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FINAL REPORT

RESEARCH AND SURVEYS

FEDERAL AID GRANT NO. F-41-R

FACTORS INFLUENCING FISH POPULATIONS IN OKLAHOMA WATERS

PROJECT 21

GENETIC STRUCTURE AND AGE AND GROWTH OF SMALLMOUTH BASS IN TWO OKLAHOMA RESERVOIRS

JULY 1, 1997 through JUNE 30, 2000

FINAL REPORT

STATE: OKLAHOMA GRANT NUMBER: F-41-R

GRANT TITLE: Factors Influencing Fish Populations in Oklahoma Waters

PROJECT TITLE: Genetic structure and age and growth of smallmouth bass in two Oklahoma reservoirs

PROJECT NUMBER: 21

OBJECTIVES:

1. To assess the relationship between genotype and growth characteristics of smallmouth bass *Micropterus dolomieu* in two situations where hatchery stock of the Tennessee "lake strain" has been introduced into reservoirs (Tenkiller and Broken Bow) supporting native stocks of the species.

2. To establish a protocol for using microsatellite DNAs for more precise stock identification among smallmouth bass populations.

ABSTRACT

A genetic survey across the natural range of smallmouth bass, *Micropterus dolomieu*, demonstrated that the native populations in eastern Oklahoma represent the two most divergent lineages of the species (referred to as the Ouachita and Neosho smallmouth basses). These results generated a need for knowledge regarding the genetic structure of smallmouth bass in reservoirs within the native range that had been stocked with non-native "Tennessee lake strain" smallmouth bass produced by the Byron State Fish Hatchery. We used microsatellite DNA loci to assess the genetic structure of smallmouth bass populations in two such reservoirs (Broken Bow and Tenkiller) and to test for genetic introgression of the native forms in upstream tributary populations. We identified and screened 14 microsatellite loci, three of which (*Mdo1, Mdo2, and Mdo3*) were diagnostic of the hatchery stock of the Tennessee lake strain within the range of the Ouachita smallmouth bass in Oklahoma and one of which (*Mdo1*) was diagnostic of the hatchery stock within the range of the Neosho smallmouth bass. Two of these three loci were diagnostically different between Oklahoma populations of the two native stocks, and the third locus showed marked differences in allele frequencies. The results indicated that alleles diagnostic of the Tennessee lake strain represent 41% of the genome in samples from Broken Bow Reservoir and 85-90% of the genome in samples from Tenkiller Reservoir. An analysis of growth versus genotype in the two reservoirs was

precluded because the Broken Bow sample consisted almost entirely of backcross progeny and the Tenkiller sample consisted primarily of non-native genotypes for the locus examined. However, in both reservoirs, the marked change in genetic structure coincided with an apparent increase in the abundance of smallmouth bass, indicating that the introduction of the Tennessee lake strain stimulated increased production of the species. A viable, but seemingly less likely alternative is that, in both reservoirs, the introductions happened to coincide with an unknown change in lake conditions that somehow favored increased production of smallmouth bass. Under the latter hypothesis, the present proportions of native and non-native genetic markers simply reflect the relative abundances of the two genomes during an earlier period of low production. Samples collected in 1999, six to eight years after the introductions, showed no evidence of genetic introgression. However, the persistent occurrence of a reservoir population harboring non-native alleles means that, with time, genetic introgression of stream populations is likely to occur. Such introgression represents the loss of native stocks and the management options they represent, and, in theory, it could adversely affect the smallmouth bass fishery in streams. The latter might occur as a result of the disruption of multilocus genotypes that are superior to non-native genotypes in streams or in short- or long-term, temporally variable conditions of the stream environment.

INTRODUCTION

Genetic diversity is an unrenewable, integral component of biological diversity that ultimately allows adaptation in a changing environment (Meffe, 1987). Traditionally, fisheries managers have not had the information to make well-informed, genetically based decisions, and preservation of genetic diversity typically has not been a priority in programs involving transplantation of stocks. The resultant genetic introgression between native and non-native stocks has been well documented and in many instances native genetic diversity has been threatened (Whitmore, 1983; Whitmore and Hellier, 1988; Hindar et al., 1991; Wilde and Echelle, 1992; Echelle and Echelle, 1997; Pierce and Van Den Avyle, 1997).

A recent allozyme survey across the natural range of smallmouth bass, *Micropterus dolomieu*, 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 basses (Stark and Echelle, 1998). The range of the Neosho smallmouth bass, as recognized from allozymes, closely corresponds with that of the morphologically recognized form that Hubbs and Bailey (1940) designated as the subspecies *M. d. velox*, and includes streams of the middle Arkansas River basin on the western edge of the Ozark Plateau in southeastern Missouri, northeastern Oklahoma, and northwestern Arkansas. The Ouachita smallmouth bass occupies streams of the Red River basin in the Ouachita mountains of southeastern Oklahoma and southwestern Arkansas. The allozyme survey indicated that most of the genetic diversity in smallmouth bass is

centered in the Interior Highlands. Populations in the remainder of the native range, northward into Canada and eastward into the Eastern Highlands, contributed little to the genetic diversity of the species.

In 1991 and 1992, the Oklahoma Department of Wildlife Conservation (ODWC) released 96,940 young-of-year fingerlings of the "Tennessee lake strain" of smallmouth bass into Tenkiller Reservoir (J. Smith, pers. comm.). Broodstock at the Byron State Fish Hatchery was the source of this introduction. In 1993, ODWC released 185,840 fingerlings from the same source in Broken Bow Reservoir (J. Harper, pers. comm.). Prior to these releases, Tenkiller and Broken Bow reservoirs supported native populations of, respectively, Neosho and Ouachita smallmouth bass. Partly in response to the findings of the allozyme survey by Stark et al. (1995), ODWC suspended releases of the hatchery stock in waters supporting native smallmouth bass. However, there is considerable pressure from anglers to re-institute releases (J. Smith, pers. comm.) because good quality smallmouth bass fisheries generally have not developed in eastern Oklahoma reservoirs (Gilliland, 1991) and the fishing public perceives that the Tennessee lake strain performs better in Oklahoma lakes than do native Oklahoma stocks.

Stocking of hatchery fish, together with the subsequent knowledge of the uniqueness of the native populations, created the need to assess the genetic structure of smallmouth bass in Broken Bow and Tenkiller reservoirs. There are two extreme possibilities for the existing genetic structure in both reservoirs. Non-native smallmouth bass might have expanded in abundance with relatively little genetic influence from the natives, producing a fishery composed primarily of non-natives. This could develop if the non-natives were better adapted to lake conditions and expanded in population density in the presence of a sparse native population. Alternatively, the population might be dominatd by natives if they represent the better adapted form or if there is no difference in fitness and natives were sufficiently abundant to genetically swamp the non-natives.

In this study we used variation at microsatellite DNA loci to resolve genetic structure of smallmouth bass in Broken Bow and Tenkiller reservoirs and their major tributaries. Microsatellite loci are tandem repeats of short DNA sequences, usually di-, tri-, or tetranucleotides, providing codominant, selectively neutral markers that are inherited in Mendelian fashion (Litt and Luty, 1989; Tautz, 1989; Weber and May, 1989). Microsatellite loci typically are highly polymorphic and have been useful in studies of various aspects of population structure, including social structure, hybridization, and parentage (Amos et al., 1993; Roy et al., 1994; McConnel et al., 1997; and Goostrey et al., 1998). Because of high rates of evolution, microsatellite loci are often species-specific (Colburne et al., 1996). For the present study, we developed microsatellite markers specifically for smallmouth bass. The allozyme study by Stark and Echelle (1998) revealed no loci that were diagnostically different between the Neosho smallmouth bass and the Byron Hatchery stock of the Tennessee lake strain and only a single locus was diagnostic between the later and the Ouachita smallmouth bass. It was expected that the more rapidly evolving microsatellite DNA would provide more markers identifying native versus non-native genetic material.

MATERIALS AND METHODS

Primer development and screening for diagnostic loci—Methods of cloning and characterization of microsatellite sequences followed Strassmann *et al.* (1996). Genomic DNA from the liver of a single specimen of *M. dolomieu* (New River, West Virginia) was extracted following Longmire *et al.* (1997) and digested to completion with *Alu*1, *Hae*III, and *Rsa*I. Fragments ranging from 200 to 600 bp were purified from a 1% agarose gel with a QIAquick gel extraction kit (Qiagen) and subjected to a phosphatase treatment. A partial genomic library was developed in a Bluescript II SK+ vector (Stratagene). The vector was cut at the *EcoR*V site and the ends were dephosphorylated. Competent JM109 cells of *Escherichia coli* (Promega) were transformed with the ligated vector and grown on agar plates. Colonies were lifted from plates using Hybond N+ nylon membranes (Amersham) and subsequently probed with a radioactively-labelled $(GT)_n$ oligonucleotide. Thirty-seven colonies screened positive for the $(GT)_n$ probe and Big Dye Terminator Cycle Sequencing Ready Reaction Mix (Perkin Elmer) was used to generate flourescently labeled sequence fragments from 35 of those colonies. Order and size of labeled fragments were assessed with a Perkin-Elmer, ABI Prism 373 automated sequencer and primers flanking microsatellite repeats were identified using software available at the Primer 3 internet site (http://waldo.wi.mit.edu/cgi-bin/primer/primer.cgi).

Polymerase chain reaction (PCR) conditions were optimized for 14 loci (Table 1) that were used to search for markers diagnostic of the Tennessee lake strain and the two native forms of smallmouth. This screening included 10 individuals of the Tennessee lake strain from the 1992 stock at the state hatchery at Byron, Oklahoma, and 10 from two populations each of native Neosho and Ouachita smallmouth bass (Figures 1, 2, and 3). The reference collections of native Neosho smallmouth were collected in 1992 from Illinois River 2.0 km N, 1.2 km E of the intersection of State Highway 10 and U.S. Highway 62, Cherokee County, Oklahoma (T 17N, R 22E, S 24 NE1/4) and from Baron Fork River 1.2 km S, 0.4 km E of Welling, Cherokee County, Oklahoma (T 16N, R 23E, S 18 NE1/4). The reference collections of native Ouachita smallmouth were collected in 1993 from Mountain Fork River near the town of Jet, McCurtain County, Oklahoma (T 1S, R 27E, S 9), and from Buffalo Creek 1.6 km S, 0.8 km E of Buffalo, McCurtain County, Oklahoma (T 2S, R 26E, S 17).

PCR was performed in 15-µl volumes with approximately 60 ng of genomic DNA extracted (Longmire et al., 1997) from muscle or liver tissue, Perkin-Elmer's True Allele premix, and primers end-labeled with $\gamma^{32}P$. All PCR reactions were carried out in a Perkin-Elmer 9600 thermal cycler using the following profile: an initial 12-min denaturation at 95 °C, followed by 10 cycles at 94 °C for 15 s, 55 °C for 60 s, 72 °C for 30 s, followed by 25 cycles at 89 °C for 15s, 55 °C for 60 s, and 72 °C for 30 s. The profile ended with a 30-min elongation at 72 °C. PCR products were separated by electrophoresis in 4% denaturing polyacrylamide gels and bands were visualized using autoradiography. Differences in relative mobility were used to identify variant alleles and

only unambiguous bands were scored. To maintain scoring consistency across gels, all known variants were included on each gel.

Analysis of Genetic structure—Gillnetting and electrofishing during the Spring and Fall of 1998 produced 167 smallmouth bass from Broken Bow Reservoir and electrofishing during the Spring and Fall of 1999 produced 110 smallmouth bass from Tenkiller Reservoir. Most of the sample from Broken Bow Reservoir was from the west side of the reservoir within 6.7 km of the dam. Collections were taken from coves "1" (n = 57), "2" (n = 28), and "3" (n = 23) (Figure 4). We do not have exact locality data for a group ("ODWC"; n = 47) collected from several sites in the spring of 1998. Finally, a group labeled "other" (n = 12) was collected from scattered localities in the lower end of the reservoir. We acquired the majority of our Tenkiller Reservoir collection (n = 96) by electrofishing rocky points on the lower west side of the reservoir within 15 km of the dam. Fourteen individuals were taken on the east side within 15 km of the dam.

To assess levels of genetic introgression in streams associated with the two reservoirs, we made a rod-and-reel collection (n = 23) of smallmouth bass in August 1999 from Baron Fork River, 0.5 km SE of Eldon at Eldon Bridge, Cherokee County, Oklahoma, and, in October 1999, we used a backpack electrofishing unit and seines to collect 20 smallmouth bass from Mountain Fork River, 1.6 km E of Smithville, McCurtain County, Oklahoma.

Liver or muscle tissue was sampled from each individual and DNA was extracted following Longmire et al. (1997). PCR amplification and visualization of diagnostic microsatellite loci followed the protocol described above for the screening process. For genotype assignment, shared alleles with markedly higher frequencies in the hatchery stock than in the reference populations were considered non-native. GENEPOP (Raymond and Rousset, 1995) was used to test for within-collection departures from Hardy-Weinberg expectations for single-locus genotypic frequencies (exact HW test; Guo and Thompson, 1992), allele frequency differences between collections (Fisher exact test), and linkage disequilibrium within collections (Fisher exact test using a Markov chain method with 1000 iterations), and to calculate pairwise F_{ST} values as a measure of genetic divergence among groups (Weir and Cockerham, 1984). In some of these analyses, the within-reservoir data were stratified by location or age. The sequential Bonferroni correction (Rice, 1989) for Type I error ($\alpha = 0.05$) in multiple tests was applied throughout the analysis.

Scales were collected from smallmouth bass from Broken Bow Reservoir smallmouth bass and both scales and otoliths were collected from those from Tenkiller Reservoir. Total length to the nearest mm was recorded for each fish. For analysis, scales were pressed on acetate slides and otoliths were set in an epoxy resin (Electron Microscopy Sciences) and sectioned laterally. Scales and sectioned otoliths were magnified, and numbers of annuli were recorded. Estimates of age for the scale samples from Broken Bow Reservoir were calibrated by comparison with estimates from scales and otoliths for each of 20 individuals collected in 1999.

RESULTS

Diagnostic Loci--Alleles at three loci (*Mdo1*, *Mdo2*, and *Mdo3*) were considered diagnostic of one or another of the three forms--Neosho and Ouachita smallmouth bass and the Byron State Fish Hatchery stock of the Tennessee lake strain (Table 2). All three loci were diagnostically useful between the hatchery stock and the Ouachita smallmouth. The reference collections of these two stocks indicated two native and four non-native *Mdo1* alleles, whereas, for both *Mdo2* and *Mdo3*, the results indicated one native and four non-native alleles. A sixth allele (F) for both *Mdo2* and *Mdo3* occurred in the reservoir population (Table 3) but not found in the reference collections; it was designated unknown in origin. Although *Mdo3* allele B was shared between reference samples of the two forms, it was designated non-native because its frequency in native populations (0.000 and 0.025; mean = 0.013) was an order of magnitude lower than in the hatchery stock (0.100).

Only one of the three loci (*Mdo1*) was diagnostically useful in separating the reference collections of Neosho smallmouth bass and the hatchery stock. The survey of reference collections indicated one native allele, two non-native alleles, and two alleles (B and E) of unknown origin (Table 2). Allele B was designated unknown in origin because it occurred at low frequencies in the two collections from native populations of Neosho smallmouth bass (frequencies = 0.00 and 0.04) and in the hatchery collection (0.05); a similar designation for allele E, which occurred at a low frequency (0.06) in the reservoir, was based on its absence in reference collections of both forms.

Two loci (*Mdo2* and *Mdo3*) were diagnostically useful in separating the reference collections of Neosho smallmouth bass from the reference collections of Ouachita smallmouth bass, although there was a low degree of allele sharing for both loci (Table 2). A third locus (*Mdo1)* showed marked allele frequency differences between the two forms, but there was broad overlap in allele frequencies.

Genetic Structure of Populations in Broken Bow Reservoir Area--All subpopulations in Broken Bow Reservoir were segregating for alleles characteristic of both the Byron Hatchery stock and native Ouachita smallmouth bass at all three diagnostic microsatellite loci (Table 3). The frequencies of native alleles were markedly reduced in all reservoir collections. Correspondingly, alleles diagnostic of the hatchery stock exhibited high frequencies in collections from the reservoir and occasionally exceeded the frequency of native alleles at individual loci. The post-introduction collection from Mountain Fork River in 1999 showed no evidence of non-native alleles at the three loci examined (*Mdo1*, *Mdo2*, and *Mdo3*).

Only one of 30 tests (10 tests for three loci) revealed a significant difference in allele frequencies among collections from the reservoir (Table 4). Correspondingly, F_{ST} values among pairs of these collections were near zero (Table 5). There also were no significant differences in allele frequencies among age classes (0+, 1+, 2+, and 3+) in the reservoir.

Pairwise tests for allele frequency differences consistently indicated significant ($P < 0.001$) differences between the hatchery collection and collections from Broken Bow Reservoir and the associated stream populations (Table 4). Differences between the reference collections from native populations in Mountain Fork River and Buffalo Creek were not significant ($P = 0.11 - 1.00$). However, 4, 10, and 6 of the 10 tests comparing those populations with the reservoir population revealed significant differences at respectively, *Mdo1*, *Mdo2*, and *Mdo3* (Table 4). In contrast, the post-introduction collection from Mountain Fork River in 1999 did not differ significantly from the pre-introduction collections taken from Mountain Fork ($P = 0.11 - 0.28$) and Buffalo Creek ($P = 0.48 - 0.73$) in 1993. Pairwise F_{ST} values (Table 5) indicated that the reservoir population was genetically intermediate between the hatchery collection and the reference collections from native populations.

None of the five collections of smallmouth bass from the reservoir showed significant deviation from Hardy-Weinberg expectation. However, combining these into a single sample produced a significant heterozygote deficiency at one of the three loci (*Mdo3*, $P = 0.01$). No significant linkage disequilibrium was detected among the three loci in any of the five subpopulations. When all subpopulations were grouped as a single sample, significant linkage disequilibrium was detected between *Mdo2* and *Mdo3* ($P = 0.007$) and between *Mdo3* and *Mdo1* ($P = 0.000$).

The ranges in frequency of non-native alleles at *Mdo1*, *Mdo2*, and *Mdo3* were respectively, 17-54%, 40-58%, and 17-40% in the five reservoir collections (Table 3). The frequency of non-native alleles in the combination of all samples in the reservoir was 38%, 50%, and 35%, respectively for *Mdo1, Mdo2*, and *Mdo3* The average of these (41%) is an estimate of overall level of genetic introgression in the smallmouth bass of Broken Bow Reservoir.

Genetic Structure of populations in the Tenkiller Reservoir Area--The Tenkiller Reservoir population was segregating for alleles diagnostic of both the Byron Hatchery stock and native Neosho smallmouth bass at *Mdo1*, the one diagnostic locus detected for this area (Table 2). The frequency of *Mdo1* allele D in the two native populations was 1.00 (Baron Fork River) and 0.93 (Illinois River). This allele was not detected in the hatchery sample and its frequency in the post-introduction reservoir population was only 0.06 (Table 6). The frequency of *Mdo1* allele F was almost identical in collections from the hatchery (0.65) and the reservoir (0.63). Correspondingly, allele frequencies in the two collections were not significantly different ($P = 0.37$).

The reservoir sample did not deviate significantly from Hardy-Weinberg expectation for genotypic frequencies. Allele frequency differences were significant between the reservoir sample and the pre- and post-introduction stream samples ($P = 0.000$), but not between samples from the reservoir and the hatchery collection ($P = 0.36$) or between pre- and post-introduction stream collections ($P = 0.14$ and 1.00). Pairwise F_{ST} values indicated high genetic divergence between the stream populations and the post-introduction lake population ($F_{ST} = 0.528 - 0.572$) and low divergence between the hatchery collection and the post-introduction reservoir population ($F_{st} = 0.017$).

The frequency of non-native alleles for *Mdo1* in the collection from Tenkiller Reservoir was 85%. The frequency may be as high as 90%, however, because of the presence of allele E. This allele was absent in all reference collections and may be a non-native allele that, as a result of sampling error, was not included in the Byron Hatchery collection.

Genotype vs Age--Age distributions in our collections from Broken Bow and Tenkiller reservoirs are presented in Figure 5. There were no significant age group differences in genotypic distributions in either of the two reservoir collections (heterogeneity chi-square test: Broken Bow reservoir, $\chi^2 = 10.3$, 6 d.f., P = 0.11; Tenkiller Reservoir, $\chi^2 = 0.235$, 2 d.f., P = 0.89). Within the Broken Bow collection, ages were estimated for 86 fish whose genotypes were scorable across all three loci. These included 80 (93%) hybrid genotypes (i.e., native/non-native heterozygotes at one or more loci across the three loci examined), and 6 homozygotes for native alleles at all loci (Table 7). No fish were homozygous for non-native alleles at all loci. Because of the rarity of parental genotypes, we were unable to make meaningful comparisons of growth rate versus genotype.

Comparison of growth rates among genotypes within the Tenkiller population was also problematic. After eliminating individuals carrying alleles of unknown origin (native vs non-native), 87% of 86 fish were homozygous for non-native alleles, 13% were heterozygous for native and non-native alleles, and none were homozygous for native alleles (Table 8). With only a single diagnostic locus, it is not possible to separate homozygous non-native fish from backcross progeny. Therefore, comparisons of growth rate versus genotype were not made.

DISCUSSION

Our results indicate that the introductions of Tennessee lake strain of smallmouth bass in Broken Bow and Tenkiller reservoirs resulted in random-mating admixtures of native and non-native stocks, with 41% and 85-90%, respectively, of the genome comprising non-native alleles. Personnel of the Oklahoma Department of Wildlife conservation report that, in both reservoirs, the smallmouth bass fishery was unsatisfactory prior to the introductions and that the fishery has improved in recent years (J. Smith , pers. comm.; J. Harper, pers. comm.). Correspondingly, unpublished data from annual surveys by the Oklahoma Department of Wildlife Conservation indicate a marked post-introduction increase in the abundance of smallmouth bass in Broken Bow Reservoir (Figure 6). Similar surveys in Tenkiller Reservoir were inadequate to assess trends in abundance of smallmouth bass because the established sampling stations were in areas where habitat conditions are not conducive to smallmouth bass production (J. Smith, pers. comm.). The high abundances of non-native alleles, together with the suggested increases in the abundance of smallmouth bass, indicate that, in both reservoirs, the introduced genome may have stimulated increased production of smallmouth bass. A seemingly less likely alternative is that environmental conditions

have changed in some unknown way to favor smallmouth bass production in both reservoirs and that the present proportions of native and non-native genetic markers simply reflect the relative abundance of the two genomes during an earlier period of poor production. Because of the age structure of our samples (mostly age 0 or 1) and the existing genetic structure in both reservoirs, we were unable to compare growth performance of the native forms with that of the non-native form and hybrids between the two.

The large changes in gene frequencies detected in Tenkiller and Broken Bow reservoirs occurred in a remarkably short period of time considering that the reservoirs supported native populations prior to the introductions. At the time of our collections, the 1991 and 1992 year-classes of fingerlings introduced into Tenkiller Reservoir would have been six to seven years old and the 1993 year-class introduced into Broken Bow Reservoir would have been five years old. If reproductive maturity is reached between ages 2 and 5 (Carlander, 1977), then the contribution of non-native genetic material to our collections reflects survival of the introduced fingerlings and a maximum of seven seasons of reproduction by non-natives and their progeny in Tenkiller Reservoir and four seasons in Broken Bow Reservoir.

The schedule of reproduction just described is somewhat inconsistent with the genetic structure of age groups in our collections of smallmouth bass from Broken Bow Reservoir (Table 7). If the introduced non-natives reached maturity as early as age 2, then fish older than the 1997 year-class (i.e., fish > age 1) should have included only the two parental types and first-generation hybrids. In contrast, six of the 10 such fish were backcross progeny. Support for this discrepancy is rather weak because of the small sample size of older fish and the potential for error in the estimates of age. Because only one diagnostic locus was available for the fish in Tenkiller Reservoir, we could not evaluate age groups for relative abundance of parental types, first generation hybrids, and backcross progeny in this population.

After a few generations of random mating, and for the moment ignoring immigration from streams, essentially all smallmouth bass individuals in both reservoirs will be "hybrids" containing mixed genetic material from native and non-native parental stocks. This is true even of Tenkiller Reservoir where the frequency of non-native alleles was 85-90% for the one available diagnostic locus for this population. The diploid chromosome number of smallmouth bass is 46 (Roberts, 1964), and if the frequency of non-native chromosomes equals the maximum estimated frequency (0.90) of non-native alleles at the one locus we examined in Tenkiller Reservoir, then the probability of encountering a genetically pure native smallmouth bass in the reservoir is roughly 8 in 1000 fish (0.90^{46}). Eventually, this probability erodes to zero because of genetic recombination, a factor that will eventually eliminate all genetically pure native and non-native chromosomes.

The estimates just given will apply once the populations have reached equilibrium frequencies for multilocus genotypes, an assumption that we could not test for Tenkiller Reservoir because only one diagnostic locus was detected in the comparison of Neosho smallmouth bass with the hatchery stock. The significant linkage disequilibrium detected

in the lumped samples from Broken Bow Reservoir might be an effect of the recent introductions and/or immigration of natives from adjacent streams. Alternatively, and because it was detected only in the aggregate sample of all specimens from the reservoir, it might be an effect of spatial heterogeneity in allele frequencies among local subpopulations.

The results indicate that, as of 1999, stream populations associated with Broken Bow and Tenkiller reservoirs supported native populations with no evidence of genetic introgression. With time, however, such introgression is likely to occur. For largemouth bass, an allozyme survey indicated that the introgression of native populations in streams by the introduced Florida subspecies was limited primarily to streams in the vicinity of reservoirs and to areas of southern Oklahoma where (Gilliland, 1994) the Florida subspecies shows higher rates of survival (Gelwick et al., 1995). It was suggested, however, that the geographical distribution of introgressed largemouth bass in streams "could be broader in the future than at present, especially where conditions favor maintenance of Florida alleles" (Gelwick et al., 1995:560). The rate of genetic introgression for native smallmouth bass populations in Oklahoma may be considerably faster than in largemouth bass because the former are restricted to relatively small streams upstream of large reservoirs.

In both Tenkiller and Broken Bow reservoirs, the highest densities of smallmouth bass apparently occur in down-reservoir areas (J. Smith and J. Harper, pers. comm.), a situation that also occurs in Lake Skiatook, another Oklahoma reservoir (Fisher et al., 2000; Long, 2000). Thus, up-reservoir reaches may present a partial barrier to populations. This would extend the time interval for any management actions aimed at protecting the genetic integrity of stream populations.

The rate of spread of non-native genes into streams would be lower when streams support denser populations of native smallmouth bass. However, population densities are temporally variable, particularly in streams where conditions are more variable than they are in reservoirs. For example, with the return of stream-flows following droughts, streams may be more susceptible to invasion from the reservoir population. This predicts that the genetic structure of stream populations will be more affected by the reservoir population than vice versa. Even in the absence of invasion of steams by hybrids, the presence of a reservoir population of hybrids potentially threatens native forms with extinction. An intervening hybrid population forms a barrier to gene flow among native populations, resulting in gradual losses of genetic diversity (Allendorf and Leary, 1988), a factor that would be especially important for small populations in streams tributary to the reservoir. In addition, models of population structure predict that long-term, local persistence of a form is dependent on immigration from elsewhere because stochastic or deterministic factors eventually cause local extinctions (Levins, 1970; Pulliam, 1988) or because aspects of the natural "aquatic landscape" (Schlosser, 1995), such as availability of refuges in harsh times (e.g., downstream areas in times of drought), are essential for persistence. The presence of a reservoir population of hybrids effectively precludes such immigration.

Although introductions of the Tennessee lake strain might improve the smallmouth bass fishery in Oklahoma reservoirs, there is some potential for adverse effects on the stream fishery as a result of outbreeding depression. In a study of outbreeding depression in *Micropterus*, native largemouth bass in Illinois had significantly greater overwinter survival and second- and third-year growth rates than did the non-native Florida largemouth, and F_1 hybrids were intermediate in these characteristics (Phillip and Whitt, 1991). Reduced fitness in hybrid and domesticated salmonids has also been documented (Chilcote et al., 1986; Skaala et al., 1996; Poteaux et al., 1998; and Hansen et al., 2000). For smallmouth bass in Oklahoma, some native elements of the genome (single or multilocus genotypes) may be superior to non-native elements in streams, or in certain short- or long-term, temporally variable conditions of the stream environment. Dilution or disruption of such genotypes by genetic introgression might cause a decline of the smallmouth bass populations in streams through reduced average fitness and /or loss of locally adaptive characteristics.

SUMMARY AND CONCLUSIONS

1. The 14 microsatellite-DNA primer-sets developed in this study will be useful in future studies of genetic structure in micropterine basses and some of the primer sets should be useful in studies of other centrarchids. [Note: The primers are being used in an ongoing study of spotted bass *M. punctulatus* (F-41-R-22: project 22). All primer sets developed for *M. dolomieu* worked successfully with *M. punctulatus* (Malloy et al., 2000).].

2. We identified three microsatellite-DNA loci (*Mdo1, Mdo2, and Mdo3*) that were diagnostic of the Byron State Hatchery stock of the Tennessee lake strain within the range of the Ouachita smallmouth bass in Oklahoma and one locus (*Mdo1*) that was diagnostic of the hatchery stock within the range of the Neosho smallmouth bass. Two loci were diagnostically different between the two native stocks, and a third showed marked differences in allele frequencies.

3. Introduction of the Tennessee "lake strain" of smallmouth bass into Broken Bow and Tenkiller reservoirs had a marked effect on the genetic structure of the reservoir populations of native smallmouth bass. The results indicate that non-native alleles represent 85-90% of the genome in samples from Tenkiller Reservoir and 41% of the genome in samples from Broken Bow Reservoir.

4. The change in the genetic structure of smallmouth bass in Tenkiller and Broken Bow reservoirs coincided with a perceived improvement in the smallmouth bass fishery in both reservoirs. This suggestion is supported by the annual ODWC black-bass survey data for Broken Bow Reservoir. Because of the placement of sampling stations, the corresponding surveys for Tenkiller Reservoir were inadequate for assessment of trends in smallmouth bass abundance. The genetic change, together with the perceived improvement in the fishery, suggests that the

introduction of the Tennessee lake strain may have stimulated increased production of smallmouth bass. A viable, but less likely alternative is that, in both reservoirs, the introduction of non-native smallmouth bass happened to coincide with an unknown change in lake conditions that somehow favored increased production of smallmouth bass. Under the latter hypothesis, the present proportions of native and non-native genetic markers simply reflect the relative abundances of the two genomes during an earlier period of low production.

5. In a few generations of random mating, all individuals in both reservoirs probably will carry mixed native/non-native genotypes except for immigration (or passive transport) of natives from streams tributary to the reservoir.

6. In 1999, six to eight years following introductions of non-native smallmouth bass, samples from stream populations associated with both reservoirs apparently represented native populations with no evidence of genetic introgression by non-native smallmouth bass. With time, however such introgression is likely to occur.

7. A concern from the fishery viewpoint is that genetic introgression of the native smallmouth bass may lead to a less productive stream fishery as a result of outbreeding depression.

8. Also of concern is that the presence of reservoir populations threatens the existence of native strains of smallmouth bass in Oklahoma and any future management options they might represent.

RECOMMENDATIONS

1. Perform lakewide surveys of the abundance and levels of genetic introgression of smallmouth bass. If the introgressed smallmouth bass population is relatively small, not growing, and primarily confined to down-reservoirs areas, then managers might be able to manage the fishery in such a way as to minimize the threat to the genetic integrity of stream populations.

2. Continue monitoring the abundance and condition of genetically introgressed smallmouth bass populations. A high quality fishery may depend on growth and reproduction of the non-native form and/or F_1 hybrids. If so, then, the quality of the fishery may decline as a result of genetic recombination. This may be particularly true of the Broken Bow population, which has a relatively high frequency of native genetic material.

3. Consider the feasibility of a stocking program that utilizes native brood stock to dilute non-native genetic material in reservoirs within the native ranges of the Neosho and Ouachita smallmouth basses. The present 60:40 ratio of native to non-native genetic material in Broken Bow Reservoir appears to be associated with an increased abundance of smallmouth bass, suggesting that higher ratios could do equally well.

4. Monitor stream populations for non-native alleles. The rate and extent of genetic introgression will be of interest in future decisions regarding introductions of non-native stocks into reservoirs.

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Table 1. Microsatellite primer sequences, size of cloned allele, and core repeat of 14 microsatellite loci isolated from *Micropterus dolomieu*.

Locus	Primer Sequence (5′ – 3′)	Size (bp)	Core Repeat
Mdo 1	GCTCTTCCCAGTGGTGAGTC*	210	$(GT)_{14}$
	ATCTCAGCCCATACCGTCAC		
Mdo 2	GCCCTTTCATATTGGGACAA*	197	$(GT)_{14}$
	CTGCTCTGGCGTACATTTCA		
Mdo 3	AGGTGCTTTGCGCTACAAGT*	135	$(CA)_{20}$
	CTGCATGGCTGTTATGTTGG		
Mdo 4	TCTGAACAACTGCATTTAGACTG*	142	$(CA)_{11}$
	CTAATCCCAGGGCAAGACTG		
Mdo 5	CAGGTTCCCTCTCACCTTCA*	200	$(CT)_9CC(CA)_{10}$
	ATGGTCTCACCAGGGACAAA		$GA(CA)_3TA(CA)_2$
Mdo 6	TGAAATGTACGCCAGAGCAG*	150	$(CA)_7(TA)_4$
	TGTGTGGGTGTTTATGTGGG		
Mdo 7	TCAAACGCACCTTCACTGAC*	172	$(CA)_{12}$
	GTCACTCCCATCATGCTCCT		
Mdo 8	GTGAGGACCAGCCAAAATGT*	220	$(CA)_{19}$
	GGAAGATTGAGGTCCCAACA		

Table 1. (cont.)

Mdo 9	TTTGATGGGCGTTTTGTGTA*	126	$(GT)_{10}$
	GACCGGTCCTGCATATGATT		
Mdo 10	GTGTCTCCGTGTGTTGATGG*	101	$(GT)_{10}$
	AACACCAGAGGCAAACAAGC		
Mdo 11	TTGTGGAGAGGGGCATAAAC*	174	$(GT)_{11}GA(GT)_3$
	GCATCCTCCCACGTTACCTA		
Mdo 12	CACCCTCCCTCTCTTCCTCT*	114	$(CA)_4CTCTAA$
	CCATCAACACACGGAGACAC		$(CA)_{17}$
Mdo 13	CCTTCACCTCACCACCACTT*	157	$(CA)_{35}$
	AACAAAGACCCACATGAGCC		
Mdo 14	GTAACACCATGCACAAGGGA*	100	$(GT)_{18}$
	GTGTGGGTGATGAGGGAGTC		

* radiolabeled primer

Table 2. Allele frequencies at three diagnostic microsatellite loci (*Mdo1, Mdo2, and Mdo3)* in reference collections of native Ouachita and Neoho smallmouth bass and the hatchery stock of the Tennessee lake strain.

	Alleles					
Mdo1	A	B	C	D	E	F
Ouachita smallmouth, 1993						
Mountain Fork (n = 19)	0.000	0.000	0.000	0.579	0.421	0.000
Buffalo Creek (n = 20)	0.000	0.000	0.000	0.375	0.600	0.025[a]
Neosho smallmouth, 1992						
Baron Fork (n = 15)	0.000	0.000	0.000	1.000	0.000	0.000
Illinois River (n = 14)	0.000	0.036	0.000	0.928	0.000	0.036[a]
Byron Hatchery (n = 10)	0.250	0.050	0.050	0.000	0.000	0.650

	Alleles					
Mdo2	A	B	C	D	E	F
Ouachita smallmouth,1993						
Mountain Fork (n = 18)	0.028[a]	0.000	0.000	0.972	0.000	0.000
Buffalo Creek (n = 20)	0.100[a]	0.000	0.000	0.900	0.000	0.000
Neosho smallmouth, 1992						
Baron Fork (n = 15)	0.000	0.000	1.000[a]	0.000	0.000	0.000
Illinois River (n = 14)	0.072[a]	0.035[a]	0.893[a]	0.000	0.000	0.000
Byron Hatchery (n = 10)	0.700	0.150	0.100	0.000	0.050	0.000

	Alleles					
Mdo3	A	B	C	D	E	F
Ouachita smallmouth,1993						
Mountain Fork (n = 17)	0.000	0.000	0.059[a]	0.000	0.941	0.000
Buffalo Creek (n = 20)	0.000	0.025[a]	0.075[a]	0.000	0.900	0.000
Neosho smallmouth, 1992						
Baron Fork (n = 15)	0.000	0.000	0.033[a]	0.967[a]	0.000	0.000
Illinois River (n = 14)	0.000	0.000	0.071[a]	0.893[a]	0.036	0.000
Byron Hatchery (n = 10)	0.100	0.100	0.300	0.500	0.000	0.000

[a] Presence of "non-native" alleles is assumed to represent allele sharing between native smallmouth basses and the hatchery stock—not results of introductions.

Table 3. Allele frequencies (*Mdo1, Mdo2, and Mdo3)* in post-introduction collections from Broken Bow Reservoir and its major tributary, Mountain Fork River. Bold values indicate assumed occurrence of introduced alleles, based on distributions of alleles in reference collections (Table 2; see text).

	Alleles						
Mdo1	A	B	C	D	E	F	Introduced
BrokenBow Reservoir, 1998							
Cove A (n = 46)	**0.098**	**0.076**	**0.065**	0.239	0.218	**0.304**	**0.543**
Cove B (n = 23)	**0.044**	0.000	0.000	0.391	0.435	**0.130**	**0.174**
Cove C (n = 6)	**0.083**	0.000	0.000	0.250	0.500	**0.167**	**0.250**
ODWC (n = 34)	**0.074**	0.000	**0.015**	0.294	0.367	**0.250**	**0.339**
Other (n = 4)	0.000	0.000	0.000	0.375	0.375	**0.250**	**0.250**
All samples (n = 113)	**0.075**	**0.031**	**0.031**	0.292	0.327	**0.244**	**0.381**
Mountain Fork, 1999 (n = 20)	0.000	0.000	0.000	0.450	0.550	0.000	0.000

	Alleles						
Mdo2	A	B	C	D	E	F	Introduced
Broken Bow Reservoir, 1998							
Cove A (n = 53)	**0.274**	0.000	**0.245**	0.396	**0.057**	0.028	**0.576**
Cove B (n = 24)	**0.292**	0.000	**0.188**	0.479	**0.021**	0.021	**0.500**
Cove C (n = 21)	**0.381**	0.000	**0.143**	0.452	**0.000**	0.024	**0.524**
ODWC (n = 45)	**0.156**	**0.022**	**0.189**	0.567	**0.033**	0.033	**0.400**
Other (n = 10)	**0.350**	0.000	**0.050**	0.500	**0.050**	0.050	**0.450**
All samples (n = 153)	**0.261**	**0.007**	**0.193**	0.474	**0.036**	0.029	**0.497**
Mountain Fork, 1999 (n = 20)	0.150	0.000	0.000	0.850	0.000	0.000	0.000

	Alleles						
Mdo3	A	B	C	D	E	F	Introduced
Broken Bow Reservoir, 1998							
Cove A (n = 48)	**0.042**	**0.063**	**0.156**	**0.135**	0.594	0.010	**0.396**
Cove B (n = 24)	**0.125**	0.000	**0.063**	**0.167**	0.604	0.042	**0.355**
Cove C (n = 9)	**0.111**	0.000	**0.056**	**0.111**	0.722	0.000	**0.278**
ODWC (n = 46)	**0.120**	**0.022**	**0.065**	**0.152**	0.620	0.021	**0.359**
Other (n = 9)	0.000	**0.056**	**0.111**	0.000	0.833	0.000	**0.167**
All Samples (n = 136)	**0.085**	**0.033**	**0.100**	**0.136**	0.628	0.018	**0.354**
Mountain Fork, 1999 (n = 20)	0.000	0.000	0.150	0.000	0.850	0.000	0.000

Table 4. Probabilities for pairwise tests of allele frequency differences at three microsatellite loci. Values in bold represent significance with sequential Bonferroni correction (tablewide $\alpha = 0.05$) for the blocks indicated.

Populations	Loci		
	Mdo1	*Mdo2*	*Mdo3*
Hatchery vs Mountain Fork	**0.000**	**0.000**	**0.000**
Hatchery vs Buffalo Creek	**0.000**	**0.000**	**0.000**
Hatchery vs Cove 1	**0.001**	**0.000**	**0.000**
Hatchery vs Cove 2	**0.000**	**0.000**	**0.000**
Hatchery vs Cove 3	**0.000**	**0.000**	**0.000**
Hatchery vs ODWC	**0.000**	**0.000**	**0.000**
Hatchery vs Other	**0.001**	**0.000**	**0.000**
Mountain Fork vs Buffalo Creek	0.113	0.355	1.000
Mountain Fork vs Cove 1	**0.000**	**0.000**	0.007
Mountain Fork vs Cove 2	0.032	**0.000**	**0.003**
Mountain Fork vs Cove 3	0.010	**0.000**	0.019
Mountain Fork vs ODWC	**0.000**	**0.000**	**0.004**
Mountain Fork vs Other	0.044	**0.000**	0.286
Buffalo Creek vs Cove 1	**0.000**	**0.000**	0.009
Buffalo Creek vs Cove 2	0.108	**0.000**	**0.001**
Buffalo Creek vs Cove 3	0.070	**0.000**	0.024
Buffalo Creek vs ODWC	**0.001**	**0.001**	**0.002**
Buffalo Creek vs Other	0.087	**0.000**	0.514
Cove 1 vs Cove 2	**0.002**	0.788	0.054
Cove 1 vs Cove 3	0.457	0.263	0.521
Cove 1 vs ODWC	0.034	0.079	0.075
Cove 1 vs Other	0.875	0.264	0.463
Cove 2 vs Cove 3	0.695	0.855	0.977
Cove 2 vs ODWC	0.379	0.490	0.956
Cove 2 vs Other	0.835	0.465	0.054
ODWC vs Cove 3	0.899	0.090	0.983
ODWC vs Other	1.000	0.230	0.134
Other vs Cove 3	0.907	0.453	0.299

Table 5. Pairwise F_{ST} values across all three loci in reference collections of native Ouachita smallmouth bass, and smallmouth bass from Byron State Hatchery and Broken Bow Reservoir.

	Hatchery	Mountain Fork	Buffalo Creek	Cove 1	Cove 2	Cove 3	ODWC
Ouachita Smallmouth Bass							
Mountain Fork	0.6256						
Buffalo Creek	0.5780	0.0260					
Broken Bow Reservoir							
Cove 1	0.1983	0.1896	0.1600				
Cove 2	0.2850	0.1497	0.1099	0.0198			
Cove 3	0.2839	0.1819	0.1088	0.0092	-0.0236		
ODWC	0.2872	0.1268	0.0942	0.0142	-0.0006	-0.0020	
Other	0.3275	0.1371	0.0873	0.0041	-0.0096	-0.0338	0.0013

Table 6. *Mdo1* allele frequencies in post-introduction collections from Tenkiller Reservoir and a major tributary, Baron Fork River. Bold values indicate frequency of introduced alleles.

	Alleles						
Population	A	B	C	D	E	F	Introduced
Tenkiller Reservoir (n = 107), 1999	**0.206**	0.033	**0.009**	0.061	0.061	**0.630**	**0.845**[a]
Baron Fork (n = 23), 1999	0.000	0.000	0.000	1.000	0.000	0.000	0.000

Table 7. Genotypic frequencies (%) by age in smallmouth bass from Broken Bow Reservoir in 1998. Native = only native alleles at all three microsatellite loci, non-native = only non-native alleles, F_1 hybrid = heterozygous native/non-native at all three loci, backcross = heterozygous native/ non-native at one or two loci. There was no significant age group differences in genotypic distributions (heterogeneity Chi-square = 10.3, 6 d.f., p = 0.11).

	Genotype across three loci			
Age	Native[a]	Non-native	F_1 Hybrid[a]	Backcross
0+ (n = 46)	6.5	0.0	17.4	76.1
1+ (n = 30)	6.7	0.0	16.6	76.7
2+ (n = 7)	0.0	0.0	57.2	42.8
3+ (n = 3)	33.3	0.0	0.0	66.7
Total (n = 86)	7.0	0.0	19.8	73.2

[a] If more diagnostic loci had been available, some of these would have been backcross progeny (see text).

Table 8. *Mdo1* genotypic percentages by age in smallmouth bass from Tenkiller Reservoir in 1999. Age groups did not differ significantly in genotypic distribution (heterogeneity Chi-square = 0.24, d.f. 2, P = 0.89).

	Genotype		
Age	Homozygous native	Homozygous non-native	Heterozygous
0+ (n = 50)	0.0	86.0	14.0
1+ (n = 29)	0.0	89.7	10.3
2+ (n = 7)	0.0	85.7	14.2
Total (n = 86)	0.0	87.2	12.8

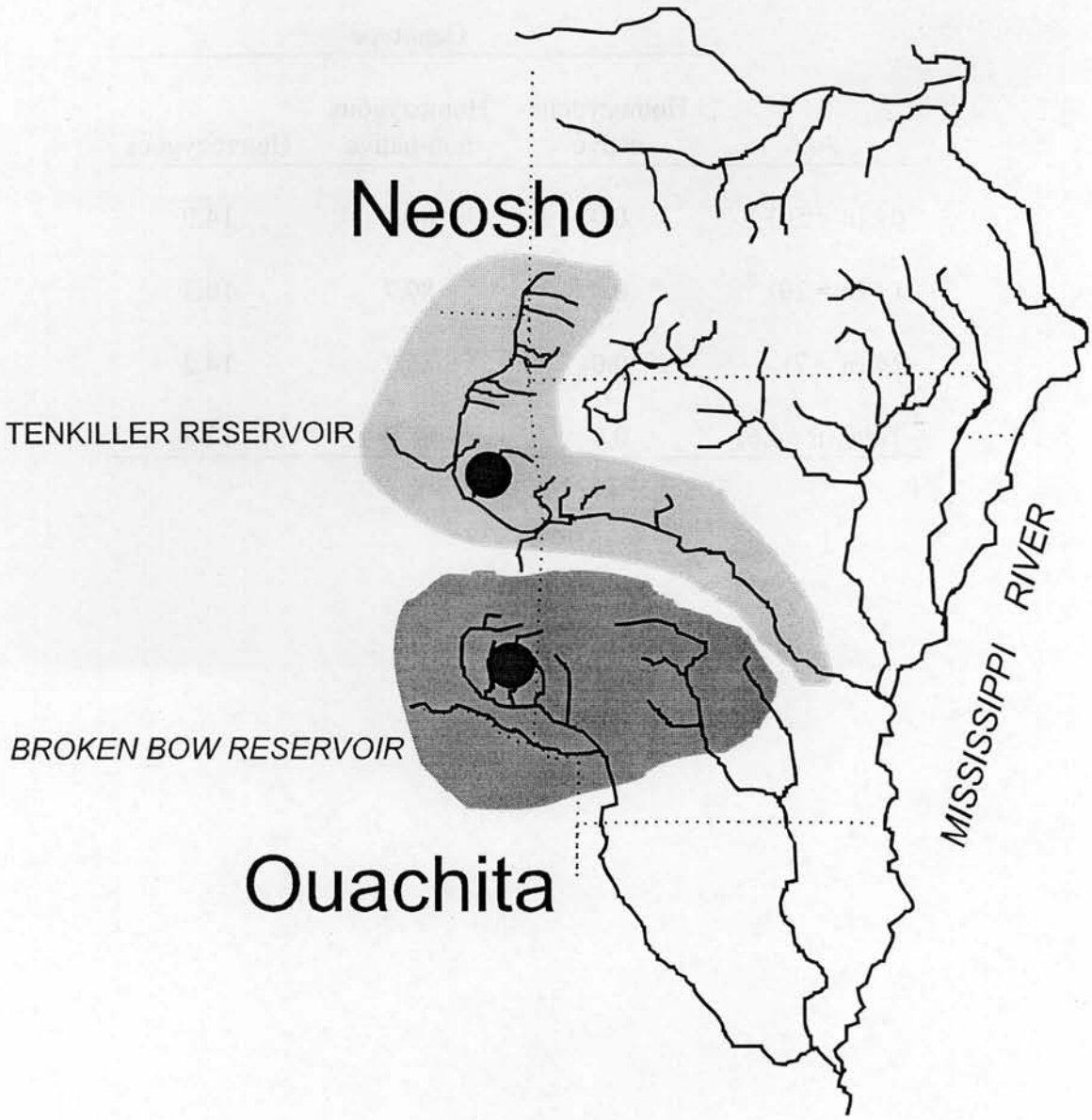


Figure 1. Native ranges of the Neosho and Ouachita smallmouth basses (Stark and Echelle, 1998). Tenkiller and Broken Bow reservoirs are represented by dots.

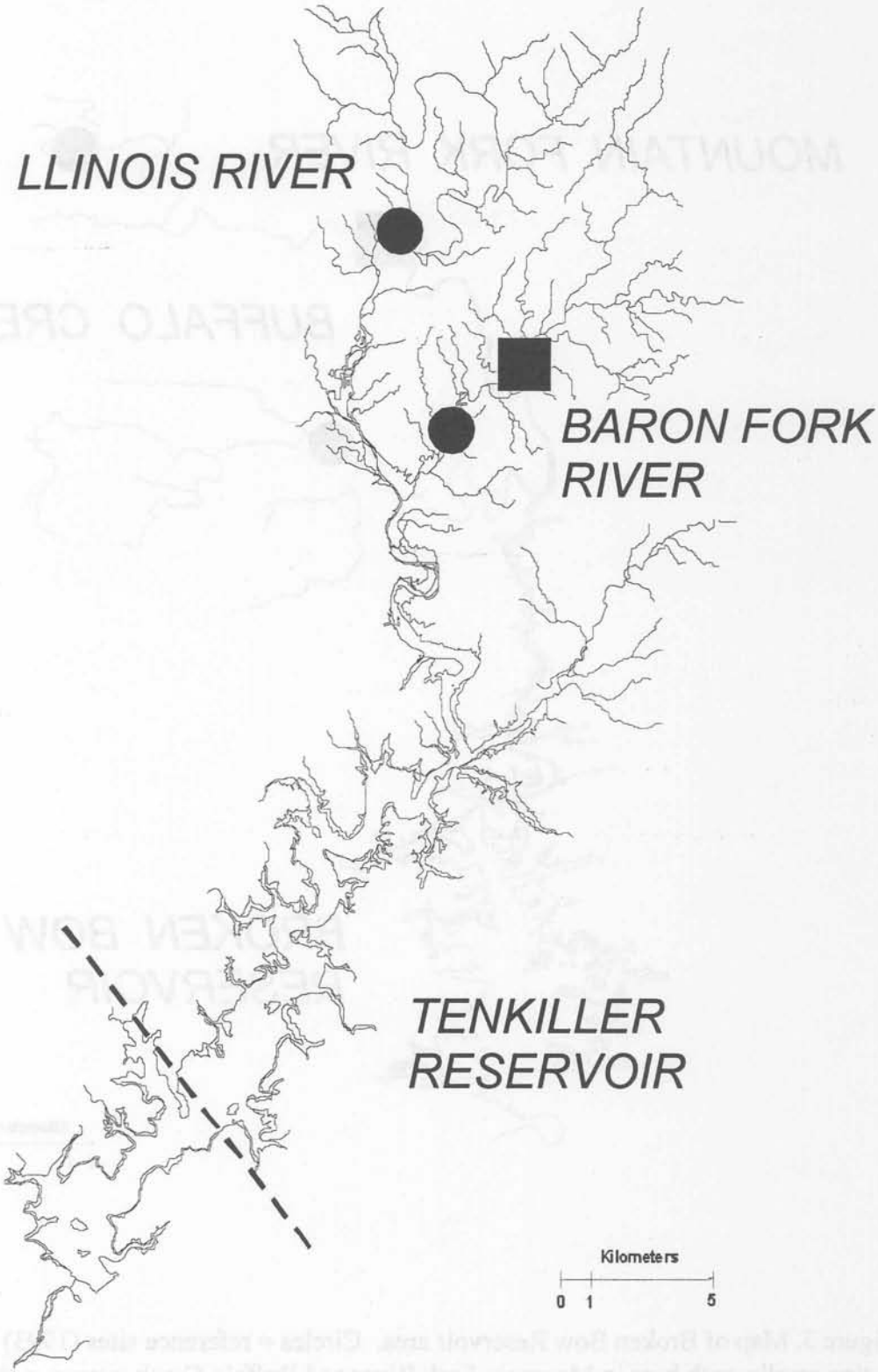


Figure 2. Map of Tenkiller Reservoir area. Circles = reference sites (1992) for native smallmouth bass in the Illinois and Baron Fork rivers, square = the 1999 collection site from Baron Fork River. Reservoir collections were taken in 1999 down-reservoir from the dashed line.

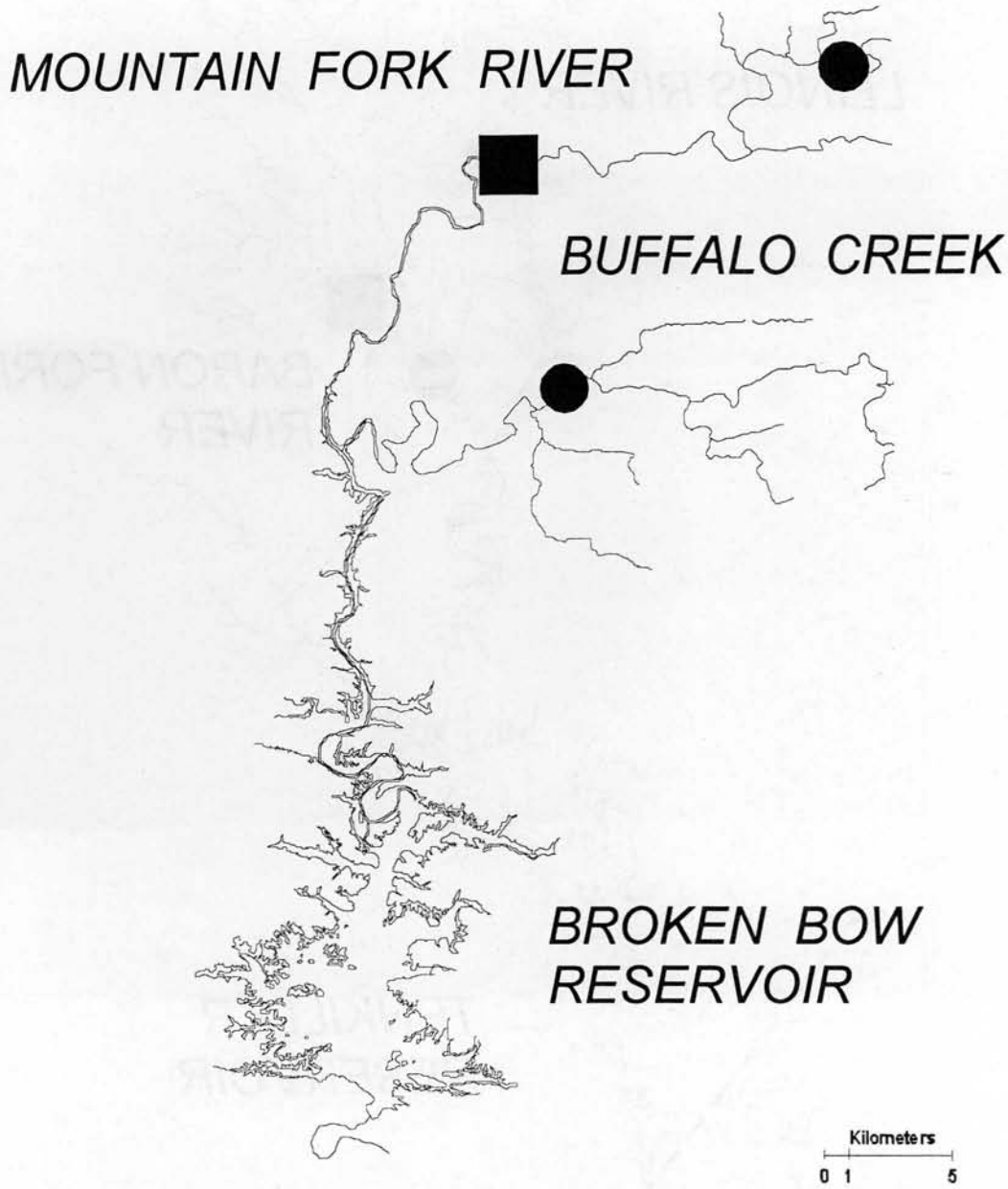


Figure 3. Map of Broken Bow Reservoir area. Circles = reference sites (1993) for native smallmouth bass in Mountain Fork River and Buffalo Creek, square = the 1999 collection site from Mountain Fork River. See Fig. 4 for areas of collection

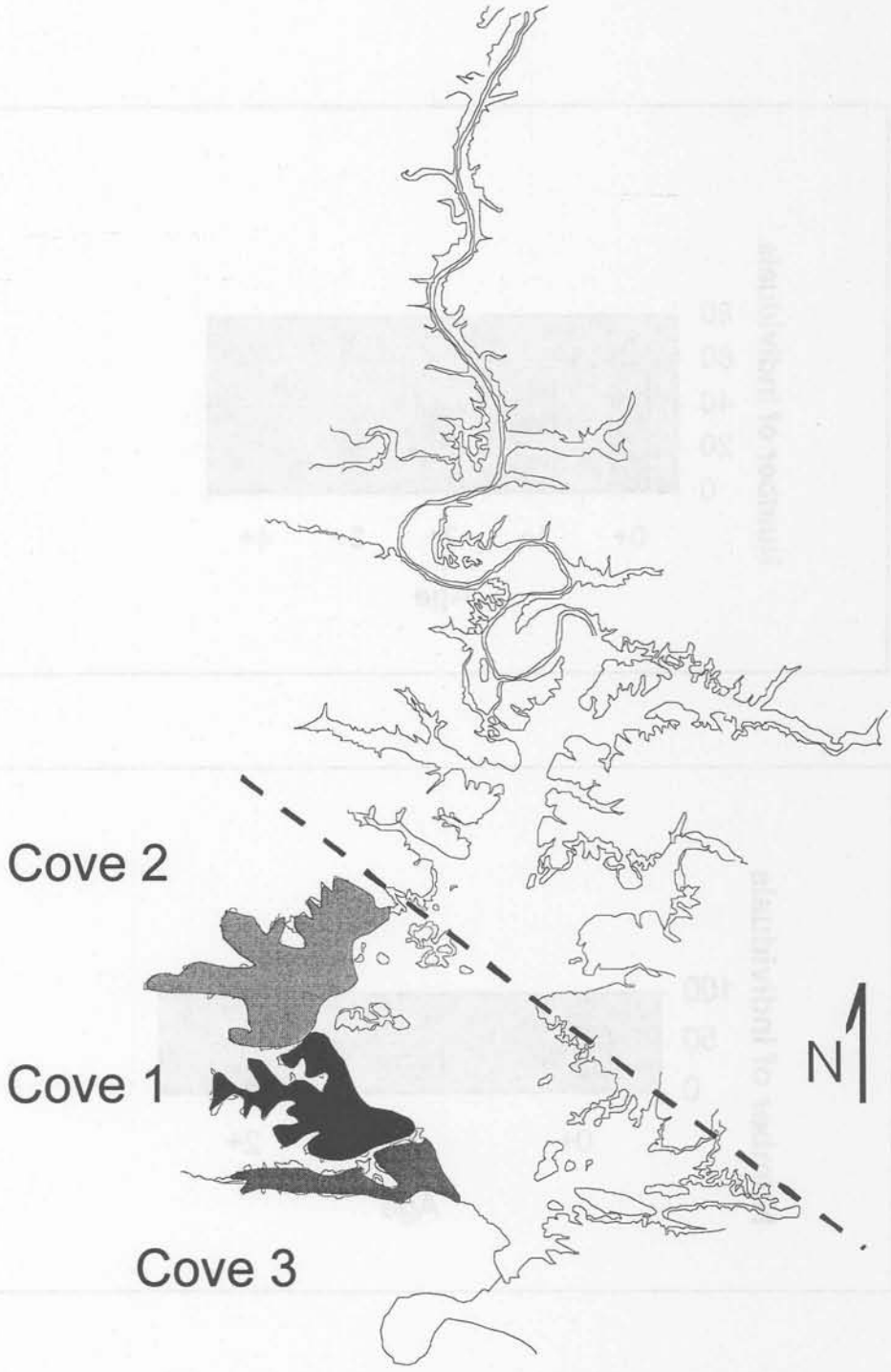
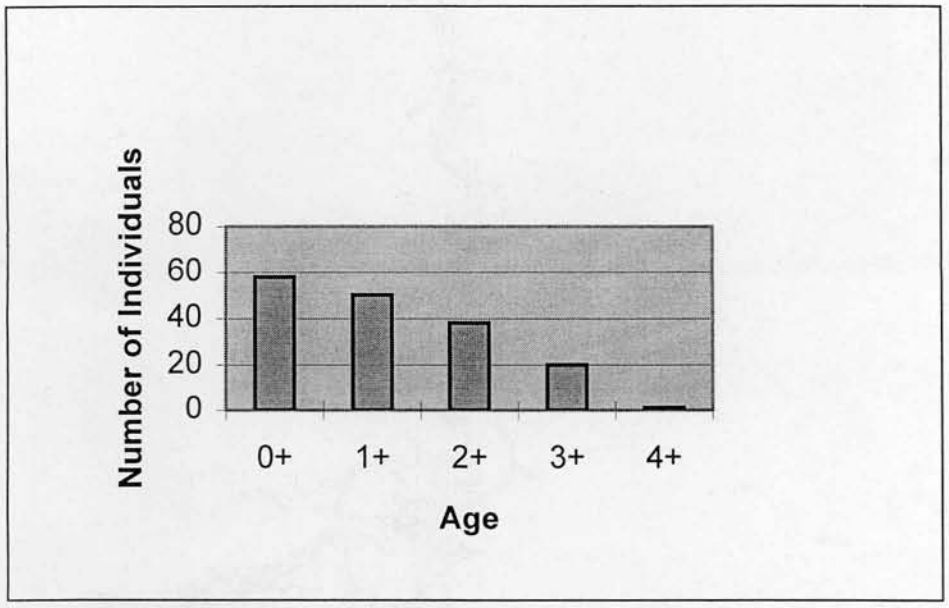


Figure 4. Localities for cove samples of smallmouth bass from Broken Bow Reservoir. The dashed line shows the up-reservoir limit of collections from the reservoir.



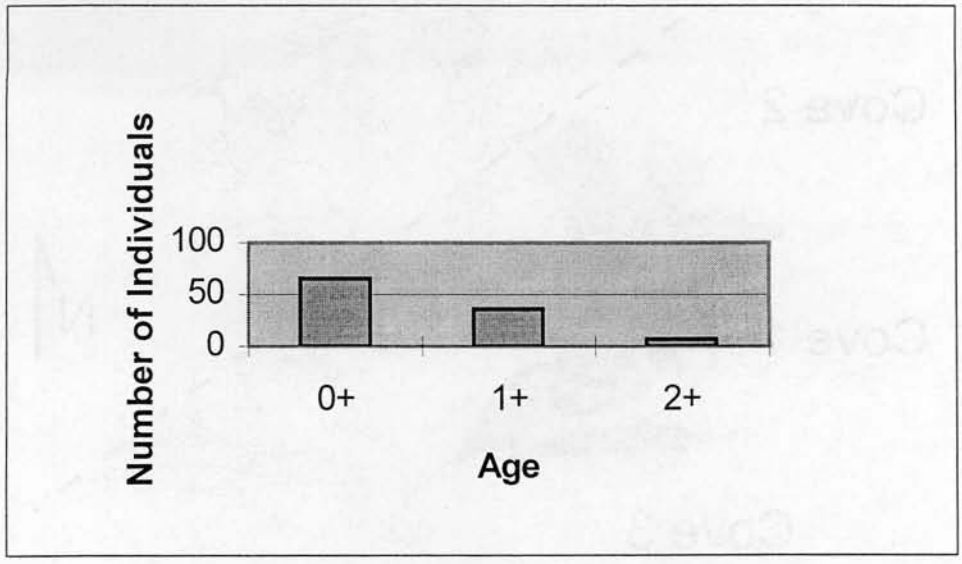


Figure 5. Age distribution of smallmouth bass from Broken Bow (Top; n = 167) and Tenkiller reservoirs (Bottom; n = 108)

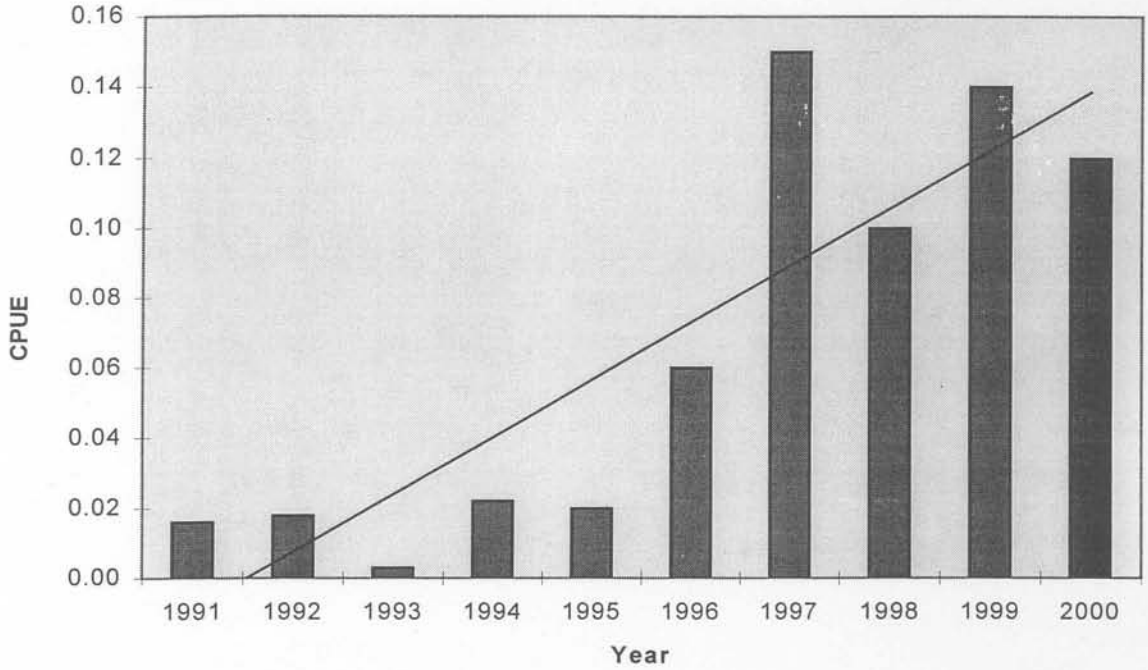


Figure 6. ODWC Fall gillnet data for smallmouth bass in Broken Bow Reservoir. CPUE = catch per unit effort expressed as catch per gillnet / hour. Solid line is the regression ($F_{(0.05;\,1,8)} = 23.9$; $P = 0.001$).

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