Genetic Marker-assisted Restoration of the Presumptive Native Walleye Fishery in the New River, Virginia and West Virginia

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Abstract: The increasing importance of the walleye (*Sander vitreus)* fishery in the New River, Virginia, and recent research findings showing persistence of a presumptive native stock motivated a seven-year program of hatchery-based restoration of the native fishery. Candidate spawners were collected from spawning areas, and DNA from fin clips was genotyped at two microsatellite loci. Candidates exhibiting alleles at the *Svi17* and *Svi33* loci that characterize the presumptive native stock were spawned. Their young were reared at one of four fish hatcheries in Virginia and West Virginia. Approximately 600,000 fry and 800,000 fingerlings were stocked in riverine sections of the New River in Virginia and West Virginia. Since stocking began, ages 0–3 walleye have become much more abundant at the upper New River spawning areas. Catch rates in spring electrofishing samples have increased from 3 to 17 fish per hour in Virginia and from 1.2 to 26.6 fish per hour in West Virginia. Walleye catch per net-night from fall gill net sets in Claytor Lake, Virginia increased from 0.2 in 2001 to 3.1 in 2006. Comparison of data from creel surveys in 2002 and 2007 showed increasing angler effort directed toward walleye from 10% to 30% and increasing catch. Native walleye allele frequencies increased from 16% and 14% at the two marker loci in the 1997 to 1999 genetic surveys to 46% and 58% in the 2004 to 2006 surveys.

Key words: walleye, *Sander vitreus*, microsatellite DNA, marker-assisted selection, supplemental stocking.

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Walleye *(Sander vitreus)* is a highly valued sportfish that inhabits the New River in southwestern Virginia and West Virginia. The New River is located on the eastern edge of the native range, and it is uncertain whether walleye is native to the drainage (Jenkins and Burkhead 1994). Hackney and Holbrook (1978) believed walleye to be native to the New River and part of a southern stock found throughout the Mississippi drainage. In the past decade, ten 5- to 7-kg walleyes were collected by anglers or the Virginia Department of Game and Inland Fisheries (VDGIF) in the upper New River above Claytor Lake.

Walleyes have been stocked outside of their native range to areas throughout the United States (Hackney and Holbrook 1978). Introductions from different geographic origins resulted in many areas containing mixtures of native and introduced stocks (Murphy et al. 1983, Fox 1993, Jennings et al. 1996, Eldridge et al. 2002, White et al. 2005, Palmer et al. 2006). Walleyes of different geographic origins were mixed in Claytor Lake and the upper New River, Virginia, as a direct result of planting in 27 stocking events from 1939 to 1996 (Jenkins and Burkhead 1994, Murphy et al. 1983, Palmer 1999). Genotype frequencies for isozyme markers indicated mixing of walleye stocks in Claytor Lake, showing that at least some of these plantings were successful (Murphy et al. 1983). All stocking was suspended in 1997. Movements of radio-tagged fish showed three spawning sites (Allisonia, Fosters Falls, and Buck Dam, Fig. 1) and suggested that lake- and river-dwelling individuals to some degree spawned in spatially distinct areas (Palmer et al. 2005).

A genetic study showed fish carrying three previously unknown mitochondrial DNA haplotypes and high frequencies of characteristic alleles at particular microsatellite DNA loci (Palmer et al. 2006). Mitochondrial haplotype 43-bearing walleye from the upper New River tended to exhibit particular alleles at two microsatellite loci. At the *Svi17* locus, the *99/99*-homozygous genotype was observed in 94% of all haplotype 43-bearing individuals. This

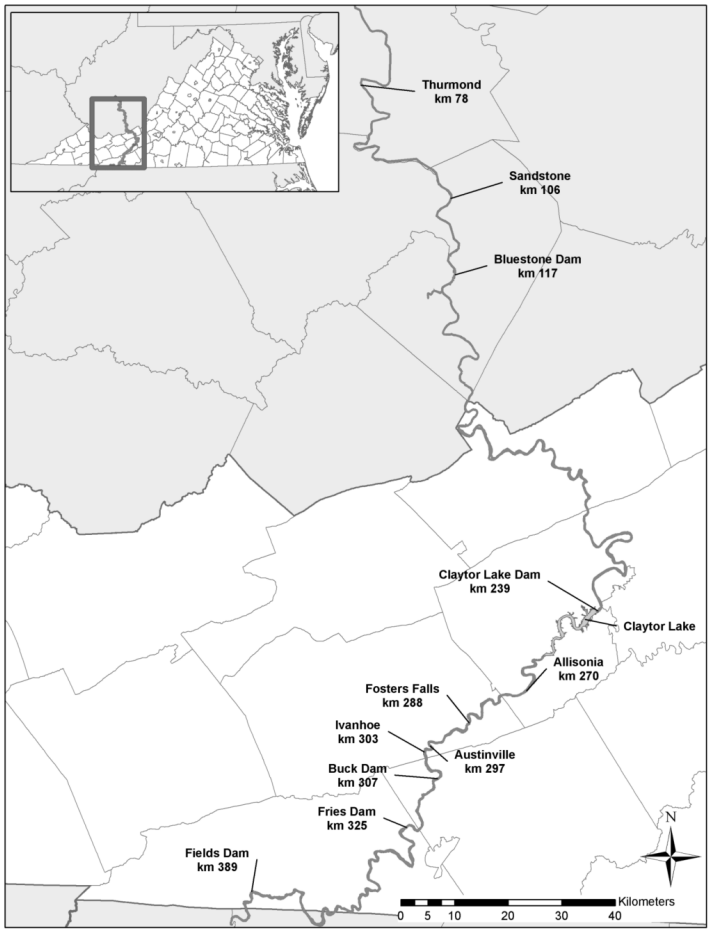

Figure 1. Study areas on the New River with key locations identified. Allisonia, Fosters Falls, and Buck Dam are spawning areas. Walleye were stocked between Fields Dam and Claytor Dam in Virginia and Sandstone and Thurmond in West Virginia.

concordance was not seen for any of the other mtDNA haplotypes identified. The *Svi33* locus also showed a unique 78bp allele in 77% of the haplotype 43-bearing walleyes. These findings suggested that a native walleye stock had persisted despite the stocking of non-native walleye.

Palmer et al. (2006) recommended that walleye management in the New River emphasize conservation of the presumptive native stock. They suggested that marker-assisted selection of spawners and stocking exclusively the river stock while restricting harvest could demographically boost the New River walleye stock. A 50.8-cm minimum size harvest limit went into effect on 1 January 2003. Here, we report hatchery-based enhancement of the unique, putatively native walleye stock based upon genetic marker-based identification of prospective broodstock.

Study Area

The upper New River study area was a 150-km segment in Virginia, beginning at the spillway of Fields Dam in Grayson County and continuing downstream to Claytor Lake Dam in Pulaski County (Fig. 1). This river section contains Fries, Byllesby, and Buck dams that block upstream and most downstream migration. A lower study area, comprising 38.6 km of the New River in West Virginia from Sandstone to Thurmond, received plantings of young walleye produced from broodstock collected in the upper river. Other waterbodies in West Virginia also received plantings.

Methods

Brood Fish Collection and Genetic Identification

Walleye were collected from the Fosters Falls and Buck Dam spawning sites by pulsed-DC electrofishing during the peak spawning run from late February through early March of 2000 to 2006. Individual fish were marked with numbered T-bar anchor tags following the procedures described by Nielsen (1992). Following a fin clip, reproductive-sized walleye were held overnight in tanks at the Buller Fish Culture Station (FCS; Marion, Virginia) while genetic analysis of each fish was carried out at Virginia Tech's Conservation Genetics Laboratory. Time of sampling and water temperature were examined to identify any effects on collection of New River stock walleye.

Microsatellite DNA variability was examined using polymerase chain reaction (PCR) to amplify two microsatellite loci (Borer et al. 1999), at which particular alleles previously were shown to occur at high frequencies only in the presumptive native New River stock (Palmer et al. 2006). Walleye with the diagnostic genetic markers, the 99bp allele at the *Svi17* locus and the 78bp allele at the *Svi33* locus, were selected for use as broodfish for hatchery production. Different degrees of stringency were applied among years depending on the number of broodstock candidates available. Male walleye were more abundant on the spawning grounds, which allowed us to select only those homozygous for both native strain-diagnostic alleles. In some years, only one or two females exhibited both diagnostic alleles, and the decision was made to accept females bearing either the *Svi17-99* or the *Svi33-78* allele.

Fish Culture and Stocking

Males and females identified to be of the New River stock were injected with human chorionic gonadotropin at Buller FCS to induce spawning. As many native walleye as practical were mated so that stocking of a limited number of genotypes would not artificially reduce the genetically effective population size of the targeted stock (Ryman and Laikre 1991, Ryman et al. 1994). Unfortunately, the limited numbers of holding pens and hatching jars did not allow the separation of eggs produced from females bearing both diagnostic alleles from those bearing only one. In 2006, production goals were reduced, allowing eggs to be segregated by

female. Fertilized eggs or fry were cultured at Buller Fish Cultural Station and Harrison Lake National Fish Hatchery (Charles City County, Virginia), and after 2003, at Palestine Hatchery (Elizabeth, West Virginia) and Apple Grove Hatchery (Mason County, West Virginia). Fry were stocked into ponds at Buller FCS and harvested in late May. The number of walleye raised to fingerling stage was maximized to the limit imposed by hatchery rearing capacity. Numbers of walleye in excess of fingerling rearing capacity were released as fry.

Riverine portions of the New River in Virginia stocked with walleye fry or 1- to 2-inch fingerlings included Fosters Falls and Ivanhoe (Table 1). Attempts were made to spread the stockings evenly throughout the river, but access was limited in some areas. Emphasis was placed on stocking the Fosters Falls and Ivanhoe locations, two major spawning and collection sites. The segregation of egg lots in 2006 presented the opportunity to stock a limited number of offspring produced from both male and female broodfish exhibiting both diagnostic alleles. These fish were reserved for the reach of New River between Fries Dam and Fields Dam, where no walleye had been stocked previously. All walleye were stocked in West Virginia as fingerlings in the New, Kanawha, Cheat, and Ohio rivers, with smaller numbers in Charles Fork and Moncove lakes.

Table 1. Summary of walleye selection and stocking activities in the New River, Virginia and West Virginia, from 2000 to 2006.

Year	Number analyzed	% New River fish	Hatchery production[a]	Number stocked
2000	283	62	9 females spawned 0.5 million eggs	0
2001	132	71	20 females spawned 2.2 million eggs	500,000 fry[b] 10,000 fingerlings[b]
2002	76	59	9 females spawned 0.9 million eggs	0
2003	92	75	20 females spawned 1.7 million eggs	100,000 fry[b] 51,840 fingerlings[b] 108,271 fingerlings[c]
2004	97	76	17 females spawned 2.0 million eggs	156,200 fingerlings[d] 101,486 fingerlings[e]
2005	85	65	23 females spawned 2.5 million eggs	90,800 fingerlings[d] 169,312 fingerlings[f]
2006	87	67	11 females spawned 1.0 million eggs	106,000 fingerlings[g] 759 fingerlings[h]

a. Eggs of each females were fertilized by milt from 2–3 males.
b. Stocked at Fosters Falls, Virginia.
c. Stocked in the Kanawha River at Marmet, West Virginia.
d. Stocked from Fries Dam to Claytor Dam in the New River, Virginia.
e. Stocked in the Kanawha River (41,120 at Marmet, 5880 at Winfield, and 8160 at London, West Virginia), New River (3126 at Sandstone, West Virginia), and 43,200 in Virginia.
f. Stocked in the New River (5080 at Sandstone, West Virginia), 39,304 in Wythe County, Virginia, Kanawha River (30,928 at Marmet, West Virginia), and 94,000 outside the New River drainage.
g. Stocked from Fields Dam to Claytor Dam in the New River, Virginia.
h. Stocked outside the New River drainage in West Virginia.

Assessment of Stocking Success

The effectiveness of the restoration program was assessed by quantifying population abundance, genetic composition, and harvest of walleye. Population abundance was indexed as catch per unit effort using electrofishing in river and gill nets in lake collections. Electrofishing was conducted using a boat-mounted pulsed DC electrofishing unit each spring from 2002 to 2006. Multiple sampling runs were conducted along riverbanks and within pool areas of the stocked portions of the river. Gill net surveys were conducted each fall in Claytor Lake. Gill nets were fished at night and retrieved in the morning. Two types of experimental gill nets were set, prey nets (30.5-m long, consisting of four 7.62-m panels with square mesh sizes of 1.27 cm, 1.59 cm, 1.91 cm, and 3.18 cm) and predator nets (30.5-m long, consisting of four 7.62-m panels with square mesh sizes of 2.54 cm, 3.81 cm, 5.08 cm and 6.35 cm). Diagnostic genetic markers were screened for electrofishing and gill net collections.

To determine whether walleye recruited to the creel, angler catch from a 2002 creel survey was compared to that from a 2007 creel survey on the walleye stocked section of the New River in Virginia (Fries Dam to Allisonia). Both surveys were stratified, two-stage sampling creel surveys focused on river fisheries. The 2002 survey was a combination roving and access point, while the 2007 survey was access point only. The 2002 survey was from 16 March–8 November, and the 2007 survey was from 1 February–31 July. Total estimated walleye fishing effort and total estimated walleye caught, harvested and released were compared between surveys.

Results

Brood Fish Collection and Genetic Identification

Time of sampling and water temperature had no clear effect upon likelihood of collecting walleye of the presumptive native stock. Water temperature fluctuated between 8–14 C during the sampling period, affected by weather systems typical in the month of March. Twice as many New River-stock walleye were caught at a water temperature of 11C than at any other temperature, although other factors, such as water clarity, flow, and time of sampling also could affect catch. Flow at the time of collection was particularly important. High flows prevented collections during the peak spawning time in 2002. Flow was augmented by Appalachian Power Company by release of water from Buck Dam to facilitate operation of boats on numerous occasions during low-flow conditions in 2001 and 2002.

Genetic data from collections made during spring spawning runs at Fosters Falls and Buck Dam showed that percentages of walleye typed as New River stock varied from year to year (Table 1). All the

female walleye 700 mm and larger ($n = 18$) belonged to the presumptive native New River stock. Genetic analysis was performed on most walleye collected at the spawning sites each year, including age 0 and immature fish, although not all walleye were sent to the hatchery for production.

Fish Culture and Stocking

Annual egg and fingerling production was dependent upon the number of New River-stock female walleye collected, and varied from year to year (Table 2). Egg mortality was high in some years, which is typical when females are injected with hormones to induce spawning (C. D. Stickley, VDGIF, personal communication). Hatchery personnel noted that eggs produced by New River-stock walleye were considerably larger (74,200/L) than eggs of non-native walleye (196,100/L). By comparison, eggs from Kansas walleye run approximately 132,500/L (C. D. Stickley, VDGIF, personal communication). New River-stock walleye were more aggressive, took feed more readily, and became cannibalistic more readily that Great Lakes-stock walleye (Rodney Null, West Virginia Department of Natural Resources, personal communication). New River-stock walleye scattered more readily when culturists worked near culture vessels.

New River walleye were stocked in the upper New River, Claytor Lake, and Byllesby Reservoir from 2000 to 2006 (Table 1). Although the upper river was stocked with fingerlings each year they were produced, Claytor Lake and Byllesby Reservoir were stocked only with fish remaining after the river received its allocation. The New River upstream of Fries Dam was stocked for the first time in 2006 and represented restoration of an extirpated population. Stockings above Fries Dam were with the offspring from parents that exhibited both the *Svi17-99* and *Svi33-78* alleles. West Virginia sites in the New and Kanawha rivers were stocked from 2003 onward, and across a wider geographic area from 2005 onwards.

Assessment of Stocking Success

Data from all methods employed to assess stocking success indicated an increasing walleye stock. Catch per unit effort for river walleye in spring electrofishing runs rose from 1 per hour in 2002 to 17 per hour in 2006 in Virginia and from 1.2 in 2004 to 26.6 per hour in 2007 in West Virginia (Table 2). Catch of walleye per net-night in Claytor Lake, Virginia, increased from 0.2 in 2001 to 3.1 in 2006 (Table 3).

Screenings of genetic markers over the course of the study showed increasing frequencies of alleles associated with the native stock. Spring collections from the spawning grounds showed varying percentages of markers for the native New River stock among years: 53% in 2000, 38% in 2001, 59% in 2002, 58% in 2003, 63% in 2004, 54% in 2005, and 52% in 2006. Fall gill net collections from Claytor Lake showed a higher percentage of native alleles in 2006 (38.5%) than in 1997–1999 (14%), likely as a result of stocking, but still lower than contemporary river collections. The latter is not surprising, as non-native walleye persist in the lake (Palmer et al. 2006), although their representation in the gene pool is decreasing. Genetic analysis of walleye collections over the upper river study site showed increasing representation of alleles characterizing the native New River stock. Data pooled across the 1997–1999 surveys showed frequencies of 16% native alleles at the *Svi33* locus and 14% at the *Svi17* locus. Data pooled across the 2004–2006 genetic surveys showed 46% native alleles at the *Svi33* locus and 58% at the *Svi17* locus. These percentages represent all walleye analyzed each year, including age 0, older juveniles, and broodstock candidates.

In 2002, an estimated 3,590 angler-hours were directed toward walleye, which constituted 10% of the total fishing effort for this section of the river during the survey period. An estimated 320 walleye were caught, 264 released, and 56 (17%) harvested. In

Table 2. Catches of walleye in spring electrofishing surveys in the New River, Virginia and West Virginia, from 2001 to 2007.

Year	Total catch	Time (hours)	Range of catch per run	CPUE (# /hr)	# Stocked in previous year
Virginia					
2001	3	1.0	0–1	3.0	0
2002	2	2.0	0–1	1.0	500,000 fry, 10,000 fingerlings
2003	2	3.0	0–1	0.7	0
2004	26	7.7	0–4	3.4	100,000 fry, 51,840 fingerlings
2005	32	7.7	0–12	4.1	101,486 fry, 156,200 fingerlings
2006	143	8.5	0–25	7.0	90,080 fingerlings
West Virginia					
2004	2	0.6	–	1.2	0
2005	5	1.6	–	9.4	3126 fingerlings
2006	75	11.2	–	15.5	5080 fingerlings
2007	184	6.9	–	26.6	9000 fingerlings

Table 3. Catches of walleye in fall gillnet surveys in Claytor Lake, Virginia, from 2001 to 2006.

Year	Nets set	Walleye caught	Catch per 100-m net	Catch per net-night
2001	43	9	0.3	0.2
2002	30	4	0.2	0.1
2003	48	15	0.4	0.3
2004	36	74	2.8	2.1
2005	36	89	3.3	2.5
2006	36	113	4.2	3.1

2007, an estimated 6,719 angler-hours were directed toward walleye, which constituted 30% of total fishing effort. An estimated 2,247 walleye were caught, 2,058 released, and 189 (8%) harvested. Clearly, angler effort and catch have increased.

Discussion

Putative Native New River Walleye Stock

Allozyme and microsatellite DNA data from the Fosters Falls and Buck Dam spawning sites in the upper New River showed evidence of the presence of more than one distinct genetic stock (Palmer et al. 2006). Furthermore, mitochondrial DNA results showed three previously unknown haplotypes, one at high frequencies, in collections from the New River, supporting the hypothesis that a unique walleye stock exists in the New River system. The New River, formerly the Teays River, flowed directly into the Mississippi River until the advance of Wisconsonian-period glaciers buried the lower two-thirds of its course (Jenkins and Burkhead 1994). The upstream portion of the river could have provided a glacial refugium for walleyes. Subsequent migration from downstream was blocked by Kanawha Falls in West Virginia. Hence, native walleye stocks in the refugium would have been genetically isolated from other stocks. Spawning habits may temporally, spatially, or behaviorally separate the presumptively native New River stock from lake-derived introduced stocks (Murphy 1981, Murphy et al. 1983, Palmer et al. 2005). Palmer et al. (2006) showed that the presumptive native stock has persisted despite decades of planting non-native stocks, suggesting an adaptive basis to its persistence.

Adaptation to their native environment may be important for the survival of walleye populations. Native populations exhibited greater hatching success than non-native populations in Georgian Bay rivers (Fox 1993). Walleye populations exhibited heritable preference for river or lake spawning habitat in Iowa (Jennings et al. 1996). Native walleye tended to increase in abundance relative to non-native stocks in three Minnesota lakes (Eldridge et al. 2002). In order to conserve the presumptive native New River stock, we recommended genetic marker-based selection of broodstock, hatchery-based demographic supplementation of the population, and a restrictive harvest policy.

Marker-assisted Selection and Hatchery-based Supplementation

Observation of genetic markers characterizing the presumptive native New River walleye stock (Palmer et al. 2006) suggested the possibility of marker-assisted artificial selection of spawners. Genetic markers have been used to select native pink salmon (*Oncorhynchus gorbuscha*) to allow supportive breeding of a population at risk of extinction (Olsen et al. 2000). To our knowledge, this is the first program attempting to identify and spawn native walleye for purposes of restoration.

The Fosters Falls and Buck Dam collection sites differed in composition of walleye collections and accessibility. The Fosters Falls site provided easy sampling access and large numbers of ripe males. Collections showed a 6:1 male-female ratio in walleye collected at Fosters Falls. It is unclear why more males were collected; females may have remained in pool areas below the Foster Falls spawning site that were not accessible to sampling gear. Sex ratio varied temporally. Males arrived first and remained at the spawning areas, while females arrived later and left soon after spawning.

The Buck Dam site had a higher percentage of females in the total catch, and a higher percentage of both sexes identified genetically as the presumptive New River stock (data not shown). Fewer walleye, 30% of total collections, were collected at Buck Dam than at Fosters Falls. However, Buck Dam was sampled only half as many times because access was dependent on moderate river flow. Based on the composition of walleye collected among the sites, we first collected walleye at the Buck Dam site when river flows allowed access because of the higher frequency of females and the higher frequency of New River-stock walleye. The percentage of New River-stock walleye among the collections was 90% at Buck Dam and only 53% at Fosters Falls. The Fosters Falls site was used when the access to the Buck Dam was limited or numbers collected were low.

Based on results from early collections, we recommended collecting more females, taking into account loss of eggs due to induction of spawning. Males could be collected readily and held until needed. In early collections, we attempted to spawn walleye onsite by stripping eggs and sperm, but attempts to collect adequate numbers of ripe females were unsuccessful. Later collections focused on taking walleye to the hatchery instead of attempting to spawn them onsite. This saved time and required the presence of fewer hatchery personnel in the field at collection times.

During the study, over 850 walleye were physically marked as described in the methods section, their genetic makeup analyzed, and over 100 females and 200 males were spawned. The genetic analysis was straightforward, with results produced and spawning recommendations made within 24 hours of receiving fin-clips. Hatchery personnel became familiar with inducing spawning and stripping the New River-stock walleye. Walleye production methods became routine after the first three years. The tags remain on the fish for several years, allowing them to be quickly identified in future years.

Management of the Sport Fishery

The walleye fishery is managed to restore the presumptive New River stock and provide recreational sport fishing. Although

there is evidence of some natural reproduction, it is not enough to sustain the fishery. Hence, in 2003, a 50.8-cm minimum size limit was placed upon harvest from Claytor Lake and the upper New River. Fishing pressure increased following stocking, stories in fishing magazines which publicized trophy catches (McCotter 2006, Ingram 2007), and new angler access.

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