Genetic Distinct Walleye Stocks in Claytor Lake and the Upper New River, Virginia

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Abstract: The increasing importance of the walleye (*Sander vitreus*) fishery in the New River, Virginia, and recent research findings motivated characterization of its genetic composition. Movements of radio-tagged fish suggested that walleyes living in Claytor Lake and the upper New River tend to spawn in different areas. In this study, allozyme, microsatellite DNA, and mitochondrial DNA genetic marker data were analyzed to assess population genetic differentiation among collections of New River walleye. The walleyes within Claytor Lake are a panmictic population, presumably resulting from years of stocking different genetic backgrounds and subsequent interbreeding. However, the genetic structure of walleyes from the New River shows the presence of more than one population. Fish in the New River system carry three previously unknown mitochondrial DNA haplotypes (43, 44, and 45), as well as high frequencies of characteristic alleles at particular microsatellite DNA loci. These observations may indicate a unique walleye stock that is native to the New River and which has remained spatially or temporally segregated by its spawning habits. The co-existence of different populations in the Claytor Lake / upper New River system justifies different management strategies. Management of the upper New River population should focus on conservation of the unique stock through marker-assisted selection of spawners with supplemental stocking of their offspring and/or strict harvest regulations.

Key words: Walleye, *Sander vitreus*, allozymes, mitochondrial DNA, microsatellites

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Walleye (*Sander vitreus*) is a highly valued sportfish (Colby et al. 1991) that inhabits the New River in southwestern 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. The New River is part of the Ohio River watershed, which is important because New River walleye may be ancestral to Ohio River stocks. Walleyes have been stocked outside of their native range to areas throughout the United States (Hackney and Holbrook 1978). Plantings from different geographic origins have 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).

Walleyes of different geographic origins have been mixed in Claytor Lake and the upper New River, Virginia, as a direct result of planting. The first known introduction was conducted in 1921 by the U.S. Fish Commission (Jenkins and Burkhead 1994). Following impoundment of Claytor Lake in 1939, the Virginia Department of Game and Inland Fisheries (VDGIF) planted over 2.2 million walleye from Lake Erie, Minnesota, and other unknown sources into Claytor Lake and the upper New River in 27 stocking

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events (Murphy et al. 1983, Palmer 1999). Genotype frequencies for isozyme markers verified mixed walleye stocks in Claytor Lake /upper New River, showing that at least some of these plantings were successful (Murphy et al. 1983). All stocking was suspended in 1997.

In the past decade, several 5- to 7- kg walleyes were collected by anglers or VDGIF in the upper New River above Claytor Lake. Movements of those 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). These observations sparked interest as to whether the river spawners may represent a distinct, native stock. The goal of this study was to characterize the population genetic structure of walleyes in Claytor Lake and

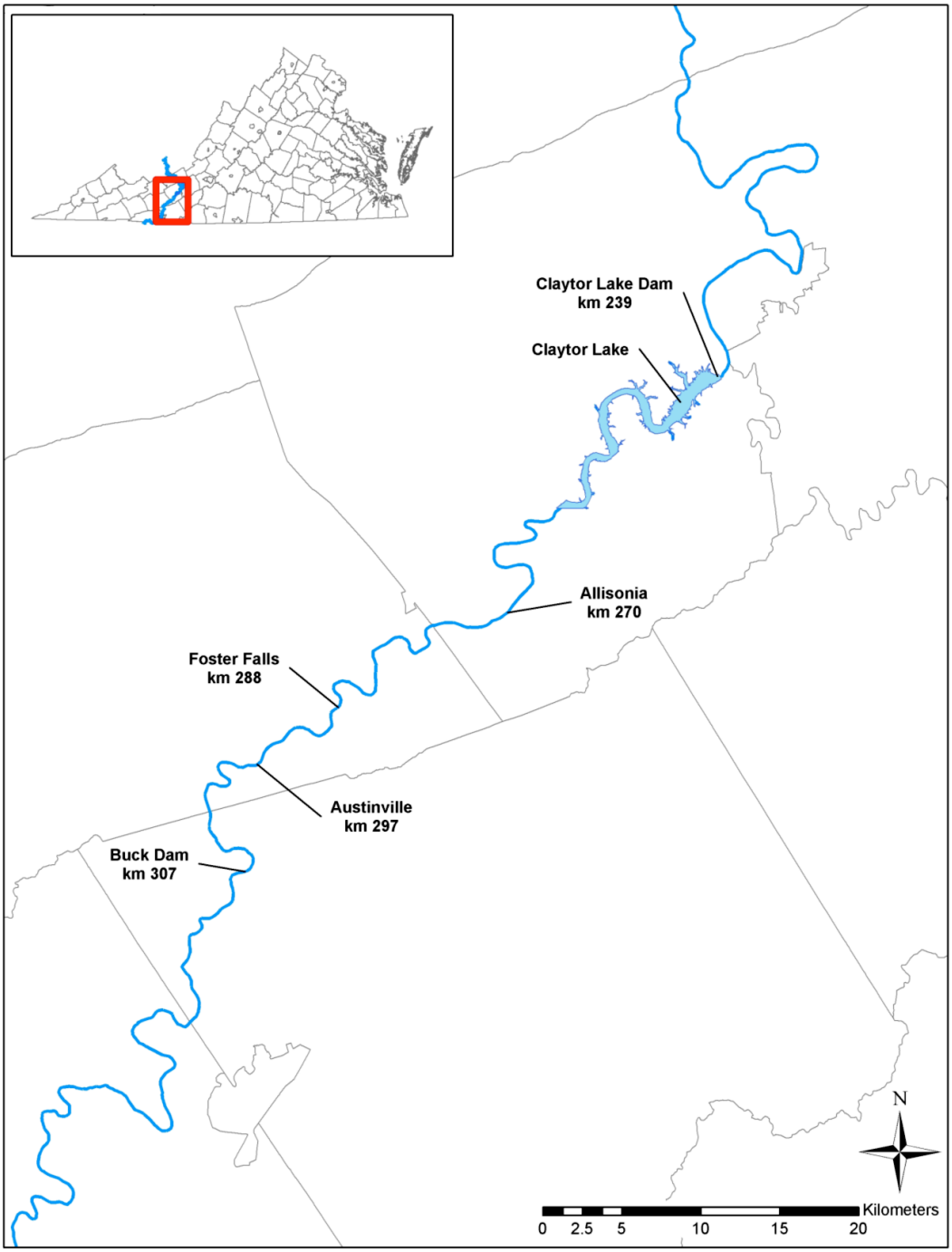

Figure 1. Study area on Claytor Lake and the upper New River, with key locations identified. Allisonia, Fosters Falls, and Buck Dam are spawning areas; Austinville is a migration corridor.

the upper New River by screening allozyme, microsatellite DNA, and mitochondrial DNA (mtDNA) genetic markers, testing the null hypothesis that a single panmictic stock of walleye lives and reproduces in the ecosystem. The intent of this study was to improve the understanding of walleye biology in the Claytor Lake / New River ecosystem, and thereby contribute to improved management of walleye fisheries.

Methods

The study area (Palmer et al. 2005) was a 68-km segment of the New River in Virginia, beginning at the spillway of Buck Dam in Carroll County and continuing downstream to Claytor Lake Dam in Pulaski County (Fig. 1). This section features 35 km of free-flowing river and 33 km of Claytor Lake at full pool.

Walleye were collected using gill nets in Claytor Lake and upstream river areas October-December 1997–1998 (N = 161). Collections were made by electrofishing in February-March 1997–1999 at spawning locations (Allisonia, Fosters Falls, and Buck Dam; N = 216), and also at Austinville (N = 35), a migration corridor for spawners at Buck Dam.

We surveyed variation at the malate dehydrogenase (*sMDHP-B**), isocitrate dehydrogenase (*IDHP-A**) and muscle myoglobin (*MYO-A**) loci (Murphy et al. 1983, Terre 1985, Murphy 1990). Data for: (1) the entire study area, (2) lake versus river collections, and (3) the three different spawning sites were tested against the null hypothesis that genotype frequencies conformed to Hardy-Weinberg equilibrium expectations using Bonferroni corrections for multiple tests.

Microsatellite DNA variability was examined using polymerase chain reaction (PCR) at six microsatellite loci (Borer et al. 1999). Deviations from Hardy-Weinberg equilibrium were assessed (Levene 1949, Guo and Thompson 1992) and F_{ST} and G_{ST} analyses (Weir and Cockerham 1984, Michalakis and Excoffier 1991) and analysis of molecular variance (Excoffier et al. 1992) were conducted using Arlequin (Schneider et al. 1997). Heterogeneity χ^2 values were calculated using Chifish (Ryman 2006); P values for Fisher's exact test were obtained using 100 batches with 5,000 iterations per batch. R_{ST} (Slatkin 1995) was calculated using Rstcalc (Goodman 1996). Pair-wise genetic distances using kinship coefficient (Dkf) and proportion-of-shared-alleles (Dps) (Bowcock et al. 1994) metrics were estimated using Microsat (Minch et al. 1995). Gene tree diagrams for distance metrics were developed using the *neighbor* function of Phylip (version 3.5c, Felsenstein 1993) and drawn using Treeview (Page 1998).

Samples of liver from 95 walleyes collected during two different spawning seasons 1998–1999 (approximately 31 samples from each of three spawning sites) were analyzed for mtDNA variation by isolation of mitochondrial DNA and restriction site analysis (Billington and Hebert 1988, 1990). The mtDNA data were analyzed in terms of haplotype frequencies and genetic distances. The χ^2 metric was used to test the null hypothesis that haplotype frequencies were independent of spawning site.

Results

Allozymes

Allozyme frequencies at three polymorphic loci were determined for 384 walleyes (Table 1). Genotype frequencies considered across the entire study area exhibited significant departure ($P < 0.001$) at all loci from frequencies expected under Hardy-Weinberg equilibrium, suggesting presence of more than one genetic stock. Genotypes at the *sMDHP-B** locus in the lake and Allisonia collections showed significant departure ($P < 0.001$) from Hardy-Weinberg equilibrium. Genotype frequencies in the river collections showed significant departures from Hardy-Weinberg equilibrium at all three loci ($P < 0.001$). Comparing data among spawning sites, genotype frequencies at the *sMDHP-B** locus in the Allisonia collection did not fit Hardy-Weinberg equilibrium ($P < 0.001$). At Fosters Falls, genotype frequencies at two loci did not fit Hardy-Weinberg expectation (*IDHP-A**, $P = 0.01$; *sMDHP-B**, $P < 0.001$). In the Buck Dam collection, genotype frequencies at all three loci did not fit Hardy-Weinberg equilibrium (*IDHP-A**, $P = 0.003$; *MDHP-B**, $P < 0.001$; *MYO-A**, $P = 0.001$). These findings collectively suggest admixture of stocks at Claytor Lake and Allisonia and lesser degrees of admixture in New River collections farther upstream.

Microsatellite DNA

Microsatellite DNA variation among 244 individuals included 60 alleles over six loci (Table 2). The number of alleles ranged from 5 to 15 per locus. Analysis of molecular variance partitioned 91.9% of variation within and 8.1% between collection sites. Most locus-by-collection tests showed significant departures of genotype frequencies from Hardy-Weinberg expectations which is not unusual for microsatellite-based surveys of populations. Heterogeneity χ^2 values were highly significant ($P < 0.001$) at each locus and across all loci, showing differentiation among collections. F_{ST} values ranged from 0.018 between the Allisonia and Claytor Lake to 0.152 between the Allisonia and Buck Dam collections. R_{ST} values ranged from 0.091 between the Allisonia and Claytor Lake to 0.435 between the Claytor Lake and Buck Dam collections. The topology of branching patterns for gene-tree associations among the collections (Fig. 2) was the same for all distance metrics. Bootstrap values of 70 or greater were considered significant (Hillis and Bull 1993).

Table 1. Sampling seasons, collection site, sample size (*N*), and allele frequencies for protein marker systems. Alleles designated 1 have slowest electrophoretic mobility, while those designated 2 and 3 have progressively more rapid mobilities.

Season / Site	IDHP-A* (1.1.1.42)	sMDHP-B* (1.1.1.37)	MYO-A*
Spring 1997			
Allisonia	1 = 0.481	1 = 0.058	1 = 0.365
N = 26	2 = 0.519	2 = 0.827	2 = 0.635
		3 = 0.115	
Buck Dam	1 = 0.231	1 = 0.154	1 = 0.846
N = 13	2 = 0.769	2 = 0.557	2 = 0.154
		3 = 0.269	
Austinville	1 = 0.764	1 = 0.000	1 = 0.794
N = 17	2 = 0.235	2 = 0.529	2 = 0.206
		3 = 0.471	
Fall 1997			
Claytor Lake	1 = 0.476	1 = 0.065	1 = 0.468
N = 65	2 = 0.524	2 = 0.701	2 = 0.532
		3 = 0.234	
Spring 1998			
Allisonia	1 = 0.393	1 = 0.107	1 = 0.464
N = 14	2 = 0.607	2 = 0.786	2 = 0.536
		3 = 0.107	
Fosters Falls	1 = 0.270	1 = 0.176	1 = 0.730
N = 37	2 = 0.730	2 = 0.338	2 = 0.270
		3 = 0.486	
Buck Dam	1 = 0.417	1 = 0.083	1 = 0.889
N = 18	2 = 0.583	2 = 0.389	2 = 0.111
		3=0.528	
Austinville	1 = 0.278	1 = 0.056	1 = 0.889
N = 18	2 = 0.722	2 = 0.638	2 = 0.111
		3 = 0.306	
Fall 1998			
Claytor Lake	1 = 0.457	1 = 0.005	1 = 0.378
N = 96	2 = 0.543	2 = 0.798	2 = 0.622
		3 = 0.197	
Spring 1999			
Allisonia	1 = 0.600	1 = 0.000	1 = 0.400
N = 25	2 = 0.400	2 = 0.680	2 = 0.600
		3 = 0.320	
Fosters Falls	1 = 0.288	1 = 0.000	1 = 0.692
N = 26	2 = 0.712	2 = 0.423	2 = 0.308
		3 = 0.577	
Buck Dam	1 = 0.097	1 = 0.000	1 = 0.778
N = 29	2 = 0.903	2 = 0.111	2 = 0.222
		3 = 0.889	

Table 2. Allele frequencies at six microsatellite loci among walleye from four collection sites in Claytor Lake (CL, *N* = 68) and the upper New River; Allisonia (AL, *N* = 57), Fosters Falls (FL, *N* = 60), Buck Dam (BD, *N* = 59).

Locus	CL	AL	FF	BD
Svi4*				
100	0.000	0.054	0.025	0.000
104	0.008	0.205	0.042	0.000
106	0.090	0.143	0.085	0.059
108	0.226	0.152	0.449	0.525
110	0.323	0.304	0.229	0.195
112	0.218	0.063	0.110	0.178
114	0.113	0.071	0.051	0.042
116	0.230	0.009	0.008	0.000
Svi6*				
130	0.019	0.011	0.020	0.000
136	0.142	0.011	0.040	0.009
138	0.038	0.000	0.000	0.000
140	0.000	0.034	0.188	0.027
142	0.387	0.517	0.188	0.063
144	0.094	0.126	0.059	0.009
146	0.038	0.023	0.010	0.009
148	0.094	0.138	0.059	0.027
150	0.066	0.057	0.069	0.107
152	0.019	0.011	0.000	0.000
154	0.047	0.011	0.287	0.018
158	0.028	0.034	0.040	0.652
161	0.019	0.000	0.010	0.071
163	0.000	0.000	0.030	0.009
165	0.009	0.023	0.000	0.000
Svi17*				
95	0.052	0.000	0.000	0.000
99	0.015	0.018	0.271	0.276
101	0.022	0.036	0.220	0.578
103	0.336	0.254	0.314	0.103
105	0.164	0.154	0.034	0.026
107	0.000	0.045	0.008	0.000
109	0.187	0.245	0.119	0.009
111	0.224	0.172	0.017	0.009
113	0.000	0.082	0.017	0.000

Locus	CL	AL	FF	BD
Svi18*				
118	0.331	0.289	0.183	0.146
120	0.169	0.070	0.258	0.345
122	0.269	0.307	0.267	0.190
124	0.231	0.298	0.167	0.078
126	0.031	0.035	0.125	0.258
Svi26*				
152	0.126	0.118	0.035	0.105
154	0.067	0.165	0.103	0.211
156	0.025	0.129	0.086	0.009
158	0.101	0.047	0.241	0.553
160	0168	0.166	0.164	0.070
162	0.025	0.035	0.129	0.000
165	0.176	0.152	0.086	0.009
167	0.118	0.141	0.026	0.018
169	0.058	0.024	0.069	0.018
171	0.042	0.000	0.026	0.009
181	0.034	0.000	0.000	0.000
183	0.017	0.012	0.017	0.000
185	0.025	0.012	0.017	0.000
Svi33*				
78	0.000	0.045	0.203	0.381
84	0.026	0.055	0.093	0.186
86	0.133	0.282	0.356	0.314
90	0.047	0.188	0.027	0.017
92	0.063	0.027	0.008	0.000
96	0.141	0.018	0.034	0.034
98	0.242	0.164	0.153	0.034
100	0.195	0.200	0.076	0.017
102	0.031	0.009	0.000	0.008
104	0.133	0.082	0.051	0.008

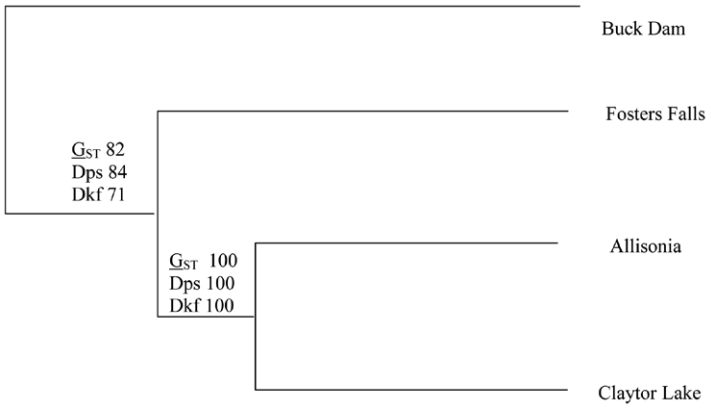

Figure 2. Topologies of population trees for walleye in Claytor Lake and the upper New River. Numbers following the different distance measures are the boot-strap values for the particular distance measure.

Mitochondrial DNA

Six haplotypes were identified among 84 walleyes characterized (Table 3). Three of the haplotypes (1, 4, and 10) have been described previously in other populations (Billington et al. 1992). The remaining three haplotypes (43, 44, and 45) had not been described prior to this study (Palmer 1999). Observation of unique genetic material supports the hypothesis that there is a unique genetic stock of walleye in this system. Haplotype 43 was the most frequently observed haplotype among spawners collected at the Fosters Falls in 1998 (90%), Buck Dam in 1998 (72%), and Buck Dam in 1999 (82%). Haplotype 4 was the most frequently observed at Fosters Falls in 1999 (41%). All six haplotypes were found among spawners at the Allisonia site (Table 3). Departure of haplotype frequencies from random distribution among spawning sites was highly significant ($\chi^2 = 35.7$, df = 2, and $P < 0.0001$) in both years combined.

The 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 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.

Table 3. Mitochondrial DNA haplotypes frequencies observed among walleye from three spawning sites in upper New River, Virginia: Allisonia (AL), Fosters Falls (FF,) and Buck Dam (BD).

Site/Year	Sample Size	Haplotype 1	Haplotype 4	Haplotype 10	Haplotype 43	Haplotype 44	Haplotype 45
AL 1998	10	7%	43%	7%	7%	29%	7%
FF 1998	10				90%	10%	
BD 1998	14		14%		72%	14%	
AL 1999	16	13%	74%	13%			
FF 1999	17	6%	41%	12%	35%	6%	
BD 1999	17		18%		82%		

Discussion

Differentiation of Claytor Lake and Upper New River Walleye Stocks

Analysis of data from Claytor Lake and Allisonia supported the hypothesis of one panmictic walleye population. This finding is not surprising, given that Allisonia is the interface between Claytor Lake and the upper New River and was the primary spawning site for Claytor Lake walleye. The Allisonia spawning collection showed all six mtDNA haplotypes observed in the study. This may be the result of mixed walleye plantings in the past plus the possible presence of fish bearing native haplotypes.

Allozyme and microsatellite 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. The mtDNA results showed three previously unknown haplotypes, one at high frequencies, in collections from the New River. The New River population includes a high proportion of genetically unique, putatively native walleyes.

The concordance of nuclear allele frequencies with mitochondrial DNA haplotype frequencies supports the alternative hypothesis that distinct walleye stocks coexist in the Claytor Lake / upper New River ecosystem. Future investigation of the unique walleye stock with newly developed microsatellite DNA primer pairs (Eldridge et al. 2002) might reveal loci that increase the concordance of mtDNA with nuclear DNA markers.

Natural History of New River Walleyes

The New River, formerly the Teays River, flowed directly into the Mississippi River until the advance of Wisconsian-period glaciers buried the lower two-thirds of its course (Jenkins and Burkhead 1994). The southeastern, 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 remained separated from other stocks, thereby preserving any genetically unique characteristics. Spawning habits may temporally, spatially, or behaviorally separate this stock from introduced stocks (Murphy 1981, Murphy et al. 1983).

The genetically unique stock of walleyes was characterized by high frequencies of previously unknown mtDNA haplotypes 43, 44, and 45. Mitochondrial DNA haplotype 43 is highly divergent from known haplotypes, differing at six restriction sites, and is most closely related to a haplotype 38 that is known from the Rockcastle River (Ohio River drainage) in Kentucky (Billington and Sloss 1998). Haplotypes 44 and 45 differ from widespread haplotypes by one restriction site. Mitochondrial DNA haplotypes of New River walleye are similar to those found in Ohio River stocks (M. White, personal communication; White et al. 2005). However, microsatellite allele frequencies from upper New River walleyes were very different from those of walleyes collected in Minnesota (Eldridge et al. 2002). Haplotype 43 may be characteristic of an ancestral strain that existed in the upper Teays River before the last Ice Age (Wisconsonian) ended approximately 10,000 years ago (Cooney et al. 1990, Pielou 1991). New River walleye may be ancestral to Ohio River stocks, may have been founded by plantings of Ohio River stocks, or both stocks may be remnants of a once-widespread form. Further investigation of these hypotheses by examination of other regional populations and by calibration of a molecular clock for the species is warranted.

Management Implications

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). Our study shows that the putative native stock has persisted despite decades of planting non-native stocks, suggesting an adaptive basis to its persistence.

The management of walleye in Claytor Lake and the upper New River should recognize the existence of a distinct, presumptively local stock that grows to large ultimate size. Newly analyzed genetic data from collections of walleye made during spring spawning runs at Fosters Falls and Buck Dam indicated that the majority of large female walleye (4 kg and larger) collected were part of the local New River stock. Eggs of New River stock walleye were two to three times larger than eggs collected from northern walleye stocks (VDGIF, unpublished data). We suggest that only the unique genetic stock found in the New River should be stocked. Stocking exclusively the river stock and restricting their harvest could demographically boost the upper New River walleye stock, thereby heightening frequencies of native genotypes.

Hatchery-based enhancements of the unique, putatively native walleye stock will depend on genetic marker-based identification of prospective broodstock. We recommend that walleye be collected from the Fosters Falls and Buck Dam spawning sites during the peak spawning run. Following physical tagging, walleye can be held overnight in pens while genetic analysis of each fish is carried out. Rapid screening of microsatellite DNA locus *Svi17** would reveal walleye bearing only the **99* or **101* bp alleles, and of the *Svi-33** locus bearing only the **78* allele. Only walleye with the diagnostic genetic markers should be used as broodfish for hatchery production. The upper New River and Claytor Lake then could be stocked with the offspring of the unique local walleye. As many native walleye as practical need to be mated so that stocking of a limited number of genotypes does not actually reduce the genetically effective population size of the targeted stock (Ryman and Laikre 1991).

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