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FINAL REPORT

RESEARCH AND SURVEYS

FEDERAL AID GRANT NO. F-50-R

FISH RESEARCH FOR OKLAHOMA WATERS

PROJECT NO. 19

DETERMINING THE DEGREE OF HYBRIDIZATION IN THE SMALLMOUTH BASS

MARCH 1, 2003 through FEBRUARY 28, 2005

FINAL REPORT

State: Oklahoma Grant Number: F-50-R

Grant Title: Fish Research and Surveys for Oklahoma Lakes and Reservoirs

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Project Title: Determining the degree of hybridization in the smallmouth bass population of Broken Bow Reservoir and the Mountain Fork River.

Contract Period: From: March 1, 2003 To: February 28, 2005

ABSTRACT

The Elk River population of smallmouth bass either carries a low frequency of non-native alleles (<7%) or the genetic structure reflects a low level of allele-sharing between natives and the Byron Hatchery stock of the Tennessee Lake strain. The genome of the Broken Bow Reservoir population is about 40% non-native, whereas the stream populations in adjacent sections of the primary tributary, Mountain Fork River, carry a low frequency (approximately 3%) of putatively non-native alleles. The upper end of Broken Bow Reservoir is at least a partial barrier to the movement of hybrids from the down-lake area into the river. But, with time the river population will carry higher frequencies of non-native alleles. The results of this study suggest that a small amount of non-native genetic material has made its way into the river. Similarly, if non-natives are introduced into Grand Lake, the Elk River population in Oklahoma and Missouri may eventually carry significant amounts of non-native genetic material.

I. PROBLEM OR NEED

Smallmouth bass *Micropterus dolomieu* is an important sportfish throughout eastern Oklahoma. A recent allozyme survey across the natural range of smallmouth bass demonstrated that the native populations on the western edge of the Interior Highlands (Ozark Plateau and Ouachita Mountains) represent the two most divergent lineages of the species; these are referred to as the Neosho and Ouachita smallmouth bass (Stark and Echelle 1998). Prior to publication of this information, the Oklahoma Department of Wildlife Conservation (ODWC) introduced fingerling "Tennessee lake strain" smallmouth bass to Tenkiller Reservoir (1991 and 1992) and Broken Bow Reservoir (1993). Prior to these stockings, Tenkiller and Broken Bow reservoirs supported native populations of Neosho and Ouachita smallmouth bass, respectively, although in low numbers. Because of concerns over loss of genetic diversity of these native populations and in response to the findings of the allozyme survey by Stark et al. (1995), ODWC discontinued stockings in waters supporting native smallmouth bass. However, improvements in smallmouth bass population structure in these two reservoirs coincided with stocking the "lake strain" fish.

Data collected under F-44-D (5) suggest that the smallmouth bass populations in both reservoirs have expanded since introductions of "lake-strain" individuals in the early 1990's. Angler reports of improved catch rates and sizes of smallmouth bass caught support these findings. Improved angler catches of smallmouth bass in Tenkiller and Broken Bow have resulted in pressure from anglers to expand stockings of "lake-strain" individuals into additional reservoirs within the native ranges of both the Neosho and Ouachita strains, Grand Lake in particular (Kim Erickson, personal communication).

II. BACKGROUND

Malloy et al. (2000a) used variation in smallmouth bass microsatellite DNA loci to determine the degree of hybridization in Tenkiller and Broken Bow reservoirs and in the impounded streams above the reservoirs. The results indicated that 85-90% of the genome in samples from Tenkiller Reservoir and 41% of the genome in samples from Broken Bow Reservoir were non-native. It appeared likely that after a few additional generations of random mating, all individuals would carry mixed/non-native genotypes. The quality of the smallmouth bass fishery may be dependent on the growth and survival of the "lake-strain" or F_1 hybrid. If so, the quality of the fishery may decline as genetic recombination increases the abundance of F_x individuals.

Malloy et al. (2000a) found no introgression of the "lake-strain" genome into the tributary streams containing the native strains. However, the authors felt that, with time, introgression is likely to occur. No samples were collected from either the lower reaches of the streams, or from the "up-lake" portions of the reservoirs, areas in which introgression into the stream populations would be most likely to occur.

Angler pressure to expand stocking of "lake-strain" smallmouth bass within the native range of the Neosho and Ouachita strains warrants further investigation of the likelihood of genetic introgression in these native populations. In addition, fitness data, namely growth and mortality rates, of existing smallmouth bass populations are limited.

Malloy et al., (2000a) noted that their samples were confined to the extreme down-lake areas of Tenkiller and Broken Bow reservoirs. They recommended expanding sampling

to include up-lake areas to determine if the "lake-strain" fish are confined to the down-lake areas and/or determine if population size is expanding. Their recommendations also included periodic genetic surveys to monitor the degree of genetic introgression (proportion of F_x individuals) in both reservoir and stream environments. If F_x individuals are "less fit" than the "lake-strain" or first generation crosses with native strains (F_1), periodic stocking of "lake-strain" individuals may be needed to maintain existing smallmouth bass population characteristics.

Sampling was confined to Broken Bow Reservoir and upstream in the Mountain Fork River for a number of reasons. First, smallmouth bass habitat is limited in the up-lake areas of Tenkiller Reservoir so collecting fish from the "transition zone" between reservoir and river would be difficult in Tenkiller. Malloy et al., (2000a) identified only a single diagnostic locus to differentiate the Neosho strain and the "lake-strain" in Tenkiller. Given a single diagnostic locus in the Tenkiller population, differentiation between F_1 and F_x individuals would not be possible and detecting introgression is more difficult. Three diagnostic loci were identified to separate "lake-strain" and Ouachita strain smallmouth bass in Broken Bow Reservoir and the Mountain Fork River. In addition, given the high degree of "hybridization" in the Tenkiller population, encountering pure "lake-strain" individuals is unlikely.

III. PROJECT OBJECTIVES:

1. Determine degree of introgression of "lake-strain" genome of smallmouth bass in the down-lake area of Broken Bow Reservoir, the up-lake "transition zone" between reservoir and stream and three stream stretches, progressively upstream from the reservoir.

2. Compare fitness (growth and mortality rates) of the different genetic strains (lake-strain, Ouachita strain, F_1 and F_x hybrids) in each of the five sampling areas.

3. The genetic composition of the Elk River, a tributary of Grand Lake, smallmouth bass population will also be determined. No previous genetic analysis has been done on the smallmouth bass populations of the Grand Lake tributaries and angler pressure to introduce "lake-strain" smallmouth bass is focused on Grand Lake.

IV. Approach:

Collection of samples.—Tissue samples (muscle preserved in 100% ethanol) were collected in the field from smallmouth bass collected during fall electrofishing sampling in 2002 under Federal Aid Project F-44-D-17 (5). Each tissue sample was individually labeled and all fish were aged using otoliths.

Locations of samples from the Broken Bow Reservoir-Mountain Fork River area are given in Figure 1. Samples from the Elk River were taken from the confluence with Grand Lake upstream to the Missouri border. Sample sizes are given in Tables 1 and 2. Reference samples from Mountain Fork River include two collections made by W. J.

Stark in 1993 from McCurtain Co., Oklahoma, one from the Mountain Fork River near Jet (n = 15), and one from Buffalo Creek, 1.6 km S, 0.8 km E of Buffalo (n = 20). No reference sample was available for Elk River. The reference collection of the Tennessee lake strain (n = 15) was from the 1992 stock at Byron State Fish Hatchery, which was the source of fish released into Broken Bow Reservoir in 1993.

DNA isolation and microsatellite survey—Isolation of genomic DNA was done with the DNeasy kit (Qiagen). Three primer pairs, Mdo1, Mdo2, and Mdo3 (Malloy et al., 2000b), were used in this analysis. In a previous study, Malloy (2001) found that all three primer pairs showed markedly different allele frequencies between native stocks in Mountain Fork River and the Byron Hatchery stock used in the introductions of lake strain bass into Broken Bow Reservoir, whereas only Mdo1 showed large differences between the Byron Hatchery stock and natives in the Illinois River and Lake Tenkiller (Malloy, 2001). All three loci were surveyed in all collections of fish.

The three microsatellite loci were amplified in polymerase chain reaction (PCR) mixtures (1.2μl DNA, 9μl true allele premix, 3.8μl dH_2O, and 1μl primer pairs) under the following conditions: an initial 12-min denaturation at 95 C followed by 10 cycles at 94 C for 15 s, 55-60 C for 60 s, 72 C for 30 s; then 25 cycles at 89 C for 15 s, 55 C for 60 s, and 72 C for 30 s; the thermal profile ended with a 30-min elongation period at 72 C. Individuals were genotyped using an ABI 377 Automated Sequencer and ABI Genescan 3.1 software. Alleles were assigned using ABI Genotyper 2.5 software.

Data analysis.—The computer program, Structure (Pritchard et al., 2000), was used to obtain plots allowing visualization of genetic structure. We used this program to compute the Bayesian probability that each "unknown" individual (all except those in the reference samples) belonged to one of two populations, one defined by the allele frequencies in the reference sample for the 1992 Byron Hatchery stock and the other defined by the allele frequencies in the two reference collections (lumped as a single sample) from streams in 1993. For this analysis, we discarded the first 20,000 replicates as "burnin" and used the results from 10,000 additional replicates. All other analyses were performed with Arlequin (Schneider et al., 2000). The sequential Bonferroni correction (Rice, 1989) for Type I error ($\alpha = 0.05$) in multiple tests was applied where appropriate.

Mean length at age data (ages 0-4) for the three sections of the Mountain Fork River were compared using a one-way ANOVA ($\alpha = 0.05$). No differences were found in mean length at age among sections so data from all sections were pooled for comparison with the respective data from Broken Bow Reservoir. Differences in mean length at age for smallmouth bass from the reservoir and the river upstream were tested using one-way ANOVA ($\alpha = 0.05$). Age and growth data from Elk River were not compared to those from the Broken Bow/Mountain Fork system.

Total annual mortality estimates of the smallmouth bass populations in Broken Bow Reservoir, Mountain Fork River, and Elk River were made using the FAST model. Age-0 smallmouth bass were not fully recruited to the gear. Therefore, catch curves were based on age-1 and older fish.

V. Results and Discussion:

General genetic structure.—Numbers of alleles per locus and allele frequencies in each collection are given in Tables 1 and 2. There were four instances of significant deviation from Hardy-Weinberg expectations for genotypic frequencies (after Bonferroni correction; critical $P = 0.05/3$ loci $= 0.017$). These include a heterozygote deficiency for Mdo3 in the Byron Hatchery sample ($P = 0.0004$), a heterozygote deficiency and a heterozygote excess for, respectively Mdo1 ($P = 0.011$) and Mdo3 ($P = 0.0001$) in Broken Bow Reservoir, and a heterozygote deficiency for Mdo1 in the Boktuklo sample ($P = 0.015$).

Tests of linkage disequilibrium among loci within individual collections, revealed only one instance of significant disequilibrium (Bonferroni correction; critical $P = 0.05/3$ pairwise tests per collection $= 0.017$). This was the Mdo2-Mdo3 combination in the Boktuklo sample from Mountain Fork River ($P = 0.015$).

Elk River.—The level of genetic introgression in Elk River is difficult to assess because of a lack of reference samples for the genetic structure of native populations. Malloy (2001) reported that the Mdo1 locus was diagnostically different between the Byron Hatchery stock and the native stocks in the Illinois River drainage, although there was a small amount of overlap (3.6%) in frequency of one allele. Because of a high degree of allele sharing, Malloy (2001) considered Mdo2 and Mdo3 (and 8 other loci surveyed) to be uninformative regarding genetic introgression. Our results for Elk River are consistent with his findings, with large allele frequency differences for Mdo1 and less divergence at both Mdo2 and Mdo3 (Table 2).

Our sample of Byron Hatchery (n = 15) was larger than Malloy's (2001; n = 10), and we used a different method of allele resolution, resulting in a greater number of Mdo1 alleles detected (7 vs 6 in his study). Regardless, our comparison of Elk River populations with the Byron Hatchery stock suggests that any genetic introgression is relatively low. If every Mdo1 allele shared with Byron Hatchery stock is a result of non-native ancestry, then the level of introgression is 6.7%. Again, however, this might be an overestimate because of the potential for allele sharing between Byron Hatchery stock and the native smallmouth bass in Elk River.

Mountain Fork River area.—There were no instances of either Hardy-Weinberg deviation or pairwise linkage disequilibrium among loci when samples from the Mountain Fork River were lumped by river zone. Further, a hierarchical analysis of molecular diversity (AMOVA) showed that 0.0% of the genetic diversity reflected variation within individual river zones. Thus, for purposes of analysis, and unless otherwise noted, all collections within each river zone were treated as a single collection.

The comparison of the 1992 Byron Hatchery stock with the reference samples for native stock in Mountain Fork River (1993 stream collections from the Mountain Fork and Buffalo Creek) indicated marked allele frequency differences for all three microsatellite loci, although none showed fixed differences (Table 1). The two reference collections of natives showed the following levels of overlapping frequencies with the hatchery stock: Mdo1, 0.00% to 10%; Mdo2, 6.2% to 12.5%; and Mdo3, 6.7% to 10.0%.

Mdo1 and Mdo3 are the clearest indicators of hybridization because, for both loci, the most common alleles in the hatchery stock were not detected in the reference collections of natives. For Mdo2, the most common allele in the hatchery stock (199; frequency = 96.7%) also occurred at moderate frequencies (6.7% and 12.5%) in the reference collections for natives.

The results from STRUCTURE verify Malloy's (2001) conclusion that the Broken Bow Reservoir population carries high frequencies of non-native genetic markers whereas the river populations are less affected (Fig. 2). Because only three loci were used, and because the reference native and non-native populations shared alleles at all three loci, no individuals received 100% confidence regarding whether or not they were genetically pure natives (Fig. 2).

Our results were in good agreement with Malloy's (2001) estimate that 41% of the genome of the Broken Bow Reservoir population is non-native. In our collections from the reservoir, the summed frequency of Mdo1 alleles detected in the hatchery stock but not in the native reference samples was 43.2%. The results for the less diagnostic loci, Mdo2 and Mdo3, are consistent with the estimate of approximately 40% genetic introgression by non-natives, but because of allele sharing between reference collections we have not attempted more exact estimates from these loci. The lack of change in the rate of introgression of non-native alleles in the Broken Bow population between the two studies can be explained by one of two scenarios. First, early in the time since introduction, pure non-natives (Tennessee lake strain), first-generation hybrids, and backcross progeny may have had an advantage over genetically pure natives (Ouachita

strain). This, together with random interbreeding and backcrossing with the natives, would result in a situation where every individual is carrying non-native alleles (not necessarily the markers used in this study). Once this happens, the markers used in this study would be "frozen" at whatever frequency existed at that time (~40%). Another possible scenario would be that the non-natives (Tennessee lake strain) and hybrids were not favored over natives (Ouachita strain). If the introduced non-native year class represented 40% of the breeding population at the time they became reproductive, then the frequency of non-native alleles would be "frozen" at roughly 40%.

Nine individuals from the three river zones had especially low probabilities of being genetically pure natives (Fig. 2; Table 3). Each of these specimens carried one or two alleles potentially representing non-native contributions to their ancestry. These alleles were detected in the hatchery stock but not in the reference collections from streams (Tables 1 and 2). Although these alleles might be non-native in origin, we cannot rule out the possibility that they were originally present in the native populations. They might have gone undetected in our relatively small sample sizes of the reference populations. The latter argument would explain the absence of clinal variation in allele frequencies in the stream variation.

Under the assumption that the alleles in question are in fact non-native and that they invaded from Broken Bow Reservoir, then the pattern of geographic variation might be expected to show clinal variation, with higher frequencies of non-native alleles downstream. Instead, the three river-zones had roughly equivalent levels of such alleles. This pattern might be explained by either of the following hypotheses: 1) individual

home ranges (or seasonal movement patterns) extend throughout the sampled portion of Mountain Fork River or 2) fish of non-native ancestry have been released by humans into Mountain Fork River. The presence of age 0 among the individuals listed in Table 3 suggests that some hybrids have reproduced.

The indicated level of genetic introgression in the three Mountain Fork river-zones seems relatively low. Frequencies of Mdo1 alleles detected in the hatchery stock but not in the two reference samples for native stock were 3.3%, 3.6%, and 3.6% for, respectively, river zones 1, 2, and 3. The corresponding values for Mdo1 were 3.3%, 2.4%, and 1.2%. This gives an average across the two loci of , respectively, 3.3%, 3.0%, and 2.4%. This is somewhat of an underestimate because other Mdo1 and Mdo3 alleles were shared between the hatchery stock and the reference samples from streams.

Efforts to collect smallmouth bass in the transition zone between Mountain Fork River and upper Broken Bow Reservoir produced only a single specimen (Table 1). This, together with the marked difference in frequency of non-native alleles between the reservoir and the three river-zones, indicates that the upper lake is at least a partial barrier to movement of hybrids from the downlake region of Broken Bow Reservoir into the river.

Mean length at age of the smallmouth bass from Broken Bow Reservoir was higher than the respective lengths at age from the Mountain Fork River population at all ages tested (one-way ANOVA; $\alpha = 0.05$; Table 4). Sample sizes of the older individuals are small so the marked differences in length at age for ages 3 and 4 may be suspect. It is not possible

to equate higher growth rates in the lake population to genetic differences previously discussed given productivity and habitat differences between stream and lake environs. Comparisons of growth rates of the Mountain Fork River smallmouth bass population reported in this study with those previously reported by Stark and Zale (1991) are difficult due to differences in methodologies. Stark and Zale (1991) reported back-calculated lengths at age using scales. Lengths at age in this study were determined using mean length of the age sample collected in the fall using otoliths (generally considered a more accurate means of determining ages of Oklahoma fishes than by using scales). No previous age and growth data for the Broken Bow smallmouth bass population are available.

Fifteen fish from the Broken Bow sample showed a high probability of being natives (Ouachita strain; Figure 2). Mean lengths at age 1 and age 2 of this subsample were 219.5 mm (N=10) and 258.0 mm (N=4), respectively. These lengths were not significantly different (one-way ANOVA; $\alpha = 0.05$) from the respective lengths of the entire sample (Table 4). Similarly, of the nine individuals from the Mountain Fork River sample showing a high probability of being non-native (Tennessee lake strain), mean length at age 0 (105 mm; N=3) and age 1 (195.7 mm; N=3) were not different from the respective lengths of the entire sample (one-way ANOVA; $\alpha = 0.05$; Table 4). These results are not surprising, given the small sample sizes and the fact that the lake population has been randomly mating for nearly a decade making it likely that all fish in the sample are hybrids and merely showing native alleles at the three loci tested. None of the stream fish had a probability >95% of being non-native so we cannot say with 95%

confidence that the fish in the sample were not native. This is because none of the three loci tested were completely diagnostic.

Growth rates of the Elk River smallmouth bass population are also given in Table 4. No statistical analyses of differences in growth between the Elk River and Mountain Fork River populations were made. However, mean lengths at age for age 0 and age 1 were similar. Sample sizes of age 2 and age 3 were low making comparisons suspect.

Annual mortality estimates were higher for the reservoir population (0.71; Figure 3) than for the river population (0.40; Figure 4). Exploitation studies have not been conducted on either Broken Bow Reservoir or the Mountain Fork River making natural mortality comparisons between the two systems impossible. However, some inferences can be made. Given the preponderance of the "catch and release" ethic practiced by today's bass anglers and the fact that a 330-406 mm protective slot length limit is in effect on Broken Bow Reservoir, it seems unlikely that angler harvest is a major contributor to the 0.71 annual mortality estimate. No size limit is in effect on the Mountain Fork River smallmouth bass population but given the "catch and release" ethic and limited public access to the stream, it also seems unlikely that angler harvest contributes substantially to the total mortality estimate (0.40) of the river population. One might infer from this that the differences in total mortality between the reservoir and river populations of smallmouth bass would then be due to differences in natural mortality rates. This could lead one to argue that the introgression of non-native genes (lake strain) into the reservoir population has contributed to a decrease in "fitness." However, one could also suggest that the improved growth rates in the reservoir population counter this argument.

The mortality estimate for the Mountain Fork River smallmouth bass population reported in this study was lower than two previously reported smallmouth bass mortality estimates, 0.96 (Fisher et al., 1997) and >0.60 (Balkenbush and Fisher 2001) for the Glover River, an unimpounded stream located approximately 50 km west of the Mountain Fork River. Mortality of the Elk River population was estimated at 0.32 (Figure 5). This estimate is based on only a 44 fish sample so must be looked at with caution.

VI. Conclusions/Recommendations:

1. The results provide baseline data on allele frequencies for smallmouth bass in Elk River and Mountain Fork River. These data will be useful in any future study of the level of non-native genetic introgression in these areas.
2. The Elk River population either carries a low frequency of non-native alleles (<7%) or the genetic structure reflects a low level of allele-sharing between natives and the Byron Hatchery stock of the Tennessee Lake strain.
3. In agreement with Malloy's (2001) previous study, the genome of the Broken Bow Reservoir population is about 40% non-native.
4. The stream populations in zones 1, 2, and 3 of Mountain Fork River carry a low frequency (approximately 3%) of putatively non-native alleles.
5. The lack of a clinal pattern in the frequency of those alleles in the river can be explained by one of the following:
    a. The putatively non-native alleles are, in fact, native to the system, but were not detected in the relatively small reference collections.

b. The alleles are non-native and the lack of a clinal pattern is a result of individuals ranging throughout most of Mountain Fork River with no significant barriers to dispersal.

c. The alleles are non-native and fish of non-native ancestry have been transported into the stream, perhaps by anglers.

6. The upper end of Broken Bow Reservoir is at least a partial barrier to the movement of hybrids from the down-lake area into the river.

7. Given time, the river population upstream of Broken Bow Reservoir will more than likely be carrying higher frequencies of non-native alleles. With dispersal and time, and assuming no selection favoring non-native alleles, gene frequencies will equilibrate such that the reservoir and the river will contain equivalent amounts of non-native genetic material. The results of this study suggest that a small amount of non-native genetic material has made its way into the river.

8. No differences in mean length at age were detected among genotypes in neither Broken Bow Reservoir nor the Mountain Fork River.

9. Similarly, if non-natives are introduced into Grand Lake, it is only a matter of time before the Elk River population in Oklahoma and Missouri are carrying non-native material. The frequency of such material is impossible to predict. It depends on the size of the introduced population relative to the native population and the relative success of hybrids. If, as seems to be the case in Lake Tenkiller (Malloy, 2001), the non-natives and hybrids compose as much as 90% of the population, then the eventual level of genetic introgression of the river population could be relatively high.

10. We recommend monitoring the genetic composition of the smallmouth bass populations in Broken Bow Reservoir and in the Mountain Fork River to assess the introgression rates of non-native alleles in both the reservoir and the river. We also recommend collecting a sample of smallmouth bass from Grand Lake to determine if non-native alleles have been introduced into that population (the most likely pathway would be angler introductions).
11. An outreach effort to inform the public of the potential harmful effects (not limited to genetics) of moving fish from one body of water to another should be instigated.

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Table 1. Allele frequencies. Bold numbers = alleles either detected in the hatchery stock but not in the two reference samples for native Mountain Fork River populations or shared alleles clearly indicating non-native influence in wild populations.

		Mdo1 alleles								
Locality	N	190	192	196	198	200	202	204	206	212
Reference sites										
Hatchery stock	15	**0.033**	**0.033**	**0.533**	-	0.100	-	**0.033**	**0.067**	**0.200**
Mountain Fork River (1993)	15	-	-	-	-	0.367	0.633	-	-	-
Buffalo Creek (1993)	20	-	-	-	0.025	0.650	0.325	-	-	-
Broken Bow Reservoir	102	-	-	**0.328**	0.010	0.240	0.319	**0.020**	**0.020**	**0.064**
Transition Zone	1	-	-	-	-	0.500	0.500	-	-	-
River Zone 1										
Dump Rd	14	-	-	-	0.036	0.393	0.571	-	-	-
Two Gates E	10	-	-	-	0.050	0.450	0.500	-	-	-
Beachton	9	-	-	**0.056**	0.056	0.500	0.389	-	-	-
Beach Crk	12	-	-	**0.042**	0.042	0.458	0.417	-	-	**0.042**
All sites, Zone 1	45			**0.022**	0.044	0.444	0.478			**0.011**
River Zone 2										
Goats Bluff	12	-	-	-	-	0.583	0.333	**0.042**	-	**0.042**
Highway 4	2	-	-	-	-	-	1.000	-	-	-
Jim's Hole	16	-	-	-	0.031	0.438	0.500	**0.031**	-	-
Gravel Pit	4	-	-	-	-	0.625	0.375	-	-	-
Pipeline	7	-	-	-	-	0.643	0.357	-	-	-
All sites, Zone 2	42				0.012	0.512	0.440	**0.024**	-	**0.012**
River Zone 3										
Grubby Smith	7	-	-	**0.071**	-	0.571	0.357	-	-	-
Busted Ford	24	-	-	**0.021**	0.083	0.500	0.375	**0.021**	-	-
Boktuklo	10	-	-	-	-	0.450	0.550	-	-	-
All sites, Zone 3	41			**0.024**	0.049	0.500	0.415	**0.012**	-	-

Table 1 continued

Locality	N	Mdo2 alleles 181	185	193	199	203
Reference sites						
Hatchery stock	15	-	**0.033**	-	**0.967**	-
Mountain Fork River (1993)	15	-	-	0.933	0.067	-
Buffalo Creek (1993)	20	-	-	0.875	0.125	-
Broken Bow Reservoir	104	0.029	**0.010**	0.431	**0.466**	0.064
Transition Zone	1	-	-	1.000	-	-
River Zone 1						
Dump Rd	14	-	-	0.929	0.072	-
Two Gates E	10	-	-	0.950	0.050	-
Beachton	9	-	-	0.778	0.167	0.056
Beach Crk	12	-	-	0.875	0.125	-
All Sites, Zone 1	45			0.889	0.100	0.011
River Zone 2						
Goats Bluff	12	-	-	0.792	0.208	-
Highway 4	2	-	-	0.750	0.250	-
Jim's Hole	16	-	-	0.719	0.281	-
Gravel Pit	4	-	-	0.750	0.250	-
Pipeline	7	-	-	0.857	0.143	-
All Sites, Zone 2	42	-	-	0.774	0.227	-
River Zone 3						
Grubby Smith	7	-	-	0.929	0.071	-
Busted Ford	24	-	-	0.917	0.084	-
Boktuklo	10	-	-	0.650	0.350	-
All Sites, Zone 3	41	-	-	0.854	0.146	-

Table 1 continued

Locality	N	Mdo3 alleles 108	114	120	122	126	132
Reference sites							
Hatchery stock	15	-	-	**0.600**	0.200	0.046	**0.133**
Mountain Fork River (1993)	15	0.933	-	-	0.067	-	-
Buffalo Creek (1993)	20	0.900	-	-	0.075	0.025	-
Broken Bow Reservoir	104	0.603	0.010	**0.211**	0.074	0.015	**0.088**
Grannys Rock	1	1.000	-	-	-	-	-
River Zone 1							
Dump Rd	14	0.821	-	-	0.143	-	**0.036**
Two Gates E	10	0.950	-	-	0.050	-	-
Beachton	9	0.944	-	-	0.056	-	-
Beach Crk	12	0.917	-	**0.042**	-	-	**0.042**
All Sites, Zone 1	45	0.900	-	**0.011**	0.067	-	**0.022**
River Zone 2							
Goats Bluff	12	0.917	-	-	0.042	-	**0.042**
Highway 4	2	1.000	-	-	-	-	-
Jim's Hole	16	0.844	-	**0.031**	0.125	-	-
Gravel Pit	4	0.875	-	-	0.125	-	-
Pipeline	7	0.714	-	-	0.286	-	-
All Sites, Zone 2	42	0.857	-	**0.012**	0.119	-	**0.012**
River Zone 3							
Grubby Smith	7	0.786	-	**0.071**	0.143	-	-
Busted Ford	24	0.938	-	-	0.063	-	-
Boktuklo	10	0.800	-	-	0.200	-	-
All Sites, Zone 3	41	0.878	-	**0.012**	0.110	-	-

Table 2. Allele frequencies for Byron Hatchery stock and Elk River population.

		Mdo1 alleles									
Locality	N	190	192	196	198	200	202	204	206	208 & 210*	212
Hatchery stock	15	0.033	0.033	0.533	-	0.100	-	0.033	0.067	-	0.200
Elk River	60	-	-	0.017	-	0.042	0.833	-	-	0.100	0.008

*Mdo1 alleles 208 and 210 were combined; respective frequencies = 0.092 and 0.008 in Elk River

		Mdo2 alleles		
Locality	N	185	193	199
Hatchery stock	15	0.033	-	0.967
Elk River	60	0.025	0.008	0.966

		Mdo3 alleles				
Locality	N	108	120	122	126	132
Hatchery stock	15	-	0.600	0.200	0.046	0.133
Elk River	60	0.083	0.900	-	-	0.017

Table 3. Specimens from Mountain Fork River with the lowest probabilities of having completely native ancestry. Alleles in bold font are considered primarily responsible.

Specimen ID	Location	Probability Of Completely Native Ancestry	Genotype indicating non-native ancestry	Age
DR14	Zone 1: Dump Road	0.18	Mdo3 108/**132**[1]	0
BEACH138	Zone 1: Beachton Crossing	0.06	Mdo1 **196**/202[2]	2
BEACRK53	Zone 1: Beach Creek	0.18	Mdo1 **196**/200 Mdo3 108/**120**[3]	1
GBL12	Zone 2: Goats Bluff	0.19	Mdo3 108/**132**[4]	0
GBL96	Zone 2: Goats Bluff	0.27	Mdo1 200/**212**[5]	4
JIM1	Zone 2: Jim's Hole	0.21	Mdo3 108/**120**[6]	1
JIM8	Zone 2: Jim's Hole	0.21	Mdo1 202/**204**[7]	1
GRUB6	Zone 3: Grubby Smith's	0.18	Mdo1 **196**/200[8] Mdo3 108/**120**[8]	0
BUST14	Zone 3: Busted Ford	0.08	Mdo1 **196/204**[9]	4

[1] Mdo3-132: absent in the reference collections of natives, present in the hatchery stock (13.3%) and in Broken Bow Reservoir (8.8%).

[2] Mdo1-196: absent in the reference collections of natives, common in both the hatchery stock (53.3%) and Broken Bow Reservoir (32.8%). Note: this was the only specimen from the three river zones carrying Mdo2 allele 203, an allele otherwise detected only in Broken Bow Reservoir at a frequency of 6.4%.

[3] See footnote 2 for Mdo1-196. Mdo3-120: absent in the reference collections of natives, common in the hatchery stock (60.0%) and Broken Bow Reservoir (21.1%).

[4] See footnote 1 for Mdo3-132.

[5] Mdo1-212: absent in the reference collections of natives, present in hatchery stock (20.0%) and Broken Bow Reservoir (6.4%). This specimen also was the only homozygote for Mdo2 allele 199 found in the three river zones.

[6] See note 3 for Mdo3-120.

[7] Mdo1-204: absent in the reference collections of natives, present in Byron Hatchery stock (3.3%) and Broken Bow Reservoir (2.0%).

[8] See notes 3 and 6 for, respectively Mdo1-196 and Mdo3-120.

[9] See note 3 for Mdo1-196. Like that allele, Mdo1-204 also was absent in the reference collections of natives but present in the Byron Hatchery stock (3.3%) and Broken Bow Reservoir (2.0%).

Table 4. Mean length at age data (ages 1-4) for smallmouth bass populations from Broken Bow Reservoir, Mountain Fork River, and Elk River. Lengths of individual ages with different superscripts from Broken Bow Reservoir and Mountain Fork River are statistically different (one-way ANOVA; $\alpha = 0.05$). Numbers in parentheses are the sample sizes.

	Age 0	Age 1	Age 2	Age 3	Age 4
Broken Bow Reservoir	(22) 114.9[a]	(60) 213.9[a]	(18) 288.4[a]	(2) 405.5[a]	(2) 465.0[a]
Mountain Fork River	(32) 101.3[b]	(45) 191.9[b]	(10) 227.4[b]	(4) 373.0[b]	(3) 347.3[b]
Elk River	(13) 132.9	(37) 189.5	(3) 280.7	(4) 338.5	

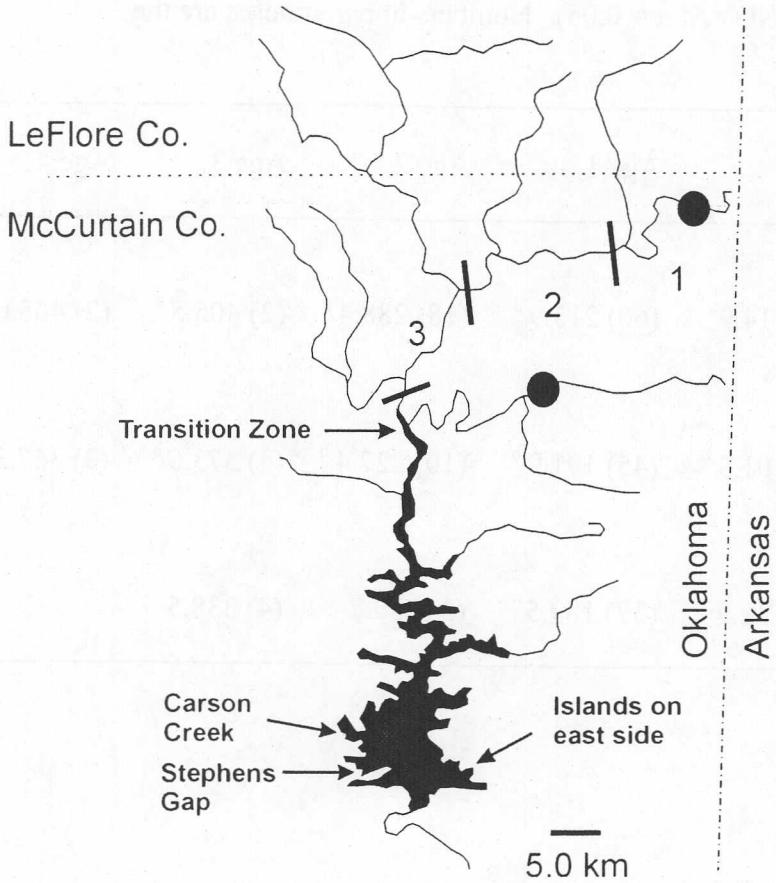

Figure 1. Broken Bow Lake-Mountain Fork River area. Dots indicate locations of reference collections from streams made in 1992. Numbers 1, 2, and 3 indicate the three river zones sampled in 2002. Reservoir collections were taken from the four areas indicated with arrows (only a single specimen was from the Transition Zone).

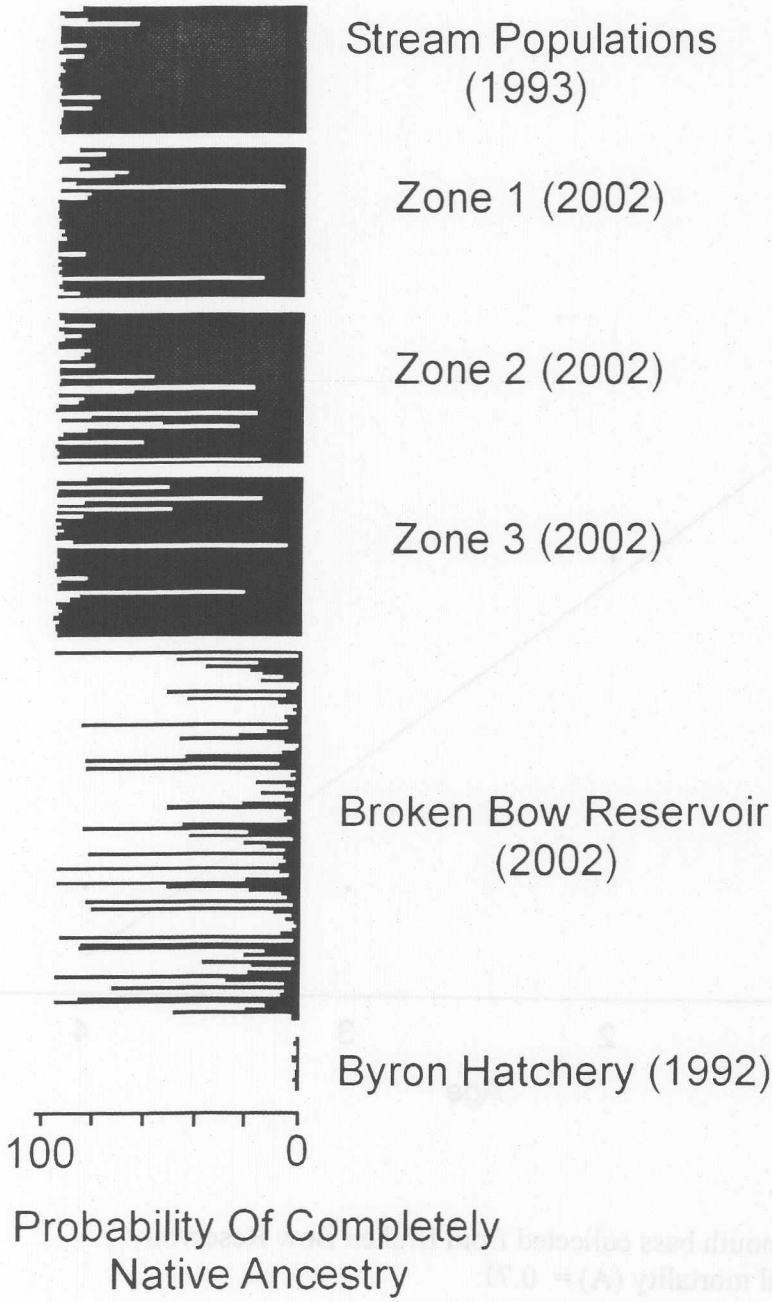

Figure 2. Probability of completely native ancestry for each specimen from the Byron Hatchery stock in 1992, two reference collections from stream populations in 1993, and Broken Bow Reservoir and three river zones in 2002. Note: this is based on only 3 loci. If more loci were sampled, the frequency of individuals with "native" genotypes would be less than indicated here, especially in Broken Bow Reservoir, where genetic structure (high frequency of non-native alleles and evidence of effectively random mating) indicates that almost every individual has non-native stock in its ancestry.

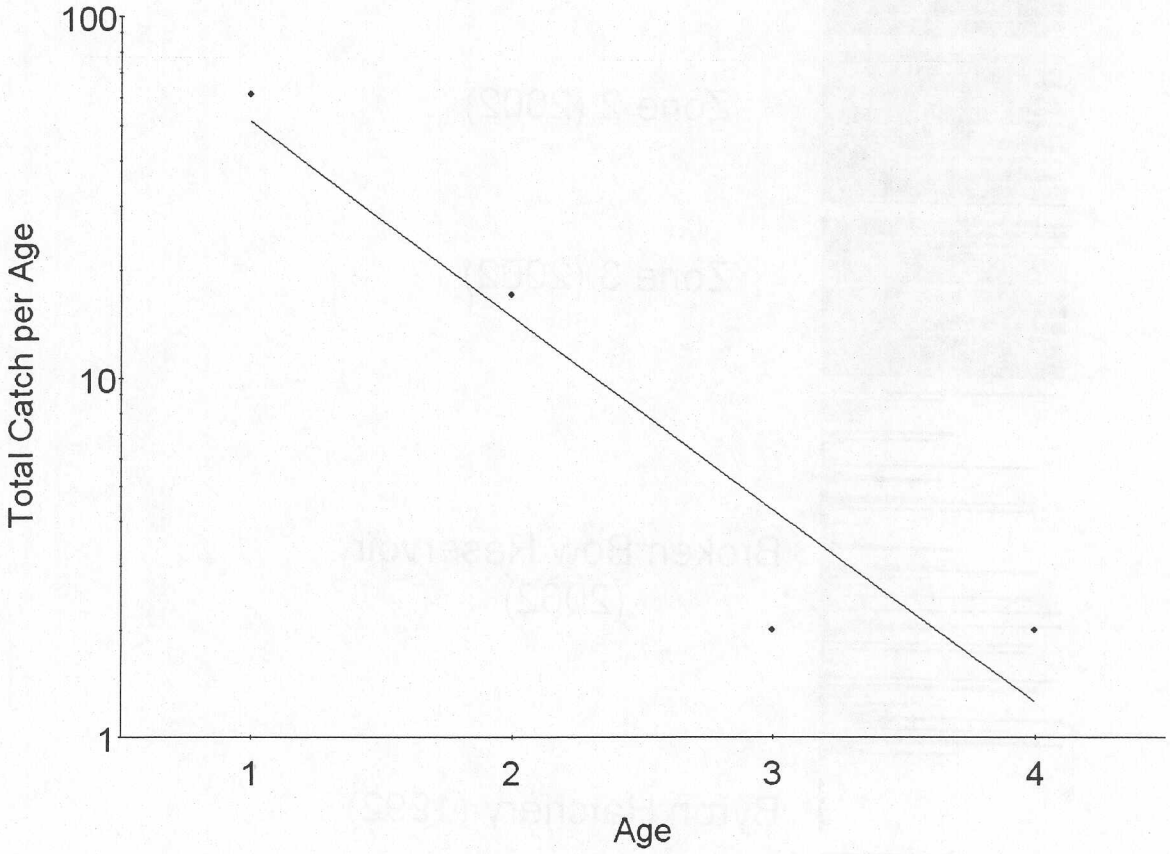

Figure 3. Catch curve for smallmouth bass collected from Broken Bow Reservoir, Oklahoma, December 2002. Total mortality (A) = 0.71.

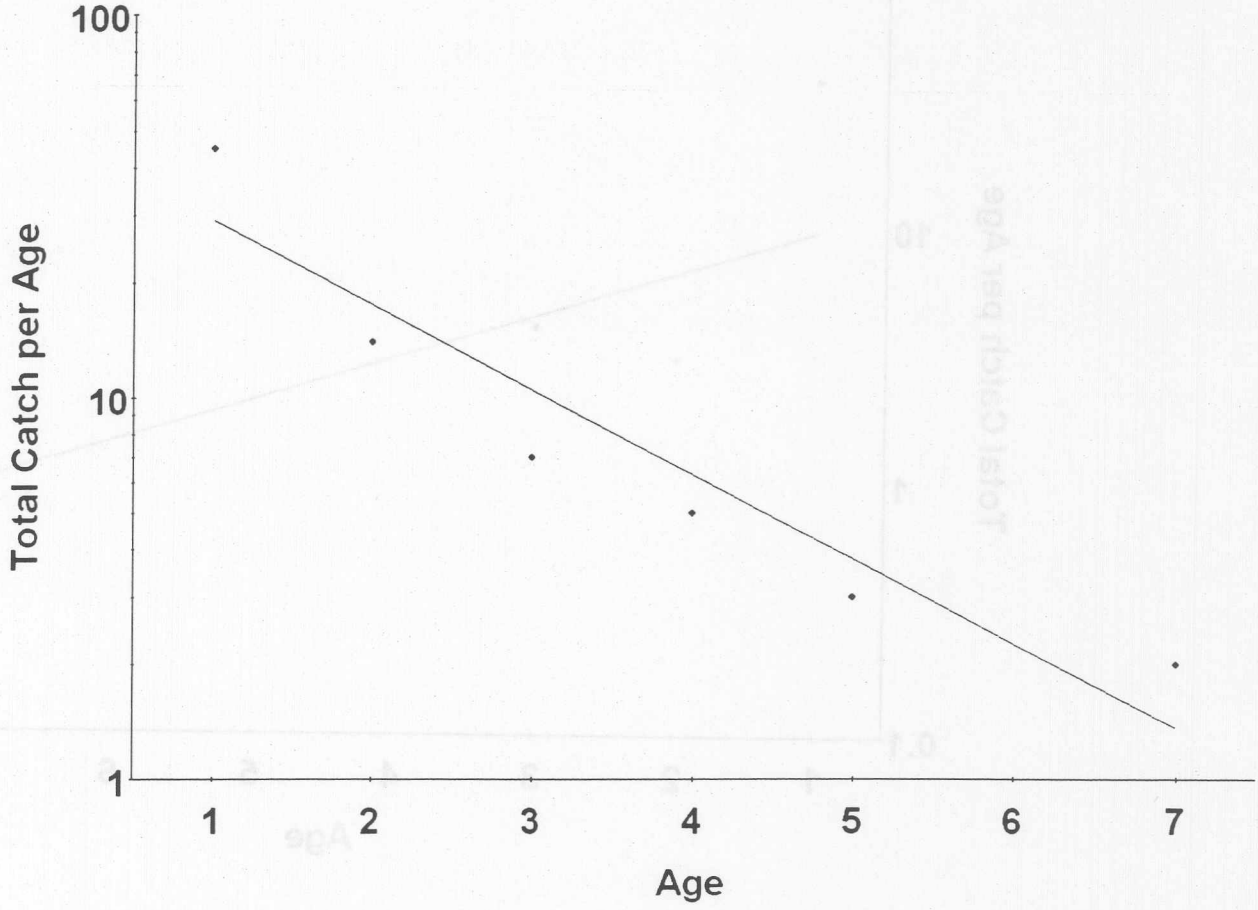

Figure 4. Catch curve for smallmouth bass collected from the Mountain Fork River upstream of Broken Bow Reservoir, Oklahoma, December 2002.
Total mortality (A) = 0.40

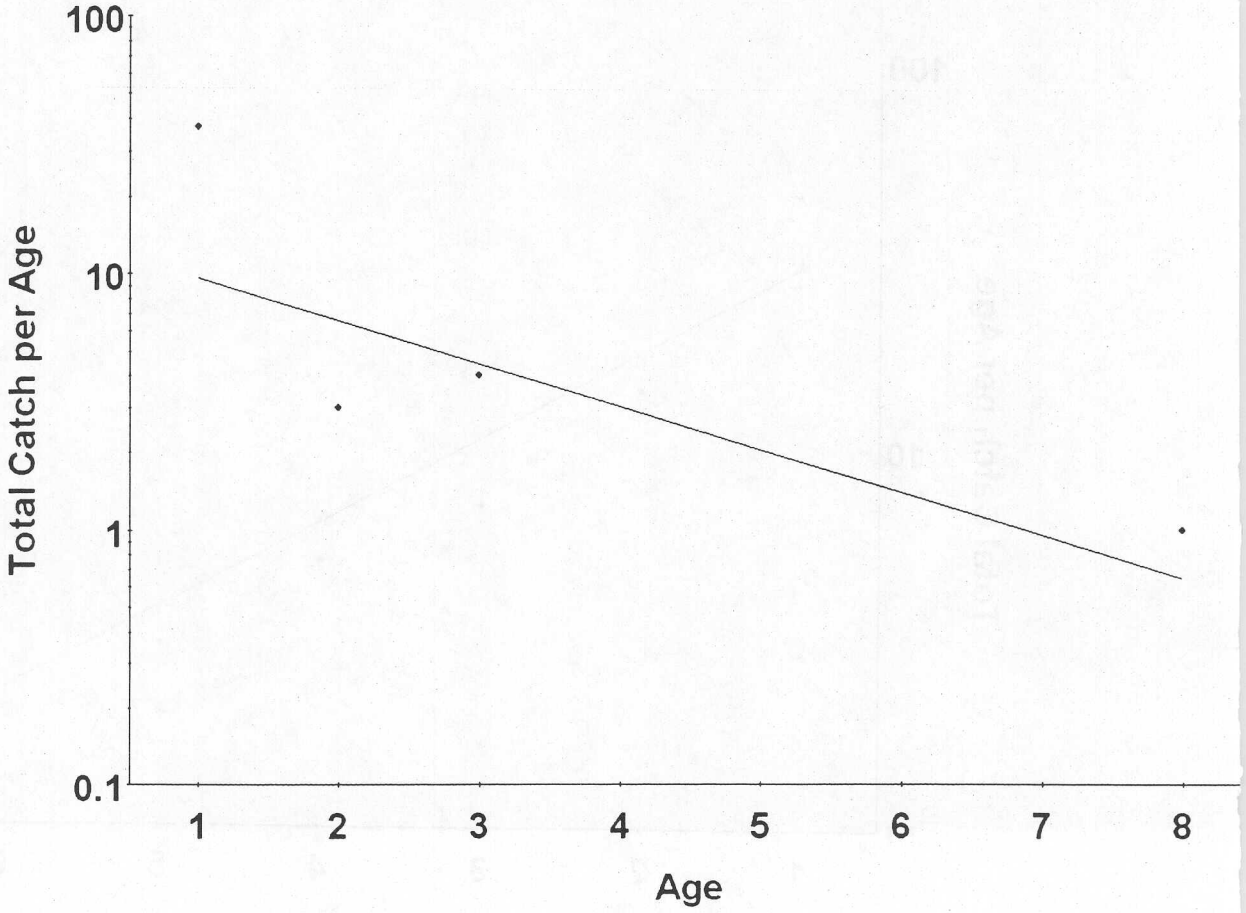

Figure 5. Catch curve for smallmouth bass from Elk River upstream of Grand Lake, Oklahoma, December 2002. Total Mortality (A) = 0.32.

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