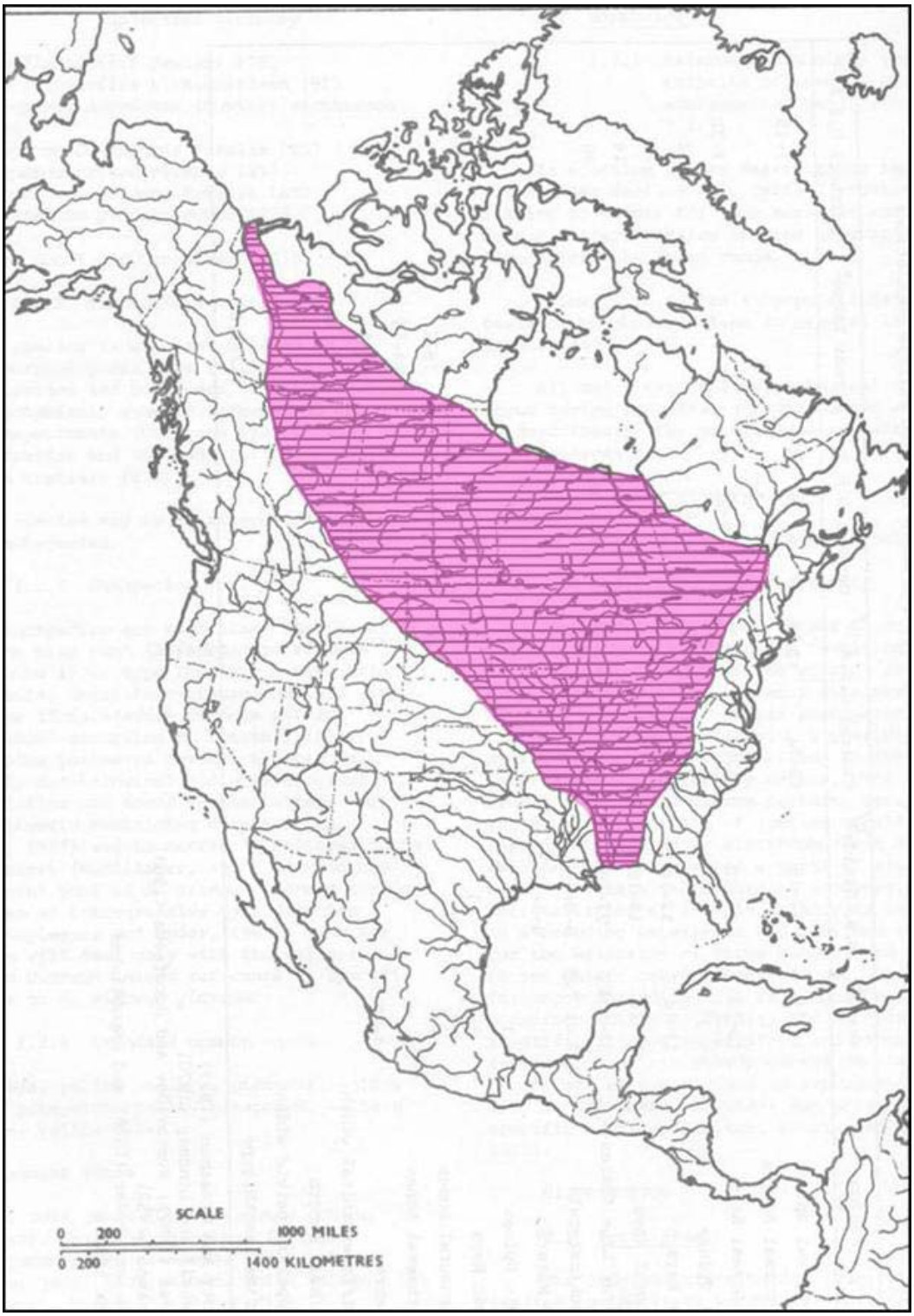

FIGURE 2.—Natural distribution of walleye (*Sander vitreus)* in North America (Adapted from Colby et al. 1979)

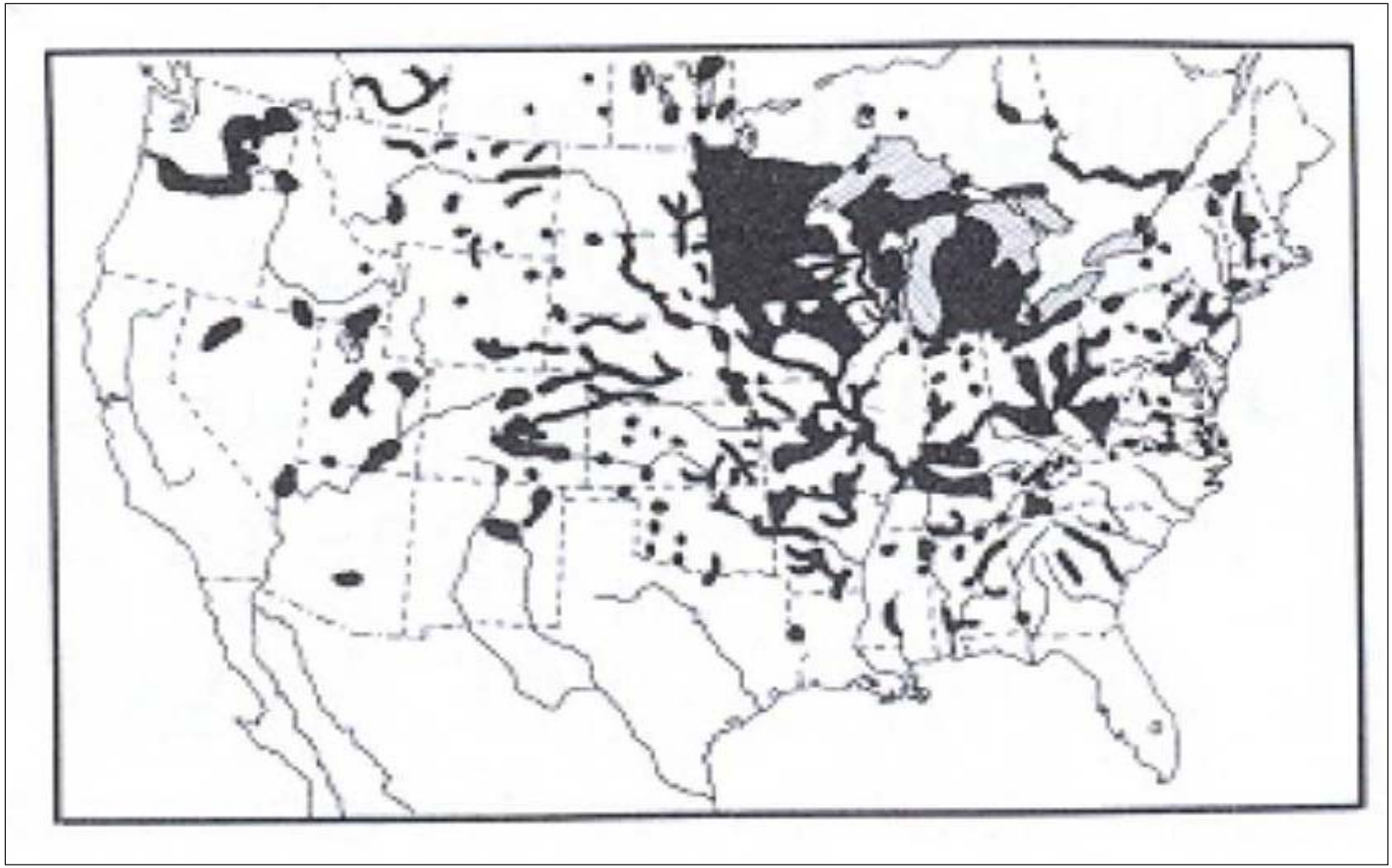

FIGURE 3.—Distribution of walleye (*Sander vitreus*) in North America, including introduced areas on the Atlantic Slope, Gulf Coast and Pacific Slope. (from Jenkins and Burkhead 1994)

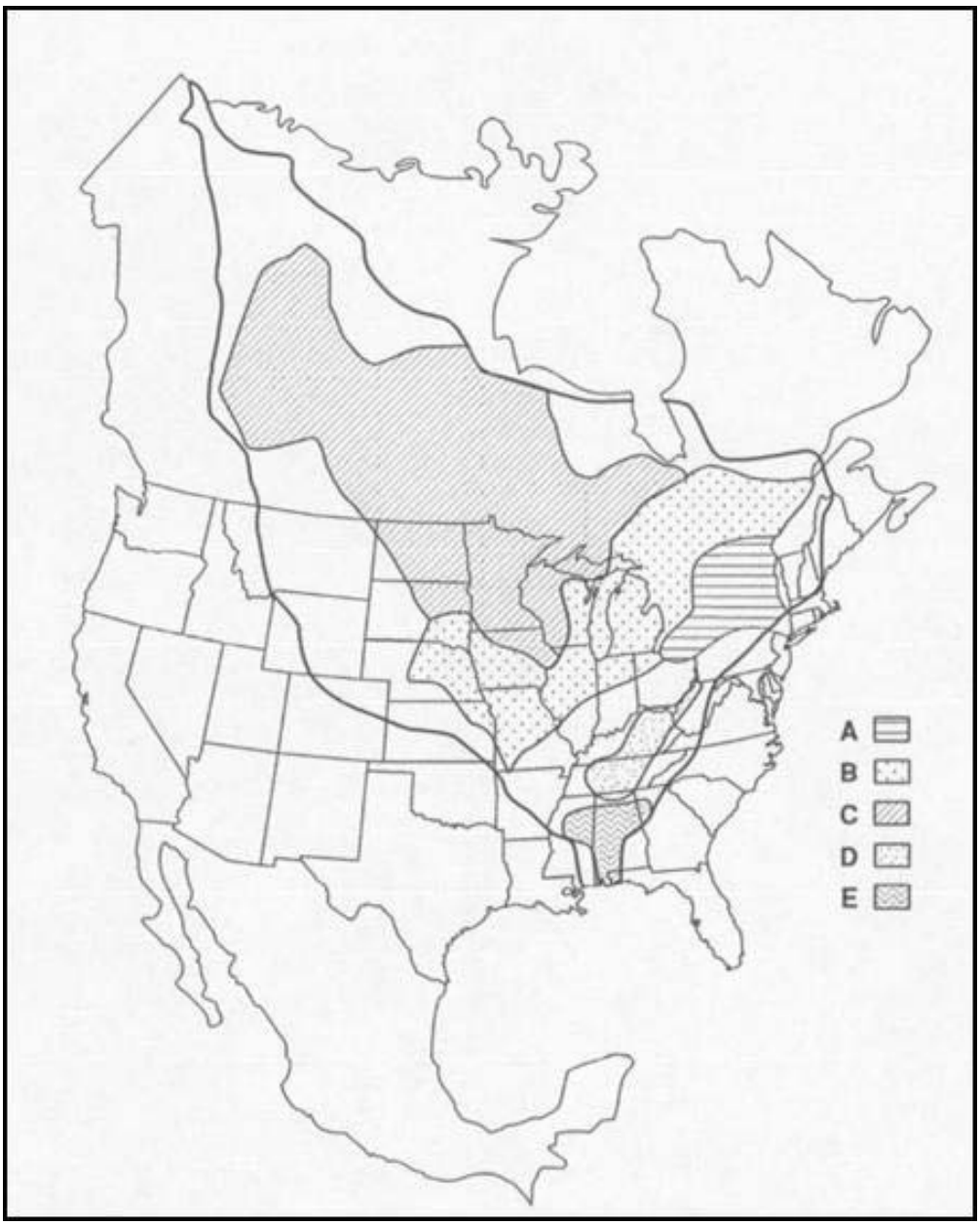

FIGURE 4.—Natural distribution of walleye (*Sander vitreus)* in bold outline, along with the generalized distribution patterns of the five main walleye mtDNA haplotype groups from Billington et al. (1996). A—Atlantic refugium origin; B—Mississippi refugium origin; C—Missouri refugium origin; D—mixture of haplotypes from stocked fish of Atlantic and Mississippi refugia origin together with other divergent haplotypes suggested to be in the area prior to stocking; E—Mobile drainage basin haplotype. (from Billington 1996)

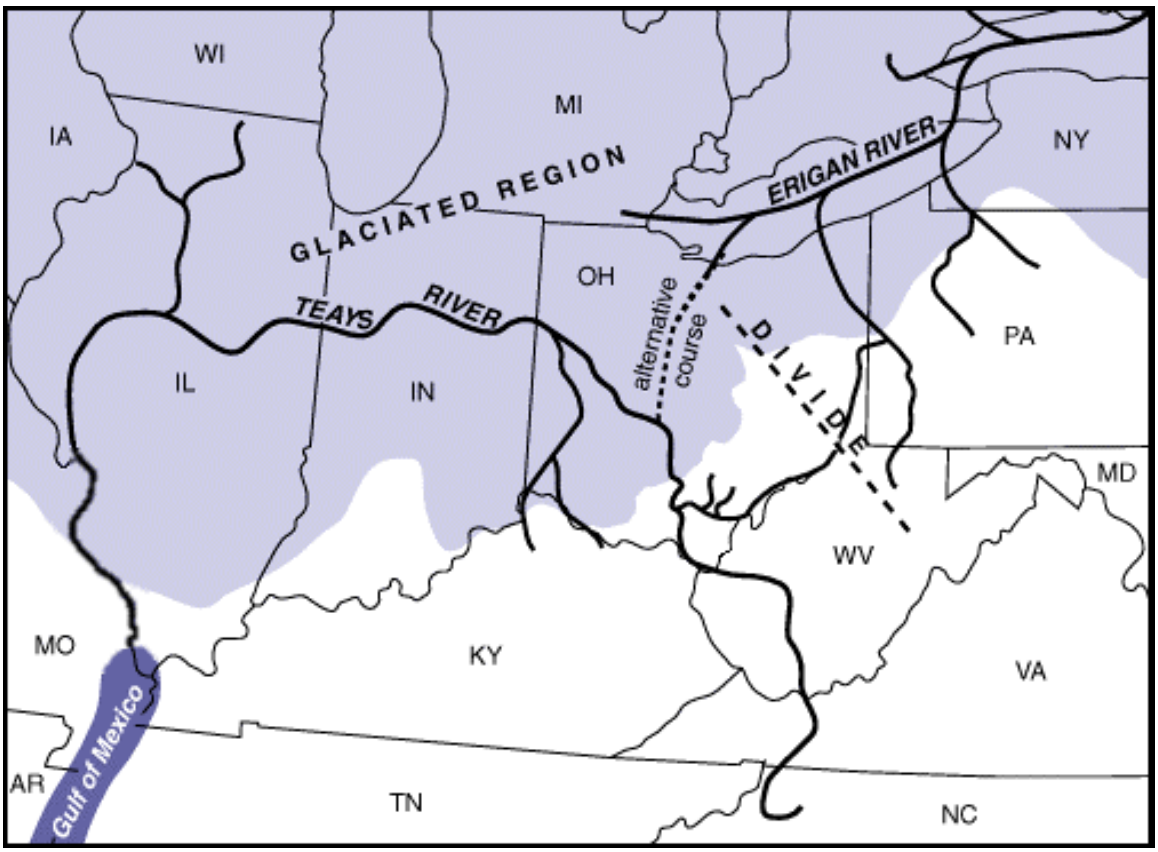

FIGURE 5.— Map showing the accepted courses of the pre-Pleistocene drainage systems of the northern U.S. and the extent of the glacial advances. Northern Ohio, Pennsylvania, and West Virginia were drained by the Erigan River. This river and the lower extent of the Teays River were buried beneath the glacial drift and were replaced with the current drainage systems we see today (from Hansen 1995).

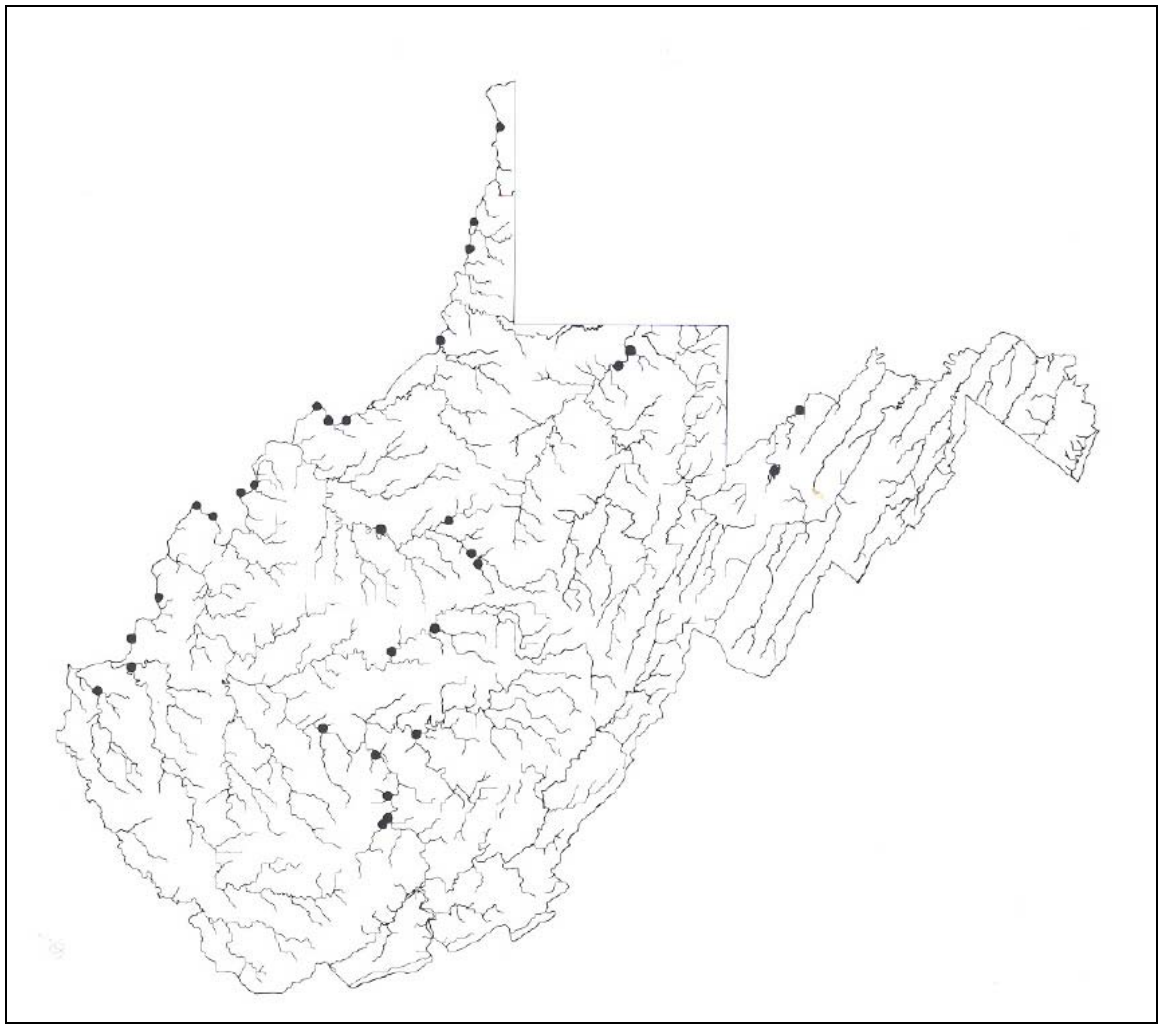

FIGURE 6.—Known distribution of walleye (*Sander vitreus*) in West Virginia. Dots indicate sampling locations from which walleye were observed. (from Stauffer et al. 1995)

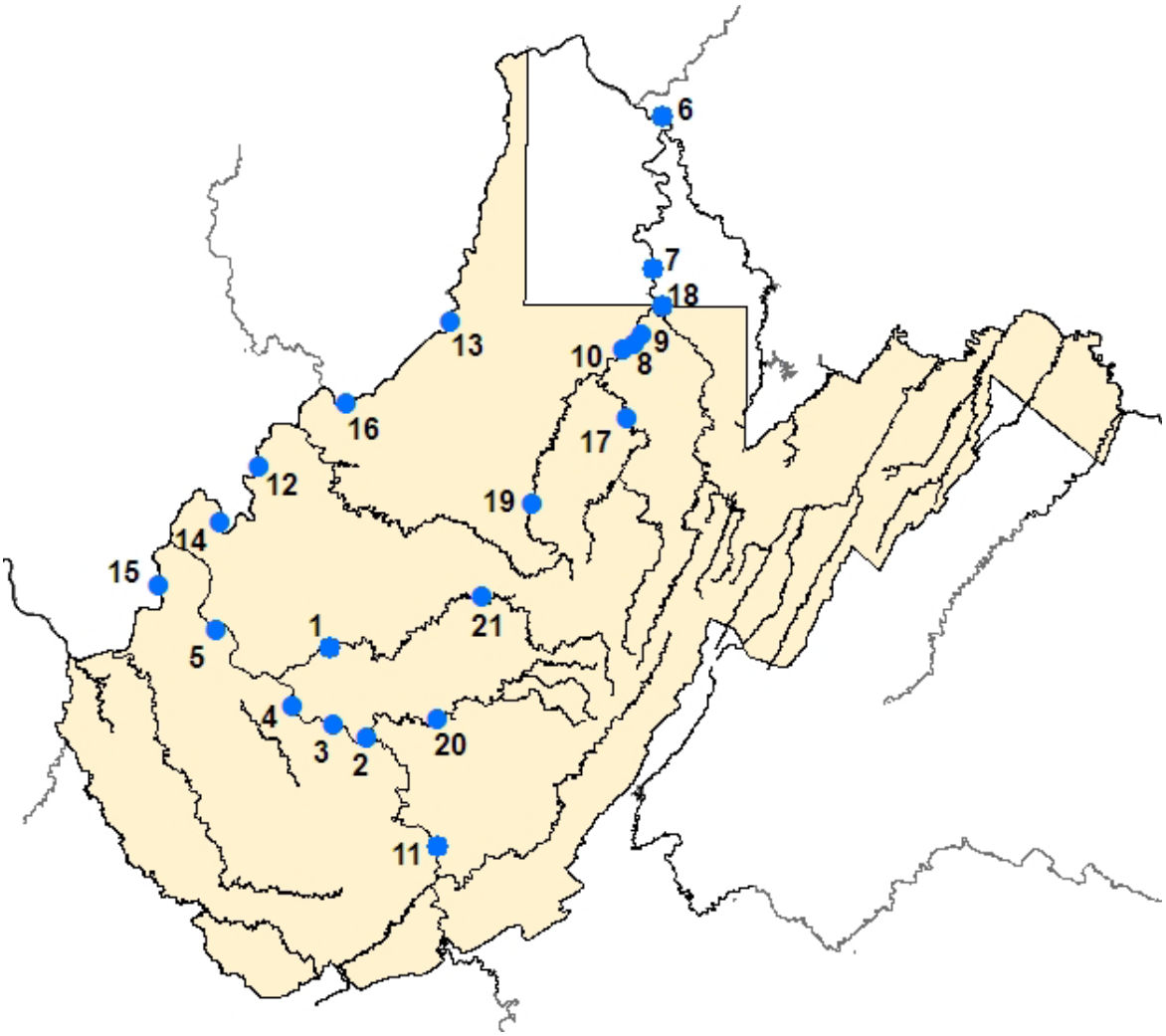

FIGURE 7.—Locations of the 21 sampling sites for walleye (*Sander vitreus)* in West Virginia. See Table 1 for details of sampling sites.

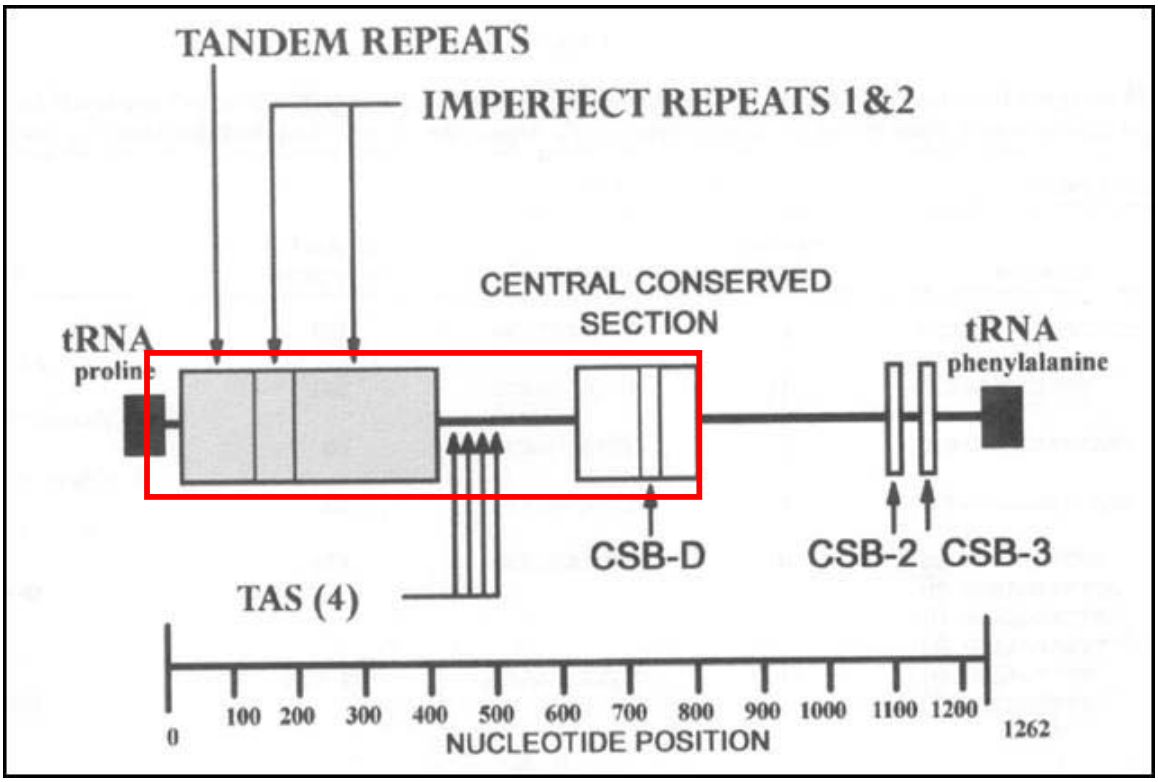

FIGURE 8.—Structure of the mitochondrial DNA control region of *Sander*. The area within the red box represents the ~800bp fragment amplified with the primers H16498 and L15926 used in this study. (Adapted from Faber and Stepien 1997)

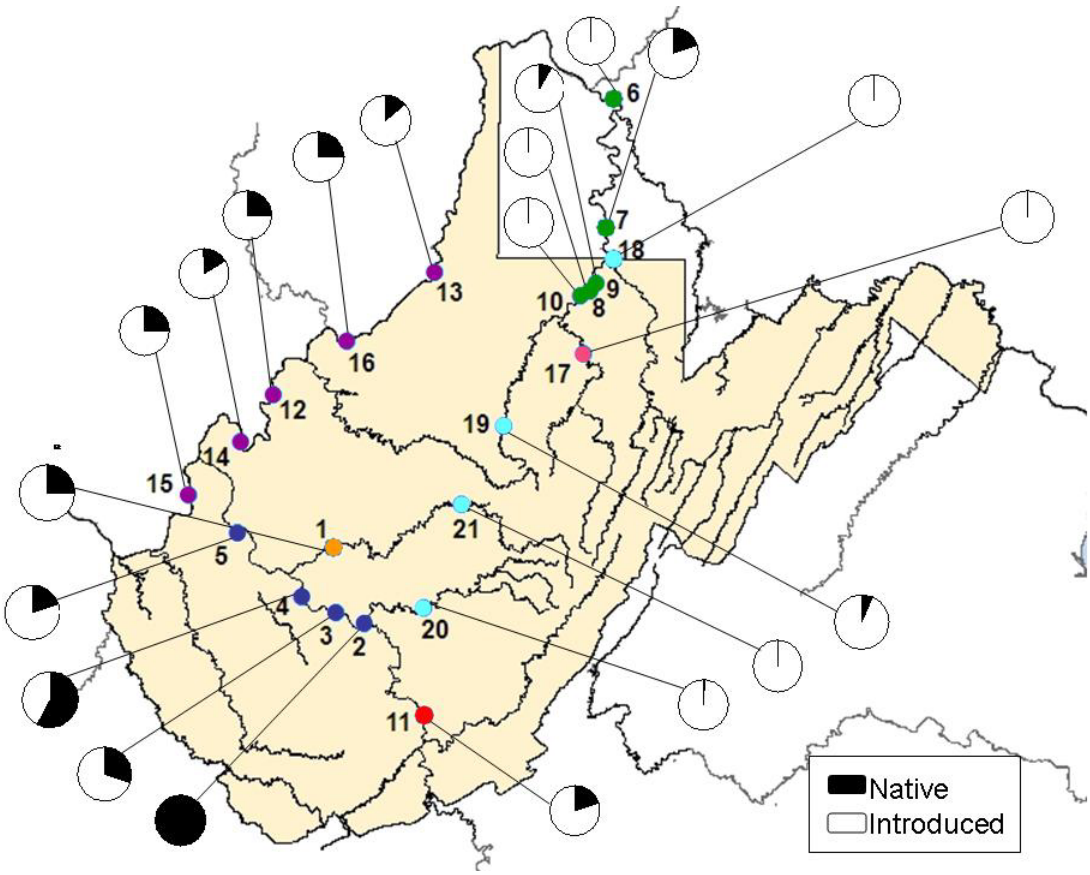

FIGURE 9.—Distribution and frequencies of native haplotypes in West Virginia. See Table 1 for locality numbers.