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Genetic Analysis of Tennessee Sport Fisheries (2000 – 2003): Largemouth Bass, Smallmouth Bass, Rainbow Trout, & Brown Trout

Final Segment Report 2000-2003

EpiGenetics

Epifanio & Associates Living Resource Consulting, Inc.
"Specializing in Resource Genetic Analysis & Planning"

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[In Cooperation with
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Submitted to

Tennessee Wildlife Resources Agency
Ellington Agricultural Center
PO BOX 40747
Nashville, TN 37204

December 2003

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Final TWRA Report (03-26) Submitted to
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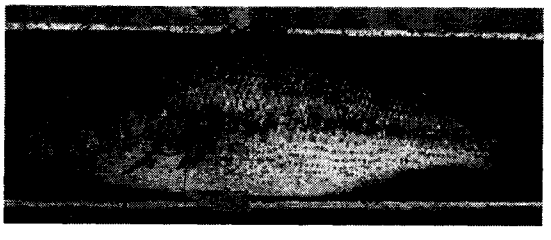


Photographs courtesy of Jim Negus

Introduction

We conducted allozyme-based genetic analyses of several important sport fisheries for the Tennessee Wildlife Resources Agency. The overall objective of the project was to describe the background genetic architecture of the *Micropterus* species and to examine the potential hatchery sources of trout (both rainbow and brown trout) to feral populations. More specific service objectives for each species included: 1) assessing the extent of the persistence of and introgression of stocked Florida bass (*Micropterus floridanus*) genes into the state's largemouth bass (*M. salmoides*) populations; 2) assessing the background variation of smallmouth bass (*M. dolomieui*) statewide and assessing introgression with other *Micropterus* species; 3) assessing the variation in the 8 "strains" of rainbow trout used for stocking state waters and a wild population occurring in Clear Creek; and, 4) assessing variation in the strain of brown trout used for stocking state waters and wild populations occurring in the S. Holston and Watauga Rivers. The analyses reported herein satisfy five specific Service Objectives requested by TWRA for Contract RFS 32801-008.

Objective 1.0: Impact of Florida (subspecies) Largemouth Bass Stocking

A. Background – Beginning in summer/fall of 2000, largemouth bass (LMB) were analyzed from two reservoirs and one state lake. Samples were subjected to protein electrophoretic analysis for the purpose of assessing the persistence of stocked fish and the reproductive impact of Florida bass (FLB). The purpose of the transfer of populations from the Florida species (formerly classified as a subspecies of *M. salmoides*; Kassler et al. 2002) to watersheds harboring primarily "intergrade" largemouth bass populations was to establish trophy Florida bass fisheries in Tennessee reservoirs on top of the native species. Such transfers may present an ecological and genetic challenge to local, native populations. It is the potential for the latter and evaluation of the success of the stocking program that motivated this and earlier reports (e.g., O'Bara et al. 1993).

B. Methods – Approximately 20-30 individual adult-size largemouth bass were collected from three localities in spring of 2000, 2001, & 2002 from the state's primary watershed (Kentucky Lake [Harmon Creek] and in the lower reaches of the Tennessee River watershed and Chickamauga Lake in the upper reaches of this watershed), and Lake Graham, a relatively small state-owned lake in the Mississippi River watershed. The samples were stored frozen and forwarded to the contractor. Samples were subjected to protein electrophoresis and histochemical staining for the presence of diagnostic alleles or variation at four enzyme loci. In largemouth bass, two of the loci are known to be diagnostic for northern LMB and FLB (designated *sAat-2** and *sIdhp-2**). Two other loci were included as a means to gather additional data, although these are only "partially diagnostic" (designated *sMdh-B** and *sSod-1**).

C. Data Analysis & Results – Allelic and genotypic scores for each individual assayed over all four loci were compiled and are reported in Appendices 1 and 2. By convention, a genotype at a locus is reported as two asterisked numbers signifying its diploid genotype (e.g., **1/*1* or **1/*2*). For each individual, a composite genotype over the four loci was used as rough identifier of individual genetic identity (to subspecies origin). In particular, any individual with any of the following genotypes would be a purported FLB (Table 1.1).

Table 1.1. Genotypes typical of Florida bass.

*sAat-2**	*sIdhp-2**	*sMdh-B**	*sSod-1**
**3/*3*	**3/*3*	**2/*2*	**1/*1*
**3/*4*	**3/*3*	**2/*2*	**1/*1*
**4/*4*	**3/*3*	**2/*2*	**1/*1*
**3/*3*	**3/*3*	**2/*2*	**1/*2*
**3/*4*	**3/*3*	**2/*2*	**1/*2*
**4/*4*	**3/*3*	**2/*2*	**1/*2*

Data were also summarized in terms of percentages of "FLB" alleles as a way of examining the incorporation (as a minimum %) of Florida alleles into the population

(Table 1.2). For comparison, included in the Appendices is a summary of frequency data and percent Florida bass alleles for the seven samples assayed and placed in comparison with data from surrounding populations analyzed in an earlier study by Philipp et al. 1982.

The presence of Florida bass alleles was not unexpected given that much of the Tennessee River lies within the suspected & previously described "intergrade" zone - a broad transitional zone of trait sharing between the Florida and largemouth species.

Harmon Creek – Stocking records (TWRA unpublished) indicate that Florida bass were stocked as fingerlings into the Harmon Creek of Kentucky Lake from 1998 to 2001. The two annual samples from Harmon Creek display widely divergent allele (and genotype) frequencies for diagnostic allozyme loci. Specifically, the year 2000 sample displays frequencies of Florida bass alleles at what might be considered more natural background levels. In support of this supposition, there are only minor differences between the frequencies observed for Harmon Creek (2000) and Guntersville Lake (AL; from Philipp et al. 1982). The year 2001 sample, however, displays a sizable increase in the presence of Florida bass alleles indicating that for the first time, Florida bass were recruited into the sampled size classes. Whether these fish will either persist, reproduce as a distinct part of fishery, or hybridize with the native species (and to what extent) is unknown at the present time.

Chickamauga Lake – Stocking records (TWRA unpublished) indicated that Florida bass were stocked as fingerlings to various locations in Chickamauga Lake in 2002 and 2001. The three annual samples from various locations in the lake display annual variation in Florida bass alleles present. As this lake occurs in the headwaters of the Tennessee River (Hiawassee) system, we anticipated the occurrence of Florida alleles. In 1981, Philipp et al. (1982) observed a high percentage of Florida bass alleles in Blue Ridge Lake (located in the upper reaches of the Hiawassee River system).

The differences in frequencies between the Chickamauga Lake sample (2000-02) are

substantial. Both of these waters are in the Hiawassee River basin. It is interesting to note that the Blue Ridge population has a much greater occurrence of the Florida bass alleles than does Chickamauga Lake. Whether this is a result of "sampling" or whether it is due to a biogeographic difference over this relatively short distance may require future exploration. Regardless, from 2000 to 2002 the percentage of Florida alleles in the sampled populations appears to be decreasing. This suggests that the Florida bass are not persisting in the lake.

Lake Graham – stocking records (TWRA unpublished) indicate that Florida bass were stocked as fingerlings into the Harmon Creek of Kentucky Lake from 1998 to 2000. The two annual samples from Lake Graham display substantial frequencies of Florida alleles, although the 2002 sample frequencies indicate a frequency decrease at both diagnostic loci. No comparable sample from Philipp et al. (1982) permits an estimate of background expectations of Florida bass alleles and whether this population is located within the "intergrade" zone. Given its location, however, we would anticipate a relatively low contribution of Florida bass alleles even if it resides within the intergrade zone. Thus, the high percentage of Florida alleles is attributed to Florida bass stocking.

Table 1.2. Percentage of Florida bass alleles in the multi-year samples of bass collected from three localities in 2000-2002.

Locality	**Year**	**Sample Size**	**% Florida Bass (FLB) alleles in sample**
Harmon Creek	2000	20	0.0% (*Aat-2**); 2.5% (*Idhp-2**)
	2001	28	39.3% (*Aat-2**); 39.3% (*Idhp-2**)
Chickamauga Reservoir	2000	30	3.3% (*Aat-2**); 13.3% (*Idhp-2**)
	2001	67	4.4% (*Aat-2**); 6.7% (*Idhp-2**)
	2002	60	0.8% (*Aat-2**); 5.8% (*Idhp-2**)
Lake Graham	2001	28	37.5% (*Aat-2**); 35.7% (*Idhp-2**)
	2002	60	16.7% (*Aat-2**); 25.8% (*Idhp-2**)

2.0: Taxonomic Certification of State Angling Records

No work was conducted under this objective.

Objective 3.0: Occurrence and Proportion of Natural Interspecific Hybrids

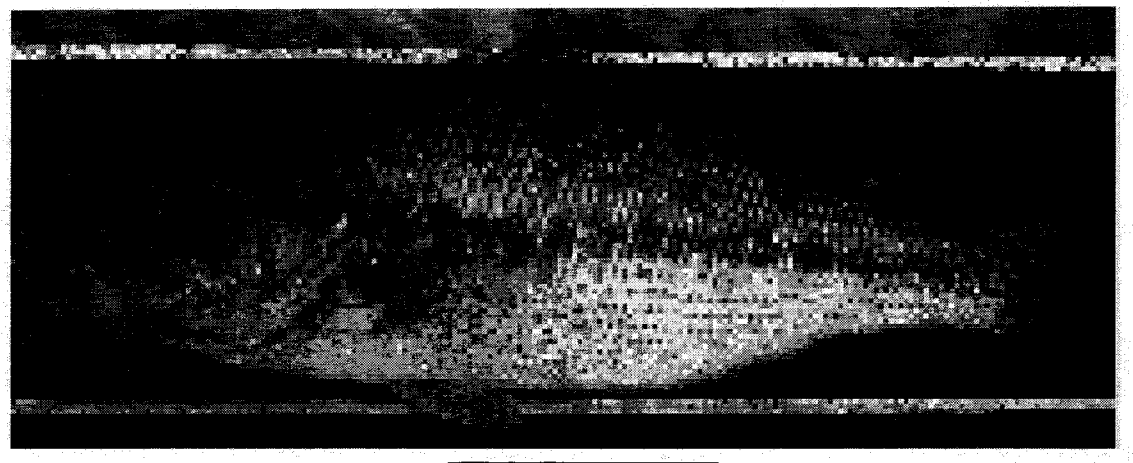
A. Background – The purpose of this Service Objective is to establish a baseline for the occurrence and proportion of pure and hybrid, and backcrossed fish populations. Similar to the investigation of LMB and FLB, these analyses aimed primarily to identify where and what level interspecific hybridization is occurring between smallmouth bass (SMB) and spotted bass (SPB). These analyses were conducted as part of the broader assessment of SMB stock variation described in Service Objective 4.0.

B. Methods – In addition to those described above in general for LMB, our analyses of SMB were somewhat broader because no comprehensive background information about variation in Tennessee populations has been published or otherwise reported. Therefore, in addition to a basic screen for variation at a standard suite of allozyme loci, we included several that are diagnostic for other species as well. The table below contains information about samples collected and assayed to date. In smallmouth bass, there are no known diagnostic alleles among the northern and Neosho subspecies, but several loci are diagnostic between smallmouth and spotted bass (*sMdh-B**, Pgm-*1**, and *sIdhp-2**) the most likely species with which interspecific hybridization might occur (Table 3.1). Samples were subjected to protein electrophoresis and histochemical staining for variation for three loci known to be diagnostic between smallmouth bass and spotted bass.

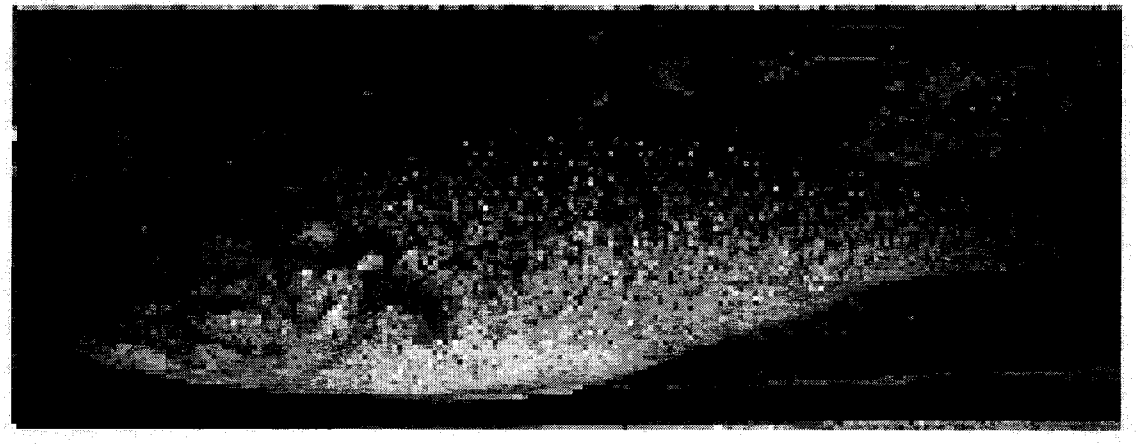
Table 3.1. Diagnostic loci assayed for the presence of hybrids in Tennessee SMB populations.

Locus Assayed	SMB Alleles	SPB Alleles
sMdh-B*	****3, *4***	****2***
Pgm-1*	****1***	****2***
sIdhp-2*	****3, *4***	****2***

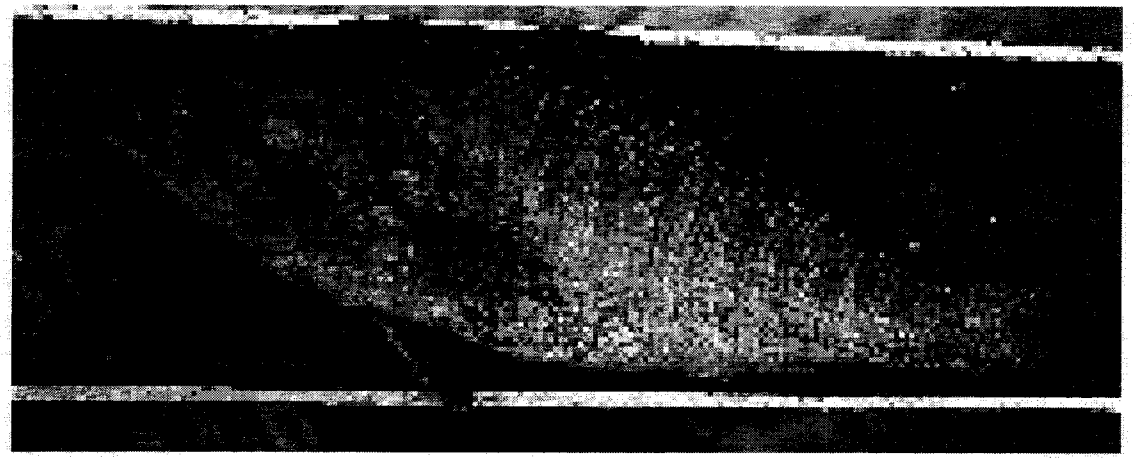
Photographs courtesy of Jim Negus



Spotted Bass



"Hybrid" Bass



Smallmouth Bass

C. Data Analysis & Results – Smallmouth bass are known to hybridize with spotted bass where they co-occur. Such interspecific hybridization – often following introduction of one species on top of another other has been documented for a number of *Micropterus* species pairs throughout the U.S. (e.g., Whitmore 1983; Morizot et al 1991; Pierce and Van den Avyle 1997). Relating to his observations with several Centrarchid species, Hubbs (1955) suggested that an increase in hybridization is expected following an ecological disturbance within a system. Broadly gathered empirical data from either "pristine" or disturbed systems is rather scant. Yet, there remains a general assumption that interspecific hybridization will be 1) rare, 2) transient, and 3) rarely proceed beyond the first generation (F1's).

Purported hybridization among *Micropterus* species has been observed in Tennessee waters (T. Churchill, TWRA, personal communication). In examining SMB samples from 15 locations throughout the state of Tennessee, seven of these contained putative hybrids (ranging from a single occurrence to a quarter of the individuals sampled in Pickwick Reservoir; Table 3.2). It is ultimately unknown whether these percentages reflect historical (or natural) levels of hybridization or whether they reflect some ecological stressor suggested by Hubbs (1955). The source of this hybridization may warrant further exploration, to determine whether this is a recent versus an historical phenomenon.

Table 3.2. Percentages of F1 or Fx hybrids in 15 SMB population samples analyzed in Tennessee watersheds.

Smallmouth bass v. SMB x SPB "hybrids" Locality	**n**	**% F1 / Fx Hybrids %**
Powell River	n=30	3.3%
East Fork Stones River	n=30	0.0%
Collins River	n=30	0.0%
Elk River	n=30	0.0%
Holston River	n=30	0.0%
Clinch River	n=30	0.0%
Pigeon River	n=30	10.0%
Duck River	n=30	16.6%
Norris Reservoir	n=23	0.0%
Boone Reservoir	n=19	0.0%
South Holston Reservoir	n=30	3.3%
Watauga Reservoir	n=45	4.4%
Lower Watts Bar Reservoir	n=30	0.0%
White Creek	n=30	3.3%
Pickwick Reservoir	n=28	25.0%
Cumberland River	n=NA	% = NA

Objective 4.0: Stock identification and characterization of Tennessee smallmouth bass populations.

A. Background – Smallmouth bass (SMB) were collected during spring 2002 to acquire an initial survey of statewide variation in this species (Figure 4.1). Historical accounts for smallmouth bass indicate that only a single subspecies occurs within the state's watersheds. Given the recreational angling and overall management interest for this species, the purpose of this Service Objective is to provide a general description of the population genetic relationships of SMB populations throughout the state.

An additional need for this work was to determine whether or not it is safe (in a local stock performance and preservation sense) to stock bass from one drainage into another. Moreover, at what level of watershed likely constitutes a "genetic management unit?"

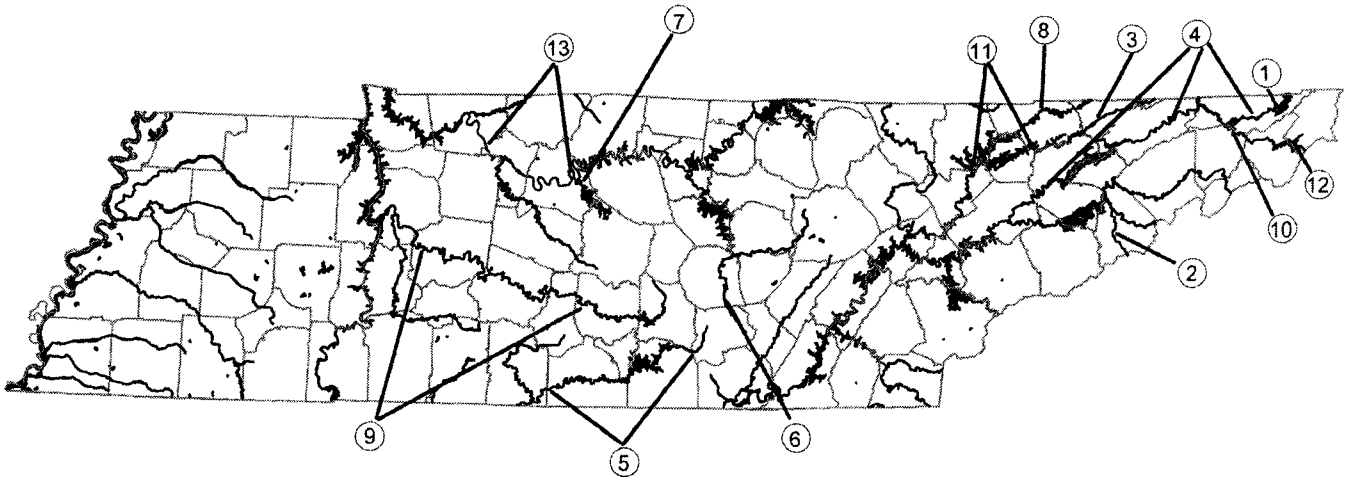


Figure 4.1. Distribution of smallmouth bass sample sites.

B. Methods – The analyses of SMB were somewhat broader than that undertaken for the LMB because no comprehensive background information about variation in Tennessee populations has been published or otherwise reported. Therefore, in addition to a basic screen for variation at a standard suite of allozyme loci, we included several that are diagnostic for other species as well. The table below contains information about samples collected and assayed to date. Samples were subjected to protein electrophoresis and histochemical staining for variation at nine loci for smallmouth bass (Table 4.1).

Table 4.1. Enzyme and loci examined for background variation in smallmouth bass. Six loci exhibited variation (more than a single "fixed" allele).

Locus Assayed	SMB Alleles
sMdh-B*	***3, *4***
Pgm-1*	***1***
sIdhp-2*	***3, *4***
Ck-A*/C*	***0, *1***
Ck-A*/C*	***1***
Gpi-A*	***1***
Gpi-B*	***1***
Ldh-C*	***1, *2***
sSod-1*	***1, *2***
sAat-2*	***2, *3, *4***
G3pdh-1*	***1***

C. Data Analysis & Results – Although the analyses uncovered a modest level of allelic variation for six of the loci assayed for smallmouth, SMB from Tennessee waters are not especially variable in terms of inter-population variation (Table 4.2). This result is consistent with the past evidence (albeit, this has been limited as no range-wide examination of the species has been published) of invariability for this species.

Table 4.2. Allelic frequencies for 15 Tennessee smallmouth population samples over nine loci. Putative hybrids from each population sample are not included in these summaries.

	Population Sample *								
Locus / Allele	PIK	WHC	WTB	WAT	SHL	BOO	NOR	DUK	PIG
*sMdh-B**									
(N)	21	29	30	43	28	19	23	25	27
**3*	1.000	1.000	1.000	1.000	1.000	1.000	0.913	1.000	0.963
**4*	0.000	0.000	0.000	0.000	0.000	0.000	0.087	0.000	0.037
*Pgm-1**									
(N)	21	29	30	43	28	19	23	25	27
**1*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*Gpd-1**									
(N)	21	29	30	43	28	19	23	25	27
**1*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ck-C*									
(N)	21	29	30	43	28	19	23	25	27
**1*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000
**2*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.980	1.000
*Gpi-A**									
(N)	21	29	30	43	28	19	23	25	27
**1*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

	Population Sample *								
Locus / Allele	PIK	WHC	WTB	WAT	SHL	BOO	NOR	DUK	PIG
*Gpi-B**									
(N)	21	29	30	43	28	19	23	25	27
*1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*Ldh-C**									
(N)	21	29	30	43	28	19	23	25	27
*1	0.714	0.534	0.741	0.791	0.714	0.684	0.674	0.640	0.759
*2	0.286	0.466	0.259	0.209	0.286	0.316	0.326	0.360	0.241
*sSod-1**									
(N)	21	29	29	43	28	19	23	25	27
*1	0.714	0.793	0.724	0.733	0.839	0.895	0.739	0.640	0.759
*2	0.286	0.207	0.276	0.267	0.161	0.105	0.261	0.360	0.241
*sAat-2**									
(N)	21	29	30	43	28	19	23	25	27
*2	0.000	0.000	0.000	0.012	0.000	0.000	0.043	0.000	0.000
*3	1.000	1.000	1.000	0.988	1.000	1.000	0.957	1.000	1.000
*sIdhp-2**									
(N)	21	29	30	43	28	19	23	25	27
*3	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Locus /Allele	Population					
	CLI	HOL	ELK	COL	STO	POW
*sMdh-B**						
(N)	30	30	30	30	30	29
*3	1.000	1.000	0.983	1.000	1.000	0.966
*4	0.000	0.000	0.017	0.000	0.000	0.034
*Pgm-1**						
(N)	30	30	30	30	30	29
*1	1.000	1.000	1.000	1.000	1.000	1.000
*Gpd-1**						
(N)	30	30	30	30	30	29
*1	1.000	1.000	1.000	1.000	1.000	1.000
*Ck-C**						
(N)	30	30	30	30	30	29
*1	0.000	0.000	0.033	0.000	0.000	0.000
*2	1.000	1.000	0.967	1.000	1.000	1.000
*Gpi-A**						
(N)	30	30	30	30	30	29
*1	1.000	1.000	1.000	1.000	1.000	1.000
*Gpi-B**						
(N)	30	30	30	30	30	29
*1	1.000	1.000	1.000	1.000	1.000	1.000
*Ldh-C**						
(N)	30	30	30	30	30	29
*1	0.583	0.633	0.717	0.600	0.800	0.638
*2	0.417	0.367	0.283	0.400	0.200	0.362
*sSod-1**						
(N)	30	30	30	30	30	29
*1	0.817	0.700	0.617	0.783	0.700	0.776
*2	0.183	0.300	0.383	0.217	0.300	0.224

*Aat-2**						
(N)	30	30	30	30	30	29
*3	0.983	1.000	1.000	1.000	1.000	1.000
*4	0.017	0.000	0.000	0.000	0.000	0.000
*sIdhp-2**						
(N)	30	30	30	30	30	29
*3	1.000	1.000	0.950	1.000	0.950	1.000
*4	0.000	0.000	0.050	0.000	0.050	0.000

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* **Key to abbreviations used in table** (note: summaries do not include putative hybrids from each sample)

PIK = Pickwick L.; WHC = White Creek; WTB = Lower Watts Barr; WAT = Watauga L.; SHL = South Holston L.; BOO = Boone L.; NOR = Norris L.; DUK = Duck River; PIG = Pigeon River; CLI = Clinch River; HOL = Holston River; ELK = Elk River; COL = Collins River; STO = Stones River; POW = Powell River.

Objective 5.0: Evaluation of genetic diversity in broodstock
Sub-Objective 5.1: Genetics of rainbow trout.

A. Background – In the mid to late 1990s a wild (or feral) rainbow trout fishery developed in Clear Creek. However, this wild population also is supplemented with individuals stocked from the state's hatchery system. Specifically, Clear Creek flows into the Clinch River as a tributary. While the Clinch River receives released propagated fish, Clear Creek does not. Tennessee currently rears eight strains of rainbow trout for stocking into state waters. The purpose of this Service Sub-Objective is to establish which of the eight strains has contributed to any significant level to the feral population in Clear Creek.

B. Methods – Samples from each of the eight hatchery strains were assayed for variation at 15 loci to characterize variation and to determine whether there was sufficient diversity among them to determine the historical origins of the feral population. As such, these samples served as the donors to the wild population that presently exist as a descendent mixture of these donors. A larger sample of young from the wild population was also assayed for variation at the same 15 loci.

Data from donor and mixture (here, stocked hatchery strains and resulting wild population) were subjected to a maximum-likelihood admixture analysis (MLE). In concept, the method iteratively simulates a set of strain mixtures that best explains the variation in the real mixture population. In using bootstrap and jackknife re-sampling methods, estimates of mean contribution (and an associated standard error and coefficient of variation) can be produced. Ultimately, the more divergent the hatchery strains are, the more precise the admixture estimates.

C. Data Analysis & Results – The various strains of rainbow trout exhibit levels of variation commonly reported for propagated rainbow trout and sufficient inter-strain variation for admixture analysis. Figure 5.1 depicts the kinds of variation among individuals in a sample for the *sIdhp-3,4** locus. Of the 15 individuals assayed in this

photograph, eight genotypes were detected. Table 5.1 summarizes allele frequency variation for the nine samples over all 15 loci.

Table 5.1. Summary of allelic frequencies over 15 loci for eight hatchery strains of rainbow trout and the Clear Creek wild (mixture) population.

Locus Sample allele	*Adh-1**	*Ck-4**	*Est-1**	*Gpd-*1*	*mIdh p-2**	*sIdhp-3,4**	*Ldh-1**	*Ldh-3**	*Ldh-4**	*Ldh-5**	*mMdh**	*sMdh-3,4**	*sSod-1**
Shasta-MI[1]	n=30	30	NA	30	30	30	30	30	30	30	30	30	30
*1		0.983		1.000		0.163	1.000	1.000					
*2	1.000	0.017			0.667	0.054			1.000	1.000	1.000	0.100	0.883
*3					0.333	0.707						0.900	0.167
*4						0.076							
Shasta-TN	n=27	21	26	27	26	27	27	27	27	27	27	27	27
*1		1.000	0.500	1.000		0.250	1.000	1.000					
*2	1.000		0.481		0.808	0.065			1.000	1.000	1.000	0.046	0.630
*3			0.019		0.192	0.658						0.945	0.370
*4						0.028						0.010	
Kamloops	n=30	16	25	30	30	25	30	30	30	30	30	30	28
*1		1.000	0.280	1.000	0.617	0.170	1.000	0.883		0.117		0.025	0.161
*2	1.000		0.720		0.350	0.130		0.117	1.000	0.883	1.000	0.133	0.714
*3					0.033	0.690						0.650	0.125
*4						0.010						0.192	
Arlee	n=21	17	21	21	21	21	21	21	21	21	21	21	21
*1		1.000	0.238	1.000	0.810	0.250	1.000	0.952		0.024		0.024	
*2	1.000		0.765		0.190			0.048	1.000	0.976	1.000	0.131	0.714
*3						0.691						0.822	0.286
*4						0.059						0.012	
*5												0.012	
EED	n=22	22	22	22	22	22	22	22	22	22	22	22	22
*1		0.932	0.114	0.955		0.113	1.000	0.977		0.045		0.022	
*2	1.000	0.068	0.886	0.045	0.659	0.034		0.023	1.000	0.955	1.000	0.978	0.568
*3					0.341	0.807							0.432
*4						0.046							

Locus Sample allele	Adh-1*	Ck-4*	Est-1*	Gpd-*1	mIdh p-2*	sIdhp-3,4*	Ldh-1*	Ldh-3*	Ldh-4*	Ldh-5*	mMdh*	sMdh-3,4*	sSod-1*
Ten Sleep	n=7	11	9	11	11	11	11	11	11	11	11	11	11
*1		0.773	0.111	1.000		0.250	1.000	1.000					
*2	1.000	0.227	0.889		0.318	0.113			1.000	1.000	1.000	0.250	0.545
*3					0.682	0.614						0.750	0.455
*4						0.023							
Fish Lake	n=27	27	24	27	27	26	27	27	27	27	27	27	27
*1		1.000	0.229	1.000		0.202	1.000	1.000		0.130		0.028	
*2	1.000		0.750		0.815	0.144			1.000	0.870	1.000	0.186	0.500
*3			0.021		0.185	0.404						0.769	0.500
*4						0.221						0.018	
*5						0.010							
*6						0.019							
Eagle Lake	n=22	22	22	22	22	19	22	22	22	22	22	22	22
*1		0.977	0.273	0.773	0.773	0.145	1.000	1.000		0.114			
*2	1.000	0.023	0.682	0.227	0.227	0.184			1.000	0.886	1.000	0.091	0.659
*3			0.045			0.553						0.909	0.341
*4						0.118							
Erwin	n=30	30	30	30	30	30	30	30	30	30	30	30	21
*1		1.000	0.017	0.900		0.325	1.000	1.000		0.083			
*2	1.000		0.983	0.100	0.650	0.042			1.000	0.917	1.000		0.714
*3					0.350	0.583						1.000	0.286
*4						0.050							
Clear Creek "Mixture"	n=107	76	96	107	107	76	107	107	107	92	107	106	107
*1		1.000		0.935	0.505	0.131	0.995	1.000		0.054			
*2	1.000		0.688	0.065	0.495		0.005		1.000	0.946	1.000	0.158	0.935
*3			0.313			0.859						0.781	0.065
*4												0.061	
*5													
*6						0.010							

[1] The Shasta strain trout from Michigan are included as a comparative reference for intra-strain variation (unpublished data).

Figure 5.1 Example of variation observed at sIdhp-3,4* for 15 rainbow trout. The genotype for each individual is observed as a series of bands in a column.

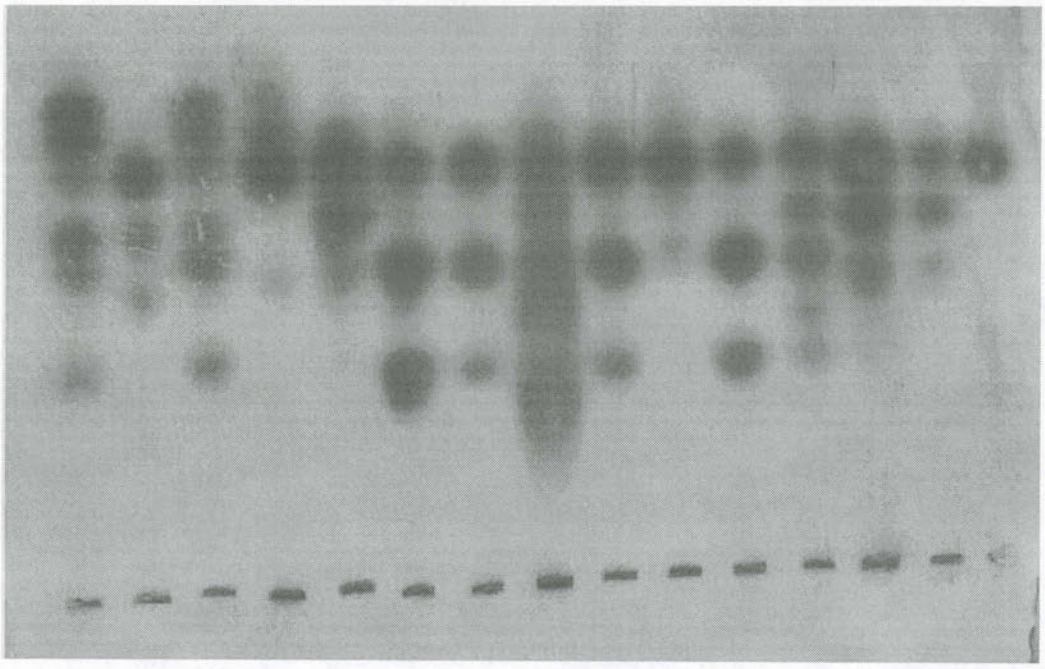


Table 5.2 summarizes the overall genetic variation of each hatchery strain and the wild population in terms of mean number of alleles observed at each locus, the percentage of loci exhibiting more than a single allele, and observed versus expected heterozygosity estimates. None of the samples are striking in terms of extremely high or low variation. In the cases of high inbreeding or low founder size, we would expect low estimates of observed (to expected) heterozygosity and low mean number of alleles, respectively. However, it is important to note that domesticated hatchery strains of rainbow trout often originated from multiple sources such that the allelic variation is due to mixing of strains (and perhaps formerly isolated evolutionary lineages). That said, it is interesting that the EED strain, which is a purposely-hybridized strain, is no more variable across loci than some of the others.

To provide an indication of the relationships among the strains and the wild samples, Figure 5.2 is a UPGMA dendrogram depicting relatedness based on genetic distances. The farther to the left a branch point is, the more divergent the two samples are. For

example, the Shasta and Arlee strains are much less divergent than the Kamloops and Ten Sleep populations are.

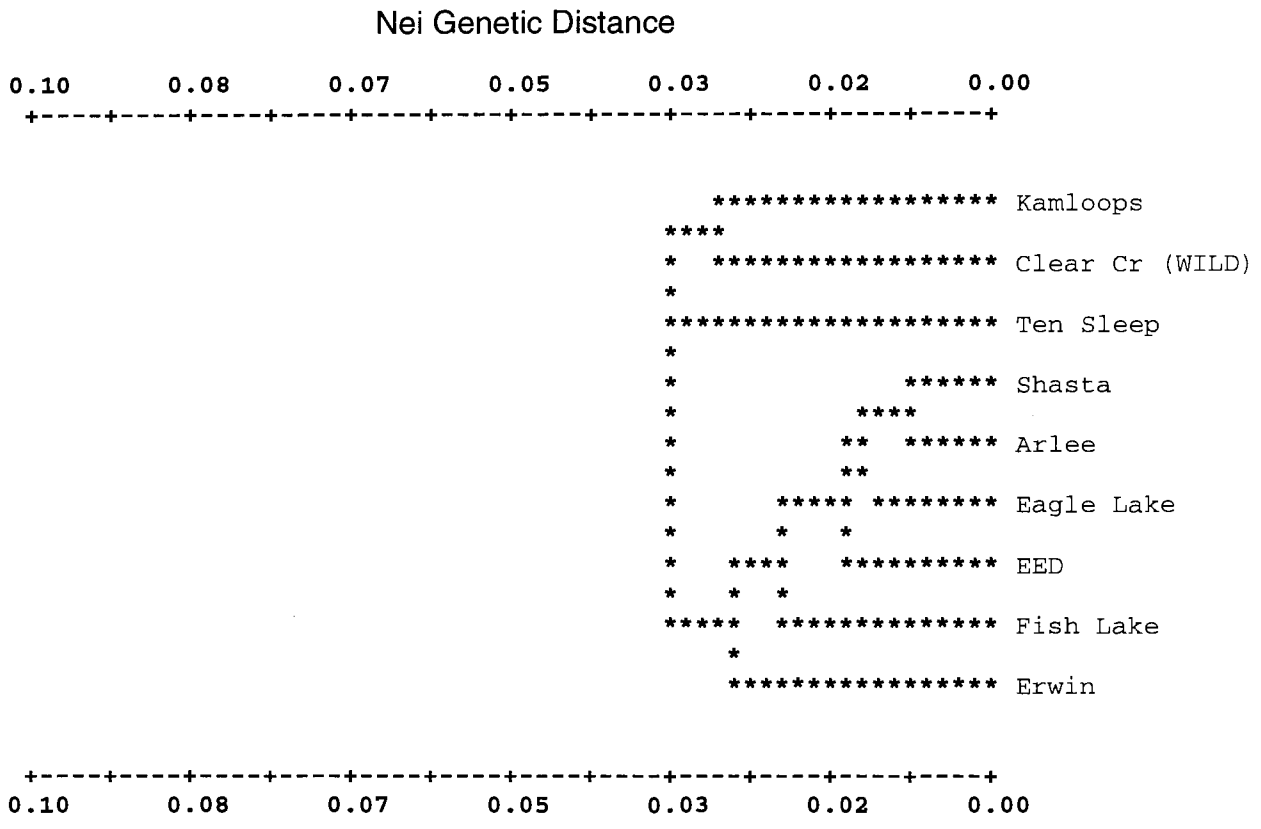
Table 5.2. Genetic variability over 15 loci in all population samples (standard errors in parantheses).

Population (or Strain)	Mean sample size per Locus	Mean no. of alleles per locus	Percentage of poly-morphic loci [1]	Mean heterozygosity Obs.	Mean heterozygosity H-W Exp. [2]
Reference Strains					
1. Kamloops	27.9 (1.0)	2.2 (0.4)	60.0	0.218 (0.069)	0.224 (0.069)
2. Shasta	26.5 (0.4)	1.7 (0.3)	40.0	0.152 (0.065)	0.151 (0.060)
3. Arlee	20.7 (0.3)	1.8 (0.3)	46.7	0.175 (0.065)	0.160 (0.058)
4. EED	22.0 (0.0)	1.8 (0.3)	60.0	0.161 (0.059)	0.146 (0.052)
5. Ten Sleep	10.6 (0.3)	1.7 (0.3)	40.0	0.121 (0.048)	0.191 (0.070)
6. Fish Lake	26.7 (0.2)	2.2 (0.5)	53.3	0.207 (0.069)	0.217 (0.068)
7. Eagle Lake	21.6 (0.3)	2.0 (0.3)	66.7	0.198 (0.054)	0.210 (0.061)
8. Erwin	29.4 (0.6)	1.7 (0.3)	46.7	0.139 (0.066)	0.133 (0.057)
Wild Population Mixture					
9. Clear Cr	98.9 (3.3)	1.7 (0.2)	60.0	0.131 (0.042)	0.158 (0.050)

[1] A locus is considered to be polymorphic if more than one allele was detected

[2] Unbiased estimate (see Nei 1978)

Figure 5.2. UPGMA dendrogram depicting relatedness of sample populations based on Nei genetic distances.



The results of the admixture analysis indicates that the feral population appears to be composed of fish derived from three primary strains and one marginal strain. Specifically, the Kamloops strain (or their ancestors) appears to have contributed an estimated 22% (+/- 5%); EED strain appears to have contributed an estimated 24% (+/- 5%); Fish Lake strain appears to have contributed an estimated 27% (+/- 6%); and, Eagle Lake strain appears to have contributed an estimated 9% (+/- 4%) (Table 5.3). Thus, these 4 strains appear to have been the most successful to date at becoming feral and contributing to wild recruitment. Note that the Kamloops strain originates from a geologically distant source (British Columbia; and therefore, probably evolutionary or genetically) than the others, which more than likely originated from central California (Sacramento or Trinity River sources). From this analysis, we do not yet know whether

the feral population is a homogeneous mixture descended from the four contributing strains or whether up to four sub-populations are being independently maintained.

Table 5.3. Estimated composition of the hatchery strains contributing to wild produced young in Clear Creek. (Admixture analysis using SPAM is an MLE-based algorithm available from Alaska Department of Fish and Game).

	Composition of Estimate	**Standard Error**	**Coefficient Variation**
Population ("strain")			
1 Shasta strain	0.0631	0.0614	0.97
2 Kamloops strain	0.218	0.0547	0.25
3 Arlee strain	0.000	0.000	0.00
4 EED strain	0.242	0.0538	0.22
5 Ten Sleep strain	0.0245	0.0316	1.3
6 Fish Lake strain	0.273	0.0645	0.24
7 Eagle Lake strain	0.0913	0.0386	0.42
8 Erwin strain	0.0323	0.0200	0.62
9 "Unknown"	0.0561		

Sub-Objective 5.2: Genetics of brown trout.

A. Background – Similar to the rainbow trout described in Sub-Objective 5.1, wild (or feral) brown trout fisheries have developed in the South Holston River and the Watauga River that has some capability for natural recruitment. However, these wild populations are also supplemented with individuals stocked from the state's hatchery system. Tennessee currently rears only a single brown trout strain for stocking into state waters (i.e., the Plymouth Rock strain). The purpose of this Service Sub-Objective is to describe genetic variation in the two feral populations and the Plymouth Rock strain. Unlike the case for rainbow trout, there was no admixture analysis performed as both feral populations were founded and descended from a single source.

B. Methods – Samples from the hatchery strain were assayed for variation at 11 loci to characterize variation.

C. Data Analysis & Results – Seven of the 11 loci exhibited more than one allele in one or more of the samples (Table 5.3). In general, there is a low level of genetic variation in these brown trout samples. This is somewhat typical of a long domesticated strain originating from a single ancestral stock as it has experienced multiple founder events. Moreover, there is not a significant amount of inter-sample variation (based on this kind of genetic analysis) to conclude that feral populations have diverged from either the stocked source or each other (although other kinds of data, such as morphometric or life-history or perhaps other kinds of genetic information, could override this conclusion). The early implication from these data is that there is no reason to think that feral populations are divergent from hatchery stocks warranting specific, but differential management approaches. Moreover, there is no evidence in Tennessee waters that identification of a special "strain" is needed for tailoring brown trout management in Tennessee to improve production or performance.

Table 5.3. Allelic variation over 11 loci for brown trout managed in Tennessee.

OTU/ Locus		*Gpi-1,2**	*Gpi-3**	*mMdh-1**	*sMdh-3,4**	*mldhp-2**	*sMdh-1**	*sIdhp-3,4**	*sSOD-1**
Tissue		WM	WM	WM	WM	WM	LR	LR	LR
Alleles = rf values		**1=-100; *2=-25*	**1=100; *2=105*	**1=100*	**3=100 *4=110*	**1=83 *2=100*	**1=100 *2=200*	**3=100*	**2=100*
Plymouth Rock "strain" - Hatchery									
n=60	**1*	0.975	0.875	1.000			0.742		
	**2*	0.025	0.125			1.000	0.258		1.000
	**3*				0.996			1.000	
	**4*				0.004				
S. Holston R. - Wild									
n=30	**1*	1.000	0.933	1.000			0.767		
	**2*	0.000	0.067			1.000	0.233		1.000
	**3*				1.000			1.000	
	**4*				0.000				
Watauga R. - Wild									
n=30	**1*	0.983	0.867	1.000			0.833		
	**2*	0.017	0.133			1.000	0.166		1.000
	**3*				1.000			1.000	
	**4*				0.000				

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